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Alginate Lyases: Substrates, Structure, Properties, and Prospects of Application

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Abstract—Alginate lyases catalyze degradation of alginic acids and their salts, alginates, which are one of the main components of brown algae cell walls and comprise up to 40% algae's dry weight. Alginates are interesting due to their high biological activity, particularly the ability of charged groups to bind tightly to opposite-charged protein amino acid residues, and chelating and jelling properties in presence of bivalent metal cations. Alginate lyases can digest substrates by β-elimination. They can be classified by the type of cleaved bonds. For today, more than 50000 amino acid sequences are referred to alginate lyases, 47000 of them belonging to bacterial genomes. Alginate lyases are one of the most common tools for degrading biofilms. Alginate digestion products display antitumor, anti-inflammatory, and antioxidant properties.

Keywords: alginate lyases, alginates, alginic acids, alginate oligosaccharides

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DISTRIBUTION AND STRUCTURE OF ALGINIC ACIDS AND ALGINATES

Alginates are one of the main components of brown algae cell walls and can comprise up to 40% algae's dry weight [1, 2]. These polysaccharides were also found in Corallinaceae family of red algae [3]. The presence of alginates is a characteristic feature of this family [4, 5]. Among terrestrial organisms, alginic acids were detected in *Pseudomonas* and *Azotobacter* soil bacteria [6]. In other marine and terraneous organisms these polysaccharides have not been found.

Alginic acids are high-molecular-weight linear polyuronides with DP 1000 to 10000 [5] containing β-D-mannuronic (M) and α-L-guluronic (G) acid residues linked with 1→4-*O*-glycoside bonds. Alginic acids from different sources can differ by not only the ratio of mannuronic and guluronic acid residues but also the distribution of these monomeric units along the polymer chain [5]. The monomers can organize regions composed of units of one type or those with alternating units [7]. The monosaccharide composition of alginic acids strongly depends on the algae type (Table 1). Bacterial alginates are *O*-acetylated at the 2- and/or 3-position of mannuronic acid residues [8, 9].

Alginates can form stable spatial structures, in which G blocks are cross-linked with Ca^{2+} or other bivalent metal ions, which form gel with strength corresponding to the $Ba^{2+} > Sr^{2+} > Ca^{2+} \gg Mg^{2+}$ order. These structures can contain polyphenols increasing the cell wall strength. It is assumed that the genes involved in the alginate synthesis were acquired by brown algae from bacteria due to horizontal transfer [10, 11].

ALGINATE LYASES

Classification

Alginate lyases degrade the substrate by β-elimination mechanism. They are divided into endo- and exo-type enzymes according to their action on substrates. Most of the alginate lyases operate by the endo-type mechanism to give a mixture of oligosaccharides.

According to the classical nomenclature based on the action specificity, endo-alginate lyases are referred to polyguluronate lyases (EC 4.2.2.11) [12, 13], polymannuronate lyases (EC 4.2.2.3) [14], and bifunctional alginate lyases specifically targeted at 1→4 glycoside bonds between both mannuronic and guluronic acid residues [15–18]. In all the cases DP values do not exceed 2–5 [19].

A special group are exo-type alginate lyases (oligoalginate lyases) (EC 4.2.2.26), which manifest exolytic activity and, therefore, are capable of cleaving alginic acid oligosaccharides to monosaccharides (https://www.brenda-enzymes.org/enzyme.php?ecno=4.2.2.26).

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Abbreviations: DP, degree of polymerization.

Table 1. Monomeric composition of alginic acids from various algae

Species	Localization	M/G	Reference
Ascophyllum nodosum	Norway	1.50	[112]
Desmarestia distans	Chile	0.58	[113]
Desmarestia ligulata	Chile	0.77	[113]
Durvillea antarctica	No data	2.13	[114]
Eisenia arborea	Mexico	1.08	[115]
Laminaria digitata	Norway	1.44	[86]
Laminaria hyperborea	Norway	0.50	[92]
Laminaria japonica	China	1.86–2.28	[116]
Lessonia flavicans	Chile	1.03	[113]
Lessonia nigrescens	No data	1.43	[114]
Lessonia trabeculata	Chile	0.34–1.21	[117]
Macrocystis pyrifera	Argentina	1.15	[118]
Macrocystis pyrifera	Canada	1.70	[119]
Sargassum asperifolium	Egypt	0.69	[120]
Sargassum dentifolium	Egypt	0.52	[120]
Sargassum enerve	Saudi (Red sea)	0.86	[121]
Sargassum filipendula	No data	0.19	[119]
Sargassum fluitans	Cuba	0.52	[122]
Sargassum fluitans	USA (Florida)	1.18	[122]
Sargassum hemiphyllum	China	1.06	[123]
Sargassum henslowianum	China	0.82	[123]
Sargassum horneri	China	0.64	[123]
Sargassum latifolium	Egypt	0.25–0.82	[120]
Sargassum mangarevense	French Polynesia (Tahiti)	1.18–1.66	[124]
Sargassum mcclurei	China	1.47	[123]
Sargassum miyabei	China	0.62–1.10	[123]
Sargassum muticum	England	0.31	[119]
Sargassum oligocystum	Australia	0.49–0.62	[122]
Sargassum pallidum	China	1.26	[123]
Sargassum patens	China	1.59	[123]
Sargassum polycystum	No data	0.21	[119]
Sargassum polycystum	India	0.56–0.74	[125]
Sargassum scoparium	Algeria	0.73	[86]
Sargassum siliquastrum	China	1.13	[123]
Sargassum tenerrimum	China	1.53	[123]
Sargassum thunbergii	China	0.78	[123]
Sargassum thunbergii	Korea	0.53	[119]
Sargassum turbinarioides	Madagascar	0.94	[126]
Sargassum vulgare	Saudi (Red sea)	0.71	[121]
Sargassum vulgare	Brazil	1.27	[127]
Turbinaria conoides	India	0.57–0.60	[128]
Turbinaria murrayana	Saudi (Red sea)	1.09	[121]
Turbinaria ornata	French Polynesia (Tahiti)	1.05–1.45	[124]
Turbinaria ornata	China	0.89	[123]

Table 2. Comparison of the number of amino acid sequences empirically characterized as alginate lyases and the number of functionally characterized enzymes for different organism groups

Group	The number of empirically characterized amino acid sequences	The number of enzymes with the EC 4.2.2.3 activity	The number of enzymes with the EC 4.2.2.11 activity	The number of enzymes with the EC 4.2.2.26 activity
Animals	34	24	2	1
Plants	448	11	1	0
Fungi	1434	6	1	0
Protozoa	153	0	0	0
Bacteria	47 719	134	66	8
Archaea	105	0	0	0
Viruses	38	6	0	0

Table 3. Ascription of alginate lyases to structural families. The number of known and structurally and functionally characterized enzymes belonging to the given family of polysaccharide lyases (PL) or possessing the given domain is shown in the cells.

EC	Type of action	Structural family or domain							
		PL5	PL6	PL7	PL14	PL15	PL17	PL18	common domain of alginate lyases and heparinases
4.2.2.3	Poly(M)	12	1	18	3	1	1	3	0
4.2.2.11	Poly(G)	0	9	16	0	0	0	3	0
4.2.2.26	Exo(oligo-)	0	0	0	1	0	2	0	2

Distribution of Alginate Lyases

Alginate lyases are widespread in nature and are found in marine algae and invertebrates, bacteria, fungi, and some viruses [7]. Alginate lyases of various specificities were isolated from mollusks *Littorina sitkana*, *Collisella* sp., and *Lambis* sp. and marine bacteria *Pseudoalteromonas citrea* KMM 3297, *Formosa algae* KMM3553, and *P. issachenkonii* KMM 3549 and studied. The first homogeneous alginate lyase VI isolated from *L. sitkana* was a polymannuronate lyase (EC 4.2.2.3) [20]. A marine bacterium *P. citrea* was shown to synthesize three intercellular alginolytic AI, AII, and AIII enzymes. All studied alginolytic enzymes belong to the endo-type and catalyze degradation of polyguluronic and polymannuronic acids to oligosaccharides with DP 3 to 5 [18]. Another alginate lyase was isolated from *Lambis* sp. hepatopancreas. Its catalytic properties were determined, and the structures of products of sodium polyguluronate enzymatic digestion were identified. Their DP varied from 2 to 5 guluronic acid residues. It was referred to poly-1→4-α-*L*-guluronate lyases (EC 4.2.2.11) [21].

Of the five recombinant *Formosa algae* alginate lyases, three display polymannuronate-lyase activity, one shows polyguluronate-lyase activity, and one is a bifunctional enzyme. Of the two recombinant *P. issachenkonii* alginate lyases, one shows polyguluronate-lyase activity, and the other, polymannuronate-lyase [22]. Using these enzymes and the collection of alginic acids differing in structures and viscosity, it is possible to obtain biologically active alginate oligosaccharides.

Currently, more than fifty thousand amino acid sequences are identified as alginate lyases, and 47 000 of them belong to bacterial genomes. Two thousand and sixty enzymes were characterized. Of them, 181 enzymes belong to mannuronate-specific alginate lyases (EC 4.2.2.3); 70 enzymes, to guluronate-specific alginate lyases (EC 4.2.2.11); and 9 enzymes, to oligo-alginate lyases catalyzing oligosaccharide cleavage by the exo-type (EC 4.2.2.26) (Table 2). Amino acid sequences are known for 72 functionally characterized enzymes: 39 for EC 4.2.2.3; 28 for EC 4.2.2.11; and 5 for EC 4.2.2.26 [12, 14, 23–29] (Table 3). If compared with other lyases, this enzyme class has been poorly studied.

Spatial Organization

Alginate lyases belong to several polysaccharide-lyase families based on the similarity of primary structures (http://www.cazy.com): PL5, PL6, PL7, PL14, PL15, PL17, and PL18 [30, 31]. Most of the enzymes belong to the PL7 family. The catalytic domain of the

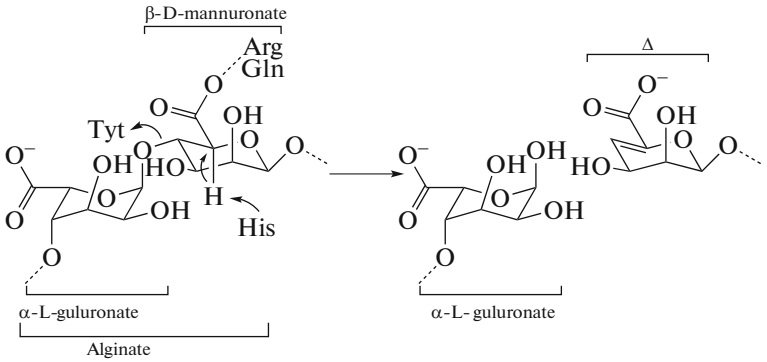

Fig 1. Mechanism of action of the alginate lyases of the PL7 family.

PL7 family is a β-cylinder, which forms a catalytic cleft with three bordering β-sheets containing catalytic amino acid residues of arginine, glutamine (asparagine), histidine, and tyrosine [32]. The catalytic domains of PL14 and PL18 families are also β-cylinders; for the PL6 family, the structure of the catalytic domain is a β-helix, whereas for PL5, PL15, and PL17 families, it is an $(\alpha/\alpha)_n$-toroid [33].

Action

Despite considerable differences in the spatial structures, the mode of alginate lyase action on polysaccharides is the same and involves three successive events (Fig. 1): neutralization of a negative charge on a carboxyl anion, elimination of the hydrogen atom at the monosaccharide C5 atom, and transfer of electrons from the monosaccharide C4 atom to give a double bond between C4 and C5 atoms. Most often, the negative charge on a carboxyl anion is neutralized by arginine and glutamine (asparagine) residues, and the electron transfer from the C4 atom occurs with a tyrosine residue [31, 32, 34, 35]. As a result, the glycoside bond is cleaved [24, 29]. The formed nonreducing end, the same for both *L*-guluronic and *D*-mannuronic acids, corresponds to 4-deoxy-*L-erythro*-hex-4-enopyranosyluronate, which is designated with a symbol Δ [36].

Apart from catalytic amino acid residues, the total charge on the surface of the catalytic cleft is also of great importance, since it provides the enzyme affinity to the substrate and enzyme specificity. In particular, it was shown for *Pseudomonas aeruginosa* alginate lyases (the PL7 family) that the K197D/K321A mutant was significantly more active due to a decrease in the positive charge around the catalytic site, facilitation of product dissociation, and, consequently, an increase in the enzyme turnover number [37].

Alginate Lyase Properties

Alginate-degrading organisms are characterized by the presence of several enzymes of this class with both homologous and heterologous specificity, which provides complete depolymerization of alginic acids. In particular, *Vibrio splendidus* 12B01 bacteria was shown to have four alginate lyases, two of which cleaved mainly MG bonds, whereas two others, mainly GG bonds [38]. The presence of such enzyme complexes is also characteristic for other polysaccharide degrading enzymes: cellulases, laminarinases, etc. For example, in the *Clostridium thermocellum* cellulase complex, one enzyme cleaves cellulose fibers to low-molecular-weight fragments with DP 2 to 5 and another one cleaves them to glucose. The β-1,3-glucanase complex from *Gaeumannomyces graminis* var. *tritici* was shown to contain two active isoforms with similar properties. Four β-1→3-glucanases with different specificity were found in the cultural medium from *Flavobacterium dormitator* var. *glucanolyticae*. This set supports the utilization by the cells of more bioavailable soluble carbohydrate-containing material at the initial stage and, after it is exhausted, the use of insoluble products, which ensures a more stable bacteria life cycle [39–42].

The activity of alginate lyases isolated from marine mollusks often depends on the presence of metal ions in the medium. For example, *Haliotis corrugate* and *H. rufescens* alginate lyases display the highest activity in the presence of 0.05–0.08 M NaCl. A high NaCl concentration (0.1–0.3 M) is also needed for enzymes from *H. tuberculata*, *Spisula solidissima*, and *Turbo cornutus* [34, 43–47]. The optimal conditions for alginate lyase from *Littorina* sp. are pH 5.6 in the presence of 0.05 M NaCl. An increase in the NaCl concentration to 0.2 M resulted in the shift of optimal activity to pH 8.0–8.5. The activity of this alginase was noticeably increased in the presence of 7.5 mM Ca^{2+} [20].

The optimal pH values of these enzymes are in a wide range of 5.0 to 10.5. For the exo-alginate lyases (oligo-alginate lyases), a narrower range is inherent, 6 to 8. Optimal temperature values are also in a wide range: 3.5 to 70°C (37 to 50°C for oligo-alginate lyases) [7, 17, 48–50]. It is noteworthy that the enzymes with hyperthermophilic properties were not

found among the alginate lyases studied and the sequences encoding alginate lyases were not found among genomes of the hyperthermophilic organism studied.

Specificity

Specificity is one of the critical properties of enzymes. Alginate lyases are generally divided into three groups: poly(M)-, poly(G)-, and poly(M/G)-specific. Some alginate lyases can catalyze degradation of both poly(M)- and poly(G)- blocks. Such enzymes are called bifunctional enzymes. All the known poly(M/G)-specific alginate lyases can also cleave one or both types of homogeneous substrates and can be referred to bifunctional enzymes. The only difference is in the activity level toward homogenous and heterogeneous substrates. In particular, the *Stenotrophomas maltophilia* KJ-2 alginate lyase of the endo-type cleaved MG-blocks ten times more effectively than polymannuronate or polyguluronate [51].

Like other polysaccharide lyases, alginate lyases are divided into enzymes of either exo- or endo-types according to the mode of action.

Alginates bind to alginate lyases at the enzyme surface, and several successive monosaccharide residues directly contact with the enzyme molecule in the active site. Amino acid residues arranging the active site interact with the substrate monosaccharide residues and form binding sites, which are numerated in accordance with their position towards the position of the cleaved bond. Thus, the enzyme active site is separated into $+n$- and $-n$-binding sites of the substrate monosaccharide residues ($n = 1, 2, 3,...$). The cleaved bond is located between +1- and −1-regions (a positive value is directed to the reducing end). For most of polysaccharide lyases, the regions within −2 to +2 are most important for manifestation of the substrate specificity and mode of action. A general requirement is also the presence of unsubstituted carboxyl groups in the monosaccharide residue adjacent to the cleaved bond from the reducing end (i.e., the end, which is transformed to 4-deoxy-L-erythro-hex-4-enopyranosyluronate during the reaction).

Polymannuronate lyases are the most abundant type of alginate lyases and are mainly represented by the PL5 structural family (the backbone is an incomplete toroid $(\alpha/\alpha)_4$) and PL7 (β-cylinder) [52, 28] (Table 3). The oligosaccharide formation as final products is related to the requirement for the enzyme to be placed onto several monosaccharide residues from the nonreducing end of the polymer molecule. In particular, *Sphingomonas* sp. A1 alginate lyase can only cleave the third and more distant bonds from the nonreducing end of the molecule [52], which results in the trisaccharide (4-deoxy-L-erythro-hex-4-enopyranosyluronate and two monosaccharide residues) accumulation. The *T. cornutus* M-specific alginate lyase can cleave the second bond from the nonreducing end, which leads to disaccharide accumulation [53]. The *Flammeovirga* sp. MY04 alginate lyase can cleave the first monosaccharide from the polysaccharide nonreducing end but requires at least three uronic acid residues from the reducing end [54].

A unique property of a shortened form of alginate lyase from *Chlorella* vAL-1(S) (PL14) virus is the formation of products with DP 2 to 6 at pH 7.0 and mainly disaccharides at pH 10.0. The authors explained this by the fact that at higher pH the substrate binds inside the catalytic cleft, whereas at a lower pH it happens at the cleft surface [55]. With the consideration that the basic structure of PL14 family is the same β-cylinder as that of the PL7 family, to which most of alginate lyases belong, the studies of the dependence on the product composition of the conditions of reactions catalyzed by the PL7 family alginate lyases is promising for the preparation of compounds with the predetermined structure.

Alginate lyases catalyzing the cleavage of polyguluronic acid (EC 4.2.2.11) are less abundant. Polyguluronate lyases mainly compose the PL7 and PL6 families. The PL6 family is characterized by a β-helix (14 turns). For PL7 and PL5 alginate lyases, the typical mechanism involves His(Tyr)/Tyr β-elimination, at which a negative charge of the carboxyl group is screened by Arg and Gln (Asn) residues followed by elimination of the C5 proton by histidine, and then a negative charge is transferred by a tyrosine residue to result in a cleavage of the *O*-glycoside bond and formation of a double bond between sugar C4 and C5 [31, 32, 34, 35, 56]. In the case of the PL6 family, the Ca^{2+}-mediated elimination prevails: a negative charge on a carboxyl group is neutralized by Ca^{2+} cation with lysine as a proton acceptor and arginine as a donor. This mechanism is characteristic for all alginate lyases bearing β-helixes [33, 51, 57].

The published data on the role of calcium ions for the polyguluronate lyase activity are in line with the concept of the three-step mechanism of β-elimination [51]. The enzymes degrading polyguluronate considerably better than polymannuronate display high diversity in their modes of action. For example, *Agarivorans* sp. [58] and *Shewanella* sp. YH1 [59] guluronate lyases degrade polyguluronate faster than heterogeneous alginate, whereas similar enzymes from *Persicobacter* sp. CCB-QB2 [60] and *Vibrio* sp. W13 [61] degrade faster a mixed M/G alginate, which may be evidence of their higher activity towards MG- or GM- than towards MM- and GG bonds.

Like polymannuronate-specific alginate lyases, for guluronate lyase from *Flammeovirga* sp. MY04 the presence of three G acid residues upstream of the cleaved bond was shown to be necessary, although the enzyme was slightly active in the presence of two residues as well. In the presence of all the required residues, the enzyme operates mainly by the endo-type

rather than by the exo-type mode [62]. An absolutely unique property is the capacity of *Zobellia galactanivorans* to attack only the bond with two guluronic acid residues from non-reducing end of the molecule [28].

Whereas the majority of polymannuronate and polyguluronate lyases belong to the PL7 family of polysaccharide lyases, a considerable part of exo-(oligo-) alginate lyases cannot be classified within the known families. Their primary structures were found to contain sequences shared by all alginate lyases and heparinases [63]. Oligo-alginate lyases of nonclassified structural membership from *Stenotrophomonas maltophilia* KJ-2 and *Sphingomonas* sp. MJ-3 preferentially effect polymannuronate than heterogeneous alginate [63, 64], whereas oligo-alginate lyases of the PL17 family from *Saccharophagus degradans* and *Shewanella* sp. possess wide specificity and prefer heterogeneous alginate [49, 65]. Exo-alginate lyase from *Haliotis discus hannai* (the PL14 family) is M-specific [66]. This allows an assumption that heterospecificity is a characteristic feature of PL17 oligo-alginate lyases.

Until recently, it was believed that, in accordance with the "lock-and-key" concept, alginate lyases can only be either M- or G-specific, and other activities were ascribed to poor purification of the preparation. Nevertheless, in recent years, many enzymes have been obtained that could cleave both polymannuronate and polyguluronate even in a highly purified state [14, 29, 67–70]. Thus, some of the alginate lyases are bifunctional enzymes with different affinities to different substrates. Therefore, it is not surprising that binding principles are often shared by different substrates. For example, alginate lyase from *Pseudoalteromonas* sp. No.272 requires three monosaccharide residues upstream and three residues downstream from the degraded bond without regard to the nature of the monosaccharide residues [16].

The use of Alginate Lyases for Biofilm Removal

Modern healthcare systems are strongly dependent on the use of biomaterials and medical devices [71]. However, the use of surgical tools and implants is associated with risks due to their long period of application, since they serve the major route for distribution of nosocomial infections [72]. Bacteria can reach the biomaterial surface, attach to it, and form multicellular aggregates within the matrix (exopolysaccharide). As a result, the structures called biofilms are formed [73]. In these cases, treatment is extremely difficult since bacteria acquire an enhanced stability to antibiotics and the attack of immunocompetent host cells [74]. The formation of biofilms is a cyclic development process initiated by bacterial adhesion to biomaterial surfaces followed by proliferation, aggregation, and maturation [75]. Among pathogens, *Pseudomonas aeruginosa* strains attract high attention due to their outstanding ability to form biofilms [76, 77]. The alginate production is one of the most well studied factors of *P. aeruginosa* virulence and is mainly associated with mucoid isolates from lungs of cystic fibrosis patients [78]. Therefore, one of the most effective ways of biofilm degradation is the use of alginate lyases [79, 80], and enzyme immobilization (for example, polydopamine-induced immobilization of *Sphingobacterium multivorium* enzymes) on the surface of medical devices is proposed as a measure for prevention of biofilm formation [81].

Another dangerous pathogen, whose virulence is due to biofilm formation, is *Helicobacter pylori*. This microorganism can form mature biofilms for three days after in vitro incubation. It was shown that a combination of alginate lyase from *Flavobacterium* sp. with clarithromycin had a synergetic effect, which allowed a 4- to 8-fold decrease in the effective antibiotic concentration [82].

Oligosaccharide Preparation and Activity Studies

Currently, commercial oligonucleotide mixtures with nonstandardized composition are most commonly used in research [83–85]. Interest in alginate oligosaccharides with regular structure is growing: during the last two years more data than during all the previous years were published [69, 86–88 vs 89–91]. Oligosaccharides are obtained by the alginate lyase action on alginates [69, 83–86, 88]. In particular, in 2016 a highly active alginate lyase (24038 U/mg) was isolated from marine bacteria *Cellulophaga* sp.NJ-1, which degraded *Macrocystis pyrifera* alginate to give products with DP 2–6. Alginate lyase from *Flammeovirga* sp. MY04 catalyzed the formation of products with DP 2–7 to give di-, tri-, and tetrasaccharides enriched with guluronic acid residues and penta-, hexa- and heptasaccharides, with mannuronic acid residues. Polyguluronate lyase from *Flavobacterium multivorum* catalyzed the alginate degradation from brown algae *Stypocaulon scoparium* to give di- and trisaccharides. The products were separated by anion-exchange chromatography and gel filtration. Basic analytical methods of both the starting material and products are NMR and mass-spectrometry, IR-spectroscopy, HPLC, and TLC [88, 89].

Another approach for alginate preparation with various M/G ratios is the use of C5 epimerases. For example, modification of polymannuronate from mutant *Pseudomonas fluorescens* (lacking is own epimerase gene) performed with epimerase AlgE6 *Azotobacter vinelandii* and its two mutant forms AlgE64 and EM1 led to alginates bearing 68, 77, and 84% G-blocks, respectively. The length of these blocks was 20 to 50 units, whereas in brown algae alginates, blocks more than 100 units long were found [92]. The effect of epimerase AlgE4 from *A. vinelandii* on natural alginates from brown algae *Durvillea antarctica*, *Lessonia nigrescens,* and *Laminaria hyperborean* resulted in an increase of guluronic acid residues from 32 to 52%, 41 to 56%, and 67 to 74%, respectively. Thus, the aug-

mentation of guluronic acid residues was inverse to the content of these residues in the starting substrate. A successive action of AlgE4 (inserting long MG sequences) and AlgE6 (inserting G blocks inside the polymannuronate chain) on the conjugate of *Pseudomonas fluorescens* polymannuronate and 1-amino-1-deoxygalactose allowed an increase in the G block content from 0 to 45% [93].

Despite each of the approaches has been studied well, there are no data on their combined effect, i.e., the possibility to obtain alginate oligosaccharides of the predetermined structure using a combination of degradation of a longer chain and epimerization of individual monosaccharide residues has not been studied.

The main issue of chemical hydrolysis is the formation of M-, G-, or M/G-blocks more than 100 residues in length [92, 86], and alginate lyases support alginate oligosaccharides with DP 2–20. In particular, in the presence of alginate lyase from *Flavobacterium multivorlum*, 3742 g/mol oligoguluronates, which corresponded to approximately 17 residues, were obtained from *Stypocaulon scoparium* alginate polyguluronate blocks [86]. Oligosaccharides obtained from *L. japonica* alginates in the presence of alginate lyase from *Vibrio* sp. 510 were separated by a combination of gel filtration and ion-exchange chromatography under moderate pressure [94].

Currently, the biological activity of alginate oligosaccharide mixtures is being studied. In particular, a conjugate of an alginate oligosaccharide mixture with salmon myofibril protein displayed an anti-inflammatory activity on a murine macrophage RAW264.7 model by suppressing the expression of TNF-α, interleukin-6, and COX-2 but not affecting COX-1 [83]. On the other hand, the effect of alginate oligosaccharides on RAW264.7 macrophages activates the toll-like receptor 4 (TLR4) and triggers TLR4-like signal pathways TLR4/Akt/NF-κB, TLR4/Akt/mTOR, and MAPK leading ultimately to the release nitric oxide (II) and TNF-α [95].

Oligosaccharides of sulfated sodium alginate with DP 2–10 stimulate proliferation of Ba/F3 cells and receptors of the fibroblast growth factor [96]. Alginate oligosaccharides are used by plants as signal molecules regulating development and protection processes. Oligosaccharide mixtures prevent the formation of *Candida albicans* colonies on a solid phase [97], decrease the content of microRNA miR-155 in blood serum [98], and can inhibit the formation of *P. aeruginosa* microcolonies [99]. They are capable of neutralizing acute doxorubicin cardiotoxicity by reducing gene expression of gp91-phox, 4-HNE, caspase-12, C/EBP-homologous protein (CHOP), markers of endoplasmic reticulum apoptosis, and Bcl-2-associated X protein (BAX) and at the same time can increase the expression of antiapoptotic Bcl-2 protein [100, 101], decrease the size and frequency of aneurism recurrence, reduce the levels of miR-29b, the toll-like TLR4 receptor, mitogen-activated protein kinase (MAPK), nuclear κB factor, IL-1β and IL-6, thus inhibiting aneurism inflammatory processes and providing conditions for recovery of injured vessels [102], stimulate the growth and migration of human endothelial cells and keratinocytes [103]. Also, they are used for cell immobilization in biotechnology or for cell differentiation [104] and are shown to exhibit more effective antioxidant properties in vitro than ascorbic acid [105].

Injection of alginic acid oligomers at a dose as low as 70 mg/kg supports a maximal concentration of granulocyte-macrophage colony-stimulating factor (GM-CSF) and other 19 cytokines including MCP-1 (CCL2), IL-6, and IL-12 in human blood serum [106, 107]. The use of alginate oligosaccharides with DP 2–4 allows an improvement in the integrity of frozen shrimps [85]. Obviously, such a different, but in all cases beneficial, effect requires further study.

Studies of the biological activity and action of individual alginate oligosaccharides are extremely limited. For the alginate lyase products with an established structure, or at least a known number of links, the only available data are as follows. Alginate oligosaccharides with DP 5 most effectively accelerate the growth of sprouting cabbage seeds, lettuce, carrot, and rice seedlings [106, 108, 109]. Oligomannuronates and oligoguluronates with DP 10 at concentrations of 100–300 mg/L support an increase in the bacitracin A production by liquid *Bacillus licheniformis* by 29% and penicillin G production by *Penicillium chrysogenum* by 50%. It is noteworthy that oligosaccharides with DP less than four are ineffective. Among alginate oligosaccharides with DP 3–9, G8 and M7 most actively increase the cytokine production (GM-CSF, TNF-α, and different interleukins) in murine macrophages. Among the products of recombinant alginate lyase from *Agarivorans* sp. L11, only the products with DP 5 inhibit the osteosarcoma MG-63 cell growth by 60–70% with long term oral administration in patients after surgery [87]. Mannuronate blocks are known to form mainly expanded ribbon-like structures, whereas guluronate ones form straight chains. Probably, it is the spatial structure that determines the Alginate oligosaccharides biological activity, whereas the monomeric composition plays a secondary role.

Although in general the mechanisms of therapeutic action of alginic acid oligosaccharides of remain unclear, the authors of the publication on the study of the antitumor activity of alginate oligosaccharides with DP 5 [87] made an assumption that it results from antioxidant and anti-inflammatory activities associated with an increase in blood serum in superoxide dismutase (SOD), glutathione (GSH), and cholesterol (high density lipoprotein, HDL-C) concentrations and a decrease in IL-1β, IL-2, triglycerides, cholesterol (low-density lipoprotein, LDL-C), and

malonic dialdehyde concentrations, as well as a reduction of AST/ALT ratio [87]. It was proved [110] that the alginate antitumor activity depended on the M/G ratio: the samples with a higher content of polymannuronates manifested a higher antitumor activity. The experiments were carried out using native alginates on Sarcoma-180 cells, Ehrlich carcinoma, and invasive breast carcinoma. Alginates with a low content of mannuronic acid residues display a high antitumor activity after the addition of 1–3 mM Ca^{2+}. Possibly, this can be explained by the requirement of a certain spatial structure for the activity [111].

CONCLUSIONS

Currently, the analysis of biological activity of the products of alginate enzymatic digestion is of great interest for both theory and practice. The data available on antitumor, anti-inflammatory, antioxidant, and antiradical activities of oligosaccharides can be associated with their ability to chelate cholesterol molecules, quench free radicals, stimulate and in other cases to inhibit cytokine synthesis. The chain length and monosaccharide composition play an important role, which may imply the necessity of a specific spatial structure for their activity. However, its precise nature remains unclear.

Alginate lyases allow the preparation of products with the desired M/G ratio, which results in a considerable improvement in the biological activity of products in comparison with initial substrates. If compared with chemical hydrolysis, enzymatic transformations of alginates lead to oligosaccharides with regular structures in high yields. Unfortunately, the studies of biological activity of alginic acid oligosaccharides fall behind the studies of their structures and physicochemical properties. As a large number of enzymes are characterized only by their amino acid sequences, the preparation and screening of their recombinant analogs looks like the most promising approach.

Preparation of alginic acid oligosaccharides requires substrate and enzyme screening and optimization of reaction conditions. Specificity of bifunctional alginate lyases toward the monosaccharide environment of the degraded bond is completely unknown. Although it was proved in many works that the presence of 1 to 3 monosaccharide residues at the degraded bond from the reducing or nonreducing ends is an obligatory condition for successful catalysis, the necessity of the presence of two guluronic acid residues at the degraded bond from the nonreducing end was demonstrated only for *Zobellia galactanivorans* alginate lyase [28]. The development of large libraries of alginate oligosaccharides with known structures is required to elucidate the fine specificity of the enzyme under study.

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