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Review Article

Adhesion in blue mussels (*Mytilus edulis*) and barnacles (genus *Balanus*): Mechanisms and technical applications

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Introduction

Representatives from all animal phyla living in the sea attach permanently or temporarily to solid surfaces, including those of other organisms. For some decades there has been an upsurge of interest in adhesion mechanisms of so-called 'fouling' organisms. These organisms also attach to artificial surfaces, e.g. ship hulls and water intake pipes, causing a loss of efficiency. The interest that has been generated centres on the fact that these fouling organisms produce adhesives, which, because they are released and glue under water, may find application in the medical and dental fields. Further understanding of their adhesion mechanisms might also lead to the development of antifouling surfaces not reliant on the leaching of toxins.

Adhesives of aquatic organisms have to fulfil several functions, including prevention of random aggregation in the secretory glands and during transport, priming underwater surfaces, dispersion of adhesive proteins and adsorption to various materials, self-organization and shielding from aqueous erosion and microbial degradation (Kamino, 2003).

The best characterized marine bioadhesive is that from the blue mussel, *Mytilus edulis*. The post-translational amino acid DOPA (3, 4-dihydroxyphenylalanine) is a common constituent of the mussel's foot protein. It functions as a cross-linking agent and mediates adhesion to the substratum. DOPA-containing adhesive proteins were also found in other mussels (Rzepecki et al., 1991) and even in other phyla (Waite, 1992; Waite et al., 1992). The recurrent DOPA motif in invertebrates promoted speculation that the chemistry of the mussel adhesive is similar to that of barnacle adhesive.

Barnacle adhesive, also called cement, can reach high adhesive and cohesive strength and is therefore desirable for technical imitation. However, barnacle cement proteins were shown to be extremely insoluble in traditional protein denaturants, which may explain the delay in studies on the characterization of these proteins. Recent investigations have shown that barnacle adhesive is different from the adhesive of *M. edulis* with regard to composition and sequence.

Due to the interest shown by industry, aquatic bioadhesives of barnacles and blue mussels were chosen to be described in this paper. Before going into detail, some general information about bioadhesion will be given.

Adhesion – a surface phenomenon

Adhesion is a surface physico-chemical phenomenon. Consequently, physical properties of the adhesive joint depend strongly on the character of the substratum surface and how the adhesive interacts with the surface.

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Bioadhesives may consist of proteins, polysaccharides, polyphenols and lipids. These substances occur mostly in combination. Adhesives of the blue mussel and of barnacles are proteinaceous materials. Other well-known proteins with adhesive properties are elastin, collagen, fibronectin, laminin, fibrinogen and keratin. Silk fibroins (â-keratin) produced by spiders and butterfly larvae belong to this group as well.

The role of chemical bonds between adhering phases is often discussed, particularly when biological materials are involved. Feldtman and MacPhee (1964), however, showed that the energy involved in physical adsorption is more than adequate to produce adhesive forces greater than the cohesive strength of either adherend. Thus, the quality of adhesion is strongly linked to the spreading of the liquid phase and wettability of the surface. Liquids will generally spread freely on solids of high surface free energy (also termed hydrophilic) but might show non-spreading behaviour on low surface energy solids (also termed hydrophobic).

Thus, it can be expected that adhering organisms preferentially settle on high-energy surfaces. However, according to numerous studies, macrofouling organisms do not favour a specific surface tension. There are some that prefer hydrophilic surfaces (Fletcher and Baier, 1984; Crisp et al., 1985; Young et al., 1988) and others hydrophobic (low energy) substrata (Eiben, 1976; Miehm et al., 1981; Brewer, 1984). Further experiments revealed a depression of fouling within the surface tension range of 20 to 25 mN m^{-1} (Becker and Wahl, 1991). This phenomenon is also known as *biocompatible range* or *Baier's Window*, for which Baier (1973) and Dexter (1979) gave a thermodynamic explanation.

Regardless of surface tension, any surface in water becomes quickly coated with a monolayer of polymeric material. This layer, commonly referred to as conditioning film, favours the adhesion of bacterial cells that multiply and form microcolonies. Some microorganisms secrete large amounts of exopolymer matrix. The matrix consists mainly of polysaccharides or glycoproteins, and it attracts settling stages (larvae and spores) of macroorganisms (Holmström and Kjelleberg, 1994).

The adhesive strength of the biofilm to the substratum is possibly crucial to the adhesion of macrofouling organisms (Mitchell and Kirchmann, 1984). On the other hand, adhesives of macrofouling organisms can penetrate the primary film to make direct contact to the substratum (Fromageot et al., 1976). The degree of penetration most likely depends on the thickness and consistency of the film. However, a direct interaction between barnacle cement or mussel adhesive and the substratum is conceivable.

Adhesion of mussels

Within the Mollusca, especially within the bivalves, various attachment mechanisms have been developed. Some bivalves are able to attach to hard substrata by elastic byssus threads. Their original function was to tie the post-larva to a surface while it undergoes metamorphosis to the adult form. In *Mytilidae* and *Dreissenidae*, the byssus is retained for permanent attachment of the adult but these mussels can still change their attachment site. The byssus threads holding the mussel in place can be torn by the mussel's foot and new threads can be produced to anchor somewhere else. The mobility diminishes with age. Byssus threads are very elastic connections to the substratum, enabling mussels to settle in high-energy environments. The number of threads increases as the mussel grows, which is reasonably due to greater mechanical stress on larger individuals compared to smaller ones.

At their proximal ends, the threads unite to a stem that is anchored via a root with numerous ramifications within the muscular tissue of the foot. Threads are produced from the byssus apparatus within the foot. At the distal end, each thread expands into an adhesive plaque adhering to the substratum (Fig. 1).

The foot possesses ventrally a groove embedded into secretory tissue that produces byssus threads: the collagen gland produces the collagen core while the accessory gland secretes the proteinaceous cortex. There are additional glands for adhesive synthesis at the distal depression of the foot: 2 gland systems produce a sulphur-rich mucopolysaccharide and phenol glands produce the proteinaceous adhesive.

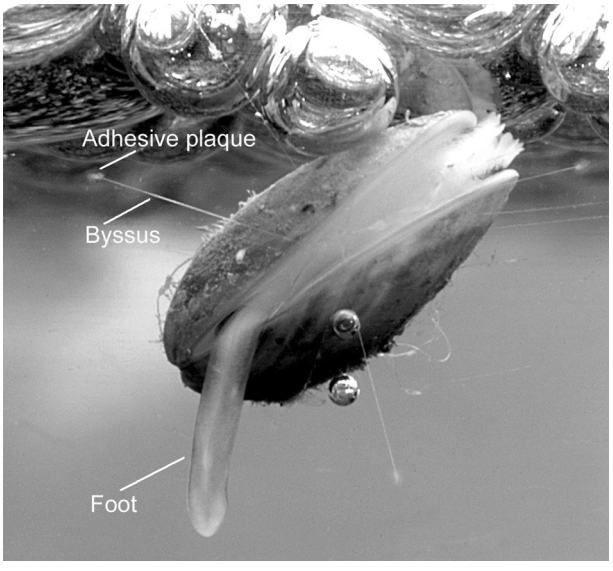

Figure 1. Juvenile *M. edulis* with newly produced byssus threads attached to glass.

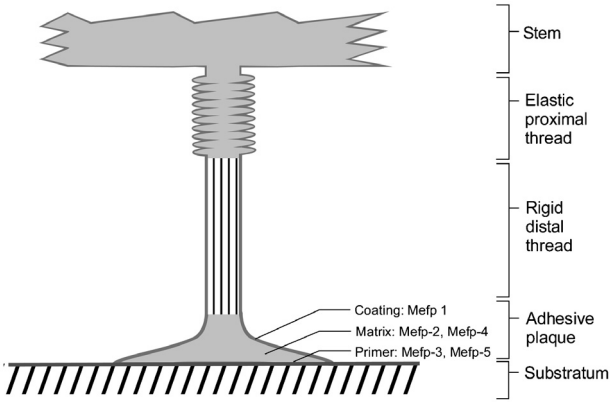

Figure 2. Schematic diagram of a byssus thread and adhesive plaque of *M. edulis*.

The coiled collagen, having a diameter of 7 – 9 nm, is packed into small vesicles that are secreted into the ventral foot groove and there formed into a byssus thread. Various collagenous proteins build the structural differentiated byssus (Qin and Waite, 1995; Coyne et al., 1997; Qin et al., 1997; Qin and Waite, 1998; Coyne and Waite, 2000). It was suggested that the protein distribution may account for the mechanical properties of the byssus. The proximal part is more elastic than the distal part that, in turn, possesses a greater cohesive strength (Fig. 2). This structural differentiation is ideally suited to absorb the energies of waves and water movement.

Prior to the attachment of a new thread, the mussel foot scrubs the surface removing weakly attached microfouling and dirt particles from the surface. Later a dispersion of phenolic vesicles and mucous substance is transferred from the foot onto the substratum and spread by specialised paddle-shaped cilia in a film approximately 50 µm thick (Waite, 1987). The adhesive plaque grows by a continuous secretion of adhesive with an increasing share of collagen fibrils eventually being confluent with the collagenous byssus.

Currently, five protein families known as *Mytilus edulis* foot proteins (abbreviated to 'Mefp' plus a number that indicates the chronological order of discovery) are known to occur within the adhesive plaque of *Mytilus* (Fig. 2): cuticular protein that forms a protective coating that covers the thread and plaque (Mefp-1) (Rzepecki et al., 1992), matrix proteins that form a solid foam in the interior of the plaque (Benedict and Waite, 1986) attributed to Mefp-2 and Mefp-4 (Rzepecki et al., 1992; Vreeland et al., 1998), and adhesive proteins that function as primers and connect the plaque to the surface (Mefp-3 and Mefp-5) (Papov et al., 1995; Warner and Waite, 1999; Floriolli et al., 2000; Waite and Qin, 2001).

Mefp-1 and Mefp-2 have widely been used as model proteins to study cohesive and adhesive properties of mussel glue. All Mefps contain post- or co-translational modified amino acids such as DOPA (3, 4-dihydroxyphenylalanine) and 4-hydroxyproline (Waite and Tanzer, 1981), 3,4-dihydroxyproline (Taylor et al., 1994), 4-hydroxyarginine (Papov et al., 1995), and *o*-phosphoserine (Waite and Qin, 2001). This is exemplified by the decapeptide Ala-Lys-Pro-Ser-Tyr-DHP-Hyp-Thr-DOPA-Lys, which is tandemly repeated approximately 75–85 times making up the majority of Mefp-1. DOPA content, some basic characteristics, and molecular weights of the Mefps are given in Table 1.

The primer proteins (Mefp-3 and Mefp-5) have unusual high DOPA contents (> 20 %). Yu et al. (1999) demonstrated that the concentration of non-oxidized DOPA at the adhesive/ substratum interface is critical for the quality of adhesion.

DOPA is not only suggested to mediate adhesion to the surface, but is a cross-linking agent contributing to the cohesive strength of the adhesive plaque. Cross-linking is pronounced in the superficial layer of the adhesive plaque formed by the cuticular protein Mefp-1 and it yields some protection to the inner vulnerable foam-like adhesive (Benedict and Waite, 1986).

The interfacial binding capacities of the Mefps compete with water and low molecular weight molecules on the surface.

Both surface primers (Mefp-3 and -5) are highly hydroxylated and have, therefore, the possibility to form numerous hydrogen bonds (Papov et al., 1995; Waite and Qin, 2001). Catechol for example, the side-chain of DOPA, is able to form strong hydrogen bonds with hydrophilic polymers. In addition, DOPA is able to form strong complexes with metal ions and metal oxide as well

Table 1. Molecular weight (MW), percentage of DOPA content and characteristic amino acids of adhesive plaque proteins of *M. edulis*.

	MW [kD]	DOPA [%]	Characteristic amino acids	Reference
Mefp-1	110	10–15	4-hydroxyproline 3,4-dihydroxyproline	Waite et al., 1985
Mefp-2	45	2–3	cysteine	Rzepecki et al., 1992
Mefp-3	~ 6	> 20	4-hydroxyarginine	Papov et al., 1995
Mefp-4	80	~ 5	histidine	Vreeland et al., 1998
Mefp-5	9.5	27	*o*-phosphoserine	Waite and Qin, 2001

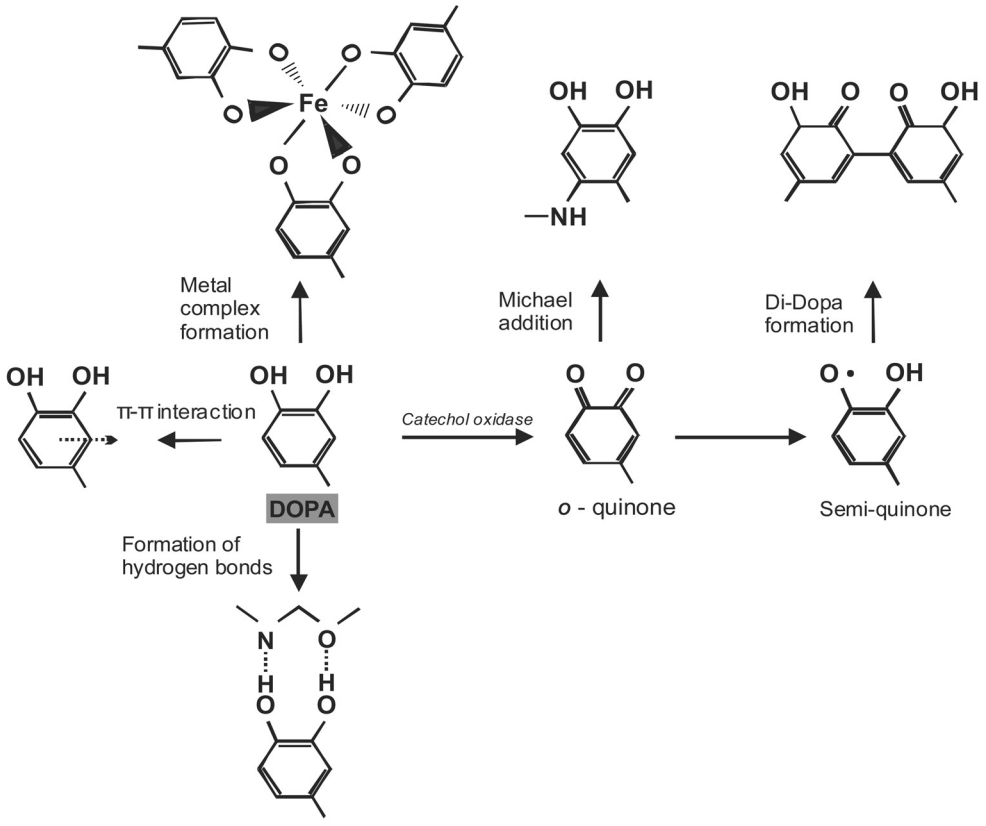

Figure 3. Adhesive and cohesive interactions of mussel adhesive via DOPA.

as silicon dioxide; all are present in mineral surfaces. A third non-covalent bond is the ∂-∂ interaction between DOPA and another aromatic group (Baty et al., 1996; Baty et al., 1997) (Fig. 3).

The amino acid *o*-phosphoserine occurring in Mefp-5 is also associated with adhesive properties of the plaque (Waite and Qin, 2001). It is likely that Mefp-5 mediates binding to calcareous minerals such as mussel shells (Fant et al., 2000).

Other possible strong binding forces are electrostatic interactions between positively charged Mefps and generally negatively charged marine mineral surfaces. Due to adsorption of acidic organic compounds, the latter are negatively charged regardless of their original polarity (Waite, 1987).

Cohesive interactions within the adhesive of *Mytilus* are based on the same chemistry as adhesive interactions. Hydrogen bonds and complex formations between DOPA and metal ions are proposed to contribute to cohesive strength within adhesive plaque and byssus (Fig. 2). The ability of metal ions to increase rigidity was shown for Fe^{3+} ions (Taylor et al., 1994; Taylor et al., 1996) and for Cu^{2+} ions (Fant et al., 2000). Recently it has been reported that transition metals – above all iron – are key reagents in protein cross-linking for mussel adhesive synthesis (Sever et al., 2004). It was proposed that an iron centre cross-links three DOPA residues (Fig. 3). The authors speculated further that metal-protein interactions may be a prevalent theme in marine biomaterials, including barnacle cement.

Prior to the report of Sever et al. (2004), the most important chemical pathway ensuring cohesive strength within the mussel adhesive was thought to be the formation of covalent cross-links between DOPA containing proteins. The byssus gland produces the enzyme catechol oxidase, which oxidizes DOPA residues to highly reactive *o*-quinones (Waite, 1985). Covalent cross-linking is initiated by a free radical mechanism between two quinones to form the quinone dimer (Fig. 3). The presence of the end products by this reaction was recently demonstrated by McDowell et al. (1999).

Though the original hypothesis was that *o*-quinones react with primary amines in a Michael addition reaction, the reaction products have not been found.

It has been shown that adhesion strength of *M. edulis* is generally greater on polar surfaces, such as glass or slate, than on non-polar surfaces, such as wax or polytetrafluoroethylene (Young and Crisp, 1982). Other investigations revealed that the area of the plaque is influenced by surface energy of the substratum: on more polar surfaces (slate and glass) smaller-sized plaques are formed (Crisp et al., 1985). However, investigations of

Mytilus foot protein-3 (Mefp-3) located in the adhesive plaque showed no correlation between variants of the highly polymorphic protein family and different substrata, e.g. steel, plastic, or glass (Floriolli et al., 2000). Remarkably, no low energy surfaces have been used in these studies. Fant et al. (2000) showed that surface chemistry is of great importance for the structure of adsorbed protein film. Mefp-1 produced a hydrogel-like film on a hydrophobic surface, while on a hydrophilic SiO_2 surface a compact rigidly attached protein layer was formed. The authors suggested that charged polar groups – making up the majority of groups in Mefp-1 – are probably highly hydrated and, therefore, repelled from the surface.

Adhesion of barnacles

Barnacles are crustaceans that show special adaptations to a sessile mode of life. Their success as a group is reflected in palaeontological records dating back to the Cambrian. Barnacles are among the commonest fouling organisms in the marine environment and are probably of great economic importance. Acorn barnacles that are cemented to the substratum with the whole calcareous or non-calcareous base are frequently found on ship hulls – most of them belong to the family Balanidae. The ability to stay attached to ships that travel with high speed gives an idea about the quality of attachment. Most studies on the adhesion mechanism of this group have been performed on acorn barnacles. Thus, speaking about barnacles in this paper refers to the acorn type.

Barnacles produce pelagic larvae (nauplii) that undergo six developmental stages before they reach the cypris stage. In its search for a suitable attachment site, the cyprid is able to sense a wide range of physical and chemical surface factors (Lindner, 1984; Yule and Walker, 1987). Permanent settlement is initiated by secretion of a small amount of strong adhering cement in which the attachment organs of the cyprid's antennules are embedded. Thereafter, the cyprid undergoes metamorphosis to a juvenile barnacle. It may take several days until the first post-metamorphic adhesive is secreted. Usually, adult cement appears as discrete rings underneath the relatively even base plate. The secretion is most likely linked to the moult cycle since cement cells are modified epidermal cells (Walker, 1999). During the growth process new cement is released at the base periphery (see Fig. 4 for the cement duct system). The liquid cement fills gaps between the base plate and the surface by capillary action and excludes water at the same time (cit.op.). In the case of *Balanus eburneus*, the adhesive cures within 6 hours (Cheung et al., 1977) to a rigid mass (hereafter called cement).

Barnacle cement is considered to be the most durable and toughest connection in the living aquatic world (Abbott, 1990). The adhesive is a proteinaceous material (> 90 % protein), while the remainder consists of carbohydrate, ash, and trace amounts of lipid (Walker, 1999). Based on the assumption that cement glands are homologous with glands that secrete the cuticle (Thomas, 1944) (cuticle of arthropoda is tanned by *o*-quinone cross-linking), and due to the existence of phenols and polyphenol oxidase in cement glands of the settling stage, it was widely postulated that quinone cross-linking also occurs in cement (Walker, 1999). Comprehensive work on cement glands of the settling stage of the barnacle clearly concluded that cypris cement contains tanned protein and, hence, quinones (Walker, 1971). Despite extensive histological and histochemical studies on cement glands of adult barnacles (Lacombe, 1970; Walker, 1970; Lindner and Dooley, 1973) a similar picture did not emerge.

Extreme resistance of hardened cement towards salt solutions, dilute acid, and alkali suggested that hydrogen or salt bonds were too weak to be the main cross-linking mechanisms. Disulphide bonds were also precluded due

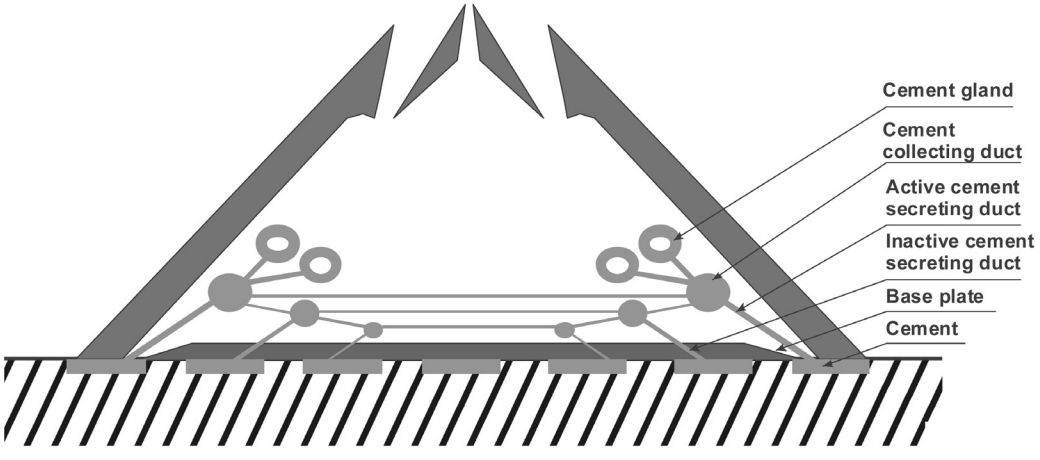

Figure 4. Schematic diagram showing cement duct system, cement glands and secretion in acorn barnacles.

to resistance of the adhesive to thioglycolate (Naldrett, 1993).

In contrast, Barnes and Blackstock (1976) and Yan and Pan (1981) showed that an anionic detergent, sodium dodecyl sulphate (SDS) containing the reductant 2-mercaptoethanol (2-ME) is sufficient to dissolve cement, and it lead to the assumption that hydrophobic interactions and sulphur cross-links are key components of the cement matrix. Though Naldrett and Kaplan (1997) found high amounts of the amino acid Tyr, which is modified to DOPA in mussel adhesive, in the major protein of *Balanus eburneus* cement, infra-red spectroscopy and NMR spectroscopy provided evidence that quinones are absent from cement of balanid barnacles (Naldrett, 1993; Naldrett and Kaplan, 1997). Still, other post-translational modifications were reported to be present, possibly being glycosylations (Naldrett and Kaplan, 1997).

In more recent studies, it was possible to render soluble at least 90 % of cement of the barnacle species *Megabalanus rosa* (Kamino et al., 2000; Kamino, 2001) and *Balanus eburneus* (Naldrett and Kaplan, 1997). The authors found that a complex of distinct proteins, cross-linked through cysteine residues, cooperatively achieves adhesion.

Naldrett and Kaplan (1997) found adhesive proteins of *B. crenatus* that have a molecular weight of ~ 3 to 40–50 kD. Reduction of the bigger aggregates with SDS in the presence of 2-ME led to the hypothesis that the lower molecular weight units are subunits and aggregate to form the higher molecular weight proteins. By comparison, cement of *B. eburneus* was only partially soluble in 2-ME and SDS (cit. op.). The 5 major proteins identified, with molecular weights of 7, 22, 36, 52, and 58 kD, however, were similar to those characterized from *B. crenatus* (cit. op.) and *Balanus perforatus* that have 39, 62, and 65 kD (Naldrett 1993). The highly hydrophobic character of residues was suggested to be the main reason for the insoluble nature of these proteins (Naldrett and Kaplan, 1997).

Kamino (2000) and Kamino et al. (2001) reported proteins of *M. rosa* cement having molecular weights of 20, 52, 68, and 100 kD. An additional 180 kD protein was believed to be an aggregate of the 57 and 60 kD proteins. The 68 kD protein resembles the 58 kD protein in *B. eburneus* cement in its molecular mass and amino acid composition. A short peptide fragment derived from *B. eburneus* cement by Naldrett and Kaplan (1997) showed some homology to a part of the 100 kD sequence of *M. rosa* (16 out of 24 amino acids in a sequence are identical) (Kamino, 2000). The 20 kD protein that possesses characteristics different from other detected components was reported to consist of six repeats of Cys-rich sequences, each between 20 and 30 residues long. Since the 20 kD protein was recovered without reduction treatment, Cys residues were suggested to form not inter- but intramolecular disulfide bonds being responsible for the proposed compactness of the molecule. A Cys-Xaa-Cys-Xaa-Cys motif occurring in one region of the 20 kD protein is also known from the 185 kD silk protein of the aquatic midge larvae *Chironomus tentans*, in which the motif is repeated after approximately 22 – 26 residues (Case et al., 1997). The protein, used in the construction of larval tubes, has been proposed to prevent random aggregation of other components in the gland (cit. op.). This was also suggested to be a function of the 20 kD molecule in *M. rosa*-cement (Kamino, 2001). The abundance of charged amino acids is another interesting characteristic of the 20 kD protein. Clusters of acidic amino acids are also present in the *Mytilus galloprovincialis* foot protein 2 (Mgfp-2). It has been suggested that Mgfp-2 undergoes protein aggregation due to reduction of the negative charge of the acidic cluster by the positive charge of basic amino acids in its central region (Rzepecki and Waite, 1995). Although no sequence similarity was found in the study of Kamino (2001), the functional relationship in underwater adhesion between these two proteins should be noted.

The ability of barnacle cement to hold metals, was reported by Walker (1972) and Yan and Tang (1981). Kamino (2001) suggested that charged amino acids have the potential to interact with metal ions. This would explain good underwater adhesion of barnacle cement to a range of substrata. However, analysing the mobility shift by SDS-PAGE with the addition of metal ions, the author found no evidence for metal binding properties of the charged amino acids.

Investigations of barnacle cement by scanning electron microscopy (SEM) and atomic force microscopy (AFM) revealed that the adhesive is composed of nano-sized globular structures (Wiegemann and Watermann, 2003). These formations might be comparable to granular structures detected by Berglin and Gatenholm (2003) using the AFM technique. Wiegemann and Watermann (2003) further reported that the globules were able to build other conglomerates, like branches, sponge-like matrices and strands or thin, dense sheets (Fig. 5a–c). The latter was seen in the case of strongly adhered barnacles grown on aluminium foil, which is a polar surface. The appearance of adhesive filaments was characteristic of barnacles grown on hydrophobic fouling-release coatings based on poly(dimethylsiloxane). On these substrata, barnacles seem to benefit from filamentous webs that provide some elasticity. Thus, the small-sized adhesive globules were suggested to be able to create specific superstructures in response to substratum characteristics, e.g. polarity.

Another interesting observation concerning the macrostructure of barnacle adhesive was the high degree of cross-linking of the outer crust forming a homo geneous smooth skin around the less dense inner part

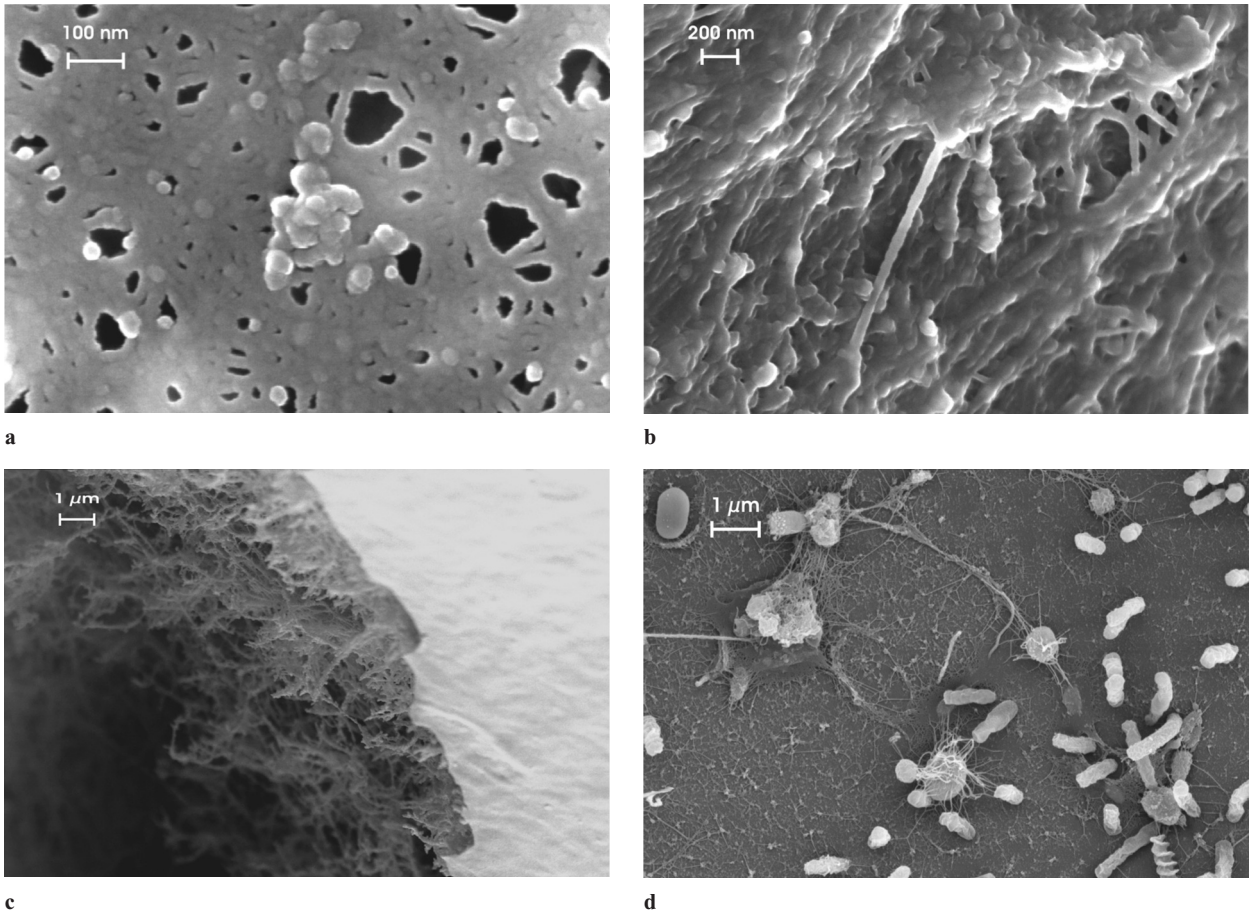

Figure 5. SEM images of adhesive of barnacles: adhesive globules forming various superstructures, like thin, dense sheets (**a**), sponge-like matrices and strands (**b**), branches (**c**). The high degree of cross-linking of the outer crust creates a homogeneous smooth skin around the less dense inner part (**c**). Mechanical interlocking is achieved by delicate adhesive filaments weaving around bacteria and dirt particles on the surface. Threads coming from different directions unite to larger strands and networks (**d**). (Credit, Wiegemann and Watermann, 2003).

(Fig. 5c, Wiegemann and Watermann, 2003). These findings correspond to reports on the *M. edulis* adhesive plaque wherein the smooth outer crust that covers the inner foam-like matrix was attributed to the cuticular protein Mefp-1 (Rzepecki et al., 1992).

Previous studies of the interface between barnacle adhesive and glass slides by electron microscopy showed delicate adhesive filaments weaving around bacteria and dirt particles on the surface (Fig. 5d, Wiegemann and Watermann, 2003). Threads coming from different directions united and formed a network. These observations suggested that mechanical interlocking, that takes advantage of surface roughness down to the nm-scale, plays an important role in the adhesion of barnacles. These narrow joints provide the required closeness for molecular forces to be active.

The principle of enlargement of the adhesion surface by projection of multitudinous structures at the µm-scale was also reported from geckos. 5000 setae/mm^2, which subdivide into 0.2–0.5 µm thick spatulae, rapidly form and break down intermolecular bonds (Autumn et al., 2000). Also, the observation that geckos get stickier with increasing surface energy of the substratum suggests that they step directly into the molecular structure of the surface they walk on.

As in the case of geckos, the adhesion strength of barnacles is generally greater on polar surfaces than on non-polar surfaces (Becka and Loeb, 1984; Holm, 1990).

It was also shown that barnacles produce a highly hydrated adhesive on fouling-release coatings that have low energy surfaces (Wiegemann and Watermann, 2003). Similar observations have been made with other organisms. Algae developed a compact discoid rhizoidal base with short, tight-jointed filaments on high surface energy glass, while on low surface energy glass the filaments were long and free, giving almost a three-fold increase in algal base area (Walker, 1987). Even conditioning films were reported to be flatter and tightly adherent to substrata with high surface energy (high polarity), while on

Table 2. Comparative characteristics of *M. edulis* and *Balanus* adhesives in comparison.

	Mytilus edulis	*Balanus ssp.*
Macrostructure	nm-sized globules observed within foam-like matrix; homogeneous coating with increased cross-linking	nm-sized globules forming various macromolecular structures (tightly packed, foam-like, branching, loosely matted strands) depending on substratum; homogeneous coating (cross-linking pronounced)
Physical characteristics		
• General	Rubber-like	Macromolecular structure determines physical characteristics forming rigid cement films or elastic hydrated adhesive plaques
• Effect of surface tension	Hydration of Mefp-1 on non-polar surfaces	Hydration of adhesive on non-polar surfaces reducing cohesion
	Adhesion strength higher on polar surfaces than on non-polar surfaces	
Biochemical characteristics		
• General	Conglomerate of several proteins, highly hydroxylated	
• Adhesion/ Cohesion	Major cross-linking pathways: post-translational amino acid DOPA forming DOPA-metal and di-DOPA complexes	Hydrophobic interactions and sulphur cross-links; Possibly charged amino acids involved; No evidence for interaction with metals though incorporation of metal ions reported

low surface energy (non-polar) surfaces they were weakly bound but thicker (Mitchell and Kirchman, 1984). These phenomena might also be comparable to observed hydrogel-like film formed by Mefp-1 on a hydrophobic surface (see above).

Low adhesion strength and the hydration of adhesive on hydrophobic, non-polar surfaces might be based on general mechanisms. It was proposed that water uptake is a repair mechanism in barnacle adhesive that increases the volume of the adhesive (Wiegemann and Watermann, 2003). This mechanism occurs whenever there are large distances between barnacle base and substratum and an adequate amount of adhesive to fill the gap is not available. Weak adhesion strength of barnacles on fouling-release coatings was observed to coincide with poor mechanical interlocking of the adhesive threads (cit. op.). Both might be a result of hydrated polar groups being repelled from the hydrophobic surface as suggested by Fant et al. (2000) for mefp-1. Similar to mefp-1, polypeptides found in the cement of the barnacle *M. rosa* were reported to be highly hydroxylated with high amounts of Ser and Thr (Kamino et al., 1996).

Table 2 summarizes and compares the main characteristics of mussel and barnacle adhesive.

Technical application and outlook

Barnacle and mussel adhesives possess numerous characteristics desirable for technical exploitation. The most important one is their outstanding adhesion in aqueous media. The facts so far known to be important for achievement of barnacle and mussel adhesion are discussed above. One interesting aspect is mechanical interlocking of barnacle adhesive combined with enlargement of the contact area down to the nm-scale. In addition, barnacle adhesive was found to respond to various substratum characteristics. A rigid structural joint that resists high tensile and shear forces is formed on easy-to-attach surfaces, while on release coatings a rubber-like adhesive is created that performs well under a peeling load. In comparison, synthetic adhesive types display wide differences in their response to different stresses, e.g. thermoplastic rubber types usually possess high peel strengths, but relatively low tensile or shear strengths. Thermosetting resins, in contrast, are often used as basic ingredients for structural adhesives, but perform relatively poor under peel or cleavage stress. A synthetic multi-purpose adhesive has not been found so far. Barnacle cement might lead the way to intelligent adhesives that adapt their structure according to demand – from gap filling to structural purposes.

Usage of mussel adhesive proteins in the medical field has been widely discussed. However, limited production has so far precluded practical application. While it is clear that DOPA-containing peptides are capable of tightly adhering to metal and polymer substrata, it has not been determined if similar techniques can be applied to biologically relevant surfaces. Efforts leading to a better understanding of adhesive and cohesive properties of mussel adhesive proteins are still required (Fant et al., 2002).

A promising approach, however, is to mimic mussel adhesive in DOPA-containing synthetic polymer materials. Such materials are especially desired for applications where water interferes with the establishment of a durable adhesive bond (Messersmith, 2003). Additionally, these mimetic polymers have surprisingly been found to have antifouling properties possibly suitable for applications in medical implants and diagnostic devices, as well as non-medical materials, in which minimization of surface fouling is desired (Hu, 2003; Dalsin et al., 2003, Patent US 1991/5 015 677). Such compounds are DOPA containing peptide-PEG (poly(ethylene glycol) conjugates or Mefp-1 decapeptides coupled with PEG. While DOPA containing peptide moieties strongly anchor PEG onto the surface, immobilized PEG chains provide steric repulsion of protein and cell adsorption. Applications might be extended to new concepts in non-toxic fouling-prevention systems for the marine environment (Messersmith, 2003, Patent US 1991/5 015 677).

Unlike the mussel adhesion system, adhesion mechanisms of barnacles have been subject to relatively few studies. However, patents on the technical exploitation of barnacle adhesive were recently applied for (Patent US 2003/0143673 A1, Patent WO 03/093413 A2). The patents claim a microorganism that is capable of expressing a barnacle adhesion protein, which could be used as high-strength protein-based adhesive to serve as an interface between implants and bone or tissue and to prevent biofouling on underwater surfaces. Interestingly, the claimed proteins named 'barnacle adhesion proteins' were not isolated from barnacle adhesive but stemmed from the base plate or the hemolymph. A protein with a size of 35 kD, however, was found to show homology to filensin, an eye protein, which is known to assemble with other proteins to form filamentous networks. Homologies were also found to the self-aggregating protein pernin recently discovered in the hemolymph of the green-lipped mussel. Earlier Japanese patents published between 1995 and 1998 claimed the gene coding of seven barnacle adhesive proteins including a metal bonding protein (JP 7265081; JP 9047288; JP 9299089; JP 10057070; JP 10327867; JP 11332572; JP 11332573). These patents, however, have never been transferred into an international or US patent.

Having separated cement proteins of the barnacle *B. crenatus*, Naldrett and Kaplan (1997) found a periodicity of ~ 3 kD between multimer bands in a high resolution gel. The 3 kD protein is thought to be the smallest subunit polymerizing to complex molecules. This observation is so far unique in biological adhesives. It makes the synthetic production of such an adhesive more probable, since the polymerization mechanism is similar to that involved in synthetic adhesives: small monomers are first cross-linked to form larger chains that further interact during the curing of the adhesive. It is actually attempted to design peptide-based materials focussing on a subunit with a size of 20 kD (Kamino, 2003).

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