Protein Adsorption on Surfaces with Grafted Polymers: A Theoretical Approach

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ABSTRACT A general theoretical framework for studying the adsorption of protein molecules on surfaces with grafted polymers is presented. The approach is a generalization of the single-chain mean-field theory, in which the grafted polymer-protein-solvent layer is assumed to be inhomogeneous in the direction perpendicular to the grafting surface. The theory enables the calculation of the adsorption isotherms of the protein as a function of the surface coverage of grafted polymers, concentration of protein in bulk, and type of solvent molecules. The potentials of mean force of the protein with the surface are calculated as a function of polymer surface coverage and amount of protein adsorbed. The theory is applied to model lysozyme on surfaces with grafted polyethylene oxide. The protein is modeled as spherical in solution, and it is assumed that the protein-polymer, protein-solvent, and polymer-solvent attractive interactions are all equal. Therefore, the interactions determining the structure of the layer (beyond the bare polymer-surface and protein-surface interactions) are purely repulsive. The bare surface-protein interaction is taken from atomistic calculations by Lee and Park. For surfaces that do not have preferential attractions with the grafted polymer segments, the adsorption isotherms of lysozyme are independent of the polymer length for chains with more than 50 ethylene oxide units. However, the potentials of mean force show strong variations with grafted polymer molecular weight. The competition between different conformations of the adsorbed protein is studied in detail. The adsorption isotherms change qualitatively for surfaces with attractive interactions with ethylene oxide monomers. The protein adsorption is a function of chain length—the longer the polymer the more effective it is in preventing protein adsorption. The structure of the layer and its deformation upon protein adsorption are very important in determining the adsorption isotherms and the potentials of mean force.

INTRODUCTION

Understanding the adsorption of protein molecules to surfaces is of primary importance in the design of biocompatible surfaces. For example, it is known that the exposure of blood to a foreign material results in the adsorption of plasma proteins to the surface (Horbett, 1993; Andrade and Hlady, 1986). Then platelets adhere to the surface, which can result in surface-induced thrombosis. The adhesion of platelets occurs by attachment to adsorbed proteins such as fibrinogen (Luscher and Weber, 1993). A requirement for a biocompatible surface is then the elimination or reduction of the initial protein adsorption. Another example of the importance of preventing protein adsorption is in contact lenses. The elimination of lysozyme build-up on the surface of the lenses can improve the quality and reduce the need for cleaning of contact lenses (Missiroli et al., 1991; Castillo et al., 1986).

One of the most successful approaches to preventing protein adsorption is to graft polymer molecules to the surface (Harris, 1992a,b; Lee et al., 1989a,b; Tan and Martic, 1990; Ishihara et al. 1991; Desai and Hubbell, 1991; Gombotz et al., 1991, 1992; Han et al., 1991; Amiji and Park, 1992; Park and Kim, 1992; Gölander et al., 1992; Bergström et al., 1992; Österberg et al., 1993; Fujimoto et al., 1993; Llanos and Sefton, 1993; Lin et al., 1994; McPherson et al., 1995). Furthermore, the grafting of polymer chains to the surface of liposomes results in increased longevity (Allen et al., 1991; Senior et al., 1991; Klibanov et al., 1991; Papahadjopoulos et al., 1991; Torchilin et al., 1994a,b,c). It is generally believed that the reduction of protein adsorption is due to the steric interaction that the grafted chains present to the protein molecules. However, a systematic understanding of the factors governing the adsorption behavior is still lacking.

There have been some theoretical attempts at understanding the effect of grafted polymers on the adsorption of proteins to surfaces. Jeon and co-workers (Jeon and Andrade, 1991; Jeon et al., 1991) calculated the free energy required for a protein to reach a surface with grafted polymers. Their theoretical work was based on the Alexander-de Gennes theory of polymer brushes. This approach is valid for very long chains in the so-called brush regime, i.e., for relatively high surface coverages. As has been discussed elsewhere (Szleifer, 1996; Szleifer and Carignano, 1996), the conditions for most experimental observations of polyethylene oxide (PEO), also called polyethylene glycol (PEG), on surfaces or liposomes are not in the brush regime. Another theoretical attempt to understand the effects of grafted polymers on protein adsorption used molecular dynamics simulations (Lim and Herron, 1992). In this study, a protein was simulated at the surface of a PEO polymer layer. The interaction between the protein and the surface was calculated for one distance and several orientations of

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the protein with respect to the surface. One could extend this approach to calculating the potential of interaction as a function of the distance from the surface. However, that is not enough to determine the adsorption behavior. The adsorption of lysozyme on surfaces with grafted PEO is a process with a time scale of seconds (Kidane et al., 1996). This time scale is more than seven orders of magnitude larger than what atomistic simulations can reach with the hardware available today, and thus different approaches are necessary. A simple model for understanding the steric repulsion induced by the polymer layer was presented by Torchilin and co-workers (1995). They simulated a single polymer chain grafted to a surface and looked at the "statistical" density cloud of the polymer. In the case of finite surface coverage of polymer, they assumed that by looking at the statistical density clouds of a single chain now separated by the distance imposed by the surface coverage, they could conclude whether there is enough space for proteins to reach the surface. The problem with this approach is that it has been shown that at finite surface coverage the structure of the grafted polymers changes from that of the single chain because of intermolecular interactions (Szleifer and Carignano, 1996). The effects of these intermolecular interactions will be shown below to be very important in determining the ability of the polymer layers to prevent protein adsorption.

In this article we present a systematic theoretical study of the behavior of proteins adsorbing to surfaces in the presence of grafted polymers. We study the effects of surface coverage, type of surface, protein configuration, and molecular weight of the grafted polymer. We concentrate our attention on the adsorption isotherms, the potential of mean force between the surface and the protein in the presence of the polymer layer, and the changes in the structure of the layer due to the presence of the protein. The work presented here considers only the equilibrium adsorption, and some discussion on what is expected for the kinetic behavior based on the potential of mean force.

This study is based on a generalization of the single-chain mean-field (SCMF) theory. This approach was originally developed to treat amphiphilic molecules in micelles and bilayers (Ben-Shaul et al., 1985; Szleifer et al., 1986, 1987, 1990; Fattal and Ben-Shaul, 1994, 1995) and later generalized to study tethered polymer molecules in contact with solvent (Carignano and Szleifer, 1993; Szleifer and Carignano, 1996). The predictions of the theory for grafted polymers have been shown to be in quantitative agreement with experimental observations (Carignano and Szleifer, 1995a; Szleifer, 1996) and computer simulations (Carignano and Szleifer, 1995b; Szleifer and Carignano, 1996) for both structural and thermodynamic properties. Therefore, we expect that the theory will be able to shed light on the microscopic origins of the adsorption behavior of proteins on surfaces with grafted polymers.

The next section shows the derivation of the theory for the case of interest here. The thirdc section presents the model to which the theory is applied. The fourth section shows results for a variety of conditions of grafted polymer. The results are for model polyethylene oxide (PEO) grafted polymers and for model lysozyme proteins. The last section includes concluding remarks and a discussion of future directions.

THEORETICAL APPROACH

The SCMF theory is based upon looking at a molecule with all of its intramolecular and surface interactions taken into account in an exact fashion, and the intermolecular interactions are considered within a mean-field approximation. For chain molecules this implies that all of the possible configurations (or a representative sample of them) of a single chain have to be calculated, and the theory provides the probability of each of these different configurations as a function of the thermodynamic variables of the system, i.e., surface coverage, temperature, and type of surface. The probability distribution function (pdf) of chain conformations depends upon the chain configuration (due to the intramolecular interactions and the surface interactions), the temperature, the surface density of polymer, and in the case of mixtures, the composition of the sample. Furthermore, because of the presence of the surface the density as a function of the distance from the surface is inhomogeneous, and therefore the mean field with which the molecule interacts is a function of the density profiles of the components in the mixture. Szleifer and Carignano (1996) have extensively reviewed the foundation of the theory and a variety of its applications. Here we present a short derivation with the purpose of highlighting the specific features that are relevant for the problem of protein adsorption.

The spirit of this theory is between that of full-scale computer simulations and that of simple analytical approaches. Computer simulations provide the exact solution of the model system. However, they are not of practical use for problems such as adsorption of proteins in the presence of grafted polymer molecules, because of the very long time of this process, which is on the scale of seconds (Kidane et al., 1996). On the other hand, analytical and scaling approaches provide a wealth of information on the "universal" properties of the systems under study. However, they cannot include detailed information on the molecular structure of the systems and they are in general derived for a particular thermodynamic regime. The SCMF theory enables us to study the behavior of these complex systems, including detailed molecular structures over the whole range of surface coverages, type of solvents, and chemical architectures of the polymer chains, by considering the intermolecular interactions in a mean-field approximation. The use of this approximation implies that intermolecular correlations are not properly taken into account by the theory. However, it has been shown that the theory provides quantitative information on detailed structural and thermodynamic properties of grafted polymer layers as compared to full-scale computer simulations and experimental observations (Szleifer

and Carignano, 1996; Szleifer, 1996). Therefore, the simplifications taken in the derivation of the theory seem not to be the ones that determine the behavior of these systems. In other words, correlations beyond the size of a polymer chain seem not to be very important in these layers. It should be stressed that although the intermolecular interactions are considered within a mean-field approximation, each chain molecule is treated "exactly," i.e., all of the single-chain conformations are taken into account. Therefore, the SCMF theory makes possible the study of detailed structural and thermodynamic properties for different chain architectures (Carignano and Szleifer, 1994) and their variation as a function of the relevant variables in the system.

Consider a system composed of a surface with tethered polymers in contact with a solution containing low-molecular-weight solvent and protein molecules, as schematically shown in Fig. 1. The solution can be thought of as a bath of solvent and protein molecules characterized by chemical potentials μ_s and μ_p for the solvent and the proteins, respectively. The surface coverage of polymer is $\sigma = N_g/A$, where N_g is the number of grafted polymers and A is the total area of the surface.

The quantities that we are interested in are the pdf of chain conformations, the distance-dependent pdf of the protein molecules, and the distribution of protein and solvent molecules. The distance dependence refers to the normal to the surface, z, and it will be measured from the closest segment of the protein to the surface. The distributions for the polymers and the protein molecules are obtained by minimizing the system's free energy. To write the relevant thermodynamic potential, we need first to describe the intra- and intermolecular interactions in the system.

The interaction of a protein molecule with the solid surface, $U_{ps}(z)$, is taken from the work of Lee and Park (1994), who considered electrostatic, van der Waals, and hydrophobic contributions. Note that $U_{ps}(z)$ does not include any interaction with the grafted polymer; it is just the interaction between the bare surface and the protein. We also consider the possibility of an attractive interaction between the surface and the monomers of the grafted polymer chains. This interaction is assumed to be short range and we call it U_{gs}. The intramolecular interactions, ϵ_{intra}, are explicitly taken into account, i.e., for each chain molecule including the proteins, a set of single-chain configurations is considered, and for each configuration the intramolecular interactions are calculated. The next step is to consider the protein-polymer, polymer-polymer, protein-solvent, polymer-solvent, and solvent-solvent intermolecular interactions. For all of these interactions we separate the attractive from the repulsive interactions. The separation is done because, as will be shown below, it makes possible the treatment of the intermolecular mean-field interactions in a straightforward way. The repulsive potential is modeled with hard-core excluded volume interactions. The attractive tails will depend on the specific nature of the two segments interacting. Let us assume, for the sake of simplicity, that all of the intersegment interactions can be modeled by the attractive tail of a van der Waals potential. Therefore, each segment (of a chain or protein) will interact with a van der Waals average potential due to the different molecules residing, on average, around that segment. At this point it is important to realize that because of the presence of the surface the segment densities of polymer, protein, and solvent are inhomogeneous in the direction perpendicular to the surface. Therefore, the mean-field interacting with the segments is different for different z, and this must be explicitly included in the calculations. In other words, the mean-field interaction is nonlocal and varies, depending on the distribution of the different molecules as a function of the distance from the surface. Thus we can write the intermolecular attractions of a grafted polymer chain

$$\langle \epsilon_{inter,g} \rangle = \sum_{\alpha} P_g(\alpha) \int_0^{\infty} \int_0^{\infty} n_g(z, \alpha) \cdot [\chi_{gg}(z, z') \langle \phi_g(z') \rangle + \chi_{gp}(z, z') \langle \phi_p(z') \rangle + \chi_{gs}(z, z') \cdot \langle \phi_s(z') \rangle] \, dz \, dz', \quad (1$$

where the subscripts g, p, and s refer to grafted polymer, protein, and solvent, respectively; $\langle\ \rangle$ represents ensemble averages; ϕ denotes volume fraction; $P_g(\alpha)$ is the pdf of grafted chain conformations, and the sum runs over all possible single-chain conformations α; $n_g(z, \alpha)\, dz$ is the number of segments that a chain in conformation α has in the volume between z and $z + dz$ (defined as the number of segments at z); and $\chi_{ij}(z, z')$ is the van der Waals (mean field) interaction between segment of type i at z and segments of type j at z'. The integral over z is to account for all of the segments of the chain, and the one over z' is to count for the "mean field" of the other molecules at different z'. The integrations are written from 0 to ∞; however, it is clear that the one over z will contain contributions only up to the distance that the grafted chain can reach, and the one over z' will be determined by the range of the interactions.

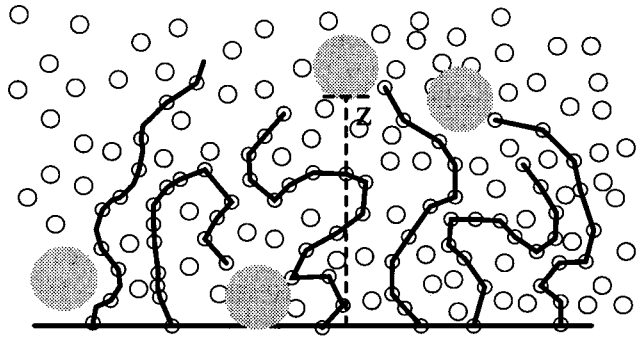

FIGURE 1 Schematic representation of a grafted polymer layer in contact with a protein solution. The connected circles represent the polymer chains, the small circles are the solvent molecules, and the large circles are the protein molecules. The z direction is defined perpendicular to the surface. The dashed line shows how the distance of the protein from the surface is defined.

Protein molecules are formed by a variety of chemically different units; thus the interaction parameters and the volume fraction of protein are shorthand notation. In the actual calculations each of the different types of segments forming the protein can be taken into account.

The protein molecules can reside anywhere in the solution; therefore we must specify their location. As mentioned above, we refer to proteins at z as those for which z is the closest distance from the surface. Then proteins at $z = 0$ are those adsorbed to the surface. We define the probability of the proteins as $P_p(\gamma; z)$, with γ denoting the configuration of the protein and z its location. The pdf of protein conformations is normalized for each z, i.e., $\Sigma_\gamma P_p(\gamma; z) = 1$ for all z. We can write the intermolecular attractions of a protein at z as

$$\langle \epsilon_{\text{inter,p}}(z)\rangle = \sum_\gamma P_p(\gamma; z)\int_z^\infty\int_0^\infty \sum_\gamma n_{p,z}(z'; \gamma) \cdot [\chi_{pp}(z', z'')\langle\phi_p(z'')\rangle + \chi_{pg}(z', z'')\langle\phi_g(z'')\rangle + \chi_{ps}(z', z'') \cdot \langle\phi_s(z'')\rangle]\, dz'\, dz'', \tag{2}$$

where the first integral now runs from z because the proteins at z do not have any segments at $z' < z$, and the rest of the symbols are as in Eq. 1, and again we note that the protein notation is a shorthand for representing each type of segment in those molecules.

The only attractive intermolecular contribution remaining is that of the solvent molecules to themselves. However, it can be shown that it is not necessary to specify that interaction, because the $\chi_{i,j}$ used above can be defined with respect to one of the interactions that we chose to be the solvent-solvent interaction (Szleifer and Carignano, 1996).

Now we turn to the intermolecular repulsive interactions. As mentioned above, we model them with hard-core excluded volume repulsions. Therefore, the repulsive interactions are taken into account by packing constraints. Furthermore, because the system is inhomogeneous in the direction perpendicular to the surface, the packing constraints must be fulfilled for all distances z from the surface. Namely, for each layer defined as the volume between z and $z + dz$, the sum of the volumes occupied by protein, polymer, and solvent must be equal to or smaller than the available volume. We assume that the equality holds, i.e., all of the volume is occupied by molecules. This implies that the system is incompressible. Although this assumption (incompressibility) is not necessary for the development of the theory or to perform the calculations, we make it here for simplicity. The properties of interest here will not be modified in a dramatic way by this approximation.

We can quantify the volume constraints by

$$N_g\langle v_g(z)\rangle dz + \sum_{z'} N_p(z')\langle v_p(z)\rangle_{z'}\, dz + \langle V_s(z)\rangle dz = A\, dz \qquad 0 \le z \le \infty, \tag{3}$$

where N_g is the total number of grafted polymers; $\langle v_g(z)\rangle\, dz = \Sigma_\alpha P_g(\alpha)\, v_g(z; \alpha)\, dz$ is the average volume occupied by a grafted chain at z; $N_p(z')$ is the number of proteins at z' with $\langle v_p(z)\rangle_{z'}\, dz = \Sigma_\gamma P_p(\gamma; z')v_{p,z'}(z; \alpha)\, dz$ being the average volume that a protein at z' occupies at z; the sum in Eq. 1 runs over all z' for which the protein contributes volume at z; $\langle V_s(z)\rangle\, dz$ is the average volume occupied by solvent molecules at z; and A is the total area of the surface. Dividing Eq. 1 by $A\, dz$, we get

$$\sigma\langle v_g(z)\rangle + \sum_{z'} \rho_p(z')\langle v_p(z)\rangle_{z'} + \langle\phi_s(z)\rangle = 1 \qquad 0 \le z \le \infty, \tag{4}$$

where we have used the surface coverage of grafted polymers, $\sigma = N_p/A$; and the density of proteins at z', $\rho_p(z') = N_p(z')/A$.

The quantities that we need to find are the pdf of grafted polymers, the z-dependent pdf of protein molecules, the distribution of protein molecules $\rho_p(z)$, and the distribution of solvent molecules $\langle\phi_s(z)\rangle$. From the knowledge of these quantities we can calculate any desired average conformational and thermodynamic quantity. For example, the amount of protein adsorbed on the surface is given by $\rho_p(0)$, and the distribution of polymer segments is given by $\langle n_g(z)\rangle = \Sigma_\alpha P_g(\alpha)\, n_g(z; \alpha)$.

The next step is to write the free energy of the system in terms of the pdf of grafted chain conformations, the z-dependent pdf of protein molecules, and the density profiles of proteins and solvent molecules. Then the minimization of this free energy will provide the functional forms of these quantities.

The ensemble that we are considering is one with a fixed number of grafted polymers at the surface in equilibrium with a bath of constant chemical potential of solvent and protein molecules. Therefore the thermodynamic potential is given by

$$W = F - N_s\mu_s - N_p\mu_p, \tag{5}$$

where $F = E - TS$ is the Helmholtz free energy, E is the internal energy, T is the temperature, and S is the entropy of the system; $N_s = \int_0^\infty N_s(z)\, dz$ and $N_p = \int_0^\infty N_p(z)\, dz$ are the total number of solvent and protein molecules, respectively, and μ_s and μ_p are their corresponding chemical potentials. The internal energy is given by the sum of the bare surface-protein, and surface-polymer interaction, the intramolecular interactions of the proteins and grafted chains and the intermolecular contributions, all of which have been described above. The entropic contributions include 1) the conformational entropy of the grafted chains, given by $S_g = -k_B N_g \Sigma_\alpha P_g(\alpha) \ln P_g(\alpha)$, ($k_B$ is the Boltzmann constant) 2) the conformational entropy of the protein molecules. This quantity must be defined for each z and is given by $S_p(z) = -k_B \Sigma_\gamma P_p(\gamma; z) \ln P_p(\gamma; z)$. The total conformational entropy of the proteins is then $S_p = \int_0^\infty N_p(z)S_p(z)\, dz$; 3) the translational entropy of the protein molecules, $S_{\text{trans, p}} =$

$-\int_0^\infty N_p(z) \ln \rho_p(z)\, dz$; and 4) the translational entropy of the solvent molecules, $S_s = -\int_0^\infty N_s(z) \ln \langle \phi_s(z) \rangle\, dz$.

The polymer chains do not have a translational contribution because of the fact that we are considering the case in which they are grafted to the surface. If the polymer chains are tethered to the surface but have translational degrees of freedom in the surface, e.g., in the case of PEO-lipid liposomes (Lasic and Martin, 1995), a contribution of the form $S_{tran,g} = -N_g \ln \sigma$ must be included. However, this term depends on fixed quantities and therefore its inclusion does not modify the functional form of the quantities of interest.

It is more convenient to write the free energy per unit area of the surface, because that transforms all of the numbers of molecules into densities. Then, summing all of the contributions, we obtain

$$\begin{aligned}\beta \frac{W}{A} = \sigma\Bigg[&\langle U_{gs} \rangle + \langle \epsilon_{intra,g} \rangle + \frac{1}{2}\langle \epsilon_{inter,g} \rangle + \sum_\alpha P_g(\alpha) \ln P_g(\alpha)\Bigg] \\ &+ \int_0^\infty \rho_p(z)\Bigg[\langle U_{ps}(z) \rangle + \langle \epsilon_{intra,p} \rangle + \frac{1}{2}\langle \epsilon_{inter,p} \rangle \\ &+ \sum_\gamma P_p(\gamma; z) \ln P_p(\gamma; z) + \ln \rho_p(z) - \mu_p\Bigg] dz \\ &+ \int_0^\infty \langle \phi_s(z) \rangle [\ln \langle \phi_s(z) \rangle - \mu_s]\, dz,\end{aligned} \tag{6}$$

where $\beta = 1/k_B T$. The ½ in front of the intermolecular interactions is included to avoid overcounting, and the dependency of the intermolecular energies on the pdf's and the distributions is given in Eqs. 1 and 2.

Now we minimize the free energy with respect to $P_g(\alpha)$, $P_p(\gamma; z)$, $\rho_p(z)$, and $\langle \phi_s(z) \rangle$, subject to the packing constraints (Eq. 4), which account for the repulsive intermolecular interactions. The minimization is done introducing Lagrange multipliers, $\beta\pi(z)$, to yield

$$\begin{aligned}P_g(\alpha) = \frac{1}{q_g} \exp\Bigg[&-\beta \epsilon_{int,g}(\alpha) - \beta U_{gs}(\alpha) \\ &- \int_0^\infty \beta\pi(z) n_g(z; \alpha)\, dz \\ &- \beta \int_0^\infty \int_0^\infty n_g(z, \alpha)[\chi_{gg}(z, z') \langle \phi_g(z') \rangle \\ &+ \chi_{gp}(z, z') \langle \phi_p(z') \rangle + \chi_{gs}(z, z') \langle \phi_s(z') \rangle]\, dz\, dz'\Bigg],\end{aligned} \tag{7}$$

where q_g is the normalization factor to ensure $\Sigma_\alpha P_g(\alpha) = 1$. The first term in the exponential comes from the intramolecular energy of the conformation, the second is the surface-polymer interaction, the third is the one associated with the repulsions and arises from the packing constraints, and the fourth is the intermolecular attractions of the chain in conformation α with the "mean field" of the environment.

For the protein pdf we obtain

$$\begin{aligned}P_p(\gamma; z) = \frac{1}{q_p(z)} \exp\Bigg[&-\beta \epsilon_{int,p}(\gamma) - \beta U_{ps}(\gamma; z) \\ &- \int_z^\infty \beta\pi(z') n_p(z'; \gamma)\, dz' \\ &- \beta \int_z^\infty \int_0^\infty n_p(z', \gamma)[\chi_{pp}(z', z'') \langle \phi_p(z'') \rangle \\ &+ \chi_{pg}(z', z'') \langle \phi_g(z'') \rangle + \chi_{ps}(z', z'') \langle \phi_s(z'') \rangle]\, dz'\, dz''\Bigg]\end{aligned} \tag{8}$$

with $q_p(z)$ ensuring normalization of the protein's pdf for each distance z, the terms in the exponential represent the intramolecular energy, the bare surface-protein interaction, the intermolecular repulsions, and the intermolecular attraction of the protein, which at z has conformation γ.

The density profile of protein molecules is given by

$$\rho_p(z) = q_p(z) e^{\beta \mu_p}, \tag{9}$$

which ensures that for all z the chemical potential of the proteins is the same.

Finally, the solvent density profile is

$$\langle \phi_s(z) \rangle = e^{-\beta\pi(z) + \beta\mu_s}. \tag{10}$$

The physical meaning of the Lagrange multipliers can be understood from Eq. 10, i.e., they are the osmotic pressures that arise in the solution to keep the chemical potential of the solvent constant at all z. Another way of looking at it is that $\pi(z)$ is the average repulsive interactions felt by a monomer. A thorough discussion of the physical significance of these quantities can be found elsewhere (Szleifer and Carignano, 1996).

Now we need to determine $\pi(z)$. This is done by replacing the functional forms of the grafted polymers pdf (Eq. 7), the proteins pdf (Eq. 8), the protein density profile (Eq. 9), and the solvent density profile (Eq. 10) in the constraint equations (Eq. 4). The input necessary to solve these equations is the grafted polymer surface coverage, σ, the temperature, the protein chemical potential, the solvent chemical potential, and the set of conformations of the grafted chain and the protein molecule. Because the intermolecular interactions include the average density profiles, these equations must be solved in a self-consistent way. For each surface coverage of polymer and bulk density of protein (or

chemical potential), one of the outputs of the theory is $\rho_p(0)$, which is the amount of protein adsorbed on the surface. Thus, obtaining $\rho_p(0)$ as a function of σ provides the protein adsorption isotherms.

It is important to note that the functional form of the pdf can also be obtained by expansion of the partition function of the system. This has been shown by Ben-Shaul et al. (1985) for chains in amphiphilic aggregates in the absence of solvent, and by Carignano and Szleifer (1993) for polymer chains in contact with solvent molecules. The derivation of the pdf from the partition function provides a better understanding of the approximations involved in the derivation of the pdf. It turns out that the functional form of the pdf is correct and the main approximation arises from the way the $\pi(z)$ are determined. However, as has been shown by comparing with full-scale computer simulations (which provide the exact solution for a given model system), the predictions of the theory are excellent (Fattal and Ben-Shaul, 1994; Carignano and Szleifer, 1995b). This implies that the "mean-field" approximation involved in the packing constraints is very good.

MODEL SYSTEM AND DETAILS OF THE CALCULATIONS

The derivation presented above is very general, and it shows the way that the adsorption of any type of molecules, including proteins, can be treated within the SCMF approach. In the case of protein molecules, one of the inputs of the theory is the set of conformations that the protein has in solution. One of the key differences between folded proteins and homopolymers is that the protein molecules in solution do not search all of their possible configurations, but they are found (mostly) in their native structure. Assuming that the protein does not fluctuate too much around its folded configuration, one can use this structure as input for the theory and then consider only the possible rotations of this fixed structure. We take a more coarse-grained model and assume that the protein in solution is a sphere, the size of which is given by the calculated radius of gyration of the protein of interest, and which has constant intramolecular energy.

Most of the results presented in the next section correspond to the adsorbed protein being spherical at the surface; however, we will specifically study the effects of different configurations when the protein is at the surface. The idea of using a hard-core model for the protein is based on the insight that has been gained in recent years on the adsorption behavior of protein molecules by the use of this type of model (Adamczyk et al., 1994). Furthermore, there is experimental evidence that some proteins at the surface behave like hard-core particles (Arai and Norde, 1990). The calculations presented in the next section correspond to model lysozyme proteins. The motivation for studying this particular protein is twofold. First, it has important practical implications (for instance, in the understanding and the prevention of lysozyme build-up in contact lenses; Missiroli et al., 1991; Castillo et al., 1986). Second, it is a small and compact protein for which the simple protein model provides good results as compared with experimental observations (Szleifer, 1996; McPherson et al., manuscript in preparation). Thus we believe that this simple model includes the main contributions determining the behavior of this small protein in grafted PEO layers.

The solid surface-protein interaction potential that we use is that calculated by Lee and Park (1994) for lysozyme with solid polypropylene surfaces. The atomistic calculated potential takes into account the native structure of the protein and the van der Waals, electrostatic, and hydrophobic protein-surface contributions. This is shown in Fig. 2. As can be seen, the potential is strongly attractive and therefore the proteins will have a tendency to be adsorbed on the surface. For the attractive interactions between the grafted polymer chains and the surface, we will consider two different cases. One is the case in which the surface does not have an effective attractive interaction with the polymer segments. This corresponds to the case of PEO molecules on the surface of liposomes, where it has been observed experimentally that PEO chains do not adsorb to the liposome surfaces (Needham et al., 1992). The second is a preferential attraction of the monomers of the polymers to the surface. We will consider a potential of the form $U_{gs} = -k_BT$ for each monomer of the grafted chain that is at a distance $0 \leq z \leq \delta$ from the surface. This will correspond to a hydrophobic surface, and the value taken for the interaction is the one that was used to quantitatively describe, with the SCMF theory, the measured pressure-area isotherms of PEO chains tethered at the air-water interface (Bessareau et al., manuscript in preparation).

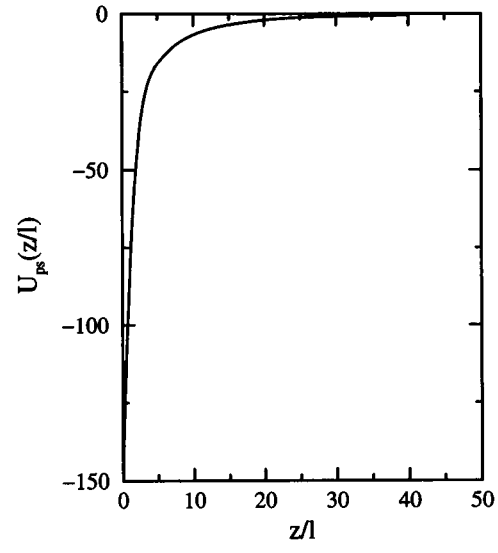

FIGURE 2 The bare lysozyme solid surface interaction, $U_{ps}(z/l)$ in kJ/mol, as a function of the distance from the surface as calculated by Lee and Park (1994). The distances are measured in units of the polymer segments length l.

The systems that we are interested in are those in which the protein and the grafted polymer are soluble in the solvent, i.e., we are considering the good solvent regime for both types of molecules. Therefore all of the interaction parameters for the intermolecular interactions are considered to be equal to zero, $\chi_{ij} = 0$, for all pairs ij.

Now that we have specified all of the detailed interactions necessary to carry out the calculations, we can write the constraint equations to be solved. From Eq. 4, using Eqs. 7–10, we obtain

$$\sigma \sum_{\alpha} \frac{1}{q_g} e^{-\beta \int_0^\infty \pi(z') n_g(z';\alpha) dz' - \beta U_{gs} \int_0^\delta n_g(z';\alpha) dz' - \beta \epsilon_{int,g}(\alpha)} n_g(z;\alpha) v_0 + \int_0^\infty e^{\beta \mu_p} \sum_{\gamma} e^{-\beta \int_0^\infty \pi(z'') v_p(z'',\gamma)/v_0 dz'' - \beta U_{ps}(z')} \frac{v_p(z;\gamma)}{v_0} dz' + e^{-\beta \pi(z) + \beta \mu_s} = 1 \quad 0 \le z \le \infty, \tag{11}$$

where the fact that our model protein has a constant intramolecular energy (and therefore, it does not affect the pdf of the proteins) has been taken into account. In this equation the only unknowns are the average repulsive interactions, lateral pressures $\pi(z)$.

To find the lateral pressures it is useful to discretize space in the z direction in parallel layers of thickness δ. Thus we call layer i the region of space between $z = (i - 1)\delta$ and $z = i\delta$. Then we transform the integral equation (Eq. 11) into the following set of coupled nonlinear equations:

$$[\sigma l^2] \sum_{\alpha} \frac{1}{q_g} e^{[-\sum_j \pi(j) n_g(j,\alpha) - \chi_{gs} n_g(1,\alpha) - \beta \epsilon_{int,g}(\alpha)]} n_g(i,\alpha) \frac{v_0}{\delta l^2} + \sum_{j=1}^{j_{max}} e^{\beta \mu_p} \sum_{\gamma} e^{[-\sum_k \pi(k) v_p(k;\gamma)/v_0 - \beta U_{ps}(j;\gamma)]} \frac{v_p(i;\gamma)}{v_0} + e^{-\pi(i) + \beta \mu_s} = 1 \quad 1 \le i \le i_{max}. \tag{12}$$

The term containing the protein contribution includes the sum over all of the layer j, from which the protein contributes part of its volume to layer i. We have written Eq. 12 with all of the quantities in dimensionless form. l is the length of a polymer segment and v_0 is the volume of a polymer segment. All of the length scales are measured in units of l and the energies in units of β. In all of the calculations presented below, $\delta = 1.86l$ and $v_0/\delta l^2 = 1$.

The chain model for the grafted polymer that we use in all of the calculations is the rotational isomeric state (RIS) model (Flory, 1989), in which each bond can take three different states: a *trans* state, which corresponds to bond i being in the same plane as bond $i - 1$, and *gauche*± states, in which the bond i is at an angle of ±120° with respect to bond $i - 1$. We take the three states to be isoenergetic, and thus $\epsilon_{int,\,g}(\alpha) = 0$ for all conformations α. In this model each segment represents a (CH_2-CH_2-O) group with an effective length l. Thus we have replaced the specific energies of rotations in the real chain by an effective segment bond length. With this model the theory is able to quantitatively predict the adsorption isotherm of lysozyme in PEO grafted surfaces (Szleifer, 1996) and the pressure-area isotherms of PEO chains (Bessareau et al., manuscript in preparation). We generate a large set of single-chain configurations by randomly chosing a sequence of bonds. This sequence of bonds is then positioned in a way such that the first segment is grafted to the surface. All of the conformations considered are self-avoiding within themselves and with the surface, i.e., no segment of the chain is allowed in $z < 0$. Once a self-avoiding conformation is obtained, the spatial distribution of segments is stored, i.e., the set of numbers $n_g(i,\alpha)$. The calculations presented in the next section include sets of 2×10^6 independent configurations for each of the chain lengths considered. For more details on the chain model, the reader is referred to Szleifer and Carignano (1996).

The spatial variation of the volumes for the proteins is obtained from the volume that a sphere with its lowest point at $z = i\delta$ overlaps with layers $j = i$ to j_{max}, where j_{max} corresponds to $z = i\delta + 2r$, with r being the radius of the protein. For the model lysozyme $r = 4.86l$.

Once the sets $n_g(i,\alpha)$ are generated for all α and the volumes of the protein are calculated, we can solve the constraint equations (Eq. 12) for any desired value of the surface coverage of polymer, σl^2, and chemical potential of the protein, μ_p. It has been shown that the chemical potential of the solvent is not needed to carry out the calculations, because of the assumption of volume filling (Carignano and Szleifer, 1994). Then the coupled nonlinear equations for $\pi(i)$ are solved by standard numerical methods for each desired value of the surface coverage.

It should be noted that we need to generate the set of single-chain conformations only once, and then this set is used for any desired conditions. Thus one can study the whole range of surface coverages of grafted polymers just by solving the corresponding sets of nonlinear equations, resulting in a methodology that is not very demanding computationally.

RESULTS AND DISCUSSION

The results presented here are for model lysozyme proteins. Fig. 3 shows the number of adsorbed proteins per unit area as a function of the surface coverage of grafted chains. These results correspond to a surface without attracting interactions with the polymer segments, i.e., $U_{gs}(\alpha) = 0$ for all α. The amount of protein adsorbed decreases as the polymer surface coverage increases. The polymer presents a higher steric barrier to the protein when more polymers are present at the surface. This repulsion, given in the theory by $\pi(z)$, competes against the bare protein-surface attraction ($U_{ps}(z)$, Fig. 2) to determine the amount of adsorbed protein. An interesting result is that from the six chain lengths shown, only $n = 25$ shows a different ability to prevent protein adsorption. From $n = 50$ the amount of protein on

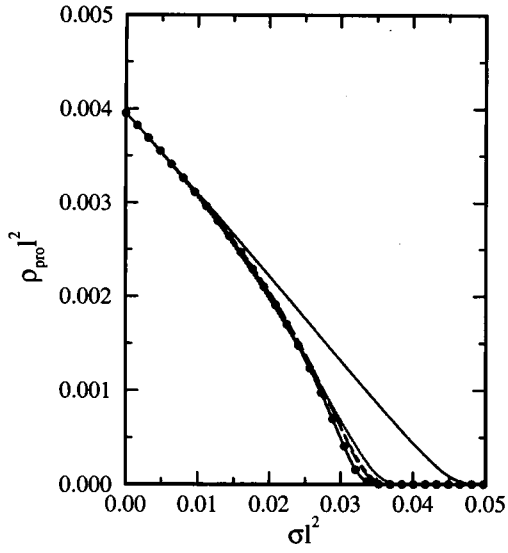

FIGURE 3 Lysozyme adsorption isotherms in PEO grafted polymer layers. The number of proteins at the surface per unit area as a function of the grafted polymer surface coverage. There are six isotherms corresponding to different chain lengths of PEO. Solid line, $n = 25$; dotted line, $n = 50$; dashed line, $n = 75$; long dashed line, $n = 100$; dot-dashed line, $n = 125$; solid line with filled circles, $n = 150$.

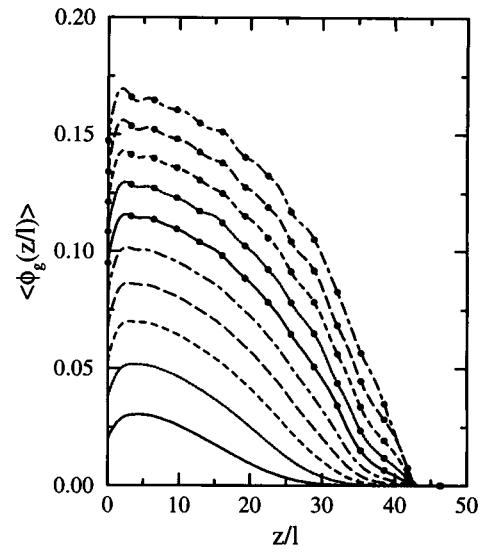

FIGURE 4 The grafted polymer density profiles in the absence of adsorbed protein. Volume fraction of polymer as a function of the distance from the surface. All the curves correspond to $n = 100$. The different surface coverages are: full line, $\sigma l^2 = 0.005$; dotted line, $\sigma l^2 = 0.01$; dashed line, $\sigma l^2 = 0.015$; long dashed line, $\sigma l^2 = 0.02$; dot-dashed line, $\sigma l^2 = 0.025$; full line with filled circles, $\sigma l^2 = 0.03$; dotted line with filled circles, $\sigma l^2 = 0.035$; dashed line with filled circles, $\sigma l^2 = 0.04$; long dashed line with filled circles, $\sigma l^2 = 0.045$; dot-dashed line with filled circles, $\sigma l^2 = 0.05$. The density profiles are normalized such that $\int_0^\infty \langle\phi_g(z/l)\rangle d(z/l) = \sigma l^2 n$.

the surface is independent of the molecular weight of the grafted chain, and it is only a function of the surface coverage.

Before moving into a detailed understanding of why the adsorption behaves as shown in Fig. 3, it is useful to translate the quantities shown into accessible experimental units. As mentioned above, all of the length scales are measured in terms of the segment length; thus if we consider PEO, the segment length is around 3 Å and the unit molecular weight is 44 g/mol. Then the chains considered here correspond to PEO of MW ranging from 1100 g/mol to 6600 g/mol, which is the protocol used in most experimental studies (Harris, 1992a,b; Lasic and Martin, 1995). We see that for chains with MW $\geq$ 2200, the surface coverage from which the protein adsorption is effectively zero is $\sigma \approx 4 \times 10^{-3}$ Å^{-2}, which is in the protocol of surface coverages for which lipid-PEO liposomes can be prepared. Therefore, the results presented here are well within the experimental accessible range of surface coverages and molecular weights.

The decrease in protein adsorption as a function of the grafted polymer surface coverage can be visualized by looking at the grafted chain density profiles. This is shown in Fig. 4 for $n = 100$ for a variety of surface coverages. These are the density profiles of the polymer layer in the absence of proteins. As the surface coverage increases, the density of polymer near the surface increases, providing a larger steric barrier to the protein molecules. However, the density profiles do not give a quantitative measure of the repulsions felt by the protein molecules. The effective interactions of the proteins with the polymer-modified surface is given by the potential of mean force of the proteins with the surface. This is the effective interaction of a protein molecule with the polymer-modified surface averaged over all conformations of the grafted chains and solvent molecules.

The potential of mean force is the work required to bring the protein from the bulk solution to a distance z from the surface (Chandler, 1987). Within the SCMF approach this quantity is given by

$$U(z) = \int_z^\infty \pi(z')v_p(z')\,dz' + U_{ps}(z), \qquad (13)$$

where we have considered only the case in which all $\chi_{ij} = 0$. The first term represents the average repulsions felt by the protein molecule due to the grafted polymers and (if present) other protein molecules, and the second term is the bare surface-protein-attractive interaction. As can be seen from Eq. 13, these potentials are easily calculated once the z-dependent repulsions are known.

Fig. 5 shows the potential of mean force that the proteins feel as a function of the distance from the surface in the case in whcih no protein is adsorbed on the surface. The figure shows the potentials that correspond to the density profiles of Fig. 4. The overall shape of the potential shows a maximum repulsion at a distance corresponding to the maximum in the density profile of the grafted polymers and then decreases at shorter and longer distances. The value of the potential when the protein is in contact with the surface is a

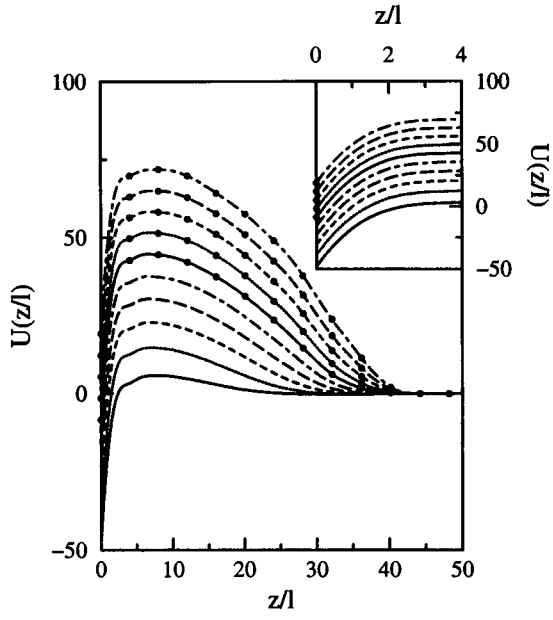

FIGURE 5 The total potential of mean force, $U(z)$ in kJ/mol, as a function of the distance from the surface for $n = 100$ and a variety of surface coverages. Lines are as in Fig. 4. The inset shows the potentials at short distances from the surface.

function of the grafted polymer surface coverage. This is shown in the inset of Fig. 5. It varies from $U(0) \approx -50$ kJ/mol for $\sigma l^2 = 0.005$ and reaches positive values (repulsive potential) for $\sigma l^2 \geq 0.035$. Clearly, if $U(0) > 0$, there will be no protein adsorption. Furthermore, the value of the potential of mean force at contact is found not to be enough to determine the equilibrium amount of adsorbed protein.

An interesting aspect of the shape of the potential of mean force is that it shows a maximum at a distance from the surface that corresponds to the maximum in the density profiles of the polymers. Although the maximum has no effect on the equilibrium amount of protein adsorbed, it is very important in determining the kinetics of the adsorption process. Actually, one can study the kinetics of adsorption by performing Brownian dynamics simulations on the potential of mean force. For the cases shown in Fig. 5, one expects the adsorption process to be slower as the surface coverage of polymer increases. This is due to the increasing value of the maxima on the potential of mean force as the surface coverage of grafted polymer increases.

Jeon and Andrade (JA) (1991) calculated the steric repulsion that a grafted layer presents to a spherical protein based on the Alexander-deGennes theory of polymer brushes. According to their approach, the protein-brush steric repulsion is given by

$$\frac{U(z)}{k_B T} = 4\pi R^2 \frac{k_1}{l^2}\left(\frac{7}{5}\frac{k_2}{k_1}\right)^{5/12} n\sigma^{11/6}\left[\left[\left(\frac{h}{z}\right)^{5/4} - 1\right] + \frac{5}{7}\left[\left(\frac{z}{h}\right)^{7/4} - 1\right]\right], \quad (14)$$

where h is the height of the brush and, according to their theory, is given by $h = (5/7\ k_1/k_2)^{1/3} ln\sigma^{1/3}$. k_1 and k_2 are constants.

Fig. 6 shows a comparison of the predicted repulsive potentials as predicted by the SCMF theory and by the JA approach using the parameters $k_1 = 0.007$ and $k_2 = 0.02$, which according to JA are the ones that correspond to PEO with 100 monomers. Also shown are the predictions of the JA approach, but with the parameters $k_1 = 0.07$ and $k_2 = 0.05$, which are the parameters from which the height predicted from their theory is in agreement with the results of the SCMF approach for $n = 100$. The steric repulsions predicted by JA are monotonically decreasing functions of the distance from the surface, and the ones obtained from the SCMF theory show a maximum. Furthermore, there is no quantitative agreement between the predictions of the theories for any set of parameters k_1 and k_2. Note that for $\sigma l^2 = 0.02$ the prediction of the JA approach for the steric repulsion at contact is a factor of ~3 larger than the SCMF theory for the parameters that fit the height of the brush, and a factor of ~3 smaller if the parameters reported by Jeon et al. are used.

The reasons for the large discrepancies in the predictions from the two theories is that the Alexander-deGennes theory for polymer brushes is intended for very long chain lengths (Alexander, 1977). Actually, it has recently been shown that the chain length for which their predictions are appropriate is for $n > 1000$ (Martin and Wang, 1995), well beyond the experimentally used PEO for biotechnological applications

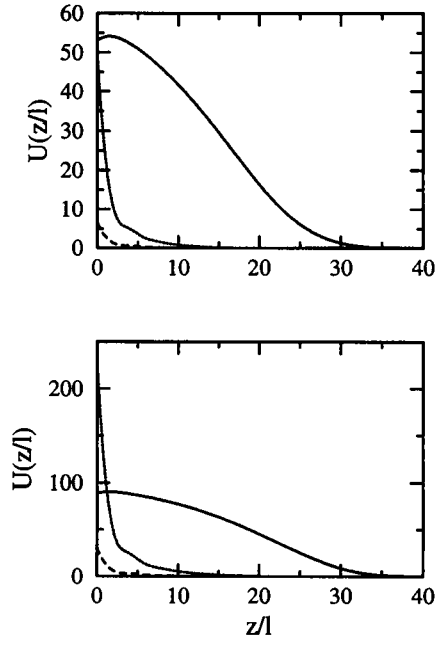

FIGURE 6 The steric repulsive contribution to the potential of mean force, in kJ/mol, as a function of the distance from the surface. The solid lines are the calculations from the SCMF theory; the dashed line corresponds to the JA approach with the parameters $k_1 = 0.007$ and $k_2 = 0.02$; the dotted line corresponds to the parameters $k_1 = 0.07$ and $k_2 = 0.05$. See text. The upper graph is for $\sigma l^2 = 0.01$, and the lower one is for $\sigma l^2 = 0.02$.

and, in particular, beyond the value used in PEO-lipid liposomes. Furthermore, the repulsive interactions were derived in the cases of interacting surfaces (Patel et al., 1988), and not the specific interactions of a relatively small sphere with the grafted layer. We believe that the predictions of the SCMF theory are correct because of the quantitative agreement found between the calculations from this theory and experimental and simulation data on thermodynamic and structural properties of grafted polymer layers composed of intermediate-chain-length molecules (Carignano and Szleifer, 1995a), and because of comparisons with experimental adsorption isotherms of lysozyme on PEO grafted surfaces (Szleifer, 1996).

All of the discussion so far has been centered on the effect of the grafted chains in the absence of any adsorbed protein. Clearly, as proteins start to adsorb there will be an additional repulsive interaction near the surface due to the presence of the protein molecules. Moreover, the average configurational properties of the grafted chains will be modified by the presence of the protein molecules. The density profiles shown in Fig. 4 correspond to the minimum free energy of the system in the absence of protein molecules. Once the proteins adsorb there will be a competition near the surface between the entropic degrees of freedom of the chains, the polymer-protein repulsions, and the gain in attractive interactions that the protein undergoes when it is in contact with the surface.

A way to visualize the effectiveness of the grafted chains in reducing protein adsorption is by looking at the average shape of the polymer with its neighboring molecules (Carignano and Szleifer, 1995a). This is quantified here by the z-dependent radius of gyration; i.e., we calculate

$$\langle R^2_{g,xy}(z)\rangle = \frac{1}{2}[\langle R^2_{g,x}(z)\rangle + \langle R^2_{g,y}(z)\rangle]$$

$$= \frac{1}{2n}\sum_{\{\alpha\}} P(\alpha)\left[\sum_{i=1}^{n}(x_i(z;\alpha) - x_{cm}(z;\alpha))^2 + (y_i(z;\alpha) - y_{cm}(z;\alpha))^2\right], \quad (15)$$

where cm denotes center of mass. This quantity provides a better measure of the lateral dimensions of the chains, as a function of z, than the density profiles.

Fig. 7 *A* shows the shape of the polymer chains in the absence of protein in the surface. The two neighboring molecules are separated by a distance $(\sigma l^2)^{-1/2}$. Also shown in the figure is the space a protein would need at the surface to adsorb between the two grafted polymers. There is a large overlap between the protein and the grafted chains. This could suggest that there will be no protein adsorption on the surface; however, as seen from Fig. 3, for this surface coverage of polymer, a relatively large amount of protein is adsorbed on the surface. The reason for this is that the structure of the polymer layer in the absence of any adsorbed protein does not provide the complete picture. Namely, the proteins can adsorb to the surface by changing the structure of the grafted polymers to achieve the best compromise between conformational entropy of the polymers, protein-polymer repulsions, and surface-protein attractions. This can be seen in Fig. 7 *B*, which shows the shape of the polymer chain when proteins are adsorbed at the density given in the isotherm of Fig. 3.

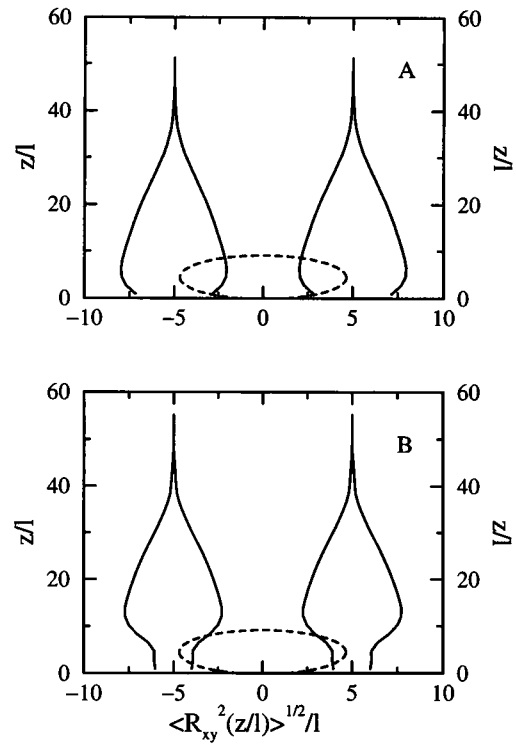

FIGURE 7 Two neighboring chains represented by their average shapes. The shape is given by the z/l-dependent lateral component of the radius of gyration as a function of the distance from the grafting wall. The distance between the tethering points is $(\sigma l^2)^{-1/2}$. The results are for $n = 100$ and $\sigma l^2 = 0.01$. (*A*) The shapes of the polymer chains correspond to those in the absence of adsorbed protein. The dashed line represents the "space" that a protein molecule will need to adsorb at the surface. Note the large overlap of the protein with the unperturbed polymers. (*B*) The shapes of the polymer chains correspond to those in the presence of adsorbed proteins on the surface. Note how the protein molecules push segments of the grafted polymers further away from the surface to accommodate themselves near the surface. See text.

The average shape of the grafted polymers in Fig. 7 *B* clearly reflects the fact that the protein molecules push the segments of the polymer that are close to the surface to make enough space to accommodate themselves near the surface. The degree of deformation of the polymer chains depends upon the polymer surface coverage.

The average shape of the molecules provides an insightful picture of how the grafted chains and the protein molecules share the available volume. It also shows the competition between the strong attractive interaction of the

proteins with the solid surface, the conformational degrees of freedom of the grafted polymers, and the polymer-protein repulsions. However, it is also clear that to understand the adsorption process it is not enough to look at the grafted layer in the absence of the protein molecules. Furthermore, as can be seen from Fig. 4, the density profiles of the grafted chains change with surface coverage because of the repulsions between the chains. Thus, looking at a single chain density map from the surface, as has been done by Torchilin and co-workers (1995), does not provide a complete picture of the ability of the grafted chains to prevent protein adsorption. Namely, the effect of surface density and the specific interactions with the protein play an essential role in the ability of the grafted chains to prevent protein adsorption, and therefore all of them should be taken into account for a proper description of the system.

The ability of the proteins to push some of the polymer segments is a function of the surface coverage. For low surface coverages fewer polymer molecules on the surface are displaced, and thus more protein is adsorbed. The density profiles of grafted polymer chains with proteins adsorbed at the equilibrium adsorption density are shown in Fig. 8 *A*. For low surface coverage there is a large change in the structure of the grafted polymers (compare with the density profiles in the absence of proteins, Fig. 4). For surface coverages $\sigma l^2 \geq 0.03$, the density profile of the grafted layer is almost identical to that in the absence of proteins, the reason being that there is almost no protein adsorption for these surface coverages (see Fig. 3). To complement this picture, Fig. 8 *B* shows the density profile of the adsorbed proteins for the same surface coverages of polymer. The shapes of the density profiles are a reflection of the spherical shape of the model proteins; namely, the proteins that contribute to these density profiles are those adsorbed at the surface. On a scale that cannot be observed in the figure, one can see that in the range $10 \leq z \leq 40$ there is a depletion of proteins at all surface coverages. This is due to the large density of polymer segments there (Fig. 8 *A*). For $z > 40$ the protein concentration goes to its bulk value (in all the calculations here ϕ_p (bulk) = 0.001).

All of the discussion of structure thus far has been centered on the effect of surface coverage at a fixed chain length. The adsorption isotherms are independent of chain length for $n \geq 50$. We analyze this behavior below, i.e., we look at the effects of molecular weight at fixed surface coverage. Fig. 9 *A* shows the density profiles for different chain lengths all at $\sigma l^2 = 0.01$ in the absence of adsorbed protein. For $n \geq 50$ the density of polymer segments in the range $0 \leq z \leq 5$ is the same for all chain lengths. The radius of the protein is 4.86, and we see that the amount of polymer segments that the protein must push further from the surface is the same for all chain lengths larger than 50, resulting in the same amount of adsorbed protein. $n = 25$ has a different profile, and its maximum is lower than for the other molec-

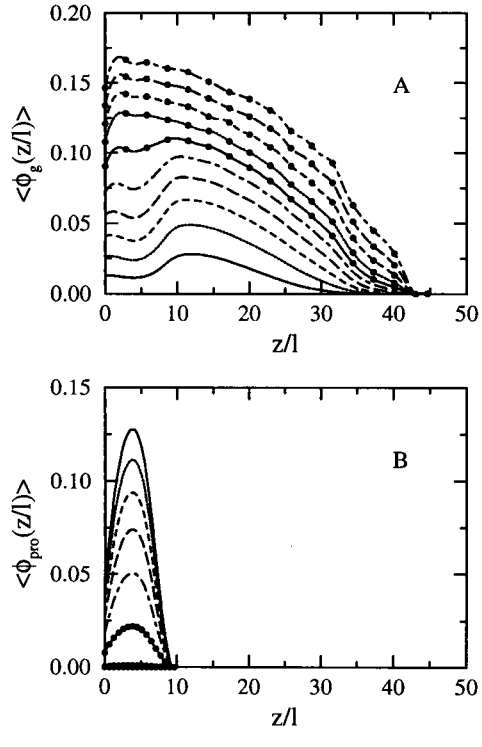

FIGURE 8 Density profiles of (*A*) grafted polymer and (*B*) protein for surfaces grafted with $n = 100$ polymers for the cases in which the amount of protein adsorbed is that given by the adsorption isotherm (Fig. 3). The lines correspond to the same cases as in Fig. 4. The protein density profile is normalized such that $\int_0^{2r/l} \langle \phi_{pro}(z/l) \rangle d(z/l) = \rho_{pro} l^2 v_p / v_0$.

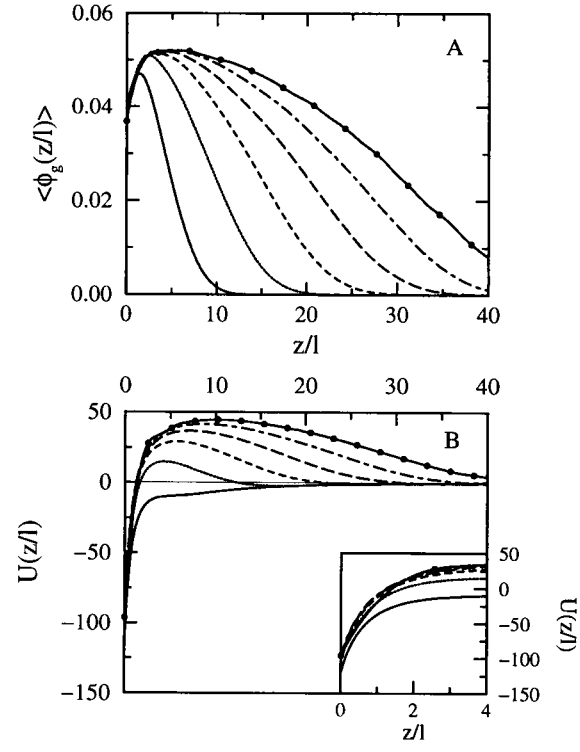

FIGURE 9 (*A*) The grafted polymer density profiles and (*B*) the total potential of mean force, in kJ/mol, as a function of the distance from the surface in the absence of adsorbed protein for a fixed surface coverage, $\sigma l^2 = 0.01$, and a variety of chain lengths. Lines are as in Fig. 3.

ular weights. Thus more proteins can approach the surface for this short-chain-length polymer, resulting in larger protein adsorption. For proteins of different sizes there will be a different molecular weight threshold from which the adsorption isotherm will be independent of the grafted polymer chain length.

The structural features described in relation to the density profiles can be seen directly in the potentials of mean force of the protein with the surface in the absence of adsorbed protein, as shown in Fig. 9 *B*. In the inset of the figure one can see that the potential at contact is the same for all chain lengths $n \geq 50$. However, the overall shape of the potentials as a function of the distance from the surface is strongly dependent on chain length, including the location and height of the repulsive barrier. For $n = 25$ the potential at the surface coverage shown is attractive for all z. Therefore, one would expect that the kinetics of protein adsorption would be rather fast. On the other hand, for all of the other chain lengths there is a marked potential barrier, and the maximum repulsion is larger the longer the chain length. Thus the rate of protein adsorption will be slower the longer the chain length.

An estimate of the rates of adsorption can be obtained by using transition state theory (TST) in which the rate is proportional to the Boltzmann factor of the maximum of the potential energy barrier (Atkins, 1994). Taking the barrier height from Fig. 9 *B*, we plot the exponential factor as a function of chain length for polymers with $n \geq 50$ in Fig. 10. Assuming that the preexponential factor is the same for all of the different chain lengths, we see that for this particular surface coverage, the rate of protein adsorption is 5 orders of magnitude slower for $n = 150$ than for $n = 50$. Thus, although the equilibrium adsorption is identical for all of those chain lengths, the kinetics may be slow enough that for practical purposes the longer chain lengths can be used for the kinetic prevention of protein adsorption.

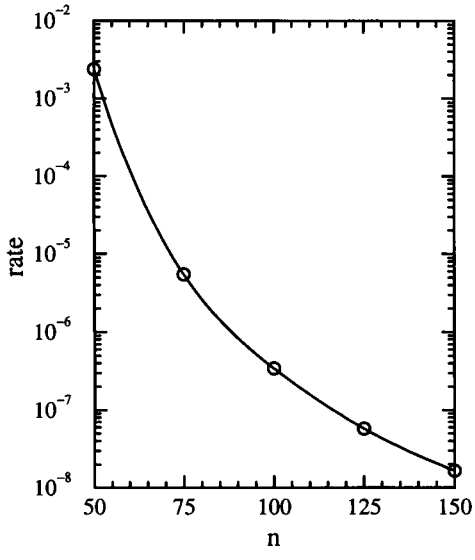

FIGURE 10 The Boltzmann factor of the maximum of the potential of mean force (rate), from Fig. 9 *B*, as a function of the grafted polymer chain length.

It should be stressed that the kinetic behavior of the adsorption process is very complex and TST will probably not be enough for a proper description of the time-dependent behavior. However, the shapes of the potentials of mean force show that the differences in rates as a function of molecular weight of the grafted polymer will be larger than those shown in Fig. 10. Therefore, the TST-like approach provides a proper lower bound to the chain length dependence of the initial adsorption rates. As proteins start to adsorb, the potential of mean force changes (see *inset* of Fig. 15 below), and therefore, to be complete, a description of kinetic behavior must consider the varying potential as a function of adsorbed protein, i.e., the effective potential is time dependent.

Conformational changes of the protein

All of the results presented above are for the case in which it is assumed that the protein keeps its structure at the adsorbing surface. This does not need to be the case, and recent experimental observations suggest that upon adsorption the T4 lysozyme protein changes its configuration from a sphere-like shape in the bulk to a pancake configuration (Billsten et al., 1995). A complete treatment of this kind of transformation would require the understanding of the whole conformational phase space of the protein and its changes in the vicinity of the surface. This is a formidable task and cannot be done, at the present time, even for small proteins in solution. Thus we assume that the protein can assume two different conformations upon contact with the surface. One is the same analyzed above, namely a spherical structure with the interaction potential shown in Fig. 2. The second is a disk-like configuration in which the height of the "pancake" is equal to the radius of the spherical configuration, and the area is such that the volume is the same as in the spherical configuration. The bare attraction of the pancake configuration to the surface is taken to be $\frac{4}{3}U(0)$, where $U(0)$ is the bare attraction at contact of the spherical configuration (see Fig. 2). The pancake configuration exists only at contact with the surface, and at all other distances from the surface the protein is spherical; its bare interaction with the surface is as in Fig. 2. Note that we are interested in the equilibrium adsorption; thus we do not need to consider the fact that there may be a high energy barrier for the change of the configuration of the protein upon contact with the surface. Such a barrier will determine the kinetic behavior of the conformational transformations on the surface.

The reason for choosing the more attractive surface-protein interaction for the pancake configuration is that experimental observations suggest that this is the configuration of adsorbed proteins (Billsten et al., 1995). However, the 4/3 factor is arbitrary, and it was chosen to look at the competition between the two possible structures of the protein at the surface in the presence of grafted polymers.

Fig. 11 *A* shows the total amount of protein adsorbed (both configurations) as a function of the grafted chain surface coverage for a variety of chain lengths. The amount of protein adsorbed is slightly larger than when only the spherical configuration is considered (Fig. 3), because the pancake configuration has a stronger attractive interaction. However, as is the case when considering only the spherical configuration, the equilibrium adsorption is independent of chain length for $n \geq 50$. The reasons for the independence of chain length are the same as discussed above.

Fig. 11 *B* shows the adsorption of each configuration as a function of the polymer surface coverage. For all surface coverages there are more pancake than spherical configurations at the surface. In the absence of grafted polymers, $\sigma l^2 = 0$, the pancake configuration has a larger population because of the stronger bare attraction to the surface.

It is important at this point to note that we are not attempting to explain directly the experimental observations of T4 lysozyme, which was suggested to adsorb in pancake-only configurations. Our goal is to study the competition between different structures of adsorbed proteins and how this competition changes with grafted polymers. We could obtain only pancake configurations on the surface (with no grafted polymers on the surface) if we assume a larger difference in the bare attractive interaction of the protein with the surface. However, because we do not have better structural information on the protein at the surface, our aim is to study how simple conformational changes at the surface modify the adsorption behavior of the protein.

For the surfaces with grafted polymers it can be observed (Fig. 11 *B*) that the pancake configuration has a constant adsorbed amount up to $\sigma l^2 \simeq 0.03$, whereas the spherical configuration shows a sharp decrease in this same range of σl^2. The different behavior of the two configurations is due to the balance between the steric repulsions that the grafted polymers present to the protein and the bare surface-protein attraction. For the pancake configuration, the repulsions arise from the segments of the chains near the grafting surface. These segments are pulled out from the surface (see Fig. 8) because of the presence of the protein, and thus they present a very high repulsion to the spherical configuration, which feels maximum repulsions at distances from the surface that the adsorbed pancake configuration does not reach. Furthermore, the solid surface-protein attraction is stronger for the pancake configuration. Therefore, the optimal surface adsorption as the grafting density increases is reached by pulling out the spherical configuration but leaving the pancake configuration constant. For $\sigma l^2 > 0.03$, the only type of protein adsorbed is the pancake, and an additional increase in grafted polymers surface coverage results in an increase in the repulsions and, thus, in a further reduction of the amount of protein adsorbed, in this case the pancake configuration.

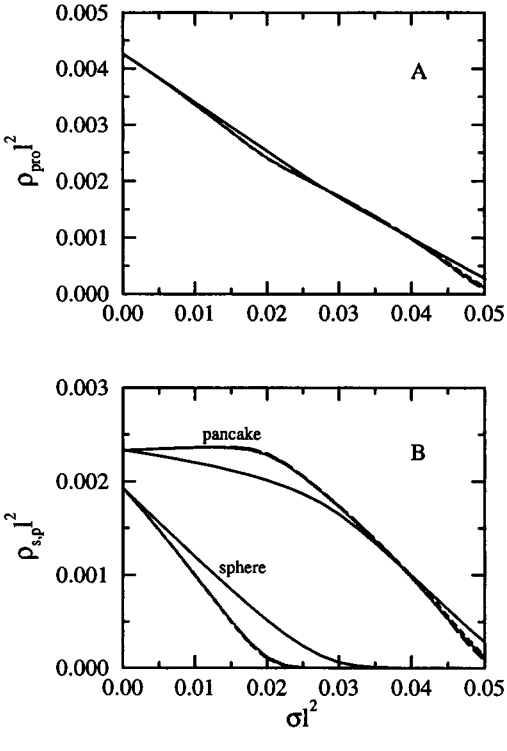

FIGURE 11 (*A*) The adsorption isotherms, amount of protein adsorbed per unit area as a function of grafted polymer surface coverage, for the case in which two possible protein configurations can adsorb at the surface. (*B*) The adsorption of each configuration, sphere and pancake, as a function of the grafted polymer surface coverage. The lines are as in Fig. 3.

Adsorbing polymer segments

The amphiphilic nature of the ethylene oxide segments induces a preferential adsorption of the monomers toward hydrophobic surfaces. Thus pure PEO molecules show adsorption from water solutions into hydrophobic surfaces (Kidane, personal communication). Furthermore, it has recently been shown that EO segments adsorb even at the water-air interface (Bijsterbosch et al., 1995; Bessareau et al., manuscript in preparation). Therefore it is important to understand the effect that the attractive interaction of EO segments with the surface has on the adsorption behavior of proteins.

All of the results shown in this section correspond to the case in which the polymer segments gain an energy of k_BT when they are at a distance $z \leq \delta$ from the surface. This particular value of the attractive interaction has been shown to correspond to the case of EO monomers at the water-air interface (Bessareau et al., manuscript in preparation). Highly hydrophobic surfaces may have a larger value. However, the results shown in this section do not change qualitatively for larger attractions. Thus this particular value of the attractive energy is enough to study the main differences between surfaces that do not have a preferential attraction to the EO monomers, which are the type of surfaces as discussed above, and those that do. Again, we use the same $U_{ps}(z)$ as given in Fig. 2 to be able to isolate the effect of the surface-polymer attractions on the adsorption isotherms. Furthermore, the results presented in Figs. 12 through 15

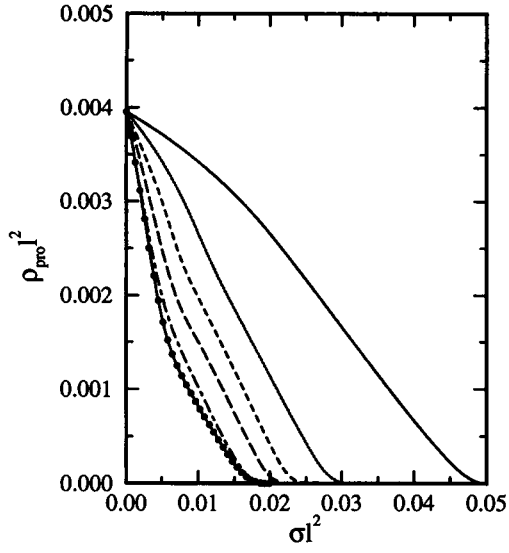

FIGURE 12 The adsorption isotherms of lysozyme as a function of PEO surface coverage in the case that the EO segments have an attractive interaction with the surface. The lines correspond to the same chain length of grafted polymers as in Fig. 3.

correspond only to spherical proteins adsorbing to the surface. Fig. 16 shows the effect of conformational changes.

Fig. 12 shows the adsorption isotherms of model lysozyme proteins as a function of the surface concentration of grafted polymer for a variety of chain lengths. The dependence of the adsorption isotherms on chain length is qualitatively different from the case in which the polymer segments experience no attraction to the surface. Namely, when the monomers of the grafted polymers have a preferential attraction to the surface, the longer the polymer, the more effective is the prevention of protein adsorption at fixed surface coverage. This behavior is very different from the one found in the case of nonattracting surfaces, in which it was shown (Fig. 3) that the adsorption is independent of chain length for $n \geq 50$.

Another interesting result obtained by comparing Figs. 12 and 3 is that for fixed chain length, and $n \geq 50$, the amount of protein adsorbed at all surface coverages is smaller in the case that the monomers of the grafted chains are attracted to the surface. The opposite behavior is observed for the shortest chains, i.e., $n = 25$.

We consider first the anomalous behavior of $n = 25$. The understanding of the differences between the attractive and nonattractive surfaces can be obtained by looking at the density profiles of the polymers for different surface coverages in these two cases in the absence of adsorbed proteins. This is shown in Fig. 13. The different shapes of the density profiles are a direct manifestation of the different surface monomer interactions. The reason that the attractive surface has more protein adsorption than the nonattractive one can be seen by looking at the density of polymer segments at $z \simeq 5$, where proteins sitting at the surface feel the largest repulsions (recall that the radius of the spherical

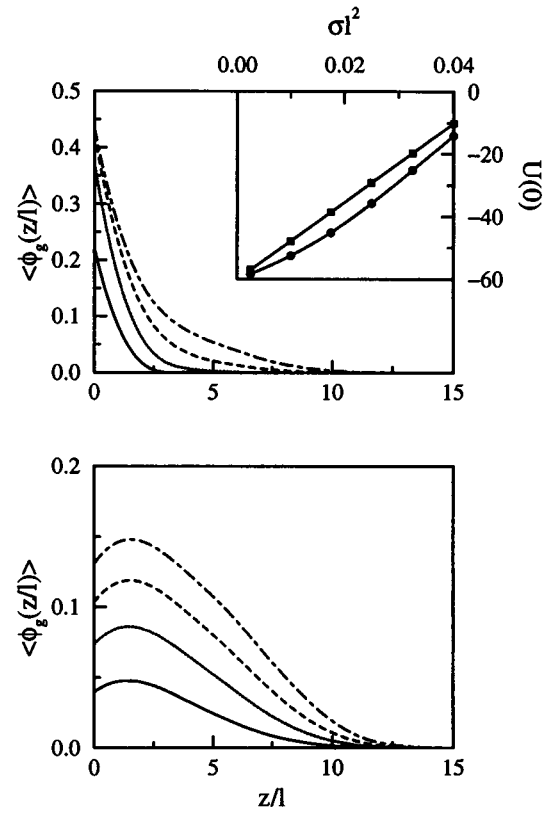

FIGURE 13 The grafted polymer density profiles for adsorbing PEO surfaces (*upper graph*) and nonadsorbing surfaces (*lower graph*) in the absence of adsorbed proteins. All for $n = 25$. The lines correspond to: solid line, $\sigma l^2 = 0.01$; dotted line, $\sigma l^2 = 0.02$; dashed line, $\sigma l^2 = 0.03$; dot-dashed line, $\sigma l^2 = 0.04$. The inset shows the potential of mean force at contact as a function of the grafted polymer surface coverage. The circles correspond to the EO adsorbing surface, and the squares to the nonadsorbing surface.

protein is 4.86). The figures show that the density of polymer monomers at that position is higher for the nonattractive surface, i.e., stronger repulsion. This can be quantified in the potential of mean force at contact, which is shown in the inset of the figure. The resulting total interaction of the surface with the protein is more attractive in the case in which the polymer segments are attracted to the surface, for all grafted polymer surface coverages.

For longer chains the nonattractive surface can adsorb more proteins, because even at low surface coverage the number of polymer segments that the proteins must pull out of the surface (segment desorption) in the attractive case requires too much energy as compared to the entropic cost necessary for pulling segments further from the surface for nonattractive surfaces (see Fig. 7). This can be seen in the density profiles of the grafted polymers for a variety of chain lengths in the absence of adsorbed protein. This is shown in Fig. 14 *A* for $\sigma l^2 = 0.01$, where it can be observed that the number of polymer segments at the surface largely increases for $n > 25$, but also that the profiles are different for the different chain lengths for $z \leq 5$ (compare with the nonattractive case, Fig. 9 *A*). This explains why the polymer

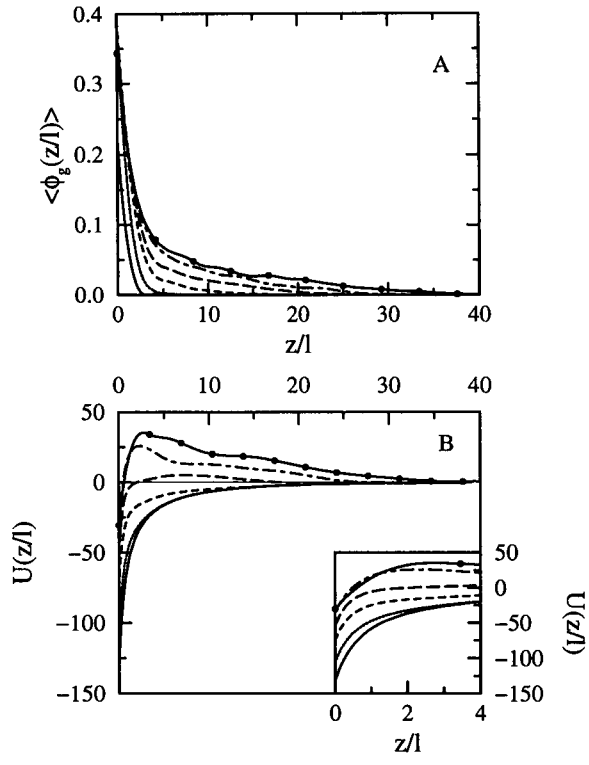

FIGURE 14 (*A*) The density profiles of PEO and (*B*) the total potential of mean force, in kJ/mol, as a function of the distance from the surface, for EO-adsorbing surfaces in the absence of adsorbed protein. All of the curves are for $\sigma l^2 = 0.01$, and the lines correspond to the chain lengths as in Fig. 3. The inset shows the potentials near the surface.

attractive surfaces with $n \geq 50$ are more effective in preventing protein adsorption than are the nonattractive ones. Furthermore, it also shows why there is a strong molecular weight dependence in the adsorption isotherms.

Fig. 14 *B* shows the potential of mean force for polymer attractive surfaces. As a result of the large monomer concentration near the surface (Fig. 14 *A*), the potential of mean force at contact is less attractive than in the case of no attraction with the grafted polymers (Fig. 9 *B*). Moreover, there is a large dependence of the potential at contact (Fig. 14 *B*, *inset*) on grafted polymer chain length. Another important result is that the barrier and range of the repulsive tail of the potential are much weaker than in the nonattracting case. This is due to the relatively small number of polymer segments not in contact with the surface as compared to the nonattractive case. Therefore, from the kinetic viewpoint it will probably be faster to adsorb the proteins in the case of attractive surfaces, but the equilibrium amount adsorbed will be smaller than in the nonattracting case.

The structure of the attracting surface grafted polymer layer is rather different from the case of nonattracting surfaces. In the former there is always a large portion of the polymer segments in the close proximity to the surface. Therefore, even though these layers are more efficient in preventing protein adsorption than the nonattracting grafted polymer layers, the steric barrier is much shorter range. If the range of these repulsions is shorter than the bare surface-protein attraction (Fig. 2), then there is the possibility of a second layer of adsorbed proteins. This is shown in Fig. 15, which displays the density profile of adsorbed proteins as a function of the distance from the surface for a variety of surface coverages. For the lowest surface coverage of grafted polymer two clear adsorbed layers can be observed. The first one is in contact with the surface, and the second is with the proteins at $z \simeq 10$, a value close to the diameter of the protein. The presence of the proteins at the surface, in addition to the grafted polymer layer, presents a steric barrier for other proteins attempting to approach the surface. This is clearly seen in the potential of mean force evaluated at the equilibrium conditions of proteins and grafted polymers shown in the inset of Fig. 15. At contact there is a pronounced minimum followed by a large barrier due to both grafted polymers and adsorbed proteins. After the barrier, a second and less pronounced minimum is found where the proteins can form this second layer of adsorption.

The second minimum in Fig. 15 has a strength of -6.5 kJ/mol. This is not a very strong binding energy, but the effect of a secondary adsorption layer may be expected to be important in very large proteins, where the bare surface-protein interactions are longer ranged than the model lysozyme that we are describing here. Preliminary experimental observations of the adsorption of fibrinogen on surfaces with grafted PEO molecules (McPherson, personal communication) show a decrease in adsorption as the surface

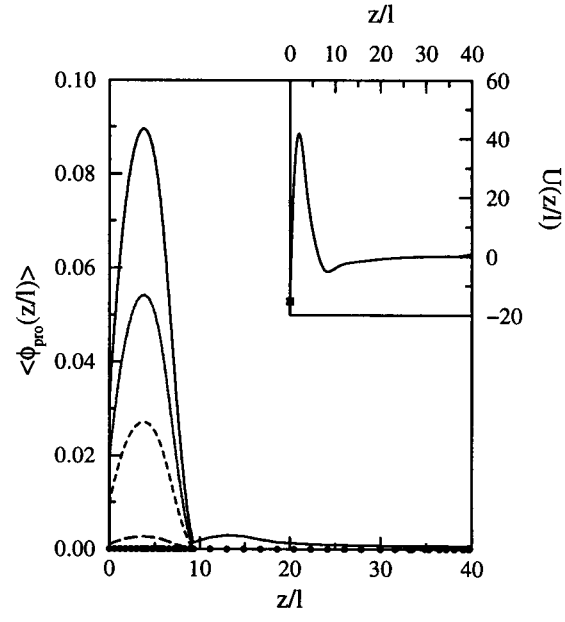

FIGURE 15 The volume fraction of protein as a function of the distance from the surface, for the densities corresponding to the adsorbed amounts from Fig. 12, i.e., for surfaces that adsorb PEO, all for $\sigma l^2 = 0.01$. The lines correspond to the same chain length as in Fig. 3. The inset shows the total potential of mean force, in kJ/mol, of the proteins with the surfaces with adsorbed proteins for $n = 25$ and $\sigma l^2 = 0.01$. Note the presence of a second minimum in the potential, which is responsible for the formation of a small second layer of adsorbed protein; see text.

coverage of polymer increases. However, it reaches a constant value, even at relatively large surface coverages of grafted polymers. This may be the result of these large proteins laying on top of the grafted layer because of long-range attractions to the surface. More experimental and theoretical work is necessary to fully understand and predict this behavior.

The partition between the pancake and spherical configuration in the nonattracting case was shown to be dominated by the stronger attractive interactions of the pancake configuration with the surface. The structure of the grafted layer when the polymer monomers are attracted to the surface is different from that in the nonattractive case. The large concentration of polymer monomers in close proximity to the surface results in stronger repulsions for the pancake configurations than for the spherical one. This is reflected in Fig. 16, in which the total adsorption isotherm and those of the pancake and spherical configurations are shown for one chain length. In the absence of any grafted polymers on the surface, the amount of pancake configurations adsorbed is larger than that of the spherical configuration. As the surface coverage of grafted polymers increases, there is a very sharp decrease in the amount of pancakes, accompanied by an increase in the spherical configurations. This interplay results from the dominant effect of the repulsive interactions that the grafted polymers attracted to the surfaces exert on the pancake configuration. Thus, even though the bare attraction of the pancake is larger than that of the spherical configuration, the strong repulsions at very short distances from the surface make the pancake configuration, which has all of its volume in the vicinity of the surface, a very unfavorable choice. The sharp decrease in the number of pancake configurations with grafted polymer surface coverage leaves some space for the number of spherical configurations to increase, even though the overall adsorption is monotonically decreasing. Once there are no pancake configurations on the surface, the number of spherical configurations adsorbed starts to decrease again.

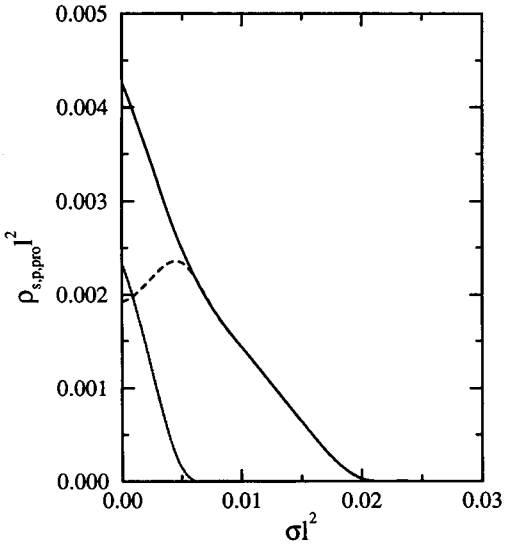

FIGURE 16 The total protein adsorption isotherm (*solid line*), the pancake configuration adsorption (*dotted line*), and the spherical configuration adsorption (*dashed line*) as a function of the grafted polymer surface coverage. The isotherms are for adsorbing PEO surfaces, and the results correspond to grafted polymers with $n = 100$.

This interplay between attractive and repulsive interactions, and the resulting qualitatively different isotherms for grafted polymers that have attractive interactions with the surfaces and those who do not, shows that each specific case of protein, surface and grafted polymer, must be treated in detail for researchers to be able to predict the overall behavior of the different systems.

CONCLUSIONS

We have presented a general theoretical approach to studying the adsorption of protein molecules on surfaces with grafted polymers. The theory provides a general framework for studying the structural and adsorption behavior of the protein-polymer mixture as a function of the thermodynamic variables of the problem: temperature, bulk protein concentration, and polymer surface coverage, among others. The basic idea of the theory is to look at a central molecule, polymer or protein, with its intramolecular and surface interactions taken "exactly" into account and the intermolecular interactions considered within a mean-field approximation. Thus, for each type of molecule in the system, grafted polymer and protein, one must look at all of the possible configurations of a single molecule; the probability of that chain conformation will be given by its intramolecular and surface Boltzmann factors and the Boltzmann factor resulting from the intermolecular repulsive and attractive interactions that are derived from the theory. The intermolecular interactions are given by an interaction "field" that depends on the distance from the surface due to the inhomogeneous arrangements of the molecules induced by the presence of a surface.

We have shown that the application of the theory provides a wealth of information on the structural and thermodynamic properties of the grafted polymer layers in the absence and in the presence of protein molecules. We have studied the adsorption isotherms and the potentials of mean force of simple model proteins in a variety of conditions. However, this is the first step toward a better understanding of the equilibrium adsorption of protein molecules on surfaces with and without grafted polymer molecules. Several important questions must be answered to see the validity of the simple model taken for the protein. Clearly, we must incorporate more information on the statistical conformations of the protein molecules close to and far from the surface (Dill and Stigter, 1995; Scheraga, 1996; Shakhnovich et al., 1996). This will enable us to predict which conformations are preferred in the vicinity of the surface as compared to the folded structure of the protein on the bulk. Moreover, more detailed configurational information will enable us to calculate a conformation-dependent surface-

protein interaction that is necessary for a more complete understanding of the thermodynamic and kinetic behavior of these systems.

None of the cases studied in this work incorporated the fact that protein molecules are inhomogeneous systems. Thus different regions of the protein may interact more or less favorably with the grafted polymers. Moreover, it may also be important to incorporate inhomogeneities parallel to the adsorbing surface. Furthermore, the kinetics of protein adsorption must be studied in more detail. Our theoretical framework makes possible the calculation of the potentials of mean force. These potentials depend on the specific configurations of the protein molecules. For example, stretched configurations of the proteins may be able to penetrate the grafted polymer layer much more rapidly than bulky configurations.

The application of the general theoretical framework presented here in a simple model is the first step toward the rational development of a more detailed understanding of how the effects enumerated above affect the adsorption behavior of protein molecules. Furthermore, this simple model provides good agreement with recent experimental observations of the adsorption of lysozyme on pluronic modified surfaces and makes possible an understanding of the experimental studies (Szleifer, 1996; McPherson et al., 1995). It is an encouraging sign that the approach can be used to understand and design biocompatible surfaces and, with the incorporation of the effects mentioned above, it should provide guidelines about the level of sophistication necessary in the theory for different purposes.

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