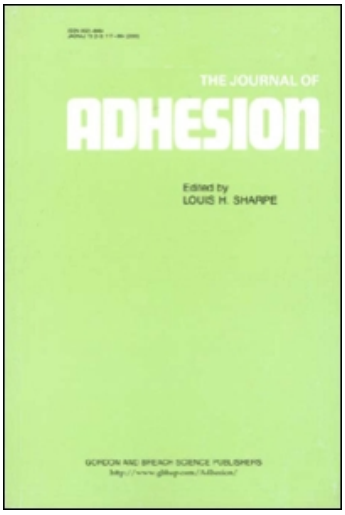
This article was downloaded by:
On: *22 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



The Journal of Adhesion
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713453635

Use of Surface-Sensitive Methods for the Study of Adsorption and Cross-Linking of Marine Bioadhesives

Mattias Berglin[a]; Julia Hedlund[a]; Camilla Fant[b]; Hans Elwing[a]
[a] Cell and Molecular Biology Interface Biophysics, Göteborg University, Göteborg, Sweden [b] Department of Applied Surface Chemistry, Chalmers University of Technology, Göteborg, Sweden

To cite this Article Berglin, Mattias , Hedlund, Julia , Fant, Camilla and Elwing, Hans(2005) 'Use of Surface-Sensitive Methods for the Study of Adsorption and Cross-Linking of Marine Bioadhesives', The Journal of Adhesion, 81: 7, 805 — 822
To link to this Article: DOI: 10.1080/00218460500189059
URL: http://dx.doi.org/10.1080/00218460500189059

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

The Journal of Adhesion, 81:805–822, 2005
Copyright © Taylor & Francis Inc.
ISSN: 0021-8464 print/1545-5823 online
DOI: 10.1080/00218460500189059

Taylor & Francis
Taylor & Francis Group

Use of Surface-Sensitive Methods for the Study of Adsorption and Cross-Linking of Marine Bioadhesives

Mattias Berglin
Julia Hedlund
Cell and Molecular Biology, Interface Biophysics, Göteborg University, Göteborg, Sweden

Camilla Fant
Department of Applied Surface Chemistry, Chalmers University of Technology, Göteborg, Sweden

Hans Elwing
Cell and Molecular Biology, Interface Biophysics, Göteborg University, Göteborg, Sweden

Downloaded At: 08:44 22 January 2011

The establishment of the bond of sessile marine organisms such as barnacles, mussels, and algae in the marine environment starts with the secretion and the adsorption of the adhesive biopolymers to the substrate. Subsequently, this is followed by the formation of cohesive interactions with the next layer of adhesive biopolymers that are deposited/adsorbed on top of the first layer. These two fundamental processes for the adhesive plaque buildup have been subjected to several investigations in recent years using model molecules, especially Mefp-1 extracted from the blue mussel Mytilus edulis. With the introduction of optical surface-sensitive methods such as ellipsometry, surface plasmon resonance (SPR), and infrared spectroscopy (IR), it has been possible to elucidate both the kinetics of adsorption and structure of the Mefp-1 film. In contrast to adsorption, the cohesive interactions or the cross-linking are not easily followed with these optical methods and new approaches and techniques are required. One such technique that has been useful is the quartz-crystal microbalance with dissipation monitoring (QCM-D), which has been used for cross-linking studies of a variety of biopolymers including bioadhesives from mussel and algae.

Received 24 January 2005; in final form 14 April 2005.

One of a collection of papers honoring Manoj K. Chaudhury, the February 2005 recipient of The Adhesion Society Award for Excellence in Adhesion Science, sponsored by 3M.

Address correspondence to Mattias Berglin, Cell and Molecular Biology, Interface Physics, Göteborg University, Box 462, SE-405 30 Göteborg, Sweden. E-mail: mattias.berglin@gmm.gu.se

Keywords: Adsorption; Cross-linking; Marine bioadhesives; Mefp-1; Quartz-crystal microbalance with dissipation monitoring (QCM-D)

Downloaded At: 08:44 22 January 2011

INTRODUCTION

Inspired by biomimetic research, there has been a growing interest in the molecular explanation of the adhesive bonding of sessile marine organisms. From a technological standpoint it is easy to understand such interest because of the differences in mode of function between synthetic adhesives and marine bioadhesives. For example, marine bioadhesives do not need surface cleaning or pretreatment before bonding [1] and they form adhesive interactions with a multitude of surfaces [2]. Moreover, the bond formation takes place with a wet surface in high ionic-strength environment and it has been shown to display self-healing capabilities and high toughness [3]. Thus, if we want to improve the performance of synthetic adhesives in situations where water is a natural component, it is the differences between synthetic and biological adhesives that should be studied and characterized in detail. This was obvious three hundred years ago when Sir Isaac Newton wrote, "There are agents in Nature able to make particles of joints stick together by a very strong attraction, and it is the business of experimental philosophy to find them out" [4].

The establishment of the adhesive joint in the marine environment starts with the secretion and the adsorption of multilayers of biopolymers at the substrate. After adsorption the adhesive biopolymers must undergo some sort of curing or cross-linking to make the adhesive joint functional. These two fundamental processes in the formation of the adhesive joint have been subjected to several investigations in recent years using a wide variety of surface-sensitive methods. For the investigations' model molecules, particularly Mefp-1, extracted from the blue mussel *Mytilus edulis*, has been used. In this review, we discuss how the introduction of surface-sensitive methods has increased the understanding of the dynamical function of marine bioadhesives, with emphasis on Mefp-1.

ADHESIVE PLAQUE OF THE BLUE MUSSEL, *Mytilus edulis*

The adhesive plaque of the blue mussel, *Mytilus edulis*, has been extensively studied and at least five different adhesive proteins involved in plaque formation have been identified [5]. *Mytilus edulis* foot protein-1 (Mefp-1) forms a protective coating around the adhesive

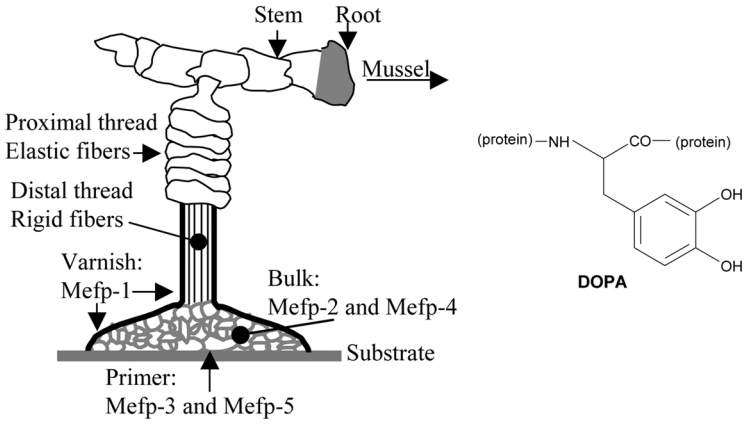


FIGURE 1 Schematic image of the attachment organ of the blue mussel, the byssus. Shown are the root, stem, thread, and plaque. The thread can be divided into proximal thread and distal thread. The building blocks in the plaque are 3,4-dihydroxy phenylalanine (DOPA)–rich proteins (*Mytilus edulis* foot protein, Mefp).

Downloaded At: 08:44 22 January 2011

plaque and the byssus thread, which is the biological structure synthesized by the mussel for the attachment (Figure 1). Mefp-2 and Mefp-4 are believed to stabilize the foam-like structure of the adhesive and, finally, Mefp-3 and Mefp-5 are believed to form the adhesive interactions with the solid substrate [6]. The characterization of the adhesive proteins from the mussel began with the pioneering work of Waite and Tanzer, who isolated the 130-kD alkaline Mefp-1 protein from phenolic glands of the blue mussel [7]. It was later shown that the dominant features of Mefp-1 are a tandemly repeated decapeptide with up to 80 repeats and a variety of posttranslational modifications [8]. The modifications include the hydroxylation of proline to 4-hydroxyproline, 3,4-dihydroxyproline, and tyrosine to 3,4-dihydroxyphenyl alanine (DOPA) [9].

In an article by Deacon and coworkers, the structure of Mefp-1 was investigated by sedimentation equilibrium studies [10]. Their semiflexible rod model for the structure of Mefp-1 consisted of a globular region with a nonrepetitive amino acid sequence and a region consisting of repeated sequences of amino acids with alternating stiff and flexible segments. This structure is advantageous for adhesion and cohesion. A flexible structure allows the establishment of many contact points with the substrate, neighboring Mefp-1 molecules, or other proteins in close proximity. As pointed out previously, Mefp-1 is the main component of the varnish that covers the byssal thread

and plaque but it has been used as a model adhesive molecule to better understand marine bioadhesion. The rational for using Mefp-1 is the similarity in chemical composition and structure between Mefp-1 and Mefp-3 and the fact that Mefp-1 is easier to extract from the mussel and gives reasonable yields. However, it should be stressed that Mefp-3 probably is a better adhesive than Mefp-1 because of the fact that Mefp-1 has only one substrate, *i.e.*, the byssus thread, whereas Mefp-3 forms interactions with a multitude of surfaces depending on where the mussel decides to settle.

ADSORPTION STUDIES OF *Mytilus edulis* FOOT PROTEIN-1 (Mefp-1)

The chemistry of industrial adhesive bonding is generally of two types: highly energetic (covalent or chelate) or a collection of weaker, noncovalent interactions. In the marine environment, it is most likely that the adhesives are dependent on the weaker noncovalent interactions across the interface. These interactions include charge–charge, hydrogen bond, dipole–dipole, induced dipole–dipole, and nonpolar coupling, among others. The latter three are more commonly known as the van der Waals forces. These interactions are very short ranged and good adsorption is mandatory for a strong adhesive joint. During the adsorption, the formation of an adhesive bond starts with the establishment of interfacial molecular contact by wetting [11]. The molecules will undergo motions toward preferred configurations at the interface and try to reach adsorption equilibrium. Thus, from an adhesive perspective, adsorption studies can reveal a preliminary indication if the molecules are able to form interactions and what type of interactions are formed with a substrate.

In this context it can be interesting to note that all surfaces in the marine environment obtain a conditioning film consisting of organic polymers such as glycoproteins and polysaccharides, which rapidly is followed by the colonization of bacteria and algae [12]. With out any cleaning of the surface the bioadhesives must form interactions with this “fuzzy” hydrated layer. The secret of this interaction must be found in the chemistry and structure of the marine bioadhesives.

The importance of the modification of Tyrosine to DOPA in Mefp-1 for the interaction with surfaces has been stressed [13]. Even though Mefp-1 has a lower molar ratio of DOPA as compared with, for example, Mefp-3, it has been demonstrated in several studies that Mefp-1 shows good adsorption to both hydrophilic and hydrophobic surfaces [14–22]. The good adsorption to hydrophilic surfaces from a

Downloaded At: 08:44 22 January 2011

water solution needs some attention because that is attractive from an adhesive perspective. The high concentration of DOPA and hydroxyarginine gives the protein an extensive H-bonding potential. Consequently, the Mefp-1 molecule is able to displace water from a hydrophilic interface. The displacement of water has two consequences. First, it makes the adhesive bond stronger because water itself can be considered a "weak boundary layer" [5]. Second, the driving force for adsorption is enhanced by the release of water. The entropy increase when water is released results in a decreased Gibbs free energy, which promotes adsorption [23]. The displacement of water and adsorption of proteins to a hydrophobic surface is a more general phenomenon even though it is still not fully understood. This results in hydrophobic surfaces generally accumulating more proteins [24–26]. This holds true even for Mefp-1, which has been shown to adsorb readily on hydrophobic surfaces [14]. A full description of adsorption theories is not within the scope of this paper but it is interesting to note the multifunctionality of Mefp-1.

Downloaded At: 08:44 22 January 2011

ADSORPTION STUDIES WITH SURFACE-SENSITIVE METHODS

For the adsorption studies a wide range of surface-sensitive analytical techniques including surface plasmon resonance (SPR), ellipsometry, attenuated total reflection infrared spectroscopy (ATR-FTIR), quartz-crystal microbalance with dissipation (QCM-D), and X-ray photoelectron spectroscopy (XPS) have been used. The different methods are able to extract different information concerning adsorption of Mefp-1.

X-Ray Photoelectron Spectroscopy (XPS)

Analysis with XPS is performed in vacuum and is used to quantify the elemental composition with a depth of the upper 90 Å of the surface. Thus, the substrate must be removed from the protein solution and dried before analysis and no time-resolved analysis of adsorption can be performed. Nevertheless, XPS has been used to elucidate the structure of Mefp-1 upon adsorption. Baty *et al*. used two different polymeric surfaces, *i.e*., polystyrene (PS) and poly(octadecyl)methacrylate (POMA), and they concluded that the differences found in the nitrogen signal as observed with XPS upon dehydration could be attributed to the strength of the interactions between Mefp-1 and the two surfaces. The adsorbed Mefp-1 was stabilized on the surface of the PS through interactions that prevent the protein layer from being disrupted upon dehydration. On the POMA surface, Mefp-1

was representative of a loosely bound protein layer that was transformed to a highly perturbed layer upon dehydration [15].

Quartz-Crystal Microbalance with Dissipation (QCM-D)

Differences in structure of Mefp-1 upon adsorption were found using QCM-D. In summary, the QCM-D is an acoustic technique where a quartz crystal is set in lateral resonance oscillation with a predefined frequency (f). Simultaneous frequency (f) and dissipation (D) measurements are made by periodically switching on and off the AC voltage over the crystal with a repetition rate of $\sim$1 Hz. The sensor decay signal is recorded and fitted to an exponentially damped sinusoidal curve [27, 28]. An acoustic evanescent wave exists at the interface between the sensor crystal and the protein solution. The magnitude of this acoustic evanescent wave decays exponentially in the direction normal to the sensor surface with a decay length dependent on the viscosity of the protein solution. For water the decay length is on the order of 200 nm, which increases with increased viscosity. The adsorbed amount can be calculated from the frequency shift (Δf) using the Saurbrey equation [29], provided that the mass is evenly distributed, does not slip on the sensor surface, and is sufficiently rigid and/or thin to have negligible internal friction. However, in the case of protein adsorption it has been shown that the Saurbrey equation overestimates the adsorbed amount because it includes the water hydrodynamically coupled to the adsorbed film [30]. In addition, the decay time (τ) of the sensor crystal is measured and is used to calculate the dissipation (d). The dissipation gives valuable information about the mechanical properties of the adsorbed layer. A very rigid material will have an increased decay time, and consequently, low dissipation. *Vice versa*, a viscoelastic material will result in fast damping of the sensor crystal, *i.e.*, a short decay time and higher dissipation, as schematically illustrated in Figure 2. Thus, the dissipation signal could be used to reveal differences in protein structure upon adsorption as shown by Fant *et al*. where Mefp-1 formed an elongated, flexible film with substantial amounts of hydrodynamically coupled water on a $-CH_3$ terminated surface, whereas Mefp-1 formed a rigidly attached adlayer with little hydrodynamically coupled water on a SiO_2 surface [14].

Ellipsometry

Ellipsometry is an optical method based on the shift in refractive index close to the sensor surface upon protein adsorption [31]. In brief, the

Downloaded At: 08:44 22 January 2011

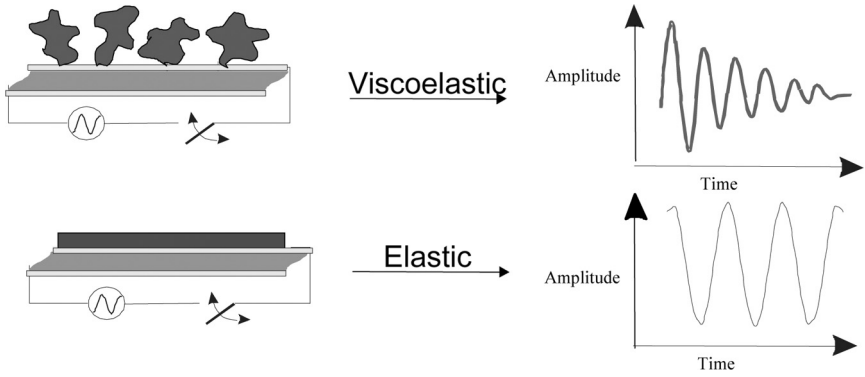


FIGURE 2 Schematic illustration of the QCM-D setup and measurement principle. The attachment of a viscoelastic layer on the gold-coated quartz sensor results in fast damping of the crystal when the electric power is shut off. When a rigid, elastic layer is interacting with the sensor surface it results in little damping of the crystal when the electric power is shut off.

Downloaded At: 08:44 22 January 2011

detection principle is based on the fact that the polarization of a light beam that is reflected at the surface changes because of changes in refractive index at the solid–liquid interface. The change in polarization is mathematically related to both the change in refractive index and the thickness of the thin organic layer. Using the thickness with the refractive index increment (the change in refractive index per unit of protein concentration) the adsorbed mass can be calculated. The drawback is that the refractive-index increment is protein specific and must be known, determined, or assumed. The advantage is the possibility to calculate the thickness of the adlayer. Ellipsometry has been used to study the adsorption kinetics and thickness of a saturated layer of adsorbed Mefp-1 [30]. The ellipsometry measurements suggested that the Mefp-1 layer is extended ($\sim$20 nm), water-rich, and hydrogel-like on a hydrophobic surface.

Attenuated Total Reflection Infrared Spectroscopy (ATR-FTIR)

ATR-FTIR is an optical method that can be used for obtaining spectroscopic information regarding adsorbed proteins [32]. In brief, IR radiation traverses through an ATR crystal and undergoes total internal reflection several times. Under total reflection, a nonradiative electric field or an evanescent wave exists at the interface between the crystal and the protein solution. The magnitude of this nonradiative field

decays exponentially in the direction normal to the ATR surface with a decay length similar to the wavelength of the light (1–2 μm). Thus, if there is something within the evanescent wave, it can interact with the IR light beam. If the frequency of the IR beam harmonizes with the frequency of the vibrational and rotational motions of the atoms, the bonds are excited, *i.e.*, the IR light is absorbed. The characteristic frequencies of such absorption are proportional to discrete energy differences between vibrational or rotational ground states and allowed excited states. These energy differences, or band gaps, are further determined by the relative motions and masses of the connected atoms, the force constant of the bonds, and their molecular geometries. An advantage of the ATR technique is the possibility to coat the ATR crystal and by this to make controlled changes of the interfacial chemistry.

Proteins have two characteristic absorption bands in the infrared spectrum, amide I ($\sim$1620–1680 cm^{-1}) and amide II ($\sim$1520–1580 cm^{-1}). Both originate from the peptide backbone, C=O stretching and N−H bending, respectively. The ATR-FTIR methodology has been used for measuring protein adsorption [33–36]. The absorbance measurements can be used to calculate the actual amount of adsorbed protein on the surface. These calculations require molar absorptivity of the protein, which can be obtained from transmission IR experiments. Another plus of the ATR-FTIR methodology is that it can be used to study changes in protein secondary structure during adsorption [37]. It has been suggested that these types of structural changes significantly enhance adsorption [23, 38]. The structural rearrangement increases the rotational freedom along the polypeptide chain. Even a small release of amino acids leads to a significant rotational freedom, increased entropy, and enhanced adsorption.

The ATR-FTIR methodology has been used to measure the adsorption of Mefp-1 [18]. The IR spectra of hydrated adsorbed Mefp-1 revealed significant differences in the amide II region and in two other bands when adsorbed to PS and POMA. This result indicated that the chemistry of the substrate film influenced the structure of Mefp-1 upon adsorption.

Surface Plasmon Resonance (SPR)

The method is based on a collective electromagnetic motion that propagates along a metal surface, associated with which there is a localized evanescent wave with a decay length of $\sim$200 nm. The surface sensing is due to the fact that the excitation of the surface plasmon is very sensitive to changes in the refractive index of the

Downloaded At: 08:44 22 January 2011

medium sensed by the evanescent wave in close proximity to the metal surface. The SPR is excited using monochromatic and plane-polarized light that, under total-internal-reflection conditions, is directed through a quartz prism at the interface between the quartz and a thin layer ($\sim$50 nm) of metal, usually gold. The SPR signal is excited at a certain angle of incidence and if the refractive index outside the gold surface changes by protein adsorption, there is a proportional change in the angle at which the SPR is generated. The SPR technique, thus, allows time-resolved measurements of mass uptake during protein adsorption. It is possible to achieve the optical thickness by more advanced analysis of the SPR response curve but for that purpose the more established ellipsometry technique is more suited.

The adsorption behavior of Mefp-1 on a hydrophilic surface was studied by SPR by Heamers and coworkers [39]. Using the SPR technique, they found that the initial rate of aggregation in solution determined the adsorption plateau value of Mefp-1 and the aggregation in solution could be increased by increased pH. Step-like adsorption curves were found, which were interpreted as the adsorption of an adlayer of Mefp-1 aggregates onto the initially adsorbed Mefp-1 layer on the surface. The rate of formation of this second layer increased with increasing pH. Thus, Mefp-1 is able to form multilayers upon adsorption, which is crucial for the adhesive plaque formation.

Downloaded At: 08:44 22 January 2011

CROSS-LINKING STUDIES OF MUSSEL ADHESIVE PROTEINS AND ANALOGUES

From a surface-science point of view, two processes must take place for an adhesive bond to function. First, as discussed previously, the biopolymers must form interactions with the underlying substrate. Second is the formation of interactions to the next layer of adhesive molecules that is deposited on top of the first layer. This strengthening of the matrix or the formation of cohesive interactions can be of a chemical nature such as covalent cross-links or of a physical nature such as entanglements or filler particles. For example, Mefp-1 is able to form aggregates and multilayers during adsorption [16]. The multilayer adsorption is a prerequisite to be able to form the micrometer-thick layers of adhesive material necessary to bond the organism to the substrate. It was shown that the multilayer formation was not diffusion controlled as the primary adsorption but determined by the establishment of specific covalent cross-links between the arriving Mefp-1 molecules and the preadsorbed layer. Thus, cross-linking was required not only for strengthening of the matrix but also for

the formation of the matrix itself. The cross-linking reaction is, therefore, fundamental to study if we want to increase the understanding of the mode of function of marine bioadhesives.

CROSS-LINKING STUDIES WITH BULK METHODS

It has been shown *in vitro* that the DOPA residue, found in varying amounts in the different mussel adhesive proteins, can be cross-linked through autooxidation, chemically using $NaIO_4$ or other simple oxidants, *via* transition metals, and enzymatically using, for example, Tyrosinase [30, 40–43]. The methods used for the analysis of the cross-linking include NMR, SDS-PAGE, penetration tests, and MALDI-TOF MS. These methods are not "surface sensitive" but because the cross-linking mainly is a bulk phenomenon, these methods have been used to elucidate the cross-linking mechanism of mussel adhesive proteins and analogues. As presented later, with the introduction of surface-sensitive methods, the insight into the kinetics and dynamic function of the process can be greatly enhanced.

Downloaded At: 08:44 22 January 2011

Nuclear Magnetic Resonance (NMR)

Rotational echo double-resonance NMR was used in a study to confirm the formation of quinone-derived cross-links in mussel byssal plaques [44]. In that study, a mussel exposed to a shear stress exhibited significantly enhanced levels of 5,5′-dihydroxyphenylalanine (di-DOPA) cross-links in the byssus plaque. However, the number of cross-links could only be studied after harvest of the plaque, *i.e.*, there is no possibility to study the formation of cross-links as a function of time.

Sodium Dodecylsulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

One common way to determine the molecular mass of proteins is by SDS-PAGE. For example, the increase in molecular mass of polyphenolic peptides extracted from the mussels *Aulacomya ater*, *Mytilus edulis chilensis*, and *Choromytilus chorus* after exposure to Tyrosinase was studied with SDS-PAGE [40]. A significant increase in molecular mass was observed after the Tyrosinase treatment, which was interpreted as formation of intermolecular cross-links between the polyphenolic proteins. The cross-linking of model peptides based on the Mefp-1 chemistry has also been studied using SDS-PAGE [45]. The "smear" detected on the SDS-PAGE gels after Tyrosinse treatments was an indication of the progressive increase in molecular mass as

polymerization proceeded. If the cross-linking reaction is under enzymatic control, the *in vivo* enzymatic oxidation might take place *via* schleroenzymes such as catechol oxidases, which are found in considerable amounts in the byssus thread [46].

Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectroscopy (MALDI-TOF MS)

Low molecular mass analogues of the mussel adhesive peptides have been used to rule out the chemistry of the cross-linking reaction. For example, MALDI-TOF MS has been used to show the polymerization of *N*-Boc-DOPA and synthetic variants of the Mefp-1 decapeptide [47, 48]. In the latter study, free Lysine and DOPA-like o-diphenols were added during the reaction. Interestingly, Lysine had no effect on the cross-linking whereas o-diphenol stopped the polymer formation. Thus, formation of di-DOPA residues seems to be the general cross-linking mechanism of mussel adhesive proteins.

Downloaded At: 08:44 22 January 2011

Penetration Test

The principle behind the penetration test is simple, *i.e.*, with increased degree of cross-linking the resistance against penetration increases. This method was used extensively to investigate the effect of different metal ions and oxidants on the cross-linking of a mussel adhesive extract [49]. Interestingly, the effect of oxidation transition metals, Mn^{3+} and Fe^{3+}, on the increased penetration resistance was striking. It was also interesting to note that no increased resistance against penetration was observed using Tyrosinase. Thus, it can be speculated if chelation and oxidation of DOPA residues *via* oxidative transition metals is the main reaction mechanism in the adhesive plaque and not an enzymatic tempering as has been suggested as the *in vivo* cross-linking process. The advantage of using metal ions instead of an enzyme is the reversibility (in case of chelation) and faster kinetics as compared with the enzymatic reaction.

CROSS-LINKING STUDIES WITH SURFACE-SENSITIVE METHODS

These methods all lack the capability to investigate the time-resolved cross-linking. This is a very important factor to study because the cross-linking reaction is under precise control by the organisms. Cross-linking in the glands would prevent the release of the glue

and with slow cross-linking after release water currents would wash away the water-soluble adhesive polymers. This precise control over the cross-linking reaction must then be transferred when it comes to the development of a synthetic water-resistant adhesive.

QCM-D

The time-resolved cross-linking reaction of Mefp-1 can be studied by using the surface-sensitive QCM-D methodology [30, 50]. The increased rigidity during cross-linking is followed as a decrease in dissipation. A typical QCM-D cross-linking experiment is shown in Figure 3 (top). The experiment was carried out as follows: (1) A baseline was established with degassed buffer, (2) Mefp-1 was added to the sample chamber, (3) the adsorption was studied, (4) it was washed with buffer, (5) the cross-linking agents was added, which in this case was $NaIO_4$. Upon addition of the cross-linking agent we observed a gradual decrease in dissipation. Note that the frequency increased concomitantly with the decrease in dissipation during the cross-linking. This suggests that the cross-linking reaction is accompanied by the loss of a reaction product, loss of the water binding capacity, or both. The strength of the QCM-D methodology is the simplicity and sensitivity on a variety of supports. The sensor surface is coated with gold, which makes the self-assembly monolayer (SAM) methodology an attractive technique for the change of sensor chemistry [25]. The sensor surface chemistry can also easily be changed by means of spin coating [51].

The advantage of the QCM-D technology is evident when comparison is made with a surface-sensitive optical method such as ellipsometry. Ellipsometry measures the "optical mass" and is not able to detect any changes in mechanical properties of the adlayer as discussed previously. In Figure 3 (bottom) the time *vs*. adsorbed mass during cross-linking of a monolayer of Mefp-1 with $NaIO_4$ is shown. The ellipsometry signal was almost stable following the addition of $NaIO_4$ as compared with the major decrease in dissipation as measured with QCM-D.

EXAMPLES OF OTHER MARINE BIOADHESIVE SYSTEMS AND FUTURE PROSPECTS

Algal Adhesion

The chemical composition and mode of function of the bioadhesive systems used by algae is unknown. To date, the adhesive system used

Downloaded At: 08:44 22 January 2011

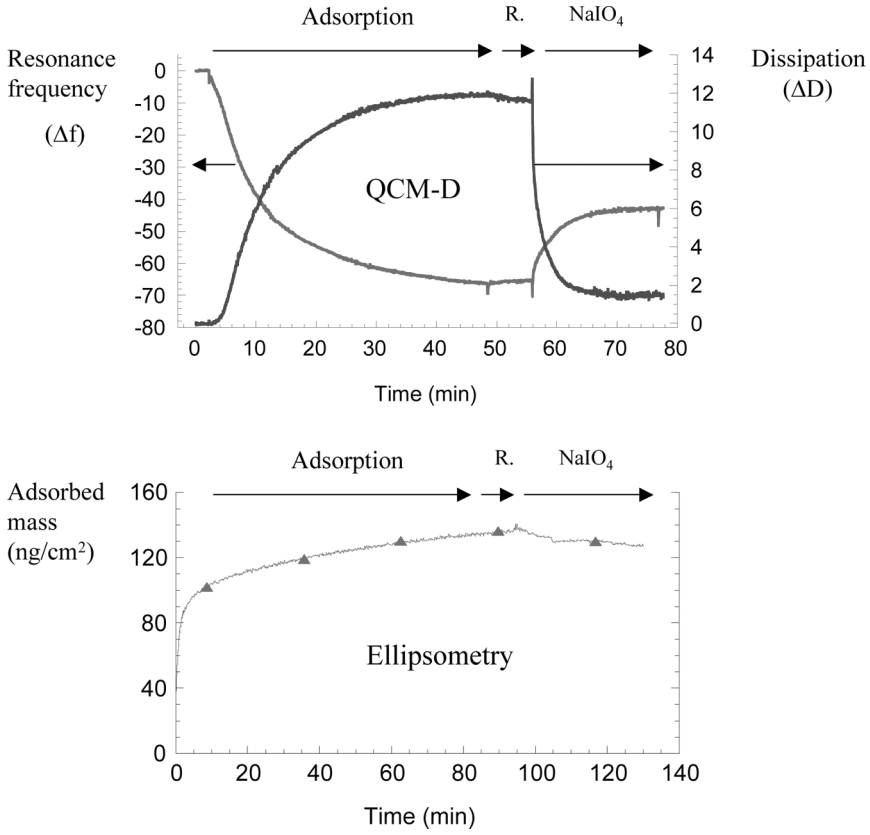


FIGURE 3 An illustration of the use of surface-sensitive methods for studying hydrodynamic effects of molecular cross-linking of Mefp-1 initiated by $NaIO_4$. Hydrophobic solid surfaces were coated with Mefp-1 (25 μg/ml) followed by rinsing with buffer (R) and exposure to 1 mM of $NaIO_4$. Upper: Continuous registration of the surface interaction was made with QCM-D, an acoustic method sensitive to the adsorbed mass and structural water of the Mefp-1 adlayer (Δf) and viscoelastic changes (ΔD) of the adsorbed layer. Adsorption of Mefp-1 could be followed continuously from time zero. Induction of molecular cross-linking with $NaIO_4$ resulted in an increase of frequency that is interpreted as that structural water disappears from the Mepf-1 layer. The D-factor decreased dramatically at cross-linking induced by $NaIO_4$. Bottom: Ellipsometry measurements of the cross-linking with $NaIO_4$ of the Mefp-1 layer did not result in much change of the adsorbed optical mass at the surface.

by swimming spores of the green alga, *Ulva intestinalis (*formerly named *Enteromorpha intestinalis)*, during settlement has been most extensively studied [52–56]. The spores secrete an adhesive

Downloaded At: 08:44 22 January 2011

glycoprotein from Golgi-derived membrane-bounded vesicles. The adhesive material undergoes rapid swelling after the release followed by a fast hardening, and a firm anchorage to the solid substrate is formed. After the curing, considerable force is needed for the detachment of the spores from both hydrophilic and hydrophobic surfaces [57–59]. After settling, a continued biosynthesis of the same, or at least a related, adhesive glycoprotein in the developing cell wall of the settled spore takes place. The adhesive-bond formation and curing process of both the primary and the secondary adhesive is, yet, still very unclear. Thus, it can be speculated whether the surface-sensitive methods could be used for the study of the attachment of the spores and the curing of the adhesive material.

Moreover, there has been speculation about the role of phenolic polymers and oxidases as key components in algal adhesion. For example, it has been shown in *Fucus* zygotes that the secretion of phenolic polymers correlated with the attachment process [6]. The secretion started a few hours after fertilization of the egg. Later, after germination, phenolic-polymer (PP) secretion was localized at the site of attachment. Based on this hypothesis the enzymatic cross-linking of a PP extracted from *Fucus serratus* was investigated using a vanadium-dependent bromoperoxidase (BPO) [60]. The methanol-extracted PP was adsorbed to a quartz-crystal sensor and the cross-linking was initiated by the addition of BPO, KBr, and H_2O_2. The decreased dissipation upon addition of the cross-linking agents, as measured with QCM-D, was interpreted as the formation of intramolecular cross-links between different phloroglucinol units in the PP (Figure 4). Thus, the QCM-D method is a versatile tool that can be used to elucidate the cross-linking kinetics of other bioadhesive systems.

Downloaded At: 08:44 22 January 2011

Barnacle Adhesion

Barnacles are another notorious macrofouler found worldwide but, in contrast to the mussel adhesive system, the adhesive system used by barnacles has been relatively little studied. The adhesion system of the adult barnacle, which was first described by Darwin [61], consists of secretory cells interlinked by a duct system, which leads in balanomorphs to a number of openings in the base. The adult cement has been analyzed and, based on the data available, it appears that the proteins involved in adhesion are significantly different from those of the blue mussel [62–65]. For example, no evidence of DOPA-containing proteins was reported. The difficulties with the solubilization of barnacle-cement proteins have long hampered the possibility of

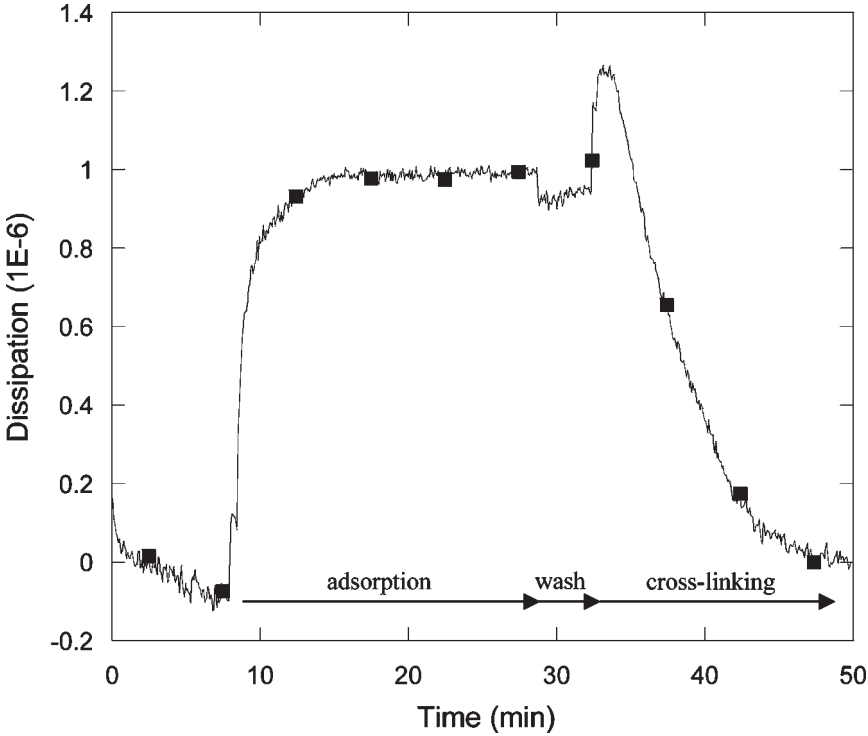


FIGURE 4 Representative QCM-D experiment showing the shift in dissipation during the adsorption and the oxidative enzymatic cross-linking of a high molecular-mass phenolic polymer (PP) extracted from the algae *Fucus serratus*. As expected, the dissipation increases during the adsorption. After adsorption and washing, the cross-linking agents vanadium-dependent bromoperoxidase (BPO), H_2O_2 (1 mM), and KBr (1 mM) were added. Upon the addition of the cross-linking agents we first observed a sharp rise in dissipation. This was interpreted as the enzyme binding to the PP film. The fast attachment of the enzyme to the substrate is notable. About a minute after the addition of BPO, KBr, and H_2O_2, we observed a gradual decrease in dissipation that continued for about 15 min before reaching steady state, *i.e.*, no more cross-linking could be observed.

carrying out any *in vitro* adsorption or cross-linking studies. Even though no *in vitro* studies of the adsorption and cross-linking of barnacle-adhesive proteins have been done, it is most likely that barnacle-adhesive proteins are able to form interactions with a variety of substrates based on the considerable force needed to detach barnacles from both hydrophilic and hydrophobic substrates as well as from release coatings [66–69]. Recently, cement from *Megabalanus*

Downloaded At: 08:44 22 January 2011

rosa was solubilized [70, 71], which makes future adsorption and cross-linking studies feasible using the surface-sensitive techniques described previously.

CONCLUSIONS

The introduction of surface-sensitive techniques such as SPR, ATR-FTIR, ellipsometry, and QCM-D have greatly facilitated the adsorption and cross-linking studies of several marine adhesive molecules such as Mefp-1 and algal bioadhesives. For example, the time-resolved kinetic aspects of both adhesion and the enzymatic or chemical cross-linking together with the structural changes taking place during the processes can now easily be studied. A fundamental understanding of adsorption and cross-linking of marine bioadhesives is required to be able to adopt strategies both for the development of new synthetic adhesives and for the development of new antifouling coatings.

Downloaded At: 08:44 22 January 2011

ACKNOWLEDGMENTS

The financial support by the European Commission GROWTH program research project, Algal Bioadhesives grant G5RD-CT-2001-00542, and MISTRA research project Marine Paint are gratefully acknowledged.

REFERENCES

[1] Waite, J. H., *Int. J. Adhes. Adhesives* **7**, 9–14 (1987).
[2] Crisp, D. J., Walker, G., Young, G. A., and Yule, A. B., *J. Colloid Interface Sci*. **104**, 40–50 (1985).
[3] Vaccaro, E. and Waite, J. H., *Biomacromolecules* **2**, 906–911 (2001).
[4] Newton, I., *Opticks* (Republished by Dover, New York, 1952), p.1704.
[5] Waite, J. H., *Integrative Comparative Biol*. **42**, 1172–1180 (2002).
[6] Vreeland, V., Waite, J., and Epstein, L., *J. Phycol*. **34**, 1–8 (1998).
[7] Waite, J. H. and Tanzer, M. L., *Science* **212,** 1038–1040 (1981).
[8] Waite, J. H., *Int. J. Biol. Macromol*. **12**, 139–144 (1990).
[9] Taylor, S. W., Waite, J. H., Ross, M. M., Shabanowitz, J., and Hunt, D. F., *J. Am. Chem. Soc*. **116**, 10803–10804 (1994).
[10] Deacon, M. P., Davis, S. S., Waite, J. H., and Harding, S. E., *Biochemistry* **37**, 14108–14112 (1998).
[11] Wu, S., *Polymer Interface and Adhesion* (Marcel Dekker, New York, 1982)
[12] Wahl, M., *Mar. Ecol. Prog. Ser*. **58**, 175–189 (1989).
[13] Ooka, A. A. and Garrell, R. L., *Biopolymers* **57**, 92–102 (2000).
[14] Fant, C., Sott, K., Elwing, H., and Hook, F., *Biofouling* **16**, 119–132 (2000).
[15] Baty, A. M., Leavitt, P. K., Siedlecki, C. A., Tyler, B. J., Suci, P. A., Marchant, R. E., and Geesey, G. G., *Langmuir* **13**, 5702–5710 (1997).

[16] Haemers, S., van der Leeden, M. C., Koper, G. J. M., and Frens, G., *Langmuir* **18**, 4903–4907 (2002).
[17] Harder, P., Grunze, M., and Waite, J. H., *J. Adhes*. **73**, 161–177 (2000).
[18] Baty, A. M., Suci, P. A., Tyler, B. J., and Geesey, G. G., *J. Colloid Interface Sci*. **177**, 307–315 (1996).
[19] Olivieri, M. P., Loomis, R. E., Meyer, A. E., and Baier, R. E., *J. Adhes. Sci. Technol*. **4**, 197–204 (1990).
[20] Frank, B. P. and Belfort, G., *Biotechnol. Progr*. **18**, 580–586 (2002).
[21] Suci, P. A. and Geesey, G. G., *Colloid Surf. B* **22**, 159–168 (2001).
[22] Suci, P. A. and Geesey, G. G., *Langmuir* **17**, 2538–2540 (2001).
[23] Norde, W. and Lyklema, J., *J. Biomater. Sci.-Polym. Ed*. **2**, 183–202 (1990).
[24] Elwing, H., *Biomaterials* **19**, 397 (1998).
[25] Prime, K. L. and Whitesides, G. M., *Science* **252**, 1164–1167 (1991).
[26] Martins, M. C. L., Ratner, B. D., and Barbosa, M. A., *J. Biomed. Mater. Res.* **67A**, 158–171 (2003).
[27] Rodahl, M., Hook, F., Krozer, A., Brzezinski, P., and Kasemo, B., *Rev. Sci. Instrum*. **66**, 3924–3930 (1995).
[28] Rodahl, M. and Kasemo, B., *Rev. Sci. Instrum*. **67**, 3238–3241 (1996).
[29] Saurbrey, G., *Z. Phys*. **155**, 206–222 (1959).
[30] Hook, F., Kasemo, B., Nylander, T., Fant, C., Sott, K., and Elwing, H., *Anal. Chem*. **73**, 5796–5804 (2001).
[31] Arwin, H., *Thin Solid Films* **377**, 48–56 (2000).
[32] Chittur, K. K., *Biomaterials* **19**, 357–369 (1998).
[33] Jung, C., *J. Mol. Recognit*. **13**, 325–351 (2000).
[34] Losche, M., *Curr. Opin. Solid St. M*. **2**, 546–556 (1997).
[35] Jeon, J. S., Raghavan, S., and Sperline, R. P., *Colloid Surf., A* **92**, 255–265 (1994).
[36] Singh, B. R. and Fuller, M. P., *Appl. Spectrosc*. **45**, 1017–1021 (1991).
[37] Ball, A. and Jones, R. A. L., *Langmuir* **11**, 3542–3548 (1995).
[38] Maste, M. C. L., Norde, W., and Visser, A., *J. Colloid Interface Sci*. **196**, 224–230 (1997).
[39] Haemers, S., van der Leeden, M. C., Nijman, E. J., and Frens, G., *Colloid Surf*. **190**, 193–203 (2001).
[40] Burzio, L. A., Burzio, V. A., Pardo, J., and Burzio, L. O., *Comp. Biochem. Phys. B*. **126**, 383–389 (2000).
[41] Monahan, J. and Wilker, J. J., *Langmuir* **20**, 3724–3729 (2004).
[42] Haemers, S., Koper, G. J. M., and Frens, G., *Biomacromolecules* **4**, 632–640 (2003).
[43] Sever, M. J., Weisser, J. T., Monahan, J., Srinivasan, S., and Wilker, J. J., *Angew. Chem., Int. Ed. Engl*. **43**, 448–450 (2004).
[44] McDowell, L. M., Burzio, L. A., Waite, J. H., and Schaefer, J., *J. Biol. Chem*. **274**, 20293–20295 (1999).
[45] Kitamura, M., Kawakami, K., Nakamura, N., Tsumoto, K., Uchiyama, H., Ueda, Y., Kumagai, I., and Nakaya, T., *J. Polym. Sci. Pol. Chem*. **37**, 729–736 (1999).
[46] Waite, J. H., *J. Mar. Biol. Assoc. U.K*. **65**, 359–372 (1985).
[47] Yu, M. E., Hwang, J. Y., and Deming, T. J., *J. Am. Chem. Soc*. **121**, 5825–5826 (1999).
[48] Burzio, L. A. and Waite, J. H., *Biochemistry* **39**, 11147–11153 (2000).
[49] Monahan, J. and Wilker, J. J., *Langmuir* **20**, 3724–3729 (2004).
[50] Fant, C., Elwing, H., and Hook, F., *Biomacromolecules* **3**, 732–741 (2002).
[51] Sellborn, A., Andersson, M., Fant, C., Gretzer, C., and Elwing, H., *Colloid. Surf., B* **27**, 295–301 (2003).

Downloaded At: 08:44 22 January 2011

[52] Callow, M. E., Callow, J. A., Pickett-Heaps, J. D., and Wetherbee, R., *J. Phycol*. **33**, 938–947 (1997).
[53] Stanley, M. S., Callow, M. E., and Callow, J. A., *Planta* **210**, 61–71 (1999).
[54] Callow, J. A., Crawford, S. A., Higgins, M. J., Mulvaney, P., and Wetherbee, R., *Planta* **211**, 641–647 (2000).
[55] Callow, J. A., Stanley, M. S., Wetherbee, R., and Callow, M. E., *Biofouling* **16**, 141–150 (2000).
[56] Callow, J. A., Osborne, M. P., Callow, M. E., Baker, F., and Donald, A. M., *Colloid Surf., B* **27**, 315–321 (2003).
[57] Callow, M. E., Callow, J. A., Ista, L. K., Coleman, S. E., Nolasco, A. C., and Lopez, G. P., *Appl. Environ. Microbiol*. **66**, 3249–3254 (2000).
[58] Finlay, J. A., Callow, M. E., Schultz, M. P., Swain, G. W., and Callow, J. A., *Biofouling* **18**, 251–256 (2002).
[59] Finlay, J. A., Callow, M. E., Ista, L. K., Lopez, G. P., and Callow, J. A., *Integr. Comp. Biol*. **42**, 1116–1122 (2002).
[60] Berglin, M., Delage, L., Potin, P., Viltner, H., and Elwing, H., *Biomacromolecules* **5**, 2376–2383 (2004).
[61] Darwin, C., *A Monograph of the Sub-class Cirripedia: Balanidae, Verrucidae, etc*. (Ray Society, London, 1854), pp. 110–123.
[62] Otness, J. S. and Medcalf, D. G., *Comp. Biochem. Physiol*. **43B**, 443–449 (1972).
[63] Walker, G., *J. Mar. Biol. Assoc. U.K* **52**, 429–435 (1972).
[64] Yule, A. B. and Walker, G., *Adhesion in Barnacles* (A. A. Balkema, Rotterdam, 1987), pp. 389–402.
[65] Naldrett, M. J. and Kaplan, D. L., *Marine Biol*. **127**, 629–635 (1997).
[66] Swain, G. W., Griffith, J. R., Bultman, J. D., and Vincent, H. L., *Biofouling* **6**, 105–114 (1992).
[67] Swain, G., Anil, A. C., Baier, R. E., Chia, F. S., Conte, E., Cook, A., Hadfield, M., Haslbeck, E., Holm, E., Kavanagh, C., Kohrs, D., Kovach, B., Lee, C., Mazzella, L., Meyer, A. E., Qian, P. Y., Sawant, S. S., Schultz, M., Sigurdsson, J., Smith, C., Soo, L., Terlizzi, A., Wagh, A., Zimmerman, R., and Zupo, V., *Biofouling* **16**, 331–344 (2000).
[68] Yule, A. B. and Walker, G., *J. Mar. Biol. Assoc. U.K*. **64**, 147–156 (1984).
[69] Yule, A. B. and Walker, G., *J. Mar. Biol. Assoc. U.K*. **64**, 429–439 (1984).
[70] Kamino, K., Inoue, K., Maruyama, T., Takamatsu, N., Harayama, S., and Shizuri, Y., *J. Biol. Chem*. **275**, 27360–27365 (2000).
[71] Kamino, K., *Biochem. J*. **356**, 503–507 (2001).

Downloaded At: 08:44 22 January 2011