MODELLING BIOLOGICAL MACROMOLECULES IN SOLUTION: THE GENERAL TRI-AXIAL ELLIPSOID

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Appendix II Illustration of use of the Λ function (equation 50) by application to data available for the tryptic subfragments of fibrinogen

It is apparent from Figure 17 that, until the harmonic mean relaxation time τ_h can be measured to a precision greater than that currently available (~ ±3% at best, assuming no significant internal rotations of the chromophore or segmental rotations of parts of a macromolecule relative to other parts), use of Λ will generally be restricted to prolate ellipsoidal particles above an axial ratio of about three.

Unfortunately, there is at present a lack of reliable steady state fluorescence depolarization data for macromolecules in this axial range. 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. 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).

Taking M_r as 95,000±2,000, $[\eta]$ as (7.18±0.07) $ml.g^{-1}$ 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 from Figure 17 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 23). The results from electron microscopy 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, be possibly ascribed to an apparent discrepancy between the viscosity data of their Figure 4 with the sedimentation data of their equation 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 gives a value for the swelling of less than 1, indicating the particle to contract 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_o 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.

Table 23. Hydrodynamic parameters and axial ratios for the tryptic subfragments of fibrinogen

Hydrodynamic Parameter	Derived Axial Ratio	Reference
ν*	7.8	Mihalyi & Godfrey (1963)
f / f_0 *	7.1	"
β	9.3	"
τ_h / τ_0 *	5.0	Johnson & Mihalyi (1965)
Λ	6.8	This study

* Assuming no particle swelling due to solvent association

BIBLIOGRAPHY.

Alexander, A.E. and Johnson, P. (1949)

'Colloid Science', Volume 2, Oxford University Press

Alpert, S.S. and Banks, G. (1976)

Biophysical Chem. 4, 287

Baghurst, P.A., Nichol, L.W., Ogston, A.G., Winzor, D.J. (1975)

Biochem. J. 147, 575

Ballinger, K.W.A. and Jennings, B.R. (1979)

Nature 282, 699

Batchelor, G.K. (1967)

'An Introduction to Fluid Mechanics', Cambridge University Press

Batchelor, G.K. (1970)

J. Fluid Mech. 41, 545

Batchelor, G.K. and Green, J.T. (1972)

J. Fluid Mech. 56, 375

Beeman, W.W., Koesberg, P., Anderegg, J.W. and Webb, M.B. (1957)

in 'Handbuck der Physik', (Flugge, S. ed.), 32, 321, Springer-Verlagg (Berlin)

Benoit, H. (1951)

Ann. Physik. 6, 561

Berne, P.J. and Pecora, R. (1974)

Ann. Rev. Phys. Chem. 25, 233

Blake, C.C.F. (1975)

Essays in Biochem. II, 37

Blake, C.C.F., Geisow, M.J. and Oatley, S.J. (1978)

J. Mol. Biol. 121, 339

Blake, C.C.F., Koenig, D.F., Mair, G.A., North, A.C.T., Phillips, D.C. and Sarma, V.R. (1965)

Nature 206, 757

Bloomfield, V.A., Dalton, W.O. and Van Holde, K.E. (1967)

Biopolymers 5, 135

Bresler and Talmud (1944)

CR Acad. Sci. URSS, 43, 310

Brinkman, H.C., Hermans, J.J., Oosterhoff, L.J., Overbeek, J. Th. G., Polder, D., Staverman, A.J. and Wiebenga, E.H. (1949)

Proc. Int. Rheol. Congress (Schveningen) II, 77

Brenner, H. (1970)

J. Coll. Int. Sci. 32, 141

Brenner, H. (1972a)

Chem. Eng. Sci. 27, 1069

Brenner, H. (1972b)

Progr. Heat and Mass Transfer 5, 93

Cantor, C.R. and Tao, T. (1971)

Proc. Nucl. Acid Res. 2, 31

Cerf, R. and Scheraga, H.A. (1952)

Chem. Revs. 51, 185

Chapman, P.F. (1913)

Phil. Mag. 25, 475

Cheng, P.Y. and Schachman, H.K. (1955)

J. Polymer Sci. 16, 19

Chu, B. (1974)

'Laser Light Scattering', Academic, New York

Chwang, A.T. (1975)

J. Fluid Mech. 72, 17

Clenshaw, C.W. and Curtis, A.R. (1960)

Num. Math. 2, 197

Creeth, J.M. and Knight, C.G. (1965)
Biochim. Biophys. Acta 102, 549

Cummings, H.Z. and Pike, E.R. (1973)
(eds.) 'Photon Correlation and Light Beating Spectroscopy', Plenum, New York

Dickerson, R.E. and Geiss, I. (1969)
'The Structure and Action of Proteins', Benjamin, California

Dougherty, J. and Kreiger, I.M. (1972)
in Kreiger, Adv. Colloid Sci. 3, 111

Edsall, J.T. (1953)
in 'The Proteins' (Neurath, H. and Bailey, K. eds) 1B, Chapter 7, Academic, New York

Edwardes, D. (1892)
Quart. J. Math. 26, 70

Einstein, A. (1905)
Ann. Physik 17, 549

Einstein, A. (1906)
Ann. Physik 34, 591

Einstein, A. (1911)
Ann Physik 34, 591

Emes, C.H. (1977)

Ph. D. thesis, University of Leicester

Emes, C.H. and Rowe, A.J. (1978a)

Biochim. Biophys. Acta 537, 110

Emes, C.H. and Rowe, A.J. (1978b)

Biochim. Biophys. Acta 537, 125

Farrant, J.L. (1954)

Biochim. Biophys. Acta 13, 569

Feldman, R.J. (1976)

'Atlas of Macromolecular Structure on Microfiche', Traco Jitco Int., Rockville, Md. USA

Gans, R. (1928)

Ann. Physik 86, 628

Garcia Bernal, J.M. and Garcia de la Torre, J. (1980)

Biopolymers 19, 628

Garcia de la Torre, J. and Bloomfield, V.A. (1977a)

Biopolymers 16, 1747

Garcia de la Torre, J. and Bloomfield, V.A. (1977b)

Biopolymers 16, 1765

Garcia de la Torre, J. and Bloomfield, V.A. (1977c)

Biopolymers 16, 1779

Garcia de la Torre, J. and Bloomfield, V.A. (1978)

Biopolymers 17, 1605

Gardner, D.G., Gardner, J.C., Laush, G. and Meinke, W.W. (1959)

J. Chem. Phys. 31, 978

Giesekus, H. (1962)

Rheol. Acta 2, 50

Gill, P.E. and Murray, W. (1976)

Nat. Phys. Lab. report NAC 72

Gold, O. (1937)

Ph. D. thesis, University of Vienna

Goodwin, J.W. (1975)

Colloid Science 2 (Chemical Society), 246

Guoy, L.G. (1910)

J. Phys. Chem. 9, 457

Hall, C.E. and Slayter, H.S. (1959)

J. Biophys. Cytol. 5, 11

Harding, S.E. (1980a)

Biochem. J. 189 (in press)

Harding, S.E. (1980b)

mss. submitted to J. Phys. Chem.

Harrison, P.M. (1959)

J. Mol. Biol. 1, 69

Herzog, R.O., Illig, R. and Kudar, H. (1934)

Z. Phys. Chem. A167, 329

Holtzer, A. and Lowey, S. (1956)

J. Am. Chem. Soc. 78, 5954

Jablonski, A. (1961)

Naturforsch 169, 1

Jeffrey, G.B. (1922)

Proc. Roy. Soc. (London) A102, 476

Johnson, P. and Mihalyi, E. (1965)

Biochim. Biophys. Acta 102, 476

Jost, J.W. and O'Konski, C.T. (1978)

in 'Molecular Electro Optics' (O'Konski, C.T. ed.) Volume 2, 529

Kartha, G., Bello, J. and Harker, D. (1967)

Nature 213, 862

Kendrew, J.C., Bodo, G., Dintzis, H.M., Parrish, R.G., Wycoff, H. and Phillips, D.C. (1958)

Nature 181, 665

Kim, S.H. (1974)

in 'Biochemistry' (Stryer, L.) 653, Freeman, San Francisco

Kirkwood, J.G. (1967)

'Macromolecules' (Aueur, P.L. ed.), Gordon and Breach

Koenig, S.H. (1975)

Biopolymers 14, 2421

Kratky, O., Leopold, H. and Stabinger, H. (1969)

Z. Angew. Phys. 27, 273

Kratky, O., Leopold, H. and Stabinger, H. (1969)

in 'Methods in Enzymology' (Hirs, C.H.W. and Timasheff, S.N. eds.), 27, 98, Academic, London

Krause, S. and O'Konski, C.T. (1959)

J. Am. Chem. Soc. 81, 5082

Kuff, E.J. and Dalton, A.L. (1957)

J. Ultrastructure Res. 1, 62

Kuhn, W. and Kuhn, H. (1945)

Helvetica Chimica Acta 38, 97

Kynch, G.J. (1956)

Proc. Roy. Soc. (London) A237, 90

Labaw, L.W. and Wycoff, R.W.G. (1957)

Biochim. Biophys. Acta 25, 263

Lauffer, M.A. (1942)

Chem. Rev. 31, 561

Laurent, T.C. and Killander, J. (1964)

J. Chromatog. 14, 317

Lipscomb, W.N. (1971)

Proc. Robert A. Welch Found. Conf. Chem. Res. 15, 134

Lloyd, P.H. (1974)

'Optical Methods in Ultracentrifugation, Electrophoresis and Diffusion',

Clarendon Press, Oxford

Lucas, C.W. and Terrill, C.W. (1970)

Collected Algorithms from CACM, No. 404

Manley, R. and Mason, S. G. (1954)

Canad. J. Chem. 32, 763

Martin, R.B. (1964)

'Introduction to Biophysical Chemistry' Chap. 11, McGraw-Hill, New York

McCammon, J.A., Deutch, J.M. and Bloomfield, V.A. (1975)

Biopolymers 14, 2479

Mehl, J.W., Oncley, J.L. and Simha, R. (1940)

Science 92, 132

Mendelson, R. and Hartt, J. (1980)

EMBO Workshop on Muscle Contraction, Alpbach Conf. Austria

Mihalyi, E. and Godfrey, J. (1963)

Biochim. Biophys. Acta 67, 90

Memming, R. (1961)

Z. Physik. Chem. (Frankfurt) 28, 169

Mooney, M. (1951)

J. Coll. Sci. 6, 162

Mooney, M. (1957)

J. Coll. Sci. 12, 575

Munro, I., Pecht, I. and Stryer, L. (1979)

Proc. Nat. Acad. Sci. (USA) 76, 56

Nichol, L.W., Jeffrey, P.D., Turner, D.R. and Winzor, D.J. (1977)

J. Phys. Chem. 81, 776

Nisihara, T. and Doty, P. (1958)

Proc. Nat. Acad. Sci. (USA) 44, 411

Oberbeck, A. (1876)

J. reine. angew. Math. 81, 62

O'Connor, D.V., Ware, W.R. and Andre, J.C. (1979)

J. Phys. Chem. 83, 1333

Offer, G., Moos, C. and Starr, R. (1973)

J. Mol. Biol. 74, 653

O'Hara and Smith (1968)

Comp. Journal 11, 213

O'Konski, C.T. and Haltner, A.J. (1956)

J. Am. Chem. Soc. 78, 3604

Oliver, J. (1972)

Comp. Journal 15, 141

Oncley, J.L. (1940)

J. Phys. Chem. 44, 1103

Oncley, J.L. (1941)

Ann. New York Acad. Sci. 41, 121

Paradine, C.G. and Rivett, B.H.P. (1960)

'Statistical Methods for Technologists', English Universities Press, London

Pearce, T.C., Rowe, A.J. and Turnock, G. (1975)

J. Mol. Biol. 97, 193

Perrin, F. (1934)

J. Phys. Radium 5, 497

Perrin, F. (1936)

J. Phys. Radium 7, 1

Perutz, M.F., Rossmann, M.G., Cullis, A.F., Muirhead, H., Will, G. and North, A.C.T. (1960)

Nature 185, 416

Powell, D.R. and MacDonald, J.R. (1972)

Comp. Journal 15, 148

Pytkowickz, R.M. and O'Konski, C.T. (1959)

J. Am. Chem. Soc. 81, 5082

Rallison, J.M. (1978)

J. Fluid Mech. 84, 237

Riddiford, C.L. and Jennings, B.R. (1967)

Biopolymers 5, 757

Ridgeway, D. (1966)

J. Am. Chem. Soc. 88, 1104

Ridgeway, D. (1968)

J. Am. Chem. Soc. 90, 18

Rowe, A.J. (1977)

Biopolymers 16, 2595

Rowe, A.J. (1978)

Techniques in Protein and Enzyme Biochem. B105a, 1

Saito, N. (1951)

J. Phys. Soc. (Japan) 6, 297

Scheraga, H.A. (1955)

J. Chem. Phys. 23, 1526

Scheraga, H.A. (1961)

'Protein Structure', Academic, New York

Scheraga, H.A. and Mandelkern, L. (1953)

J. Am. Chem. Soc. 79, 179

Simha, R. (1940)

J. Phys. Chem. 44, 25

Simha, R. (1952)

J. Appl. Phys. 23, 1020

Shaw, D.J. (1970)

'Introduction to Colloid and Surface Chemistry' (2nd edn.), Butterworths

Small, E.W. and Isenberg, I. (1977)

Biopolymers 16, 1907

Sorenson, N.A. (1930)

CR Lab. Carlsberg 18, No. 5

Squire, P.G. (1970)

Biochim. Biophys. Acta 221, 425

Squire, P.G. (1978)

in 'Molecular Electro Optics' (O'Konski, C.T. ed.) Volume 2, 565

Squire, P.G., Moser, L. and O'Konski, C.T. (1968)

Biochemistry 7, 4261

Stacey, K.A. (1956)

'Light Scattering in Physical Chemistry', Butterworths, London

Stokes, Sir G. (1851)

Trans. Cambridge Phil. Soc. 9, 8

Stokes, Sir G. (1880)

'Mathematical and Physical Papers', Cambridge University Press

Svedberg, T. and Pedersen, K.O. (1940)

'The Ultracentrifuge', Oxford University Press

Tanford, C. (1955)

J. Phys. Chem. 59, 798

Tanford, C. (1961)

'Physical Chemistry of Macromolecules', Wiley, New York

Theorell, H. (1934)

Biochem. Z. 268, 46

Vand, V. (1948)

J. Phys. Coll. Chem. 52, 277

Van de Hulst, H.C. (1957)

'Light Scattering by Small Particles', Wiley, New York

Van Holde, K.E. (1971)

'Physical Biochemistry', Prentice Hall, New Jersey

Wahl, P. (1966)

Compt. Rend. Acad. Sci. (Paris) 263D 1525

Wales, M. and Van Holde, K.E. (1954)

J. Polymer Sci. 14, 81

Wilde, D.J. (1964)

'Optimum Seeking Methods', Prentice Hall, New Jersey

Williams, R.C., Ham, W.T. and Wright, A.K. (1976)

Anal. Biochem. 73, 52

Wilson, R.W. and Bloomfield, V.A. (1979a)

Biopolymers 18, 1205

Wilson, R.W. and Bloomfield, V.A. (1979b)

Biopolymers 18, 1543

Yang, J.T. (1961)

Adv. Protein Chem. 16, 323

Zimm, B.H. (1948)

J. Chem. Phys. 16, 1093

Zimm, B.H. (1956)

J. Chem. Phys. 24, 269