22. Cell Wall Polysaccharides of Marine Algae

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Marine algae are interesting as a plentiful source of many bioactive compounds, including polysaccharides, which represent various structurally different polymers of high diversity in monosaccharide composition, absolute and anomeric configuration, glycosidic linkages, molecular mass, and the presence and distribution of various functional groups. These polysaccharides are used by algae as cell wall structural components or food reserve. Algal polysaccharides are highly indicative for main algal taxa. Sulfated galactans of periodical unit/linkage sequence (agars, carrageenans) are typical for red algae, alginates, and fucoidans for brown algae; sulfated glucuronoxylorhamnans (ulvans) and other sulphated glycans for green algae. Algal cell wall polysaccharides are extracted from the raw material and further purified by preparative chromatography and/or chemical treatment. Many algal polysaccharides are assigned as phycocolloids due to their good solubility in water and their ability to create colloid systems, including gels and films. They also demonstrate various biological activities (immunomodulation, antitumor, anticoagulant, antiviral, and many other activities), which are prerequisites of pharmaceutical and medicinal applications. Finally, initially inactive or weakly active natural algal polysaccharides can be partially degraded or structurally modified to be fitted to various medicinal applications.

22.1 Overview

Marine algae are a plentiful source of many bioactive compounds. Among these chemical components, polysaccharides are of interest because of their physical and chemical properties, which are useful for food and medicinal applications. Algae synthesize various types of polysaccharides as cell wall structural components or energy reserves. Algal polysaccharides demonstrate a high specificity to raw algae, which has been used by phycologists to clarify the phylogenetic relationship between main algal taxa and higher (vascular) plants [22.1–3]. Several species of common marine algae that are the source for the isolation of polysaccharides for food and medicinal purposes are shown in Fig. 22.1.

Fig. 22.1a–i Marine algae producing polysaccharides of medicinal importance: **(a)** *Enteromorpha (Ulva) linza* (Ulvales, Chlorophyta); **(b)** *Capsosiphon fulvescens* (Ulotrichales, Chlorophyta); **(c)** *Sargassum muticum* (Fucales, Phaeophycea); **(d)** *Ecklonia cava* (Laminariales, Phaeophycea); **(e)** *Undaria pinnatifida* (Laminariales, Phaeophycea); **(f)** *Laminaria japonica* (Laminariales, Phaeophycea); **(g)** *Carpopeltis affinis* (Cryptonemiales, Rhodophyta); **(h)** *Chondrus ocellatus* (Gigartinales, Rhodophyta); **(i)** *Scinaia japonica* (Nemaliales, Rhodophyta). Algal photos were kindly provided by Dr. Myounglae Cho, East Sea Institute of the Korea Institute of Ocean Science and Technology (KIOSTI), Korea

22.1.1 Structure of Algal Cell Walls

Algal cell walls are composed of a highly integrated network of biopolymers, mainly polysaccharides, which interact with water, metal cations, and other molecules [22.2]. Structural specificities, including substitution patterns and degrees, are prerequisites of the physical and functional properties of cell wall polysaccharides in marine algae. Crystalline and fibrous parts (cellulose, xylans, hemicelluloses, etc.) are embedded in a gel-like matrix made of carboxylic and/or sulfated polysaccharides. Other biopolymers (proteins, proteoglycans, and polymeric phenolics) may also participate in cell wall formation.

22.1.2 Polysaccharides as Taxonomic Markers of Marine Algae

The great variability of cell wall polysaccharides in marine algae is determined by the specie/taxa [22.3–7], anatomical part of the alga [22.7], the developmental and life-cycle stage [22.7–11], and season and habitat [22.12, 13]. Algae represent a diverse group of photosynthetic eukaryotes with a wide range of cell wall types [22.2]. Besides pigmentation and food reserve specificity, matrix cell wall polysaccharides are useful as structural and taxonomical markers of marine algae. Cell wall polysaccharides from various marine algae of three main divisions, i. e., *Clorophyta* (green algae), *Rhodophyta* (red algae), and *Phaephyta* (brown algae), are summarized in Table 22.1.

Green algae produce various sulfated and/or carboxylic cell wall polysaccharides of specific structure. Red algae have complex composite cell walls made of cellulose, xylan, or mannan fibrils and sulfated galactans (agars, carrageenans) as the main matrix components [22.14]. Brown algae produce cell walls containing cellulose fibrils and two main types of matrix polysaccharides (alginates, fucoidans). Cell walls of all marine algae are rich with sulfated matrix polysaccharides. This is an adaptation to marine habitats that has also been observed for many other marine organisms, including angiosperms and invertebrates [22.15]. There are two groups of algal sulfated polysaccharides (SPS) [22.16], i. e., (i) uronic acid-rich (UA: uronic acid) and (ii) UA-limited ones. In the green algae of Ulvophyceae [22.3], the first group is represented by ulvans (genera *Ulva*, *Monostroma*, *Gayralia*, and *Acetabularia*) [22.5]; the second

Table 22.1 Major cell-wall polysaccharides in the main groups of algae [22.1–3]

Polysaccharides	Chlorophyta	Rhodophyta	Phaeophyta (Phaleophycea)
Crystalline	Cellulose – (1 → 4)-β-D-glucan		
	(1 → 4)-β-D-mannan		
		(1 → 4)-β-D-xylan	
		(1 → 3)-β-D-xylan	
Hemicelluloses	Xyloglucan Mannans (1 → 4)-β-D-glucuronan (1 → 3)(1 → 4)-β-D-glucan	Glucomannan (1 → 3)(1 → 4)-β-D-xylan	Sulfated xylofucoglucan Sulfated xylofucoglucuronan (1 → 3)(1 → 4)-β-D-glucan
Matrix	Ulvans Sulfated glycans	Sulfated galactans (agars, carrageenans)	Alginates Fucoidans

one includes sulfated (1 → 3)-β-D-galactans [22.17–22], (1 → 3)-β-L-arabinopyranans [22.23], and β-(1 → 4)-D-mannans [22.24] (genera *Caulerpa*, *Codium*, and *Bryopsis*). In addition, alkali-soluble linear (1 → 4)-β-D-glucuronan found in some green algae plays the role of hemicellulose [22.25]. Red algae contain linear and branched sulfated galactans having no uronic acids. By contrast, brown algae have specific kinds of carboxylic and sulfated polysaccharides, namely alginates and fucoidans; some of the latter ones may also contain galactose or glucuronic acid residues. More complex highly branched sulfated heteropolysaccharides (xylofucoglucans, xylofucoglucuronans, etc.) of brown algae were assigned as hemicelluloses.

22.2 Structural Diversity of Algal Polysaccharides

Algal cell wall polysaccharides are structurally variable polymers showing significant differences in molecular mass, monosaccharide composition, absolute and anomeric configuration of sugar units, glycosidic linkages, presence and distribution of functional groups (carboxylates, methyl ethers, acetyl esters, sulfate semiesters, 3,6-anhydro and others). There are linear and branched polysaccharides, the latter may have stubs, longer side chains or even very complex branching with defined core, inner and peripheral parts. Basic structure of common algal polysaccharides is demonstrated in Fig. 22.2. Structural variability of specific algal polysaccharides is reviewed below.

22.2.1 Alginates

Alginates are matrix cell wall polysaccharides of brown algae (Phaeophyceae). They are linear co-polymers of (1 → 4)-linked units of β-D-Man*p*A and its C-5 epimer α-L-Gul*p*A. These uronic acids are distributed in three types of blocks containing (i) only β-D-Man*p*A (M-blocks, M: mannuronic acid), (ii) α-L-Gul*p*A (G-blocks, G: guluronic acid), or (iii) alternating sequences of both these residues (MG-blocks) [22.44]. In algal cells alginates are synthesized as homomannuronan polymeric chains and then β-D-Man*p*A units are partially converted to α-L-Gul*p*A ones by enzyme C-5 epimerase. This conversion is irreversible and both types of blocks are formed. The $^4C_1$ and $^1C_4$ conformations were confirmed for β-D-Man*p*A and α-L-Gul*p*A alginate units, respectively [22.30, 45]. In the homopolymeric blocks the units are bound with diequatorial (M-blocks) and diaxial (G-blocks) glycosidic linkages, while in the alternating sequence the units are bound by mixed equatorial-axial (MG) and axial-equatorial (GM/MG – alternating blocks in alginates) linkages (Fig. 22.2a). The homopolymeric blocks form helical structures stabilized by intra-molecular hydrogen bonds involving hydroxyls, ring oxygens, and/or carboxyls; the MG-blocks take up disorder conformations. These helices demonstrate different stiffnesses: G-blocks look like *buckled ribbons* and they are stiffer than *flat-ribbon* M-blocks [22.41, 46, 47]. The M/G ratio (molar ratio between mannuronic and glucuronic acids in alginates) and distribution of co-monomer residues along an alginate chain depend on the source and geographic location of raw algal material, season of collection, the age of the parts of the plant, and extraction methodology used. The M/G ratio of alginates originating from Indian algae (the coast of Ki-

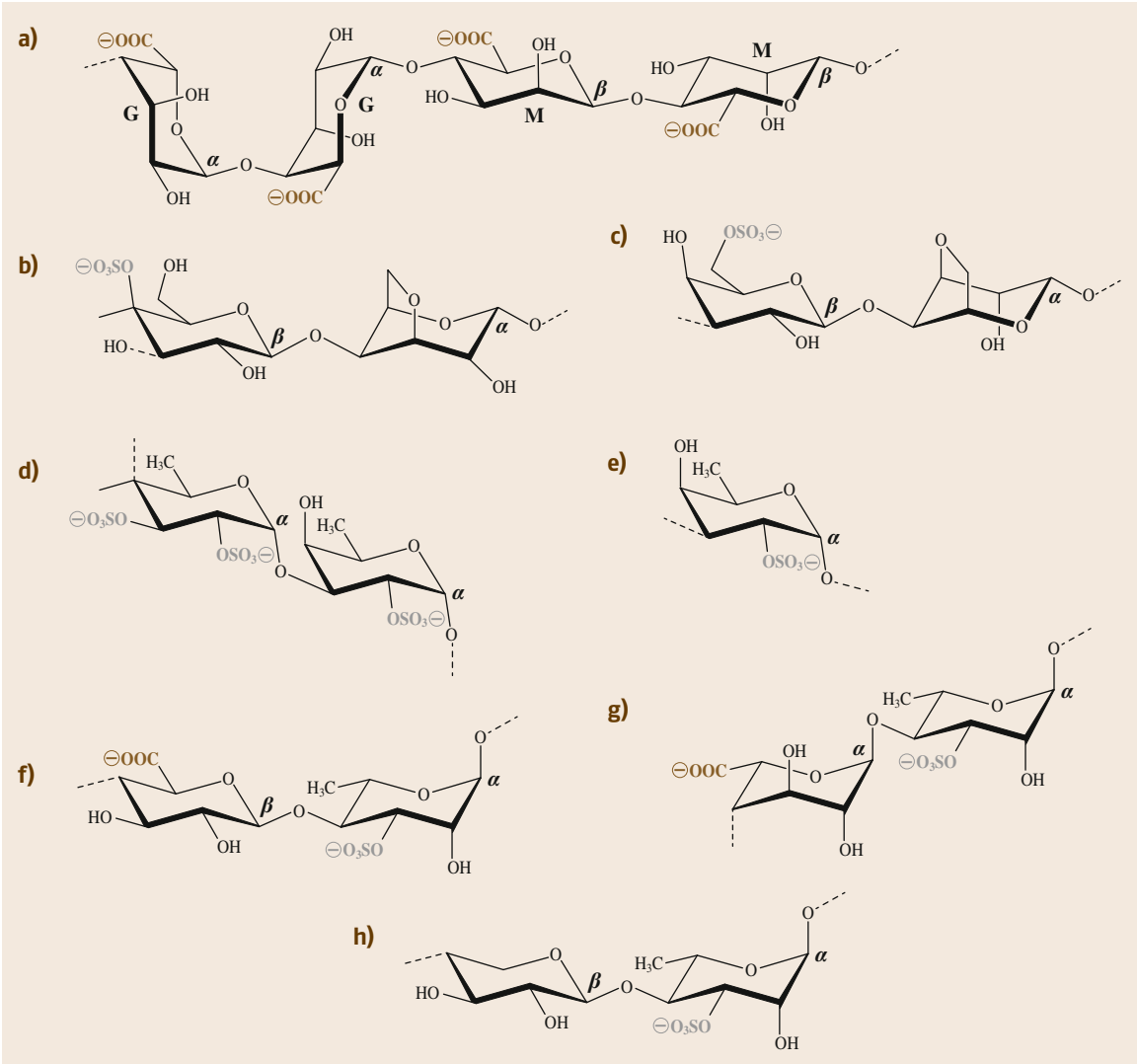

Fig. 22.2a–h Basic structures of marine algal polysaccharides: **(a)** alginate (G – guluronic acid, M – mannuronic acid); **(b)** κ-carrageenan; **(c)** agarose-6-sulfate; **(d,e)** fucoidans; **(f–h)** ulvans

lakarai, the Gulf of Mannar, South India) was reported to be maximal in summer and minimal in premonsoon seasons; this value also depends on the anatomical part of algal thalli used for isolation [22.12, 13]. The high variability of the M/G ratio for alginates from various brown algae is illustrated in Table 22.2. The block composition of alginates was investigated by a partial acidic hydrolysis (0.3 mol L^{-1} HCl, 100 °C, 2 h) followed by precipitation of the resistant fragments [22.11, 32]. Strong acidic hydrolysis of alginates from *Laminaria brasiliensis* gave rise mainly to M and G-oligosaccharide blocks, indicating preferential hydrolysis of glycosidic bonds in the MG-regions [22.42]. Two types of alginates were defined based on the distribution of monomers [22.32], i. e., those containing (i) many G-blocks, less MG-blocks, and even less M-blocks (GG > MM > MM), and (ii) a small proportion of GG and near equal proportions of MG and M-blocks (GG < MG≈MM). Alternatively, algal alginates could be classified as being high-M, low-M (or high-G), or intermediate type [22.27]. Well-defined MG-blocks were detected only for the intermediate alginates, whereas

Table 22.2 M/G values obtained for alginates from various algal sources

Order	Species	M/G value	References
Chordariales	*Myriogloeia intestinalis*	0.33	[22.26]
	Papenfusiella lutea	0.53	
	Splachnidium rugosum	0.56	
	Spermatochnus paradoxus	1.3	[22.11]
	Chordaria flagelliformis	0.9	
Desmarestiales	*Desmurestiu aculeatu*	0.85	
Dictyosiphonales	*Dictyosiphon foeniculaceus*	0.85	
Durvillaeales	*Durvillaea antarctica*	3.0–4.0	[22.26, 27]
	Durvillaea potatorum	3.17	[22.11]
	Durvillaea willana	2.57	
Dictyotales	*Zonaria* sp.	0.41	[22.28]
	Spatogossum sp.	0.75	
Ectocarpales	*Chnoospora* sp.	0.51	
	Stilophoru rhizodes	0.44–0.47	[22.29]
	Leathesia difformis	0.37	
Fucales	*Ascophyllum nodosum*	1.29–1.56	[22.11, 29–32]
	Cystophora retroflexa	1.08	[22.11]
	Cystophora torulosa	0.99	[22.26]
	Captophyllum maschalocarpum	0.94	
	Xiphpophore chondrophylla	1.36	
	Pelvetia canaliculata	1.50	[22.11]
	Sargassum sp.	0.44–1.09	[22.28]
	Sargassum fluitans	0.19–1.18	[22.33–35]
	Sargassum oligocystum	0.49–0.62	[22.34]
	Sargassum filipendula	0.19	[22.36]
	Sargassum muticum	0.31	
	Sargassum thunbergii	0.53	
	Sargassum dentifolium	0.52	[22.37]
	Sargassum asperiifolium	0.69	
	Sargassum latifolium	0.82	
	Sargassum tenerrimum	high-G	[22.38]
	Sargassum trichophyllum	1.88	[22.39]
	Sargassum siliquosum	0.72	[22.33]
	Sargassum vulgare	1.27–1.56	[22.40]
	Sargassum polycystum	0.54–0.74; 0.21	[22.13, 36]
	Turbinaria conoides	0.60–0.77	[22.12]
	Hormosira banksii	1.50; 1.33	[22.11, 26]
Laminariales	*Laminaria hyperborea*	0.62	[22.32]
		0.54–1.35 (fronds)	[22.11, 29, 31, 32, 41]
		0.40–0.65 (stipes)	[22.11, 30, 32]
	Undaria pinnatifida	1.45–2.65	[22.7]
	Laminaria japonica	2.26	
		2.34–3.18 (basal)	
		1.61–2.02 (apical)	
	Laminaria brasilliensis	1.2	[22.42]
	Kjellmaniella crassifolia	13	[22.43]

Table 22.2 (continued)

Order	Species	M/G value	References
Laminariales	*Laminaria pallida*	2.33 (stipes)	[22.48]
	Laminaria digitata	1.45	[22.11]
		1.35–2.08 (blade)	[22.29]
	Laminaria longicruris	1.44–2.17	
	Agarum cribosum	1.30 (blade)	
	Macrocystis pyriphera	1.38–1.70	[22.7, 11, 29, 32, 49]
		1.52 (frond)	
		1.02 (stipes)	
		1.41 (blade)	
	Macrocystis boryana	0.79	[22.11]
	Lessonia trabeculata	1.73	[22.50]
	Lessonia variegata	1.95	[22.26]
	Ecklonia radiata	1.60	
Scytosiphonales	*Scytosiphon lomentaria*	1.15; 0.67	

the high-M and low-M alginates consist mainly of homopolymeric blocks (GG, MM), which are almost directly connected and contain few or no heteropolymeric blocks. It was also found that young algal tissues are rich in M-blocks, and that the difference between the alginates from different species was mainly due to the contribution of the older parts of thalli [22.11].

22.2.2 Sulfated Galactans

Sulfated galactans are common cell wall matrix polysaccharides of green (*Clorophyta*) and red (*Rhodophyta*) algae. They are polymers of partially sulfated β-D- sand/or α-D(L)-galactopyranosyl (Gal*p*) units.

Sulfated galactans from cell walls of green algae (genera *Codium* and *Bryopsis*) were reported to be both homo and heteropolysaccharides, depending on the species [22.17, 19, 20, 22, 51]. Polysaccharides isolated from *Codium isthmocladum* [22.20] and *Codium yezoense* [22.17, 52] are sulfated homogalactans containing similar backbones, composed mainly of (1 → 3)-linked β-D-Gal*p* units and short oligosaccharide side chains bound at *O*-6. The units were sulfated mainly at *O*-4 and rarely at *O*-6, and nonreducing terminal residues were pyruvylated forming cyclic 3,4-ketals. Sulfated galactan isolated from *Bryopsis plumosa* (Bryopsidales, Chlorophyta) consist of a (1 → 3)-linked β-D-Gal*p* partially sulfated on *O*-6 and substituted with pyruvic acid, forming 4,6-ketals [22.19]. A branched sulfated (1 → 3), (1 → 6)-β-D-galactan was isolated from *Codium fragile*. This polysaccharide has a (1 → 3)-linked partially 4-*O*-sulfated β-D-Gal*p* backbone with some 3,4-pyruvated β-D-Gal*p* stubs at *O*-6 positions [22.21]. By contrast, galactans isolated from *Codium fragile* [22.51] and *Codium cylindricum* [22.22] contain other sugar units – arabinose and glucose, respectively. Nevertheless, the →3)-β-D-Gal*p*4S-(1→ units still predominate in these polysaccharides. Water-soluble highly sulfated arabinans, galactans, and/or arabinogalactans were isolated from *Codium fragile* and *Codium vermilara* [22.18]. These polysaccharides are composed of (1 → 3)-linked β-D-Gal*p* and β-L-Ara*p* residues; they are partially substituted with pyruvic acid ketals.

Red algal cell walls contain (1 → 3), (1 → 4) – mixed linkage α, β-D(L)-galactans. Most of these polysaccharides are nonbranched. Their backbone is made of disaccharide repeating fragments of alternating (1 → 3)-β- and (1 → 4)-α-linked Gal*p*. The former units (G) always have D-configuration, whereas the latter ones (D or L) are present as the D- (the carrageenan family) or L- (the agaran family) enantiomers (Fig. 22.2b,c). Sulfate hemiesters, methyl ethers, and pyruvic acid are commonly bound to these polysaccharides. Sulfate and/or occasionally methyl groups may occur at the *O*-2 and/or *O*-4 position(s) of the (1 → 3)-β-linked Gal*p* units and at *O*-2, *O*-3-, and/or *O*-6 position(s) of the (1 → 4)-α-linked Gal*p* units; pyruvic acid may be attached to (1 → 3)-β-D-Gal*p* residues as ketals at *O*-4 and *O*-6 positions [22.81, 82]. The 3,6-anhydro derivatives of D(L) units may be present in the backbone of carrageenose (agarose) after elimination of sulfate from *O*-6 by enzymatic or alkali treatment. Agarose, a simplified structure of agar, is a sequence of

Table 22.3 Disaccharide repeating structure and abbreviation [22.14] of some agarans and carrageenans

Agaran	Formulae (IUPAC[a])	Abbreviation
Agarose	[3)-β-D-Gal*p*-(1 → 4)-3,6a-α-L-Gal*p*-(1→]$_n$	**G**-LA[b,c]
Agarose precursor	[3)-β-D-Gal*p*-(1 → 4)-α-L-Gal*p*6S-(1→]$_n$	**G**-L6S[d;f]
Agarose sulfate	[3)-β-D-Gal*p*6S-(1 → 4)-3,6a-α-L-Gal*p*-(1→]$_n$	**G6S**-LA
Agarose sulfate precursor	[3)-β-D-Gal*p*6S-(1 → 4)-α-L-Gal*p*6S-(1→]$_n$	**G6S**-L6S
	[3)-β-D-Gal*p*-(1 → 4)-α-L-Gal*p*-(1→]$_n$	**G**-L
Carrageenan	**Formulae (IUPAC)**	**Abbreviation**
β-family	β [3-β-D-Gal*p*-(1 → 4)-3,6a-α-D-Gal*p*-(1→]$_n$	**G**-DA[c]
	γ [3-β-D-Gal*p*-(1 → 4)-α-D-Gal*p*6S-(1→]$_n$	**G**-D6S[e]
	ψ [3-β-D-Gal*p*6S-(1 → 4)-3,6a-α-D-Gal*p*2S-(1→]$_n$	**G6S**-DA**2S**
	ω [3-β-D-Gal*p*6S-(1 → 4)-α-D-Gal*p*2,6diS-(1→]$_n$	**G6S**-D**2S**6S
λ-family	α [3-β-D-Gal*p*-(1 → 4)-α-D-Galp2S-(1→]$_n$	**G**-DA**2S**
	δ [3)-β-D-Gal*p*-(1 → 4)-α-D-Gal*p*2,6diS-(1→]$_n$	**G**-D**2S**6S
	θ [3)-β-D-Gal*p*2S-(1 → 4)-3,6a-α-D-Gal*p*2S-(1→]$_n$	**G2S**-DA2S
	λ [3)-β-D-Gal*p*2S-(1 → 4)-α-D-Gal*p*2,6diS-(1→]$_n$	**G2S**-D2S6S
	ξ [3)-β-D-Gal*p*2S-(1 → 4)-α-D-Gal*p*2S-(1→]$_n$	**G2S**-D2S
	π [3)-β-D-Gal*p*Pyr2S-(1 → 4)-α-D-Gal*p*2S-(1→]$_n$ [g]	**GP2S**-D2S
κ-family	κ [3-β-D-Gal*p*4S-(1 → 4)-3,6a-α-D-Gal*p*-(1→]$_n$	**G4S**-DA
	ι [3-β-D-Gal*p*4S-(1 → 4)-3,6a-α-D-Gal*p*2S-(1→]$_n$	**G4S**-DA2S
	μ [3-β-D-Gal*p*4S-(1 → 4)-α-D-Gal*p*6S-(1→]$_n$	**G4S**-D6S
	ν [3-β-D-Gal*p*4S-(1 → 4)-α-D-Gal*p*2,6diS-(1→]$_n$	**G4S**-D2S6S
	o [3-β-D-Gal*p*4S-(1 → 4)-α-D-Gal*p*2S-(1→]$_n$	**G4S**-D2S

[a] International Union of Pure and Applied Chemistry (IUPAC); [b] first unit in agarans and carrageenans (G); [c] 3,6-anhydro units (LA, DA); [d] second unit in agarans (L); [e] second unit in carrageenans (D); [f] sulfation of hydroxyls (S); [g] pyruvation of hydroxyls (Pyr)

identical disaccharide (agarobiose) fragments without sulfates. By contrast, real agars have some α-L-Gal*p*6S (porphyran), pyruvic acid 4,6-ketals, *O*-methyl, or sulfate groups attached to β-D-Gal*p* residues [22.81, 83]. Similarly, carrageenans may have a structure described by one of the defined disaccharides (carrabioses) and thus are classified by Greek prefixes according to their structural specificity, i. e., sulfation patterns and the presence of 3,6-anhydro groups (Table 22.3). More often, however, they contain several types of disaccharide fragments, i. e., hybrid types (Table 22.4). Structural and conformational analysis of κ/ι hybrid carrageenans from *Sarcothalia crispala*, *Mazaella laminarioitles*, and *Chonrlrus crispus* showed that they are not a mix of pure carrageenans but have a mixed chain containing both κ and ι-disaccharide fragments [22.84]. Gametophyte and sporophyte generations of the algal biological cycle contain carrageenans of different structure, especially sulfate and 3,6-anhydride groups. Carrageenans of *Phyllophoraceae* and *Gigartinaceae* were described to be hybrids of κ, ι, and ν forms for gametophytes and rather λ subfamily forms (λ, ξ, and π) for tetrasporophytes [22.9, 10, 64, 68, 69, 85, 86]. Carrageenans usually contain more sulfates than agarans, while the latter often contain more methyl and glycosyl groups (Table 22.3). The structural variability of red algal galactans, especially of their substitution patterns, has been described for different species, harvesting location, or seasons. Sometimes the structure of red algal galactans (α/β anomers, D/L enantiomers, sequence of linkages, substitution patterns, etc.) deviates from the defined structural types of agars and carrageenans. Other sugars (Xyl, Glc, GlcA, Ara, or Man) are bound to some of them as backbone or side chain parts [22.87]. There are also some polysaccharides, named DL-galactan hybrids, having both D and L-units distributed within the same polysaccharide chain [22.88]. The real structure of red algae sulfated galactans is probably a result of the following stepwise biosynthesis: (i) formation of the regular polymer backbone, (ii) sulfation, methylation, and/or pyruvation of specific hydroxyls, and (iii) enzymatic

Table 22.4 Hybrid carrageenans and DL-galactans

Algal species	Structure	References
Mastocarpus stellatus	κ/ι [a]	[22.53]
Kappaphycus striatum	$\kappa/\iota/\kappa_{6Me}/\iota_{6Me}$ [b]	[22.54]
Furcellaria lumbricalis	κ/β	[22.55]
Tichocarpus crinitus	$\kappa/\beta/\mu$	[22.56]
Kappaphycus alvarezzi	κ/μ	[22.57]
Sarconema filiforme	$\iota/\alpha/\alpha_{Pyr}$ [c]	[22.58]
Stenogramme interrupta	$\iota/\alpha/\alpha_{Pyr}$ (cystocarpic) λ/ξ (tetrasporophyte)	[22.8]
Eucheuma denticulatum	ι/ν	[22.59, 60]
Coccotylus truncatus	$\iota/\nu/\alpha_{Pyr}$	[22.61]
Botryocladia occidentalis	Unusual	[22.62]
Meristiella gelidium	$\kappa/\iota/\nu$	[22.63]
Gigartina pistillata	$\kappa/\iota/\nu$ (gametophyte) $\lambda/\xi/\pi$ (tetrasporophyte)	[22.10, 64]
Gigartina alveata, Gigartina clavifera	κ/ι (gametophyte) ξ/θ (tetrasporophyte)	[22.9]
Gigartina chapmanii	$\xi/\lambda/\theta/\pi$ (tetrasporophyte)	[22.10]
Gigartina skottsbergii	$\lambda/\xi/\delta$, μ/ν, D/L κ/ι, μ/ν (gametophyte) λ/θ (tetrasporophyte)	[22.65] [22.66, 67] [22.68] [22.69]
Sarcothalia crispate	κ/ι, μ/ν (gametophyte) λ/θ (tetrasporophyte)	[22.69]
Gigartina atropurpurea	κ/ι, μ/ν (gametophyte) λ/θ (tetrasporophyte)	[22.70]
Schizymenia binderi	D/L	[22.71]
Gymnogongrus torulosus	D/L	[22.72, 73]
Cryptonemia seminervis	D/L	[22.74]
Cryptonemia crenulata	D/L	[22.75, 76]
Halymenia durvillei	D/L	[22.77]
Gloiopeltis furcata	D/L	[22.78]
Digenea simplex	D/L	[22.79]

[a] More κ/ι hybrid carrageenans are reviewed by [22.80]; [b] methylation of hydroxyls (Me); [c] pyruvation of hydroxyls (Pyr)

conversion of some (1 → 4)-α-linked Gal*p*6S residues to 3,6-anhydro derivatives. According to this sequence, porphyran is a precursor of agarose, and, similarly, it is true for the pairs of carrageenans. Formation of 3,6-anhydrogalactose, similar to the final step of biosynthesis, is possible by the treatment with heated dilute alkali on the corresponding precursor. The corallinans, which are agar-like branched sulfated and methylated galactans from red algae of the order *Corallinales*, have (a) an agaran backbone, (b) a β-D-Gal*p* unit almost completely substituted at *O*-6 mostly with β-D-xylosyl side chains and less by sulfate, and (c) α-L-Gal*p* units partly substituted by methoxyl and sulfate groups at *O*-2 [22.89]. In addition, the latter units can be substituted at *O*-3 or *O*-6 with partially *O*-methylated α-D- or α-L-Gal*p* side chains (Table 22.5). Polysaccharides from various algae may differ in the position of these branches. There is a balance between the hydrophobic (methoxyl) and the hydrophilic (sulfate) groups, so the algal enzymatic system equilibrates their proportions to fulfill their biological design [22.90]. Decalcified tissue from *Calliarthron cheilosporioides* contains sulfated galactan, highly methoxylated but lowly glycosylated with xylose at *O*-6 of the β-D-Gal*p* units [22.91]. By contrast, calcified segments of this alga produce xylogalactans with high levels of xylose and low levels of 6-*O*-methyl ethers. Therefore, decalcification of algal tissues led to replacing xylosyl branches by methyl groups. These structural changes are caused by specific methoxyl transferase blocking xylosylation in decalcified tissue.

22.2.3 Fucoidans

Fucoidans are sulfated matrix cell wall polysaccharides of brown algae (Phaeophyceae). Simple fucoidans are sulfated homofucans, i. e., polysaccharides consisting of sulfated fucose only. However, in some reports this term is used to describe complex fucose-containing heteropolysaccharides. The chemical composition and structure of fucoidans are complex and variable, reflecting the differences in biosynthesis [22.6, 152]. Very often the exact structure of these polysaccharides remains unclear even when modern methods of structural analysis like selective enzymatic cleavage or correlation NMR analyses are applied. This problem is caused by significant irregularities in the structure of algal fucoidans, including the presence of numerous minor sugar and nonsugar components, random sulfation, and/or acetylation [22.6, 153]. There are two main types of backbone structure

Table 22.5 Side chain positions in corallinans and other agar-like branched sulfated xylogalactans

Source	Side chain	Position	References
Bossiella cretacea	β-D-Xyl*p*	*O*-6 of β-D-Gal*p*(2S) [a]	[22.82, 92]
Gymnogongrus torulosus	β-D-Xyl*p*	*O*-6 of β-D-Gal*p*(2S)	[22.93]
Pachymenia lusoria	β-D-Xyl*p*	*O*-6 of β-D-Gal*p*(2S)	[22.94]
Calliarthron cheilosporioides	β-D-Xyl*p*	*O*-6 of β-D-Gal*p*(2S)	[22.91]
Polysiphonia nigrescens	β-D-Xyl*p*	*O*-6 of β-D-Gal*p*(2S)	[22.95]
Joculator maximus	β-D-Xyl*p*	*O*-6 of β-D-Gal*p*(2S)	[22.96]
Corallina pilulifera	β-D-Xyl*p*	*O*-6 of β-D-Gal*p*(2S)	[22.97, 98]
Cryptonemia crenulata	β-D-Xyl*p*	*O*-6 of β-D-Gal*p*(2S)	[22.75]
Bostrychia montagnei	β-D-Xyl*p*,β-D-Gal*p*	*O*-6 of β-D-Gal*p*(2S)	[22.99]
Kappaphycus alvarezii	β-D-Xyl*p*,β-D-Gal*p*	*O*-2 and/or *O*-4 of β-D-Gal*p* *O*-3 and/or di-*O*-2,3 of α-D(L)-Gal*p*	[22.100]
Lithothamnion heterocladum	β-D-Xyl*p* 2,3-di-*O*-Me-D-Gal*p* [b]	*O*-6 of β-D-Gal*p* *O*-3 and *O*-6 of α-L-Gal*p*	[22.89]
Jania rubens	β-D-Xyl*p* 2,3-di-*O*-Me-D-Gal*p* 3-*O*-Me-D-Gal*p* 3-*O*-Me-L-Gal*p*	*O*-6 of β-D-Gal*p* *O*-3 and/or *O*-6 of α-L-Gal*p*	[22.101]
Corallina officinalis	β-D-Xyl*p* 4-*O*-Me-D-Gal*p*	*O*-6 of β-D-Gal*p* *O*-6 of α-L-Gal*p*	[22.90, 102]
Gracilaria tikvahiae	4-*O*-Me-L-Gal*p*	*O*-6 of β-D-Gal*p*	[22.103]
Gracilaria verrucosa	4-*O*-Me-L-Gal*p*	*O*-6 of β-D-Gal*p*	[22.104]
Acanthophora spicifera	β-D-Xyl*p*	*O*-3 of α-L-Gal*p*2,6diS	[22.105]
Palisada flagellifera	β-D-Xyl*p*, β-D-Gal*p*, 2,3-di-*O*-Me-D-Gal*p*	*O*-3 of α-L-Gal*p*6S	[22.106]
Phacelocarpus peperocarpos	β-D-Xyl*p*	*O*-3 of α-L-Gal*p*6S	[22.107]
Laurencia nipponica	β-D-Xyl*p*	*O*-3 of α-L-Gal*p*6S	[22.108]
Nothogenia fastigiata	β-D-Xyl*p*,β-D-Gal*p*	*O*-3 of α-L-Gal*p*6S	[22.109]
Chondria macrocarpa	β-D-Xyl*p*	*O*-3 of α-L-Gal*p*6S	[22.110]
Ceramium rubrum	β-D-Xyl*p*	Not defined	[22.110]
Okamurina (Laingia) pacifica	β-D-Xyl*p*	*O*-2 of α-L-3,6aGal*p*	[22.111]
Pterocladiella capillacea	β-D-Xyl*p*	*O*-6 of β-D-Gal*p* *O*-3 of α-L(D)-Gal*p*	[22.112]

[a] Sulfation of hydroxyls (S); [b] methylation of hydroxyls (Me)

found in fucoidans [22.152–154] (Fig. 22.2d,e, Table 22.6). The first type of fucoidan backbone structure consisting of (1 → 3)-linked partially or completely sulfated α-L-Fuc*p* units revealed fucoidans from orders *Ectocarpales* and *Laminares*: *Adenocystis utricularis* [22.141], *Analipus japonicus* [22.114], *Chordaria flagelliformis* [22.140], *Chorda filum* [22.127], *Ecklonia kurome* [22.123], and *Saccharina latissima* [22.128]. The second type is characterized by alternating the (1 → 3) and the (1 → 4)-linked α-L-Fuc*p* sequence. This backbone structure is common for fucoidans from *Stoechospermum marginatum* (order Dictyotales) [22.116] and from algae of the order Fucales, i. e., *Ascophyllum nodosum* [22.117], genera *Fucus* [22.118–120] and *Sargassum* [22.121]. Single α-L-Fuc stubs or short fuco-oligosaccharide sequences are present in many fucoidans as side chains attached mainly at *O*-2 of the backbone units. Structurally different fucoidans are commonly produced by the same algal species. For example, fucoidan isolated from *Ascophyllum nodosum* has a linear structure of alternating (1 → 3) and (1 → 4)-linked sulfated α-L-Fuc*p* [22.116]. By contrast, another fucoidan isolated from this alga is defined as a highly-

Table 22.6 Basic structures of sulfated fucans (fucoidans) from brown algae

Order	Source	Backbone	Side chains	Position	Reference
Dictyotales	*Stoechospermum marginatum*	→ 3)-α-L-Fuc*p*(2S,4S)-(1 → 4)-α-L-Fuc*p*(2S)-(1→			[22.113]
Ectocarpales	*Analipus japonicus*	→ 3)-α-L-Fuc*p*(2S,4Ac,S)-(1→	α-L-Fuc*p*	*O*-4;*O*-2	[22.114]
Fucales	*Ascophyllum nodosum*	→ 3)-α-L-Fuc*p*2S(4S)-(1→ ; →4)-α-L-Fuc*p*2S-(1→	α-L-Fuc*p*	*O*-2	[22.115]
			FOS[a]		
	Fucus vesiculosus	→ 3)-α-L-Fuc*p*2S-(1 → 4)-α-L-Fuc*p*2,3diS-(1→			[22.116, 117]
	Fucus distichus	→ 3)-α-L-Fuc*p*2,4diS-(1 → 4)-α-L-Fuc*p*2S-(1→			[22.118]
	Fucus evanescens	→ 3)-α-L-Fuc*p*2S(4S)-(1 → 4)-α-L-Fuc*p*2S-(1→			[22.119]
	Fucus serratus	→ 3)-α-L-Fuc*p*2S4Ac-(1 → 4)-α-L-Fuc*p*2S3Ac-(1→	FOS[a]	*O*-4	[22.120]
	Sargassum horneri	→ 3)-α-L-Fuc*p*2S(4S)-(1 → 4)-α-L-Fuc*p*(2S)3S-(1→	α-L-Fuc*p*3S	*O*-2	[22.121]
		→ 3)-α-L-Fuc*p*2S-(1→; →4)-α-L-Fuc*p*2S-(1→ → 3)-α-L-Fuc*p*-(1 → 4)-α-L-Fuc*p*-(1→ (three fractions)			[22.122]
Laminariales	*Ecklonia cava*	→ 3)-α-L-Fuc*p*2S-(1 → 4)-α-L-Fuc*p*2S-(1→			
	Ecklonia kurome	→ 3)-α-L-Fuc*p*4S-(1 → 3)-α-L-Fuc*p*-(1→ (major) →?)-α-L-Fuc*p*-(1→2)-Gal-(1 → 4)-α-L-Fuc*p*-(1→ (minor)	α-L-Fuc	*O*-4	[22.123]
			α-L-Fuc4S	*O*-2	
	Laminaria cichorioides	→ 3)-α-L-Fuc*p*2S(4S)-(1→			[22.124]
		→ 4)-α-L-Fuc*p*2,3diS-(1→			[22.125]
		→ 3)-α-L-Fuc*p*(2S,4S)-(1→; →4)-α-L-Fuc*p*(4S)-(1→	α-L-Fuc	*O*-2;*O*-4	[22.126]
	Chorda filum	→ 3)-α-L-Fuc*p*(2S,Ac)4S-(1→	α-L-Fuc*p*	*O*-2	[22.127]
	Saccharina latissima	→ 3)-α-L-Fuc*p*(2S)4S-(1→	α-L-Fuc*p*	*O*-2	[22.128]

[a] FOS – fucooligosaccharides

branched polysaccharide with mainly (1 → 3) and a few α-(1 → 4)-linked sulfated α-L-Fuc in the core region; branch points are at *O*-2 position of the (1 → 3)-linked backbone units [22.115]. Three fucoidans from *Sargassum hornery* have a different backbone structure: (1 → 3), (1 → 4), and mixed (1 → 3),(1 → 4)-linked α-L-fucans; the latter polysaccharide was nonsulfated [22.122].

Some fucoidans were identified as not true fucans but rather complex heteropolysaccharides containing fucose as the major or minor component of the backbone (core), stubs, or longer side chains [22.152–154]. The structural features of these polysaccharides are summarized in Table 22.7. The core of such fucoidans is commonly built of alternating hexuronic acids–hexose sequences. This backbone is very sta-

Table 22.7 Basic structures of fucose containing heteroglycans from brown algae

Order	Source	Backbone	Side chains	Position	Reference
Laminariales	*Kjellmaniella crassifolia*	→ 4)-β-D-Glc*p*A-(1→2)-α-D-Man*p*(6S)-(1→	α-L-Fuc*p*(2S,3S,4S)	*O*-3 of α-D-Man*p*	[22.129]
	Saccharina (Laminaria) japonica	→ 4)-β-D-Glc*p*A-(1→2)-α-D-Man*p*-(1→	α-L-Fuc*p*(2S,4S) fucooligosaccharides		[22.130]
		→ 3)-β-D-Glc*p*A-(1→	α-L-Fuc*p*(2S,4S) fucooligosaccharides		
		→ 3)-α-L-Fuc*p*(2S,4S)-(1→ (major)	α-L-Fuc*p*4S	*O*-2 (major)	[22.131]
		→ 4)-α-L-Fuc*p*(2S)-(1→ (minor)	β-D-Gal*p*3,4diS-(1→6)-β-D-Gal*p*4S	*O*-4	
	Saccharina latissima	→ 6)-β-D-Gal*p*-(1→	α-L-Fuc*p*, β-D-Gal*p*	*O*-2	[22.128]
		→ 4)-β-D-Glc*p*A-(1→2)-α-D-Man*p*-(1→	α-L-Fuc*p*	*O*-3 of α-D-Man*p*	
		→ 3)-β-D-Glc*p*A-(1→	α-L-Fuc*p*	*O*-3	
	Saccharina longicruris	→ 3)-α-L-Fuc*p*4S-(1→; →6)-β-D-Gal*p*3S-(1→			[22.132]
	Undaria pinnatifida (sporophyll)	→ 3)-α-L-Fuc*p*2S-(1→; →3)-β-D-Gal*p*6S-(1→ → 4)-β-D-Gal*p*(3S,6S)-(1→; →6)-β-D-Gal*p*3S-(1→			[22.133–137]
	Costaria costata	→ 3)-α-L-Fuc*p*(2S,4S)-(1 → 3)-β-D-Gal*p*(2S,6S)-(1→			[22.122, 138]
		→ 3)-β-D-Glc*p*A-(1→2)-α-D-Man*p*-(1→ (core)	Galacto- and fucooligosaccharides	*O*-4 of β-D-Glc*p*A	
Ectocarpales	*Cladosiphon okamuranus*	→ 3)-α-L-Fuc*p*(4S)-(1→	α-D-Glc*p*A	*O*-2	[22.129, 139]
	Chordaria flagelliformis	→ 3)-α-L-Fuc*p*(2S,4S)-(1→	α-D-Glc*p*A	*O*-2	[22.140]
			α-L-Fuc*p*, α-L-Fuc*f*-(1→2)-α-L-Fuc*f*	*O*-4	
	Adenocystis utricularis	→ 3)-α-L-Fuc*p*4S-(1→; →3)-β-D-Gal*p*4S-(1→ → 6)-β-D-Gal*p*4S-(1→	α-L-Fuc*f* , α-L-Fuc*p*(2S)	*O*-2	[22.141]
Dictyotales	*Spatoglossum schroederi*	→ 4)-β-D-Gal*p*3S-(1 → 4)-β-D-Gal*p*-(1→ (core)	Xylofuco-oligosaccharides	*O*-2 of β-D-Gal*p*	[22.142]
		→ 3)-β-D-Glc*p*A-(1→ (core)		*O*-4	[22.143]
	Padina gymnospora	→ 3)-β-D-Glc*p*A-(1→; →4)-β-D-Glc*p*A-(1→	α-L-Fuc*p*3S, β-D-Xyl*p*	*O*-2	[22.144]

ble and other sugars including sulfated or nonsulfated α-L-Fuc*p* can be attached to it as stubs or longer side chains. The complete highly branched molecule is thus structurally similar to rhamnogalacturonans of higher plants and may perform similar functions in algal cell walls. For example, highly branched fucoidan (92.7 kDa) isolated from *Hizikia fusiforme* contains a fucose-free core composed of the alternating sequence → 2)-α-D-Man*p*6S-(1 → 4)-β-D-Glc*p*A-(1 → with a little amount of → 4)-β-D-Gal*p*-(1 → [22.146]. The α-D-Man*p*6S units are sulfated or glycosylated (branching points) at the *O*-6 position. Side chains are formed by α-D-Xyl*p*, sulfated α-L-Fuc*p*, fuco and xylo-oligosaccharides, and longer → 6)-β-D-Gal*p*3S-(1→

Table 22.7 (continued)

Order	Source	Backbone	Side chains	Position	Reference
Fucales	*Ascophyllum nodosum* (ascophyllan)	→ 4)-β-D-Man*p*A-(1→; other UA	β-D-Xyl*p*-(1 → 3)-α-L-Fuc*p*4S		[22.145]
	Hizikia fusiforme	→ 4)-β-D-Glc*p*A-(1→2)-α-D-Man*p*(4S)6S-(1→ → 4)-β-D-Gal*p*-(1→ (core)	→ 6)-β-D-Gal*p*3S-(1→ sulfated α-L-Fuc*p*; α-D-Xyl*p* fuco- and xylooligosaccharides	*O*-2 of β-D-Gal*p* *O*-3 of α-D-Man*p*	[22.146]
	Sargassum stenophyllum	→ 6)-β-D-Gal*p*-(1→2)-β-D-Man*p*-(1→ (core)	→ 3)-α-L-Fuc*p*4S-(1→ → 4)-α-L-Fuc*p*4S-(1→ → 4)-α-D-Glc*p*A-(1→ → 4)-α-D-Glc*p*-(1→; β-D-Xyl*p*		[22.147]
	Sargassum linifolium (sargassan)	→ 4)-β-D-Glc*p*A-(1 → 4)-β-D-Man*p*-(1→ (core)	→ 4)-β-D-Gal*p*(3S,6S)-(1→ → 2)-β-D-Xyl*p*-(1→ → 3)-α-L-Fuc*p*4S-(1→	internal peripheral	[22.148–151]
	Sargassum trichophyllum	→ 3)-α-L-Fuc*p*(2S,4S)-(1→; →6)-β-D-Gal*p*-(1→			[22.39]

Table 22.8 Basic structures of ulvans from *Ulva* sp. (after [22.5])

Name	Structure	Abbreviation	Species
Ulvanobiuronic acid 3-sulfate A	→ 4)-β-D-Glc*p*A-(1 → 4)-α-L-Rha*p*3S-(1→	$\mathbf{A_{3s}}$	*Ulva rigida*
Ulvanobiuronic acid 3-sulfate B	→ 4)-α-L-Ido*p*A-(1 → 4)-α-L-Rha*p*3S-(1→	$\mathbf{B_{3s}}$	*Ulva* sp.
Ulvanobiose 3-sulfate	→ 4)-β-D-Xyl*p*-(1 → 4)-α-L-Rha*p*3S-(1→	$\mathbf{U_{3s}}$	*Ulva rigida*
Ulvanobiose 2′, 3-sulfate	→ 4)-β-D-Xyl*p*2S-(1 → 4)-α-L-Rha*p*3S-(1→	$\mathbf{U_{2's,3s}}$	*Ulva rigida*
Ulvanobiuronic acid 2′-glucuronic acid 3-sulfate A	→ 4)-β-D-Glc*p*A[β-D-Glc*p*A-(1→2)]-(1 → 4)-α-L-Rha*p*3S-(1→	$\mathbf{A_{2'g,3s}}$	*Ulva rigida*

chains. Another variant of the core was found in fucoidan of *Padina gymnospora* [22.144]. The backbone of this polysaccharide is glucuronan, consisting mainly of (1 → 3)- or (1 → 4)-linked β-D-GlcA. Ascophyllan, a branched xylofucoglycuronan isolated from *Ascophyllum nodosum*. It has a backbone made of uronic acid, mainly (1 → 4)-linked β-D-Man*p*A, and branches containing sulfated α-L-Fuc*p* and β-D-Xyl*p* [22.145]. Sargassan, a complex polysaccharide from *Sargassum linifolium*, has a glucuronomannan core with internal side chains of (1 → 4)-linked sulfated β-D-GlcA and peripheral side chains made of sulfated α-L-Fuc*p* and β-D-Xyl*p* [22.148–151].

Complex highly branched polysaccharides have been isolated from *Sargassum stenophyllum* [22.147]. One of these had a linear galactomannan core formed by (1 → 6)-β-D-Gal*p* and/or (1 → 2)-β-D-Man*p* units with side chains containing β-D-Xyl*p*, (1 → 3)- and/or (1 → 4)-linked α-L-Fuc*p*, (1 → 4)-linked α-L-Glc*p*A, and α-D-Glc*p*. Two polysaccharides obtained from *Adenocytis utricularis* were identified as galactofucan and glycuronofucan [22.141].

22.2.4 Ulvans

Ulvans are principal matrix cell wall polysaccharides of green algae, genus *Ulva* [22.155]. The composition of these polysaccharides varied according to species, harvest season, growth conditions, and method of isolation [22.5, 156]. Four monosaccharides, i. e. α-L-Rha*p*, β-D-Xyl*p*, β-D-Glc*p*A and α-L-Ido*p*A, and sulfate are the main constituents of ulvans [22.155, 156]. Like in the case of alginates, these two uronic acids are C-5 epimers. All these types of sugar units are commonly arranged in the backbone, while single β-D-Glc*p*A can also constitute the side chains. The basic structure of ulvans is described by several repeating disaccharide fragments called ulvanobiuronic acids or ulvanobioses. Ulvanobiuronic acids (Fig. 22.2f,g) contain α-L-Rha*p* and uronic acid residues, while ulvanobioses contain

β-D-Xyl*p* instead of uronic acid (Fig. 22.2h). Ulvans isolated from several Ulvales species are composed of variable proportions of these disaccharides. Table 22.8 summarizes various oligosaccharides defined as structural fragments of ulvans.

22.2.5 Other Polysaccharides

Cell walls of many green algae contain alkali soluble linear (1 → 4)-β-D-glucuronans [22.25, 157–159]. Sulfated rhamnans have been isolated from *Monostroma nitidum* and *Monostroma latissinum* (Ulotrichales, Chlorophyta) [22.25, 160–165] and from *Gayralia oxysperma* (Ulvales, Chlorophyta) [22.25, 166]. Similar polysaccharides but in smaller amounts have been detected in *Bryopsis plumosa* (Bryopsidales, Chlorophyta) [22.19]. These polysaccharides have mainly (1 → 2)-linked, but also (1 → 3) and/or (1 → 4)-linked partially sulfated α-L-Rha*p* units. Moreover, they have single stubs of uronic acid residues as side chains. Rhamnan sulfate from *Monostroma nitidum* consists of (1 → 3)-linked α-L-Rha*p* residues, some of which are sulfated mainly at *O*-2 [22.162]. Minor amounts of internal (1 → 2)-linked and branched α-L-Rha*p* linkages have also been found. Branched sulfated glucorhamnan has been isolated from the same alga [22.162]. This polysaccharide is mainly composed of (1 → 2) and (1 → 3)-linked α-L-Rha*p* (1 : 2) partially sulfated at the *O*-3 and *O*-2 positions, respectively. In addition, (1 → 4)-linked β-D-Glc*p* fragments are bound at *O*-2 of some (1 → 3)-linked backbone units. By contrast, polysaccharide from *Monostroma latissinum* is defined as highly sulfated rhamnan composed mainly of (1 → 2)-linked α-L-Rha*p* residues with sulfate groups substituted at *O*-3 and/or *O*-4 [22.163]. Sulfated heterorhamnan isolated from *Gayralia oxysperma* has (1 → 3) and (1 → 2)-linked α-L-Rha*p* units in the backbone [22.166]. The latter units are partially substituted at C-3 by side chains containing single Glc*p*A, Gal*p*A or Xyl*p*. Both types of Rha*p* units are partially sulfated at *O*-2 and/or *O*-4; side chain uronic acids are also sulfated but only at *O*-2.

Sulfated xylomannans, linear or containing single stubs of β-D-Xyl*p*, or 3-*O*-methyl-β-D-Xyl*p* at *O*-2, have been described for many red algae. Branched sulfated xylomannans (33–222 kDa) have been isolated from two species of the genus *Chondrophycus* (Ceramiales, Rhodophyta) [22.167]. These polysaccharides consist of (1 → 4)-linked β-D-Man*p*2S backbone carrying stubs (15–25%) of single β-D-Xyl*p* (70–80%) and β-D-Man*p*2S (20–30%). Several fractions from *Nothogenia fastigiata* (Nemaliales, Rhodophyta) have been identified as xylomannans with a backbone of (1 → 3)-linked α-D-Man*p* sulfated at the *O*-2 and *O*-6 positions and single stubs of β-D-Xyl*p* at *O*-2 [22.168, 169]. Sulfated xylomannan (150 kDa) has been isolated from *Sebdenia polydactyla* (Sebdeniales, Rhodophyta) [22.170]. This polysaccharide contains a backbone of (1 → 3)-linked α-D-Man*p* units, partially substituted with a single stub of β-D-Xyl*p* at *O*-6. The main polysaccharide fraction isolated from *Nemalion helminthoides* (Nemaliales, Rhodophyta) has been identified as (1 → 3)-α-D-mannan sulfated at the *O*-4 and *O*-6 positions; other fractions are similar mannans but they contain single stubs of β-D-Xyl*p* [22.171, 172]. Sulfated arabinopyranan has been isolated from the green alga *Enteromorpha clathrata* [22.173]. The backbone of this polysaccharide is mainly composed of (1 → 4)-linked β-L-Ara*p* residues, partially sulfated at the *O*-3 position. Two sulfated polysaccharides, i. e., arabinan and arabinogalactan, have been isolated from *Codium dwarkense* (Bryopsidales, Chlorophyta) [22.174]. Two water-soluble sulfated polysaccharides from *Codium vermilara* (Bryopsidales, Chlorophyta) have been identified as (i) partially 2-*O*-sulfated (1 → 4)-β-D-mannan and (ii) (1 → 3)-β-L-arabinopyranan highly sulfated at the *O*-2 and *O*-4 positions [22.23, 24].

22.3 Isolation from Algal Raw Material

Crystalline, fibrilar, and matrix polysaccharides of algal cell walls can be successfully isolated from raw material by subsequent extractions. The isolation is based on the difference in solubility of these polysaccharides in various media at neutral, acidic, or alkali conditions. Usually matrix polysaccharides are well soluble in hot water, hemicellulose components are soluble in alkali solutions, while crystalline cellulose and, in some cases, xylans or mannans, remain insoluble at these conditions. Before subsequent extraction of polysaccharides, raw material is usually washed with organic solvents to remove lipids, pigments, and other small molecules, and then washed with distilled water to remove small hydrophilic molecules. Finally, raw material is dried and milled.

22.3.1 Extraction Procedures

The subsequent extraction steps vary depending on whether the polysaccharides contain sulfate and/or carboxylic groups. Routine procedures of common extraction of polysaccharides from various algae are outlined in Table 22.9. Extraction with cold water is followed by extractions with hot water and alkali solutions at different pH and temperatures [22.175]. This approach could be defined as universal because it is effective for the isolation of major polysaccharides from red algae (sulfated galactans, agars, and carrageenans), brown algae (sulfated fucoidans and xylans), and green algae (heteroglycuronans, ulvans). Neutral sugar (NS) and UA compositions vary significantly for crude water and alkali extracts, so it is possible to separate water and alkali soluble polysaccharides. For example, sulfated galactans predominate in water extracts, while floridean starch predominates in the alkali extracts. Heterofucan was assigned as the major polysaccharide in extracts from brown algae; however, alginates were not analyzed in this report. Alternatively, a combination of other media can be used for extractions.

In other work [22.176], brown algae (*Ascophyllum nodosum*, *Fucus vesiculosus*, and *Saccharina longicruris*) were subsequently treated with selective extraction media at 70 °C to obtain three fractions: fucoidan in a mixture with laminaran (2% aq. $CaCl_2$), fucoidan (0.01 mol L^{-1} aq. HCl) and alginate (3% aq. Na_2CO_3). Three different ways of sodium alginate isolation from brown algae *Macrocystis pyrifera* (Table 22.10) were compared in [22.49]. Acid pre-treatment was used in order to eliminate polyvalent cations prior to the extraction.Alginate was then solubilized under basic conditions and purified in different ways. Three methods of precipitation/purification (ethanol, HCl, and $CaCl_2$ routes) were used to study the influence of process conditions on the final products. Both HCl and $CaCl_2$ routes give low M_w alginates of poor mechanical properties owing to the partial acidic hydrolysis of the polymeric chain by the treatment with 1 mol L^{-1} HCl. By contrast, the ethanol route showed the highest yield and good rheological properties of the products. The diluted acid pre-treatment improved the yield of the ethanol route, avoiding the acidic hydrolysis of alginate.

Extraction of agars and carrageenans needs different conditions because these polysaccharides have a marked difference in sulfate contents [22.178]. Carrageenophytes (*Mastocarpus stellatus, Chondrus crispus, Calliblepharis jubata, Chondracanthus acicularis*, and *Chondracanthus teedei*) have been used for carrageenan extraction by alkali treatment (1 mol L^{-1} of NaOH, 80 °C, 3 h), whereas agars have been isolated from agarophyte (*Gracilaria gracilis*) by hot water extraction (90 °C, 4 h). Two major cell-wall polysaccharides have been extracted from the green seaweed *Ulva rigida* sequentially with oxalate and KOH containing media [22.157]. Sulfated glucuronorhamnoxylan (ulvan) has mainly been extracted with oxalate, while two hemicelluloses, i. e., (1 → 4)-β-D-glucuronan and glucoxylan, have been isolated at alkali conditions.

Kinetic modeling (KM) is often used for optimization of extraction procedures and to obtain good quality products. For example, a kinetic model has been proposed for the alkaline extraction of alginate from the fresh brown alga *Laminaria digitata* in order to predict the yield as a function of the stirring level and the size of algae pieces [22.179]. Such models may help to

Table 22.9 Extraction of marine algal polysaccharides

Extraction media/reagents	Main polysaccharides			References
	Red algae	Green algae	Brown algae	
Sodium oxalate		Ulvan		[22.157]
$CaCl_2$			Laminaran/fucoidan	[22.176]
Diluted HCl			Fucoidan	
Cold water	Sulfated galactans	Ulvan Sulfated glycans	Fucoidan	[22.175]
Hot water	Sulfated galactans Agar	Ulvan Sulfated glycans	Fucoidan	[22.175, 177]
Na_2CO_3			Alginate	[22.176]
	Floridean starch	Glucuronan	Fucoidan	[22.175]
NaOH	Floridean starch Carrageenan	Glucuronan	Fucoidan	[22.175] [22.177]
KOH		Glucuronan		[22.157]
$NaClO_2$/AcOH		Glucoxylan		[22.157]

Table 22.10 Three ways of sodium alginate isolation from algal material according to [22.49]

Steps	Conditions/routes		
	I	II	III
Soaking	**0.1 mol l^{-1} HCl**		
Extraction	**1 mol l^{-1} Na_2CO_3**		
Precipitation			**1 mol l^{-1} $CaCl_2$**
Washing			Water reflux
Precipitation		**1 mol l^{-1} HCl**	
Solubilization		**1 mol l^{-1} Na_2CO_3**	
Precipitation	**Ethanol (1 : 1)**		
Washing	Ethanol reflux		

reduce the extraction time of high-quality alginate production. The proposed model was defined as a first step in the more complete extraction kinetic model, taking into account the effect of temperature. The effects of various variables (alkali treatment, relative amount of water, soaking/extraction time, and temperature) have been investigated on agar extraction from the red alga *Gracilaria cliftonii* [22.180]. The agar yield was significantly affected by all these variables.

Response surface methodology (RSM) has been used to determine the optimum extraction conditions for yield and further parameters of agar extraction from *Hydropuntia cornea* [22.177] and carrageenan extraction from *Kappaphycus alvarezii* [22.181]. This method explores the relationships between several explanatory (reaction conditions of designed experiments – temperature, extraction time, pH, concentration of reagents, etc.) and response (yield, molecular weight, structural features, and physical properties of extracted polysaccharides) variables [22.182]. Among the parameters of reaction conditions, the extraction temperature is supposed to be the most important for increasing of the yield. Extraction procedures can be also optimized by the assistance of other additional factors like extrusion, microwaves (MW), or specific enzymes. Alkaline extraction of alginates from *Laminaria digitata* has been modified by reactive extrusion, which enables process time savings, yield increase, and improvement of the rheological properties of the products [22.183].

Microwave-assisted extractions (MAE) of algal polysaccharides have been tested and optimized [22.183]. A combination of mathematical and statistical techniques allowed us to determine the optimal experimental conditions for MAE extraction of agar from *Gracilaria vermiculophylla*. Enzyme-assisted extraction has also been used in the processing of marine algae [22.184] using endoprotease (Neutrase) for enzymatic digestion. A novel enzymatic extraction of marine algal polysaccharides with cellulase using RSM to search for the optimal conditions (enzyme concentration, ratio of medium/raw material, and extraction time) has been developed to reach high yields [22.185].

22.3.2 Purification of Crude Extracts

The crude extracts of algal polysaccharides are usually concentrated, dialyzed against distilled water, and then freeze-dried or precipitated with an excess of ethanol. Crude extracts usually contain a mixture of two or more polysaccharides, for example, pairs of carboxylic and sulfated ones like an alginate/fucoidan mixture from brown algae or an ulvan/glucuronan mixture from green algae. These components are effectively separated by changing the pH or by treatment with Ca^{2+} cations [22.49]. Alginic acids are insoluble due to protonation of carboxyls. In addition, G-blocks in alginates are able to form egg-box complexes with Ca^{2+}, which leads to calcium alginate precipitation. By contrast, fucoidans remain soluble at low pH and weakly interact with divalent cations. Glucuronans are often co-extracted together with ulvans from the cell wall of *Ulva* sp. with the use of hot water that often contains a calcium chelating agent such as sodium oxalate; ion-exchange chromatography has been used to eliminate glucuronans from the ulvan preparations [22.157, 158].

By contrast to ulvans, $(1 \rightarrow 4)$-β-D-glucuronans are insoluble at lower pH. Based on this difference, acidic precipitation (pH 2) has been used to separate ulvan and glucuronan from the hot oxalate buffer extract (0.05 mol L^{-1}, pH 6, 90 °C, 3 h) of *Ulva lactuca* [22.159]. The high amount of uronic acids (94% m/m) and the absence of sulfate groups have confirmed the purity of precipitated glucuronan. Extracts of crude algal polysaccharide have further been fractionalized and/or purified on various preparative columns using gel permeation and/or ion exchange chromatography with isocratic or gradient elution [22.83, 173]. These procedures lead to separation of polysaccharides according to their specific retention times and to removal of impurities like proteins, phenolics, or other polysaccharide admixtures. Proteins are also removed by the treatment with various chemical reagents or proteases; phenolics

by the treatment with oxidative agents. Fucoidan was purified from its natural complex with polyphenols by the treatment with 5% H_2O_2 (pH 8.3–8.7, 18 °C), and this treatment did not lead to marked desulfation of the polysaccharide [22.83, 186]. Fucoidans treated with H_2O_2 have not demonstrated any significant changes in backbones or sulfation degrees compared with the raw polysaccharide [22.83, 187]. However, a decrease in M_w is possible at higher reaction temperatures, H_2O_2 concentration, and reaction time.

22.4 Algal Polysaccharides such as Phycocolloids

Algal matrix cell wall polysaccharides can be assigned as phycocolloids (or phycohydrocolloids) because they are soluble in water and can create colloid systems in aqueous media or participate in their formation. Some of these polysaccharides can form gels and films at appropriate conditions; the films are commonly obtained by solution casting and solvent evaporation. The physical and chemical properties of phycocolloids, including gel formation, have been reviewed in connection with their structure [22.188]. The geometry of glycosidic linkages and the ability to form intra and intermolecular junctions are prerequisites of gel and film formation. These associations may be affected by (i) negatively charged groups (sulfates and carboxylates), which tend to expend a polysaccharide chain due to mutual electrostatic repulsion; (ii) various substituents (methyls, acetyls, pyruvate ketals, glycosylation, etc.), which cause steric hindrance and/or participate in various intra and intermolecular interactions; and (iii) dissolved small molecules or metal cations that influence the hydratation property of water. Conditions such as temperature, pH, and ionic strength may also influence gel formation.

22.4.1 Alginic Acid/Alginate Gels and Films

Alginates of alkali metals and alginic acids form transparent, flexible, and water soluble films by casting of their aqueous solutions. Films prepared from Gul*p*A rich alginate demonstrate lower plasticity and have proved to be better moisture barriers than those prepared from Man*p*A rich alginate under the same conditions [22.189, 190]. Alginate films are effective cation exchangers. Their solubility may significantly decrease in the presence of multivalent metal cations [22.191, 192]. The Ca^{2+} and Zn^{2+} cations make alginate film water insoluble, increase its tensile strength, and decrease elongation. These effects are less pronounced for Cu^{2+} and Al^{3+} cations and little pronounced for Fe^{3+} and Mg^{2+} cations. Metal cations of smaller ionic radius cause tighter cross-linking of alginate films [22.193]. Cross-linking by Ca^{2+} produces significantly thicker and stronger, but less elastic alginate films; water vapor permeability decreases significantly only for highly cross-linking films [22.194]. The incorporation of multivalent metal cations into the alginate films may partially replace the weak polar interactions between polysaccharide chains by stronger cation mediated electrostatic junctions [22.195].

Alginates are able to form two types of gels: (i) alginic acid gel at acidic conditions (pH < pK_a of uronic carboxyls) and (ii) cross-linking thermostable gels at neutral conditions in the presence of Ca^{2+}. Acidic gels are stabilized by intermolecular hydrogen bonds and the junction zones of these gels are probably composed of random aggregates [22.196]. G-blocks are the most important fragments for both acidic and Ca^{2+} gel formation, whereas M-blocks support only acidic gel formation. Alternating or random sequences destabilize alginic acid gel formation due to their lack of repeating structure. In the case of neutral gels, cations of other alkaline earth metals, excluding Mg^{2+} or other divalent metal cations, can be used instead of Ca^{2+}. G-blocks, but not GM or M-blocks, of alginates interact strongly and cooperatively with these cations, forming *egg-box* type structures [22.47, 197]. Ca^{2+} ions can replace carboxylic protons in the G-blocks, which are zipped into cavities [22.198, 199]. The Ca^{2+} binding capacity does not depend on the M/G ratio of alginates, but Gul*p*A exhibit much stronger affinity to these cations than Man*p*A [22.200]. In this model, the cations (*eggs*) are situated in the cavities inside *puckered boxes* formed by four Gul*p*A units of the two superimposed chains. The individual *boxes* potentially possess oxygen ligands from COO^-, 2-OH, 3-OH, O-5 pyranoid rings, and O-4 of glycosidic bonds in the G-blocks; some of these ligands directly interact with Ca^{2+}, others participate in intra and intermolecular hydrogen bonds stabilizing the *egg-box* complex [22.201]. Based on FTIR (Fourier transformed infrared) spectroscopic analysis of ionic alginate gels with various divalent metal cations, different coordination geometry was proposed for the metal-carboxylate complexes in G-blocks (*pseudo-bridged* unidentate) and M-blocks (bidentate

bridging) [22.202]. The strength of alginate gels depends on the M/G ratio and distribution of GG, MM, and GM-blocks in the polysaccharide chain [22.203, 204]. Alginates with a low M/G ratio and a high content of G-blocks form dense and brittle gels, whereas alginates with a high M/G ratio and a high content of M-blocks give more elastic gels [22.12, 13, 205].

There are three distinct and successive steps in the binding of Ca^{2+} to alginate with increased concentration of Ca^{2+} ions [22.206]: (i) interaction of Ca^{2+} with a single Gul*p*A unit forming mono-complexes, (ii) propagation and formation of *egg-box* dimers via pairing of these mono-complexes, and (iii) lateral association of these dimers, generating *egg-box* multimers. In freshly prepared gels, junction zones are assumed to consist of *egg-box* dimers only. At higher Ca^{2+} concentration upon drying, these dimers may associate laterally into ordered domains (*egg-box* multimers) by different ways, including the most probable electrostatic interactions between Ca^{2+} and carboxylates [22.198, 207]. Lateral association of alginate chains is reduced by removing Ca^{2+} excess from the gel beads in a washing step prior to air drying. The *egg-box* multimers are disrupted by noncross-linking cations (Na^+, K^+ or Mg^{2+}), which replace the Ca^{2+} bound to the alginate. The result is swelling of the alginate gel. Ca^{2+} alginate gelation has been described as random cross-linking of multifunctional monochains [22.208]. Alginate gels showed strain-hardening behavior at large deformations, which was explained by (i) the re-orientation of chain segments longer than the distance between cross-links [22.209], (ii) structure densification or highly cross-linked polymer systems [22.205], and (iii) the deformation of rod-like junction zones [22.210].

22.4.2 Gels and Films Based on Sulfated Galactans

Some red algal sulfated galactans (agars and carrageenans) are able to form films and thermally reversible hydrogels by cooling of their hot aqueous solutions. Agar films demonstrate a lower tensile strength, water vapor permeability, swelling ratio, and water solubility than κ-carrageenan films. Each property of the mixture agar/κ-carrageenan films varies depending on the ratio of each component [22.211]. Gelation of sulfated galactans is a complex process that depends on temperature, structure, and concentration of polysaccharide, and on the presence of metal cations, which can induce conformational changes in the polymer chain. As a result, the initial coil-to-helix transition is followed by subsequent aggregation of these helices [22.212–215].

The final gel structure is the result of mutual interactions between conformational transition, molecular cross-linking, and phase-separation processes [22.216]. A honeycomb-like network has been observed for aqueous solutions both of agars and carrageenans at various temperatures. Its structure depends on the gelling stage, the structural type (D- or L- absolute configuration of the 3,6-anhydro-α-Gal*p* units), and the concentration of sulfated galactan. Honeycomb-like structures are gradually replaced by more homogeneous networks during the formation of brittle and strong agar-type hydrogels; they are retained, however, in the case of highly elastic carrageenan-type hydrogels. Therefore, the gelation mechanisms should be different for agars and carrageenans. In addition, sulfated galactan gels of high polysaccharide concentration may be structurally different from those of low polysaccharide concentration. Three possible mechanisms of carrageenan/agar gel formation focussing on the specificity of junction zones have been proposed: (i) the double-helical model based on intertwined double helices [22.217, 218], (ii) the domain model with cation-mediated aggregates of double helices [22.212, 219], and (iii) the nested, single-helix model with cation-specific salt bridges between ordered chain segments [22.220, 221]. To date, the step-by-step mechanism of gel formation as well as corresponding conformational transition, i. e., coil-helix or coil-double helix are still unclear [22.216]. Metal cations may be involved in gel formation depending on the presence and distribution of sulfate semiester groups. The shielding effect of alkali metal cations prevents electrostatic repulsion of sulfate groups in κ-carrageenan gels and thus stabilizes the double helices. Agarose gel is not influenced so much by these cations because of the lack of sulfate groups [22.222].

Carrageenan films have randomly distributed sets of pores [22.223]. Their mechanical properties depend on the sulfate/anhydro substitution of carrageenan. Pure κ-carrageenan films are stronger, less flexible, and exhibit a higher tensile strength than pure ι-carrageenan films. The tensile strength of the mixture of κ/λ and κ/ι-carrageenan films decreases with an increasing contribution of κ-carrageenan and is lower than that of pure λ and ι-carrageenan films. Reciprocal relations have been demonstrated for the elongations and water vapor permeability [22.224]. The gelation properties of carrageenans strongly depend on their structure, first of all on the presence of sulfate semiester and 3,6-anhydro groups. The high density of charged sulfates

supports extensive conformation of the carrageenan chain. The presence of the 3,6-anhydro-link in λ-carrageenan allows the α-D-Gal*p* residues to revert to their $^1C_4$ conformation, which is necessary for the initial double helix formation required for gelling. Alkali treatment of carrageenans or their precursors during processing increases the gelling properties of the final polysaccharide and the strength of gels due to conversion of α-D-Gal*p*6S to the 3,6-anhydride. As a result, κ-carrageenans and furcellaran (κ/β-carrageenan) with less sulfates but more 3,6-anhydro groups form a firm gel with K^+. By contrast, more sulfated but less or no anhydrated ι- and λ-carrageenans are slightly affected by these cations; ι-carrageenan needs Ca^{2+} to form soft elastic gels, and λ-carrageenan cannot form gels at all. Hot solutions of κ and ι-carrageenans form gels after cooling of their hot aqueous solutions to 40–60 °C in the presence of the appropriate metal cations. These gels are stable at room temperature, can be re-melted by heating to 5–20 °C above the gelling temperature, and re-gel after repeated cooling. Highly flexible carrageenan molecules are able to form helical structures when their concentration increases or temperature decreases. The mechanism of κ-carrageenan gelation in the presence of K^+ and other alkali metal cations (Na^+, Rb^+) is thus based on the cation supported aggregation of helical dimers, which consist of two single [22.225] or double [22.210] helices.

In any case, a two-step gelation mechanism has been proposed for both ι and κ-carrageenans [22.212, 214]. Thermally reversible carrageenan gels exhibit hysteresis or a difference between setting and melting temperatures. The gelation of κ-carrageenan solutions is independent of liquid-liquid phase separation due to the rapid formation of cross-linking points [22.226]. Furcellaran (κ/β-carrageenan) and κ-carrageenan gels are relatively rigid and undergo syneresis, but ι-carrageenan yields soft and elastic gels with very little tendency to undergo syneresis. In this case, the 2-*O*-sulfate groups on the 3,6-anhydro-α-D-Gal*p* units prevent the tightly-packed aggregation responsible for the rigidity of κ-carrageenan gels.

Agar is commonly defined as the mixture of neutral agarose and highly sulfated agaropectin. The ratio between these parts depends on the algal source of agar. Agarose demonstrates a much higher gelling ability than amylopectin. Agarose is well soluble in hot water (up to 80 °C) because it has chains at stiff random coil conformation [22.227–229]. Upon cooling below the gelation temperature (40 °C), the coils reorder to form helices that subsequently laterally aggregate into a network of thick bundles to form a gel. It is unclear, however, if the proposed high-ordered structure is made of a single helix [22.228–230] or a double helix [22.218]. Agarose gels (≈ 4 m/v %) are typically rigid and turbid, and undergo hysteresis between the melting and setting temperatures. The length of helices suitable for stable association increases with heating. Slow cooling leads to longer helices than rapid cooling, and longer helices promote better aggregation. As a result, the gels formed by slow cooling are stronger and more turbid than those obtained by rapid cooling [22.231]. The gelation process is followed by liquid–liquid phase separation because the network formation based on aggregated bundles leads to the dilution of solute agarose in the network interspaces [22.226, 232]. The agar chains form bundles of aggregates, generating a network at the temperature of sol–gel transition. Longer agar chains preferably aggregate, while shorter agar chains are retained in the solute and form isolated aggregates upon further cooling. Reheating leads to easy dissociation of the loose aggregates, whereas bundles of aggregates are thermostable like the whole gel [22.233].

22.4.3 Gelling/Film Forming by Fucoidans and Ulvans

In contrast to other phycocolloids, there is a little evidence about the gelling and film forming properties of fucoidans. There are, however, several reports that confirm that this polysaccharide by itself or in a mixture with other polymers is able to form gels and films [22.234]. Varying amounts of fucoidan in a liquid medium may provide specific rheological properties of the resulting gel.

Green seaweed polysaccharide ulvans are well soluble in water. However, despite their high negative charge, they demonstrate condensed conformation and low intrinsic viscosity in aqueous saline or acidic solutions, which is probably due to the hydrophobic contribution of CH_3 in Rha*p* [22.156]. This polysaccharide is probably not fully dissolved but forms free condensed spherical nanostructures (10–18 nm) dispersed in the solvent. At strong alkali conditions (pH 13) these structures collapse into a dense homogeneous network, which is the basis for strong films and gels. Gelation and film formation at lower pH levels needs divalent cations and/or boric acid. Ulvan forms thermoreversible gel in the presence of boric acid, Ca^{2+}, or other divalent metal cations (but not Mg^{2+}), and narrow region of pH around 7.5 [22.11]. The mechanism of gel formation

Table 22.11 Composite algal polysaccharide (phycocolloid) films and gels containing other hydrocolloids

Phycocolloids	Other hydrocolloids	Composite films	Composite gels
Alginate	Starch	[22.235, 236]	[22.235, 237–239]
	Pullulan	[22.240, 241]	
	Cellulose derivatives	[22.241, 242]	
	Pectin	[22.243, 244]	[22.245–247]
	Arabic gum		[22.248]
	Konjac gum	[22.249, 250]	
	Xanthan gum	[22.251]	
	κ- or ι-carrageenan	[22.252–256]	[22.257]
	Phosphorylated chitin	[22.258]	
	Chitosan	[22.259–264]	[22.265–268]
	Mucin		[22.269]
	Proteins	[22.249, 270–274]	[22.248, 275, 276]
Agar	Starch and arabinoxylan	[22.277]	
	κ-carrageenan	[22.211]	
	Galactomannans		[22.278]
	Proteins	[22.279, 280]	[22.281]
Carrageenans	Native or modified starch	[22.282]	[22.283–285]
	Nanocellulose fibres	[22.286]	
	Pectin	[22.287, 288]	
	Galactomannans	[22.289]	[22.290–292]
	Chitosan	[22.293]	[22.294]
	Proteins	[22.273, 295]	[22.296]
Fucoidan	Starch		[22.297]
	Chitosan	[22.298]	
	Protein	[22.299]	
Ulvan	Chitosan		[22.300]

is unique, very complex, and not well understood. The role of borate and cations in ulvan gelation is unclear. Both these reagents probably promote the aggregation of ulvan nanostructures mentioned above, which are interconnected by more hydrophilic parts of non-condensed polysaccharide chains. Ulvan gels should be stabilized by weak reversible interactions, so borate ester bridges between ulvan macromolecules are improbable [22.301, 302]. NMR spectroscopy has not confirmed formation of ulvan borate esters [22.303]. On the other hand, Ca^{2+} may link borate ester, sulfate semi-ester, and/or carboxylate groups forming junction zones. The unique properties of ulvan gel are interesting for systems of controlled gelation, for example, in drug delivery systems [22.303, 304].

22.4.4 Interaction with Other Hydrocolloids

Phycocolloids can interact with other natural and chemically modified hydrocolloids and between each other, forming mixed films, gels, and other structures. Some composite films and gels containing phycocolloids are overviewed in Table 22.11. Among these examples, the alginate–pectin mixtures are interesting due to their ability to form thermoreversible synergistic gels at pH < 3.8 without the addition of sucrose [22.246, 247]. The strength and melting points of these gels increase at lower pH and M/G ratios of alginates. Gelation depends on the sequential distribution of monomeric units in the alginate chain and needs tetrameric or longer G-blocks. Mixtures containing alginates with low M/G ratio and high methoxy (HM) pectins show the strongest synergism, the highest storage modulus, and the fastest kinetics of gel formation [22.245]. By contrast, gels based on alginates with high M/G ratio and low methoxy (LM) or low methoxy amidated (LMA) pectin demonstrate lower storage modulus and slower kinetics. Pectin, but not alginate, affects the network density and the strand characteristics of mixed gels, whereas the homogeneity in the gel microstruc-

ture decreases with the addition of alginate content but is independent of the pectin. Specific interactions between hydrocolloids may significantly change the properties of hydrogels. For example, the addition of starch, alginate, or κ-carrageenan decreases the strength of agar gels, while plant seed galactomannans demonstrate marked synergistic effects on the strength of agar and κ carrageenan gels [22.278, 290, 292].

Rheological properties of starch colloid systems can be regulated by the addition of fucoidan [22.297]. At lower concentrations, fucoidan probably damages the junction zones between starch macromolecules, but these polysaccharides are not mutually excluded. By contrast, at higher concentrations, fucoidan may induce considerable mutual exclusion through a phase separation process. Mixed films and gels can be prepared based on the electrostatic interaction between negatively charged phycocolloids and positively charged chitosan or protein. For example, a combination of sodium alginate and gelatine provides synergism in the rheological properties of the resulting mixed gels [22.276]. Chitosan-alginate films exhibit low solubility, good tensile properties, permeability to water vapor, and biocompatibility, which is important for biomedical applications [22.259, 260]. The addition of fucoidan to chitosan films increases their mechanical robustness and elasticity, making composite chitosan-fucoidan films more suitable as wound dressings than pure chitosan films [22.298]. Ulvan-chitosan ionic complex gels are also prepared and characterized as more stable than alginic acid (alginate)-chitosan gel under the appropriate conditions [22.300].

22.5 Biological Activities and Medicinal Applications

Besides hydrocolloid properties, both sulfated and carboxylated algal matrix cell wall polysaccharides are known to have various specific biological activities, which are prerequisites of pharmaceutical and medicinal applications. Partially sulfated algal polysaccharides exhibit immunomodulatory, anticoagulant, antithrombotic, antimutagenic, anti-inflammatory, antitumor, antiprotozoal, antimicrobial, and antiviral activities [22.305]. Generally, these biological activities are related to the structure, composition, and sulfate substitution of polysaccharide macromolecules. Among the activities mentioned above, the immunomodulating and anticoagulant activities seem to be immediate and, therefore, the most important for understanding the mechanism of action. As potential anticoagulants, algal polysaccharides are able to compete with the commonly used heparin. Because they affect the immune system, they may enhance antitumor, antibacterial, and antiviral resistance. In medicaments they can be used as an active substance if they possess the specific biological activity necessary for curing, or as a drug carrier if they are able to interact with an active substance (commonly small or nonpolymeric molecules), and thus improve its activity or provide its delivery to specific parts of the organism.

22.5.1 Immunomodulation and Related Activities

Immunomodulation is based on the specific enhancement or suppression of the host response with the aim to reach the desired immune effect [22.306]. This approach allows enhanced natural mechanisms of the immune response without the use of therapeutics like antibiotics. Immunomodulators are substances that affect the immune system. They can stimulate, suppress, or modulate any specific part of the immune system, including both adaptive and innate arms of the immune response [22.307]. Immunomodulators can be classified into three categories: (i) immunoadjuvants or specific immune stimulants, (ii) nonspecific immunostimulants, and (iii) immunosuppressants. Immunoadjuvants are true modulators that select between various types and mechanisms of immune responses. Thus, these agents are used to design vaccines or enhance their efficacy. Nonspecific immunostimulants enhance the basic level of immune response and are thus used for prophylactic purposes or for strengthening the impaired immune system. Both specific and nonspecific immunostimulants activate immunity directly or indirectly to be effective against cancer cells and various infection agents (prions, viruses, bacteria, or microscopic *Eukaryota*). Therefore, antitumor, antiprion, antiviral, antimicrobial, antifungal, or antiprotozoal activities of algal polysaccharides are often mediated by immune stimulation. By contrast, immunosuppressants are able to weaken the immune response that is often applied to reduce inflammation in various pathological states. Sulfated algal polysaccharides (fucoidans, galactans, etc.) may stimulate/inhibit cell immunity by activation/inhibition of macrophages and/or other cells of the immune systems and enhance/suppress the production of specific antibodies. Macrophages are critical in reg-

ulating innate immunity as well as adaptive immune responses by producing an array of cytokines such as interleukins (IL) IL-1β and IL-6, tumor necrosis factor (TNF)-α and interferon-γ (INF-γ), and various types of chemokines such as RANTES (regulated on activation, normal T-cell expressed and secreted), monocyte chemotactic protein (MCP)-1, macrophage inflammatory protein (MIP)-1α, thymus and activation regulated chemokine (TARC), etc. [22.308, 309]. Polysaccharide macromolecules may interact with specific membrane receptors of macrophages including Toll-like receptor (TLR)-4, cluster of differentiation (CD)-14, complement receptor (CR)-3 and scavenger receptors (SR). As a result, the intracellular signaling pathways are promoted, involving mitogen-activated protein kinases (MAPK) and transcription factors. Activation of macrophages via MAPK induces the production of both nitric oxide (NO) and cytokines. Partially, interleukin-12 (IL-12) stimulates the development of T-cells, which produce interleukin-2 (IL-2) that, in turn, activates proliferation of natural killer (NK) cells, representing a first line of defence organism against both primary tumors and metastases. NK cells produce other cytokines, including interferon (INF)-γ, which can further provoke macrophages in their stimulation of T-cells via induction of IL-12. NK-mediated killing of target cells by apoptosis is facilitated by activation of caspase cascades [22.310]. Many algal polysaccharides have been reported for their immunomodulatory activities as they stimulate the activity of macrophages. Polysaccharides from the red algae *Porphyra yezoensis* [22.311, 312] and *Gracilaria verrucosa* [22.313] are reported to have macrophage-stimulating activity in vitro and in vivo. A fucoidan isolated from the sporophyll of brown alga *Undaria pinnatifida* (Korean *Miyeokgui*, Japanese Mekabu) stimulated macrophages to produce cytokines (IL-6, TNF-α) and chemokines (RANTES, MIP-1α) from macrophages and splenocytes [22.125]. Carrageenan isolated from red algae promotes mice leukocytes to produce TNF-α [22.314]. A sulfated water-soluble polysaccharide isolated from the green alga *Capsosiphon fulvescens* is reported to stimulate macrophage to release TNF-α and IL-6, and induce the expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2). These enzymes are responsible for the production of NO and prostaglandin E2 (PGE2), respectively [22.315]. A sulfated glucuronorhamnan, an ulvan, of the green algae *Ulva rigida*, is also reported to stimulate macrophage to secrete PGE_2 and induce an increase in COX-2 and NOS-2 expression [22.316]. Immunomodulation and other biological activities of marine algal polysaccharides are reviewed in Table 22.12.

22.5.2 Antitumor and Antimutagenic Activities

The antitumor effect of fucoidans and other sulfated algal polysaccharides that are nontoxic for tumor cells is commonly associated with the following indirect activities:

1. The inhibition of tumor cell proliferation
2. The stimulation of the apoptosis of tumor cells
3. Blocking tumor cell metastasis
4. The inhibition of blood vessel formation
5. The enhancement of various immune responses [22.393].

Partially, antitumor and antimetastatic activity of these polysaccharides is mediated by increased NO production by stimulated macrophages [22.364], activation of NK cells via specific cytokines, and/or induction of caspase-dependent cancer cell apoptosis via inhibition of specific MAPK [22.310, 381, 394]. Fucoidan may also activate caspase-independent apoptosis via activation of ROS-mediated MAPK and regulation of the Bcl-2 family protein-mediated mitochondrial pathway [22.395]. Fucoidan from *Cladosiphon okamuranus* significantly inhibited in vivo and in vitro growth and induced apoptosis of human T-cell leukaemia virus type 1 (HTLV-1) infected T-cells. This fucoidan also inhibited in vivo growth of model HTLV-1-induced tumors based on subcutaneously transplanted infected T-cell lines in immune deficient mice [22.396]. Both Miyeokgui (*Undaria pinnatifida* sporophyll) and commercial (*Fucus vesiculosus*) fucoidans showed antitumor activity against four types of cancer, i. e., PC-3 (prostate cancer), HeLa (cervical cancer), A549 (alveolar carcinoma), and HepG2 (hepatocellular carcinoma) cells [22.137]. Fucoidans blocked tumor cell adhesion that could be important for antimetastatic effects [22.397, 398]. Fucoidan from *Fucus evanescens* produced an inhibitory effect against metastasis and potentiated antimetastatic, but not antitumor, effects of cyclophosphamide [22.348]. Fucoidans suppress angiogenesis and thus reduce the supply of tumor tissues [22.399, 400]. Fucoidans stimulate proliferation of macrophages [22.401] and mediate tumor destruction through Th1 cell and NK cell responses [22.380]. Fucoidans activate lymphocytes and macrophages mediated by the production of NO, H_2O_2, and cy-

Table 22.12 Biological activities of algal cell wall polysaccharides

Polysaccharide	Algal species	Specific biological activities	References
Ulvans	*Enteromorpha prolifera*	Activation of T cells and macrophages	[22.317, 318]
	Ulva lactuca	Antioxidant (biomembrane protection)	[22.319]
	Ulva pertusa, *Ulva rigida*	Activation of macrophages	[22.316, 320]
	Monostroma nitidum	Activation of macrophages, anticancer	[22.321]
Sulfated rhamnan	*Monostroma nitidum*	Anticoagulant	[22.164]
	Monostroma latissimum	Anticoagulant	[22.163, 321]
	Monostroma latissimum	antiviral (HSV-1, HCMV-1, HIV-1)[a,b,c]	[22.322]
Sulfated heterorhamnan	*Gayralia oxysperma*	Antiviral (HSV-1, 2)	[22.166]
Sulfated arabinopyranan	*Enteromorpha clathrata*	Anticoagulant	[22.173]
Sulfated galactan	*Codium fragile*	Activation of macrophages, antiviral (HSV-2)	[22.21, 160]
	Codium cylindricum	Anticoagulant	[22.22]
	Schizymenia binderi	Antiviral (HSV-1, 2)	[22.71]
	Bostrychia montagnei	Antiviral (HSV-1)	[22.147]
	Chaetomorpha aerea	Antibacterial (*Staphylococcus aureus*)	[22.323]
Sulfated xylogalactan	*Nothogenia fastigiata*	Antiviral (HSV-1)	[22.324]
Sulfated xylomannans	*Nothogenia fastigiata*	Antiviral (HSV-1 and other), anticoagulant	[22.168, 169, 325, 326]
	Sebdenia polydactyla	Antiviral	[22.170]
	Nemalion helminthoides	Antiviral (HSV-1)	[22.171]
Sulfated heteromannan	*Capsosiphon fulvescens*	Activation of macrophages	[22.327]
Sulfated mannan	*Codium vermilara*	Anticoagulant	[22.24]
Carrageenans	*Stenogramme interrupta*	Antiviral (HSV-1, 2)	[22.8]
κ-, ι-, λ-carrageenans	*Gigartina acicularis*, *G. pisillata* *Eucheuma cottonii*, *E. spinosa*	Antioxidant (free radical scavenging)	[22.328]
ι-carrageenan	Nonspecified	Antiviral	[22.329–332]
Carrageenan	*Gigartina skottsbergii*	Antioxidant (ABTS, FCA, ORAC, ROS scavenging)[d]	[22.65]
λ, κ/ι and μ/ν carrageenans	*Gigartina skottsbergii*	Antiviral (HSV-1)	[22.333]
$\kappa/\iota/\nu$ carrageenans	*Meristiella gelidium*	Antiviral (HSV-2, DENV-2)[e]	[22.63]
Unusual hybrid carrageenan	*Botryocladia occidentalis*	pro/anticoagulant, antithrombotic	[22.62, 334–338]
Sulfated galactans	*Gelidium crinale*	pro/anticoagulant, antithrombotic	[22.337, 338]
	Gelidium crinale	Anti-inflammatory, antinociceptive	[22.339]
	Grateloupia indica	Antiviral (HSV-1, 2)	[22.340]
	Sphaerococcus coronopifolius *Boergeseniella thuyoides*	Antiviral (HIV-1, HSV-1)	[22.341]
$\kappa/\iota/\nu$ carrageenans	*Cryptonemia crenulata* *Gymnogongrus griffithsiae*	Antiviral (HSV-1, 2; DENV-1-4)	[22.76, 342, 343]
DL-galactans	*Gymnogongrus griffithsiae*		
	Schizymenia binderi	Antiviral (HSV-1, 2)	[22.71]
	Gymnogongrus torulosus	Antiviral (HSV-2, DENV-2)	[22.73]

[a] HSV: herpes simplex virus; [b] HCMV: human cytomegalovirus; [c] HIV: human immunodeficiency virus;
[d] ABTS: 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), ORAC: oxygen radical absorbance capacity, FCA: ferrous ion chelating ability, ROS: reactive oxygen species; [e] DENV: dengue virus

Table 22.12 (continued)

Polysaccharide	Algal species	Specific biological activities	References
Agarocolloid	*Gracilaria birdiae*	Antioxidant (DPPH)[a]	[22.344]
Alginates alginic acid	*Sargassum vulgare*	Antitumor (sarcoma 180)	[22.328]
	Macrocystis pyrifera	Immunostimulant (leucocytes, *Gadus morhua*)	[22.345]
Fucoidan (sulfated fucan)	Nonspecified	Antiprotozoal (*Leishmania donovani*)	[22.346]
	Fucus evanescens	Antitumor, antimetastatic	[22.347, 348]
	Fucus vesiculosus	Antioxidant (ROS scavenging, lipid peroxidation)	[22.328]
	Fucus vesiculosus	Immunostimulant (leucocytes, *Gadus morhua*)	[22.345]
	Fucus vesiculosus	Antitumor (mouse breast cancer)	[22.349]
	Fucus vesiculosus	Anti-inflammatory (suppression of microglia and macrophages)	[22.350–352]
	Fucus vesiculosus	Gastric protection anti-ulcer	[22.353, 354]
	Fucus vesiculosus	Anticancer (induction of apoptosis, activation of NK cells)	[22.310, 355–357]
	Fucus vesiculosus	Anti-obesity (stimulation of lipolysis)	[22.358]
	Laminaria angustata var. *longissima*	Activation of macrophages	[22.359]
	Laminaria japonica	Antibacterial (*Escherichia coli, Staphylococcus aureus*)	[22.360]
	Laminaria japonica	Anti-inflammatory (inhibition of NO production)	[22.361]
	Laminaria japonica	Antioxidant	[22.360, 362, 363]
	Cladosiphon okamuranus	Antitumour, activation of macrophages	[22.364–366]
	Cladosiphon okamuranus	Antiviral (HCV-1b)[b]	[22.367]
	Cladosiphon okamuranus	Prophylactic against prion infection	[22.368]
	Cladosiphon okamuranus	Antibacterial (*Helicobacter pylori*)	[22.369]
	Cladosiphon okamuranus	Anti-inflammatory (inhibition of IL-6 production)	[22.370]
	Cladosiphon okamuranus	Anti-ulcer	[22.371]
	Laminaria cichorioides	Anticoagulant	[22.125]
	Sargassum horneri	Antiviral (HSV-1, HCMV, HIV-1)[c]	[22.372]
	Sargassum hemiphyllum	Anti-inflammatory (inhibition of IL-6 production)	[22.373]
	Sargassum sp.	Anticancer (activation of NK cells)	[22.310]
	Ecklonia cava	Anticoagulant (inhibition of proteases II, VI and X)	[22.374]
	Ecklonia cava	Anti-inflammatory (suppression of macrophages)	[22.375]

[a] DPPH: (2,2-diphenyl-1-picrylhydrazyl); [b] HCV: hepatitis C virus; [c] HSV: herpes simplex virus, HCMV: human cytomegalovirus, HIV: human immunodeficiency virus

tokines (TNF-α, IL-6), contributing to their effectiveness against tumors [22.402].

22.5.3 Anti-Inflammatory Activity

Inflammation is a complex physiological phenomenon that responds to injury, infection, and stress. Anti-inflammatory activity is the ability to reduce inflammation and is used, partially, for pain remedying. Cytokines, such as interleukins IL-1β, IL-6, TNF-α, and NO have been known to play important roles in pro-inflammatory responses involving various types of cells [22.393]. NO is synthesized by inducible nitric oxide synthase (iNOS) isophorm, which is expressed in response to activation of nuclear factor κB (NF-κB) and MAPK induced by various effectors, such as bacterial lipopolysaccharides (LPS) or Concanvalin A (ConA). PGE2, COX-2, and monocyte chemoattractant protein-1 (MCP-1) are also related to inflammatory states. Sulfated polysaccharide from *Sargassum hemiphyllum* significantly reduces secretion of NO and pro-inflammatory cytokines (IL-

Table 22.12 (continued)

Polysaccharide	Algal species	Specific biological activities	References
Fucoidan (sulfated galactofucan)	*Adenocystis utricularis*	antiviral (HIV; HSV-1, 2)	[22.141, 376]
	Undaria pinnatifida (sporophyll)	Antioxidant	[22.377]
	Undaria pinnatifida (sporophyll)	Anti-obesity (suppression of adipogenesis)	[22.378]
	Undaria pinnatifida (sporophyll)	Anti-allergic (regulation of Th1/Th2 cell response)	[22.379]
	Undaria pinnatifida (sporophyll)	Antitumor	[22.137, 380–383]
	Undaria pinnatifida (sporophyll)	Anticoagulant	[22.355, 384]
	Undaria pinnatifida (sporophyll)	Antiprotozoal	[22.380, 385]
	Undaria pinnatifida (sporophyll)	Antiviral (HSV-1,2; human cytomegalovirus)	[22.133, 134, 386]
	Saccharina japonica	Antitumor	[22.383]
	Lobophora variegata	Anticoagulant, anti-inflammatory	[22.387]
	Spatoglossum schroederi	Antithrombotic	[22.142]
Heterofucan	*Spatoglossum schroederi*	Antithrombotic	[22.143]
	Padina gymnospora	Antioxidant, free radical scavenging	[22.388]
	Sargassum filipendula	Antioxidant, antiproliferative	[22.389]
Ascophyllan	*Ascophyllum nodosum*	Activation of macrophages, anticancer	[22.390, 391]
		Growth promoting effect on mammalian cell line	[22.392]

1b, IL-6, TNF-α) by LPS-activated macrophages; it also inhibits LPS-triggered messenger ribonucleic acid (mRNA) expressions of IL-1b, iNOS, and COX-2, which may be attributed to the downregulation of NF-jB in the nucleus [22.373]. Similarly, fucoidan from *Ecklonia cava* inhibits NO and PGE2 production by LPS-stimulated macrophages via the inhibition of enzymes iNOS and COX-2 [22.375]. Microglial activation plays an important role in the pathogenesis of neurodegenerative diseases, and fucoidans significantly inhibit LPS-stimulated microglial production and mRNA/protein expression of the mentioned inflammatory factors involving suppression of specific MAPK [22.350, 361]. Mekabu (*Undaria pinnatifida* sporophyll) fucoidan enhanced Th1 cell response and inhibited Th2 cell response in normal mice, reducing the immunoglobulin E (IgE) level in mice serum [22.379]. Fucoidan also induces IL-10 production in plasma and liver, inhibits the production of ConA-induced proinflammatory cytokines, and thereby prevents liver injury [22.352]. Fucoidan improves murine chronic colitis by inhibition of IL-6 production in colonic epithelial cells [22.370]. The gastro-protective (anti-ulcer) effect of fucoidan against aspirin-induced ulceration may take place through the prevention of the elevation of proinflammatory cytokines IL-6 and IL-12 [22.353]. The anti-ulcer property of fucoidan might contribute to protecting inflammatory cytokine-mediated oxidative damage to gastric mucosa [22.354]. The anti-obesity effect of fucoidan is also related to its anti-inflammatory activity. Miyeokgui (Mekabu) fucoidan suppresses adipogenesis via the inhibition of major markers and inflammation-related cytokines in adipocytes. Hence, it may afford some potential to control or reduce obesity [22.378]. Fucoidan from *Fucus vesiculosus* can be useful for the prevention or treatment of obesity due to its stimulatory lipolysis [22.358].

22.5.4 Activities Against Infection Agents

Marine algal polysaccharides, especially sulfated ones, have attracted considerable attention in recent years for their antiviral activities against animal viruses [22.325, 403, 404], such as HSV types 1 and 2 [22.113, 133, 170, 405], human immunodeficiency virus type-1 (HIV-1) [22.406, 407], the influenza virus [22.408], and the human cytomegalovirus (HCMV) [22.133]. Although the use of these polysaccharides and oligosaccharides as drugs is in its infancy, currently, some are already in various phases of clinical trials [22.409–412]. An *Undaria pinnatifida* extract comprising predominantly sulfated galactofucan (60−70% in mass and with relatively higher molecular weight of over 30−1000 kDa) has been patented for its in vivo antiviral activity including HSV-1 [22.413]. The antiviral activity of algal sulfated polysaccharides may have a direct or an indirect mechanism, i. e., based on (i) hindering the adsorption of viral

particles onto the host cell surface and/or direct inhibition of viral replication, or (ii) activation of immune pathways. Many viruses are able to bind to cell surface proteoglycans (heparan sulfates), which is important for the entrance of viral particles into the cell. Because they have a strong affinity to viruses, algal sulfated polysaccharides may inhibit the adhesion of viral particles onto the cell surface and thus put obstacles into the viral life circle [22.170]. If the antiviral effect of polysaccharides is significant only at the early stage of the viral replication cycle, viral adsorption is proved. By contrast, antiviral activity after infection confirms that the polysaccharide directly inhibits the viral replication and transition of virions between uninfected and infected cells (virucidal effect). For example, sulfated galactans from red algae *Sphaerococcus coronopifolius* and *Boergeseniella thuyoides* inhibit in vitro replication of both HIV and HSV-1. However, in the case of the HSV-1 cycle, these polysaccharides block the adsorption step, while a direct virucidal effect was observed for HIV-1 replication [22.341]. Antimicrobial and antiprotozoal activities of algal sulfated polysaccharides may be caused by direct interaction with microorganisms, blocking of microorganism-binding cell receptors, or by modulation of the immune response. For example, fucoidan (sulfated galactofucan) from *Undaria pinnatifida* sporophyll might inhibit cryptosporidiosis by direct binding to the *Cryptosporidium parvum*-derived functional mediators in the intestinal epithelial cells of neonatal mice [22.385]. This polysaccharide also exhibits anti-malarial activity against cultured *Plasmodium falciparum* parasites in vitro and on *Plasmodium berghei*-infected mice in vivo [22.414].

22.5.5 Anticoagulant and Antithrombotic Activities

The anticoagulant activity of sulfated polysaccharides, including those from algal sources is based on the interactions with plasma cofactors, which are natural inhibitors of coagulation proteases [22.415]. These interactions are complex and depend on the monosaccharide composition, the molecular weight, and the structure and distribution of sulfate groups. Sulfation is always important for both anticoagulant and antithrombotic activities, but the charge density is not always critical. Commonly anticoagulant activity of algal hydrocolloids, i. e., sulfated galactans, mannans, rhamnans, fucans, or more complex polysaccharides, is described as *activated partial thromboplastin time* (aPTT), *thrombin time* (TT), *prothrombin time* (PT), and *antithrombin to anticoagulation factor Xa* activities in comparison with corresponding values of heparin obtained at the same in vitro and/or in vivo experimental conditions [22.416]. The anticoagulant activity of algal sulfated polysaccharides is mainly attributed to thrombin inhibition mediated by antithrombin and/or heparin cofactor II, while other mechanisms have also been proposed. For example, several different fucoidan preparations from various algal species, including *Fucus versiculosus* [22.417, 418], *Laminaria brasiliensis* [22.417], *Ecklonia kurome* [22.418], *Ascophyllum nodosum* [22.419], *Pelvetia caniculata* [22.420], and the sporophyll of *Undaria pinnatifida* [22.384], have been reported for their anticoagulant activity. Fucoidans have potent anticoagulant activity mediated by antithrombin and/or heparin cofactor II [22.421]. The differences in anticoagulation mechanisms could be explained by the structural diversity of these polysaccharides and the interaction with several target proteases. The driving force for the formation of the sulfated polysaccharide/protein complex is the nonspecific electrostatic interaction between these macromolecules; then the complex is stabilized by other (polar or nonpolar) short-range interactions [22.422]. Anticoagulant polysaccharide should undergo appropriate conformational transitions to possess distorted ordered conformation suitable for protein binding. Model invertebrate sulfated α-L-fucans and sulfated α-L-galactans of regular linear structure show in vitro anticoagulant activity; some of them also express antithrombotic activity on in vivo models [22.421]. More complex algal sulfated polysaccharides having similar structural patterns may exhibit similar activities, which are not determined by charge density, but rather depend on monosaccharide composition and sulfation pattern. Partially, disulfated internal monosaccharide residues probably have the highest potential for anticoagulant activity due to their ability to place sulfate groups in spatial positions adequate for interaction with proteins. Slight differences in the proportions and/or distribution of sulfated residues may be critical for the interaction between proteases, inhibitors, and activators of the coagulation system, resulting in a distinct pattern in anti and pro-coagulant activities and in the antithrombotic action of algal sulfated galactans [22.337]. Sulfated galactans need much longer chains for the anticoagulant effect than heparin. Macromolecules of $\approx$15–45 kDa are able to bind antithrombin but are too short to link the plasma inhibitor and thrombin. Binding to different sites on antithrombin, sulfated galactans are less effective than heparin at promoting antithrombin conformational ac-

tivation. A different mechanism predominates over the conformational activation of antithrombin in ensuring the antithrombin-mediated anticoagulant activity of the sulfated galactans. Possibly, sulfated galactan connects antithrombin and thrombin, holding the protease in an inactive form. The conformational activation of antithrombin and the consequent formation of a covalent complex with thrombin appear to be less important for the anticoagulant activity of sulfated galactan than for heparin [22.338]. The $(1 \rightarrow 3)$-α-L-galactan, but not α-L-fucan, sulfated at *O*-2 is a potent thrombin inhibitor mediated by antithrombin or heparin cofactor II; these two polysaccharides show similar inhibitions when factor Xa is used instead of thrombin [22.423]. The proportion and/or distribution of 2,3-disulfated α-Gal*p* units along the galactan chain may be a critical structural motif to promote the interaction of the protease with specific protease and coagulation inhibitors [22.338, 424]. In contrast to the linear fucans from echinoderms and mammalian glycosaminoglycans, which need thrombin inhibitors (antithrombin or heparin cofactor II) for a reasonable anticoagulant effect, branched fucans (fucoidans) from brown algae are direct inhibitors of thrombin [22.425]. The occurrence of 2,4-di-*O*-sulfated units is an amplifying motif for 3-linked α-fucan-enhanced thrombin inhibition by antithrombin. If we replace antithrombin by heparin cofactor II, then the major structural requirement for the activity becomes single 4-*O*-sulfated fucose units, while the presence of 2-*O*-sulfated fucose residues always inhibits anticoagulant activity [22.424]. Therefore, the anticoagulant activities of sulfated galactans and fucans are not determined only by their charge density. The structural requirements for interaction of sulfated galactans and sulfated fucans with coagulation cofactors and their target proteases are stereospecific and not merely a consequence of their charge density and sulfate content [22.423, 424]. The unusual sulfated D-galactan (hybrid carrageenan) from *Botryocladia occidentalis* demonstrate marked anticoagulant activity via enhanced inhibition of thrombin and factor Xa by antithrombin and/or heparin cofactor II [22.62]. Disulfated α-Gal*p* units seem to be necessary for effective anticoagulant action, while high negative charge density itself is not so important. Sulfated galactofucan from *Spatoglossum schroederi* does not show any in vitro anticoagulant activity but demonstrates a potent antithrombotic activity in vivo and strong stimulation of the synthesis of heparan sulfate by endothelial cells. These effects have not bee observed after desulfation [22.142].

22.5.6 Antioxidant Activities

Because they are important for living organisms, enzymatic or nonenzymatic oxidative reactions can be destructive in some cases. Many products, including reactive oxygen species (ROS) and other free radicals are commonly produced by the oxidation of lipids and other biomolecules. These products promote chain reactions that cause the destruction of cellular structures and hence cell death. Antioxidants, in turn, may terminate chain reactions by free radical scavenging. Living organisms, including marine algae, have developed regulator systems based on natural antioxidants, both enzymatic (peroxidases, superoxide dismutase, and catalase) and nonenzymatic (glutathione, vitamins A, C, and E). If such systems do not work well, oxidative stress may occur and lead to various pathological states. Therefore, antioxidants might prevent many diseases, including degenerative ones, and thus promote human health and longevity. Algal antioxidants including sulfated polysaccharides have been reviewed in the context of algal biology and utilization [22.426]. The antioxidant activity of algal polysaccharides is defined as their ability to inhibit model oxidation processes; some examples are given in Table 22.12. The antioxidant properties of algal polysaccharides and associated compounds are commonly established as (i) DPPH (2,2-diphenyl-1-picrylhydrazyl) free-radical scavenging, (ii) oxygen radical absorbance capacity (ORAC), (iii) ferric ion reducing antioxidant power (FRAP) or ferrous ion chelating ability (FCA), (iv) ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), and (v) ROS scavenging assays [22.427]. Structure and sulfation patterns may influence these antioxidant effects of algal polysaccharides. It has been reported that ι-carrageenan demonstrates higher hydroxyl radical scavenging than λ and κ-carrageenans [22.328]. Alternatively, algal polysaccharides have been tested in accordance with in vitro and in vivo responses to experimental oxidative stress. For example, it has been reported that ulvan-like sulfated polysaccharide from *Ulva lactuca* stabilizes mitochondrial and microsomal membranes by prevention of the oxidative stress induced by D-GalN [22.319].

22.5.7 Medicinal Materials Based on Algal Polysaccharides

Algal cell wall polysaccharides have great potential for medicinal applications because of their biocompatibility with human tissues, their biodegradability, and

their nontoxicity. Among these polysaccharides, commercially available, low-cost alginates are known to be the most suitable for the preparation of biomedical materials based on pure and composite films, gels, and fibers [22.428, 429]. Due to the high water content, porosity, elasticity, permeability, and the ability to create a moist environment, alginate gels are effective in wound healing, drug delivery, and tissue engineering. Partially, calcium alginate hydrogels demonstrate both haemostatic activity due to Ca^{2+} and promotion of the aggregation of platelets and erythrocytes. The hydration, swelling, and erosion behavior of alginate matrices depend on pH, which is also true for drug release mechanisms [22.430]. As was mentioned above, alginic acid/alginate is able to form two types of gel at acidic and neutral conditions, which is a good prerequisite for the construction of controlled-release systems [22.431, 432]. Two types of diffusion systems have been prepared based on alginates [22.431]: (i) the polymer membrane system, in which the drug formulation (solid, suspension, or solution) is inside semipermeable gel-like polymeric capsules, and (ii) the polymer matrix system, in which the drug is homogeneously dispersed in a rate-controlling polymeric matrix of swelling microspheres or conventional tablets. Sodium alginate matrices can effectively sustain drug release even for highly water-soluble drugs [22.433, 434]. Alginate-based dressings are widely used to treat various surgery wounds [22.435–437]. The medicinal effect of calcium alginate as a wound dressing is based on its cation exchange properties [22.435]. After application, Ca^{2+} of the film/fibre surface is partially replaced by Na^{+} from blood serum, which leads to swelling, partial dissolution of the polysaccharide, and gelation. The effectiveness of this process depends on the structure and source of the alginate. Some properties of alginate films like transparency, thermal stability, water absorption, and swelling behavior improve with the presence of various additives, for example *Aloe vera* [22.438]. Composite materials consisting of alginate and other natural or modified polysaccharides have been shown to be potentially useful in drug delivery systems and other medicinal applications [22.131, 439]. Much fewer references in the literature are devoted to using fucoidan for wound healing. Because it has various biological activities, this polysaccharide is interesting as a component of wound dressing materials. Fucoidan-chitosan films might be a potential treatment system for dermal burns [22.298]. Local administration of fucoidan films safely reduced post-surgical adhesion in model animals [22.440]. Fucoidan improves the physical/chemical properties (roughness, hydrophilicity and surface sulfate content) of silk fibroin films, and brings about anticoagulant activity and enhanced endothelial cell affinity to silk fibroin [22.441]. Thus, fucoidan/silk fibroin films can be applied as blood-contacting biomaterials.

22.5.8 How to Improve Algal Polysaccharides

There are many ways to improve initially inactive or weakly active natural algal polysaccharides to fit various medicinal applications. First of all, partial degradation of algal polysaccharides leads to obtaining polymeric molecules of lower molecular mass, smaller oligomeric molecules (oligosaccharides), and small molecules (monosaccharides or their fragments). Smaller fragments of algal polysaccharides are more soluble in water, less viscous, and may interact with various biological targets like cell receptors or viral particles. Marine algal polysaccharides or derived oligosaccharides can be also chemically modified by the addition of polar or nonpolar groups. Partially, sulfation of nonsulfated polysaccharides (alginate) or oversulfation of sulfated algal polysaccharides (galactans, fucoidans) is the way to increase the negative charge density of the macromolecules, which may have a beneficial effect on their immunomodulation, anticoagulant, and other properties.

22.5.9 Partial Degradation

Marine algal polysaccharides can be degraded by the use of chemical or enzymatic treatment. Examples of microbial enzymes and microorganisms that are able to cleavage algal polysaccharides are shown in Table 22.13. Biological functions of oligosaccharides originating from marine algae were reviewed by [22.454]; some of these activities are similar to the corresponding activities of native polysaccharides. For example, both native algal polysaccharides and their oligomeric fragments may stimulate defence responses and protect plants against various pathogens [22.455]. However, in other cases, algal oligosaccharides exhibit novel activities that are nontypical for the initial polysaccharides, even the way of cleavage could be important. Partially, alginate oligosaccharides obtained by enzymatic degradation promote growth and influence the fatty acid composition of the biohydrogen producer of the green algae

Table 22.13 Examples of microbial enzymes and microorganisms degrading marine algal polysaccharides

Polysaccharide	Enzyme	Microbial source	References
Alginate	Alginate lyases	*Vibrio* sp. 510	[22.442]
		Pseudomonas sp. HZJ 216	[22.443]
		Pseudoalteromonas sp. Y-4	[22.444]
Carrageenans	Carrageenases	*Cytophaga* sp. MCA-2	[22.445]
		Pseudoalteromonas carrageenovora IFO12985	[22.446]
		Pseudoalteromonas carrageenovora	[22.4]
		Alteromonas fortis	[22.4, 59, 60]
Agar	Agarase	Unidentified marine bacteria	[22.447]
		Bacillus sp. MK03	[22.448]
		Agarivorans sp. HZ105	[22.449]
		Pseudomonas vesicularis MA103	[22.450]
		Aeromonas salmonicida MAEF108	[22.450]
Fucoidan	Fucoidanase	*Sphingomonas paucimobilis* PF-1	[22.135, 136]
		Fusarium sp. LD8	[22.451]
		Luteolibacter algae H18	[22.452]
	Acetyl esterase	*Luteolibacter algae* H18	[22.452]
Ulvan	Ulvan lyases	*Persicivirga ulvanivorans*	[22.453]

Chlamydomonas reinhardtii, which may significantly enhance biomass production [22.456]. By contrast, partial acidic hydrolysis of alginate does not lead to products that are able to affect the growth of these algal cells. Alginate oligosaccharides also promote the growth of the microalga *Nannochloropsis oculata*, which is used as a feed for sea animals and zooplankton [22.457]. Alginate oligosaccharides stimulate epidermal growth factor (EGF)-dependent growth of human keratinocytes [22.458] and vascular endothelial growth factor (VEGF)-mediated growth and migration of human endothelial cells [22.459]. Alginate oligosaccharides stimulate the in vivo growth of probiotic bacteria of the genus *Bifidobacterium* [22.460]. Alginate oligosaccharides inhibit colonization of *Salmonella enteritidis* in broiler chickens [22.461]. Alginate oligomers affect the growth and quality of biofilms, as well as the state of bacterial cells inside and also reduce the tolerance of wound biofilms to antibiotics [22.462]. Alginate oligosaccharides have a high scavenging •OH activity, while fucoidan oligosaccharides demonstrate good Fe^{2+} chelation [22.463]. Low-molecular weight fuco-oligosaccharides (< 3749 Da) have been prepared by enzymatic degradation of fucoidan (sulfated and acetylated galactofucan) from the Korean *Undaria pinnatifida* sporophyll [22.135, 136]. These fucoidan oligomers exhibit strong anticoagulant activities at which APTT and TT are significantly prolonged. Unlike intact fucoidan, these fuco-oligosaccharides do not affect PT. Therefore, the molecular weight and/or sulfate content may be important factors for the anticoagulant activities of fucoidans. Fucoidan oligosaccharides can markedly reduce the arterial blood pressure and plasma angiotensin II of the resting heart rate (RHR), and the respiration of RHR has the same change trend as blood pressure [22.464]. Carrageenan and agar oligosaccharides have also been prepared and tested on various bioactivities. Various biological activities of these oligosaccharides have been observed, and often these activities depend on the molecular weight and degrees/patterns of sulfation. An oligosaccharide (2 kDa) obtained from κ-carrageenan effectively inhibits influenza A H1N1 virus replication, as well as mRNA and protein expression inside the cell [22.465]. Both κ and λ-carrageenan oligomers demonstrate antitumor activity via a complex modulation of the immune response [22.466, 467] or organ-mediated defence reactions [22.445]. The κ-carrageenan oligosaccharide fraction exhibits a relatively higher antitumor activity against the three types of cancer cells (KB, BGC, and Hela) than the initial polysaccharide [22.466]. The κ-carrageenan oligosaccharides inhibited in vitro growth of five microorganisms, i. e., *Escherichia coli*, *Staphylococcus aureus*, *Saccharomyces cerevisiae* (maximal effect), *Penicillium citrinum*, and *Mucor* sp. [22.465]. The short agaro-oligosaccharides (2–4 units) suppress TNF-α production and iNOS expression [22.468], while somewhat longer oligosaccharides (6–8 units) can elicit a physiological response in red algae [22.469, 470]. Agar oligosaccharides obtained by enzymatic cleavage demonstrate antioxidative activities in ROS scavenging (•OH, $\bullet O_2^-$) and inhibit lipid peroxida-

tion [22.447]. Sulfated and longer fragments show stronger antioxidant activities than nonsulfated and shorter ones.

22.5.10 Chemical Modification

Sulfation (or oversulfation) of marine algal polysaccharides is an effective way of obtaining biologically active derivatives that are especially interesting as potential anticoagulants. The patterns of sulfation are often an important factor of expected biological activity. The inactive partially sulfated and 3,6-anhydro-substituted κ, ι, and θ-carrageenans have been oversulfated regioselectively, and the anticoagulant activity of the derivatives obtained has been studied by aPTT *in vitro* assay [22.471]. The results confirmed that sulfation at *O*-2 of 3,6-anhydro-α-D-Gal*p* and at *O*-6 of β-D-Gal*p* increases the anticoagulant activity. The oversulfated κ-carrageenan showed a 30 times higher anticoagulant activity on doubling the PT time in comparison with the native polysaccharide [22.472]. This derivate also gave a threefold enhancement of the glutamic plasminogen (Glu-Plg) activation by tissue plasminogen activator (t-PA) or by urokinase (u-PA), while both native κ-carrageenan and crude heparin were less active. Similarly, oversulfated fucoidan showed four times higher anticoagulant activity in doubling PT time and gave higher stimulations of Glu-Plg activation by t-PA and by u-PA in comparison to the native fucoidan [22.473]. Oversulfation of fucoidan also enhances its antiangiogenic and antitumor activities [22.474]. Similarly what was mentioned above, oligomers of marine algal polysaccharides can be sulfated or oversulfated as well. Alginate oligosaccharides obtained by treatment with alginate lyase from *Vibrio* sp. 510 [22.442] have been sulfated by the formamide-chlorosulfonic acid method [22.475]. One of the derivatives obtained showed significant inhibition of tumor growth without direct cytotoxic effect, which was proposed to be caused by immunomodulation. Oversulfated, acetylated, and phosphorylated derivatives of κ-carrageenan oligosaccharides have been prepared and their in vitro antioxidant activities tested [22.476, 477]. In certain antioxidant systems some of these derivatives exhibit higher activities than the initial poly and oligosaccharides. The κ-carrageenan oligosaccharide derivatives inhibit tumor growth and stimulate macrophage phagocytosis and spleen lymphocyte proliferation [22.478]. Among them, the oversulfated derivative shows the highest antitumor activity expressed both in the decrease of tumor weight and in eliciting NK cell activity. These effects are significantly higher than those of the unmodified oligosaccharides. There are two ways to obtain novel materials based on marine algal polysaccharides: (i) covalent cross-linking via previously added substituents and/or cross-linking agents (dialdehydes, epichlorohydrin, phenolics, etc.), and (ii) interaction with synthetic polymers or their fragments. Porous scaffolds based on alginate derivative bearing phenolics has been prepared using horseradish peroxidase catalyzing cross-linkage formation between phenolic hydroxyls [22.479]. These materials have been shown to be suitable for cell immobilization. Cross-linking carrageenan beads (microgels) have been prepared for controlled release delivery systems using epichlorohydrin as the cross-linker [22.480]. The swelling/shrinking behavior of these cross-linked microgels in saline solutions has shown great potential for the application in food or pharmaceutical products. Highly stretchable and tough hydrogels have been prepared from cross-linked algal polysaccharide and synthetic polymers [22.481]. Both types of macromolecules form networks based on ionic (alginate) and covalent (polyacrylamide) cross-linkages. Because they contain $\approx$ 90% water, composite gels can be stretched beyond 20 times their initial length, and notched samples exhibit a stretch of 17 times. These novel material containing marine algal polysaccharides (cross-linked alginate) significantly enlarge the area of hydrogel applications.

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