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Sedimentation equilibrium analysis

Sedimentation equilibrium experiments were performed in an analytical ultracentrifuge (Optima XLA; Beckman Coulter), using an An-60Ti rotor, with the protein in 100 mM Hepes, pH 7.4, 100 mM NaCl, 2 mM dithiothreitol. Experiments were performed at 10.0°C and at speed 16,000 rev/min, with scanning at 280 nm. Cells were largely filled (400-μl sample) to give data over a wide range of concentration in each cell, at a variety of initial concentrations. After an initial scan, the centrifuge was over-speeded at 24,000 rev/min for 6 h, to reduce the time taken to reach equilibrium (Van Holde and Baldwin, 1958), and then the speed was reduced and another scan was taken, followed by further scans at intervals of 24 h. When successive scans were indistinguishable, the later scan was taken as being operationally at equilibrium, and scans, averaging over 100 readings, were taken for analysis. Subsequently, the centrifuge was overspeeded to sediment the macromolecule away from the meniscus, before slowing to equilibrium speed and taking a further scan to establish the effective base-line absorbance for each cell.

Data were analyzed initially by taking overlapping sets of 41 datum points to calculate the apparent weight average molecular mass $\overline{M}_{w,app}$, taken to be at the concentration of the middle point, by using the equation:

$$\overline{M}_{w,app} = \frac{d\ln(c)}{dr^2}\ \frac{2RT}{\omega^2(1-\bar{\nu}\rho)}$$

where c is the concentration (as optical density) at radius r, and ω is the angular velocity (in radians/sec), $\bar{\nu}$ is the partial specific volume and ρ is the solvent density. These latter values were calculated from the amino acid composition of the protein and the buffer composition using the program SEDNTERP (provided by John Philo to the RASMB archive, based on Laue et al., 1992) and gave $\bar{\nu}$ = 0.6988 ml/g and ρ = 1.00673 g/ml. Plots of $\overline{M}_{w,app}$ against c were made, using the program Profit version 5.1.2 ppc (Quantum Soft, Zürich, Switzerland). Concentrations were calculated from the optical densities using the molar extinction coefficient for the protein $\varepsilon_{280} = 6790 M^{-1}$, calculated from the amino acid composition).

Visual inspection of these plots showed that an appropriate model for further analysis, by directly fitting the absorbance against radius data (Johnson and Straume, 1994), was a non-ideal monomer model. This was tested using Profit with the Levenberg-Marquardt algorithm (with error estimates in absorbance from the recorded data). A non-ideal solute can be described by the equation (Casassa and Eisenberg, 1964; Eisenberg, 1975: assuming that only the second virial coefficient need be considered):

$$\frac{(1+2Bc_r)}{c_r}dc_r = \frac{M_1(1-\bar{\nu}\rho)}{RT}rdr$$

where B is the molar second virial coefficient (in M^{-1}), c_r is the molar concentration of the macromolecules at radius r, and M_1 is the monomer mass. This leads to the equation:

$$c_r = c_0\exp\left(\frac{M_1(1-\bar{\nu}\rho)\omega^2}{2RT}(r^2-r_0^2) - 2B(c_r-c_0)\right)$$

where c_0 is the reference concentration of macromolecules at r_0. This expression can be fitted to the data by evaluating c_r against r and then converting into absorbance with the equation:

$$A_r = \varepsilon_1 c_r + \delta A$$

Since the non-ideality equation is transcendental (i.e., c_r appears on both sides), it was evaluated numerically for each radius, by fitting c_r successively until the change was less than 10^{-5} of the final value. The reference radius (r_0) was taken two-thirds down the column, and the reference concentration (c_0) was evaluated here. Fitting was carried out with parameters for error in the baseline, as well as for B.

Residuals were plotted against radius, to assess the goodness of fit and, particularly, to see any systematic deviation rather than random error due to noise in the data. Theoretical curves for $\overline{M}_{w,app}$ against c were also plotted onto the experimental plots, using the fitted parameters, so that the fit could be seen.

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