Notes

Do Denatured Proteins Behave Like Polymers?

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Received December 20, 1993

Introduction

Since proteins *in vivo* are usually confined to very small spaces (specific regions of the cell) and since proteins *in vitro* are often associated with surfaces (catheters, dialysis membranes, separation devices such as chromatography packings and synthetic membrane filters, and marine surfaces such as hulls), much recent research has been focused on the folding behavior of proteins adsorbed at various interfaces and under different conditions.[1,8,10,11] Many different experimental methods have been used in these studies to probe the kinetic and equilibrium behavior of adsorbed proteins. Little attention has, however, been devoted to the behavior of unfolded or denatured proteins. Although it has been implicitly assumed that unfolded or denatured proteins are linear in structure, solely dependent on their covalent amino acid sequence, experimental evidence confirming this has been wanting. We address this question here by using methods that have been widely used for studying adsorbed linear polymers with unfolded denatured human serum albumin as a model protein.

Experimental Section

Normalized force–distance measurements were obtained for two experiments with adsorbed human serum albumin (HSA; molecular weight 66 500, #A 3782, Sigma, St. Louis, MO) in a 10^{-2} M KCl solution at pH 2 (see the data points in Figure 1). In the first experiment, HSA was adsorbed onto mica for 2 h from a 5 mg/L protein solution containing 10^{-2} M KCl at pH 2. After the adsorption step, the protein-containing solution was replaced with a 10^{-2} M KCl solution at the same temperature (20 ± 0.5 °C) and the forces were measured as a function of separation. This solution was then replaced with a 10 M urea and 10^{-2} M KCl solution at pH 2, and the forces were again measured as a function of separation. We assume that constrained or restricted equilibrium existed, i.e., the protein was irreversibly adsorbed, and that on replacement of the protein solution with the KCl solution little if any protein desorbed into the solution. This was confirmed by the absence of proteins in the KCl solution after the experiments. The technique involves the direct measurement in a surface force apparatus of the intermolecular forces between two cross-mica cylinders onto which HSA has been preadsorbed for 2 h and uses interferometry and the spring constant to determine both the separation distance (D) and the forces.[6] Dividing the measured force by the radius of curvature of the mica cylinders (R = 2 cm) obtains the interaction energy between the two surfaces using the Derjaguin approximation.[5]

Results and Discussion

For constrained equilibrium with polymers, the amount of noninteracting linear polymer molecules between two mica surfaces is constant and independent of D. Hence, for small D, where the polymer concentration between the surfaces is constant, de Gennes[3,4] has shown that the free energy of interaction between the surfaces scales with $D^{-1.25}$. For large D, the polymer concentration in the solution is negligible as compared to that adsorbed on the surfaces, and the free energy of interaction between the surfaces scales with D^{-2}. Note that these exponents are independent of the number of monomers in the polymer chains. Since these and other relationships have been tested for polymer adsorption under constrained equilibrium,[2,9] we decided to use them to determine whether denatured proteins (under acid transition at pH 2 and a combination of low pH with a strong denaturing agent, 10 M urea) also follow these scaling laws.

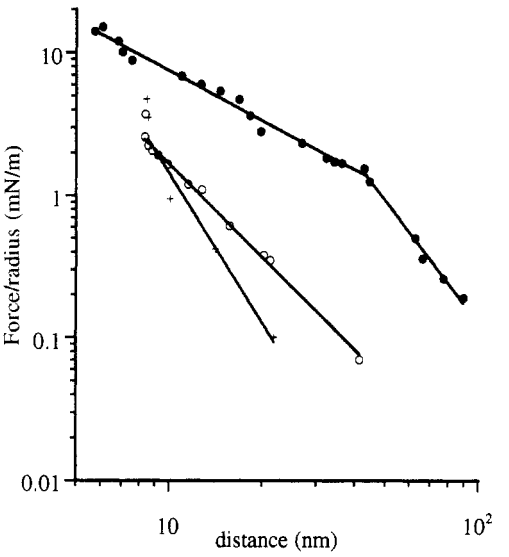

Figure 1. Normalized force versus distance of separation for mica surfaces immersed in a 5 mg/L human serum albumin (HSA) and 10^{-2} M KCl solution at 20 ± 0.5 °C. The solid circles represent the force–distance profile at pH 2 with 10 M urea added to the solution to denature the protein. A linear regression gives a $D^{-1.15}$ dependence for the short-range forces and a $D^{-2.8}$ dependence for the long-range forces. The open circles represent the force–distance profile at pH 2 without urea. A linear regression gives a D^{-2} dependence for the forces. At short distance (8.5 nm) the profile is vertical on a log–log scale. The decay length is 5.6 nm, and the deposited layer thickness is 4.2 ± 0.5 nm. The crosses represent the force–distance profile at pH 9 without urea. A linear regression gives a $D^{-3.4}$ dependence for the force–distance profile. At short distance (8.5 nm) the profile is vertical on a log–log scale. The decay length is 3.7 nm, and the deposited layer thickness is 4.3 ± 1.0 nm. The lines represent the linear regression for each case.

In the presence of 10 M urea and at pH 2, HSA behaves according to the near and far distance scaling laws predicted for a polymer and is most likely fully unfolded and denatured (solid circles and lines in the upper curve in Figure 1). Without urea at pH 2, the globular protein HSA undergoes acid transition that produces partially unfolded states reminiscent of the globular state.[7] This could explain the observed $D^{-2.0}$ dependence at shorter distances for the far field and the *much lower* energy of interaction between the adsorbed molecules (open circles). The measured decay length on a semilog plot for this case (without urea) is 5.6 nm which is larger than that expected for electrostatic interactions of 2.2 nm (Debye decay length)

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0024-9297/94/2227-3424$04.50/0

at pH 2. The deposited layer thickness on one surface was about 4.2 ± 0.5 nm, about the size of the side-on dimension of 4 nm for HSA. This suggests, in addition to electrostatic interactions, the existence of weak steric forces probably resulting from the interaction between partially denatured HSA. As a control, we measured HSA/HSA interactions without urea in 10^{-2} M KCl and at pH 9 (crosses in Figure 1). For this case, the normalized force showed a $D^{-3.4}$ on separation with a decay length of 3.7 nm and a deposited layer thickness on one surface of 4.3 ± 1.0 nm. The decay length is close to that expected from classical DLVO theory, while the thickness again suggests a side-on orientation at this pH.

Using the surface force apparatus and the scaling rules of de Gennes, we show that unfolded (denatured) human serum albumin behaves essentially as a linear polymer while adsorbed onto molecularly smooth mica in 10^{-2} M KCl at pH 2 in the presence and absence of 10 M urea. This is the first confirmation on a molecular scale that unfolded (and adsorbed) protein molecules can indeed behave as flexible polymer molecules and that the interactions between the adsorbed molecules, the adsorbent, and the solvent determine their molecular state.

Acknowledgment. The authors thank Ed Uzgiris and Jeffrey Koehler for comments on the manuscript.

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