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High-Resolution Atomic Force Microscopy Study of Protein Surface Conformations

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Background

Protein adsorption on a surface is a part of many relevant biomolecular processes taking place in living organisms (e.g., during immune response) and in biotechnological applications (e.g., during biosensor operation). An adsorbed protein molecule assumes a particular surface conformation, which may differ from the conformation in the bulk [1]. Since the three-dimensional structure of a protein molecule determines its ability to specifically bind to other biomolecules and defines its functional activity [2], understanding of a protein's surface conformation has a great biomedical and biotechnological relevance. In this work, we have used atomic force microscopy (AFM) to characterize the adsorption and conformation of several proteins, such as ferritin, fibrinogen and RNA polymerase, on model surfaces at a single-molecule level.

Methods

Bare or modified highly oriented pyrolytic graphite (HOPG) and freshly cleaved or soaked mica surfaces were used as model surfaces. A freshly cleaved HOPG surface was modified with stearyl amine or stearic acid isopropanol solution (100 μg/ml) by spin coating or N,N'-(decane-1,10-diyl)bis(tetraglycineamide) (GM) water solution (10 μg/ml) by drop casting for ~10 seconds. The duration of protein deposition varied from less than 1 s to several hours. AFM investigations were performed in semi-contact or Peakforce tapping mode either in air or in aqueous solution using standard commercially available cantilevers or super-sharp home-built cantilevers with a tip radius of ~1 nm [3].

Results

We have characterized the morphology of individual adsorbed protein molecules, such as ferritin, fibrinogen and RNA polymerase (RNAP), including their shape and dimensions. Our results have demonstrated that the surface conformation of the proteins depends not only on the underlying substrate but also on the duration of protein deposition (adsorption) and other conditions (such as the ionic strength of the solution or temperature).

On a bare HOPG surface the height of adsorbed protein globules decreased during the time after adsorption until a partial (ferritin) or complete (fibrinogen, RNAP) transformation into a thin adsorbate, which tended to aggregate on the surface and formed a continuous layer (Figure 1). RNAP and fibrinogen molecules adsorbed on a GM-HOPG surface revealed unfolding into fibrillary structures during several minutes, whereas ferritin did not significantly change its globular conformation. We have

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observed a slight decrease in height of ferritin molecules and fibrinogen globular regions and a reorientation of fibrinogen globular regions within ~10 s after adsorption on a stearylamine modified HOPG surface. Moreover, the fibrinogen conformation on a stearylamine or stearic acid modified HOPG surface is more compact than on a GM-HOPG surface, indicating differences in the protein interaction with these surfaces. The observed fibrinogen conformation was more extended a soaked than it was on a freshly cleaved mica surface. The observed surface conformations of the proteins may be rationalized by their different properties such as hydrophilic and charge characteristics [4].

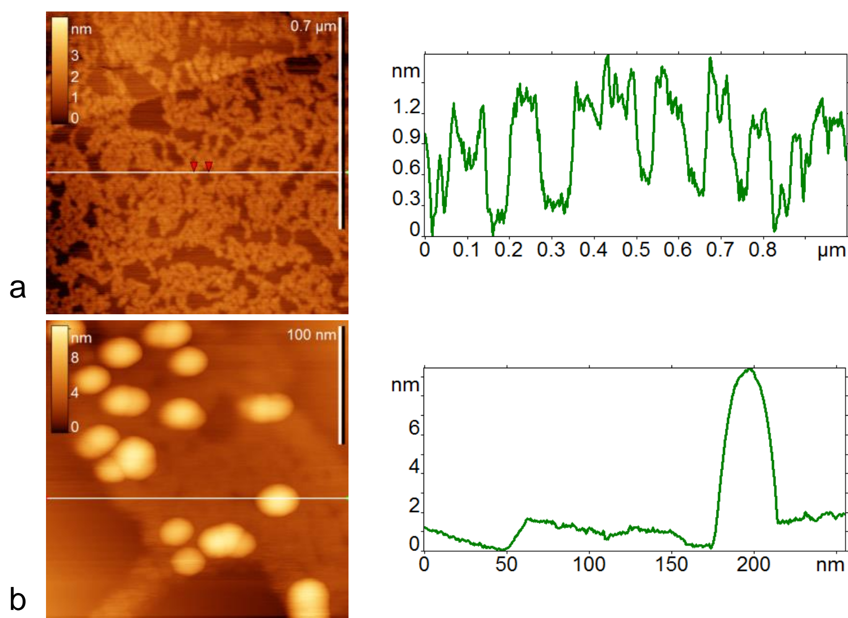

Figure 1. (a) AFM image of a denatured fibrinogen layer formed on a bare HOPG surface. (b) AFM image of ferritin molecules on a denatured ferritin layer formed on a bare HOPG surface. The profiles along the lines in the images are shown on the right.

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