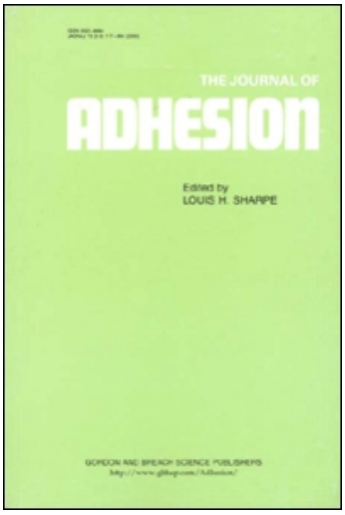

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Interaction of the Adhesive Protein Mefp-1 and Fibrinogen with Methyl and Oligo(Ethylene Glycol)-terminated Self-assembled Monolayers*

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The interaction of fibrinogen and Mefp-1, the adhesive protein of the common blue mussel *Mytilus edulis*, with methyl- and oligo(ethylene oxide) (OEG)-terminated self-assembled monolayers (SAMs) has been investigated by Fourier Transform Infrared Reflection (FT-IRAS) analysis. The measurements on the hydrophobic surfaces show that the underlying SAM is structurally undisturbed when the proteins adhere. Mefp-1 is used as an attachment factor (Cell-Tak™) in cell cultures and in biomedical applications, and it is of interest to determine if OEG-terminated surfaces are inert towards Cell-Tak-mediated cell adhesion. We find that, when Langmuir transfer of a protein film at the liquid/air interface is avoided, the moderately hydrophilic hydroxyhexa (ethylene oxide) and methoxytri(ethylene oxide) undecanethiolate SAMs prepared on Au substrates are protein resistant. The inertness of the OEG-terminated surfaces does not depend on any specific protein present in solution, but rather appears to be a general phenomenon that is independent of the specific structure or chemistry of the macromolecule.

Keywords: Adhesion of proteins on alkanethiol self assembled monolayers (SAM); Oligo (ethylene oxide) terminated SAMs; Fibrinogen; FT-IRRS; Adhesive protein Mefp-1; Protein resistant surfaces

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1. INTRODUCTION

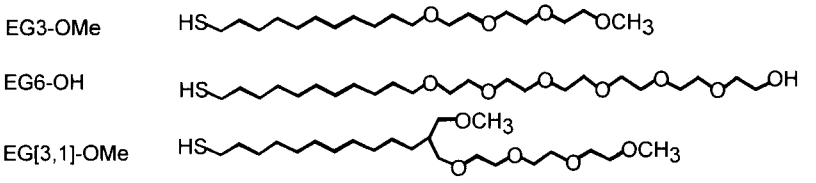
In this paper we present data on two important aspects of the interaction of proteins with artificial organic surfaces: First, we address the nature of interaction of the protein with a hydrophobic organic film and, second, we extend our studies on inert surfaces to proteins other than fibrinogen to probe the resistance towards adsorption and adhesion.

It is well established that large globular proteins adsorb rapidly onto hydrophobic surfaces and that the desorption process is sufficiently slow to make the adsorption kinetically irreversible [2–4]. Maximum coverage, despite the irreversibility of adsorption, usually increases with the concentration in solution because interfacial relaxations determine the surface area per molecule [5, 6]. Little, if any, attention, however, has been given to whether the protein adhering to the surface can induce structural changes in the underlying film and, hence, exert a lateral pressure that might change the mobility and permeability of the organic film substrate. Structural changes could be induced by the attractive forces acting between the protein and the substrate, or simply by entanglement. Here we use a very simple and rigid prototype organic film to investigate the structure of a hydrophobic film, *i.e.*, a self-assembled alkanethiolate monolayer, in direct contact with fibrinogen and Mefp-1.

For the experiments on inert surfaces reported here to be in the proper context of efforts to understand the ability of some organic monolayers to prevent adsorption and adhesion of macromolecules, we must first summarize previous work. A common method for the preparation of an inert surface is to coat it with end-grafted hydrophilic polymer brushes. The resistance of end-grafted poly(ethylene glycol) (PEG), $(—O—CH_2—CH_2)_n$, for example, towards nonspecific protein and cell adsorption is well explained by the "steric repulsion" theory [7, 8] which associates the inertness of the polymer brushes with the high conformational freedom of the PEG chains in the near-surface region. This concept and explanation, based on the statistical mechanics of polymer brushes, is well established. However, as shown by Prime and Whitesides [9] in a series of systematic experiments, the ability to resist protein adsorption is also exhibited by oligo(ethylene glycol) (OEG)-terminated alkanethiol self-assembled

monolayers (SAMs). In some of these films the OEG chain consists of only two EG units which are too short to act as an entropic barrier against adsorption, suggesting that a mechanism other than the steric repulsion effect (involved in end-grafted polymer films) causes the observed inertness of the dense and rigid SAM.

In our previous studies [10] we investigated the mechanism by which SAMs formed from oligo(ethylene oxide) (OEG)-derivatized undecane alkanethiols on Au and Ag surfaces resist adsorption of fibrinogen, which is a typical high molecular weight human plasma protein. Three different OEG termini were tested: a tri(ethylene glycol) with an $-OCH_3$ end group (EG3-OMe), an OH-terminated hexa(ethylene glycol) (EG6-OH) and a tri(ethylene glycol) with a $-CH_2-O-CH_3$ side chain (EG[3,1]-OMe) [10].



These studies of the methoxy tri(ethylene glycol) (EG3-OMe)-terminated alkanethiol monolayers self-assembled on Au and Ag revealed a fundamental aspect of the mechanism of inertness, *i.e.*, that the ability of the SAMs to resist protein adsorption is determined by the molecular conformation of the oligo(ethylene glycol) moieties. Whereas the helical and amorphous conformers[1] of EG3-OMe, assembled on the gold surfaces, are inert towards protein adsorption (Fig. 1), the planar all-*trans* conformers present on the silver substrates are not [10]. Due to the subtle balance between the lattice energy and the electrostatic stabilisation of the helical conformation, the longer chain oligo(ethylene oxides) cannot be compressed into a planar all-*trans* structure [10, 11] and, in the case of EG (3, 1)-OMe, steric

[1]The helical structure of PEG is characteristic for the crystalline state of the polymer. In the helical structure of PEG the sinuousness is 2/7 and the bonds of the backbone are arranged in a *trans*-gauche-*trans* (tgt) order, where the gauche angle is rotated uniformly with respect to the —C—C—O plane over the length of the helix, either clock- or counterclockwise ((+) or (−)). Introduction of gauche rotations other than those characteristic for the uniform helix leads to a gauche defect and, ultimately, to an amorphous conformation in which the sense of rotation is arbitrary.

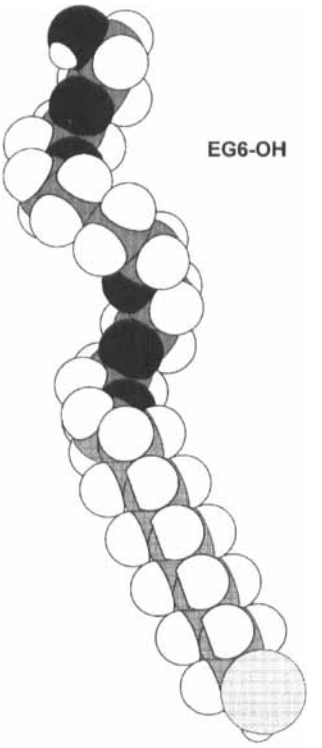


FIGURE 1 Oligo(ethylene glycol)-terminated alkanethiolates form well-ordered monolayers on polycrystalline Au surfaces. The alkyl-tails and the OEG end-groups both try to maximize their intermolecular interaction. The latter prefer a helical structure with an $O—CH_2—CH_2—O$ gauche-*trans*-gauche conformation and the helical axis parallel to the surface normal. The alkyl-tails adopt a planar all-*trans* structure tilted by $\sim 30°$.

constrains prevent ordering into the planar phase on the silver substrates [10].

The difference between the different conformers with respect to their fibrinogen adsorption characteristics was later verified by force/distance measurements between differently derivatized AFM tips and the OEG-terminated SAM surfaces on Au and Ag surfaces [12]. The repulsive interaction between helical and amorphous conformers on gold and a hydrophobic tip is of extremely long range (60 nm) in deionized water, and is reduced to a hard-wall repulsion of about 4–6 nm range with increasing electrolyte concentration consistent with the decrease in Debye length. In these AFM model experiments

both surfaces are electrically neutral, *i.e.*, no electrostatic surface charges are present which could explain the long-range interaction. The long-range repulsion over 60 nm, which is not caused by electrostatic repulsion of point charges, can not be explained by present theories. In the same experiment the interaction of a hydrophobic tip with an end-grafted poly(ethylene oxide) brush was measured [12]. In contrast to the short oligomers, the range of the repulsive forces observed for the thiol-coupled poly(ethylene glycol) of molecular weight 2000 ($n \approx 45$), is of the order of 6 nm (approximately the brush thickness) and is not scaled to the Debye length, emphasizing that a different mechanism is responsible for resistance of protein adsorption to the oligomeric and polymeric surfaces, respectively.

To explain the experimental observation that the helical and amorphous conformers on Au render the surface inert, whereas the planar all-*trans* layer adsorbs protein, Wang *et al.* [1] employed *ab initio* calculations (HF with a 6-31 G* basis set) to simulate the adsorption of water molecules on a cluster of EG3-OMe strands. The structure of the cluster was such that the EG3-OMe strands were oriented perpendicular to the surface, as determined experimentally. It turned out that the cluster of helical EG3-OMe strands, corresponding to the SAM on the Au substrate, easily accommodates water molecules due to the formation of strong hydrogen bonds. In addition, the arrangement of the water molecules directly in contact with the SAM is such that the total electrostatic potential created by these water molecules, together with the SAM itself, is similar to that created by the SAM alone for the first water layer. Hence, the EG3-OMe-terminated SAM on Au can be considered to serve as a template for adsorption of water. In the calculations, the resulting water layer is tightly bound to the helical EG3-OMe surface thereby preventing protein adsorption. By contrast, no water-layer-hindering protein adsorption was found to form on the cluster composed of the all-*trans* planar EG3-OMe strands. This water-template hypothesis is experimentally supported by recent Neutron Reflectivity measurements [13].

In our present study we investigate whether the inertness of the OEG-terminated SAM surfaces is unique to plasma proteins, or if it is a general phenomenon which can also be observed with structurally and chemically different proteins known to form strong adhesive bonds to natural and artificial surfaces. Because of its extraordinary

ability to facilitate strong adhesion to substrate surfaces, we chose to investigate the interaction of an adhesive protein of the common blue mussel (*Mytilus edulis*) with methyl- and OEG-terminated alkanethiolate SAM surfaces.

The foot protein (Mefp-1) of *M. edulis* is a prominent protein in the byssus (a fibrous holdfast structure) and is used by marine mussels for underwater adhesion to hard surfaces. Purified Mefp-1 is a flexible, rod-shaped molecule in solution with a mass of 110 kDa [14, 15]. It is the main component in formulations for cell and tissue attachment (Cell-Tak, BioGlue) because it has excellent adhesive strength, is nontoxic, and does not interfere with cell division and spreading. Mefp-1 consists of more than 50% hydroxy-containing amino acids in its primary structure. Eighty percent of the primary sequence consists of decapeptide repeats that have conserved lysines at positions 2 and 10, hydroxylated prolines at positions 3, 6 and 7 and 3,4-dihydroxyphenylalanines (DOPA) at positions 5 and 9 [16–18]. The ortho-diphenolic DOPA residues are easily oxidized to quinones which then couple to form diDOPA cross-links [19]. Mefp-1 adsorption was studied onto stainless steel [20], pyrolyzed graphite [21], polystyrene and poly(octadecylmethacrylate) [22]. Adsorption, in all cases, is ostensibly Langmuirean, rapid and irreversible. The adhesive strength of bulk Mefp-1 depends on the formation of a protein clot by aggregation, oxidation, and subsequent intra- and intermolecular condensation reactions, or on the high stability of iron ion complexes with the DOPA residues [23].

Fibrinogen has equal amounts of hydroxylated residues, and residues with basic amino-groups and carbonic acids, respectively. Fibrinogen also has about twice the fraction of non-polar residues as Mefp-1 has and exposes different domains to attach to surfaces which are either hydrophilic or hydrophobic [24]. Despite these fundamentaly different primary structures both proteins are very sticky.

2. EXPERIMENTAL

4 mg freeze-dried Mefp-1 from purified Mussel adhesive protein, Sigma A-2707, was mixed with 20 mL PBS buffer (PBS = phosphate

buffered saline, Sigma P4417) at pH 7.0 (adjusted by addition of sodium dihydrogen-phosphate). About half of the protein could be dissolved and the solution was stored at 4°C for two months before the adsorption experiments. The solution at this time was still clear and colorless and did not show signs of quinone tanning.

The surfaces for the protein adsorption studies were $2.5 \times 2.5\,cm^2$ silicon substrates with a thermally-evaporated 10 nm Cr adhesion layer and 200 nm Au film. The samples were immersed for 24 h into 1 mmol solutions of oligo(ethylene glycol)-terminated alkanethiols EG3-OMe, EG6-OH or a 1 mmol perdeuterated hexadecanethiol solution in ethanol. Details of the monolayer preparations and properties have been described elsewhere [10]. An *ex-situ* IR-spectrum of the individual samples was recorded immediately before and after the adsorption experiment. Adsorption experiments were done following two different protocols: 10 mL Mefp-1 solution were either filled into a 50-mL-screw-cap glass vial with the *dry* sample on the bottom, or into a glass vial containing the sample and just enough water (~ 5 mL) to cover the SAM surface (*prehydrated* sample). The latter recipe avoids an LB-like transfer of protein onto the dry sample surface at the cost of a dilution of the protein. After 30 min in the protein solution the non-prehydrated samples were taken out of the protein solution and immediately rinsed with ~ 200 mL of deionized water. The prehydrated samples were again protected against an LB-like transfer of Mefp-1 from the surface of the aqueous solution onto the substrate by further dilution and exchange of the protein solution with deionized water before the sample removal.

Fibrinogen (F-4883, 94% clottability) was purchased from Sigma and filtered through 0.22 μm filter units. The adsorption of fibrinogen on prehydrated OEG-terminated monolayers has already been reported [9, 10] and we discuss only the results for adsorption on dry films for comparison with the Mefp-1 adsorption.

The FT- IRRAS Spectra were recorded with a dry-air-purged BIO-RAD spectrometer (model FTS 175c), equipped with a liquid nitrogen cooled MCT detector, a polarizer, and an accessory for grazing reflectance spectroscopy. Spectra were taken with a resolution of $4\,cm^{-1}$ and reproduced on several independent substrates.

3. RESULTS AND DISCUSSION

3.1. Adsorption of Mefp-1 and Fibrinogen on Perdeuterated Alkanethiolate SAMs

We describe first the adsorption of fibrinogen and Mefp-1 on methyl-terminated hexadecanethiolate monolayers on Au substrates to calibrate relative coverages and to elucidate if the two different proteins induce conformational changes in the substrate SAM.

Figure 2 shows the difference spectra (with respect to the clean SAM surface) obtained from a perdeuterated hexadecanethiolate monolayer (not pre-hydrated) before and after a 30 min immersion time in Mefp-1 (0.1 mg/mL) and fibrinogen (0.5 mg/mL) solution, respectively. Perdeuterated alkanethiolates have the same hydrophobicity and exhibit protein adsorption properties identical to protonated alkanethiolates. However, in FT-IRAS experiments the frequency shift of

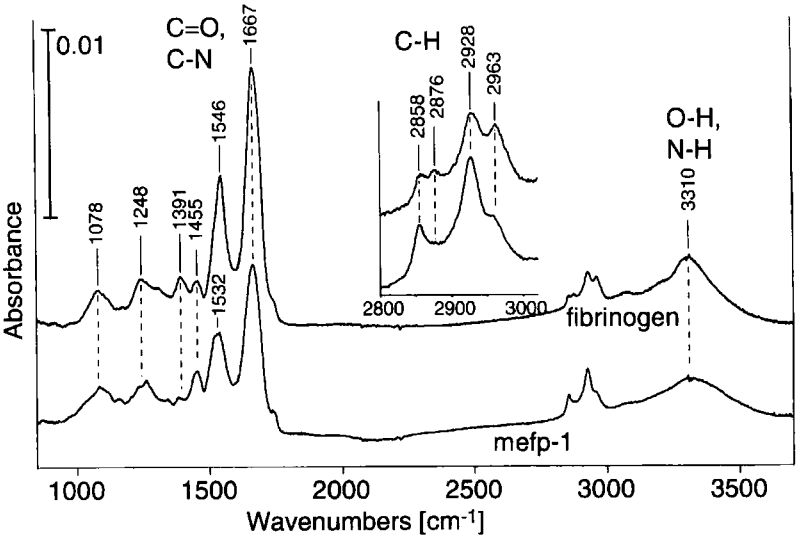


FIGURE 2 FTIR spectra of adsorbed Mefp-1 fibrinogen on a perdeuterated hexadecanethiolate d_{33} monolayer on Au. Both protein spectra are dominated by the NH, CO and CN stretching modes of the polyamide backbone. The very different Mefp-1 and fibrinogen amino-acid sequence affects only second tier absorption bands like the 1391 cm^{-1} carboxylate stretch that is nearly absent in the Mefp-1 spectrum with a low amount of carbonic acid residues.

the methylene stretching modes from 2800 – 3000 cm^{-1} (CH_2, CH_3) to 2050 – 2250 cm^{-1} (CD_2, CD_3) allows separation of the vibrational C-D stretching modes of the deuterated alkanethiolate from the methylene vibrations of the adsorbed protein. The fibrinogen and Mefp-1 spectra are both dominated by the absorption bands of the polypeptide backbone's amide I and amide II vibrations. The amide I band at 1667 cm^{-1} is mainly associated with the stretching vibration of the C=O bond, while amide II, found at 1532 cm^{-1} (Mefp-1) and 1546 cm^{-1} (fibrinogen), respectively, is associated with coupled C—N stretching and N—H bending vibrations of the peptide groups. The amide III bands are around 1248 cm^{-1}; the other vibrational bands can not be unambiguously identified in the proteins. The different absorption maxima for fibrinogen and Mefp-1 are caused by the different intensities of the overlapping aromatic ring stretch band at $\sim$1515 cm^{-1} from aromatic residues. 20% of the Mefp-1 amino-acid sequence are aromatic residues (tyrosine, DOPA), whereas fibrinogen has less than 7% tyrosine and phenylalanine residues. A symmetric carboxylate stretch at 1390 cm^{-1} is observed only in the fibrinogen spectrum, but not in the spectrum of Mefp-1 which contains no residues with carboxy sidechains.

The similar position and halfwidth (68 and 61 cm^{-1} for Mefp-1 and fibrinogen, respectively) of the amide I band indicates that the integrated intensity per mole residue is the same for Mefp-1 and fibrinogen. The average molecular weight per residue is 122 Da in the Mefp-1 decapeptide repeat sequence [17] and 115 Da per residue for human fibrinogen [14] with a MW of 340,000 Da. We, therefore, use the same amide-I-band-area calibration for the Mefp-1 coverages in ng protein per mm^2 as for the fibrinogen coverages. The latter have been determined for methyl-terminated-surfaces as a function of fibrinogen concentration with surface plasmon resonance [4] and calibrated with radiolabeled proteins [24].

The surface coverages as calculated from Figure 2 are 6 ng/mm^2 for fibrinogen (0.5 mg/mL PBS) and 2.8 ng/mm^2 for Mefp-1. The higher surface coverage for fibrinogen agrees with the higher molecular weight (340 kDa *versus* 120 kDa for Mefp-1) and the higher concentration in solution. The fibrinogen surface coverage of 6 ng/mm^2 is more than a monolayer (3.5 ng/mm^2) and indicates multilayer adsorption.

Protein adsorption on non-charged alkanethiolate surfaces is explained by a hydrophobic effect. However, not much is known about the interaction between protein and organic monolayers on a molecular level, *e.g.*, if fibrinogen and/or Mefp-1 entangle with the alkanethiolate chains, replace them on the surface, or adsorb without perturbing the underlying organic film. The two former possibilities could cause a rearrangement in the film and disorder of the alkanethiolate chains and should be observable with infrared spectroscopy. In Figure 3 the difference spectra of a perdeuterated alkanethiolate monolayer before and after Mefp-1 and fibrinogen adsorption are displayed. They reveal that protein adsorption mainly reduces the band intensities of the CD_3-end-group and slightly reduces the CD_2-stretching-bands. Reduced band intensities can be due to an orientational change or simply result from the lower absorption of a methyl group at the thiolate-protein-interface in comparison with the thiolate-air-interface.

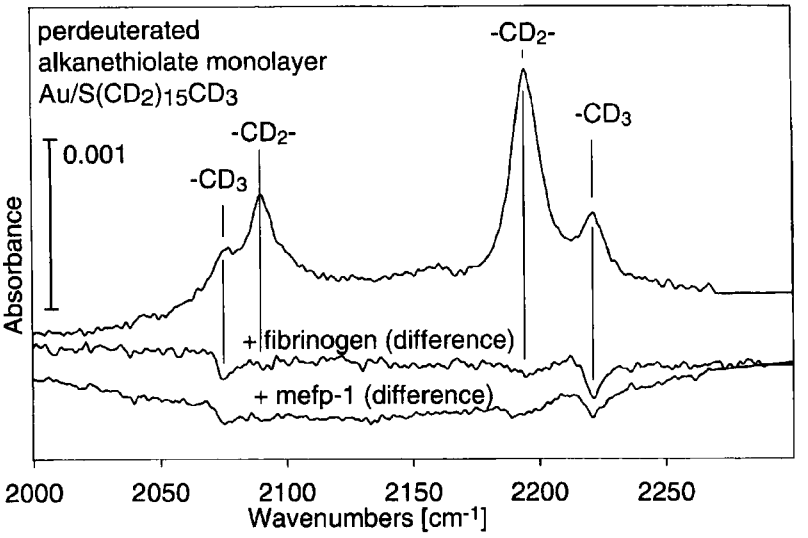


FIGURE 3 Changes in the spectra of the perdeuterated alkanethiolate monolayer after Mefp-1 and fibrinogen adsorption. The protein overlayer reduces both absorption bands of the outer methyl (—CD_3) groups. The unchanged intensity of the inner 15 methylene (CD_2) indicates that the protein adsorbs on top of the monolayer but does not entangle between the perdeuterated alkyl tails. If the latter would reorient from their alignment close to parallel to the surface plane, their intensity would increase according to the cosine square dependency of the FT-IRAS method.

In FT-IRAS experiments, the band intensities are proportional to the square of the cosine of the normal components (with respect to the surface plane) of the dynamic dipole moments. Vibrations strictly parallel to the surface give no contribution to the spectrum. The molecular axes of long-chain alkanethiolates on Au are tilted by $\sim 30° - 35°$ from the surface normal, so that the 15 CD_2-groups of the perdeuterated hexadecane thiolate are oriented nearly parallel to the surface plane and their symmetric (in-phase) and antisymmetric (out-of-phase) stretching bands are relatively weak. The terminal CD_3-group has an orientation perpendicular to the surface plane and the symmetric CD_3-stretching band at 2075 cm^{-1}, with a weaker intrinsic intensity, is as strong as the two-fold degenerated antisymmetric mode with higher intrinsic intensity. Disorder in the alkanethiolate chains would increase the average tilt angle, and we would expect an increase of the CD_2-band intensities, a decrease of the symmetric CD_3-stretching mode and an increase of the anti-symmetric CD_3-mode. However, the presence of a fibrinogen or Mefp-1 overlayer reduces both CD_3-modes simultaneously (see above) and has basically no effect on the CD_2-mode intensities. The *simultaneously* reduced intensity for the outermost CD_3-groups, therefore, must be caused by changes of the local field strength due to the higher refractive index of the protein in going from an alkanethiolate-air to an alkanethiolate-protein interface, whereas the CD_2-groups within the film are basically unaffected by the protein overlayer.

In summary, the difference spectra of the perdeuterated monolayer reveal no differences between fibrinogen and Mefp-1 adsorption, and both protein overlayers do not affect the molecular conformation or order of the underlying alkyl tails. Hence, adsorption and adhesion is due to unspecific attractive van der Waals interactions between the protein and the alkanethiolate gold substrate.

3.2. Exposure of Mefp-1 to EG6-OH and EG3-OMe Terminated SAMs

Hydroxy-hexa(ethylene oxide) (EG6-OH)-terminated undecane thiolate SAMs are hydrophilic (advancing water contact angle ca. 35°) monolayers with helical and amorphous conformations of the hydroxy-terminated hexa(ethylene glycol) unit. EG6-OH monolayers

were shown to be resistant to fibrinogen adsorption even when the packing density was diluted by short-chain unfunctionalized alkanethiols [9]. However, Langmuir transfer onto the surface during inserting or removing the sample from solution can obscure the results. During our efforts to develop an experimental protocol in which only adsorption of the dissolved macromolecule is possible, we found that a noticable effect is observed depending on whether the SAMs are dry or prehydrated in PBS buffer. Figure 4 shows an experiment where no precautions were taken to avoid Langmuir transfer of Mefp-1 onto the SAM surface and the absorbed amount of Mefp-1 was about $0.2 \pm 0.05\,ng/mm^2$, as calculated from the amide I band height. The difference spectrum shows not only positive amide bands, but also a decrease of all the infrared absorption bands of the hexa(ethylene glycol) groups, *e.g.*, the C—O—C stretching mode at 1114 and the CH_2-wagging mode at $1348\,cm^{-1}$. The only band which is not suppressed is the antisymmetric methylene stretching mode of the alkyl chains at $2921\,cm^{-1}$ (the symmetric stretching mode at

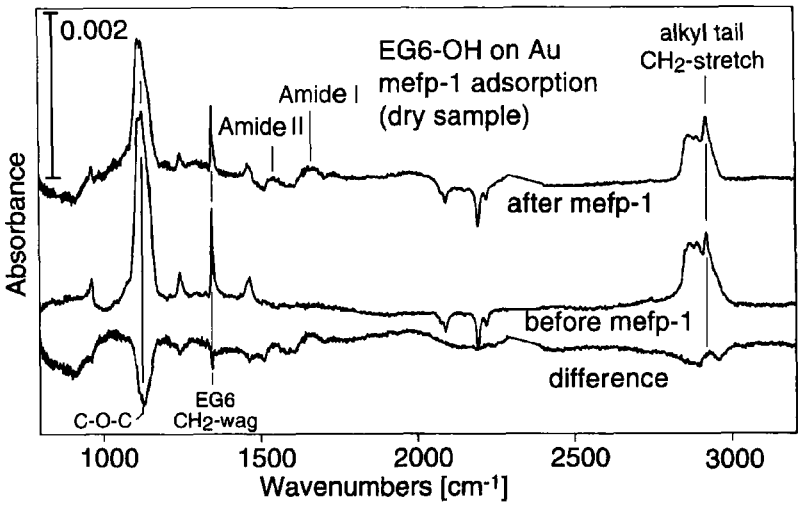


FIGURE 4 A small amount of Mefp-1 irreversibly adheres to the EG6-OH surface when the sample is immersed directly into or withdrawn from the protein solution (see text). The protein adsorption can be detected by the amide I and amide II bands at 1667 and $1532\,cm^{-1}$. The protein overlayer buries the hexa(ethylene glycol) units underneath and the transfer from the film-air-to the monolayer-protein-interface reduces the intensity of the COC-stretching modes at $1130\,cm^{-1}$.

$2850\,cm^{-1}$ is hidden under the strong methylene stretching modes of the ethylene glycol units). The reduced intensity of the infrared bands of the outmost OEG modes indicates the presence of adsorbed protein as discussed for the alkanethiolate films. The effect of the dielectric properties of the protein, however, vanishes over a short distance since the Mefp-1 adsorption has no effect on the infrared intensities of the alkyl tails, which are beneath the hexa(ethylene glycol) end group.

In the experiments shown in Figure 5 the sample was prehydrated and an LB-like transfer was avoided. Clearly, the EG6-OH film is resistant against Mefp-1 adsorption from solution when immersion into and withdrawal from the adsorption solution avoided transfer through the air-Mefp-1-solution-interface. The difference spectrum shows that the vibrational bands associated with hexa(ethylene glycol) did not change by immersion in the Mefp-1 solution, further eliminating the possibility of an Mefp-1 film on the surface.

The methoxy-terminated EG3-OMe SAM on Au with only three ethylene glycol units forms monolayers with a more disordered helical conformation of the end group as compared with the EG6-OH

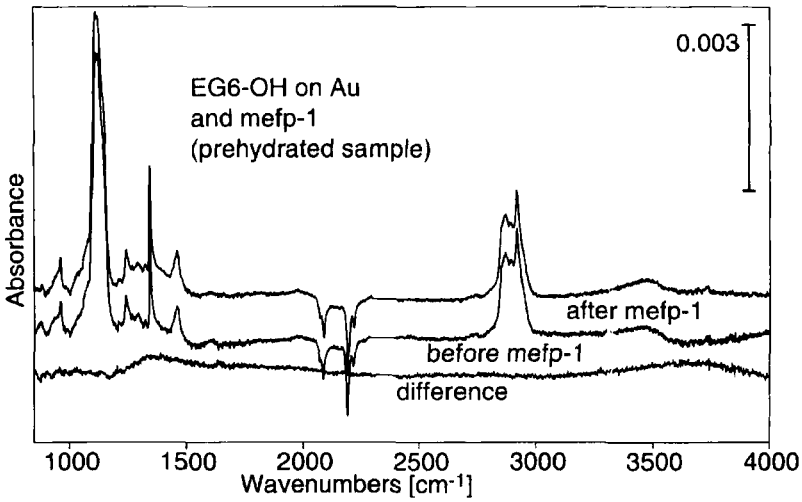


FIGURE 5 EG6-OH monolayers show no Mefp-1 adsorption after 30 min in a 0.1 mg/mL solution. The irreversible adhesion of dried-up protein when the solution de wets from the monolayer surface can be avoided by prehydration of the sample before addition of the protein and exchange of the aqueous solution before sample removal.

alkanethiolates with six ethylene glycol units [10]. The contact angle of water is 63°, and the helical and amorphous films on Au are fibrinogen-resistant as shown before [10]. The same observation as with the EG6-OH film was made with EG3-OMe SAMs, that a protein film is transferred onto the SAM if no precautions are taken (see Tab. I, spectra not shown). Mefp-1 adsorbed onto the dry EG3-OMe monolayers during insertion, or dried-up Mefp-1 adhering to the surface after removal from the aqueous solution, can no longer be washed from the surface with PBS buffer or deionized water.

As with the experiments described above for the EG6-OH terminated SAMs, adsorption of Mefp-1 on a prehydrated EG3-OMe sample on Au was significantly reduced. The small amide bands detected (see *e.g.*, Fig. 6, in this particular case corresponding to 0.05 ng/mm^2) were not due to a homogeneous adsorbate layer, but to patches of protein that dried up on the surface during removal from the solution. This specific sample also exhibits a contamination caused by some organic material with carboxylic ester groups (band at 1750 cm^{-1}) which might be responsible for the adsorbed protein, and it demonstrates the variability in the results when extreme precautions are not taken to avoid LB transfer or contamination.

On Ag, the higher packing density forces the EG3-OMe into a planar conformation that shows no protein resistance [10, 11]. In

TABLE I Mefp-1 and fibrinogen adsorption on dry and prehydrated alkanethiolate monolayers

		Mefp-1	*Fibrinogen (0.5 mg/mL)*
	Concentration	*Adsorbed amount*	*Adsorbed amount*
EG3-OMe on Au, dry sample	0.1 mg/mL	0.15 ng/mm^2	0.3 ng/mm^2
EG3-OMe on Au, prehydrated sample	0.05 mg/mL	0.05 ng/mm^2	0.0 ng/mm^2
EG3-OMe on Ag, dry sample	0.1 mg/mL	1.0 ng/mm^2	1.0 ng/mm^2
EG6-OH on Au, dry sample	0.1 mg/mL	0.2 ng/mm^2	0.0 ng/mm^2
EG6-OH on Au, prehydrated sample	0.07 mg/mL	0.0 ng/mm^2	0.0 ng/mm^2
pd alkanethiolate, dry sample	0.1 mg/mL	2.8 ng/mm^2	6.0 ng/mm2

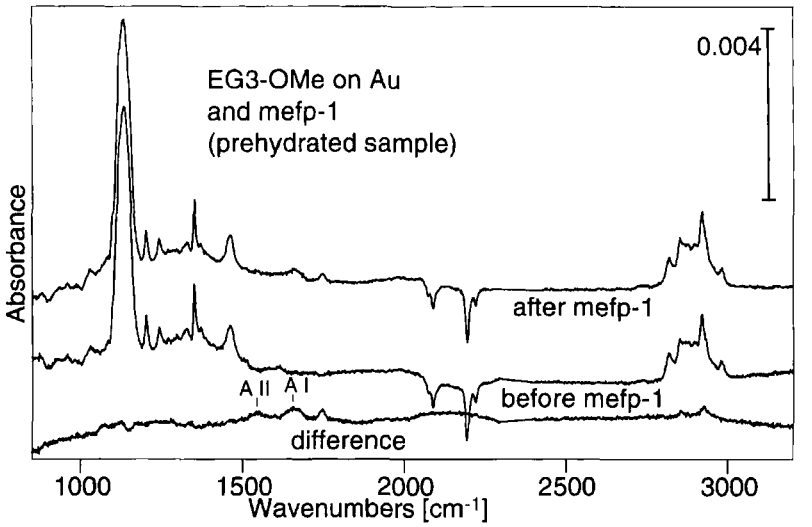


FIGURE 6 Prehydrated EG3-OMe samples show weak protein bands after 30 min in the aqueous Mefp-1 solution. This specific sample exhibits a contamination caused by some organic material with carbonic ester groups (band at 1750 cm^{-1}) which might be responsible for the adsorbed protein.

the present studies, EG3-OMe on Ag adsorbed significantly higher amounts of both Mefp-1 and fibrinogen than the helical films on Au [25]. In Table I we compiled the results for Mepf-1 adsorption on the dry and prehydrated EG3-OMe and EG6-OH films on Au and Ag substrates. Obviously, prehydration reduces protein adsorption and, in the case of EG6-OH films, prevents adsorption.

Our measurements, at least on the EG6-OH surfaces, confirm that the resistance of the OEG-terminated rigid SAMs is independent of the specific macromolecules in solution. As with the non-specific hydrophobic attractive interactions, there obviously is a nonspecific repulsive interaction which does not depend on the nature of the macromolecule. As discussed in Ref. [1], we propose that this repulsive force is related to the way water can interact with the different conformers present in the OEG termini in the organic monolayers, since it does not depend on the terminal group in the OEG SAMs (— O—CH_3 or —OH) nor on the nature of the macromolecule in solution.

4. SUMMARY

Fibrinogen or Mepf-1 adhering to a hydrophobic, methyl-terminated SAM do not induce any structural changes in the alkanethiolate film, confirming a purely non-specific van der Waals interaction between protein and substrate. Adsorption of Mefp-1 resulted in the same 1–2 layer thick protein films as was observed for most other proteins, including fibrinogen. The OEG-terminated SAMs were found to be resistant to both fibrinogen and Mefp-1 adsorption from solution. Care had to be taken in these experiments to avoid transfer of a protein film onto the surface during dipping or withdrawing the sample through the liquid/air interface. Hydroxy-hexa(ethylene glycol)-terminated monolayers adsorbed no Mefp-1 from solution, but irreversibly bound the protein if it came in contact with the dry surface upon immersion into or withdrawing from the protein solution. Hydrated EG3-OMe films on Au are probably resistant, also, but the experimental results are not unambiguous and further experiments are necessary to prove that the small amount of adsorbed Mefp-1 was caused by contamination. When we compare the adsorption properties of Mefp-1 with those of fibrinogen, we conclude that Mefp-1 (at least in the absence of iron) shows similar adsorption properties.

The inertness of OEG-terminated SAMs seems to be both independent of the terminal function of the OEG units (as confirmed in Ref. [10]) and the chemical composition and structure of the macromolecule. Inertness depends only on the conformation of the OEG moieties in the SAM and, hence, the static and dynamic electric fields in the interphase generated by the molecular conformers, suggesting that the phenomena of rigid inert organic surfaces can be explained by a general physical model rather than by empirical relationships based on wettability measurements.

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