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The combination of the viscosity increment with the harmonic mean rotational relaxation time for determining the conformation of biological macromolecules in solution

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A new hydrodynamic shape function, Λ, is derived for determining the conformation of biological macromolecules in solution, adding to the increasing number of shape parameters whose experimental determination does not require a knowledge of the particle swelling due to solvation in solution. Λ can be found from a knowledge of the molecular weight, intrinsic viscosity and the harmonic mean rotational relaxation time. A table of values and a plot of Λ as a function of axial ratio for both oblate and prolate ellipsoids of revolution are given.

The idea of combining together two hydrodynamic measurements in order to eliminate the requirement of a knowledge of the swollen molecular volume for determining the conformation of macromolecules in solution, initially proposed by Oncley (1941) and Sadron (1942), has been extensively applied (Scheraga & Mandelkern, 1953; Scheraga, 1961; Squire *et al.*, 1968; Squire, 1970; Nichol *et al.*, 1977; Rowe, 1977). Of these, Squire (1970, 1978) has eliminated the swollen molecular volume, V_e, by combining simultaneously the harmonic mean rotational relaxation time ratio, τ_h/τ_0, with the translational frictional ratio, f/f_0:

$$\frac{\tau_h}{\tau_0} = \frac{1}{3}\frac{\tau_a}{\tau_0} + \frac{2\tau_b}{\tau_0} \equiv \frac{\tau_h kT}{3\eta_0 V_e} \qquad (1)$$

$$\frac{f}{f_0} \equiv \frac{M_r(1-\bar{v}\rho_0)}{N_A 6\pi\eta_0 s}\left(\frac{4\pi}{3V_e}\right)^{\frac{1}{3}} \qquad (2)$$

where τ_h is the harmonic mean of the two rotational relaxation times τ_a and τ_b for ellipsoids of revolution, f is the frictional coefficient and τ_0 and f_0 are the corresponding coefficients for a sphere of the same volume and molecular weight, k is the Boltzmann constant, T is the absolute temperature, ρ_0 is the solvent density, η_0 is the solvent viscosity, M_r is the relative molecular mass (molecular 'weight), N_A is Avogadro's number, s is the sedimentation coefficient and $\bar{v}$ is the partial specific volume. The swollen volume, V_e, was eliminated by taking the cube root of the inverse of eqn. (1) and multiplying it by eqn. (2):

$$\Psi = \frac{f}{f_0}\left(\frac{\tau_0}{\tau_h}\right)^{\frac{1}{3}} \equiv \frac{M_r(1-\bar{v}\rho_0)}{\tau_h^{\frac{1}{3}} sA} \qquad (3)$$

where

$$A = 6\pi\eta_0 N_A \left(\frac{kT}{4\pi\eta_0}\right)^{\frac{1}{3}}$$

At 25°C, A is equal to 7.243×10^{18}, and at 20°C, A is equal to 7.492×10^{18}. The explicit expressions for τ_a/τ_0, τ_b/τ_0 (and hence τ_h/τ_0) and f/f_0 in terms of the axial ratio for ellipsoids of revolution are given by Perrin (1934, 1936) with a correction for the relaxation-time ratios given by Koenig (1975). The corresponding variation of Ψ as a function of axial ratio could thus be evaluated (Squire, 1970). It is apparent, however, that Ψ is extremely insensitive to axial ratio. This is probably largely due to the presence of the cube root of the harmonic mean relaxation time. Squire (1970) has pointed out on the other hand that this extreme insensitivity can be used for checking the internal consistency of the data, particularly for low axial ratios (<4) where Ψ differs from its spherical value (=1) by less than 3% for prolate ellipsoids and by less than 7% for oblate ellipsoids. This may be of particular importance for checking τ_h, generally the most difficult to determine, since the method used for its determination, i.e. steady-state fluorescence depolarization (Weber, 1953), encounters several uncertainties: (i) most macromolecules do not possess a naturally fluorescent group or chromophore, thus one has to be

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introduced artificially, (ii) the decay time of the chromophore itself is required, (iii) internal rotation of the chromophore, or of a fragment of the macromolecule to which the chromophore is attached, with respect to the rest of the macromolecule may occur (Johnson & Mihalyi, 1965).

The swollen molecular volume, V_e, can also be eliminated from either eqn. (1) or eqn. (2) by combining instead with the viscosity increment, ν, given by:

$$\nu = \frac{[\eta]M_r}{N_A V_e} \quad (4)$$

(Yang, 1961), where $[\eta]$ is the intrinsic viscosity ($ml \cdot g^{-1}$) and where $N_A V_e$ is the effective molar volume. The explicit expression for ν as a function of axial ratio for ellipsoids of revolution is given by Simha (1940). Scheraga & Mandelkern (1953) eliminated V_e by combining eqn. (2) with eqn. (4):

$$\beta = \frac{N_A^{\frac{1}{2}}}{(16\,200\pi^2)}\nu^{\frac{1}{2}}\left(\frac{f_0}{f}\right) \equiv \frac{N_A s[\eta]^{\frac{1}{2}}\eta_0}{M_r^{\frac{1}{2}}(1-\bar{\nu}\rho_0)100^{\frac{1}{2}}} \quad (5)$$

The resulting β-function is, however, again very insensitive to axial ratio, especially for prolate ellipsoids of axial ratio < 10 and for oblate ellipsoids < 100.

Alternatively, the viscosity increment (eqn. 4) can be combined with the harmonic mean rotational relaxation time ratio (eqn. 1) to eliminate V_e and produce a swelling-independent Λ function:

$$\Lambda = \nu\left(\frac{\tau_0}{\tau_h}\right) \equiv \frac{[\eta]M_r}{\tau_h B} \quad (6)$$

where

$$B = \frac{N_A kT}{3\eta_0}$$

At 25°C, B is equal to 9.2442×10^{11}, and at 20°C, B is equal to 8.08251×10^{11}. Λ is plotted as a function of axial ratio in Fig. 1 and a table of values is given in Table 1. It is apparent that Λ is much more sensitive to axial ratio than Ψ. Even so, until τ_h can be measured to a precision greater than that currently expected ($\sim \pm 3\%$ at best, assuming no significant internal or segmental rotations), the function is generally restricted, at the moment at least, to prolate ellipsoidal particles above an axial ratio of about 3.

Unfortunately, there is at present a lack of reliable steady-state fluorescence-depolarization data for macromolecules in this range of axial ratios. Use of the function may, however, be illustrated by application to data available for the tryptic fragment of bovine fibrinogen. By using a steady-state fluorescence-depolarization technique, Johnson & Mihalyi (1965) reported a harmonic mean relaxation time for fibrinogen of 195 ± 5 ns, a value lower than the corresponding value for a sphere of the same volume (299 ns); the value for τ_h of the tryptic subfragment was 178 ns, strongly suggesting that the tryptic subfragments had rotational freedom within the fibrinogen molecule itself. Assuming there is still no further internal rotation within the subfragment itself, one can combine this result with viscosity and molecular-weight data obtained previously by Mihalyi & Godfrey (1963).

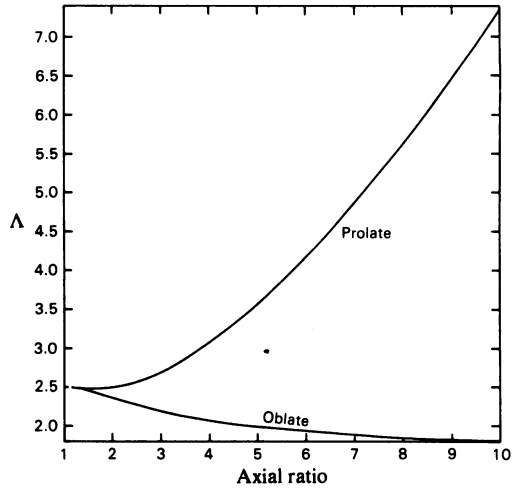

Fig. 1. *Plot of Λ as a function of axial ratio for prolate and oblate ellipsoids of revolution*

Table 1. *Variation of Λ with axial ratio of prolate and oblate ellipsoids of revolution*

	Λ	
Axial ratio	Prolate	Oblate
1.0	2.500	2.500
1.5	2.478	2.497
2.0	2.490	2.356
2.5	2.564	2.265
3.0	2.692	2.187
3.5	2.864	2.123
4.0	3.071	2.070
4.5	3.310	2.026
5.0	3.575	1.989
5.5	3.865	1.957
6.0	4.177	1.931
6.5	4.510	1.907
7.0	4.862	1.887
7.5	5.234	1.869
8.0	5.624	1.854
8.5	6.032	1.840
9.0	6.457	1.827
9.5	6.900	1.816
10.0	7.359	1.805

Table 2. *Hydrodynamic parameters and derived axial ratios of the tryptic subfragment of fibrinogen*

Hydrodynamic parameter	Derived axial ratio	Reference
ν^*	7.8	Mihalyi & Godfrey (1963)
f/f_0^*	7.1	Mihalyi & Godfrey (1963)
β	9.3	Mihalyi & Godfrey (1963)
τ_h/τ_0^*	5.0	Johnson & Mihalyi (1965)
Λ	6.8	

* Assuming no particle swelling due to solvent association.

Taking M_r as 95000 ± 2000, $[\eta]$ as $7.18 \pm 0.07\,ml \cdot g^{-1}$ and assuming a ± 5 ns standard error in τ_h, Λ is calculated to be 4.74 ± 0.17, where the method for calculating the standard error in Λ is given by Paradine & Rivett (1960). This corresponds to a prolate ellipsoid of axial ratio 6.8 ± 0.3, consistent with the estimates of the axial ratio derived from four other hydrodynamic parameters, three of which assume no particle swelling due to solvent association (Table 2). The results from electron-microscopic studies suggest, however, that the subfragments are nearly spherical (Hall & Slayter, 1959); as Mihalyi & Godfrey (1963) have previously stated, this difference is probably too large to be explained by drying effects alone. At least part of this difference can, however, possibly be ascribed to an apparent discrepancy between the viscosity data of their Fig. 4 with the sedimentation data of their eqn. (2); the latter suggests a sedimentation regression coefficient, k_s, of ~3.6 (after correction to solution density; Rowe, 1977), whereas the viscosity regression coefficient, k_η, is only ~2.5. Rowe (1977) has shown that the ratio k_η/k_s is equal to the swelling ratio $\bar{v}_s/\bar{v}$, where $\bar{v}_s$ is the swollen specific volume in solution. Mihalyi & Godfrey's (1963) data apparently give a value for the swelling of less than 1, indicating that the particle contracts in solution, an unlikely event. Unfortunately, although the pH values of the solutions used for the sedimentation and harmonic-mean-relaxation-time measurements are given and are near (6.5 and 7.1 respectively), that for the viscosity is not given, so this is a possible source of error.

It is hoped that the availability of the new Λ function will encourage the production of more reliable data in order to resolve these difficulties, and also accelerate improvement in the methodology so that τ_h/τ_0 can be measured with much greater precision, enabling application of the Λ function to prolate ellipsoids of axial ratio less than three and also to oblate ellipsoids.

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CORRECTIONS

The primary structure of the calcium ion-transporting adenosine triphosphatase protein of rabbit skeletal sarcoplasmic reticulum: peptides derived from digestion with cyanogen bromide, and the sequences of three long extramembranous segments

G. ALLEN, B. J. TRINNAMAN and N. M. GREEN

Volume 187 (1980)

p. 614, Fig. 9, sequence 3:

for $\overset{100}{\text{B}}\text{SLLDFNETKGVYEKVGEADET}\overset{120}{\text{A}}$

read $\overset{100}{\text{B}}\text{SSLDFNETKGVYEKVGEATET}\overset{120}{\text{A}}$

The kinetic properties and reaction mechanism of histamine methyltransferase from human skin

D. M. FRANCIS, M. F. THOMPSON and M. W. GREAVES

Volume 187 (1980)

p. 825, Table 2, column 2:

for *S*-Adenosylmethione *read* *S*-Adenosylmethionine

p. 821, para. 1, l. 18:

for Substrae kinetic *read* substrate kinetics

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p. 359, Eqn. 1:

for $$\frac{\tau_h}{\tau_0}=\frac{1}{3}\frac{\tau_a}{\tau_0}+\frac{2\tau_b}{\tau_0}\equiv\frac{\tau_h kT}{3\eta_0 V_e}$$

read $$\frac{\tau_h}{\tau_0}=\frac{3}{\left(\frac{\tau_0}{\tau_a}+\frac{2\tau_0}{\tau_b}\right)}\equiv\frac{\tau_h kT}{3\eta_0 V_e}$$

p. 360, Eqn. 5:

for $$\beta=\frac{N_A^{\frac{1}{2}}}{(16\,200\pi^2)}v^{\frac{1}{2}}\left(\frac{f_0}{f}\right)\equiv\frac{N_A s[\eta]^{\frac{1}{2}}\eta_0}{M_r^{\frac{1}{2}}(1-\bar{v}\rho_0)100^{\frac{1}{2}}}$$

read $$\beta=\frac{N_A^{\frac{1}{2}}}{(16\,200\pi^2)^{\frac{1}{2}}}v^{\frac{1}{2}}\left(\frac{f_0}{f}\right)\equiv\frac{N_A s[\eta]^{\frac{1}{2}}\eta_0}{M_r^{\frac{1}{2}}(1-\bar{v}\rho_0)100^{\frac{1}{2}}}$$