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Mechanism of Hen Egg White Lysozyme Adsorption on a Charged Solid Surface

Karina Kubiak-Ossowska[†,‡] and Paul A. Mulheran*[,†]

[†]*Department of Chemical and Process Engineering, University of Strathclyde, James Weir Building, 75 Montrose Street, Glasgow G1 1XJ, United Kingdom, and* [‡]*Institute of Physics, Faculty of Physics, Astronomy and Informatics, Nicolaus Copernicus University, ul. Grudziadzka 5/7, 87-100 Torun, Poland*

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The mechanism of hen egg white lysozyme (HEWL) adsorption on a negatively charged, hydrophilic surface has been studied using atomistic molecular dynamics (MD) simulation. Sixteen 90 ns trajectories provide adequate data to allow a detailed description of the adsorption process to be formulated. Two distinct adsorption sites have been identified. The main one is located on the N,C-terminal protein face and comprises Arg128 (the crucial one), supplemented by Arg125, Arg5, and Lys1; the minor one is used accidentally and contains only Arg68. Adsorption of this protein is driven by electrostatics, where the orientation of the protein dipole moment defines the direction of protein movement. The diffusion range on the surface depends on protein side-chain penetration through the surface water layers. This is facilitated by the long-range electric field of the charged surface, which can align polar side chains to be perpendicular to the surface. A simulation of adsorption onto a neutral ionic surface shows no such surface water layer penetration. Therefore, protein flexibility is seen to be an important factor, and to adsorb the HEWL has to adjust its structure. Nevertheless, at a flat surface only a slight loss of α-helical content is required. The adsorbed HEWL molecule is oriented between side-on and end-on ways, where the angle between the protein long axis (which mostly approximates the dipole moment) and the surface varies between 45° and 90°. Simulations with targeted mutations confirm the picture that emerges from these studies. The active site is located on the opposite face to the main adsorption site; hence, the activity of the immobilized HEWL should not be affected by the surface interactions. Our results provide a detailed insight into the adsorption mechanism and protein mobility at the surface. This knowledge will aid the proper interpretation of experimental results and the design of new experiments and functional systems.

Introduction

Control of protein adsorption, which follows a deep understanding of its mechanism, is crucial in modern biology, medicine, food processing, and technology.[1] Protein immobilization on medical implants is extremely important for functionality, while adsorption on nanoparticles may be used in well-addressed drug or nutrient delivery systems.[2] Proteins and peptides easily adsorb onto various surfaces,[3−10] including nanoparticles,[11−13] and then aggregate[8,14,15] or even fibrillate.[7,16]

It has already been found that protein adsorption may be affected by the solute properties (pH, temperature, composition, ionic strength), as well as protein properties (hydrophobicity, charge, shape, size).[17−20] It also appears that the adsorption mechanism strongly depends on surface characteristics. In the case of polymeric surfaces, the major role seems to be played by hydrophobic interactions,[19,21] while adsorption on materials like gold, magnetic particles, diamond nanocrystals, mica, or silica is probably driven by electrostatics.[8,9,12−15,22,23] However, a detailed understanding of these differences in behavior is yet to be achieved.

One of the most widely used proteins for model system study is hen egg white lysozyme (HEWL). This inexpensive, small (129 residues), and globular protein is convenient to use in both experimental and theoretical studies. HEWL was one of the first proteins to have its three-dimensional structure revealed.[24] This ellipsoidal protein is composed of six α-helices and three β-sheets connected by a few flexible loops and organized in two domains, α and β (see Figures 1, 2). The active site is located in the cleft

*Corresponding author. E-mail: paul.mulheran@strath.ac.uk. Tel.: +44 (0)141 548 2385.

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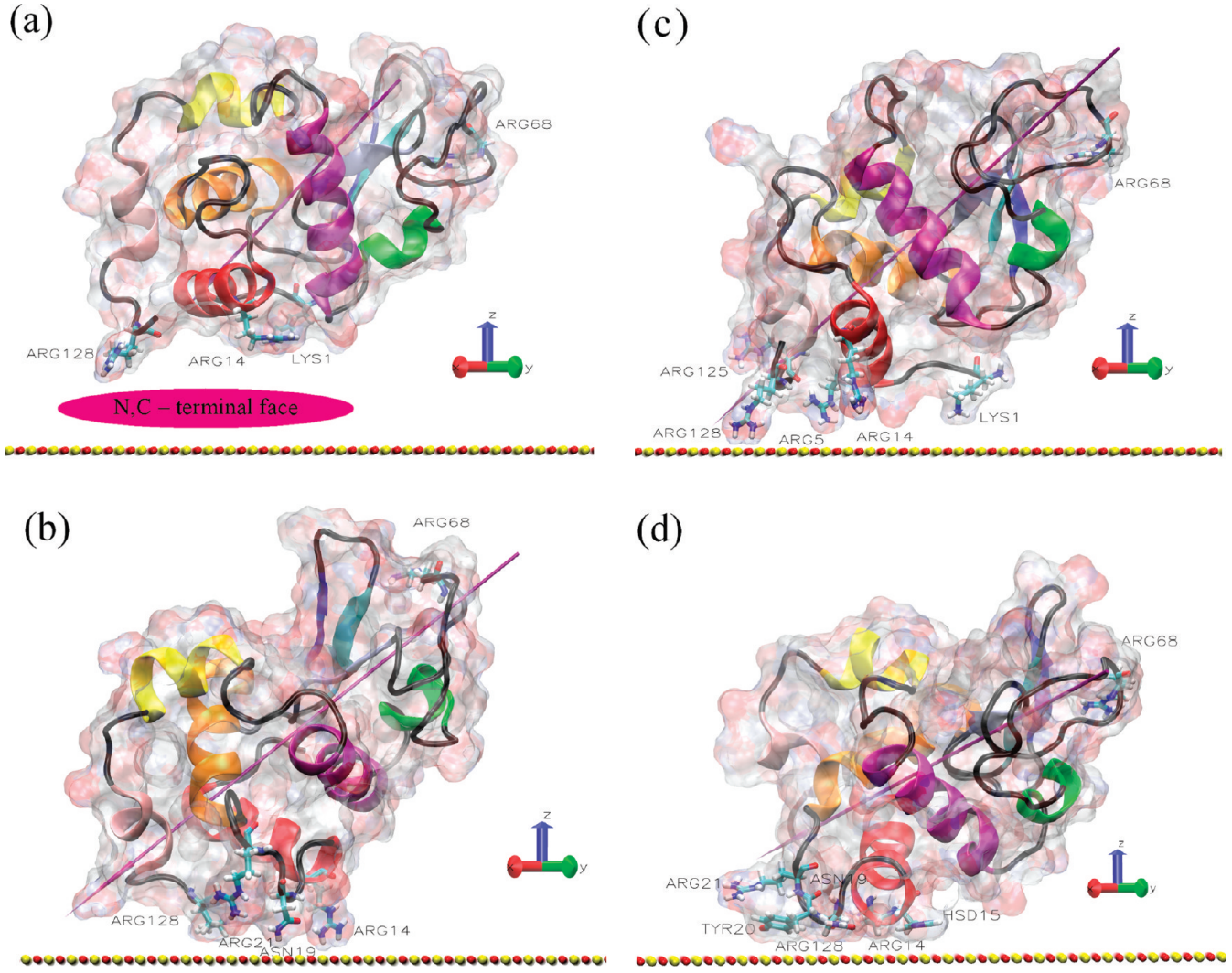

Figure 1. Protein−surface system in orientation 1. (a) An initial structure in orientation 1. Protein surface is shown as a ghost surface colored by charge, while the solid surface atoms are shown as red and yellow CPKs for oxygen and silicon atoms. The secondary structure is indicated by a cartoon, black parts indicate loops, red helix represents α-helix A, orange − α-helix B, purple − α-helix C, yellow − α-helix D, pink − α-helix 3_{10} from the α domain. The β domain contains structures: βB1 shown in blue, βB2 (cyan), βB3 (ice blue), and α-helix 3_{10} (green). The closest residues to the surface, as well as Arg68, are shown by licorice with coloring scheme: white − hydrogen, red − oxygen, cyan − carbon, blue − nitrogen. N- and C-termini are not annotated, but the location of the N,C-terminal face is indicated. The magenta arrow crossing through the protein represents its dipole moment. (b) The final structure in trajectory O1_05M. (c) The final structure from the O1_002M_0 trajectory. (d) The final structure from the O1_001M_N trajectory. The notations used to differentiate the trajectories are explained in the Methods section.

between the domains, which lies on the long protein side between the middle and one end of the ellipsoid. On the opposite face, close to the opposite end, the N and C termini are located. This part of the protein is usually called the N,C-terminal face. The protein long axis is oriented approximately from the active site toward the N,C-terminal face.

The ellipsoidal shape begs the question of how the adsorbed protein is oriented on the surface: is a side-on, end-on, or any intermediate orientation favored? However, the differentiation among side-on, end-on, and all intermediate orientations may be difficult, since the protein ellipsoid is not particularly elongated; the long axis is 45 Å, while the short one is 30 Å. The task is also complicated by possible protein rotations and translations on the surface. Because of limited resolution, experimental studies have focused on distinguishing only between the side-on and end-on modes (e.g., deciding if the observed layer thickness is closer to 45 Å or to 30 Å). Of these two options, it has been found that the preferred orientation on the surface is side-on,[21,25−28] although end-on adsorption followed by reorientation to side-on orientation is also possible.[21,27,28] Atomic force microscopy (AFM) experiments indicate that protein orientation on the surface depends on the coverage; at low coverage, lysozyme preferentially adsorbs side-on, with adsorption in the end-on way becoming apparent at higher coverage.[15] Nevertheless, the intermediate orientation is also not excluded.[29]

Irrespective of the estimated orientation on the surface, the protein is always expected to use the same region in its interactions with the surface. By using a lysine labeling method, the

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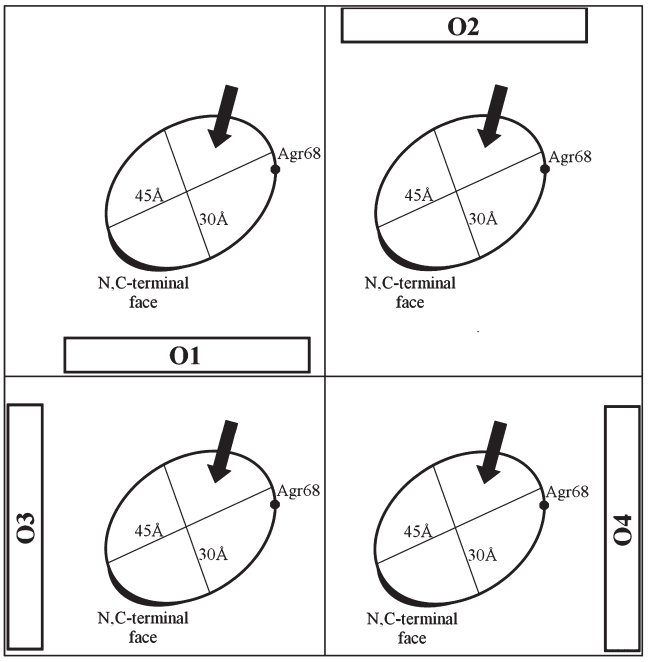

Figure 2. Schematic representation of the four initial relative protein−surface orientations studied. The protein is shown as an ellipsoid with its two major axes; the N,C-terminal face and the minor adsorption site (Arg68) position are also illustrated. The arrow points to the active site location.

approximate location of the binding site has been revealed, and the role of the N,C-terminal face residues in the binding of the protein to a surface has been indicated.[5,25,26,28−31] According to this picture, the HEWL adsorbs to the surface using its largest positively charged patch on the protein surface, which lies on the N,C-terminal face and comprises Lys1, Lys13, Lys95, Lys97, Arg14, and Arg128,[5,28,31] although an exact list of crucial residues for adsorption is not known. Limited resolution together with problematic definition of protein orientation on the surface, as well as the adsorption binding site composition, presents questions that can perhaps uniquely be answered by molecular dynamics (MD) studies.

As was recently demonstrated with human serum albumin,[32] theoretical studies can elucidate such details and therefore have a crucial role to play in developing a deep understanding of protein surface adsorption that will underpin future exploitation of the process. Previous MD studies have already confirmed the importance of the HEWL N,C-terminal face and proposed the residues which may be crucial for adsorption at a charged surface, at least during the early stages.[8,9] However, a detailed mechanism of the adsorption, orientation on the surface, and sequence of events still needs to be described. We do this in this present paper, bearing in mind the usual limitations of atomistic modeling work. The main fully atomistic MD limitation is the simulation time; currently for proteins, tens of nanoseconds are usually reached with commonly available computational resources. The 90 ns time scale we use here is relatively long from the simulation perspective, but is extremely short when compared to the experimental time scale. The second limitation is the system size one can easily simulate; the larger the number of atoms, the shorter the accessible simulation time. For this reason, only single protein simulations are usually studied. The final limitation of our simulations comes from the choice of potentials between nonbonded atoms. In this work, we utilize the Charmm27 force field, including some parametrization of the interactions between the protein, water molecules, and our model surface in an attempt to provide a realistic mimic of the potentials encountered at a real surface.

Another unclear issue in HEWL adsorption is the extent of its conformational changes. Some work has shown that denaturation is necessary for adsorption,[4] while in other works, it has been concluded that the tertiary structure is maintained.[3,11,33,34] Evidence that adsorption induces an increase in random-coil and β-structure content and a decrease in the α-helix content has been found.[3,33] According to Larsericsdotter et al., it might indicate that HEWL adopts a more flexible conformation on the solid surface.[11] Our theoretical investigations have partially explained a source of the inconsistency in the experimental conclusions;[8,9] nevertheless, more systematic studies are needed and are presented here.

As a final justification of this theoretical study, we note that, as has been observed in AFM experiments, HEWL adsorbed on mica is still mobile.[8,14,15,23] An insight into HEWL motion on the surface may be provided by appropriate atomistic molecular dynamics simulations of sufficient duration.[10]

In a previous paper, we presented a series of 20 ns trajectories for the adsorption of HEWL at a model charged silica surface.[9] These highlighted not only the role of electrostatics in driving the adsorption, but also the important role played by the penetration of the surface water layers by key residues at the N,C-terminal face. In subsequent work, we have reinforced this picture, presenting results from some longer 90 ns trajectories, where we have identified Arg128 as the crucial residue for immobilizing the protein at the surface.[10] In this current paper, we shall present more details of the behavior we observe in these trajectories, supplemented by further designed simulations. Together, these provide a comprehensive overview of the adsorption process we observe for HEWL at our model charged surface.

We have extended the simulated time scale to 90 ns for six trajectories we presented in ref 9, focusing on the adsorption of single proteins on a flat surface. Ten additional 90 ns trajectories are also presented and discussed. These include a new orientation between the protein and the surface; three further, statistically independent examples of the most important orientation; five trajectories with targeted mutations to confirm the key residues involved in anchoring the protein at the surface; and one simulation for the most representative initial orientation interacting with a neutral surface. Some of these 90 ns trajectories have been briefly discussed in ref 10, but here we provide further key details in a systematic manner, allowing us to draw out any similarities or contrasts between trajectories.

As a consequence of our analysis of this set of trajectories, we can provide a detailed description of the protein adsorption mechanism on a charged solid surface for our model system. In particular, the list of crucial residues and the order of events during adsorption are provided. The description also focuses on the role of electrostatics, protein conformational changes, and the impact anchoring residues have on the protein surface mobility. We believe that the results presented here provide new insights into the protein adsorption process and will help both the

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interpretation of existing data and the design of new experiments, as well as show a new direction in engineering with proteins.

Material and Methods

All simulations were run under the NAMD 2.6[35] package with the Charmm27 force-field. 1iee.pdb[36] with all four disulfide bridges kept was used as the starting HEWL structure. We prepared seven simulation systems and ran sixteen 90 ns trajectories. Two simulation systems were simply the "isolated" protein embedded in a rectangular water box, with ionic strength 0.5 M (I_05M system) and 0.02 M (I_002M system), respectively. The varying ionic strengths were achieved through a protein neutralization procedure with Na^+ and Cl^- ions. The abbreviations O1 through to O4 denote protein−surface systems in four different orientations visualized in Figure 2. In the case of orientation 1 (O1), the solid surface was located below the N,C-terminal face, in the (x, y) plane. By moving the surface up by 80 Å, orientation 2 (O2), in which the surface was located above the protein, was achieved. Orientations 3 and 4 (O3 and O4) denote the situation in which the charged surface is located at either end of the ellipsoid. In O3, the surface lies in the (y, z) plane close to the end nearest to the N,C-terminal face, while in O4, the surface lies close to the opposite protein end nearby the minor adsorption site. Calculations for systems O2−O4 were run under 0.02 M ionic strength, while in the case of O1, both ionic strengths (0.5 M and 0.02 M) were used. This yielded systems denoted O1_05M and O1_002M, respectively. Moreover, to obtain better statistics, we have run the O1_002 M system four times and obtain four different trajectories denoted as O1_002M_0 through to O1_002M_3. In one simulation denoted as O1_001M_N, a neutral ionic surface model was used. The solvent ionic strength in this case was 0.01 M, this lower value being chosen to ensure adsorption at this neutral model surface.

In the case of O1 and O4 systems, we have also performed calculations for mutated versions of the HEWL. Four mutations (O1_R128A, O1_R128G, O1_R68G_R128G, and O4_R68G) have been created from the starting structure, and then the same simulation protocol used for the native protein was applied. In the fifth mutation, O1_90 ns_R128G, we have mutated the already adsorbed protein (the final structure of the O1_002M_0 trajectory) followed by the usual simulation protocol. The abbreviation R128A(G) means that Arg128 has been substituted by alanine (glycine), and so forth.

The surface has been parametrized to mimic properties of the natural mica surface at pH 7. We have used a single layer of static oxygen and silicon atoms organized into a square array, with O−Si distance of 1.6 Å as found in SiO_2. To better model the natural mica surface widely used in experimental work, we reproduce the mica surface charge density $\sigma = -0.021$ e/Å^2 by using partial charges +1.11 e and −0.66 e on Si and O, respectively (yielding $\sigma = -0.0217$ e/Å^2). The surface dimensions used in the O1 system were 86.4 Å × 92.8 Å. Other parameters required in the force-field were adjusted to achieve the best approximation of the mica surface.[9,10] In the case of the neutral surface simulation (O1_001M_N), we used charges +1.00 e and −0.50 e on Si and O, respectively, with the other parameters remaining unchanged.

The production MD simulations, preceded by water equilibration, minimization, heating to 300 K, and equilibration, were pursued for 90 ns at 300 K in the *NVT* ensemble. The integration step was 2 fs; the SHAKE algorithm and PBC were used. The cutoff distance for both van der Waals and Coulomb interactions was 12 Å. For ionizable residues, the most probable charge states at pH 7 were chosen. The accuracy of the chosen protocol has been already discussed.[9]

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Results

Isolated Proteins (I_05M and I_002M) Systems. As observed previously in our 20 ns trajectories,[9] the dynamics of isolated proteins seem to not be affected much by the solute ionic strength. The protein was stable in both conditions (Figure 3). The root mean square deviation (rmsd) calculated with respect to the initial structure (using the standard calculation which removes the effects of global translations and rotations) achieved a horizontal character after about 20−25 ns at the values 2.0 ± 0.1 Å and 2.3 ± 0.1 Å for I_05M and I_002M, respectively. The rmsd for I_05M is shown in Figure 4. Protein structural changes were limited to the loops, mainly the long loop 61−78, and the secondary structure was well-maintained (Figure 3a,b). In both cases, the protein was flexible and the loops were working as hinges connecting relatively rigid secondary structural elements. The N- and C-termini remained attached to the protein surface. Both trajectories were treated as references for HEWL-surface systems.

HEWL−Surface Orientation 1, 0.5 M Ionic Strength (O1_05M). The 90 ns trajectory confirms the picture reported in ref 9 that the character of the rmsd in this simulation was very similar to that observed for an isolated protein. The HEWL structure was stable after ∼20 ns of trajectory, following which the rmsd was steady at 1.85 ± 0.1 Å. The protein was flexible, but the flexibility was again limited mainly to the loops. Figure 3c demonstrates that protein structure was well-maintained.

As can be seen in the Supporting Information movie O1_05M.avi, during the first 10 ns the protein moved away from the surface by a few angstroms. Following this, the conformation of the long loop 61−78 has changed and so the relative orientation of the secondary structure elements has altered too (Figure 3c). Next, the protein started to slowly translate toward the surface. At about 14 ns, the N,C-terminal face residues Arg128, Ans19, Arg21, and Arg14 started to interact with the surface because their side chains exposed the positively charged and polar groups to the negatively charged surface. The electrostatic attraction between these groups and the surface enhanced the protein translation toward the surface. Between 14 ns and 18 ns, the translation was accompanied with a slow, anticlockwise (as viewed in Figure 2) rotation of the protein. This caused protein reorientation with respect to the surface. The dipole moment achieved an orientation toward the surface with an angle 45° and then at 20 ns protein adsorbed. Figure 1b shows adsorbed HEWL after 90 ns dynamics.

For the purposes of this discussion, we will adopt the following descriptive terms. We consider that a contact with the surface is made when any side change is oriented toward the surface, and its distance to the surface remains stable and lower than 5 Å, indicating that it is interacting at least with the outer water layer.[9] With a single contact of this type, the protein movement is not greatly constrained. We will consider adsorption to occur when contacts between side chains and the surface are stable for at least 1 ns, the protein center-of-mass (COM) is located close (the precise distance depends on protein orientation) to the surface for at least 2 ns, and the protein mobility is visibly constrained.

Once adsorbed in trajectory O1_05M, the HEWL was stable on the surface and global protein translations or rotations were strongly constrained, with only fluctuations in protein structure observed thereafter. Arg128, Asn19, Arg21, and Arg14 side chains were penetrating both surface water layers, and as Figure 1 and Table 1 show, their distances to the surface were relatively small. Their interactions with the surface were strong enough to efficiently constrain protein diffusion.

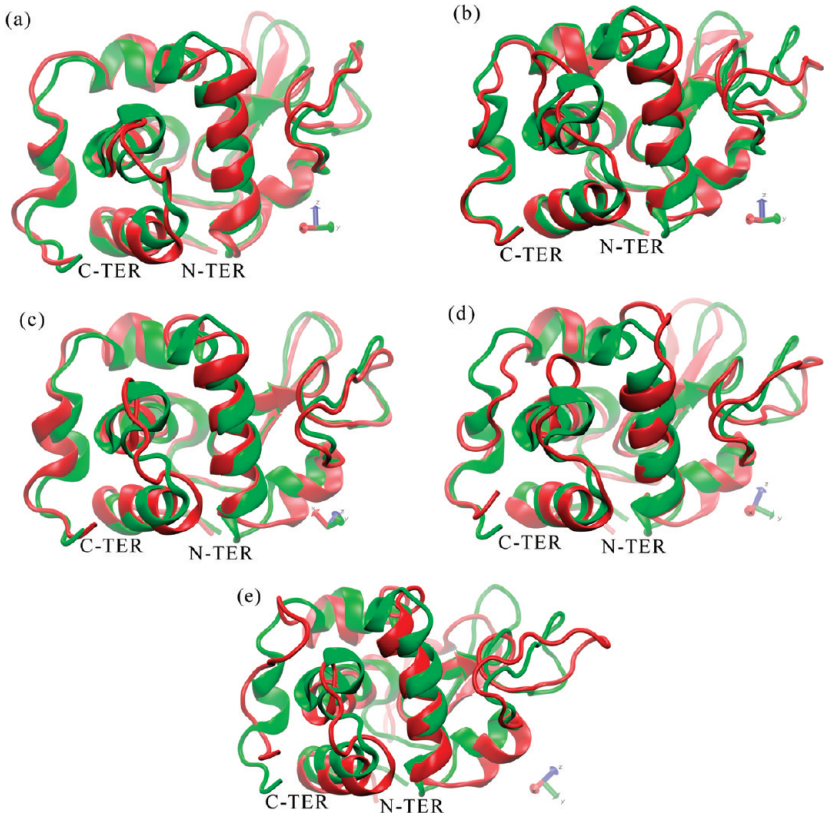

Figure 3. Overlap of the initial (green) and final (red) structures obtained after 90 ns of the trajectory. (a) I_05M. (b) I_002M. (c) O1_05M. (d) O1_002M_0. (e) O1_002M_3. Locations of N- and C-termini are indicated.

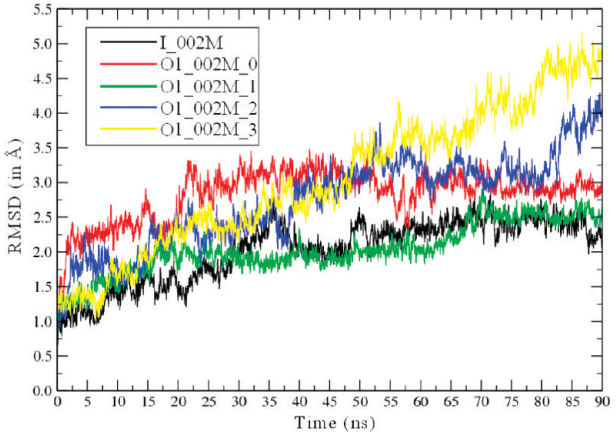

Figure 4. Rmsd calculated for trajectories: I_002M (the black line), O1_002M_0 (the red line), O1_002M_1 (the green line), O1_002M_2 (the blue line), and O1_002M_3 (the yellow line). As a reference, the initial structure has always been used.

The protein dipole moment and the long protein axis had almost equivalent directions. The angle between these and the surface was ~45°, and did not change much during the trajectory. The adsorbed protein was between the end-on and side-on orientations discussed above. The COM came closer to the surface by about 4 Å (Table 2) and traveled on the surface the distance of 7 Å (data not shown); for this reason, the adsorbed protein has to be considered as still mobile.

HEWL−surface Orientation 1, 0.02 M Ionic Strength, Trajectory 0 (O1_002M_0). In this trajectory, the protein structure was influenced by the surface. From the rmsd plot (Figure 4), it can be seen that protein reached a stable structure after about 25 ns of the trajectory, but the value of the rmsd (3.0 ± 0.1 Å) is ~1 Å higher than in the case of the isolated HEWL in the same ionic strength. As with the isolated protein, the main contribution to the rmsd comes from the loop regions (Figure 3d). Moreover, during the first 20−25 ns a partial unfolding was observed in α-helix A (5−16, the first helix) and in α-helix 3_{10} (120−125, the last helix) from the α domain (see Figure 1 and Figure 3d). Both helices are a part of the N,C-terminal face. Unfolding was not observed in the β-sheet structures.

As we already described elsewhere,[9] the HEWL started to move toward the surface from the beginning of the trajectory. The movement was accompanied by small rotations and translations in the plane parallel to the surface (data not shown). As Figure 1 shows and Table 1 lists, the initial distance between the protein and the surface was about 7 Å, while the final was less than 2 Å; the distance traveled by COM toward the surface was 4.0 ± 0.5 Å (Table 2). Attraction between the HEWL and the surface started to be visible after about 3.5 ns of the trajectory. At this moment, Arg14 and Arg128 side chains, initially parallel to the surface, started to be perpendicular to the surface. Arg128 was involved in the first contact with the surface, which has appeared at 5 ns, but Arg14 did not reach a similar close approach and conformation at the surface. From 5 ns, the attraction became stronger and more directed, and the protein adsorbed at 14 ns. At this stage, just one contact (with Arg128) was maintained. Other interactions involving Arg14, Arg5, Arg125, and Lys1 were unstable. The second contact has appeared at 17.8 ns with Arg5, followed at 21 ns by Arg125. The former has disappeared at 34 ns, and then, at 46.1 ns, the last surface contact with Lys1 has appeared. From 14 ns, the protein was almost immobilized on the surface. Adsorption suppressed the unfolding but not the loop flexibility. The final protein orientation on the surface is shown in Figure 1.

Table 1. Shortest Initial and Final Protein−Surface Distances Obtained in the Trajectories[a]

Orientation 1 (O1)

start		end									
		O1_05M		O1_002M_0		O1_002M_1		O1_002M_2		O1_002M_3		O1_001M_N	
res.	dist.	res.	dist.	res.	dist.	res.	dist.	res.	dist.	res.	dist.	res.	dist.
K1	10.48	R14	2.26	K1	3.76	Q121	2.52	K1	3.59	K1	2.73	R14	2.79
R14	10.46	N19	2.37	R5	3.10	R125	2.25	R5	3.18	R5	4.89	H15	2.99
R128	7.89	R21	4.34	R14	4.20	R128	3.23	R125	4.06	R125	2.16	N19	2.61
		R128	2.57	R125	7.42			R128	2.31	R128	1.91	Y20	3.09
				R128	1.86							R21	6.19
												R128	2.79

Orientation 2 (O2)				Orientation 3 (O3)				Orientation 4 (O4)			
start		end (image)		start		end		start		end	
res.	dist.	res.	dist.	res.	dist.	res.	dist.	res.	dist.	res.	dist.
T47	8.13	R14	∼2.0	Q121	10.16	R5	4.51	R45	8.77		
N103	8.57	R128	∼3.5	R128	9.56	R125	2.65	R68	9.07	R68	3.04
R112	7.29					R128	2.66				

O1_R128A				O1_R128G				O1_R68G_R128G			
start		end		start		end (image)		start		end (image)	
res.	dist.	res.	dist.	res.	dist.	res.	dist.	res.	dist.	res.	dist.
K1	10.48	K1	3.58	K1	10.48	R125	∼3.5	K1	10.48	R125	∼4.0
R14	10.46	R5	4.44	R14	10.46			R14	10.46		
A128	10.02	R125	3.31	G128	11.14			G128	11.14		

O1_90ns_R128G				O4_R68G			
start		end		start		end (image)	
res.	dist.	res.	dist.	res.	dist.	res.	dist.
K1	3.86	K1	4.09	R45	8.77	R21	∼2.0
R5	3.15	R5	3.29	G68	10.26	R125	∼5.0
R14	4.21					R128	∼2.5
R125	7.47	R125	4.39				
G128	6.73	G128	8.98				

[a] The closest residues and their distances to any surface atom is given. Acronyms used: A − alanine (Ala), G − glicyne (Gly), H − histidine (Hsd), K − lysine (Lys), N − asparagine (Asn), Q − glutamine (Gln), R − arginine (Arg), T − threonine (Thr), Y − tyrosine (Tyr).

Table 2. Minimum Distance between Protein COM and the Surface[a]

	distance (in å)			
trajectory	initial	final	difference	commentary
O1_05M	26.6 ± 0.4	22.4 ± 0.2	4.2 ± 0.6	
O1_002M_0	25.9 ± 0.3	21.9 ± 0.2	4.0 ± 0.5	
O1_002M_1	26.7 ± 0.8	29.4 ± 0.2	3.3 ± 1.0	rotations
O1_002M_2	25.2 ± 0.4	23.8 ± 0.2	1.4 ± 0.6	
O1_002M_3	26.5 ± 0.3	21.7 ± 0.1	4.8 ± 0.4	
O1_001M_N	25.6 ± 0.7	19.0 ± 0.2	6.6 ± 0.9	weak
O2	24.6 ± 0.8	31.0 ± 0.1	−6.4 ± 0.9	image
O3	28.7 ± 0.5	24.1 ± 0.2	4.6 ± 0.7	
O4	29.6 ± 0.4	31.8 ± 0.3	−2.2 ± 0.7	R68
O1_R128A	28.2 ± 0.5	22.6 ± 0.2	5.6 ± 0.7	
O1_R128G	26.1 ± 0.5	34.8 ± 0.4	−8.7 ± 0.9	image
O1_R68G_R128G	26.9 ± 0.2	41.4 ± 0.2	−14.5 ± 0.4	image
O1_90ns_R128G	22.0 ± 0.2	23.3 ± 0.2	−1.3 ± 0.4	diffusion
O4_R68G	30.9 ± 0.7	45.0 ± 0.2	−14.1 ± 0.9	image

[a] To account for normal fluctuations, the initial as well as final distances are averaged over 1 ns.

The dipole moment was always oriented toward the surface; at 14 ns, a 45° angle between its direction and the surface was established which remained unchanged for the next 76 ns. The N, C-terminal face was turned toward the surface. The protein dipole moment and the long axis had almost equivalent directions, and the protein adsorbed between a side-on and an end-on way.

The residues involved in contacts to the surface are a part of the N,C-terminal face; Arg5, Arg14, and Arg125 belong to the unfolding α-helix 3_{10}. Arg128 seems to be a crucial one, while others may be described as auxiliary. Lys1, Arg5, Arg14, and Arg125 side chains at best perturbed only the outer water layer, whereas the Arg128 side chain is seen to thrust through the outer and inner layers (see also ref 10). The first group was interacting with the surface mainly via water molecules, with direct interactions fleetingly observed. In contrast, Arg128 interacted with the surface directly. An extended hydrogen-bond network between the penetrating side chains and the water layers has always been observed. This means that at least one H-bond between the considered residue and a water molecule belonging to the water layers is evident. Sometimes, the most important arginines could create four H-bonds with water molecules, while Lys1 could create as many as six (three by the NH_3 group in the side chain and three by the terminal NH_3 group). Water molecules involved in interactions with the penetrating residue were simultaneously engaged in water−water interactions creating the structured water layers on the surface.

The reduction of protein mobility on the surface was discussed elsewhere.[10] In Figure 6, we show the protein movement toward the surface (z-direction) and the distance traveled during the trajectory for comparisons with other trajectories. Arrows on the

Table 3. Adsorption Mechanism[a]

			simulation time 0 ns → 90 ns					
trajectory	angle (°)	comment	residue					*T* (ns)
O1_05M	45 (45)	jump and rotation before adsorption	Arg128 (13.3)	Asn19 (14.6)	Arg21 (14.6)	Arg14 (14.7)		20
O1_002M_0	45 (45)	rotation before adsorption	Arg128 (5.0)	Arg5 (17.8)	Arg125 (21.0−34.0)	Lys1 (46.1)		14
O1_002M_1	80 (90)	rotation before and after adsorption	Arg128 (3.6)	Arg125 (6.8)	Gln121 (24.0)			12
O1_002M_2	80 (60)	rotation before adsorption	Arg128 (6.0)	Arg125 (10.6)	Lys1 (12.4)	Arg5 (23.3)		18
O1_002M_3	60 (45)	rotation before adsorption	Arg128 (4.6)	Arg5 (12.4)	Arg125 (12.6)	Lys1 (22.7)		14
O1_001M_N	30 (45)	weak adsorption	Arg128 (8.8)	Arg14 (11.7)	Hsd15 (21.3)	Asn19 (58.1)	Tyr20 (58.1)	10
O2	30 (20)	adsorption to the image	Arg128 (2.5)	Lys1 (3.4)	Arg14 (10.2)			21
O3	80 (90)	rotation before adsorption	Arg128 (6.0)	Arg125 (17.7)	Arg5 (51.7)			52
O4	60 (80)	no rotation nor translation	Arg68 (3.0)					8
O1_R128A	80 (45)	rotation, weak adsorption	Lys1 (16.4)	Arg125 (38.7)	Arg5 (75.6)			40
O1_R128G	60 (90)	rotation, weak adsorption to the image	Arg68 (8.0−53.0)	Arg125 (82.0)				9−53, 85
O1_R68G_R128G	60 (45)	diffusion, weak adsorption to the image	Arg125 (61.5)	Arg112 (80.0)				88
O1_90ns_R128G	60 (80)	adsorption is weaker	Arg5 (0.0)	Lys1 (0.0)	Arg125 (39.3)			0
O4_R68G	60 (60)	adsorption to the image	Arg128 (26.7)	Arg21 (36.2)	Arg125 (39.3)			31

[a] Residues are given in the order which agrees with order of creating contact with the surface. The time moment (in nanoseconds) of contact is given in the bracket. The contact is considered if the distance between the surface and the side chain is smaller than 5 Å. The approximate a_1 angle between the protein dipole moment and the surface (the a_2 angle between surface and protein long axis is in brackets) at the final stage of adsorption is listed, as well as the time *T* at which adsorption is considered to occur.

plots indicate the points by which we consider the protein to be adsorbed.

HEWL−surface Orientation 1, 0.02 M Ionic Strength, Trajectories 1, 2, and 3 (O1_002M_1, O1_002M_2, and O1_002M_3). Three other runs of the same orientation, namely, trajectories O1_002M_1, O1_002M_2, and O1_002M_3, in general gave the same adsorption pattern as described above. Partial unfolding of the α-helix 3_{10} from the α domain and maintenance of other secondary structure elements were again observed. In versions 2 and 3, the N-terminus became detached from the protein and moved toward the surface. The slight conformational changes in secondary structure regions, together with extended conformational changes in the loop regions, observed in all O1_002M trajectory versions indicate that HEWL cannot be described as unfolded or denaturated, but rather as a very flexible protein, agreeing with the concept of HEWL as a "hard" protein. Moreover, in each version the protein adsorbed to the surface using the same adsorption site containing Arg128, Arg125, Arg5, and Lys1, with Arg128 playing a key role. As described above with version 0, in the further O1 versions the protein also moved in the direction defined by the dipole moment. Generally, the final orientations on the surface may be described as intermediate between the side-on and end-on ways, with a possible reorientation to the end-on way as observed in version 1. As listed in Table 3, the time needed to adsorb varied between 12 ns and 18 ns. Some more details about protein interactions with the surface and its COM movement are provided by Table 1 and Table 2, respectively.

A more detailed description of trajectories O1_002M_1, O1_002M_2, and O1_002M_3 can be found in the Supporting Information. One can also find there the movies visualizing these trajectories.

HEWL−surface Orientation 1, 0.02 M Ionic Strength, Neutral Surface (O1_001M_N). In this simulation, the protein structure slowly changed from the beginning of the trajectory until about 40 ns when the rmsd reached a value of 2.38 ± 0.18 Å and then remained stable during next 50 ns (data not shown). The rmsd increase is related to changes in the loop regions, as well as small, partial unfolding of the α-helix 3_{10} from the α domain. The location of the protein ends remained unchanged, and the overall protein shape and structure were well-maintained.

At 8.8 ns, the initial protein movement toward the surface resulted in the first contact made by Arg128. The resulting orientation was stable, and the adsorption occurred at 10 ns. However, it is notable that the side chain of Arg128 was then oriented approximately parallel to the surface rather than perpendicular to it, a consequence of the absence of the long-range electric field with the neutral surface. The protein itself was oriented in a side-on way, and the dipole moment and the long protein axis were almost parallel to the surface with the active site exposed to the bulk water. Subsequently, the Arg14 side chain changed its orientation and made a contact with the surface (11.7 ns). The third contact involving Hsd15 appeared at about 21.3 ns. None of these residues were able to directly interact with the surface atoms; therefore, the adsorption was relatively weak, resulting in an enhanced protein mobility. Much later, between 53 ns and 58 ns, the HEWL suddenly rotated anticlockwise by about 60° (12°/ns) which allowed Asn19 and Tyr20 interactions with the surface (both at 58.1 ns). Even with five residues interacting with the surface, the HEWL was not anchored well (see Supporting Information movie O1_001M_N.avi). Rotations were still possible, and between 66.8 ns and 70.8 ns, the HEWL rotated clockwise by ∼30° and reached its final orientation shown on Figure 1d. This orientation may be described as an intermediate between the side-on and end-on ways.

Orientations 2, 3, and 4 (O2, O3, and O4). The detailed description of these trajectories is provided in the Supporting Information. All of them support our previous observations classifying HEWL as a "hard" protein. In O2 and O3, the HEWL again adsorbed using its N,C-terminal face (see Table 1 and Table 3) with the dipole moment oriented toward the adsorption surface. To expose the N,C-terminal face, the protein either rotated (in O3) or adsorbed to the image of the surface (in the O2 trajectory). Trajectory O4 is an exception, revealing that a secondary adsorption site comprising Arg68 may be used in some particular conditions. However, adsorption at this site is not strong, since here the dipole moment is directed away from the

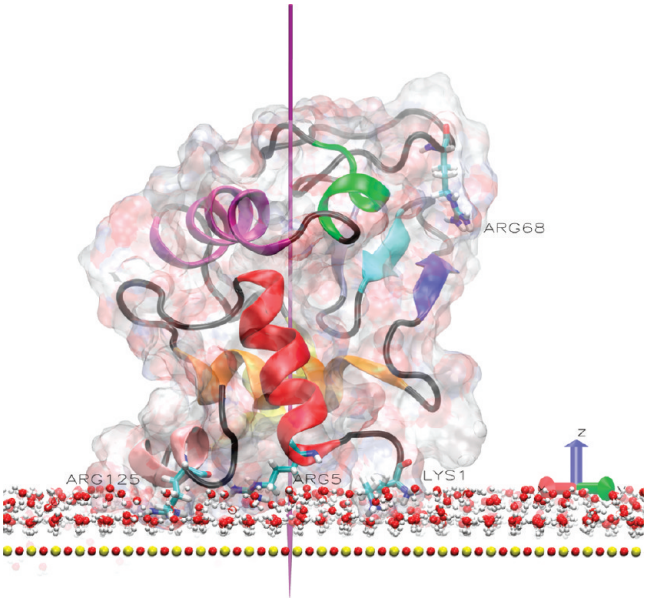

Figure 5. Protein penetration through the surface water layers observed at the final stage of the trajectory O1_R128A. General coloring scheme is the same as in Figure 3; additionally, surface waters are shown by CPKs.

charged surface, and the protein is observed to be mobile when adsorbed in this orientation; see also ref 10.

Orientation 1, Mutation Arg128→Ala128 (O1_R128A). In the case of Arg128→Ala128 mutation in Orientation 1, after the initial growth and jump from 1.5 ± 0.1 Å at 13 ns to 3.4 ± 0.2 Å at 20 ns the rmsd plot had a horizontal character (data not shown). The jump reflects the detachment of the N-terminus and partial unfolding of α-helix 3_{10} from the α-domain. Despite this alteration, the protein maintained its structure quite well.

During the first 10 ns of trajectory, the HEWL rotated anticlockwise by 15° and a 60° angle between protein long axis and the surface was reached. At 13 ns, the detachment of the N-terminus exposed Lys1, which was able to interact with the surface after further protein rotation by 10° (resultant angle ∼70°). Simultaneously, the α-helix 3_{10} started to unfold, which exposed Arg125. At 16.4 ns, Lys1 made contact with the surface. The protein continued its rotation and finally reached an angle of ∼80°. This enabled Arg125 to contact the surface at 38.7 ns. This contact stabilized the HEWL position, which adsorbed at 40 ns. Further stabilization was achieved at 75.6 ns, when Arg5 created a contact with the surface (Table 3). The described rotations and structural changes were accompanied with protein translation toward the surface (Table 2).

This mutation changed a partial charge distribution so that the dipole moment direction is now different from the protein long axis orientation. As Figures 5 and 6 indicate, the O1_R128A adsorption is weaker than the native version. Residues Lys1, Arg5, and Arg125 were not able to interact with the surface directly; Lys1 and Arg125 were usually penetrating the inner water layer, while Arg5 just penetrated the outer one (Figure 5). For this reason, O1_R128A was more mobile on the surface than the native protein (Figure 6). The Supporting Information movie O1_R128A.avi gives a qualitative confirmation of the above observations.

Orientation 1, Mutations Arg128→Gly128 (O1_R128G), and Mutation Arg68→Gly68 + Arg128→Gly128 (O1_R68G_R128G). Details of both these trajectories are given in the Supporting Information. Similar to the trajectory O1_R128A, trajectories O1_R128G and O1_R68G_R128G indicate that the lack of Arg128 makes the adsorption process slower, weaker, and more difficult to achieve. Nevertheless, still the N,C-terminal face was used by the protein to adsorb, verifying the importance of the whole region. In the adsorbed state, the protein dipole moment faced toward the surface (or its image in the case of O1_R68G_R128G) and the protein orientation may be described as the end-on way (O1_R128G) or intermediate between the side-on and end-on ways (O1_R68G_R128G).

Orientation 1, Mutation Arg128→Gly128 on Adsorbed Protein (O1_90ns_R128G). Details of this trajectory are also provided in the Supporting Information. The mutation on the previously adsorbed protein indicates that Arg128 is important not only for creating contacts with the surface and the overall adsorption process, but also in constraining the mobility of the adsorbed protein (see Figure 6 and ref 10). The lack of Arg128 again makes adsorption weaker (Tables 1−3).

Orientation 4, Arg68 → Gly68 (O4_R68G). This trajectory provides evidence that the secondary adsorption site contains only one residue, namely, Arg68. The point mutation on this position introduces a local change in the protein surface and its electrostatic field, which resulted in adsorption (to the image of the surface) using the N,C-terminal face. The adsorption mechanism appears very similar to those observed in trajectory O3 and other orientations as well. A detailed description of the O4_R68G trajectory is given in the Supporting Information.

Discussion

Role of Electrostatics. The role of electrostatics in the adsorption process has previously been discussed in ref 9 with the conclusions drawn from the set of 20 ns trajectories. All of the conclusions drawn in that paper stand in light of the longer trajectories presented here, and are supported by the mutation simulations too.

In general, protein translation toward the surface (or its image) has proceeded through achieving a favorable orientation of the dipole moment. For this reason, employing sufficient simulation time, as we have done here, to allow protein rotations is essential to obtain a thorough understanding of the adsorption process. If necessary, the HEWL rotates and changes its structure to obtain a dipole moment oriented toward the surface. Of course, the dipole moment direction and value (∼200 D) are sensitive to protein structural alterations. The protein movement direction is usually defined by the dipole moment orientation.

The only exception is trajectory O4 in which the dipole moment was oriented away from the surface. Nevertheless, in this case it was clearly visible that the adsorption was not strong and that the protein was captured in a local energy minima. The total energy in O4 was higher than in all O1 simulations by $\sim 55 \times 10^3$ kcal/mol (on average) and higher than in O2 and O3 simulations by $\sim 25 \times 10^3$ kcal/mol. The protein as a whole body moved away from the surface (Table 2). The most likely explanation is that the local attraction was stronger than the long-range Coulomb repulsion. Given more time, it is probable that the protein would escape from this weakly bound state. Mutation at position 68 changes the local electric field, and thereafter, the protein is able to use the typical adsorption scenario in which the translation direction agrees with the dipole moment orientation.

It should be emphasized that the list of residues important for the adsorption, penetrating the water layers and interacting with the surface, is composed of arginine and lysine residues. Both amino acids in neutral pH (as studied here) are polar and carry a positive charge. For adsorption on a flat surface, the contribution of residues other than arginine and lysine is negligible, although this is not the case for a nonflat surface geometry.[9]

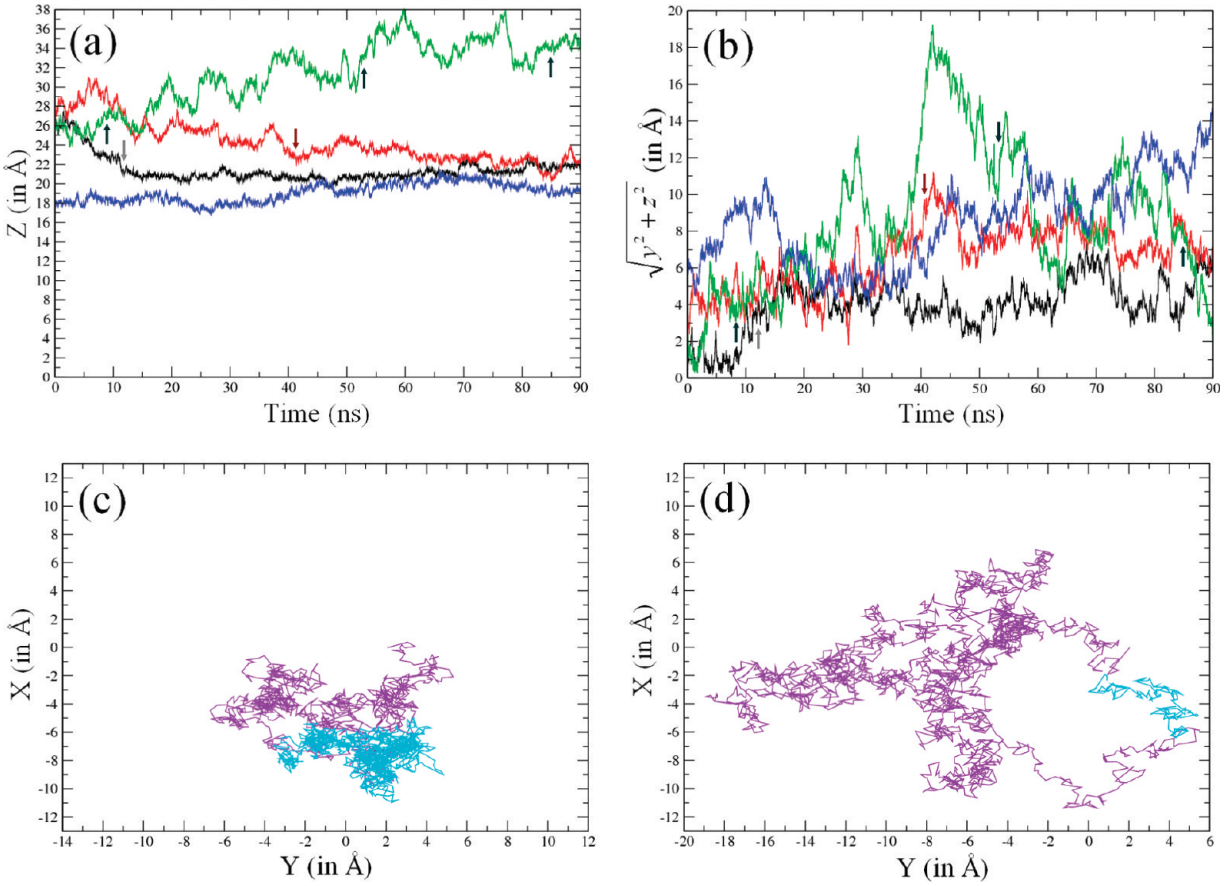

Figure 6. Protein center of mass (COM) diffusion. (a) Evolution of the COM distance perpendicular to the surface plane. (b) Projection of the COM on the surface plane. The coloring scheme is as follows: black − O1_002M_0, red − O1_R128A, green − O1_R128G, blue − O1_90 ns_R128G. Arrows indicate the times of adsorption or desorption. (c) Plan views of the COM projection onto the surface plane for O1_R128A and (d) O1_R128G. The violet part of the plots indicate the diffusion before and during adsorption, and the cyan parts indicate the diffusion on the surface/image following adsorption.

The electrostatic field created by the charged surface is important not only for the overall protein positioning, but also for appropriate orientation of the amino acids' side chains. If this field is weaker, as is the case with the neutral surface, the polar residues are not oriented optimally and so are not able to directly interact with the surface. The partial charge distribution on the surface is not enough to act as a driving factor for side-chain orientation, but it is sufficient to produce the two highly organized water layers observed in all the trajectories studied herein. These water layers, discussed previously in ref 9, mediate the protein−surface interactions. To achieve strong adsorption, both the protein dipole moment and the polar side chains must be oriented toward the surface, which is only achieved through the long-range electric field produced by the charged surface. Otherwise, the adsorption is weak and the protein diffuses more readily on the surface.

Taken together, these results imply that electrostatic interactions are the main force governing adsorption, at least during the deposition of the first molecules on the first protein layer. As mentioned previously, this conclusion is reinforced by the slowing down of the adsorption process at higher ionic concentrations, consistent with the experimental observation that high ionic strength prevents adsorption.[25] Hydrophobic or hydrophilic forces may work at most as supplementary forces during the adsorption of isolated HEWL. However, the driving force for second and further protein layer formation in multilayer adsorption, as well as in higher protein concentration and higher surface coverage, may involve hydrophobic or hydrophilic interactions between two protein molecules and/or layers. For this reason, simulations with a few protein molecules located in the primary simulation cell are required and are currently underway. The importance of electrostatic interactions for HEWL adsorption was indicated in previous Brownian dynamics studies[22] and in numerous experimental investigations.[13,20,23,37,38]

Conformational Changes Induced by Adsorption. Comparison of trajectories for HEWL in the same ionic strength but with and without the surface shows that the overall dynamical features such as protein flexibility, including the mobility of the loops, have in general the same character. Moreover, the protein is much more responsive to the presence of the surface in low ionic strength; at 0.02 M, the adsorbing protein is more flexible than the isolated one. This agrees with previous experimental observations that the adsorbing protein adopts a more flexible structure.[11] It has already been agreed that HEWL belongs to the "hard" protein grouping (see, for example, ref 27), which means that the protein is flexible but does not significantly change its structure at any level. This is exactly the behavior we see in the presented trajectories.

In most trajectories, the α-helix 3_{10} from the α-domain displayed only partial unfolding, and in two of them, some changes also appeared in α-helix A (trajectories O1_002M_0 and O3). Both helices contain residues building the N,C-terminal face and

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the main adsorption site. The other α-helices were much more stable. The last substantial conformational change was connected with the N-terminus location. Inspection of the protein shape reveals that those changes are probably part of the protein flattening process on the surface. As has already been assumed in a recent theoretical model of adsorption kinetics,[39] the adsorbing protein undergoes slight, local changes of its shape. The part of the protein surface which is facing toward the solid surface becomes more flat to make the protein−surface interface wider, creating more protein−surface contacts and further lowering the total interaction energy. Examination of the final protein structures (Figure 1b,c and Figure 5) reveals that the HEWL surface in the N,C-terminal face region is indeed flattened as a result of the adsorption. This HEWL flattening cannot be assumed as an initial step of protein spreading on the surface. The protein does not lose its overall structure, but only slightly reduces its α-helix content with respect to β structures, which has been observed experimentally upon HEWL adsorption.[3,11,25,33,34] All the aforementioned changes were rather marginal, and the proteins adsorbed on the flat surface were far away from an unfolded state even after a 90 ns trajectory (confirming HEWL as a "hard" protein). Moreover, it appears that there is a correlation between the adsorption strength and the range of conformational changes. With fewer residues interacting with the surface (Table 1), or weaker interactions, the protein was more mobile on the surface (Figure 6) and its structure was closer to the native one. In turn, it may be concluded that adsorption requires enhancement of internal HEWL flexibility and even partial unfolding of two terminal α helices: A and 3_{10} from the α domain, which leads to a flattening of the protein shape at the N,C-terminal part of its surface.

Conformational change starts in the very early stages of the adsorption trajectories and causes the exposure of residues important for the adsorption. The protein has then been able to adsorb and after some more structure adjustments has reached a relatively stable structure on the surface (see Figure 4). A few nanoseconds after adsorption, the unfolding ceases with both α-helices just partially unfolded; further structural changes can be caused by N-terminus position changes and the usual loop mobility. Nevertheless, the possibility that over a longer time scale unfolding might continue cannot be excluded by this work.

Mobility on the Surface. We have discussed the mobility of the adsorbed HEWL across the surface previously,[10] where we focused on the key role played by Arg128 in immobilization. Here, we can provide a more detailed discussion of surface mobility drawing on our full set of 90 ns trajectories.

Monitoring the COM motions gives an insight into HEWL mobility during and after adsorption. Sometimes the distance between the closest residue and the surface has decreased by 7 Å, whereas the protein as a whole has moved with respect to the surface by only 4.0 ± 0.6 Å during adsorption to the surface and by 7.9 ± 0.7 Å to the image (Table 2). The difference is caused by the fact that the location of the COM does not always reflect the conformational changes in the way the distance to the closest residue does. As Figure 6 indicates, the COM mobility on the surface is very sensitive to adsorption site composition. Lack of the Arg128 side chain makes protein diffusion on the surface much easier.

It has already been observed experimentally that adsorbed protein is still mobile on the surface.[14,15,23] We have also noticed diffusive motion with range and intensity depending on the adsorption site used. If the HEWL has adsorbed at the main adsorption site (Arg128, as in trajectory O1_002M_0), its mobility is strongly constrained (Figure 6), contrary to absorption at the minor site (Arg68, trajectory O4), which does not much impede the HEWL mobility. The second factor governing the mobility is the composition of the main binding site. If Arg128 has been substituted by alanine or glycine, the number of residues interacting with the surface was lower, so the adsorption was weaker and the diffusion on the surface was easier (Figure 6). Hence, adsorption strength is reflected by protein mobility on the surface. The question of dimer or larger cluster mobility on the surface is currently under further investigation.

As mentioned, the higher the number of interacting residues, the lower the protein mobility. Residues that penetrate the surface water layers (e.g., Figure 5) and create an extended hydrogen bond network are most effective at immobilizing the protein. Recently, the importance of surface water has been reported for HSA (human serum albumin) adsorbing on a TiO_2 surface,[41] and the role of an extended hydrogen bond network was reported for BMP-2 (bone morphogenic protein) adsorbed on a hydroxyapatite surface.[42] The existence of the highly organized surface water layers on mica, as discussed previously,[9] largely prevents the HEWL from interacting directly with the surface. Most interactions are indirect via water molecules. Only Arg128 could change its conformation (through side chain straightening) sufficiently to enable direct and permanent contacts with the surface atoms (see Table 1). We do not consider chemical bonding in this study, finding that the adsorption can be driven solely by physical interactions.

In the majority of cases, the protein side chains penetrate just the outer water layer. The insertion of one side chain into one water layer is connected with relaxation of three water molecules from the layer and the creation of a protein−water hydrogen bond network. Adsorption seems to be easier than protein movement on the surface. The first process is connected with the exclusion of a few water molecules, whereas the second one requires reorganization of water−surface, protein−surface, and protein−water interactions. Therefore, residues forming the binding site were acting as anchors. Mutation of the strongly interacting Arg128 made the diffusion easier; the protein could then slide on the inner water layer. A similar effect was observed in the simulation with the neutral surface, where there is no long-range electric field. All this together explains protein immobilization on the surface and the dependence between the range of surface translation and the number of residues interacting with the surface.

Adsorption Mechanisms and the Binding Site(s). The main sequence of events during the adsorption trajectories is summarized in Table 3, which gives a list of residues creating contacts with the surface over time and indicates the adsorption moment, as well as giving insight to protein orientation on the surface. On the basis of our results, we can propose the following HEWL adsorption mechanism on a charged solid surface. In the initial stages, the protein is far from the surface and freely diffuses in the bulk environment. If during its diffusion the protein orients in an appropriate way, the adsorption starts. The requirements are the following: (i) the protein dipole moment is oriented toward the surface; (ii) the N,C-terminus faces toward the surface; and (iii) the separation is small enough for the protein to feel the surface's electrostatic field. The protein is then attracted to the surface and

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can simultaneously rotate to achieve an angle between the long axis and the surface of ~45°. For the native protein, the directions of the long axis and the dipole moment are almost equivalent. The Arg128 side chain is subsequently exposed and attracted to the surface, which enhances the protein attraction to the surface. At this moment, three actions are possible: (i) the rotation ceases; (ii) the rotation slows down; or (iii) the rotation is continued. In the first case, the final angle between protein long axis and the surface remains at 45°; in the second case, the protein slowly rotates and achieves the angle 60°; while in third case, the final angle is about 90°. The rotation does not influence further events such as exposing other residues at the N,C-terminal face (Arg125, Arg14, Arg5, and Lys1) and small conformational changes due to protein flattening. Sometimes, the exposure of Lys1 is preceded by a conformational change of the N-terminus, which detaches from the protein surface, bends, and comes toward the solid surface. Trajectories obtained for mutated proteins confirmed that Arg128 is the crucial residue for the adsorption. Residues Arg125, Arg14, Arg5, and Lys1 belong to the N,C-terminal face and assist protein immobilization and anchoring on the surface. All five mentioned residues create the main adsorption binding site.

In two trajectories (O1_002M_1 and O1_001M_N), Arg21, which does not belong to the N,C-terminal face, was detected as a residue that can contact the surface. Nevertheless, Arg21 activity seems to be marginal, acting as an additional stabilizing factor rather than offering a new and distinct adsorption site. We note that, in trajectories O3 and O4, Arg21 was initially exposed toward the surface or its image but was not subsequently involved in the protein−surface (or image) interactions. This confirms the idea that Arg128 dominates strong adsorption, while an adsorbed protein may be sometimes stabilized by other residues due to protein flexibility.

HEWL may adsorb in three main orientations described as side-on way, end-on way, and the intermediate state between side-on and end-on ways. The differentiation is based on the angle (a_1, for example) between the protein dipole moment and the surface. The angle a_2 is defined by the protein long axis and the surface. The value estimation is not, and does not have to be, accurate, since both angles strongly fluctuate. The criterion we can use to decide which orientation is observed is as follows: (a_1, a_2) $\ll 45°$ for the side-on way, (a_1, a_2) $\approx 45°$ for an orientation between side-on and end-on, and (a_1, a_2) $\gg 45°$ for the end-on adsorption (Table 3).

In the early stages, HEWL is adsorbed in an end-on way or between end-on and side-on ways. In the latter case, subsequent rotation to achieve an end-on orientation was possible; nevertheless, the intermediate orientation between end-on and side-on ways seems to be preferred (Table 3). This intermediate orientation is equivalent to the back-on orientation obtained by Xie et al. from parallel tempering Monte Carlo simulations,[40] the orientation proposed by Robeson et al. revealed by total internal reflection fluorescence (TIRF) method,[29] and, in principle, HEWL orientation observed on ion exchange resins.[5,31] Moreover, our intermediate orientation seems to be the same as the side-on orientation revealed by other experimental studies, such as indirect estimations coming from reflection fluorescence experiments[27,28] or quartz crystal microbalance (QCM) technique[21] and inferences from protein layer thickness measurements using neutron reflection experiments.[25,26] Adsorption in a genuinely side-on way with the long axis almost parallel to the surface was observed just once (trajectory O2). If the main adsorption site was used, desorption was not possible in the 90 ns time scale of our simulations. Long time-scale simulations as well as simulations for higher protein concentration are required to complete the picture.

The second adsorption site is composed from one residue, Arg68, which is part of a long loop sequence. That is why adsorption using this binding site is relatively weak. This second adsorption site appears to be used accidentally, after a conformational change in the long loop, which exposes Arg68 to the surface. The adsorbed protein is oriented between end-on and side-on ways, with desorption and further adsorption possible (as was observed in O1_R128G, Table 3).

The adsorption mechanism we describe here largely agrees with those proposed before from experiments.[15,21,28,29] It provides a detailed description of the protein behavior, gives a sequence of events during the HEWL adsorption, highlights the adsorption site composition, and elucidates the protein orientation on the surface. The existence of two adsorption binding sites agrees with previous experimental observations.[5] Moreover, their location and approximate composition is generally consistent with previous assumptions.[5,22,30,31,40] Nevertheless, our selection is much more detailed and well-documented.

Since the HEWL active site is located opposite the main adsorption binding site and the protein structure is not changed much, its activity should be maintained at quite a high level when HEWL is adsorbed on a flat, charged surface. It has already been found experimentally that lysozyme molecules immobilized on silica may exhibit the same or even higher activity than in solution as long as only a monolayer is formed on the surface.[43] Nevertheless, further investigations on immobilized HEWL activity are desirable. In addition, it would be interesting to test experimentally our hypothesis that Arg128 is crucial for the HEWL immobilization at the surface.

Conclusions

HEWL adsorption cannot be described simply as a protein landing on the surface. In order to adsorb, the protein has to adjust its structure, and in particular has to make a flat contact with the surface. To achieve this, the N,C-terminal α-helices have to partially unfold and expose charged polar residues oriented toward the surface. Hence, the internal protein flexibility is crucial for the adsorption. HEWL behaves as a "hard" protein, which is free to adjust its structure but does not unfold, spread, or denature on the surface. Monitoring of protein orientation on the surface reveals that HEWL adsorbs between side-on and end-on ways or in an end-on way, but not in a side-on way. Two adsorption sites may be used: the major one located on the N,C-terminal face (Arg128 plus Arg125, Arg5, and Lys1) and the minor one with Arg68. The desorption probability, as well as protein mobility on the surface, strongly depends on the adsorption site used. The active site composition drives protein diffusivity on the surface. The presence of Arg128 strongly slows down this diffusion. In agreement with intuition, if more residues are involved in the protein−surface contacts the protein mobility is lower. Another correlation observed is that, the more pronounced the protein's surface flattening, the more residues are able to create a contact with the surface. Finally, the net surface charge influences the adsorption strength, since it orients polar side-chains enabling them to penetrate the surface water layers. Therefore, the adsorption has to be considered as a multifactor process.

On the basis of our atomistic MD studies, we are able to give a detailed, step-by-step description of the adsorption mechanism and to interpolate the results to longer time scale in which protein

(43) Ding, H. M.; Shao, L.; Liu, R. J.; Xiao, Q. G.; Chen, J. F. *J. Colloid Interface Sci.* **2005**, *290*, 102.

unfolding is not expected. What we can expect is protein diffusion on the surface in its native-like form and cluster creation. The latter possibility has to be examined by multiprotein adsorption simulations, which would also address questions about which mechanisms govern second-layer formation.

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Supporting Information Available: Movies showing protein dynamics in illustrative orientations. Detailed description of trajectories: O1_002M_1, O1_002M_2, O1_002M_3, O2, O3, O4, O1_R128G, O1_R68G_R128G, O1_90ns_R128G, and O4_R68G. This material is available free of charge via the Internet at http://pubs.acs.org.