A Brownian Dynamics Study of the Initial Stages of Hen Egg-White Lysozyme Adsorption at a Solid Interface

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We investigate the initial stages of hen egg-white lysozyme (HEWL) adsorption at a charged solid interface using Brownian Dynamics simulation. The protein is modeled at the atomistic level and the adsorption surface is represented by a planar array of positively charged sites. Adsorption reactions are simulated at neutral pH and at low and high salt concentrations. We find that the HEWL, which has a net positive charge, can adsorb onto the positively charged surface. The results are consistent with an electrostatically driven adsorption process. While the orientational distribution of the adsorbed protein is nonuniform, there is no dominant patch composed of two or more oppositely charged residues. The electric field distribution around the protein is the best predictor of the adsorption behavior.

I. Introduction

Protein adsorption is a rich phenomenon with several important applications in chemical, industrial, and medical areas.[1−5] Despite its significance, the process is not fully understood due to the interplay of several complex chemical and physical factors.

Modeling protein adsorption has progressed mainly along the fronts of continuum model calculations[6,7] and static numerical simulations.[8−11] Atomistically detailed simulations are rare because of the extensive computational requirement.[6,8−11] As far as we know, the only dynamical study reported is the molecular dynamics (MD) simulations of lysozyme and myoglobin adsorption on a poly(ethylene glycol) surface.[12]

Lesins and Ruckenstein[13] carried out anion-exchange chromatography experiments on four basic proteins, lysozyme, α chymotrypsinogen A, cytochrome *c*, and ribonuclease A that showed that a positively charged protein can adsorb onto a like charged interface. They attributed this anomalous behavior to a distribution of amino acid residues on the protein surface that results in patches of charge opposite to the net charge of the surface. Other experimental observations also show that the net charge of the protein does not always play a deterministic role in the adsorption.[14]

The hypothesis of Lesins and Ruckenstein[13] is supported by various simulation studies. Noinville et al.[11] used detailed molecular models and employed static methods to study the chromatographic retention behavior of acidic α-lactalbumin (ALC) and the basic hen egg-white lysozyme (HEWL) on a poly(vinylimidazole) polymer. Their calculations showed that there is an energetic preference for some orientations of the adsorbed proteins. Asthagiri and Lenhoff[6] adopted a continuum approach and used numerical calculations to compute the free energy of interaction of ribonuclease A and cytochrome *c* with different positively charged model adsorption surfaces. They used an atomistic description for the proteins and modeled the adsorption surfaces with three different levels of idealization. They identified protein orientations that are energetically favorable near these model adsorption surfaces. The preference for certain protein orientations with respect to the adsorption surface results from the favorable position[6,11] demonstrate the importance of incorporating details of both the protein structure and the adsorption surface in the model in order to account for some adsorption phenomena.

The above-mentioned studies amply confirm the role played by electrostatic interactions in the adsorption process. For example, a decrease in the capacity factor as the ionic strength is raised is unmistakable evidence for the dominance of attractive electrostatic interactions.[13] Various studies have provided some insight into the effect of salt concentration and the pH of the surrounding medium on these interactions. But until now, the dynamical aspects of the adsorption process have not been fully explored. In this article we present Brownian dynamics (BD) simulation results of the initial stages of HEWL adsorption on a charged solid interface. The adsorption surface is modeled in microscopic detail and is similar to one of the models considered by Asthagiri and Lenhoff.[6] We demonstrate that this simulation methodology is a useful tool with which to investigate the dynamical aspects of the early stages protein adsorption.

II. Model and Simulation Method

The HEWL is modeled in atomistic detail and is constrained to be rigid in our simulations, a choice which is consistent with the hard nature of this protein.[15−18]

The structure of HEWL was obtained from the Protein Data Bank (Entry−7LYZ) and polar hydrogens were explicitly added. The partial charges of the atoms in the amino acid residues were calculated using the partial equalization of orbital electronegativities (PEOE) formalism,[19] as implemented in MOE.[20] The results are at neutral pH for which the protein has a net charge of +8.

The model adsorption potential consists of screened Coulombic and van der Waals contributions:

$$E = \sum_{i,j} \frac{e^2 q_i q_j e^{-\kappa r_{ij}}}{4\pi\epsilon_0 \epsilon_r(r_{ij}) r_{ij}} + \sum_{i,j} \left(\frac{A_{ij}}{r_{ij}^{12}} - \frac{B_{ij}}{r_{ij}^{6}} \right) \qquad (1)$$

where r_{ij} is the distance between atom i of the protein and j of the adsorption surface, q_i is the partial charge of the atom, and

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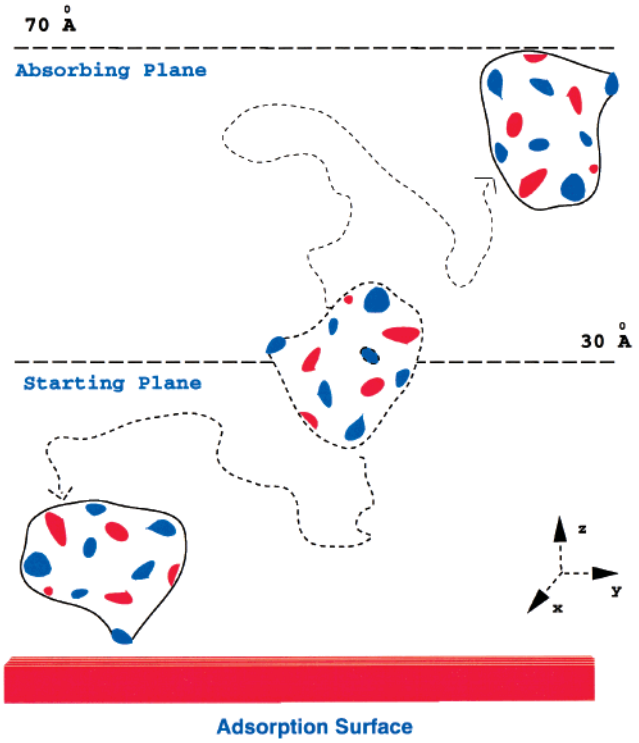

Figure 1. Schematic diagram showing the details of the simulation method. In this figure the protein molecule is represented as an arbitrarily shaped object with patches corresponding to both positively charged (blue) and negatively charged (red) amino acid residue collections. The figure also shows the conditions for the adsorption and the removal of the protein from the system.

κ is the inverse Debye length. The electron charge, vacuum permittivity, and relative permittivity are denoted by e, ϵ_0, and ϵ_r, respectively. Relative permittivity is assumed to be distance dependent and is taken as $\epsilon_r \approx r$. A_{ij} and B_{ij} are the van der Waals interaction parameters and were obtained from the AMBER force field.[21,22] The adsorption surface is modeled as a 2-dimensional square array of atoms, each assigned a charge of +e and having radius and well depth as 1.85 Å and 0.152 K cal mol^{-1}, respectively. The van der Waals parameters for the cross interactions were obtained from those of the pure components using the Lorentz−Berthelot mixing rules. Each atom is separated by 30 Å yielding a surface charge density of $\sigma = 1.8\ \mu$C/cm^2. The adsorption surface side length was chosen as 240 Å to be consistent with the earlier studies.[9−11]

The salt effects are introduced in the simulation by using the Debye parameter corresponding to a 2:1 salt.[11,13] Ionic strengths of 0.1 M and 0.3 M were chosen to enable (eventual) comparisons with the available experimental results.[13]

Each trajectory starts by randomly placing the protein's center of mass at a height of 30 Å above the adsorption surface in a random orientation (Figure l). A trajectory is terminated when any of the protein's atoms reaches a height of 70 Å or comes within 3 Å of the adsorption surface[6] (see Figure 1). In the latter case, corresponding to successful adsorption, the atoms within 3 Å of the surface and the associated amino acid residue are recorded for later analysis.[6] To avoid edge effects periodic boundary conditions were employed in the lateral directions (see Figure 1). All simulations were performed using the Molecular Operating Environment (MOE) software developed by Chemical Computing Inc.[20] The Brownian dynamics algorithm of Ermak and McCammon[23] was implemented into MOE using Scientific Vector Language (SVL). A multiple time-step method was used in the Brownian dynamics simulations.[24] The full potential (eq 1) without a cutoff was used in the calculation of force in the simulations.

TABLE 1: Fraction of Sucessful Trajectories for Two Different Salt Concentrations

I (M)	
0.1	0.71 ± 0.03
0.3	0.64 ± 0.02
[a]	0.62 ± 0.03

[a] Correspond to simulations carried out with no electrostatic interactions. Note that the total number of trajectories for each case was 100. The deviations are reported in the form of standard error obtained from 5 sets of 20 trajectories each.

TABLE 2: Average Distance $\langle r \rangle$ the Protein Diffuses before Adsorption

I(M)	$\langle r \rangle$ (Å)
0.1	19.5 ± 1.6
0.3	25.1 ± 2.2
[a]	23.1 ± 1.8

[a] Correspond to simulations carried out with no electrostatic interactions. Note that the total number of trajectories for each case was 100. The deviations are reported in the form of standard error obtained from 5 sets of 20 trajectories each.

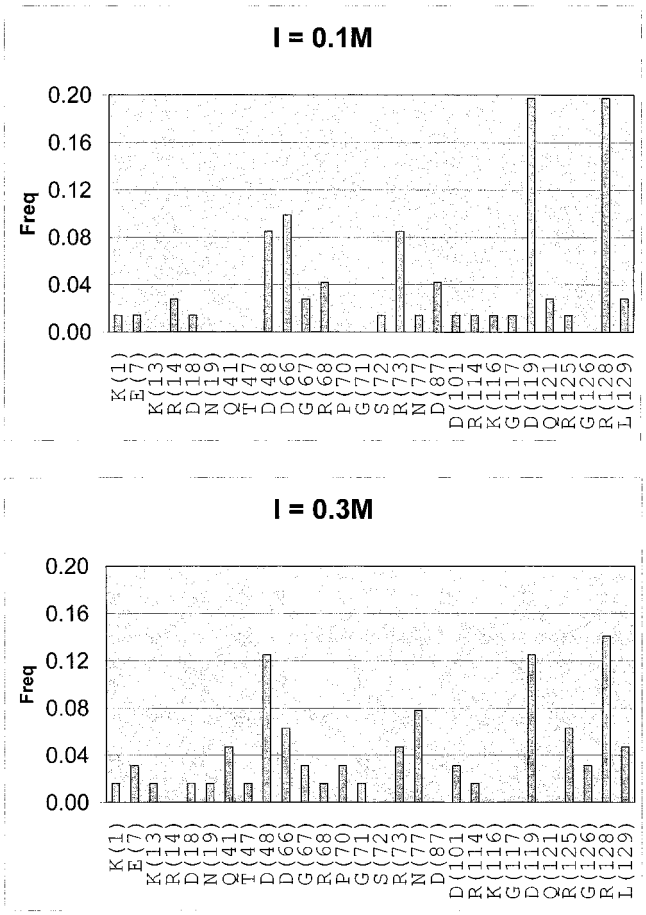

Figure 2. (a) The frequency of the amino acid residues closest to the adsorption surface. The salt concentration is O.1 M. Note that the amino acid residues are represented by one-letter symbols followed by the residue number. (b) Same as Figure 2a except for $I = 0.3$ M.

The translational and rotational diffusion coefficients for HEWL are 4.3×10^{-7} cm^2 s^{-1} and 8.9×10^{-2} ns^{-1}, respectively.[25] The simulation algorithm was tested by performing simulations in the bulk phase and verifying that the diffusion coefficients calculated from the mean square displacement and

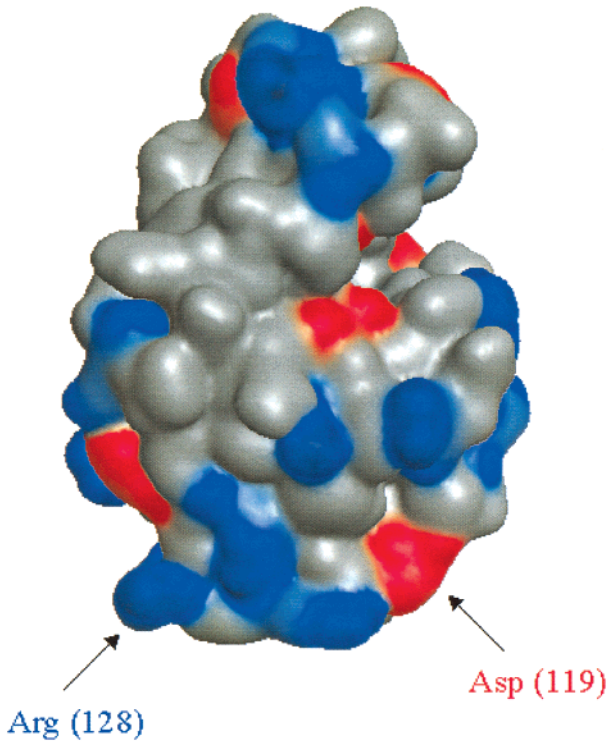

Figure 3. HEWL is shown in molecular surface (GaussConnolly) representation. The blue, red, and white colored surface represents the location of the positive, negative, and neutraly charged amino acids on HEWL.

the reorientational correlation function, respectively, were consistent with these values. To obtain reasonable statistics we carried out 100 separate simulations for each ionic strength.

We also computed the electrostatic field around the protein by solving the Poisson−Boltzmann equation (PB)[24,26] as implemented in MOE.[20] Only the protein and not the adsorption surface was included in these calculations. The potential distribution is shown in a plane drawn through the residues of interest and parallel to the (space fixed) *yz*-plane.

III. Results and Discussion

Table 1 shows the number of trajectories which resulted in adsorption for ionic strengths 0.1 and 0.3. That the number of successful trajectories decreases with increasing ionic strength is consistent with the idea that attractive electrostatic interactions govern the adsorption process. An increase of the ionic strength attenuates these interactions and results in a decrease in the number of trajectories leading to adsorption. This behavior is consistent with the chromatography experiments[13] that indicate that the adsorption of HEWL on a positively charged interface in the low ionic strength regime is controlled primarily by attractive electrostatic interactions. The strength of electrostatic interactions is also reflected in the average distance the protein diffuses from its starting point until it reaches a height of 7 Å. The calculated values, shown in Table 2, decrease with decreasing ionic strength indicating the strengthening of attractive electrostatic interactions. We also performed simulations in which the electrostatic contributions were switched off. The fraction of successful trajectories leading to adsorption (see Table 1) is less than for simulations at low salt concentrations which include both the electrostatic and van der Waals interactions. The average time the protein spends in the bulk phase is also significantly more (5.8 ns/4.0 ns) than in the presence of the electrostatic contributions supporting the above arguments.

If the net charge were the major criterion then HEWL would not adsorb on a positively charged surface. But it is clearly the distribution of charged amino acid residues on the protein surface that is the dominant factor in determining the electrostatic interaction between the protein and the surface. Figures 2a and 2b show the distribution of the amino acid residues closest to the adsorption surface for the two ionic strengths studied. At I = 0.3 the distribution is more uniform than at I = 0.1. In the latter case, there is a considerable preference for Asp(119) and Arg(128). These residues, however, do not form part of a larger patch of negatively charged amino acid residues (see Figure 3). This should be compared with the observed patch-controlled behavior of basic proteins such as ribonuclease and cytochrome *c* on a positively charged surface.[6,13]

To develop additional insight into the adsorption behavior we calculated the electrostatic potential around the protein by solving the Poission−Boltzmann equation, see Figure 4. Note that the low and high energy regions are shown in red and blue, and the intermediate ones are represented by interpolating between these colors. The figure clearly shows the dominance of low energy field near the surface. Moreover, there is a relatively intense region of negative electric field around the

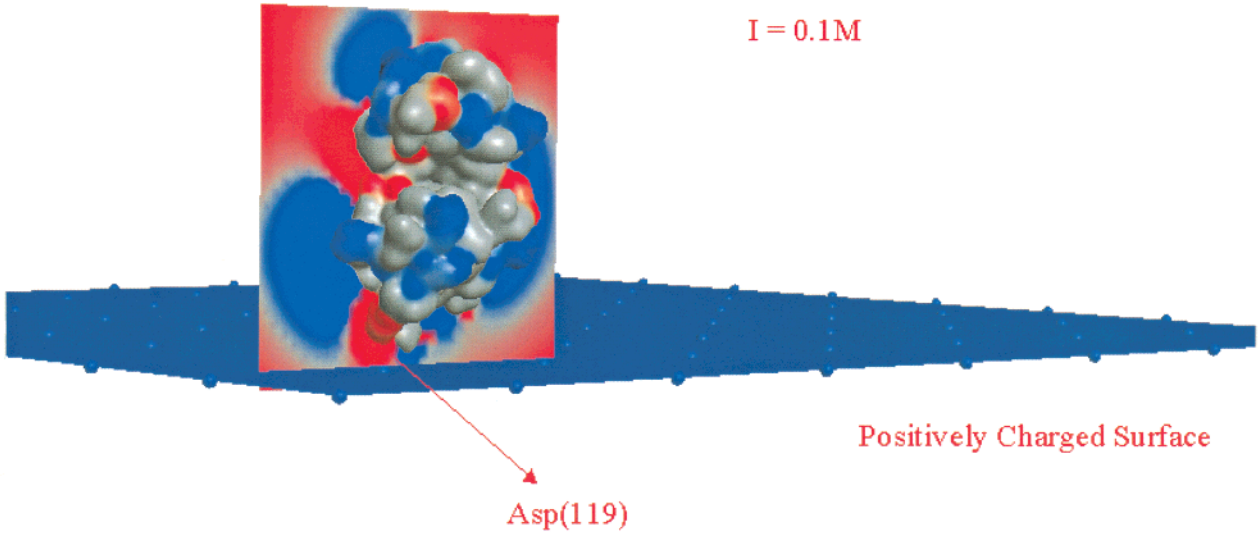

Figure 4. Calculation of the potential field around the adsorbed protein using PB calculation for $I = 0.1$ M. The calculation is done only on the protein and the adsorption surface is not included. Note that the plane drawn through the lattice sites of the adsorption surface is shown for a better representation and is not a part of the simulated system. The protein molecule is shown in molecular surface (see Figure 3) representation.

residues Asp(119) and Arg(128) that would favor their positioning next to the positively charged surface. This explains the high frequency of these groups in the histograms shown in Figure 2 for both $I = 0.1$ and 0.3 M. We conclude that even a single amino acid residue can be responsible for driving the protein from the interfacial region to the adsorption surface.

Our results, consistent with other studies,[11,27] suggest that the effective net charge of small subsets of amino acid residues (smaller than would normally be considered as defining a patch) can control the adsorption behavior. Ultimately, of course, it is the electric field distribution that provides the most accurate predictor of the adsorption behavior. A detailed analysis will be presented in a future publication.

The results presented here are specific for low surface coverage and hard proteins, i.e., those that do not undergo significant conformational changes following adsorption. The model adsorption surface has a low surface charge density to avoid multi-ligand interactions and to search for patch-controlled mechanism.[6] We are currently expanding the model to include protein−protein interactions to study the effect of crowding using this microscopic model.

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References and Notes

(1) Andrade, J. D. *Surface and Interfacial Aspects of Biomedical Polymers. Vol- ume 2 Protein adsorption*; Plenum: New York, 1985.
(2) Andrade, J. D.; Hlady, V. *Adv. Polym. Sci.* **1986**, *79*, 1.
(3) MacRitchie, F. *Protein Chem.* **1978**, *32*, 283.
(4) Biopolymers at interfaces; Malmsten, M., Ed.; Marcel Dekker, Inc.: NewYork, 1998.
(5) Proteins at interfaces II, Fundamentals and Applications; edited by Horbett, T. A., Brash, J. L., Eds.; ACS Symposium Series 602, 1995.
(6) Asthagiri, D.; Lenhoff. A. M. *Langmuir* **1997**, *13*, 6761.
(7) Yoon, B. J.; Lenhoff. A. M. *J. Phys. Chem.* **1992**, *96*, 3130.
(8) Juffer, A. H.; Argos, P.; De Vlieg, J. *J. Comput. Chem.* **1996**, *17*, 1783.
(9) Lu, D. R.; Park, K. *J. Biomater. Sci. Polym. Ed.* **1990**, *4*, 243.
(10) Lu, D. R.; Lee, S. J.; Park, K. *J. Biomater. Sci. Polym. Ed.* **1991**, *3*, 127.
(11) Noinville, V.; Vidal-Madjar, C.; Sebille, B. *J. Phys. Chem.* **1995**, *99*, 1516.
(12) Lim, K.; Herron, J. N. In *Biomedical Applications of Polyethylene Glycol Chemistry: Biotechnical and Biomedical Applications*; Harris, J. M., Ed.; Plenum: New York, 1992.
(13) Lesins, V.; Ruckenstein, E. *Colloid Polym. Sci.* **1988**, *266*, 1187.
(14) Kopaciewicz, W.; Rounds, M. A.; Fausnaugh, J.; Regnier, F. E. *J. Chromatogr.* **1983**, *266*, 3.
(15) Norde, W.; Favier, J. P. *Colloid Surf.* **1992**, *64*, 87.
(16) Robeson, Tilton, R. D. *Langmuir* **1996**, *12*, 6104.
(17) Kondo, A.; Oku, S.; Higashitani, K. *J. Colloid Interface Sci.* **1991**, *143*, 214.
(18) Billsten, In *Biopolymers at interfaces*; Malmsten, M., Ed.; Marcel Dekker: Martin New York, 1998; page 627.
(19) Gasteiger, J.; Marsili, M. *Tetrahedron* **1980**, *36*, 3219.
(20) http://www.chemcomp.com.
(21) Weiner, S. J.; Kollman, P. A.; Case, D. A.; Singh, U. C.; Ghio, C.; Alagona, G.; Profeta, S.; Weiner, P. *J. Am. Chem. Soc.* **1984**, *106*, 765.
(22) Weiner, S. J.; Kollman, P. A.; Nguyen, D. T.; Case, D. A. *J. Comput. Chem.* **1986**, *7*, 230.
(23) Ermak, D. L.; McCammon, J. A. *J. Chem. Phys.* **1978**, *69*, 1352.
(24) Madura, J. D.; Briggs, J. M.; Wade, R. C.; Davis, M. E.; Luty, B. A.; Ilin, A.; Antosiewicz, J.; Gilson, M. K.; Bagheri, B.; Scott, L. R.; McCammon, J. A. *Computer Phys. Comm.* **1995**, *91*, 57.
(25) Smith, P. E.; van Gunsteren, W. F. *Mol. Biol.* **1994**, *236*, 629.
(26) Santavy, M.; Labute, P. Electrostatic Fields and Surfaces in MOE. *J. Chem. Comput. Group* **1998**.
(27) Melander, W. R.; El Rassi, Z.; Horvath, C. *J. Chromatogr.* **1989**, *469*, 3.