389d Protein Interaction Forces at High Salt Measured Using Atomic Force Microcopy

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Protein interactions in concentrated salt solutions relevant to protein crystallization are commonly characterized in terms of the osmotic second viral coefficient, B_{22}, measured by a number of methods including static light scattering, membrane osmometry and self-interaction chromatography. The B_{22} value quantifies the protein pair-wise interactions at a given solution condition, although detailed knowledge of the functional form for the underlying interaction is often difficult to obtain. In this study we have employed atomic force microscopy to measure these protein interaction forces directly in high ionic strength conditions for bovine serum albumin (BSA) and bovine pancreas ribonuclease A at varied pH with a number of inorganic electrolytes. Colloidal probe microscopy was used to measure these protein interactions for both physisorbed and covalently immobilized proteins on silica surfaces. In contrast to previous measurement of protein interactions using AFM, the ionic strengths span a greater range from 0.01 molar to 4 molar, which is more relevant to the high salt conditions used for protein crystallization. The interaction forces at such high ionic strengths are small in magnitude, as well as short-ranged, and consequently can be obscured by thermal noise using conventional colloidal probe microscopy. This experimental limitation can be overcome by modification to the methods of Cleveland *et al.* (*Phys Rev B* 52, R8692 (1995)) and Hienz *et al*. (*J Phys Chem B*, 104, 622 (2000)), where the probe-sample potential is isolated from the total potential based on perturbations to the thermal motion of the cantilever.

The force interactions exhibit an expected electrostatic repulsion at low ionic strengths. At higher ionic strengths a short-range repulsion is observed, but for some solution conditions a transition to a smoothly varying attraction is observed with an increase in salt concentration. The magnitude of the attraction continues to increase with increasing ionic strength. The occurrence and onset of this attraction is dependent on the protein, pH, the type and concentration of salt in solution. For a given protein and set of solution conditions the onset of attraction corresponds to the transition of literature B_{22} values from positive to negative. A qualitative correlation is observed with an increase in the magnitude of this attraction and an increasingly negative B_{22} value. Quantitative correlations between B_{22} from the literature and B_{22} values calculated using the measured AFM interaction forces will also be discussed.