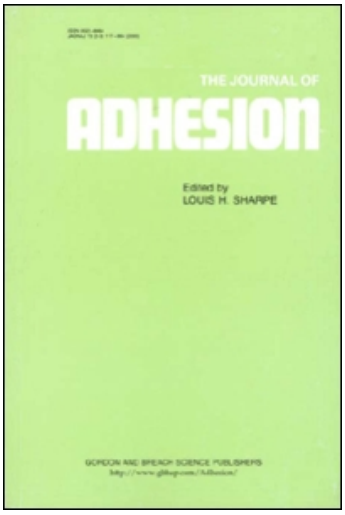

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Molecular Design of Barnacle Cement in Comparison with Those of Mussel and Tubeworm

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Molecular Design of Barnacle Cement in Comparison with Those of Mussel and Tubeworm

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Various organisms are known to attach themselves to a wide range of foreign materials in water as one of their essential physiological functions. To accomplish this, these organisms have acquired their specialized molecular systems in the process of their evolution. The molecular systems of sessile organisms are excellently designed for the purpose of underwater adhesion from the macroscopic scale to the molecular level. This review focuses on the unique sessile crustacean, the barnacle, in which a molecular system called cement was found for the underwater adhesion, which is completely different from the molecular system found in the mussel and tubeworm. The components, properties, and unique functions of the cement proteins from barnacles in comparison with those of the mussel and tubeworm are discussed.

Keywords: Barnacle; Cement; Molecular design; Protein based materials; Sessile organisms; Underwater adhesive

INTRODUCTION

Our lives are surrounded by various man-made adhesives, and become more and more convenient by their use. Many types of adhesives have been developed to facilitate every aspect of our lives and activities. This situation, however, does not apply for adhesives in water. It is generally considered that underwater attachment is a troublesome and largely unachieved technology. This idea on the difficulty of underwater

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One of a Collection of papers honoring J. Herbert Waite, the recipient in February 2009 of *The Adhesion Society Award for Excellence in Adhesion Science, Sponsered by 3M*.

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attachment is apparently not appropriate if we consider that many sessile aquatic organisms often attach themselves to various surfaces, whether being of their own kinds or of foreign origin, in water.

In practice, there have been huge developments in both the theory and applications of adhesives in air, whereas limited endeavors have been made for the development of applications and theory of underwater adhesive technology. This may be related to the fact that almost all conventional man-made adhesives are petrochemical products. Petrochemical products are outstanding in their low price and are numerous. However, since they are usually produced/manipulated in organic solvents instead of water, they may not be best fitted to function in water, in spite of the fact that water is a physically peculiar solvent [1]. Thus, I suggest that development of underwater adhesives should be separated from the extension of principles to design adhesives in air. Molecular designs specific to underwater attachment should be investigated and examined thoroughly. Such activities may also lead to future materials compatible with environmentally-friendly products.

What are the principles for the design of underwater adhesive technology? Aquatic sessile organisms may provide clues to this question. A variety of organisms are known to attach their bodies to various materials in water during a specific, or the whole, stage of their life. This attachment process has become one of the essential physiological functions for these organisms; thus, they have acquired the molecular systems required for the underwater attachment in the process of their evolution. Prof. Herb. Waite and his colleagues have undoubtedly led this field of research by their important and continuous investigations on the molecular systems of mussel holdfast, byssus, and tubeworm cement in the last three decades. Their researches on the "DOPA-system" in the mussel and the tubeworm have also encouraged studies on the underwater adhesive systems of other sessile organisms such as echinoderm, mollusk, and crustacean [2]. This review focuses on the underwater attachment by the unique sessile crustacean, the barnacle, in which a molecular system completely different from those of the mussel and tubeworm was found. Based on the comparison of the barnacle system with other systems from mussel and tubeworm, the differences and consistencies in the design of individual holdfast is discussed.

DIFFERENT MODES OF ATTACHMENT AMONG DIFFERENT ORGANISMS

Mussels, tubeworms, and barnacles inhabit the seashore. They attach to various surfaces, and withstand the turbulent action of waves to

sweep them away. They have developed, improved, and secured their adhesive joint during their evolution, resulting in the different modes of attachment among them (Fig. 1). Mussels attache to various surfaces by forming several tens of byssal threads as if the animal sits

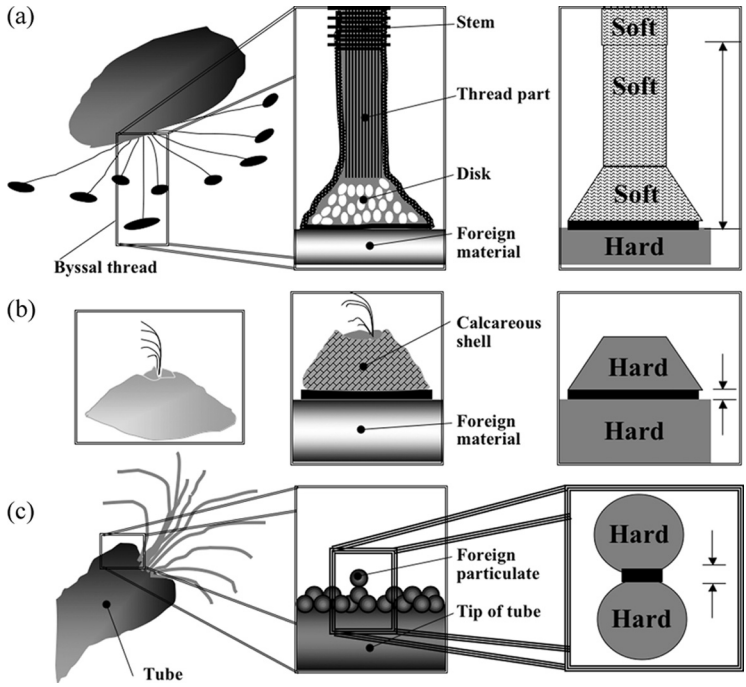

FIGURE 1 Modes of attachment of mussel, barnacle, and tubeworm. Schematic illustrations of adhesive joints: (a) Mussel makes several tens of byssal threads that have macroscopically modular structures. The byssal thread as a whole functions as the holdfast of the animal, with a distance between the animal and foreign materials on the order of cm. The coupling layer at the tip of the byssal disk actually bonds hard matter (foreign materials) and soft matter (the mussel's own byssal thread). A simplified illustration is shown on the right side. (b) Barnacle attaches to foreign materials by secretion of the cement underneath their own calcareous base. The cement layer on natural or easy-to-attach surface usually has a thickness of a few μm. Thus, the barnacle bonds two hard materials, and the distance between the two hard materials is of the order of micrometers. (c) Tubeworm dwells in their inhabiting tube made of natural particulates. The particles are bonded together *via* tubeworm cement to construct the tube. In the process of construction, the animal picks an appropriate particulate, puts the cement onto it, and pushes it onto the edge of the building tube.

on a trampoline made by the byssus. Thus, mussels take advantage of "threads" to strengthen their adhesive joint. Occasionally, mussels move to change their habitat by cutting off the bundle of byssal threads at the proximal stem. The thread shape of their holdfast may, therefore, be appropriate to the physiology. The distance between a foreign substratum and the animal, linked by byssus, is in the order of cm. The byssal thread has a modular structure that includes a proximal part and a distal part of the thread, and an adhesive disk, all of which are proteinaceous [3]. Microscopically, the byssus is coated all over by a few microns of a cuticle layer [4], and the adhesive disk is further separated by a bulk and a tip layer, the latter being directly coupled to foreign materials. Simply, a thin coupling layer in the tip of the disk bonds together foreign substrata which are usually "hard" materials and the thread part of the byssal thread which is a "soft" material (Fig. 1). The hard materials are diverse and full of changes, while the soft material is always their own byssal thread. Because the animal is tossed by the sweeping action of turbulent waves, this mode of holdfast with cm distance would latently include several breakpoints. Mussels, however, overcome the obstacle by optimizing the design of the byssal thread from the mezo-/microscopic range to the molecular range. The most reliable design is a mechanical gradient along the longitudinal direction of the byssal thread [5], which seems to be achieved by a gradient distribution of different proteins along with the longitudinal direction. Additionally, the solid foam-microstructure in the adhesive disk [6], and intermolecular metal coordination bonding [7], may provide additional sustainability to the molecular design of the byssal thread.

In contrast to the byssus, barnacles and tubeworms join two hard materials in water with an order of μm-distances through the use of cement complexes. Barnacles attache their own calcareous base[1] to foreign materials with a cement layer of a few μm (Fig. 1) [8]. Thus, the cement joints two hard materials, the animal's own calcareous base, and another hard material which could be any foreign substrata, together in water. In the case of the tubeworm, the cement is used for constructing their inhabiting tubes. The animal grasps and "kisses" an environmental particulate at a distance of several hundreds of μm to put the cement onto the particulate, and presses the tip of their building tube against the particulate for attachment [9,10]. The cement, in

[1]Some barnacle genus have a membranous base. They do not have a calcareous base, and the membranous bases with chitinous lining is directly attached to foreign materials. No reports have been published on their cement proteins.

a several tens of μm layer, thus connects two hard particulates in water.

The adhesive layer of barnacle and tubeworm cements seems to have, macroscopically, a rather simple structure in which there exist at most two portions, namely, a coupling layer on the surface and an inner bulk layer of the adhesive [9,11,12]. This simple design of holdfasts would link two materials at a shorter distance, and the formation of a thinner adhesive layer might be a prerequisite for their secure attachment. This kind of thinner adhesive layer is apparently made possible by the action of the animal [8,12]. Simple macroscopic structures of the holdfasts are also a result of the secretory organ of the adhesives in both barnacles and tubeworms [8,10,13,14]. Barnacle adhesive seems to be biosynthesized only in a giant unicellular organ localized in the soft body, and is transported through a duct system to the site for attachment; thus, the animals can eject the total adhesive complex to the outer environmental space (Fig. 2). Underwater attachment after secretion from their duct system is, thus, a molecular event. On the other hand, byssus is made by a specialized organ, the foot [15]. The peculiar motion of the foot allows the process of injection molding to the ventral groove of the foot to proceed. Several glands specialized in the biosynthesis of individual foot proteins are appropriately aligned along the longitudinal axis of the ventral groove in the foot, making a macroscopically modular structure of the byssal thread possible. For these reasons, the byssal thread depends to a significant extent on the skillfulness of the foot.

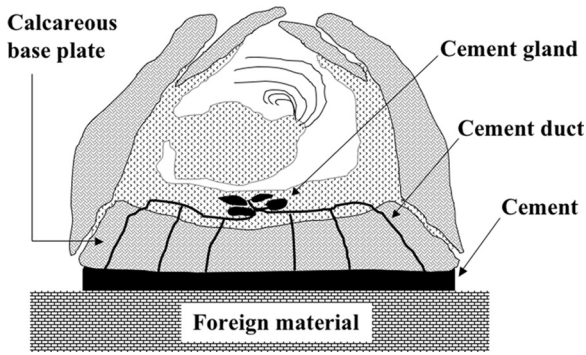

FIGURE 2 Cross-sectional view of the barnacle. The cement is biosynthesized in the cement glands which are laid on connective tissue in close association with ovarian tissue and joined together by ducts. These ducts eventually lead to the base of the animal. The cement is underneath the calcareous base.

How do the modes of attachment in the barnacle and tubeworm make the strong and sustainable attachment possible between two hard materials by soft adhesives at a few μm distance? The barnacle adhesive layer would have a much lower stiffness than both the calcareous base and foreign materials such as steel and rock. If we, in general, sandwich two materials having high stiffness *via* a soft adhesive material with lower stiffness, the adhesive joint would be easily broken by horizontal forces against the adhesive layer. Barnacles are basically fixed to a place on which they settled in their larval stage, and their adhesive layer must be maintained for several years. The barnacle adhesive must have a special design to overcome the obstacle of different stiffness. This is an intriguing question to be solved in the future.

BARNACLE UNDERWATER ADHESIVE, CEMENT

As described above, the barnacle attaches itself to foreign materials underwater by means of secretion of an extra-cellular adhesive substance, cement. The cement is proteinaceous with more than 90% of its content occupied by proteins [16,17]. In barnacles with a calcareous base, the cement joins their calcareous plate and foreign materials such as mineral, metal, wood, and synthetic polymer in water. Although the cement layer is macroscopically simple as mentioned above, the cement complex itself is composed of more than six different proteins [18]. All these cement proteins, except for an enzymatic one, are novel without significant homologues in databases currently available. Neither homology to mussel foot proteins/tubeworm cement proteins nor modification to DOPA [11,17] was found in barnacle cement proteins. Foot proteins in the mussel byssus have been found to undergo multiple post-translational modifications. In contrast, no indication of protein-modification has been found in the barnacle cement proteins. Two barnacle cement proteins have been verified to be of a simple type with no post-translational modifications [19,20], and the other two proteins also seem not to be modified. Such molecular systems that do not rely on post-translational modifications have not been found in other biological underwater adhesives.

Each cement protein of the barnacle has its own remarkable characteristics, and probably has specific functions in the underwater attachment which, as a whole, is a multi-functional process [21]. The functions involved in the underwater attachment can be roughly classified into surface functions and bulk functions. The former includes displacing the bound-water layer on a foreign substratum with the adhesive, as well as spreading and coupling of the adhesive with a variety of material surfaces. On the other hand, the latter functions

include self-assembly of the adhesive, curing to make the holdfast stiff and tough, and protecting the adhesive layer from microbial degradation. Possible proteins responsible for each category of the functions are summarized in the following sections.

Preparation, analysis of samples employed in the cement study, and the methods to render the cement complex soluble have been explained elsewhere [18,22]; thus, they will not be discussed in detail here.

CEMENT PROTEINS WITH SURFACE FUNCTIONS

Two proteins with apparent molecular weights of 19 and 20 kDa, namely, the 19 kDa- and 20 kDa-cement proteins (cp19k and cp20k, respectively), are possible candidates for surface functions in underwater attachment. Both proteins are smaller than other cement proteins and are hydrophilic (Fig. 3). These proteins have been verified to be not post-translationally modified [19,23]. Recombinant forms of both proteins in *E. coli* have been homogeneously prepared in solution under physiological conditions [19,20], enabling direct analyses of their physical properties and functions possible. These are the only examples in biological adhesive proteins reported so far.

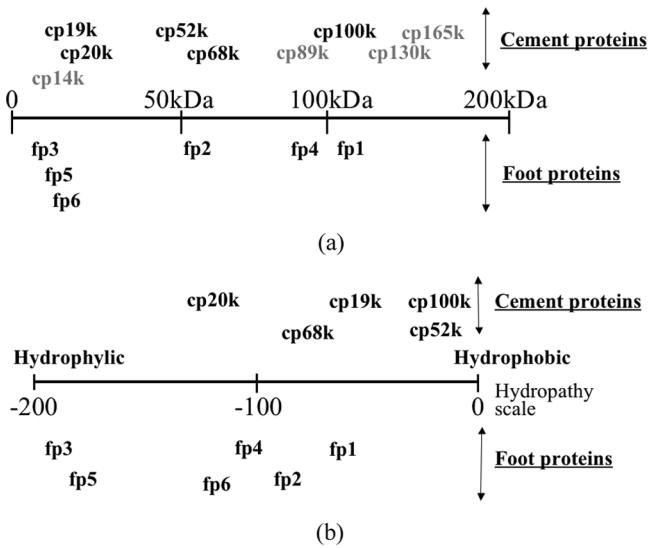

FIGURE 3 Ranges of molecular weight and hydrophobicity/hydrophilicity of various barnacle cement proteins and mussel foot proteins. Hydrophobicities of proteins have been calculated using hydrophobic parameters of amino acids [42].

The cp19k [19] is rich in six amino acids, Ser, Thr, Gly, Ala, Lys, and Val, which comprise almost 70% of the total 173 amino acids. The primary structure is interpreted to have four alternating repetitions of two segments, a former segment rich in four amino acids (namely, S, T, G, and A, which occupy 65–86% of the segment, 17–24 amino acids long, abbreviated as "STGA-segments"), and a latter segment comprises amino acids except for the four amino acids (with a content of 13–40% of the four amino acids in the segment, 15–24 amino acids long, abbreviated as "non-STGA segments"). The non-STGA segments are not simple in their amino acid composition, and contain residues such as K, L, V, D/E, and so on. The purified protein was found to be adsorbed to surfaces with various characteristics, including hydrophilic, negatively charged, positively charged, and hydrophobic surfaces, in seawater. Surface plasmon resonance [19], quartz crystal microbalance, and dynamic contact angle measurements [24] confirmed that the materials on which the proteins were adsorbed in seawater include bare gold, alkylated gold, titanium oxide, silicic acid, alumina, polystyrene, polyethylene, polypropylene, poly-oxy-methylene, and hydroxyapatite. The adsorption layers were stable in water for at least 1 week, and were also stable upon exposure to solutions with various pHs. The adsorption amounts seemed not to exceed that of a single molecular layer, suggesting the formation of a monolayer of the protein on various surfaces. Because the barnacle attaches itself to diverse, naturally occurring surfaces which are actually a patchwork of different surface characteristics, the protein is thought to be responsible for coupling with foreign materials in underwater attachment of the cement. Circular dichroism (CD) Spectra in solution and computer aided structure prediction suggested that the protein is poor in secondary structure. This may not mean that the protein has a random-polymer like conformation. We would rather speculate that the two segments mentioned above have individual conformation and constitute the minimum structural unit, although the protein as a whole lacks a regular secondary structure. From the biased amino acid compositions in each segment, the non-STGA segments may have the responsibility to couple with foreign materials, and the STGA segments may be useful to serve as a flexible linker. When the protein is extruded from the animal and comes across materials on which to be adsorbed, the arrangement of the segments may be optimized to ensure a strong coupling to occur. This idea arises from difficulty in the controlling of the conformation of hydrophobic residues in aqueous environment, if the protein has a random-polymer-like conformation. The non-STGA segments of the protein contain several amino acids including hydrophobic and charged ones. Although synthetic chemists may use organic solvents to maintain the conformation of hydrophobic residues, these have to be achieved in an

aqueous environment in nature. Because the protein must keep fluidity and avoid random aggregation before extrusion from the animal, the protein should have a folded structure at its secretory gland. This may not be a problem in the case of mussel, because foot proteins for surface functions have a limited number of hydrophobic residues.

The cp20k protein is characterized by the abundance of charged amino acids such as His, Glu, and Asp [23]. The richest amino acid residue is Cys, comprising 18% of the total residues. All Cys-residues are present in the intra-molecular disulfide form, and are essential in maintaining the molecular conformation [20]. The recombinant form of the protein was adsorbed to limited types of materials, and the adsorption isotherm indicated that calcite is the best material on which to be adsorbed in seawater. The cement always attaches its own calcareous plate, which is made of calcite. Thus, the barnacle seems to have acquired the specific coupling agent to the indispensable surface, calcite. The protein is the only underwater adhesive protein that was verified to have a 3-D structure [25]. Therefore, the specific function of adsorption to calcite might be optimized by its 3-D structure. This molecular design is familiar to biochemists and protein scientists, because the functions of many cellular proteins are designed for specific interactions, and the specific interactions are based on the 3-D structure of proteins. This is true for almost all proteins such as receptors, enzymes, antibodies, and so on. Conversely, the function (coupling to non-specified materials) and molecular design (dynamic change of the protein structure) suggested for cp19k might be unusual. In either case, the protein-design for surface functions in barnacle cement is very different from that of the mussel. The mussel adhesive proteins found at the interface of the adhesive layer (FP-3 and FP-5) [1,26] are typically small (6–10 kD) (Fig. 3) with substantial post-translational modifications to DOPA, *O*-phosphoserine, and 4-hydroxyarginine. Many studies have shown that the catechol group in the DOPA residue is essential in the surface coupling [27,28]. *O*-phosphoserine might have the special function of coupling to calcareous material such as the shell surface of other mussels [26]. This is also known for the tubeworm cement [29]. On the other hand, the barnacle employs commonly used amino acids and/or protein conformation in compensating for the remarkable and attractive properties that DOPA and *O*-phosphoserine possessed by the mussel.

From the application point of view, studies on the mussel system have made great impacts through the introduction of the coupling ingredient DOPA to metal/metal oxides [30]. The DOPA-residue found in foot proteins was simplified to functional units of synthetic polymer mimics, thereby greatly facilitating the practical usage. On

the contrary, application of barnacle cement proteins with surface functions points to the utilization of protein-based materials. Both cp-19k and cp-20k are functional in their recombinant forms produced in *E. coli*, and fusion with other functional proteins or biological motifs enabled immobilization of them on material surfaces [31]. The functional proteins such as the antibody binding motif of *Staphylococcal* protein A anchored by the cement protein tag gave higher activity than random adsorption of the antibody. Thus, fusion with cement protein seems to have resulted in a better orientation of the fused protein for its functioning on material surfaces. This may be useful in the design of an anchoring tag for protein micro-array technology.

The block copolymer-like structure of cp19k is intriguing for synthetic polymer mimics. Especially, the STGA (Ser, Thr, Gly, and Ala)-rich segment is noteworthy. These amino acids are also remarkably enriched in another cement protein of 68 kDa, in which the four amino acids comprise 60% of the total residues [32]. These four amino acids have relatively smaller side chains; in particular, Gly may make the structure flexible. The OH-groups of Ser and Thr may replace the water boundary layer formed on underwater surfaces [21], and may facilitate spreading of the protein onto the surface. Although these amino acids do not have remarkable functional groups like DOPA, the other segment of cp19k is rich in charged and hydrophobic amino acid residues that may function in coupling with foreign materials. Combination of the OH-group rich/flexible segments with the other coupling segment to materials may be essential in the barnacle molecular design for surface functioning. It is suggested that we should learn how it works well in water in addition to how it strongly couples with materials. Biological adhesives must be biological molecules, which are produced and used under mild conditions such as physiological temperature, pH, solvent, and so on. Continuous investigations on the mechanisms of biological adhesion should bring useful clues to the creation of new materials.

Cement proteins with surface functions have also been reviewed elsewhere [32], and interested readers are suggested to refer to that for additional information.

CEMENT PROTEINS WITH BULK FUNCTIONS

Two other proteins, a 100 kDa- and a 52 kDa-cement protein (cp100k and cp52k, respectively), form the bulk layer of the adhesives. The contents of the two proteins in the cement are remarkably high compared with all cement proteins and are similar with each other. In fact, the insoluble nature of the cement is due to the properties of the two proteins [18]. A combination of non-proteolytic [33] and

proteolytic methods [17] to render the cement soluble has revealed that the cement proteins, including cp100k and cp52k, are composed of single polypeptide chains without inter-molecular cross-linking except for disulfide bond. Although both protein-denaturant and reducing agent were indispensable for the non-proteolytic method, it is not known whether Cys-residues in cp-100k and cp-52k are cross-linked intermolecularly. The cp100k and cp52k proteins have rather lower contents of Cys-residues (1.4 and 1.1% of total residues, respectively). Although careful inspection must be continued to be able to draw conclusions about any possible existence of cross-linking, in either case the contents of cross-linkage would be limited in the two proteins.

Both bulk proteins must keep their fluidity before being fixed within the cement, in order to be transported from the cement gland to the site of attachment, and the cement seemed to be initially almost liquid [34]. This initial lower viscosity may be useful to fill small gaps on the foreign surface and to fill cracks in the old cement layer. Filling and hardening at extremely small gaps may contribute to coupling to surfaces by a nano-anchoring effect [35]. Although cp100k and cp52k have low sequence homology with each other, both of them are characterized by their hydrophobicity (Fig. 3). This is in contrast to the other cement proteins which were identified to be rather hydrophilic. Due to abundant hydrophobic amino acid residues, the bulk proteins should be adequately folded to bury hydrophobic residues into their inner space. Actually, the recombinant forms of both proteins in *E. coli* gave rise to inclusion bodies in the cytosolic space, and are difficult to solubilize [36]. It is logical that the bulk proteins have a regular conformation at least when they are secreted from the animal. If so, it may be a reasonable idea that the structures of the bulk proteins after secretion play a role to optimize protein-protein interactions that are important for the formation of the insoluble bulk layer of the cement. These protein-protein interactions might include intermolecular hydrophobic interactions among the bulk proteins. An investigation of self-assembly peptides of a design based on the primary structure of the bulk cement proteins indicated that one of the bulk cement proteins actually included amyloid-like sequences [37]. This is intriguing because the amyloid-like sequences form a beta-sheet structure and homogeneously interact with each other to form an insoluble self-assembly. Direct ATR-IR measurements of the natural cement layer also indicated the existence of abundant beta-sheet structure [38]. This type of interaction is stronger than one would expect. Thus, the cement bulk layer may be formed by optimization of molecular interactions based on the structure of their individual proteins.

This proposed mechanism is a complete contrast to the mussel byssus. Byssi are also extremely resistant to solubilization, and no method is known yet to make them soluble. The insoluble nature is due to several intermolecular cross-linking interactions [27]. The bulk portion of the adhesive plaque in the byssus is formed by FP-2. No hydrophobic protein has so far been found in the mussel foot proteins (Fig. 3), so cross-linking would be the major contributor to organize the bulk portion. This complete difference of bulk formation between the barnacle and mussel may be derived from the different places where each of the holdfasts is formed. The mussel must form a single byssal thread with almost the full strength immediately after the secretion of their components, because turbulent and unexpected wave action would challenge even a just-formed byssal thread. Therefore, all processes to form the byssal thread, including curing, must be completed as soon as possible. In contrast, barnacle cement is added to an enlarged marginal area of calcareous base as the animal grows or repairs a cement layer that has already been formed. In this case, the cement layer which has already been formed would assist the holdfast to mature even under the splashing of strong waves. Actually, the expression levels of the cement mRNAs increase upon approaching its molting stage, which is also the time that the calcareous shell grows. Thus, the curing process by which the new cement layer reaches full strength may be relatively slow in the barnacle cement. In a different way, the mussel occasionally discards old byssi by their sequential behavior of new formation of byssi and cutting off of old byssi at the stem, whereas adult barnacles never move once they have attached. Thus, the cement layer of barnacle must be kept functioning for a long period. It is not known, however, how they keep the adhesive strong for such a long time.

Other cement proteins such as cp19k and cp68k, which are partially soluble even in other solutions, could be completely solubilized by destroying the bulk structure formed by cp100k and cp52k [18,33]. This indicates that cp100k and cp52k have the responsibility to link the other cement proteins together in the cement. As mentioned before, all cement proteins are probably not cross-linked to any other proteins in the barnacle cement. Although the linking mechanism between bulk proteins and other proteins are not yet known, molecular interactions and/or metal-coordinate bonds are possible candidates for their functioning.

In spite of the fact that cement proteins such as cp19k, cp20k, and cp68k are rather hydrophilic, the barnacle also attaches onto hydrophobic surfaces. Thus, hydrophobic cp100k and cp52k can not be excluded from a contribution to surface functions. It might be too

simplified an idea that each cement protein has a single function in the process of underwater attachment.

The cement proteins are fated to interact tightly with other materials for surface coupling or homo-/hetero-cement proteins for self-assembly to form the adhesive bulk materials. This suggests that

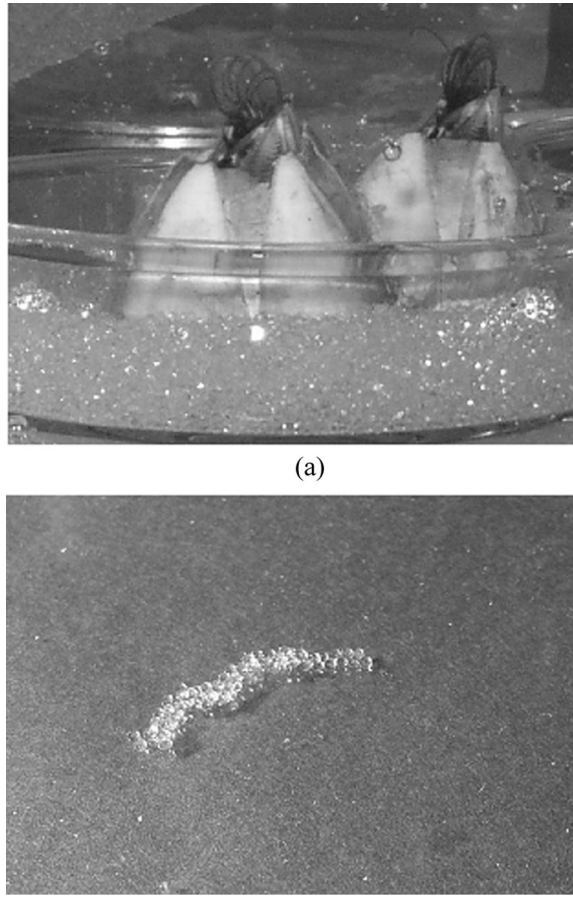

FIGURE 4 Bonding of materials by barnacle cement in water. Barnacles which had been carefully dislodged from their substratum were placed on small particles spread all over the bottom of a tank in water. Barnacles secrete the cement through their calcareous bases (Fig. 2), whereby foreign particles are attached together in water. When particles made up of different materials are mixed, the cement bonds the different materials together. In the bottom photo, glass particles were bonded together by the barnacle cement in water.

the cement proteins may have structural units for self-assembly. In addition to the bulk proteins, cp-20k [39] and cp-19k [40] seemed to have such structural units for self-assembly. Peptide-based materials have now opened a new trend in material science during the last decade [41]. Biological adhesive proteins might be a good source to design peptides with self-assembling functions.

CONCLUDING REMARKS

Biological holdfasts have a great potential for the targeted design of man-made adhesives from the macroscopic to the molecular range. Although unraveling the comprehensive design in the barnacle cement is on the halfway, we now have revealed that the biological adhesive is composed solely of bio-molecules. The molecular system is based on the full cast found in cellular proteins; for example, applying various functional groups in common amino acids, specific molecular interactions to couple with defined materials, protein-protein interactions for assembly, and optimization of the function by specific 3-D structures. What is worth mentioning is that all of these functions are accomplished in the outside of the cells. This makes the task of underwater adhesion more difficult, since the bulk cement proteins contain many hydrophobic residues. Technology to manipulate and utilize materials with conflicting natures, namely, a hydrophobic component and water, is a great challenge. Overcoming this obstacle may, therefore, put underwater adhesives into practice.

As mentioned previously, barnacle cement attaches calcareous materials to a wide range of foreign materials. Would it be possible to attach two different materials other than the calcareous materials in water? The answer is yes, and the cement has actually been shown to do so (Fig. 4). It is hoped that we may mimic this function of the cement in the near future.

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