6 Phenolic-based Adhesives of Marine Brown Algae

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6.1 Introduction

Brown algae, such as kelps and fucoids, occur over large areas of the subtidal and intertidal rocky shores, including tropical reef habitats, producing high biomass and determining the structure of the ecosystem (i.e. kelp forests). Brown algae live firmly attached to the substratum and are often exposed to high gradients of turbulence. Therefore, they experience drag and lift forces of currents and waves with velocities that may exceed 10 m/s. As other sessile marine organisms, to disperse in certain phases of their life histories and to survive in such a stressful environment, they had to evolve strategies to adhere strongly and durably underwater from the microscopic reproductive cell stages to the large thalli of giant kelps. Brown algal filamentous species such as *Ectocarpus* were also identified as major ship fouling organisms in the 1960s, when the use of anti-fouling paints containing organo-metallic compounds–which was successful against fouling green algae–enabled *Ectocarpus* to bloom (Baker and Evans 1973).

Unfortunately, studies on adhesion mechanisms of marine brown algal adhesives have consisted mostly of analogy and hypothesis (Vreeland et al. 1998). Better characterization of these adhesives will eventually lead to new strategies to prevent biofouling or to produce or design synthetic water-resistant adhesives of commercial value. The properties of the adhesives in terms of spreading and curing underwater are crucial to successful colonization of substrata. However, much of the evidence on the composition of algal adhesives is circumstantial and based on methodologies such as histochemistry (Fletcher and Baier 1984; Gonzales and Goff 1989; Fletcher and Callow 1992; Callow and Callow 2002). These studies indicate that macroalgal adhesion involves carbohydrate and glycoprotein-containing mucilage (Fletcher and Callow 1992) that 'cure' with time after discharge, thereby increasing the strength of attachment to the substratum. In contrast with the progress in the chemical characterization of diatom adhesives (Chamberlain 1976; Stossel 1993; Lind et al. 1997; Wustman et al. 1997; Wetherbee et al. 1998; Higgins et al. 2002), the composition and physicochemical nature of adhesives used by different macroalgae remain unknown. Therefore, it is difficult to infer by which processes they

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Biological Adhesives
(ed. by A.M. Smith and J.A. Callow)

consolidate attachment through 'curing' reactions of adhesives, without knowing their basic components. However, some studies of the formation of adhesives in brown algal zygotes indicated that this mechanism shares common processes with the settlement of intertidal invertebrates such as the oxidase-mediated polymerisation of phenolic compounds (Vreeland et al. 1998).

This chapter summarizes recent work the results of which strengthen the hypothesis that brown algal phlorotannins could be one of the components of the bioadhesive system of brown algae and that vanadium-dependent haloperoxidase may regulate its curing process. It also highlights the recent development of genomic and genetic approaches in brown algae as an inspiring source of new research strategies to understand adhesion processes and to design biomimetic materials.

6.2 Adhesion of Brown Algal Propagules

Brown algae propagate mostly by sexual reproduction with at least swimming male gametes (antherozoids) and by motile spores. Female gametes may be either motile bi-flagellated cells or non-motile eggs in oogamous species (oospheres of the Fucales). The change from a motile to a permanently adhered spore, or the adhesion of a non-motile fertilized egg, is fundamental to the colonization of a new substratum.

6.2.1 Settlement and Attachment of Brown Algal Spores

In kelps, actively swimming spores can choose whether or not to settle and can terminate the settling process after contacting a surface (Reed et al. 1992). In contrast, *Ectocarpus siliculosus* spores do not seem to be attracted (either chemotactically or chemokinetically) nor stimulated to settle by nutrients (Amsler et al. 1999). Nevertheless they did settle differentially to substrates of different surface energy (hydrophobicity or wetability) with fourfold higher rates of settlement on hydrophobic surfaces compared to either positively- or negatively-charged hydrophilic surfaces (Amsler et al. 1999). *Hincksia irregularis* also settles at faster rates on hydrophobic surfaces (Greer and Amsler 2002, 2004; Greer et al. 2003) as do spores of ulvoid green algae (e.g., Callow and Callow 1998a,b, 2000; Ista et al. 2004 and see Chap. 4). Spores attach more readily to hydrophobic surfaces, probably because such a surface more readily allows the exclusion of water molecules from the adhesive/substratum interface (Maggs and Callow 2003, Chap. 4, this volume). However, consistent with most of published data that show that attachment strengths of a range of marine organisms to hydrophobic low-energy surfaces is weakened, most spores appear to be more easily removed from hydrophobic than hydrophilic surfaces. Motile flagellated spores of *E. siliculosus* were also shown to attach to substrata through a mechanism that involves the secretion of a fibrous

material from vesicles (Baker and Evans 1973). After settlement for 1–2 h, the attached cell develops a thin cell wall, but a thin cushion of a fibrillar material underlies the settled cells when viewed by TEM (transmission electron microscopy) (Baker and Evans 1973). The fibrillar nature of this adhesive material is questionable in the light of the recent results obtained with *Ulva* spores using Environmental Scanning EM whereby the adhesive appears as a gel-like material rather than the fibrillar appearance previously shown by TEM, suggesting that the latter may be an artefact of dehydration during specimen preparation (see Chap. 4 in this volume). When combined with cytochemical investigation, this study concluded that most of the *Ectocarpus* spore adhesive seems to be a polysaccharide (Baker and Evans 1973). While we have some information about spore settlement and attachment in brown algae, which allow comparisons with ulvoid spore adhesion processes (see Chap. 4 in this volume), the composition and physicochemical nature of adhesives used by brown algal spores and how they consolidate attachment through 'curing' reactions have been hardly investigated.

6.2.2 Adhesion of Fucoid Zygotes

Since earlier studies like those of Thuret and Bornet (1878) and Levring (1947), the rapid and firm attachment of the zygotes and embryos of fucoid brown algae has been mentioned and it was reinvestigated in the 1970s using scanning electron microscopy techniques (Moss 1981). The non-motile eggs of the Fucales are some of the largest reproductive cells to be found amount seaweeds. As soon as they are released they sink and, immediately after fertilization, the zygotes adhere to the substratum and select a growth axis according to environmental cues (Fig. 6.1). Indeed, fucoid algae exhibit an early developmental pattern in which zygotes generated from symmetric eggs undergo the formation–and eventual fixation–of a polar axis in response to external stimuli, including unilateral light (reviewed in Quatrano 1997; Brownlee et al. 2001). Fucoid zygotes develop cortical and cell surface asymmetries as polar development progresses (Fig. 6.1).

Surprisingly, in this extensively-investigated model, little attention has been paid to the origin and chemical composition of the sticky jelly that surrounds the entire zygote (Fig. 6.1). The chronology of cell adhesion, adhesive deposition, and polar growth axis selection induced by light (photopolarization) have been investigated with regard to zygotes of *Pelvetia fastigiata* (Schröter 1978) and *Silvetia compressa* (Hable and Kropf 1998), respectively. As proposed by Vreeland et al. (1993), the requirements for secreted compounds and the involvement of cytoskeleton in these processes were also confirmed by Hable and Kropf (1998). Adhesive deposition occurred in two distinct stages: a first uniform deposition took place on the outer surface of young zygotes (uniform primary adhesive), simultaneously with cell adhesion and photopolarization, and shortly thereafter an asymmetrical deposition (polar secondary adhesive) occurred at the future growth site of the

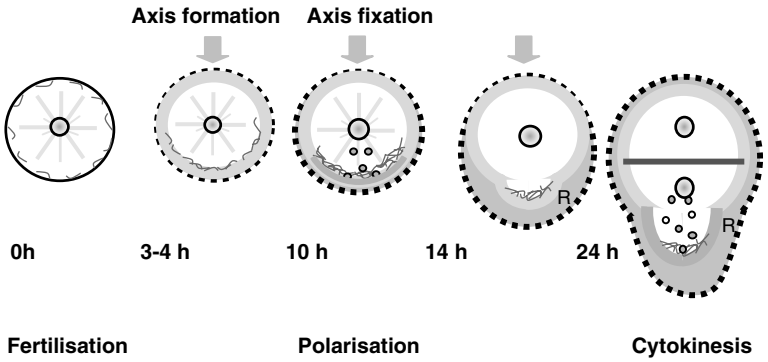

Fig. 6.1. Pattern of adhesive deposition during the early development of fucoid brown algae as revealed by non-fluorescent (Schröter 1978) or fluorescent microbeads (Hable and Kropf 1998) and imaging by light or confocal microscopy. At fertilization (0 h), no adhesive is present. It becomes visible at about 3 h after-fertilization (AF) as a thin layer of uniformly distributed adhesive. From 10 to 14 h AF, adhesive is apparent at both poles of the zygote, but is becoming thicker at the rhizoid pole (R). Cell wall is drawn in *dark line* and the *dotted line* symbolises the microspheres indicating the surface of the adhesive. Some of the key cytological events during the photopolarisation (*arrow*) and asymmetric division of the fucoid zygote are schematized, such as the localisation of actin microfilaments and the targeted secretion of Golgi at the future rhizoid pole

rhizoid cell. Associated with the formation of the polar axis is an asymmetric targeting of molecules, creating a localized accumulation of specific polypeptides (Wagner et al. 1992; Shaw and Quatrano 1996; Pu et al. 2000) and carbohydrates (Quatrano and Crayton 1973; Novotny and Forman 1974; Brawley and Quatrano 1979; Hable and Kropf 1998) at the cell surface prior to the visible morphological changes associated with asymmetric cell growth (Quatrano and Shaw 1997). Based on various cytological methods, it was proposed that some of the components of this jelly are alginate and fucan polysaccharides interacting with other macromolecules (Vreeland et al. 1998).

Following initial adhesion, algae may eventually become tightly bound to the substratum by a range of processes involving complex extracellular polymeric substances or extracellular matrix, including crosslinking mechanisms that have been proposed for diatoms and brown algae (Wang et al. 1996, 1997; Wustman et al. 1997; Vreeland et al. 1998).

A possible mechanism of adhesion, although not directly proved in brown algae, involves a vitronectin-like protein, a so-called Cell Adhesion Molecules (CAM) protein in mammalian adhesion processes (Felding-Habermann and Cheresh 1993). It has been shown that polyclonal antibodies against the human Vitronectin (Vn) recognized a vitronectin-like glycoprotein (Vn-F), exclusively secreted in the cell wall of an elongating rhizoid tip of the zygotes and embryos of *Fucus distichus*. It was also shown in an adhesive assay that, in the presence of the Vn-antibody, the rhizoid was unable to adhere to the

glass substratum, suggesting that the Vn-like glycoprotein might have a functional role in *F. distichus* adhesion (Wagner et al. 1992). If algal adhesion is promoted via a Vn-like glycoprotein, it displays similarities with focal adhesions in animal cells (Yamada and Miyamoto 1995). The heparin binding sequence of this molecule might also interact with the anionic polysaccharides in the adhesive material.

In another study on the adhesion of zygotes of *F. distichus*, it was shown that the primary adhesion was initially noncovalent in nature based on the loss of adhesive properties in high salt or with chelators (Vreeland and Laetsch 1990). Secondary adhesion is thought to involve a number of common processes, including a fiber-phenolic-catalyst mechanism that cross-links extracellular matrix components, including those of the above-mentioned focal adhesion complex (Vreeland et al. 1998). The model of phenolic cross-linking is strengthened by the accumulation of osmiophilic bodies that may contain phenolic compounds, at the site of rhizoid initiation in *Fucus* zygotes (Fig. 6.1).

6.3 Secretion of Brown Algal Phenolics and Adhesion

Brown algal phenolics (Fig. 6.2) are structural analogues of terrestrial condensed tannins. These so-called phlorotannins are known only from brown algae (Phaeophyceae) and are present at detectable concentrations across almost all brown algal orders. Soluble phenolics can constitute up to 25% dry weight (e.g., Targett et al. 1992; van Alstyne et al. 1999). These tannins are acetate-malonate derived polymers of phloroglucinol (1,3,5-trihydroxybenzene). Their chemical structure which is based on aryl-aryl and/or diaryl ether linkages of phloroglucinol units is rather complex and polymerisation processes lead to a wide range of molecular sizes (126 Da to 650 kDa). Depending on the nature of the structural linkages binding the phlorotannin polymers and on the number of hydroxyl groups present, six major groups of structure-based motifs have been defined: fucols, phlorethols, fucophlorethols, fuhalols, isofuhalols and eckols (Ragan and Glombitza 1986). These specific groups are often characteristic of specific algal genera, for example, fucols in *Fucus* and eckols in *Ecklonia*. Phenolic compounds represent about 7–9% of the dry weight of *Fucus* young stages. They are mainly formed of four to seven phloroglucinol units, primarily ether linked with a maximum of three phenyl units per oligomer (Ragan and Glombitza 1986). Branching occurs in about 25% with a degree of polymerization of greater than 100 units. The most striking feature of the polymer in Fucales is that most of the branches terminate with a bi-hydroxyl or tri-hydroxyl phenol group, which could contribute both to the potential oxidative cross-linking of the polymer and to the adhesive properties of the polymer by the ability to replace water at the interface (Waite 1987). It is suggested that they play multiple ecological roles, such as

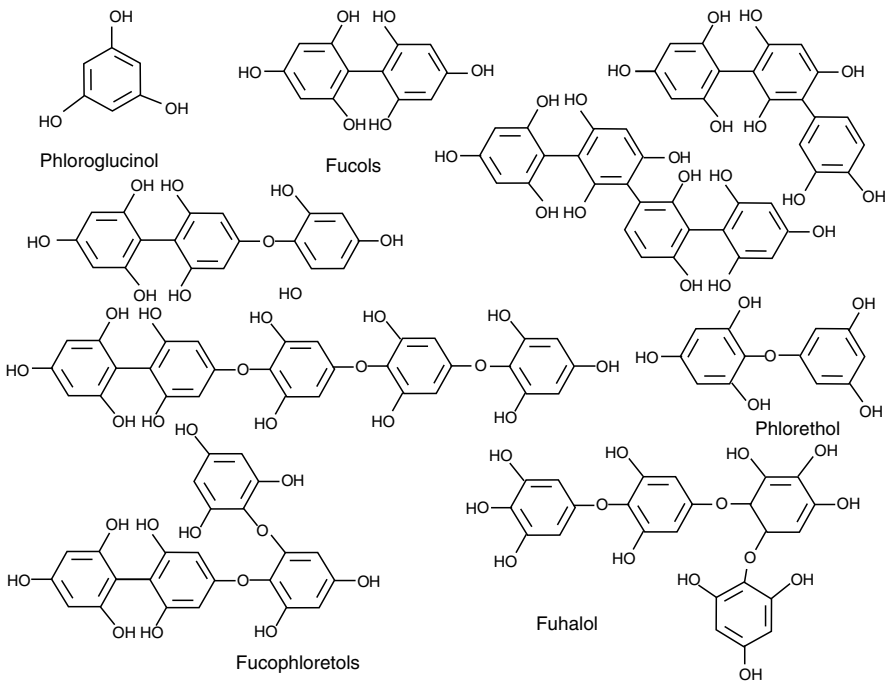

Fig. 6.2. Chemical structure of the main encountered phloroglucinol-based polyphenols in brown algae. Fucols: phloroglucinol units linked through aryl-aryl bonds, occurring in Fucales and in Ectocarpales; Fucophloretols: dehydrooligomers of phloroglucinol which contain both direct carbon-carbon and diaryl ether bond, occurring primarily in Fucales and sporadically in Laminariales. Phlorethols: phloroglucinol units linked through diaryl ether bonds, occurring both in Fucales and Laminariales. Fuhalols: ether-linked phloroglucinol linked through para and ortho ether bonds with an extra hydroxyl group on one unit, occurring primarily in Fucales

antifouling substances and chemical defences against grazers (Arnold and Targett 2002).

Phlorotannins are stored in cell organelles, the so-called physodes, which are round to elliptical, highly mobile, vesicle-like, strongly refractive bodies, observed in the cytoplasm of brown algae (Ragan and Glombitza 1986; Schoenwaelder 2002). Once they have crossed the cell membrane, phenolic bodies break up, presumably because they are no longer bound to the cell membrane, and small particles of phenolic material become embedded in the zygote cell wall. Physodes accumulate at the zygote periphery early in development and are secreted into the primary zygote wall. During germination, physodes accumulate at the rhizoid tip. Phenolic compounds are also involved in the formation of the cell plate and cross-walls of *Hormosira* and *Acrocarpia* (Schoenwaelder and Clayton 1998a) and also in several species of *Fucus* (Schoenwaelder and Wiencke 2000).

It has also been shown in *Fucus* zygotes that the secretion of phenolic polymers correlate with the attachment process (Vreeland and Epstein 1996). The secretion starts a few hours after egg fertilization. Later, after germination, phenolic polymer secretion is localized at the site of attachment. The osmiophilic bodies likely accumulate at the site of rhizoid initiation and one antibody to alginate gelling subunit was shown to label phenolic vesicles, as well as Golgi vesicles in *Fucus* zygotes (Vreeland and Laetsch 1990).

6.4 Curing Mechanisms Involving Brown Algal Vanadium Peroxidases

A model of oxidative cross-linking of secreted phenolics (Fig. 6.3) mediated via the catalysis of a vanadium bromoperoxidase was proposed based on some indirect evidences, such as vHPO immunolocalization in adherent cells

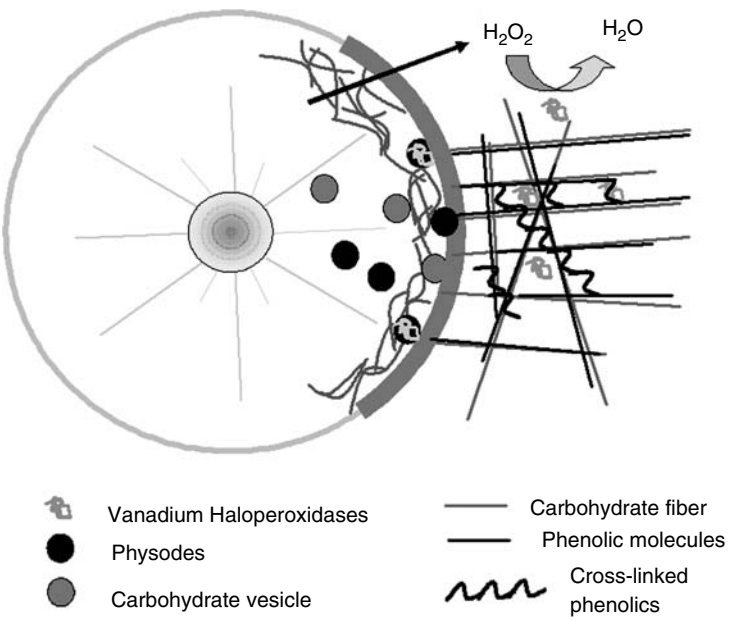

Fig. 6.3. A hypothetical model of adhesive deposition and phlorotannin crosslinks in the adhesive jelly surrounding the cell wall of fucoid zygote at the future rhizoid pole (adapted from Vreeland et al. 1998). This model is not based on the direct testing of cross-links formation, nor on studies of microstructure. It is deduced from the observation of fibrillar material at the site of adhesive deposition in fucoid zygotes by Scanning Electron Microscopy. It takes into account the secretion of phenolic-rich and carbohydrate-containing vesicles and the extracellular location of vanadium haloperoxidases in brown algae. The steady-state release of H_20_2, which is continuously produced during the early development of *Fucus* zygotes and embryos is indicated

of several brown algae (Vreeland and Laetsch 1990; Vreeland and Epstein 1996). Indeed most of our current knowledge is based on this review paper by Vreeland et al. (1998) and on the information displayed in a US Patent (Vreeland and Grotkopp 1996). Simple aggregation experiments were shown, but no details were given on the cross-linking processes.

6.4.1 Brown Algal Vanadium-dependent Haloperoxidase

The first vanadium-dependant haloperoxidase (vHPO) was discovered in *Ascophyllum nodosum*, a brown alga belonging to the Fucales (Vilter 1984). Whereas vHPO activities have been detected in a very large number of the classes of the Phaeophyceae (for a review see Vilter 1995), very few data were available on their biochemical properties notably because of the difficulties in purifying such enzymes from algal matrices extremely rich in anionic polysaccharides and polyphenolic compounds. The improvement of an aqueous two-phase extraction protocol by Jordan and Vilter (1991) for Laminariales, later extended to Fucales (Vilter 1994), allowed the acquisition of biochemical data on some partially- or fully-purified enzymes. At the molecular level, only five cDNAs of vHPOs have been cloned in the three species *A. nodosum*, *F. distichus* and *Laminaria digitata* (Table 6.1).

Haloperoxidases catalyze, in the presence of hydrogen peroxide, the oxidation of halides (X^-: iodide, bromide or chloride) to their corresponding hypohalous acids or a related electron oxidized halogenating intermediate such as OX^-, X_3^- and X^+. A variety of halocarbons can subsequently be generated if the appropriate nucleophilic acceptors are present (for review see Butler and Carter-Franklin 2004). They are named according to the most electronegative halide that they can oxidize: chloroperoxidases can catalyze the oxidation of chloride as well as of bromide and iodide, bromoperoxidases (BPO) react with bromide and iodide, whereas iodoperoxidases (IPO) are specific for iodide. Because of these halogenating properties, extensive research has been conducted on *A. nodosum* vBPO, which has become a laboratory model for understanding catalytic mechanisms of vHPOs (Butler and Carter-Franklin 2004). Its crystal 3D structure has been resolved from native enzyme preparation (Weyand et al. 1999) and compared to those of the vCPO from the fungus *Curvularia inaequalis* and of the vBPO from the red algae *Corallina*, whereas the complete catalytic cycle is still uncertain and the origin of halide selectivity remains one of the unanswered question regarding these enzymes (For reviews see Littlechild and Garcia-Rodriguez 2003; Butler and Carter-Franklin 2004). Very recently, new post-translational bromination and iodination of tyrosine residues of *A. nodosum* vBPO have also been reported (Feiters et al. 2005).

Laminaria digitata features two distinct vanadium-dependent haloperoxidase gene families (Colin et al. 2003, 2005). Iodoperoxidases could be involved in the highly efficient mechanism of iodine accumulation in kelps (Küpper

Table 6.1. Biochemically-characterized vanadium-haloperoxidases in Phaeophyceae

Classification[a]	Species	Activity	References
Order: Fucales	*Ascophyllum nodosum*[b]	vBPO	Vilter (1984); Krenn et al. (1989); Weyand et al. (1999)
	Fucus distichus[c]	vBPO	Soedjak and Butler (1990, 1991); Vreeland et al.[d]
	Pelvetia canaliculata	vIPO	Almeida et al. (2000)
Order: Laminariales	*Macrocystis pyrifera*	vBPO	Soedjak and Butler (1990, 1991)
	Ecklonia stolonifera	vBPO	Hara and Sakurai (1998)
	Laminaria saccharina	vBPO	De Boer et al. (1986); Almeida et al. (2001)
	Laminaria hyperborea	vBPO and vIPO	Almeida et al. (2001)
	Laminaria ochroleuca	vIPO	Almeida et al. (2001)
	Laminaria digitata[c]	vBPO and vIPO	Colin et al. (2003, 2005)
Family: Phyllariaceae	*Phyllariopsis brevipes*	vIPO	Almeida et al. (1996)
	Saccorhiza polyschides	vIPO	Almeida et al. (1998)

vBPO: vanadium-bromoperoxidase; vIPO: vanadium-iodoperoxidase
[a] According to Draisma et al. (2003)
[b] Resolution of the crystal structure from the native protein
[c] Characterization at the molecular level with full-length cDNAs available
[d] Vreeland V, Ng K, Epstein L (unpubl.), GenBank accession no. AF053411

et al. 1998; Colin et al. 2003). The vBPOs form a large multigenic family, whose members should have evolved towards specialized functions such as halocarbon production in relationships with algal chemical defense (Potin et al. 2002) or oxidative detoxification, as seen in protoplasts (Roeder et al. 2005). In the context of cross-linking events, these enzymes should also take part in cell wall assembly during regeneration of protoplasts (Roeder et al. 2005).

6.4.2 *In vitro* Investigations of Haloperoxidase-mediated Oxidative Cross-linking

In fact, vBPO was proven to be involved in the cross-linking of brown algal phlorotannins only very recently (Berglin et al. 2004). Phlorotannins were adsorbed to a quartz crystal sensor and the cross-linking was initiated by the addition of vBPO, KBr and H_2O_2. The decreased dissipation upon addition of the cross-linking agents, as measured with the quartz crystal microbalance

with dissipation monitoring (QCM-D) method, was interpreted as intramolecular cross-links formed between different phloroglucinol units in the phlorotannins. With surface plasmon resonance (SPR) it was shown that no desorption occurred from the sensor surface during the cross-linking. UV/Vis spectroscopy verified the results achieved with QCM-D that all components, i.e. vBPO, KBr and H_2O_2, was necessary in order to achieve intramolecular in vitro oxidative cross-linking of the polymers.

In the light of the recent report that iron is the key reagent in protein cross-linking for mussel adhesive synthesis (Sever et al. 2004), it will be interesting to question the prevalence of this theme of protein-transition metal interactions in marine biomaterials such as those of coral reef structures, kelp adhesives, and barnacle cements. In none of these systems, however, is there available a detailed picture of the bonding schemes employed for material construction. Assuming that the oxidative cross-linking of phlorotannins shares common mechanisms with the bonding of plant phenolics, it may involves a one-electron oxidation and subsequent deprotonation (Oudgenoeg et al. 2002). The formed phenoxyl radical species can combine with other radical species to produce a covalent cross-link (Gross and Sizer 1959). This putative mechanism may also be catalyzed by transition metals in brown algae. Alternatively, considering that the dimerization and rearrangement or fragmentation of phenoxyls is stimulated if the phenols were substituted with a halogen (Eickhoff et al. 2001), a speculative cross-linking mechanism could thus start with the bromination of the phenols (as has been suggested as the main task for vBPO), followed by the oxidation and condensation, and finally, the rearrangement and release of HOBr. Based on the occurrence of halogenated phlorotannins in brown algae, while in low abundance (Ragan and Glombitza 1986), the involvement of a vBPO in the catalysis of phenolic cross-linking is our favourite hypothesis. The occurrence of a transition metal-catalyzed oxidation may eventually lead to the degradation of alginate polymers by generating the Fenton reaction and to the subsequent loosening of attachment (Larsen and Smisrod 1967).

Shear-lap tests have shown that oxidation of the polyphenol is important for the formation of contact points with the surface, thus improving the adhesion strength of the glue (Bitton et al. 2006). However, hardening with alginate and/or calcium is essential for high cohesive strength. Further insight into the spatial arrangement of the glue was obtained from small angle X-ray scattering, light scattering and cryo-Transmission Electron Microscopy (Bitton et al. 2006). The phenolic polymer rearranges and forms flexible chain-like objects. This structure does not change upon oxidation, addition of calcium ions or alginate. However, once the alginate is cross-linked with calcium ions, a rigid network is formed (Fig. 6.4). Presumably this network is responsible for the cohesive strength of the glue. When coupled with results showing that vBPO brings about curing of adhesive extracts more than other catalysts, these data implicate vBPO to be the key reagent in controlling the cross-linking of phenolic polymers for the assembly of brown algal adhesives.

6.4.3 Requirement for an Efficient Oxidation Mechanism *In Situ*

As discussed above, cross-linking of the water-soluble adhesive polymers must take place rapidly or they will be washed away from the site of action. At the interface, all the catalysts of the reaction have therefore to be secreted simultaneously with the phenolic and polysaccharide polymers or already present in the seawater.

In *L. digitata* vBPO, the presence of peptide signal sequences suggested an exportation of the proteins in the cell wall (Colin et al. 2003) and some vBPO were effectively reported to be extracellular in giant kelps (Butler et al. 1990; Jordan et al. 1991). Moreover, in Fucales, an isoenzyme has been more precisely located in the cell wall of surface cells (Krenn et al. 1989). Thus, our observations support a role for haloperoxidase in the differentiation of *Fucus* embryo by cell wall cross-linking. Abnormal localization or excess of

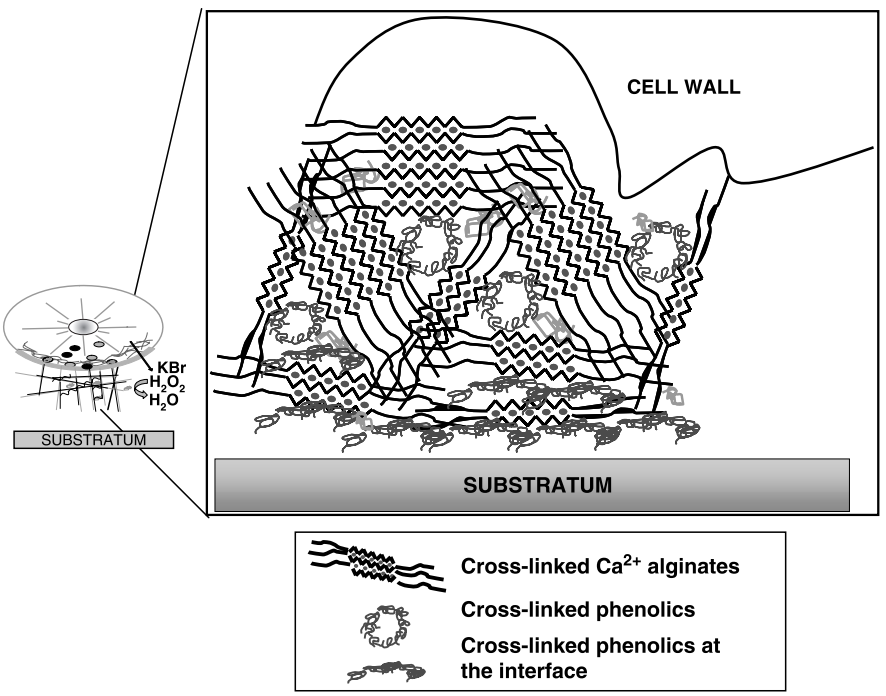

Fig. 6.4. A speculative hypothetical model of spatial arrangement of the deposition of brown algal adhesives at the level of a single vesicle discharged into the extracellular medium. This magnification of a localized region from the model displayed in Fig. 6.3 takes into account the embedding of oxidized phlorotannins in a calcium alginate gel network as deduced from the investigation of microstructure (Bitton et al. 2005) and of surface cross-linking measurements (Berglin et al. 2004). Cross-linked phenolic polymer forms flexible chain-like objects which might form micelles or globular aggregates in aqueous media. We postulate that at the interface with the substratum, these aggregates tend to exclude water and might squash to the surface

exogenous vBPO activity result in preventing cell wall expansion and tip growth initiation (Potin et al., unpubl. res.). The same pattern of abnormal morphogenesis was previously reported in co-cultures of the fucoid alga *A. nodosum* and spores of its endophytic fungus *Mycosphaerella ascophyllii* (Garbary and McDonald 1995). This associated fungus possesses a vanadium-haloperoxidase, which might interfere with normal cross-linking of *A. nodosum* zygote cell walls and prevents wall expansion and consecutive rhizoid emission. In accordance with this precise control of wall assembly by vBPO in *Fucus*, preliminary molecular data have shown a specific expression of a vBPO gene during the first hours after fertilization (Delage et al., unpubl. res.).

Cross-linking of cell wall material by the catalysis of haloperoxidase also requires halogen and H_2O_2. Assuming that the concentrations of halides in the cell wall at the surface of the thallus are close from those found in seawater, the level of bromide (3 mmol/l) should be quite sufficient for the enzymatic reaction, whereas iodide concentration could be limiting (around 0.25 µmol/l) (Saenko et al. 1978). In *Fucus*, we have shown that, during normal embryogenesis, zygotes constitutively secrete H_2O_2 in the extracellular medium (Potin et al., unpubl. res.). This oxidative response was previously reported as an increase in O_2 consumption following fertilization of *Fucus* eggs (Whitaker 1931). Contrarily to the redox changes occurring during fertilization of some marine invertebrates eggs (Schomer and Epel 1998), this ROS (Reactive Oxygen Species) production is not transient but persists for at least 24 h, and then seems to decrease, concomitantly with the elongation of the rhizoid. This steady-state H_2O_2 emission may likewise be under developmental control.

As already mentioned, phenolic compounds are released into the wall of fucoid brown algae from the time of fertilization (Schoenwaelder and Clayton 1998a,b) and then, in *Fucus* zygotes, all the components of the glue are secreted simultaneously under developmental control to permit the efficient attachment of the cells.

6.5 Industrial Potential of Brown Algal Adhesives

The constraints to make an adhesive that can form bonds to a variety of substrates in wet and in high ionic strength environments have led to considerable research in diverse marine organisms (Deming 1999). To date, the mussel adhesive has been most mimicked and several papers investigating such adhesives have been published (Deming 1999; Kitamura et al. 1999; Ninan et al. 2003; Tatehata et al. 2000, 2001; Yamamoto et al. 1995, 2000). The major drawback with the extraction of adhesive proteins directly from the mussel is the cumbersome procedure and the low yield of material. Until recently, the post-translational modification of tyrosine to L-DOPA was con-

sidered as a major challenge affecting the production of recombinant functional adhesive proteins. However, the successful expression in *E. coli* and subsequent modification of two functional mfp5 and mfp3 peptides was recently reported (Hwang et al. 2004, 2005). Therefore synthetic routes to produce peptide mimics are no longer the only alternative for producing mussel adhesives (Statz et al. 2005).

However, alternative marine sources of extracted material with different adhesive properties are suitable. Seaweeds are harvested or maricultured in large quantities and are used as raw material in colloid industry. Therefore, by-products of the seaweed industry are rich in phenolic polymers, inspiring new uses for algae.

Adhesives constitute an important component in the manufacture of a wide array of products derived from the forest industry. At present, most of these adhesives are based on synthetic phenolic compounds cross-linked using toxic catalysts such as formaldehyde. In recent decades, various political, economical and environmental concerns have caused the supply of wood adhesives to decrease dramatically from time to time, producing a concomitant marked increase in price. This situation has led to recognition of the importance of research on alternative sources of starting materials for the production of wood adhesives (Umemura et al. 2003). In general, the materials chosen for study as alternative sources of adhesives feedstock are structurally similar to materials already used for this purpose. For example, tannins and lignins have been studied as replacements for phenol in phenol-formaldehyde adhesives because they contain phenolic moieties within their chemical structures. However, chemical reactivity is dependent in very subtle ways on the chemical structure of a given compounds. Chitosan/phenolics systems were investigated as wood adhesives (Yamada et al. 2000). Adhesion between two pieces of wood veneer developed only when all three components chitosan, a phenolic compound, and laccase (or tyrosinase) were present. The adhesion mechanisms of these chitosan/phenolics systems were proposed to be similar to those of mussel adhesive proteins (Yamada et al. 2000; Peshkova and Li 2003).

When using algal phenolics as adhesives, an industrial catalysis process would require large amounts of enzymes. Similarly, with the mussel adhesive proteins it will require massive production of recombinant enzymes. The heterologous overexpressions have been developed for the vHPO of the fungus *C. inaequalis* (Hemrika et al. 1999) and of the red algae *Corallina* (Carter et al. 2002; Ohshiro et al. 2002). For brown algae, the recombinant expression of an active 198 amino-acid polypeptide derived from *Fucus* vBPO has been described in an international patent (Vreeland 2002). It has already often been reported that the ability of vHPOs to halogenate a broad range of organic compounds of both pharmaceutical and commercial interest as well as their high stability towards high temperatures, oxidative conditions and the presence of organic solvents make them good candidates for use in industrial biotransformations (Littlechild 1999; Dembitsky 2003; Butler and Carter-Franklin 2004). However, given their role in oxidative cross-linking,

they should also be included as strong catalysts in the composition of synthetic polyphenolic adhesives. Another potential application of these enzymes is the development of anti-biofouling processes, which is economically important (Callow and Callow 2002). As seen above, incorporation of vHPO in a product intended for under-seawater uses should prevent the attachment of zygotes thus preventing the growth of brown algae on surfaces.

6.6 Conclusions and Future Prospects

Recent phylogenetic analyses of the eukaryotes have shown that brown algae belong to an independent lineage (Heterokonta) that diverged about one billion years ago during the eukaryotic crown radiation (Baldauf 2003). Phaeophyceae (brown algae) belong in the same lineage as Bacillariophyceae (diatoms) and oomycetes. They are thought to have arisen from a secondary endosymbiosis between a plastid-less protist and an ancestral unicellular red alga. Both Phaeophyceae and oomycetes form the same type of flagellate zoospores displaying the characteristic heterokont paired flagella, one of which is short and smooth and points backwards when swimming, the other being forward-pointing, long and tinsel-like (Maggs and Callow 2003).

Worth noting is that bromide is required for the stalk assembly and the adhesion of the diatom *Achnanthes longipes,* and it could be that haloperoxidases are components in the oxidative cross-linking (Johnson et al. 1995; Wustman et al. 1997). Bromoperoxidase activity was characterized in *Nitzschia* sp., but as a heme-containing enzyme (Moore et al. 1996). Indeed, recent molecular data from the complete genome of *Thalassiosira pseudonana* and EST from *Phaeodactylum tricornutum* do not reveal the presence of vHPO homologues in these two diatom species. Vreeland et al. (1998) also suggested that haloperoxidase-mediated polyphenol-carbohydrate glues could be involved in adhesion of red and green algae. If in *Corallina* red algae, vBPO have been characterized (Shimonishi et al. 1998; Isupov et al. 2000; Ohshiro et al. 2002; Carter et al. 2002), no vHPO activity has been clearly identified in chlorophytes. Adhesion mechanisms are not likely to be conserved processes in the different algal lineages (see Chaps. 4 and 5 in this volume dealing with the chlorophyte *Ulva* and diatoms).

Inside the brown algal phylum, genomic approaches on the newly-established genetic model, *E. siliculosus*, revealed some vHPO homologues in preliminary EST data (Cock et al., pers. comm.). This genetic model could therefore be used to validate the role of vHPO in oxidative cross-linking, using complementary approaches, such as specific invalidation of vHPO genes using RNA interference or general screening of mutants deficient in adhesion processes. Cell adhesion is a criterion of choice to differentiate *E. siliculosus* sporophyte from gametophyte, as the latter floats while the former is attached to the substratum in Petri dishes. A global screen could be con-

ducted on the *Ectocarpus* mutagenised gametes population to examine germination for unattached sporophyte filaments. In these adhesion-deficient mutants, the pattern of vHPO activities, halogen, phenolics and alginate compositions should be modified in comparison with wild type sporophytes. This should allow the identification of new key-actors of the processes involved in biosynthesis and assembly of phlorotannins. They are thought to arise from the condensation of acetate and malonate units via a polyketide synthase enzyme complex although, to date, no such complex has been described in brown algae (Arnold and Targett 2002). It might also point out the key role of vBPO in the control of the oxidative condensation of phlorotannins.

Integrating the data obtained from surface chemistry, physico-chemistry and of knock-down experiments appear to be a necessary strategy to be able one day to elucidate the molecular regulation of cross-linking mechanisms in brown algae.

Acknowledgments. We are grateful to the colleagues of our research group (Ludovic Delage, Carole Colin and Stéphane Labarre) and of the Algal Bioadhesives consortium for stimulating exchanges, and particularly to Mattias Berglin and Havazelet Bianco-Peled, who contributed most of the ideas that gave rise to the hypotheses for mechanisms and to the design of a model for the spatial arrangement of the brown algal phenolic-based adhesives. We also warmly thank Michael Friedlander and Jim Callow for introducing us in the field of algal adhesives. The financial supports by the European Commission "GROWTH" program, Research project; Algal Bioadhesives, Grant G5RD-CT-2001-00542 and CNRS are gratefully acknowledged.

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