Toward a Molecular Understanding of Protein Solubility: Increased Negative Surface Charge Correlates with Increased Solubility

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Supporting Material

Materials and Methods

Thermal denaturation experiments

Thermal denaturation experiments were performed on AVIV spectrophotometers with either a 62DS or a 202SF model using a 3 minute equilibration time, a 30 second averaging time, and a 1 cm pathlength cuvette. Experiments were run in pH 7.0, 50 mM sodium phosphate for all proteins except RNase Sa which was run in pH 7.0, 30 mM Mops due to the fact that it binds phosphate ions. Elipticity was monitored at 222nm for ovalbumin, α-chymotrypsin, lysozyme, and human serum albumin with a protein concentration of 0.025 mg/mL. Elipticity was monitored at 234nm for RNase Sa and 270nm for α-lactalbumin with protein concentrations of 0.1 mg/mL and 0.5 mg/mL respectively. The data were analyzed using KaleidaGraph version 3.52. The analysis of denaturation curves has been described elsewhere.(29)

Preparation of protein stock solutions

Human fibrinogen, human serum albumin, bovine α-chymotrypsin, and bovine α-lactalbumin were obtained from Calbiochem; chicken lysozyme and ovalbumin were obtained from Sigma, and RNase Sa was purified as described elsewhere.(30) Protein samples were dialyzed overnight into 5 mM pH 7.0 citric acid, which had been pH adjusted using NaOH, in Slide-A-Lyzer dialysis cassettes from Thermo Scientific. Samples were then concentrated using Amicon Ultra centrifugal filters from Millipore. Concentrations were determined using the following extinction coefficients (ε_{280}= L cm^{-1} mg^{-1}) 1.55, 0.53, 2.04, 2.09, 2.60, 1.16, 0.71 for fibrinogen, human serum albumin, α-chymotrypsin, α-lactalbumin, lysozyme, and ovalbumin respectively. An extinction coefficient (ε_{278}= L cm^{-1} mg^{-1}) of 1.16 was used for RNase Sa.

Solubility measurements

Solubility measurements were performed similarly to as described previously.(16) In short, three samples were prepared: pH 7.0, 5 mM citrate, a protein stock solution, and a solution of either ammonium sulfate (obtained from Sigma) or PEG-8000 (obtained from Hamilton) in pH 7.0 5 mM citrate. These solutions were mixed together and allowed to equilibrate for approximately 10 minutes unless otherwise noted. Samples were then centrifuged in an Eppendorf 5417R microcentrifuge at 16,000 RPM for 10 minutes. Aliquots from the supernatant were taken,

diluted as necessary, and the concentration was determined using an Agilent 8453 UV-Visible spectrophotometer.

Accessible surface area calculations

Accessibility data were determined using pfis(31) for all proteins except fibrinogen for which the program Naccess(32) was used. Both programs are based on the algorithm by Lee and Richards(33) and provide accessible surface area data for all atoms in the protein; however, pfis is only able to accept single peptide chains as input. The PDB files 3GHG,(34) 1E78,(35) 1YPH,(36) 1F6R,(37) 2VB1,(38) 1OVA,(39) and 1RGG(40) were used for fibrinogen, human serum albumin, α-chymotrypsin, α-lactalbumin, lysozyme, ovalbumin, and RNase Sa respectively. For fraction polar and nonpolar ASA, carbon and sulfur atoms were defined as nonpolar and nitrogen and oxygen atoms as polar. For fraction charged, positively charged, and negatively charged ASA calculations, the nitrogen atoms of the lysine and arginine side chains and the N-terminus were defined as positively charged and the oxygen atoms of the aspartic acid and glutamic acid side chains and the C-terminus were defined as negatively charged.