

Analytical ultracentrifugation Sedimentation equilibrium experiments were performed at 25°C on peptides solubilized in C14-betaine (Ultrapure, Sigma) by using a Beckman Coulter XL-I analytical ultracentrifuge. The contribution of the detergent to the buoyant molecular weight of the peptide/detergent complexes was eliminated by using D₂O to adjust buffer density to that of the detergent. The samples were centrifuged in three-compartment carbon-epoxy centerpieces by using sapphire windows, at times sufficient to achieve equilibrium. Data obtained by UV absorption at 280 nm were analyzed by nonlinear least-squares curve fitting of radial concentration profiles by using the Marquardt Levenberg algorithm implemented in Igor Pro (Wavemetrics, Oswego, OR) with a user-defined function coding the equations describing reversible association in centrifugation.

$$S(r) = \frac{n(1.2\varepsilon)[C_o]^n \exp\{n\chi M_w\}}{K_n} + baseline$$

$S(r)$ = Signal due to all sedimenting species ($n=1,2, \dots,n$) at radial position r

1.2ε = Path length times the monomer molar extinction coefficient for absorbance data

$3.3 * M_w$ for interference data (assumes $dn/dc=0.187$)

C_o = Molar concentration at r_o of monomer of molecular weight M_w

$$\chi = \frac{(1-\bar{v}\rho)\omega^2}{2RT} (r^2 - r_o^2) ; r_o = \text{arbitrary fixed radius reference}$$

K_n = N -mer dissociation constant in units of M^{-1}

\bar{v} = Partial specific volume of sedimenting species (cm^3/gm)

ρ = Density of supporting buffer (gms/cm^3)

ω = Angular velocity of rotor (radians/sec)

M_w = Molecular weight of sedimenting species (gms/mole)

R = Ideal Gas constant (8.315×10^7 ergs $K^{-1} mol^{-1}$)

T = Temperature (K)

Monomeric molecular masses and partial specific volumes were calculated by using the program Sedinterp modified to include revised values for individual amino acid residues and corrected for hydrogen deuterium exchange by using averaged H-D exchanged amino acid residue weights. The calculated values of these parameters were held constant in fitting the absorbance vs. radius profiles to various equilibrium models. Data at different speeds were globally fit to a function describing sedimentation equilibrium of a monomer in equilibrium with a trimer (solid line) or dimer (dashed line). Baselines were constrained to be identical for all three speeds. A comparison of experimental and calculated (by integration over preset limits of the cell compartment) concentrations within the cell is shown in the material balance histograms as an additional measure of model consistency. The algorithm for performing these calculations is available on request.