Prevention of Protein Adsorption by Tethered Poly(ethylene oxide) Layers: Experiments and Single-Chain Mean-Field Analysis

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Prevention of protein adsorption by the surface-grafted poly(ethylene oxide) (PEO) chains has been well-known. We have examined the mechanisms of how the grafted PEO prevents protein adsorption. PEO−poly(propylene oxide)−PEO (PEO−PPO−PEO) triblock copolymers were used to graft PEO to the trichlorovinylsilane (TCVS)-modified glass by γ-irradiation. The surface density of the PEO chains was varied up to 60 pmol/cm^2 and the number of the ethylene oxide (EO) units of the PEO segment was varied from 75 to 128. The adsorption of lysozyme and fibrinogen to the PEO-grafted glass was examined using radiolabeled proteins. The surface protein concentration decreased as the surface density of the grafted PEO increased, but surface protein concentration never reached zero. The experimental data were compared with the predictions by the single-chain mean-field theory. There was very good agreement between the predictions of the theory and the experimental observations. It was found that the mechanism for prevention of protein adsorption by the grafted PEO chains in the hydrophobic surfaces was due to the blocking by the PEO segments of the adsorbing sites of the proteins. The mechanism of the grafted chains to prevent protein adsorption was shown to depend upon the interactions of the surface with the segments of the grafted polymers. Surfaces that did not attract the polymer segments present effective kinetic barriers but were not very good for equilibrium prevention. On the other hand, hydrophobic surfaces, such as the ones used in the experimental work, were very effective for reducing the equilibrium amount of proteins adsorbed. It was found that the most important parameter in preventing protein adsorption by grafted polymers is the surface density of the grafted polymer. The polymer molecular weight, or the chain length, was found to have a weak effect.

Introduction

Surface-induced thrombosis, which is one of the major problems in the clinical application of blood-contacting materials, begins with adsorption of plasma proteins to the surface.[1,2] Preventing protein adsorption should thus improve biocompatibility. It is known that protein adsorption may be significantly decreased by surface modification with hydrophilic polymers, such as poly(ethylene oxide) (PEO).[3−9] It has been generally thought that the prevention of protein adsorption is due to the steric repulsion by the surface-grafted (or tethered) PEO chains.[10,11] The steric repulsion picture emerged from the view that the grafted polymer layer forms a brush on the surface. However, the usual grafting densities and molecular weights of the PEO used for improved biocompatibility are such that the polymer layer is not in the so-called "brush" regime.[12,13] In most cases the surfaces of interest are hydrophobic, and therefore, one should expect the PEO to be attracted to the surface.

Although the ability of grafted PEO chains to reduce protein adsorption has been observed frequently, its theoretical analysis has been difficult for a few reasons. First of all, theoretical analysis on the behavior of the tethered PEO chains requires the information on the surface density of the grafted PEO chains. In most of the

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published studies, however, the information on the amount of the grafted PEO is not available. This is mainly due to the difficulties in measuring the amount of the grafted PEO chains. Second, the surface densities and molecular weights of PEO used in experiments require the use of molecular approaches, since the analytical "brush" theories[14,15] are for a regime of surface coverages and polymer molecular weight that is well beyond the range of those used in experimental studies. Recently, the single-chain mean-field (SCMF) theory has been used successfully to analyze the systems with relatively short PEO chain length ($n \leq 200$) and with wide range of the densities of the tethered PEO chains.[12,13,16,17]

The objective of this study was to determine the effect of grafted PEO chain length and surface chain density on protein adsorption. The combination of experimental and theoretical approaches enables us to obtain a microscopic understanding of the ability of PEO to prevent protein adsorption on hydrophobic surfaces. Fibrinogen and lysozyme adsorption profiles were measured using radiolabeled proteins. Fluorescence studies were performed to probe the behavior of PEO-containing polymer molecules in solution, since it affects polymer adsorption. To prepare a monolayer of PEO chains on the surface with different densities, Pluronic surfactants were used in this study. They are triblock, amphiphilic copolymers of the general formula (ethylene oxide)$_n$−(propylene oxide)$_m$−(ethylene oxide)$_n$, where the subscripts indicate the number of repeating units. Such triblock copolymers readily adsorb and form a self-assembled monolayer on hydrophobic surfaces via the hydrophobic poly(propylene oxide) (PPO) segment with the PEO segments thought to be extended into the aqueous medium[18,19] (see the Discussion section). The grafting of Pluronics and other PEO-containing hydrophilic−hydrophobic block copolymers is known to be similar to the end grafting of PEO chains to the substrate.[20,21] Since adsorption of block copolymer surfactants to hydrophobic surfaces occurs mainly by individual molecules through its hydrophobic segment,[22−24] they have been used widely in modifying various surfaces.[8,25−27] Pluronic surfactants are available with a variety of PEO block lengths, facilitating the surface modification with varying PEO chain lengths. The PEO chain length was varied from 75 to 128, and the surface density of the adsorbed PEO chains was determined using the radiolabeled PEO chains.

The next section describes the single-chain mean-field (SCMF) theory as applied to the study of protein adsorption on surfaces with grafted polymers. Experimental methodology, experimental observations, and their comparison with the theoretical predictions are presented in later sections.

Theoretical Approach

The theoretical approach that we applied to predict the adsorption of proteins in the presence of grafted polymers is the SCMF theory as recently generalized[17] for these type of systems. The basic idea of the theory is to look at the chain molecules (both proteins and polymers) with their intramolecular and surface interactions "exactly" taken into account, and the intermolecular interactions are modeled within a mean-field approximation. This approach enables the incorporation of detailed conformational and chemical structure of the molecules through the exact treatment of the single-chain conformations. At the same time, this approach enables systematic studies because of the incorporation of the intermolecular interactions within a mean-field approximation. The theory has been shown to provide quantitative agreement with full-scale computer simulations[12,13,28] and with experimental observations[12,13,16,29] for conformational and thermodynamic properties of tethered polymer layers. For example, a recent study showed the ability of the theory to quantitatively predict the pressure−area isotherms of PEO−polystyrene diblock copolymers at the water−air interface.[16] The excellent agreement with the experimental observations shows that the theory is very suitable for the modeling of the structure and interactions of grafted PEO layers. A detailed derivation of the theory as applied to protein adsorption on grafted polymer layers can be found elsewhere.[17] Here, we present a short derivation that highlights the main features of the theory.

Single-Chain Mean-Field Theory. We consider a surface of total area A with N_g polymer molecules grafted at one of their ends to the surface in equilibrium with a solution containing protein molecules. The solution is characterized by solvent molecules with chemical potential μ_s and by protein molecules with chemical potential μ_p. We assume that the protein and the polymer molecules are both soluble in the solvent, i.e., we are considering the good solvent regime. Thus, the interactions determining the behavior of the system are the effective (hard-core) repulsion between solvent and protein, solvent and solvent, protein and protein, polymer and solvent, and polymer and protein. In addition, we have to consider the bare surface−protein, ϵ_{sp}, and the bare surface−grafted polymer, ϵ_{sg}, interactions.

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The repulsive interactions are taken into account through packing constraints. Namely, the volume available to the molecules at each interval between z and $z + dz$ from the surface must be filled by polymer segments, protein, or solvent molecules. This can be expressed as

$$\sigma\langle v_g(z)\rangle + \int \rho_p(z')\langle v_p(z)\rangle_{z'}\, dz' + \phi_s(z) = 1 \quad 0 \le z \le \infty \tag{1}$$

with $\sigma = N_g/A$ being the surface coverage of grafted polymer. $\langle v_g(z)\rangle\, dz$ is the average volume per grafted chain at z; $\rho_p(z') = N_p(z')/A$ is the density of protein at z', the location of the protein molecules is defined by the point of closest distance from the surface. Thus, $\rho_p(0)$ is the density of adsorbed proteins. $\langle v_p(z)\rangle_{z'}\, dz$ is the average volume that the proteins at z' have at z, and $\phi_s(z)$ is the volume fraction of solvent at z. The $\langle\,\rangle$'s denote ensemble averages, and they are calculated over the probability distribution function (PDF) of chain conformations of the grafted polymer $P_g(\alpha)$ and of the protein $P_p(\gamma_{z'})$. Thus, $\langle v_g(z)\rangle\, dz = \sum_{\{\alpha\}} P_g(\alpha) v_g(z;\alpha)\, dz$ where the sum runs over all possible conformations α, and $v_g(z;\alpha)\, dz$ is the volume that a grafted polymer in conformation α occupies at distance z from the surface. For the proteins, $\langle v_p(z)\rangle_{z'}\, dz = \int P_p(\gamma_{z'}) v_p(z;\gamma_{z'})\, dz\, dz'$ is the volume that proteins at all z' contribute to z. The protein's PDF is normalized for each z', and $\gamma_{z'}$ denotes the set of possible protein configurations at z'.

The interactions between the polymer chains and the surface as well as the protein molecules and the surface have two contributions. One is the bare attractive interactions with the surface. For the PEO this contribution is modeled as $\epsilon_{att,gs}(\alpha) = \chi_{att,gs} n_g(z \le \delta; \alpha)$ where $\chi_{att,gs}$ is the strength of the attraction and $n_g(z \le \delta; \alpha)$ is the number of polymer segments in conformation α that are adsorbed to the surface, i.e., at a distance smaller than δ from the surface. For the protein one can use atomistic derived interactions[30] or use the strength of the attraction to determine the amount of protein adsorbed in the absence of the grafted polymer (see next section).

The other contribution is the repulsive interaction arising from the fact that the polymers used are triblock copolymers. The PPO block attached to the surface exerts a repulsive interaction to the PEO and also to the protein molecules. One evidence for these repulsive interactions between the PEO and PPO is that the Pluronic molecules form micelles in solution. Therefore, as the coverage of grafted polymer on the surface increases, one needs to include effective surface−polymer and surface−protein repulsions that are proportional to the surface coverage of grafted polymers. It is reasonable to assume that the only effect of the PPO is the effective repulsion with the PEO and the proteins. This is due to the high hydrophobicity of the PPO, and therefore, it is assumed that it has all its segments grafted on the surface.

The total polymer−surface interaction is now $\epsilon_{gs}(\alpha) = \epsilon_{att,gs}(\alpha) + \chi_{rep,gs}\sigma n_g(z \le \delta; \alpha)$ where $\chi_{rep,gs}$ is the strength of the PEO−surface repulsion induced by the presence of the PPO. For the protein−surface interaction we have $\epsilon_{ps}(\gamma_z) = \epsilon_{att,ps}(\gamma_z) + \chi_{rep,ps}\sigma v_p(z \le \delta; \gamma_z)$ with $\epsilon_{att,ps}$ denoting the bare protein−surface attraction and $\chi_{rep,ps}$ is the strength of the protein−surface PPO-induced repulsion.

The next step is to determine the PDF of chain conformations of grafted polymer and proteins, the density profile of the proteins, $\rho_p(z)$, and the density profile of solvent molecules $\phi_s(z)$. They are found by minimizing the systems free energy subject to the packing constraints (eq 1). The ensemble that we need to consider is that of a surface of area A with N_g grafted polymers in contact with a bath of protein and solvent molecules with chemical potentials μ_ρ and μ_s, respectively. The relevant free energy density, i.e., free energy per unit area, is given by

$$\beta\frac{W}{A} = \sigma\sum_{\{a\}} P_g(\alpha)[\ln P_g(\alpha) + \beta\epsilon_{gs}(\alpha)] + \int_0^\infty \rho_p(z) \times [\ln \rho_p(z) - \beta\mu_p + \sum_{\gamma_z} P_p(\gamma_z)[\ln P_p(\gamma_z) + \beta\epsilon_{ps}(\gamma_z)]]\, dz + \int_0^\infty \phi_s(z)[\ln \phi_s(z) - \beta\mu_s]\, dz \tag{2}$$

where $\beta = 1/(k_B T)$ is the inverse of the absolute temperature multiplied by the Boltzmann constant.

The minimization of the free-energy density, eq 2, subject to the packing constraint, eq 1, is carried out by introducing the Lagrange multipliers, $\beta\pi(z)$, conjugated to the packing constraints to give for the PDF of the grafted chains

$$P_g(\alpha) = \frac{1}{q_g}\exp[-\beta\epsilon_{gs}(\alpha) - \int \beta\pi(z) v_g(\alpha; z)\, dz] \tag{3}$$

where q_g is the normalization constant. The two terms in the exponential correspond to the surface−polymer interaction and to the intermolecular repulsive interactions associated with the packing constraints.

For the protein molecules we find

$$P_p(\gamma_z) = \frac{1}{q_p(z)}\exp[-\beta\epsilon_{ps}(\gamma_z) - \beta\int \pi(z') v_p(z'; \gamma_z)\, dz'] \tag{4}$$

where the distribution is normalized for each z. The two terms of the exponential correspond to the protein−surface interaction and the intermolecular repulsion, respectively. The density profile of proteins is given by

$$\rho_p(z) = q_p(z)\exp[\beta(\mu_p - 1)] \tag{5}$$

and that of the solvent molecules is

$$\phi_s(z) = \exp[-\beta(\pi(z) v_s - \mu_s)] \tag{6}$$

The PDF's and density profiles are all determined by the knowledge of the Lagrange multipliers $\pi(z)$. These quantities represent the average repulsion arising from the neighboring molecules. They are related to the solvent (lateral) osmotic pressures arising from the thermodynamic condition that the chemical potential of the solvent molecules be constant at all z. A thorough explanation of the physical meaning of the lateral pressures and their origin can be found in refs 12, 13, and 17.

The distribution of proteins $\rho_p(z)$ from the surface to the bulk solution is determined by the thermodynamic condition of constant chemical potential of the protein molecules throughout the system. The factors that will change the amount of protein adsorbed (and the whole distribution) can be understood by looking at eq 5 in the form

$$\beta\mu_p = \ln\frac{\rho_p(z)}{q_p(z)} + 1$$

with

$$q_p(z) = \sum_{\gamma_z} \exp[-\beta(\epsilon_{ps}(\gamma_z) + \int \pi(z') v_p(z'; \gamma_z)\, dz')] \tag{7}$$

Therefore, to keep μ_p constant at its bulk value, the density

of the proteins adsorbed, $\rho_p(0)$, will decrease if the partition function, $q_p(0)$, decreases. Thus, in order to design the proper polymer layer to prevent protein adsorption, we have to look at the factors that will decrease the value of the partition function of the proteins in contact with the surface. The partition function is determined by two contributions: (i) the protein−surface interaction and (ii) the intermolecular repulsion of the protein with the grafted polymers and the other adsorbed proteins. As the density of grafted polymer increases on the surface, the repulsion increases, $\pi(z)$ increases, and the partition function decreases resulting also in a lower adsorption of the protein. We will see the quantitative effects in the following section.

The lateral pressures $\pi(z)$ are determined by inserting the PDF of grafted polymers (eq 3), the pdf of the protein molecules (eq 4), the density profile of the protein (eq 5), and the solvent density profile (eq 6), into the constraint equations, eq 1. The resulting equations for the lateral pressures are solved by discretizing space into layers parallel to the surface. The resulting set of nonlinear coupled equations (see eq 12 of ref 17) is solved by standard numerical methods. The inputs necessary to solve the equations are (i) the set of single-chain conformations of the grafted polymers, (ii) the set of single-chain conformations of the protein molecules, (iii) the surface coverage of polymer, (iv) the chemical potentials of the solvent and protein molecules, (v) the bare surface−protein and surface−polymer attractions; and (vi) the strength of the PPO−PEO and PPO−protein repulsions.

For the conformations of the grafted chains we use the rotational isomeric state model as explained in detail in refs 12, 13, and 17. The choice of this model for the PEO chains is based on the excellent quantitative agreement of the predictions of the SCMF theory with experimentally measured pressure−area isotherms for these polymer chains.[16,31]

Parameters Used in the SCMF Analysis of Protein Adsorption on PEO-Grafted Surface. Our study has concentrated on two types of proteins: lysozyme and fibrinogen. For lysozyme we consider the protein in the bulk solution to be spherical. It is assumed that the protein is compact in solution. The radius of the protein is taken to be $r_l = 15.5$ Å. Upon contact with the surface we assume two possible configurations of the lysozyme: spherical and pancake-shape. This is based on recent experimental observations that suggest that adsorbed lysozyme has a pancake configuration.[32] Thus, we will consider the pancake configuration to have a stronger adsorption energy with the surface than the spherical configuration. The interaction between the bare surface and the protein can be taken from the atomistic calculations of Lee and Park.[30] However, those calculations were carried out for a surface that is different from the one used in the experimental work presented below. Furthermore, they did not consider the possibility of conformational changes when the protein sits at the surface. Therefore, we used the same dependence of the potential as calculated by Lee and Park, but the strength of the interaction was fitted to obtain reasonable agreement with the experiments for the amount of protein adsorbed in the absence of grafted polymer. We have found that a good value is $\epsilon_{ps,sphere}(0) = -18.75$ kJ/mol and $\epsilon_{ps,pancake}(0) = -375$ kJ/mol. The thickness of the adsorbed pancake configuration is taken to be 11 Å, and the area is such that the protein volume is the same as in the spherical configuration. A detailed description of how this is incorporated in the calculations can be found in ref 17.

Table 1. Three Pluronic Surfactants Used for the Surface Grafting

polymer	mol wt (Da)	EO units	PO units	cmc (μM) (our study)	cmc (μM) (lit)	ref
PF68	8400	75	30		12000−34000	34, 35
PF127	12600	98	67	150	80−560	22, 36
PF108	14600	128	54	900	550−3000	22, 36

The structure of fibrinogen is assumed to correspond to three spheres connected by straight strings. The two outer spheres have a radius of 27.5 Å, and the central one has a radius of 24 Å. The distance between the central and outer spheres is 150 Å. We assume that the fibrinogen can adsorb in two possible conformations: (i) end-on (or head-on) conformation, which implies that one of the outer spheres is in contact with the surface and the rest of the molecule is perpendicular to the surface; (ii) side-on (or lying-down) conformation in which the three spheres are in contact with the surface. We take $\epsilon_{ps,end-on}(0) = -480$ kJ/mol and $\epsilon_{ps,side-on}(0) = 2.5\epsilon_{ps,end-on}(0)$. The side-on conformation has a stronger attraction to the surface because it has more surface exposed to it.

The chain model for the PEO chains has been chosen as in refs 16 and 31 where it has been shown that the predictions of the theory are in excellent agreement with pressure-area isotherms of PEO at the water−air interface. The strength of the protein−surface attractive interactions has been selected so that the adsorption of protein in the absence of grafted polymer shows good agreement with the experimental observations. For the attractive and repulsive interactions of the PEO with the surface and the repulsions of the protein with the surface due to the PPO, there are no available data that provide the strengths of these interactions. We have studied a variety of parameters and how they affect the agreement with experiments as it will be described in the following sections. Furthermore, in ref 17 a thorough discussion of how the different parameters affect the predicted adsorption isotherms was presented.

Experimental Section

Preparation of Surfaces and Polymer Grafting. Glass capillary tubes (1.5 mm i.d. × 100 mm long; Kimble Products, Vineland, NJ) were cleaned by immersion in chromic acid solution overnight. They were rinsed with running deionized distilled water (DDW) and dried at 60 °C. The inner surfaces of clean capillaries were modified by immersing the tubes in a solution of 5% trichlorovinylsilane (TCVS, Aldrich Chemical Co., Milwaukee, WI) in chloroform (analytical grade, Mallinkrodt, Paris, KY) at room temperature. After 3 h they were rinsed sequentially in fresh chloroform, absolute ethanol, and DDW. Finally, the silanized tubes were dried and cured at 60 °C overnight. Silanized samples were stored covered at room temperature until use.

Pluronic surfactants used were F127, F108, and F68 (referred to hereafter as PF127, PF108, and PF68). Solutions of PF108, PF127, and PF68 at the concentration ranging from 0.01 to 10 mg/mL were injected into the capillaries. The information on these three polymers are listed in Table 1. After 1 h of adsorption, the excess polymer was rinsed away with DDW. The polymer-adsorbed tubes, immersed in DDW, were exposed to γ-radiation from a ^{60}Co source for a total dose of 0.3 Mrad for grafting.[33] Nongrafted polymers were removed by washing in 1% sodium

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dodecyl sulfate (SDS) for 1 h. The tubes were then rinsed thoroughly in running deionized water and dried at 60 °C until use.

Surface Density of the Grafted PEO Chains. PF108, PF127, and PF68 were radiolabeled by a method described in our previous publication.[37] Briefly, the terminal hydroxyl groups of PEO chains were reacted with a 5-fold molar excess of *p*-methoxyphenyl chloroformate (MPC, Aldrich, Milwaukee, WI) in acetonitrile for 48 h at room temperature. The ester product, referred to as, for example, MP-PF108, was precipitated in cold anhydrous ether and filtered. It was resuspended in fresh ether and refiltered twice to remove unreacted MPC. The modified polymers were then radiolabeled with ^{125}I using the Enzymobead reagent and purified by gel filtration over Sephadex G-10 (exclusion limit = 700 Da, Pharmacia, Piscataway, NJ). The radiolabeled polymer was diluted with DDW to prepare adsorption solutions with concentrations ranging from 0.01 to 10 mg/mL. Surface-modified capillaries were injected with solutions of radiolabeled polymer at room temperature. After 1 h for adsorption they were rinsed of the excess polymer with DDW and the surface bound radioactivity determined.

Protein Adsorption. Human fibrinogen and hen egg lysozyme (Sigma, St. Louis, MO) in phosphate-buffered saline (PBS, pH 7.2) were radiolabeled with ^{125}I using the Enzymobead reagent (Bio-Rad, Richmond, CA). The radiolabeled protein was purified by gel filtration over a column packed with Bio-Gel P6-DG (Bio-Rad, exclusion limit 6000 Da), which was equilibrated with PBS and calibrated with blue dextran (Sigma). Radiolabeled protein was mixed with native protein in a 1:39 mass ratio to yield the adsorption solution. The final protein concentrations were 0.1 and 0.15 mg/mL for fibrinogen and lysozyme, respectively. These protein concentrations produced monolayer coverages on the control TCVS−glass.[38,39]

PEO-grafted capillaries were filled with PBS to hydrate the surface for 1 h prior to protein adsorption experiments. Hydrated capillaries were injected with either fibrinogen or lysozyme adsorption solution, displacing the PBS, at room temperature. After 1 h for adsorption the protein solution was displaced with PBS. Care was taken to avoid introducing air bubbles. Surface-bound radioactivity was determined on a γ scintillation counter (Beckman Gamma 5500B). The raw γ counts were divided by the sample surface area and protein specific activity to yield data in μg/cm^2 of the adsorbed protein.

Critical Micelle Concentration Measurement by Pyrene Fluorescence. The critical micelle concentrations (cmc) of Pluronic surfactants (BASF Corp., Wyandotte, MI) were measured from the fluorescence spectra of pyrene. A stock solution of 0.2 mM pyrene (Sigma, St. Louis, MO) in absolute ethanol was prepared. Stock solutions of Pluronic surfactants at a concentration of 100 mg/mL were prepared in DDW. Samples for fluorescence measurement were prepared by pipetting 50 μL of the pyrene solution into a flask followed by aliquots of the polymer stock solution. They were diluted to the final 10 mL volume with DDW and gently mixed. The finished samples thus consisted of a binary solution of 1 μM pyrene and polymer at a concentration ranging from 0.001 to 100 mg/mL. They were set aside and shielded from light for at least 2 h prior to analysis. All samples were prepared in chromic acid-cleaned volumetric glassware and analyzed the same day.

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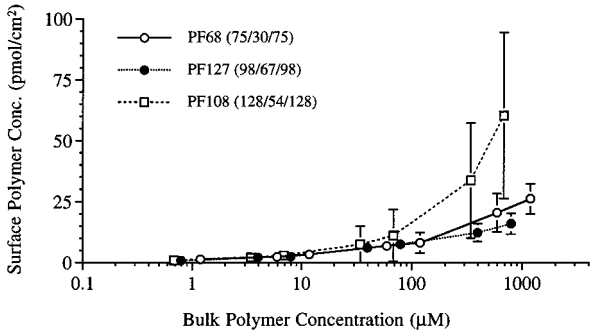

Figure 1. Surface polymer concentrations of PF68 (○), PF127 (●), and PF108 (□) on the vinylsilane-treated glass as a function of bulk polymer concentration used for adsorption (average ± standard error of the mean (SEM), $n = 6$).

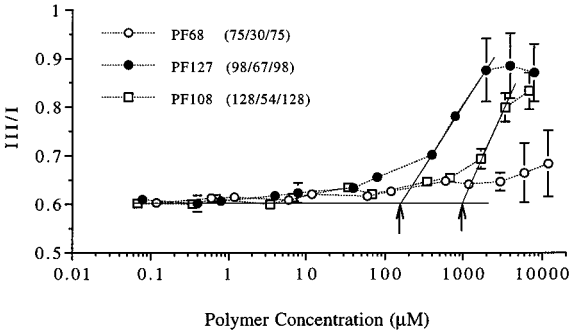

Figure 2. III/I values of pyrene fluorescence spectra as a function of polymer solution concentration for PF68 (○), PF127 (●), and PF108 (□) (average ± SEM, $n = 3$).

Fluorescence spectra were collected on an SLM 8000 spectrofluorometer (SLM, Rochester, NY). Pyrene was excited at 343 nm, and its fluorescence was measured over the range 360−420 nm with 1.0 nm step size at 1 s/nm. Emission slits were set to 0.5 mm. The intensities of the peaks centered at 372 and 384 nm, referred to as peak I and peak III, respectively, were noted. The ratio of peak III to peak I (III/I) for each spectrum was calculated and plotted against bulk polymer concentration.

Results

Adsorption of Pluronics to the Surface. The adsorption isotherms of three Pluronics, PF68, PF127, and PF108, to the TCVS-treated glass were examined. The isotherms in Figure 1 are plotted in molar units on both axes. When the solution concentration was about 1 μM, the surface concentrations of three polymers ranged from 0.9 to 1.4 pmol/cm^2. The differences in the adsorption isotherms became prominent when the solution concentrations were above 100 μM. There were little differences between the adsorption of PF68 and PF127 ($p < 0.05$). The surface concentration of PF68 reached 26 pmol/cm^2 when the solution concentration was 1190 μM. The adsorption of PF108, however, increased substantially more than the other two. The surface concentration of PF108 reached 60 pmol/cm^2 when the solution concentration was slightly less than 700 μM.

To understand the differences in the adsorption isotherms, the critical micelle concentrations (cmcs) of Pluronic surfactants were measured from the III/I ratio of pyrene fluorescence peaks. Pyrene III/I ratio was measured as a function of the polymer concentration (Figure 2). The *x* axes in Figure 2 are shown in molar concentration units so that the polymers with varying molecular weights can be compared. The III/I ratios of all three Pluronics were the same at 0.61 when the polymer concentrations were below 1 μM. This value is charac-

teristic of pyrene in an aqueous environment.[40] For PF127, the III/I ratio remained at 0.61 until the polymer concentration reached 40 μM. It slowly increased as the polymer concentration increased up to more than 1000 μM, where it reached a plateau of 0.88. The plateau value of 0.88 is characteristic of pyrene in a low dielectric environment, i.e., the environment of PPO aggregates.[40] PF108 exhibited a low III/I ratio until the polymer concentration reached about 200 μM. It sharply approached a value of 0.8, when the PF108 concentration was 3500 μM. The III/I values of PF68 remained relatively flat until the bulk concentration reached 1000 μM. Even then the III/I value was still below 0.7. Because this value does not represent the hydrophobic environment, it was not possible to estimate the cmc for PF68. The cmc values for PF108 and PF127 were estimated from the intercepts (arrows shown in Figure 2) of the line connecting the III/I value of water and the linear portion of the III/I values at high polymer concentrations. The cmc values estimated from Figure 2 were 150 and 1000 μM for PF127 and PF108, respectively. The cmc values for PF127 and PF108 found in the literature are 80−560 and 550−3000 μM, respectively.[22,36] Our cmc values are in the ranges observed by others. The cmc value for PF68 in the literature ranges from 12 000 to 34 000 μM.[34,35] Such a high cmc value was probably why we were not able to observe the increase in the III/I value of PF68 above 0.7, since the concentration used in our study was less than 12 000 μM.

The cmc values can explain, at least qualitatively, the differences in adsorption isotherms of PF108 and PF127 in Figure 1. Since the cmc value of PF127 observed in our study was 150 μM, at the polymer concentrations higher than this value, polymers start to form micelles and the monomer concentration does not increase as much as the total polymer concentration increases. PF108, on the other hand, had a cmc of 1000 μM. Thus, most of PF108 exist as monomers up to that concentration. The presence of abundant monomers in solution may have resulted in a higher surface concentration of PF108. Thus, micelles do not appear to be responsible for the adsorption of Pluronic surfactant molecules to the surface. The adsorption of PF68 was not as good as that of PF108, despite the very high cmc concentration (12 000 μM to 34 000 μM) in the literature. This is probably due to the shorter PO segment (30 units) of PF68 compared to the PO segment (54 units) of PF108. Our previous study has shown that Pluronics adsorb to the hydrophobic surface more readily as the length of the PO segment increased.[41] Thus, despite the abundance of the monomeric PF68, the affinity of PF68 to the surface was not as high as that of PF108.

Prevention of Protein Adsorption by Tethered PEO Chains. The adsorptions of lysozyme and fibrinogen onto the surfaces grafted with PF68, PF127, and PF108 are shown in Figure 3. The data are plotted as protein surface concentration versus polymer concentration (in mg/mL) used for the adsorption step of surface grafting. This is the usual way for presenting the protein adsorption data in the literature. The surface lysozyme concentration on the control surface was 0.19 ± 0.04 μg/cm^2. PF68 grafting reduced lysozyme adsorption to 0.1 μg/cm^2 when the polymer concentration was only 0.01 mg/mL (Figure 3A). The surface lysozyme concentration steadily decreased with increasing polymer concentration, reaching a minimum of approximately 0.05 μg/cm^2 when the PF68 concentration was 5 mg/mL. PF127 at a concentration of 0.1 mg/mL decreased lysozyme adsorption to 0.13 μg/cm^2. It further decreased lysozyme adsorption with increasing polymer concentration, reaching a minimum of approximately 0.075 μg/cm^2 at 5 mg/mL. Lysozyme adsorbed to the PF108-grafted surface to 0.15 μg/cm^2 when the polymer concentration was 1 mg/mL. It decreased steadily with increasing polymer concentration to a minimum of 0.06 μg/cm^2 at approximately 10 mg/mL PF108. All polymers were effective at decreasing lysozyme adsorption to the treated glass at polymer concentrations of 5 mg/mL and above. Figure 3B shows fibrinogen adsorption to the PF68, PF127, and PF108 grafted glass. Fibrinogen adsorbed to the control-treated glass at 0.35 ± 0.05 μg/cm^2. When the PF68 concentration was 0.01 mg/mL, fibrinogen adsorption was reduced to 0.27 μg/cm^2. It remained at that level until the PF68 concentration reached 1 mg/mL. It then decreased steadily until the PF68 concentration was 10 mg/mL where it was 0.09 μg/cm^2. PF127 grafting reduced fibrinogen adsorption to 0.15 μg/cm^2 at 0.05 mg/mL. Above this value, fibrinogen adsorbed to approximately 0.10 μg/cm^2, irrespective of PF127 concentration. PF108 was most effective in reducing fibrinogen adsorption. Fibrinogen adsorption was reduced to 0.27 μg/cm^2 by PF108 at a concentration of 0.01 mg/mL. It decreased steadily throughout the range of PF108 concentrations studied to a minimum of 0.02 μg/cm^2. The lysozyme adsorption profiles were very similar for all of the grafted surfaces. Both the magnitude of adsorption and dependence on polymer concentration were similar, irrespective of the polymer PEO block length. Fibrinogen adsorption, on the other hand, showed dif-

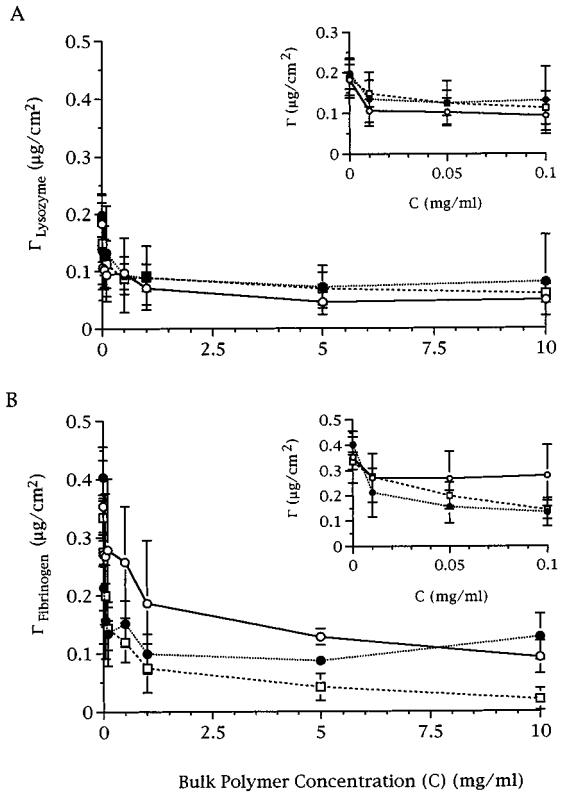

Figure 3. Surface concentrations (Γ) of lysozyme (A) and fibrinogen (B) adsorbed onto glass grafted with PF68 (○), PF127 (●), and PF108 (□) as a function of the bulk polymer concentration (*C* in mg/mL) used for adsorption. Molecular weights of PF68, PF127, and PF108 are listed in Table 1 (average ± SEM, $n = 3$).

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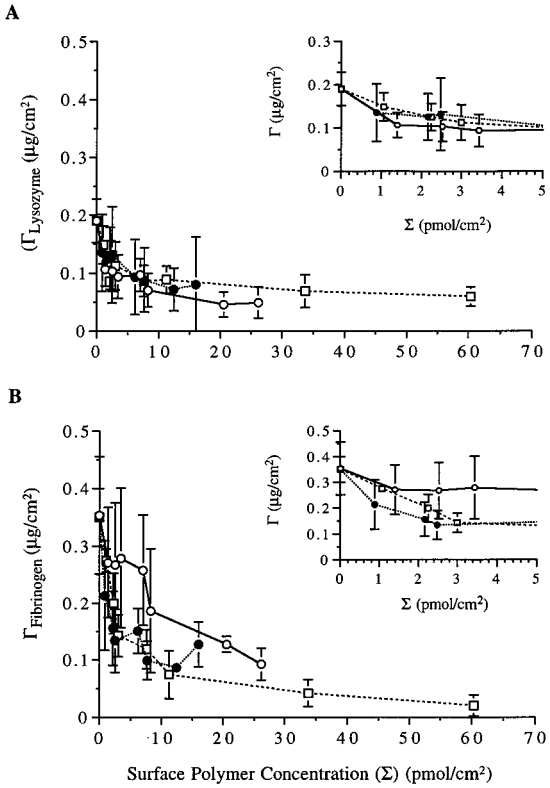

Figure 4. Surface concentrations (Γ) of lysozyme (A) and fibrinogen (B) adsorbed onto glass grafted with PF68 (○), PF127 (●), and PF108 (□) as a function of the surface polymer concentration (Σ in pmol/cm²) after grafting (average ± SEM, $n = 3$).

ferences between the polymers used. PF108, which has the longest PEO chain, was the most effective.

Figure 4 shows the protein adsorption data plotted as a function of the surface polymer concentration instead of the bulk polymer concentration used for adsorption. Since the prevention of protein adsorption is related to the PEO concentration on the surface rather than that in the adsorption solution, the data in Figure 4 present more useful information than those in Figure 3. The information in Figure 4 is also necessary for the theoretical analysis of the steric repulsion behavior based on the SCMF theory. Figure 4A shows the lysozyme adsorption to the PEO-grafted surfaces. As expected, lysozyme adsorption decreased with increasing amount of polymer on the surface for all polymers, irrespective of PEO chain length. All of the polymers used have rather long PEO blocks (75 or more repeating units), so there was no difference among them based on the PEO block length. Thus, lysozyme adsorption to PEO-grafted surfaces appears to be primarily dependent upon the surface polymer density. Fibrinogen adsorption versus polymer surface density is shown in Figure 4B. PF68 shows little decrease in fibrinogen adsorption until the surface concentration reaches more than 10 pmol/cm². Fibrinogen adsorption then decreases with increasing PF68 surface density. PF127 showed a sharp reduction in fibrinogen adsorption, which leveled off at about 0.05 μg/cm² when the polymer density was 10 pmol/cm². PF108 shows a steady decrease in fibrinogen adsorption with increasing polymer surface density throughout the concentration range. It produced the greatest reduction in fibrinogen adsorption of all the polymers used.

Comparisons of the Single-Chain Mean-Field Predictions with Experimental Observations. Figure 5 shows the theoretical analysis of the adsorption of

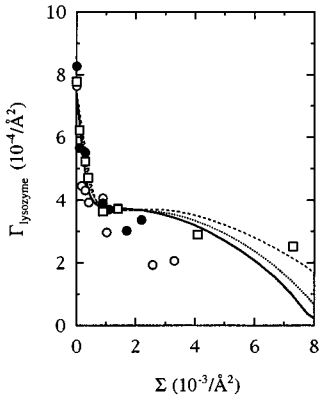

Figure 5. Surface concentration (Γ) of lysozyme (in molecule/Å²) adsorbed onto glass grafted with PF68 (○), PF127 (●), and PF108 (□) as a function of the surface polymer concentration (in number of PEO chains/Å²) after grafting. The lines represent predictions of the protein adsorption by the SCMF theory and correspond to the surfaces grafted with PF68 (dashed line), PF127 (dotted line), and PF108 (solid line). The calculations correspond to $\chi_{att,gs} = -5.4$ kJ/mol, $\chi_{rep,gs} = 810$ kJ Å²/mol, $\chi_{rep,ps} = 0$. The error bars of data points are not shown for easier visualization. The error bars are the same as those shown in Figure 4.

lysozyme to the PEO-grafted surface. The surface density of protein (Γ in number of protein molecules/Å²) is plotted as a function of the surface coverage of PEO chains (Σ in number of PEO chains/Å²). The agreement between the theory and the experimental observations is rather excellent, in particular the initial sharp decrease of the amount of protein adsorbed as the surface coverage of Pluronic increases. The theoretical predictions, in agreement with the experimental observations, show that there is almost no effect on the adsorption isotherms on the polymer chain length. Furthermore, the theoretical predictions show that there is a change in the behavior of the adsorption isotherm at $\sigma > 0.001$ Å². The isotherms show a change in slope at this surface coverage. In order to understand the source of this effect, we need to discuss in more detail the effect of the different interactions involved in determining the structure of the polymer−protein mixture at the surface.

The calculations presented in Figure 5 correspond to the strength of the PEO−PPO interaction, $\chi_{rep,gs} = 810$ kJ Å²/mol, while the protein does not feel any PPO-induced repulsive interaction, i.e., $\chi_{rep,gs} = 0$. The plateau-like region of the adsorption isotherm turns out to correspond to surface coverages in which the interplay between the repulsive PPO−PEO interactions and the PEO−surface attractions are such that in the absence of the proteins the polymer layer is very close to the region where a pancake to brush first-order phase transition is predicted. Namely, on the dilute side the PEO chains are completely adsorbed to the surface. As the surface coverage increases, the PPO−PEO repulsions start to dominate, and thus, the PEO desorbs from the surface. This can be observed in Figure 6, which shows the volume fraction of PEO adsorbed at the surface as a function of polymer surface coverage in the absence of adsorbed proteins. The amount of PEO adsorbed increases very sharply for low surface coverages, i.e., the bare PEO−surface attractions dominate. This is the range of surface coverage for which the amount of lysozyme adsorbed decreases very sharply (see Figure 5). There is a maximum in the fraction of PEO adsorbed that corresponds to the same surface coverage at which the amount of adsorbed protein in Figure 5 shows a large change in slope. For larger surface coverages of polymer, the fraction of adsorbed PEO decreases. This

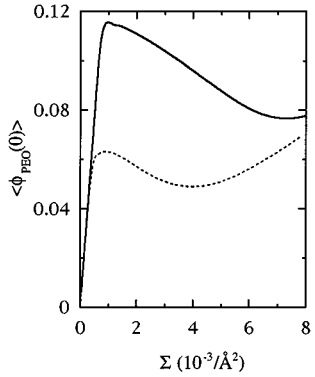

Figure 6. Volume fraction of PEO in contact with the surface as a function of the surface coverage of grafted polymer for PF68. The theoretical predictions are for grafted layers without proteins on the surface (full line) and with adsorbed proteins (dashed line). The amount of adsorbed protein is the equilibrium amount, as predicted in Figure 5.

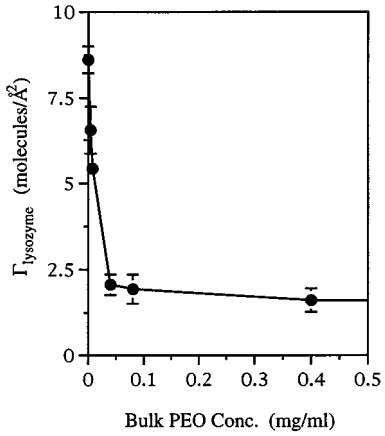

Figure 7. Surface concentration (Γ) of lysozyme (in molecule/Å^2) adsorbed onto glass as a function of the bulk PEO homopolymer concentration. The molecular weight of PEO was 8000.

results in a more accessible surface when the proteins attempt to adsorb and thus in the slow decrease of protein adsorption seen in Figure 5. Figure 6 also shows the fraction of PEO adsorbed in the presence of adsorbed proteins (with the surface density of proteins corresponding to that in Figure 5). For protein surface coverages $\Gamma_{lysozyme} > 0.0005$/Å^2, the presence of the adsorbed protein results in an effective repulsive interaction of the PEO (due to steric effects) that reduces the fraction of PEO in contact with the surface in order to allow more surface space for the protein.

The excellent agreement between the predicted isotherms and the experimental observations gives confidence that the parameters chosen in the calculations are appropriate. However, it is important to discuss the physical source of those values and also how a variation of these parameters will change the predicted isotherms. First, we consider the bare PEO−surface attraction. The reason for having such interaction, as discussed in the presentation of the theory, is that the ethylene oxide monomers are amphiphilic. Furthermore, it has been recently shown that in order to describe the pressure−area isotherms of tethered PEO at the water−air interface, one needs to consider an attraction between the EO monomers and the interface with a strength $\chi_{att,gs} = -2.4$ kJ/mol.[16] The calculations presented above were done with $\chi_{att,gs} = -5.4$ kJ/mol. The choice for this stronger attraction is due to the more hydrophobic nature of the modified surface used in the experiments compared to the air−water interface.

To verify that the PEO is strongly attracted to the surface, we have measured the adsorption of protein when the surface was grafted with PEO homopolymers instead of Pluronics. The adsorption of lysozyme as a function of the bulk concentration of PEO used for grafting is shown in Figure 7. The isotherm shows a sharp decrease in lysozyme adsorption at the bulk PEO concentration of 5 μM. Clearly, χ_{gs} must be large enough in order for the PEO to be adsorbed on the surface even at these small bulk concentrations.

The effect of varying the strength of the grafted polymer−surface attraction has been studied in detail previously.[17] The study showed that the stronger the attraction between PEO and surface, the more effective the polymer layer in preventing protein adsorption. However, the kinetic barrier presented by grafted polymers *not* attracted to the surface is much larger than for the attractive case. Thus, the conditions for kinetic and thermodynamic control are different. Namely, when the segments of the grafted polymers have attractive interactions with the surface, the polymer layer is more effective in the thermodynamic control of protein adsorption. However, the time scale for protein adsorption is much larger when the polymer segments are not attracted to the surface.[17,31]

Second, we need to consider the repulsive interaction parameter between the PEO and the grafted PPO and between the protein and the grafted PPO segments. The good agreement with the experimental observations was obtained with $\chi_{rep,gs} = 810$ kJ Å^2/mol and $\chi_{rep,ps} = 0$. Although it is clear that there should be a repulsive interaction between the PEO and the PPO, it is not clear that this should be the case for the proteins. Furthermore, there is no easy way to quantify the value from independent experiments. Therefore, we show what would be the changes in the adsorption isotherms if the strengths of those interactions are changed. Figure 8A shows the lysozyme adsorption isotherms for a variety of PEO−PPO repulsive interaction parameters when there is no repulsive PPO−protein interactions, i.e., $\chi_{rep,ps} = 0$, as in the experimental comparison in Figure 5. As the strength of the PEO−PPO repulsive interaction decreases, the ability of the polymer layer to prevent protein adsorption does not change up to $\Gamma_{lysozyme} = 0.001$/Å^2. For higher surface coverages, the PEO layer becomes more effective in preventing lysozyme from adsorbing. The lower the PPO−PEO repulsion the better prevention abilities of the polymer layer. The reason for this behavior is that if the PPO does not exert a repulsive interaction over the PEO, then there is a higher fraction of PEO on the surface providing a more repulsive steric interaction to the proteins. The effect of a PPO−protein repulsion, at constant PPO−PEO repulsion, can be seen in Figure 8B. Clearly, as the protein−PPO repulsion, increases there is less adsorption of protein as the surface coverage of polymer increases. Note that the initial decay of the protein adsorption does not depend on the strength of the PPO−protein or PPO−PEO repulsions. That initial decay, which is very well predicted by the theory, depends only on the properties of the grafted PEO molecules and their ability to occupy the surface, diminishing the amount of available surface to the protein and at the same time the steric repulsion that the adsorbed PEO segments present to the protein.

Figure 9 shows the adsorption isotherms for fibrinogen. There is good agreement between the theoretical predictions and the experimental observations for low surface

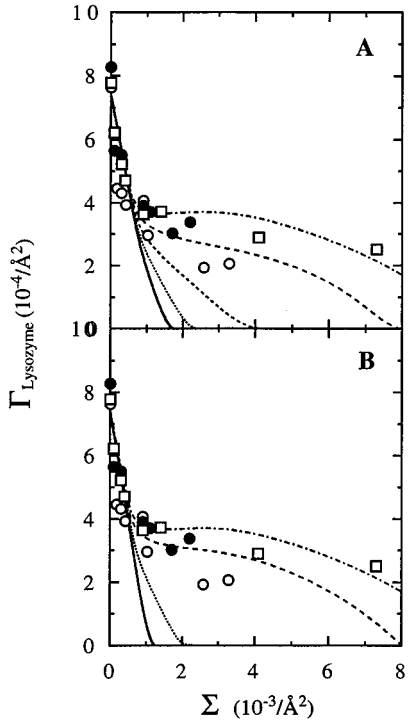

Figure 8. Same as in Figure 5 with theoretical predictions only for PF68 with $\chi_{att,gs} = -5.4$ kJ/mol and for a variety of different repulsive interaction parameters. (A) All the theoretical predictions are for $\chi_{rep,ps} = 0$. The different lines correspond to $\chi_{rep,gs} = 0$ (full line), $\chi_{rep,gs} = 202.5$ kJ Å^2/mol (dotted line), $\chi_{rep,gs} = 405$ kJ Å^2/mol (dashed line), $\chi_{rep,gs} = 607.5$ kJ Å^2/mol (long dashed line), and $\chi_{rep,gs} = 810$ kJ Å^2/mol (dot−dashed line). (B) All the theoretical predictions are for $\chi_{rep,gs} = 810$ kJ Å^2/mol. The different lines correspond to $\chi_{rep,ps} = 810$ kJ Å^2/mol (full line), $\chi_{rep,ps} = 410$ kJ Å^2/mol (dotted line), $\chi_{rep,ps} = 16.2$ kJ Å^2/mol (dashed line), and $\chi_{rep,ps} = 0$ (long dashed line).

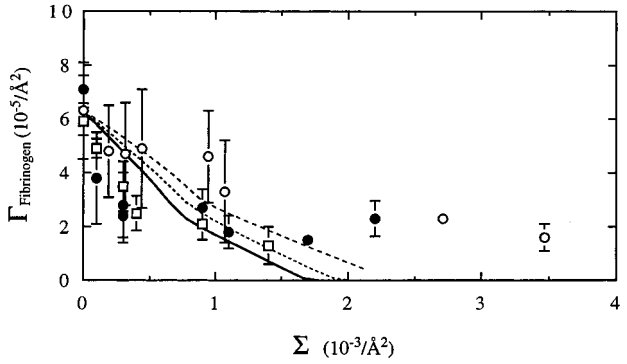

Figure 9. Surface concentration (Γ) of fibrinogen (in molecule/Å^2) adsorbed onto glass grafted with PF68 (○), PF127 (●), and PF108 (□) as a function of the surface polymer concentration (in number of PEO chains/Å^2) after grafting. The lines represent predictions of the protein adsorption by the SCMF theory and correspond to the surfaces grafted with PF68 (dashed line), PF127 (dotted line), and PF108 (solid line).

coverages. The predictions of the theory show total prevention of fibrinogen adsorption, while the experimental observations show a plateau. The theory predicts that from the two possible configurations, end-on (or head-on) and side-on (or lying-down), the first is the most favorable for all surface coverages of grafted PEO (data not shown). It turns out that the adsorption isotherms of fibrinogen are less sensitive to the strength of the PEO−surface attraction, PEO−PPO repulsion, and PPO−protein repulsion than in the case of lysozyme. This is due to the huge size of fibrinogen compared to PEO chains. The net result is that the fibrinogen isotherms look almost identical for all the range of parameters studied. The determining factors of the isotherm are the volume distribution of the fibrinogen and the bare fibrinogen−surface attraction at contact.

The qualitative features of the experimental isotherms for both proteins are very similar to each other. In both cases there is a very sharp decrease of protein adsorption for low surface coverage of PEO that is properly predicted by the theory. As the surface coverage increases, the theory predicts the following: (i) for the case of fibrinogen a continued decrease of the amount of adsorbed protein up to total prevention; (ii) for the case of lysozyme a plateau (in agreement with the experimental observations) or complete prevention of lysozyme adsorption, depending upon the strength of the PEO−PPO repulsion and of the PPO−protein repulsion. However, the experimental measurements show that for both proteins there seems to be a leveling off (plateau) in the adsorption isotherm.

Discussion

Prevention of Protein Adsorption by the Grafted PEO Layers. Our study using the hydrophobic surfaces suggests that the ability of the PEO layers to prevent protein adsorption stems from the direct coverage of the surface by the PEO segments, resulting in blocking of the adsorption sites for the proteins. The usual steric repulsion theory based on the picture of the polymer brush is not appropriate for hydrophobic surfaces. This is particularly important at low surface coverage of polymer, where the slope of the isotherm is the sharpest. At higher surface coverages the details of the interactions between all the components in the system can result in different types of the isotherms for small proteins, but they seem to be of no influence for very large proteins. The measure of the size of the protein is relative to the volume of the polymer molecules.

The steric repulsion theory has been used to explain the prevention of protein adsorption and cell adhesion onto the surfaces modified with a variety of molecules such as PEO, albumin, heparin, dextran, and other hydrophilic macromolecules.[11,42] It was not easy, however, to use the steric repulsion theory for explaining the prevention of protein adsorption and cell adhesion by rather rigid molecules such as albumin, heparin, or dextran. This appears to be explained by the concept developed from this study (i.e., prevention of protein adsorption is due to the blocking of the protein adsorption sites). It is very reasonable to expect that rigid molecules such as albumin, heparin, or dextran, can adsorb to the surface and block the protein adsorption sites. In fact, it is well-known that albumin adsorbs readily to the surface, especially to the hydrophobic surface.

Comparison between the SCMF Theory and Experimental Observations. As shown in Figures 5 and 9, SCMF theory can explain well the decrease in protein adsorption for lysozyme at all polymer surface coverages and for fibrinogen at low PEO coverage on the surface. Unlike the theoretical predictions, however, fibrinogen adsorption occurred, albeit in small quantities, to the PEO-grafted surfaces. This difference raises the question of whether the theory is unable to predict the adsorption isotherms for fibrinogen or there is a physical process in the experimental observations that has not been taken into account in the theory. The most probable sources of the difference are (i) kinetically controlled adsorption, (ii)

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long-range interactions between the protein and the surface, (iii) attractive interactions between the protein and the polymer segments, and (iv) inhomogeneous grafting of the polymers on the surface.

To examine the effect of kinetically controlled adsorption, we have performed experiments measuring the protein adsorption kinetics.[39] Our kinetic studies have shown that after 5 min the adsorption reaches its final value, which does not change even after several hours. Although this is not proof that the measured amount of protein is in equilibrium, it strongly suggests that the solution has reached a local equilibrium in which the amount of protein adsorbed is determined by the equality of chemical potential between the bulk and surface proteins. Furthermore, the suggestion that the experimental measurements are not kinetically controlled is supported by the fact that the measured amount of adsorbed protein is larger, at high polymer surface coverages, than the calculated one, while there is agreement at low surface coverages. Kinetically controlled adsorption is expected to give lower amounts of protein on the surface than equilibrium adsorption.

For the effect of long-range interactions between the protein and the surface, we have performed calculations using long-range interactions. Any reasonable choice of the long-range potential gives an overestimation of the amount of the protein adsorbed at low and intermediate surface coverages of polymers. Moreover, we do not find agreement with the experimental observations at any range of surface coverages. The possibility of weak attractive interactions between proteins and PEO layers has been predicted[43] and observed experimentally for avidin.[44] The calculated interactions suggest the possibility of adsorption of protein on top of the grafted layer and not by direct contact with the surface. We have carried out calculations considering this possibility and found that such weak attractive interactions may not result in any change of the adsorption isotherms (for relatively weak EO monomer−protein interactions). For stronger EO monomer−protein interactions, a large increase in the adsorption of proteins for all surface coverages of polymers is found. Again, there was no reasonable agreement with the experimental observations in any range of polymer surface coverages.

The last possibility that at high surface coverages the grafted polymers do not form an homogeneous layer seems to be the most reasonable one. In fact, we believe that this is the case owing to the following facts. First, the "plateau" of the experimental adsorption isotherm starts at the same surface coverage of polymer for both proteins. The inhomogeneously covered surfaces will have patches of uncovered surface and domains (or two-dimensional micellar aggregates) covering the surface, allowing a free surface for these two very different proteins to adsorb. Second, the theory predicts that in the absence of proteins (for the parameters that the lysozyme isotherm is well predicted at all surface coverages) the polymer layer is very close to a mushroom to brush transition region. Third, there is an independent set of experimental measurements of the adsorption isotherms of lysozyme and fibrinogen in homogeneous covered surfaces with short chain length EO oligomers performed by Prime and Whitesides.[45] They used self-assembled monolayers of varying surface coverage of chains with a number of monomers varying from zero to 17. They found that even for chains with two EO units one can totally prevent the protein from adsorbing at the surface. It has recently been shown that their experimental observations are very well predicted by SCMF theory.[31] Finally, our recent studies using atomic force microscopy (AFM) indicated inhomogeneous surface coverage by the grafted PEO chains (unpublished data).

In summary, the Pluronic-covered surfaces at high surface coverages do not appear to be homogeneous. This suggestion is supported by (i) the agreement between the SCMF predictions and the experimental observations of Prime and Whitesides at all surface coverages of polymers, (ii) the fact that the self-assembled monolayers are homogeneous systems, and (iii) the agreement between the theory and the experimental observations at low and intermediate surface coverage of long chains of PEO shown above (Figures 5 and 6). It is highly possible that two-dimensional micelles or microphase separation is underway on the surface. We are currently carrying out experimental measurements to check such possibilities, and our preliminary studies using AFM support the possibility of microphase separation.

Conclusion

We have carried out systematic studies of the ability of grafted PEO layers to prevent protein adsorption. The experiments provided macroscopic information on the importance of the surface density of the grafted PEO. The theoretical analysis by the SCMF approach provided further insights into the mechanisms responsible for the prevention of protein adsorption by the grafted polymers. With this information, the modification of biomaterials no longer has to be based on trial-and-error to prepare protein- and platelet-resistant surfaces.

It is important to realize that the PEO layer on the surface does not exist as the commonly referred brush. The surface coverages and polymer chain lengths used here (and in other experimental systems) are far from the ones necessary to reach the true brush regime, which requires nonattracting surfaces, high surface coverages, and very large molecular weights of the grafted polymer. The adsorption of proteins is determined by the interplay between the attractive interaction of the protein upon contact with the surface and the attractive interaction of the adsorbed PEO segments with the surface, and with the entropic changes associated with the deformation of the PEO layer due to the adsorbing proteins. Furthermore, even for the surfaces that do not attract the PEO chains, the amount of protein adsorbed depends upon the ability of the adsorbing protein to deform the tethered PEO layer. This points to the needed revision of the "brush picture" and the steric repulsion theory in the understanding of protein adsorption on grafted polymer layers. Therefore, the theoretical description of the adsorption behavior of proteins on surfaces with tethered polymers requires the use of molecular theories such as the SCMF used in this study.

The mechanism responsible for prevention of protein adsorption by grafted PEO in hydrophobic surfaces results from the ability of the grafted polymer to block the adsorption sites for the proteins. Therefore, the fact that PEO is attracted to the surface results in more effective prevention. The cases in which the grafted PEO are not

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attracted to the surfaces, as in lipid−PEO layers[46] and liposomes,[47] the polymer chains are more effective in presenting a kinetic barrier to the proteins in solution rather than in thermodynamically preventing protein adsorption.

Our analysis showed that experiments on protein adsorption on PEO grafting become rather meaningless if the critical information, such as the density of the grafted PEO chains, is not known. Our experimental results and the SCMF analysis suggest that for prevention of protein absorption the density of the grafted PEO chains should be above a certain level, which varies depending on the type of grafted PEO.

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