

NMR properties of Sedimented Solutes

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Supporting Information

Persistency of the sediment in MAS rotors and in ultracentrifuges

Assuming that all the protein is in a compact layer with a limit density of 700 mg/ml, for a 60 mg/ml solution in a 4 mm rotor (internal radius 1.5 mm and height 7.1 mm), the protein is confined in a 4.3 mm^3 volume, and the solvent exposed surface is 64 mm^2 , so that the surface/volume ratio is about 15 mm^{-1} . If the same protein solution is sedimented in a 200 μl centrifuge tube of 5 mm inner diameter, the protein volume is 17 mm^3 and the contact surface is about 20 mm^2 . The latter corresponds in this case to the area of the section of the tube, which is perpendicular to the direction of the centrifugal force. Therefore, the surface/volume ratio decreases to about 1.2 mm^{-1} . Under these conditions the sediment takes a large amount of time to re-dissolve and, if the supernatant solution is removed, it will not redissolve at all, permitting easy collection of the sample. When the volume of the sediment is comparable to the volume devoid of protein, as in a 250 mg/ml solution of BSA, the sample in a 3.2 mm rotor (height 4.4 mm) forms 7.1 mm^3 of sediment, with a contact surface of 27 mm^2 , yielding a surface/volume ratio of 3.8 mm^{-1} . Under these conditions, even in the rotor the sediment may take some time to re-dissolve.

Theoretical analysis of the sedimentation process

For a protein of molecular weight M , density ρ_{protein} in a solvent of density ρ_{solvent} , that can reach a maximum concentration of c_{limit} , sealed in a rotor of radius b spinning at ω_r , the equation describing the concentration $c(r)$ as a function of the distance from the rotation radius is the following:

$$c(r) = \frac{RT}{M(1 - \rho_{\text{solvent}} / \rho_{\text{protein}})(1 - c / c_{\text{limit}})\omega_r^2 r} \frac{dc}{dr} \quad (2)$$

Introducing for the sake of simplicity

$$K^{\text{Sed}} = \frac{M(1 - \rho_{\text{solvent}} / \rho_{\text{protein}})\omega_r^2}{RT} \quad (\text{S1})$$

and $x=c/c_{\text{limit}}$, equation S1 is recast into:

$$\frac{dx}{dr} = K^{\text{Sed}} xr(1-x). \quad (\text{S2})$$

so that

$$\frac{dx}{x(1-x)} = K^{\text{Sed}} r dr. \quad (\text{S3})$$

Eq. (S3) is integrated as:

$$\ln \left| \frac{x}{1-x} \right| = \frac{K^{\text{Sed}}}{2} r^2 + \tilde{A}, \quad (\text{S4})$$

where \tilde{A} is an integration constant, so that

$$c = \frac{c_{\text{limit}}}{Ae^{-\frac{K^{\text{Sed}}}{2}r^2} + 1}. \quad (3)$$

From this equation, the integration constant A is evaluated by imposing the principle of conservation of mass as follows:

$$2 \int_0^b rc dr = c_0 b^2 \quad (\text{S5})$$

that yields

$$\ln \left[\frac{-e^{-\frac{K^{\text{Sed}}}{2}b^2} - A}{-1 - A} \right] = \frac{K^{\text{Sed}} c_0 b^2}{2c_{\text{limit}}}, \quad (\text{S6})$$

from which the constant A can be obtained:

$$A = \frac{e^{-\frac{K^{\text{Sed}}}{2}b^2 \left(1 - \frac{c_0}{c_{\text{limit}}}\right)} - 1}{1 - e^{-\frac{K^{\text{Sed}}}{2}b^2 \frac{c_0}{c_{\text{limit}}}}}. \quad (4)$$

Fraction of sedimented solute and thickness of the sediment layer

From equation (3) the amount of sedimented protein can be calculated as

$$f(\omega_r) = \frac{2 \int_a^b rc(r) dr}{c_0 b^2} \quad (\text{S7})$$

where the integration limit $a(\omega)$ is the distance from the rotor axis at which the concentration is a fraction k of c_{limit} , large enough to produce a detectable signal. $a(\omega)$ is given by

$$a(\omega_r) = \sqrt{-\frac{M(1 - \rho_{\text{solvent}} / \rho_{\text{protein}})\omega_r^2 r}{2RT} \ln\left(\frac{1-k}{kA}\right)} \quad (\text{S8})$$

so that equation S7 finally reads:

$$f(\omega_r) = \frac{c_{\text{limit}} \left[\ln\left(-e^{-b^2 K_s(\omega_r)} - A\right) - \ln\left(-e^{-\ln[(1-k)/kA]} - A\right) \right]}{K_s(\omega_r) c_0 b^2} \quad (\text{S9})$$

with

$$K_s(\omega_r) = \frac{2RT}{M(1 - \rho_{\text{solvent}} / \rho_{\text{protein}})\omega_r^2} \quad (\text{S10})$$

The estimation of the threshold k should reflect the physical limit imposed by the minimum concentration at which the rotational correlation rate is slower than the MAS rate. Such estimate can only be empirical, as it is strictly related to protein self-interactions or to the interactions with molecular to macromolecular crowders that are added to the solution.¹⁻⁴ All these interactions are strongly dependent on molecular concentrations in a highly nonlinear way.^{5;6}

Reference List

- (1) Y. Wang, C. Li and G. J. Pielak, *J.Am.Chem.Soc.*, 2010, **132**, 9392-9397.
- (2) T. L. James, G. B. Matson and I. D. Kuntz, *J.Am.Chem.Soc.*, 1978, **100**, 3590-3594.
- (3) C. Luchinat and G. Parigi, *J.Am.Chem.Soc.*, 2007, **129**, 1055-1064.
- (4) M. Iino, *Magn.Reson.Med.*, 1994, **32**, 459-463.
- (5) S. H. Koenig and R. D. Brown III, *Progr.NMR Spectrosc.*, 1990, **22**, 487-567.
- (6) L. Groesh and F. Noack, *Biochimica et Biophysica Acta*, 1976, **453**, 218-232.