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# Hen Egg White Lysozyme Adsorption on a Mica Surface: A Fully Atomistic Molecular Dynamics Study

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The interactions between proteins and solid surfaces are essential for a number of applications, such as functionalising biomaterials and for medical implants. The understanding of fundamental forces and processes involved in protein adsorption has a great importance in the construction of new, biocompatible materials. Our recent effort has been focussed on adsorption processes and protein dynamics on the surface, including protein cluster formation and cluster diffusion. Hen egg white lysozyme adsorption on a mica surface is an excellent model system for these investigations. Despite recent theoretical and numerical investigations, including Brownian Dynamics (BD) and Monte Carlo (MC) simulations, many atomistic details of lysozyme adsorption, cluster formation and protein/cluster(s) diffusion on the mica surface remains unknown.

Here, for the first time, we present results of fully atomistic, Molecular Dynamics (MD) simulations of hen egg white lysozyme (1iee.pdb) located in the neighbourhood of a mica surface. Protein adsorption is driven by electrostatic forces and so strongly depends on ionic strength. We have therefore examined two systems: solvated, neutral hen egg white with ionic strength equal 0.5 M and 0.02 M respectively. As a reference, a trajectory obtained for the isolated and solvated protein in ionic strength 0.5 M is also used. Careful analysis of four 20 ns trajectories provides an insight into early events during lysozyme adsorption on the mica surface, as well as influence of the surface and different ionic strength on the protein structure and stability.

## 1 Introduction

Interactions between proteins and solid surfaces are essential for a number of applications, such as functionalising biomaterials and for medical implants. The understanding of fundamental forces and processes involved in protein adsorption has a great importance in the construction of new, biocompatible materials. Our recent effort has been focused on adsorption process and protein dynamics on the surface, including protein cluster formation and cluster diffusion<sup>1</sup>. Hen egg white lysozyme (HEWL) adsorption on a mica surface is an excellent model system for these investigations. Despite recent theoretical and numerical investigations, including Brownian Dynamics<sup>2,3</sup>, Monte Carlo simulations<sup>4</sup> and numerous Molecular Dynamics studies<sup>5</sup>, many atomistic details of lysozyme adsorption, cluster formation and protein/cluster diffusion on the mica surface remains unknown.

Here, for the first time, we present results of fully atomistic Molecular Dynamics simulations of HEWL (1iee.pdb<sup>6</sup>) located in the neighbourhood of a mica surface. Protein adsorption onto a charged surface is driven mainly by electrostatic forces and so strongly depends on ionic strength. We have therefore examined two systems: solvated, neutral HEWL at pH=7 with ionic strength equal to 0.5 M and 0.02 M, respectively. As references, trajectories obtained for the isolated, solvated protein in these solutions are also

used. Careful analysis of these four 20 ns trajectories provides an insight into the early events during lysozyme adsorption on the mica surface, as well as the influence of the surface and different ionic strength on the protein structure and stability.

## 2 Methods

The crystal structure of HEWL (1iee.pdb<sup>6</sup>) was the starting structure of all our simulations, with all four disulphide bridges kept. The calculations were performed using the NAMD package<sup>7</sup>. The protein was placed in a rectangular box of water molecules (TIP3) that extend 8 Å from any protein atom. In the case of protein-surface system, a SiO<sub>2</sub> surface (mimicking a mica surface) with dimensions  $x=86.4$  Å and  $y=92.8$  Å was placed about 9 Å away from the closest HEWL side chain and 12 Å away from the HEWL backbone. The surface was created from silica and oxygen atoms, charged +1.11 e and -0.66 e respectively, located 1.6 Å away from each other, in a square array. The resulting surface charge density  $\sigma = -0.0217$  e/Å<sup>2</sup> is almost equal to that of mica at pH=7 (see ref. 4). The HEWL-surface system was solvated in a water box that extends at least 20 Å from any protein atom. The protein or protein-surface systems were then neutralised by adding NaCl salt with ionic strength 0.5 M and 0.02 M. Most probable charge states at pH=7 were chosen for ionizable residues. These systems, composed from more than 15000 and 55000 atoms for HEWL and HEWL-surface respectively, were subject to 100 ps water equilibration, 10 000 steps of whole system minimisation, 30 ps heating to 300 K and 270 ps equilibration at this temperature. The production MD simulations were pursued for 20 ns at 300 K in the NVT ensemble. The integration step was 2 fs, and the SHAKE algorithm and periodic boundary conditions were used. The cutoff distance for both van der Waals and Coulomb interactions was 12 Å.

## 3 Results and Discussion

During the 20 ns trajectory for the HEWL-mica system with 0.5 M ionic strength solution, no attraction between the HEWL and surface was observed. Comparisons with the trajectory for the isolated HEWL show that the interactions and forces acting on the protein are not changed by the mica surface. Overall dynamical features such as protein mobility, including the mobility of the loops and changes in their conformation, show virtually the same interactions with water and salt ions. Based on this we can conclude that the electrostatic interactions are the main forces driving the adsorption process, and the solvent ionic strength 0.5 M effectively screens any electrostatic attraction between the negatively charged mica surface and the positively charged HEWL. Therefore the protein dynamics are not affected by the mica surface (data not shown) under these conditions.

A different picture emerges from the analysis of the 20 ns trajectory of the HEWL-mica system with 0.02 M ionic strength. In this case a strong attraction between the HEWL and mica surface is clearly visible, and the protein moves about 4-5 Å closer to the mica surface during the simulation. Graphical analysis reveals two simultaneous processes: the protein is moving as a whole towards the mica surface, whilst a conformational rearrangement occurs without losing secondary structure. This observation is supported by RMS and RMSF analyses. The RMS calculated for the HEWL-mica system stabilises after 1.4-2.0

ns at the 2.0-2.5 Å level, which means that the HEWL doesn't change its fold. The average fluctuations for the whole protein (Ca atoms) are 2.18 Å<sup>2</sup>, for loop regions 2.47 Å<sup>2</sup> and for secondary structures 1.83 Å<sup>2</sup>. From these RMSF results we can conclude that all helices and beta-sheets are maintained, and that conformational changes are accommodated only by loop and H-bonded turn rearrangement.

The HEWL conformation is more stable when the mica surface is absent; the RMS for the isolated HEWL reaches 1.5 Å after 20 ns (c.f 2.0-2.5 Å found above). Therefore the negatively charged mica surface placed close to the positively charged HEWL induces conformational changes accompanying the adsorption process.

The most important interactions between the mica surface and the HEWL are electrostatic attractions between the negatively charged surface and the positively charged Lys1, Arg14 and Arg128 residues. The distance between the surface and the Lys1 side chain is 11.3 Å at the beginning and 5.4 Å after 20 ns of simulation. In the case of the Arg14 side chain these distances are 11.3 Å and 7.9 Å after 20 ns, and for the Arg128 side chain 8.6 Å and 2.9 Å respectively.

The Lys1, Arg14 and Arg128 side chains form a triangle (side lengths 11 Å, 10 Å and 14 Å) whose conformation is better maintained when the mica surface is located close to the protein. All of these residues are strongly attracted by the surface and most probably the observed HEWL conformation changes are forced by this triangle maintaining its planar conformation.

## 4 Conclusions

We have found that the protein adsorption strongly depends on ionic strength, and conclude that the dominant interactions driving adsorption are the electrostatic interactions between the mica surface and the HEWL protein. Close proximity of the mica surface to the HEWL induces conformational rearrangement mostly confined to the loop regions, so that the HEWL secondary structure is maintained. The most important residues for the HEWL adsorption are Lys1, Arg14 and Arg128, whose rigid planar topology in close proximity to the mica surface appears to drive the loop conformational changes.

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