Rotational and translational dynamics of human albumin

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Laser-light-scattering and electron-spin-resonance spectroscopies have been used to measure the translational and the rotational diffusion coefficients of human albumin. In the framework of the Debye-Stokes-Einstein theory, the two coefficients yielded two significantly different values for the hydrodynamic radius of the biomolecule. The discrepancy is discussed in connection with the involvement of the solvent internal degrees of freedom.

The determination of both translational (D_T) and rotational (D_R) diffusion coefficients has often been employed to get information on the size of globular biomolecules.[1,2] The connection between the diffusion and size is obtained through the translational (f_T) and rotational (f_R) friction coefficients, which are simply related to D_T and D_R:

$$D_T = k_B T/f_T; \quad D_R = k_B T/f_R \ . \tag{1}$$

The relationship between f_T and f_R and the molecular size is generally obtained using a naive model of the molecular motion within the solvent. In fact, a perfect-gas model of the solvent is employed, thus neglecting the microscopic structure of both molecule and solvent. Using such a model, the only source of dissipative effects is the shear viscosity (η_s) of the solvent, so that the following relationships hold:

$$f_T = 6\pi\eta_s R; \quad f_R = 8\pi\eta_s R^3 \ . \tag{2}$$

On the other hand, for a spherical shape the radius of the biomolecule can be calculated through

$$R = (3M\bar{v}/4\pi N)^{1/3} \ , \tag{3}$$

where M is the molecular weight, $\bar{v}$ is the partial specific volume, and N is Avogadro's number.

In the course of a study to ascertain the reliability of the above-mentioned relationships for the radius of human serum albumin (HSA), a globular protein of 69 000 daltons molecular weight (1 dalton $= 1.659\,79\times10^{-24}$ g), we found significant differences in the estimate of its radius, depending on which of the two friction coefficients was used.

To measure f_T we employed the well-established technique of intensity fluctuation spectroscopy (IFS),[1,3] using a 5-mW He-Ne laser as light source and a Malvern digital correlator, working in single clipping mode, to analyze the scattered photon flux. Experiments performed at 20 °C on aqueous HSA solutions at different concentrations and extrapolated to zero concentration yielded $D_T = (6.48 \pm 0.10)\times10^{-7}$ cm^2 s^{-1} and $f_T = (6.24 \pm 0.10)\times10^{-8}$ g s^{-1}. This result is in close agreement with previous estimates.[4] It should also be remarked that little, if any, HSA-concentration dependence of D_T was found in the range 0 to $10^{-3}M$, while the shear viscosity changes from 0.010 to 0.016 g cm^{-1} s^{-1} in the same concentration range and at the same temperature. A similar trend of D_T as a function of the biomolecule concentration was found by Dubin, Clark, and Benedek[5] in the case of lysozyme.

Owing to the fast rotational motion of these biomolecules (characterized by a rotational correlation time τ_R, smaller than 0.5 μs), standard IFS is no longer suitable to reliably estimate f_R. In this respect, a spectroscopic technique which has been successful in detecting rotational motions in a wide time domain (10^{-11}–10^{-4} s) is electron spin resonance (ESR).[2] In the spin-labeling ESR approach, the rotational correlation time of the macromolecules is provided by the analysis of the ESR anisotropic spectrum displayed by the unpaired electron-bearing small molecule (spin label), which is suitably bound to the biomolecule so that no motional freedom is retained by the spin label with respect to the tertiary structure of the biomolecule itself. On the other hand, the rotational friction coefficient f_R is connected to τ_R through the relationship

$$f_R = 6k_B T\tau_R \ . \tag{4}$$

Using, then, this technique in the experimental and theoretical framework of a previous study,[6] we obtained $\tau_R = (2.0 \pm 0.2)\times10^{-8}$ s and $f_R = (4.9 \pm 0.5)\times10^{-21}$ g cm^2 s^{-1} for a spin-labeled HSA solution at 20 °C and presenting $\eta_s = 0.011$ g cm^{-1} s^{-1}. In this case τ_R and f_R were found to be highly dependent on the protein concentration, the trend being controlled by the shear viscosity of the solution.

From Eqs. (2) and (3) the following three values have been deduced for the hydrodynamic radius of the HSA molecule: $R_T = 33.1 \pm 0.5$ Å, $R_R = 26.4 \pm 0.9$ Å, and $R_v = 27.6$ Å, where the last value has been obtained with $\bar{v} = 0.733$.[4] We can see R_R and R_v are equal within the experimental errors, while R_T is significantly higher.

In order to get more consistent results, we could admit that the HSA molecule is better described by an ellipsoid of revolution, so that, within the same approximation used to derive Eq. (2), the corresponding relationships given by Perrin[7] apply. However, no solution of Perrin's equations can be found using the measured f_T and f_R values. It is worth noting that, employing Perrin's equations, f_R should be larger than 8.8×10^{-21} g cm^2 s^{-1} to be consistent with the present value of f_R. Therefore, we have to admit that internal degrees of freedom in the solvent are relevant in determining the friction coefficients. A similar conclusion can be derived also from the work of Paul and Pusey[8] dealing with translational diffusion of large particles (1.7-μm diameter) in water. Their results indicate a translation friction coefficient higher than that predicted by means of Eqs. (2). Recently, a detailed description of the friction coefficient has been made by Reichl,[9,10] still within a continuous model of the solvent, by introducing the microscopic structure

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throughout various macroscopic fluid parameters. The limit of validity of Eqs. (2) can be deduced from the numerical results[11] obtained within Reichl's model. From that work it is seen that $f_R/f_T < \frac{4}{3}R^2$, when internal degrees of freedom are taken into account. Our results can be analyzed following Reichl's model. Assuming, according to Ref. 11, that the rotational friction coefficient is almost independent of the internal degrees of freedom of the solvent, we can use Eqs. (2) and our experimental value for f_R to get $R = 26.4 \pm 0.9$ Å, a value which is in close agreement with that deduced from the specific volume. Accordingly, using Eqs. (2) we can derive a translational friction coefficient appropriate to an *unstructured* solvent $f_T^0 = 5.1 \times 10^{-8}\ \mathrm{g\,s^{-1}}$. Then we have $f_T/f_T^0 = 1.22$, to be compared with $f_T/f_T^0 = 1.05$ deduced according to Refs. 11 and 12. As we can see, the correct trend of f_T/f_T^0 is deduced from Reichl's model, though the quantitative agreement is lacking. However, this last point is not surprising as the boundary conditions between the protein and water should play an important role in determining the translational diffusion of the molecules. Finally, we can conclude that present results give a strong indication that internal degrees of freedom must be taken into account to properly describe the diffusion process. Therefore great care must be used in deducing the molecular radius from f_T, while the present experimental approach can be used to get useful experimental information on the water structure as well as on the protein solvation.

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