

aldehydes has been studied. Greater reactivities are shown by the Schiff's bases compared with the corresponding *N*-acylchitosans, despite the hydrolysis and accompanying *N*-acetylation that occurs with the Schiff's bases prepared from aliphatic aldehydes. This, together with the previously demonstrated fact¹ that *O*-acetylation of *N*-acylchitosans virtually ceases with ~50% of the hydroxyl groups still unreacted, can be explained on the basis of intramolecular steric hindrance between the imino hydrogen of the *N*-acyl group and the 3 or 6' hydroxyl group.

Acknowledgements

The authors wish to thank Hercules Inc. for the gift of a sample of Kytex H, Mr B Marshall (Dept. Mech. Eng., Trent Polytechnic), for assistance with the X-ray diffraction studies, and Mrs F. A. Wood for preparing the Figures. One of the authors (GKM) thanks the Science Research Council for the award of a Research Studentship).

A compound hydrodynamic shape function derived from viscosity and molecular covolume measurements

Stephen E. Harding*

The Biophysics Laboratory, Department of Biochemistry, Leicester University, Leicester LE1 7RH, UK
(Received 22 January 1981)

Introduction and theory

In order to determine the gross conformation of a macromolecule in solution it is generally necessary to consider its hydrodynamic properties; scattering of light or X-rays can also be used, but with the serious limitation that any interpretation of the results in terms of shape assumes the macromolecule to be of uniform density.

The experimental determination of the hydrodynamic shape functions, for example the viscosity increment¹ v , the translational frictional ratio^{2,7} f/f_0 and the molecular covolume³ U , requires a knowledge of the swollen molecular volume in solution, V_c :

$$v \equiv \frac{[\eta]M_r}{N_A V_c} \quad (1)$$

$$\frac{f}{f_0} \equiv \frac{M_r(1 - \bar{v}\rho_0)}{6\pi\eta_0 s N_A} \left[\frac{4\pi}{3V_c} \right]^{1/3} \quad (2)$$

$$U_{\text{red}} \equiv \frac{U}{N_A V_c} \quad (3)$$

where $[\eta]$ is the intrinsic viscosity (ml g^{-1}), M_r the molecular weight, N_A Avogadro's number, \bar{v} the partial specific volume, ρ_0 the solvent density, η_0 the solvent viscosity and U the molecular covolume, which it has been assumed can be determined for rigid particles from

* Present address: Department of Biochemistry, Bristol University, Bristol BS8 1TD, UK.

References

- 1 Moore, G. K. and Roberts, G. A. F. *Int. J. Biol. Macromol.* 1981, **3**, 292
- 2 Moore, G. K. and Roberts, G. A. F. *Int. J. Biol. Macromol.* 1980, **2**, 73
- 3 Plisko, E. A., Nud'ga, L. A. and Danilov, S. N. *Chem. Abstr.* 1972, **76**, 154705
- 4 Nud'ga, L. A., Pliska, E. A. and Danilov, S. N. *Chem. Abstr.* 1974, **80**, 96252
- 5 Stanley, W. L., Watters, G. G., Chan, B. and Mercer, J. M. *Biotechnical Bioeng.* 1975, **17**, 351
- 6 Stanley, W. L. and Watters, G. G. U.S. Pat. 3,909,358 (1975)
- 7 Muzzarelli, R. A. A., Barontini, G. and Rocchetti, R. *Biotechnol. Bioeng.* 1976, **18**, 1445
- 8 Hirano, S., Yamaguchi, R., Matsuda, N., Muira, O. and Kondo, Y. *Agric. Biol. Chem.* 1977, **41**, 1547
- 9 Lee, V. Univ. Microfilms, Ann Arbor 1974, **74/29**, 446
- 10 Hall, L. D. and Yalpani, M. J. *Chem. Soc. Chem. Commun.* 1980, 1153; *Carbohydr. Res.* 1980, **78**, C4
- 11 Rimini, E. *Chem. Zentr.* 1898, **69** (Pt. II), 132
- 12 Suydam, F. H. *Anal. Chem.* 1963, **35**, 193
- 13 Bellamy, L. J. in 'The infrared Spectra of Complex Molecules', Methuen, London, 1960, p. 71
- 14 Freedman, H. H. J. *Am. Chem. Soc.* 1961, **83**, 2900
- 15 Harada, K. in 'Chemistry of the Carbon-Nitrogen Double Bond' (Ed. S. Patai), Interscience Publishers, New York, 1970, p. 265
- 16 Moore, G. K. and Roberts, G. A. F. *Int. J. Biol. Macromol.* submitted for publication

sedimentation equilibrium, osmometry or light scattering experiments in terms of the activity coefficient at the isoelectric point³⁻⁵. This assumption is suggested by Jeffrey *et al.*³, to be reasonable, at least for globular proteins. U has been 'reduced'⁶ in equation (3) to obtain an explicit expression in terms of axial ratio alone, and the expressions giving v , f/f_0 , and U as functions of axial ratio for ellipsoids or revolution have been given, respectively, by Simha¹, Perrin² and Jeffrey *et al.*³.

In order to eliminate the requirement of knowledge of the swollen volume it is necessary to combine the expressions for two of the hydrodynamic shape parameters; this naturally assumes that the macromolecule has the same shape and 'swelling' in both types of experiment. Scheraga and Mandelkern⁷ eliminated V_c by combining equation (1) with equation (2); the resulting β function is, however, sensitive to experimental error and very insensitive to axial ratio. Several workers have eliminated V_c by combining equation (1) or equation (2) with rotational frictional or rotational relaxation functions but these are generally very difficult to determine^{8,15,16}. Jeffrey *et al.*³ combined equation (2) with equation (3):

$$\psi = \frac{U_{\text{red}}}{162\pi^2} \left[\frac{f_0}{f} \right]^3 \equiv \frac{U\eta_0^3 N_A^2 s^3}{M_r^3 (1 - \bar{v}\rho_0)^3} \quad (4)$$

ψ also is very sensitive to experimental error, primarily because of the number of terms on the right-hand side of equation (4), many of them cubed. A function less sensitive to experimental error can be found by combining the molecular covolume, equation (3) instead with the viscosity increment, equation (1):

$$\Pi = \frac{U_{\text{red}}}{v} \equiv \frac{U}{[\eta]M_r} \quad (5)$$

Application

From Figure 1 it is seen that use of the Π function is apparently restricted to prolate ellipsoidal macromolecules because of the relative insensitivity, like the β

function. to axial ratio for the oblate case; it can, however, be measured with a greater precision than either β or ψ , and is more sensitive to axial ratio than ψ for prolate ellipsoids.

The result can be illustrated by application to molecular covolume and viscosity data available for haemoglobin, a protein well characterized both in crystalline form⁹ and in solution^{5,10,11}. The molecular covolume U is related to the 2nd virial coefficient B employed in osmometry by⁴ $U=2BM_r^2$. Baghurst *et al.*⁵, using a molecular weight of 64 500, found the value of BM_r to be 4.8 ml g^{-1} . By using instead the more exact value for the molecular weight of 64 793 from the amino acid composition⁸, we find $BM_r=4.78 \text{ ml g}^{-1}$; this gives the ratio U/M_r to be 9.56 ml g^{-1} . From the plot of reduced specific viscosity vs. concentration (Figure 2) an intrinsic viscosity $[\eta]$ of 2.99 ml g^{-1} has been determined by a least-squares fit to a new universal equation for transport coefficients at all solute concentrations^{12,16}, this being an extension by Rowe of his earlier theory¹³. The value of Π is thus 3.20, corresponding to a spherical particle. The value of ν for a spherical particle is the Einstein value of 2.5; back substitution into equation (1) gives a value for V_c of $1.286 \times 10^{-19} \text{ cm}^3$. This corresponds to a Stokes radius of 31.3 Å, in agreement with the result of 32.3 Å calculated from the diffusion coefficient determined by laser correlation spectroscopy by Alpert and Banks¹⁰, and 31.3 Å calculated by Laurent and Killander¹¹ from the diffusion coefficient determined by gel filtration, both groups assuming a 'hard sphere' model. The Stokes radius can also be found directly from the molecular covolume and molecular weight if a hard sphere model is assumed: Baghurst *et al.* determined a value of 31.3 Å, again in exact agreement. The corresponding radius of the sphere calculated from the crystallographic dimensions of $64 \times 55 \times 50 \text{ Å}$ is 28.0 Å, indicating haemoglobin to be swollen in solution by $\sim 40\%$ (ν/ν).

If one uses standard errors of ± 0.03 ($\equiv 1\%$) and ± 0.096 ($\equiv 1\%$) in $[\eta]$ and U/M_r , respectively, the calculated standard error¹⁴ in Π is ± 0.045 . The maximum error corresponds to an axial ratio of 1.8 for a prolate model but

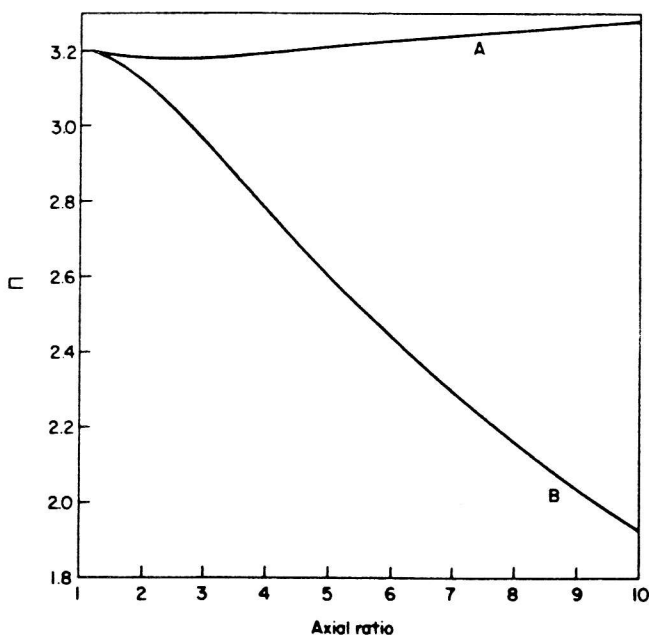


Figure 1 Plot of Π as a function of axial ratio for ellipsoids of revolution: A, oblate; B, prolate

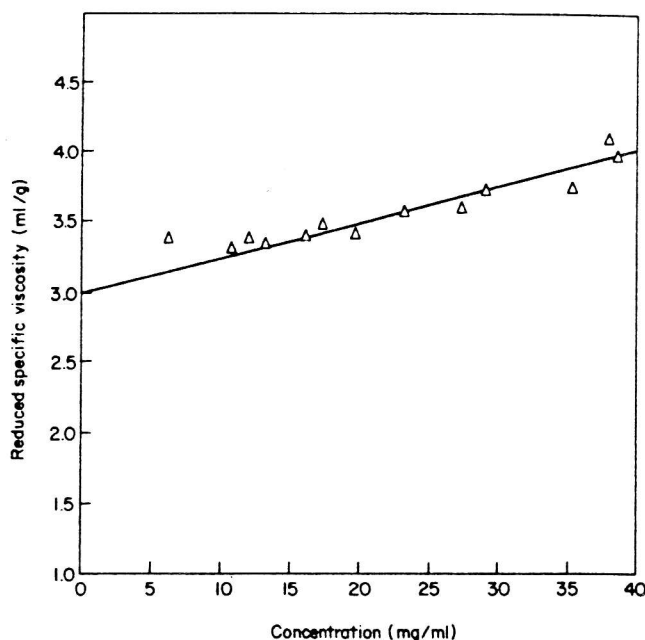


Figure 2 Plot of reduced specific viscosity vs. concentration for human oxyhaemoglobin (in 0.1 M KCl, buffered to pH 6.0)

as high as 6.8 for an oblate model, indicating the difficulty in applying Π to macromolecules that are oblatoid. Although these large error bounds make it very difficult to determine the axial ratio of near-spherical proteins like haemoglobin, at least the results above are not inconsistent with previous findings. The most important use of Π will perhaps be in conjunction with the sensitive R -parameter, which can be determined from the ratio of the sedimentation concentration regression coefficient to the intrinsic viscosity¹³. This latter function has the disadvantage in not being able to distinguish between an oblate or prolate ellipsoid. A given value of Π , however, uniquely specifies the geometry of the ellipsoid.

Acknowledgement

I thank Mr A. Pancholi and Dr A. J. Rowe for allowing me to use their viscosity data for haemoglobin and Dr Rowe for permission to use the universal equation for the concentration dependence of transport coefficients prior to publication.

References

- 1 Simha, R. *J. Phys. Chem.* 1940, **44**, 25
- 2 Perrin, F. *J. Phys. Radium* 1936, **7**, 1
- 3 Jeffrey, P. D., Nichol, L. W., Turner, D. R. and Winzor, D. J. *J. Phys. Chem.* 1977, **81**, 776
- 4 Tanford, C. 'Physical Chemistry of Macromolecules', Wiley, New York, 1961
- 5 Baghurst, P. A., Nichol, L. W., Ogston, A. G. and Winzor, D. J. *Biochem. J.* 1975, **147**, 575
- 6 Ridgeway, D. *J. Am. Chem. Soc.* 1968, **90**, 18
- 7 Scheraga, H. A. and Mandelkern, L. *J. Am. Chem. Soc.* 1953, **79**, 179
- 8 Squire, P. G. *Mol. Electro Optics* 1978, **2**, 565
- 9 Perutz, M. F., Rossmann, M. G., Cullis, A. F., Muirhead, H., Will, G. and North, A. C. T. *Nature (London)* 1960, **185**, 416
- 10 Alpert, S. S. and Banks, G. *Biophys. Chem.* 1976, **4**, 287
- 11 Laurent, T. C. and Killander, J. *J. Chromatogr.* 1964, **14**, 317
- 12 Rowe, A. J. manuscript in preparation
- 13 Rowe, A. J. *Biopolymers* 1977, **16**, 2595
- 14 Paradine, C. G. and Rivett, B. H. P. 'Statistical Methods for Technologists', English Universities Press, London, 1960
- 15 Harding, S. E. *Biochem. J.* 1980, **189**, 359
- 16 Harding, S. E. *PhD Thesis* Leicester University (1980)