Stabilization of surface-immobilized enzymes using grafted polymers

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(Received 20 January 2004; published 13 September 2004)

We introduce a two-dimensional lattice model of immobilization and stabilization of proteinlike polymers using grafted polymers. The protein is designed to have a specific bulk conformation reproducing a catalytic cleft of natural enzymes. Our model predicts a first order denaturing adsorption transition of free proteins. On the other hand, for an immobilized protein we observe a more gradual disappearance of the hydrophobic centers accompanied by adsorption. We show that, using hydrophilic grafted polymers of proper length and grafting density, the conformation as well as the hydrophobic centers of the protein can be restored.

DOI: 10.1103/PhysRevE.70.032902 PACS number(s): 87.15.−v, 82.35.Pq, 82.35.Gh

The rapid development of biomedical engineering and biotechnology demands development of sophisticated bioactive modules with large contact areas and management of physiological conditions with a high degree of liability. A relatively well established approach that improves the biocompatibility of an artificial surface is grafting polymers such as polyethylene glycol (PEG) [1–5]. Localization of enzymes on or in the vicinity of the surface can impart biofunctionality and significantly enhance bioseparation processes [2,6–8]. However, although enzymes confined to the surface have been found to be more stable than bulk enzymes, their catalytic activity is often drastically affected by nonspecific biological and immunological reactions [9]. In addition, adsorption of the enzyme on the supporting matrix can lead to conformational changes resulting in partial or complete inactivation of catalytic centers [10,11]. The enzymatic properties may further deteriorate due to nanoenvironmental changes such as partitioning of solvents in the immediate vicinity of the surface, accumulation of products of catalytic activity, presence of charged species, and presence of other adsorbed macromolecules [8,10,12].

Various stabilization strategies aimed at improving the activity of the immobilized enzyme are being studied, including multipoint binding [7,10], mutagenesi [9,10,12], and grafted polymers on the surface of the enzyme [2,12]. These methods involve direct modification of the protein and are in general accompanied by partial deactivation even prior to contact with the destabilizing environment. Using a mean-field model, we study an alternative approach where grafted polymers on the surface *only in the vicinity* of the immobilized enzyme are used in order to stabilize it by screening, e.g., the surface-protein or solvent-protein interactions.

Several theoretical models on the interaction of proteins with surfaces and grafted polymers can be found in the literature. However, these models in general look at nonspecific interactions of rigid objects [13–16]. Szleifer [17] developed a somewhat more sophisticated mean-field theory that includes a transition from the globular bulk protein conformation to the adsorbed flat configuration, which, however, is imposed by the model. More complex models of proteinlike polymers having random or designed sequences have captured the unique freezing and adsorption behavior of proteins [e.g., [18–21]]. We use lattice mean-field theory to model a proteinlike heteropolymer having a unique bulk conformation. Near an adsorbing surface, the protein undergoes a sharp adsorption transition to a flat conformation. An anchored protein, on the other hand, may be found in a partly denatured state near the surface. The designed conformation can be restored using grafted hydrophilic polymers in the vicinity of the immobilized protein [20,21].

We specify a rigid protein structure by defining a compact copolymer made up of hydrophobic and hydrophilic groups that form a hydrophobic core and active sites through strong interactions between the hydrophobic segments (e.g., disulfide bonds). In addition, two active regions are defined at a certain distance from the center of mass of the hydrophobic core, thus leading to a unique two-dimensional conformation in the bulk, reproducing, e.g., the catalytic cleft of natural enzymes [6,8]. In principle, we can model spherical proteins, elongated proteins, or other shapes in the native state.

We extend the model developed by Scheutjens *et al.* [22,23] to two dimensions and include interactions with a free or anchored protein. The segment density distributions for the grafted polymers, solvent, and proteins are obtained from the Boltzmann weighting factor $G_j(x,z)$ of excluded volume particles, where x is the coordinate parallel to the surface and z is the distance from the surface,

$$G_j(x,z) = \exp[-u_j(x,z)/kT], \tag{1}$$

where $u_j(x,z)$ includes both energetic and entropic contributions and is in general a function of the average monomer density $\langle\phi(x,z)\rangle$, averaged over the nearest neighbor monomers only. The surface-protein interactions are modeled as exponential decay while the entropic contribution is calculated from the number of ways that the polymers can be arranged on the lattice under the given constraints [22].

The monomer density is obtained from

$$\phi(z,s) = C_j G_j(x,z,s|x',z',1) G_j(x,z,s|N)/G_j(x,z), \tag{2}$$

where the index j refers to the different species [i.e., protein (1), polymer (2), or solvent (3)]. C_j is a normalization con-

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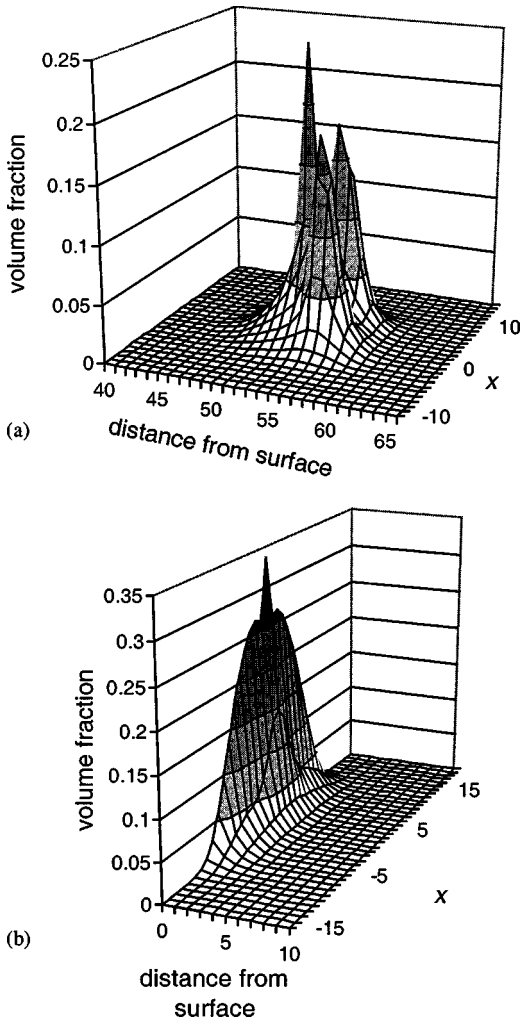

FIG. 1. Density profiles of a dimeric protein as a function of x-z location in simulation box (length in dimensionless units): (a) bulk and (b) adsorbed conformation. $\chi_{23}=\pm 0.5$, $\chi_{2s}=\pm 2.0$(s =surface).

stant. $G_j(x,z,s|x',z',1)$ is the probability that segment s is at position (x,z) given that the first segment of the chain (protein or polymer) is at position (x',z'). For the grafted polymers, $z'=0$ and thus $G_2(x,0,1|x',z',1)=1$, while for the protein both x' and z' are fixed if it is covalently attached to the surface at x_{fix} using a rigid space of length z_{fix}. We chose to fix the first monomers of the dimer and therefore $G_1(x_{\mathrm{fix}},Z_{\mathrm{fix}},1|x',z',1)=1$. $G_j(x,z,s|N)$ is the probability that segment s is at position (x,z) given that the last segment of the chain is anywhere on the lattice. Clearly, $G_j(x,z,N|N)=1$ for both protein and grafted polymers. In each iteration the positions of the active centers of the protein are relocated according to the position of the hydrophobic core (maximum density). The segment densities of the grafted polymers, the protein, and the solvent are obtained self-consistently by solving Eqs. (1) and (2) with these boundary conditions. The presented results were obtained for lattice width $W=98$, lattice height $H=100$, and grafted polymer length $N_2=20$.

In Fig. 1(a) the density profile of a dimeric protein far from the surface designed as two copolymers each one consisting of 27 segments of which 21 are hydrophobic is shown. When the protein is located at a critical distance from the surface, it undergoes a sharp adsorption transition into a flat (denatured) conformation [Fig. 1(b)].

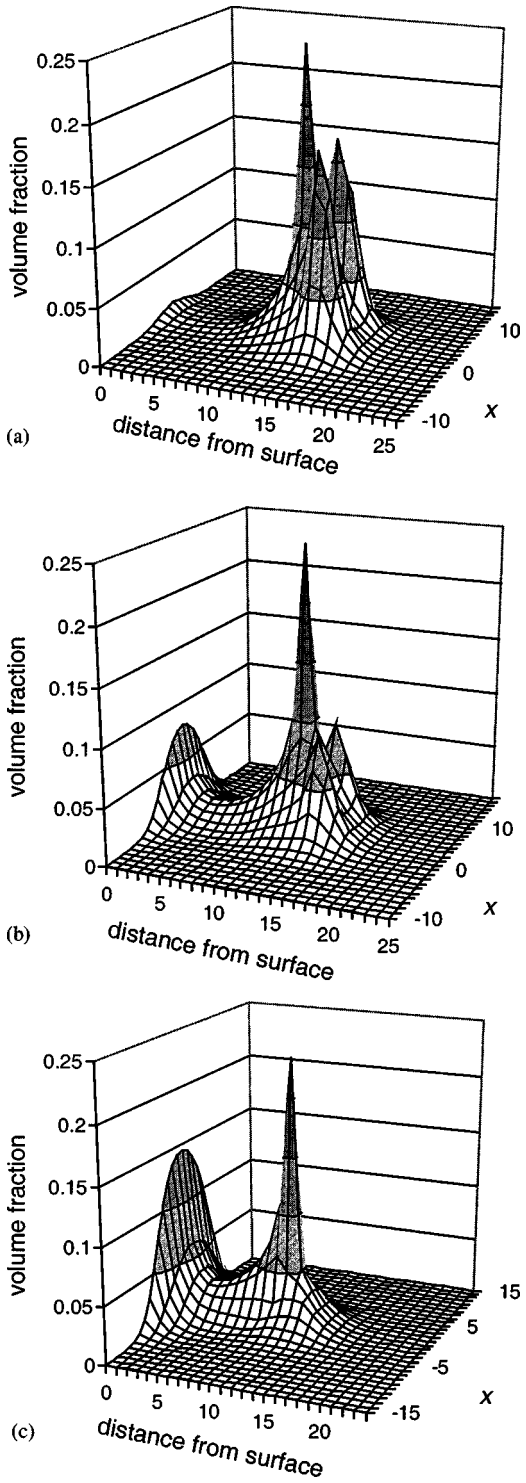

FIG. 2. Density profiles of an immobilized protein as a function of x-z location in simulation box (length in dimensionless units). $z_{\mathrm{fix}}=12$, (a) adsorption begins; (b) 11; and (c) 10, peaks designating active sites have essentially disappeared.

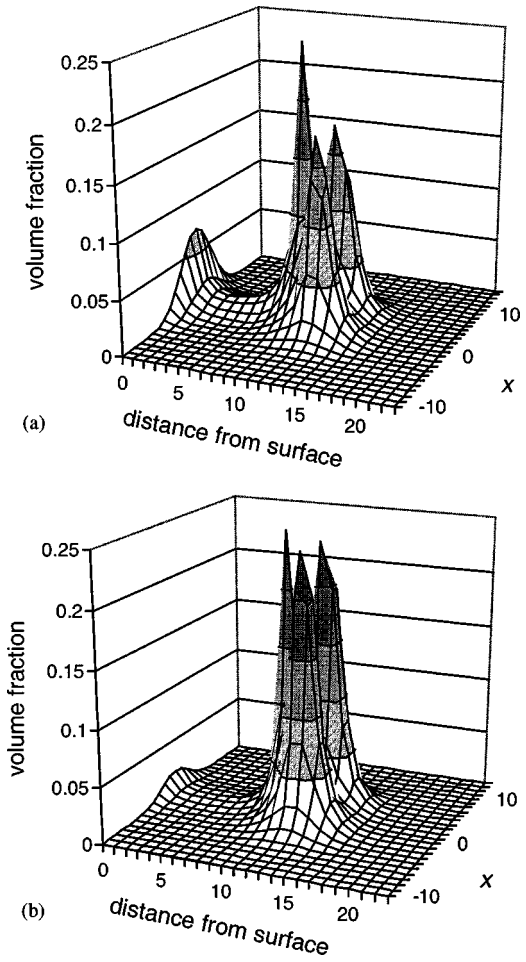

FIG. 3. Density profiles of the protein immobilized at $z_{fix}=9$, stabilized by hydrophilic grafted polymers as a function of x-z location in simulation box (length in dimensionless units). (a) Noninteracting polymers ($\chi_{12}=0$); (b) hydrophilic polymers ($\chi_{12}=\pm 0.2$).These results are for $N_1=20$ and $\sigma=0.1$.

A protein that is covalently attached to the surface also adsorbs at a critical spacer length. However, in this case, adsorption and "denaturation" is more gradual since the covalently bound segments are physically kept away from the surface. In Fig. 2 it is seen that adsorption begins at $z_{fix}=13$, where the density of the hydrophobic peaks, presumably, begin to shift onto the surface; at $z_{fix}=12$, the hydrophobic peaks have substantially diminished, and significant adsorption of the protein can be seen; denaturation is completed at $z_{fix}=10$, at which the hydrophobic sites have completely collapsed.

Grafted hydrophilic polymers in the vicinity of the immobilized protein have a dramatic influence on the protein's conformation. In Fig. 3(a) it is seen that entropic effects alone (i.e., neutral polymers with respect to the surface and protein) can partially regenerate the conformation, while fine-tuning grafting parameters (polymer length and grafting density) can nearly restore the native conformation, shown in Fig. 3(b). However, the optimal range of grafting density, chain length, and protein-polymer specific interaction parameter depend on the protein's conformational stability, which in turn is determined by the specific interactions between the

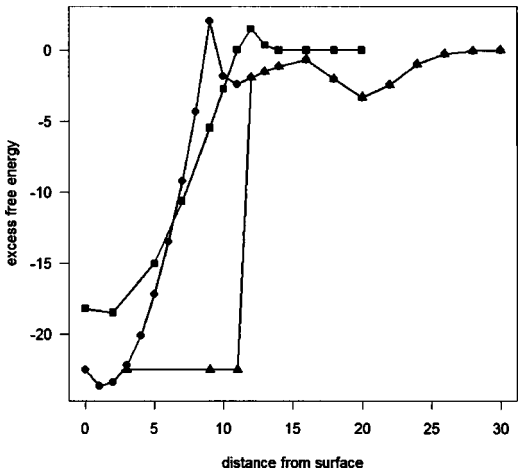

FIG. 4. Excess free energy as a function of dimensionless distance of the center of the protein from the surface for a free protein (triangles), an immobilized protein (squares), and an immobilized protein stabilized by hydrophilic grafted polymers (circles) for $N=20$, $\sigma=0.1$, and $\chi_{12}=\pm 0.2$.

monomers in our model. We find that a rather narrow range of these parameters results in a stabilized protein. While noninteracting long chains and high grafting densities lead to adsorption, short chains and low grafting densities do not provide sufficient screening. However, when hydrophilic protein-polymer interactions are present that are on the same order of magnitude as the interactions between the segments of the protein, the activity and conformational stability of the protein are nearly fully restored at substantially lower grafting densities.

We define the excess Helmholtz free energy as

$$A^*/kT = (A - A^0)/kT = \sum_z \sum_x \sum_{i=1}^{m} W\langle\phi_i(z,x)\rangle \times \left\{ \frac{\ln C_i N_i}{N_i} + \ln G_i(z,x) + \sum_{j=i+1}^{m} \chi_{ij}\langle\phi_j(z,x)\rangle \right\}, \quad (3)$$

where A^0 is the free energy of the protein in a hydrophilic solvent, W is the width of the simulation box, and χ_{ij} is the Flory χ interaction between species i and j. The third summation is over the different species. A^* is plotted as a function of distance from the surface in Fig. 4 for a free polymer in the presence of grafted polymers, a protein immobilized on a clean attractive surface, and an immobilized protein in the presence of a grafted layer. For a free protein (triangles), a sharp adsorption and denaturing transition is seen when the location of the protein is initiated at distances less than 12 lattice units from the surface. That is, for $z \geqslant 12$, the protein remains in the bulk, while for smaller distances, the protein adsorbs onto the surface. An immobilized protein, on the other hand, undergoes a more gradual adsorption transition, which, however, begins at the same critical distance ($z=12$). A highly unstable state is observed at this critical distance

when the configuration is highly stretched [Fig. 2(a)] and the hydrophobic segments span a large distance between the core and the surface. As the protein is fixed closer to the surface, adsorption is accompanied by denaturation and a drop in the free energy toward a minimum near the surface. A grafted polymer layer, however, shifts the transition closer to the surface. The fluctuations in energy seen at $z>9$ are presumably due to the interactions between the polymer and the different segments of the protein. At $z=26$, the hydrophilic monomers begin to interact favorably with the grafted polymers; thus there is a decrease in energy, reaching a minimum at $z=20$. As we move closer to the surface, the hydrophobic monomers begin to overlap with the grafted polymers, leading to an increase in the free energy of the protein. For $z<16$, the outer hydrophilic monomers enter the grafted region, again leading to a decrease in the free energy.

Grafted polymers are frequently used to enhance the biocompatibility of surfaces by presenting an entropic barrier to undesirable adsorption of bulk proteins and macromolecules that can lead to thrombosis. In addition, we show that they can provide biofunctionality by screening an embedded enzyme from the surface and thus stabilizing its native conformation and active centers.

Theis research was funded in part by the Matilda Barnett Revocable Trust and the New York Metropolitan Research Fund. S.S. acknowledge support from the Koebner-Klein Foundation and the Israel Science Foundation.

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