Modelling biological macromolecules in solution: 3. The Λ-R intersection method for triaxial ellipsoids

Stephen E. Harding
Department of Biochemistry; University of Bristol; The Medical School; Bristol BS8 1TD; UK
and Arthur J. Rowe
Department of Biochemistry, University of Leicester, Adrian Building, Leicester LE1 7RH, UK
(Received 10 February 1982; revised 8 June 1982)

The general triaxial ellipsoid model for the gross conformation of macromolecules in solution represents a significant advance over the previously, almost ubiquitously used ellipsoid of revolution model. A new method is presented which involves the graphical intersection of two triaxial hydrodynamic functions (Λ and R) involving viscosity, sedimentation and fluorescence depolarization. The method is restricted to macromolecules asymmetric enough for the functions to be sufficiently sensitive but not so asymmetric for there to be problems of internal rotations between parts of the macromolecules. The method is illustrated by application to data for neurophysin II monomers and dimers.

Keywords: Mathematical models; ellipsoidal model; hydrodynamic shape functions; numerical inversion; neurophysin II

In the first paper of this series[1] the use of the ellipsoid of revolution as a model for biological macromolecules in solution was discussed and the various hydrodynamic shape functions for such a model compared. Hydrodynamicists have, for a long time, recognized that the more general triaxial ellipsoid with its extra degree of freedom would be a much more realistic model for many macromolecules. In paper 2 of this series[2] we have shown that application of the triaxial model is possible by combining new functions involving viscosity, sedimentation and electric birefringence decay data. In this paper we present an alternative method involving combination of viscosity, sedimentation and harmonic mean relaxation data. Although this method is experimentally and numerically simpler and requires data of less precision, it is applicable only to a restricted class of macromolecules, i.e. those asymmetric enough for a particular function (Λ) to be sensitive to axial ratio but not *so* asymmetric for segmental rotation of parts of the macromolecule with respect to others, or internal rotation of the fluorescent chromophore, to become a problem.

In paper 2[2] we stated that analytic solutions for the Simha-Saito viscosity increment ν, the Perrin translational frictional function P, and the various rotational frictional and relaxation functions in terms of the two axial ratios (a/b, b/c) characterizing a general triaxial ellipsoid of semi-axes $a \geqslant b \geqslant c$ were now available. We described how these functions required for their experimental evaluation a knowledge of the (swollen) macromolecular volume in solution and how this requirement could be eliminated by combining any two of these functions to give new volume independent functions. All these functions have the 'line solution' property, i.e. although a given value of (a/b, b/c) uniquely specifies a value for a particular function the converse is not true and a given value of the function has a *line solution* of possible values of (a/b, b/c).

By plotting two of these (swelling independent) functions in the (a/b, b/c) plane a unique solution can in principle be found from the intersection. The choice of a pair of functions for this plot is affected by (i) their practical experimental measurability, (ii) their sensitivity to axial ratio and insensitivity to experimental error, and (iii) the orthogonality of the intersection. In paper 2[2] we showed that use of the swelling independent R function was desirable, combined with the swelling independent $\delta\pm$ functions. Measurement of $\delta\pm$ requires resolution of two decay constants from an electric birefringence decay. For globular macromolecules in particular, these can be very close and resolution requires a complicated 'R contrained' technique and birefringence apparatus with a very fast response time[3] ($< \sim 8$ ns).

For some macromolecules we have now found a much simpler approach is possible. Although the general principle is the same [viz. intersection of the R function with some other suitable function in the (a/b, b/c) plane], measurement of the Λ function used for the intersection can be much easier.

Theory

The Λ and R functions

The R function[4] can be measured experimentally from the ratio of sedimentation regression coefficient, k_s to intrinsic viscosity $[\eta]$. It is related to the axial ratios (a/b, b/c) of a general ellipsoid by:

$$R \equiv \frac{k_s}{[\eta]} = \frac{2}{\nu}(1 + P^3) \qquad (1)$$

0141-8130/82/060357-05$03.00

where the viscosity increment ν[5-7] and the Perrin frictional function, P[2,7,9] are themselves explicit functions of axial ratios. Firstly:

$$\nu = \frac{1}{abc}\left\{\frac{4(\alpha_0''+\beta_0''+\gamma_0'')}{15(\beta_0''\gamma_0''+\gamma_0''\alpha_0''+\alpha_0''\beta_0'')} + \frac{1}{5}\left[\frac{\beta_0+\gamma_0}{\alpha_0'(b^2\beta_0+c^2\gamma_0)} + \frac{\gamma_0+\alpha_0}{\beta_0'(c^2\gamma_0+a^2\alpha_0)} + \frac{\alpha_0+\beta_0}{\gamma_0'(a^2\alpha_0+b^2\beta_0)}\right]\right\}$$

$$-\frac{1}{5abc}\left\{\frac{\left[\frac{a^2-b^2}{a^2\alpha_0+b^2\beta_0}+\frac{b^2-c^2}{b^2\beta_0+c^2\gamma_0}+\frac{c^2-a^2}{c^2\gamma_0+a^2\alpha_0}\right]^2}{\left[\frac{a^2+b^2}{a^2\alpha_0+b^2\beta_0}+\frac{b^2+c^2}{b^2\beta_0+c^2\gamma_0}+\frac{c^2+a^2}{c^2\gamma_0+a^2\alpha_0}\right]}\right\} \quad (2)$$

where the α_0 etc are elliptic integrals defined by Jeffrey[7,10]. The last term in this expression[8] is negligible for globular particles (axial ratio $\lesssim 3$) and contributes $< \sim 1\%$ towards ν for particles of higher asymmetry. Secondly:

$$P = \frac{2}{(abc)^{1/3}\int_0^\infty \frac{d\lambda}{[(a^2+\lambda)(b^2+\lambda)(c^2+\lambda)]^{1/2}}} \quad (3)$$

The R function is extremely sensitive to axial ratio and, because systematic errors in concentration measurement disappear in the ratio $k_s/[\eta]$, can be measured to a precision of up to 1%[1,4].

The Λ function[11] can be measured experimentally from a knowledge of the intrinsic viscosity $[\eta]$, the molecular weight, M_r, and the harmonic mean rotational relaxation time τ_h. It is related to the axial ratios of a general ellipsoid by:

$$\Lambda \equiv \frac{3\eta_0[\eta]M_r}{N_A kT\tau_h} = \frac{\nu}{(\tau_h/\tau_0)} \quad (4)$$

where τ_h/τ_0 is the harmonic mean relaxation time ratio[7,12,13].

$$\frac{\tau_h}{\tau_0} = \frac{3}{\left(\frac{\tau_0}{\tau_a}+\frac{\tau_0}{\tau_b}+\frac{\tau_0}{\tau_c}\right)} \quad (5)$$

and where

$$\frac{\tau_a}{\tau_0} = \frac{1}{3abc\left(\frac{c^2\gamma_0+a^2\alpha_0}{c^2+a^2}+\frac{a^2\alpha_0+b^2\beta_0}{a^2+b^2}\right)}$$

$$\frac{\tau_b}{\tau_0} = \frac{1}{3abc\left(\frac{a^2\alpha_0+b^2\beta_0}{a^2+b^2}+\frac{b^2\beta_0+c^2\gamma_0}{c^2+a^2}\right)} \quad (6)$$

$$\frac{\tau_c}{\tau_0} = \frac{1}{3abc\left(\frac{b^2\beta_0+c^2\gamma_0}{b^2+c^2}+\frac{c^2\gamma_0+a^2\alpha_0}{c^2+a^2}\right)}$$

and the α_0 etc are those defined by Jeffrey[10].

The Λ and R functions can thus be evaluated for any given value of the two axial ratios (a/b, b/c) with the aid of high speed computers for the numerical solution of the elliptic integrals α_0 etc.[15].

The line solutions of (a/b, b/c) corresponding to experimentally evaluated values for Λ and R can also be plotted using simple numerical inversion techniques[14] and this has been done for a typical asymmetric particle with true axial ratios (a/b, b/c)=(5.0, 5.0) in *Figure 1a*. *Figure 1b* gives the same plot allowing for errors of $\pm 1\%$

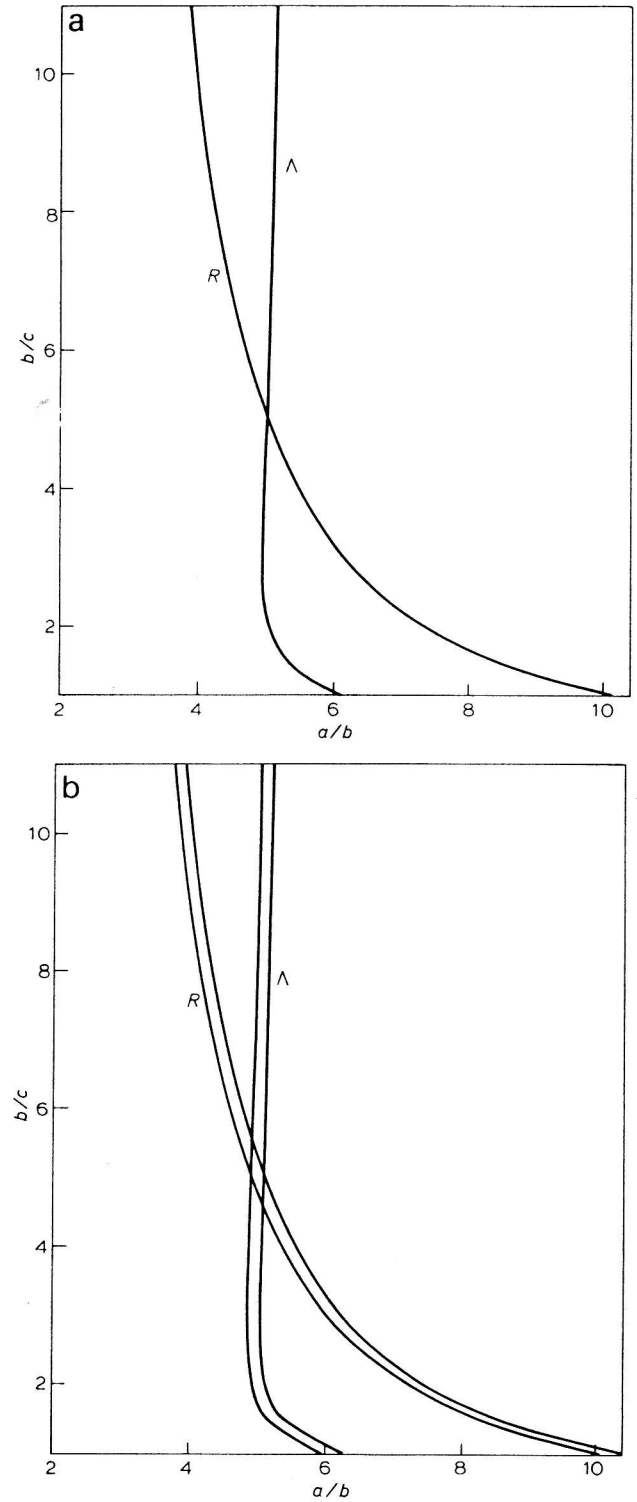

Figure 1 (a) Plots of constant R and Λ in the (a/b, b/c) plane corresponding to $a/b = 5.0$, $b/c = 5.0$. (b) As (a) but allowing for $\pm 1\%$ error in the measured value of R, $\pm 2\%$ in Λ

in the measured value of R and $\pm 2\%$ in Λ. It is seen that the '$\Lambda - R$ intersection method' reproduces the true value of $(a/b, b/c)$ to very reasonable limits of precision.

Limitations of the method

The Λ–R intersection method cannot be applied to all macromolecules. Not only must a triaxial ellipsoid be a reasonable approximation to the overall shape of the macromolecule[2] but:

(i) the macromolecule must be sufficiently asymmetric so that Λ is sufficiently sensitive. For a precision in Λ of $\sim \pm 2\%$ one axial ratio $\gtrsim 3$ is usually sufficient.

(ii) the macromolecule must not be *so* asymmetric that segmental rotation of parts of the macromolecule with respect to others occurs.

(iii) there must be negligible internal rotation of the fluorescent chromophore[16,17].

If (ii) and (iii) occur the measured τ_h will not be accurate. This has been discussed in some detail elsewhere[16,17]. A startling example of segmental rotation occurring has been shown for fibrinogen by Johnson and Mihalyi[18], thus some form of test to check (ii) and (iii) are not occurring is desirable. τ_h can be determined using two essentially independent methods[21] or results using more than one chromophore can be compared[23].

The Λ–R intersection method applied to neurophysin II monomers and dimers

One system that appears to satisfy the criteria outlined in the previous section is neurophysin II (monomers and dimers)[19–21]. Rholam and Nicholas[21] have recently determined the Λ function for both monomer and dimer ($\Lambda_M = 3.16$, $\Lambda_D = 2.69$, where the subscripts M and D denote monomer and dimer, respectively). Two independent methods were used to obtain the τ_h values (steady state fluorescence and fluorescence depolarization) and found to be in close agreement. Furthermore these τ_h values were shown to be consistent with data from sedimentation velocity and viscosity measurements.

Normally, for homogeneous non-associating systems in ionic media where 'charge effects' have been suppressed k_s can be found reasonably easily and accurately from the limiting slope of a plot of the sedimentation coefficient, s *versus* concentration c:[4,7]

$$s = s_0(1 - k'_s c) \tag{7}$$

(where s_0 is the sedimentation coefficient at infinite dilution) and then corrected to solution density[4,7]:

$$k_s = k'_s - \bar{v} \tag{8}$$

However for a monomer–dimer associating system the situation is not so simple. k_s may still be evaluated from the relation[4,7]:

$$k_s = 2\bar{v}\left[\frac{4\pi}{3\bar{v}} \cdot \frac{M_r^2}{N_A^2}\left(\frac{1 - \bar{v}\rho_0}{6\pi\eta_0 s_0}\right)^3 + \frac{v_s}{\bar{v}}\right] \tag{9}$$

where ρ_0 and η_0 are the solvent density and viscosity, respectively, normally corrected to water at 20 or 25°C, v_s is the (swollen) specific volume of the particle + associated solvent and $\bar{v}$ is the partial specific volume. In order to use this equation an estimate for the 'swelling' $v_s/\bar{v}$ is required. Rholam and Nicolas[20] use a hydration value of 0.38 g g^{-1} for both monomer and dimer. This is equivalent to a $v_s/\bar{v}$ of 1.54.

Neurophysin II monomer. For the monomer, Rholam and Nicolas[19,21] have found $M_r = 10041$ g mole^{-1}, $\bar{v} = 0.709$ ml g^{-1} and $s_0 = 1.25$ S. Taking $\bar{v}_s/\bar{v}$ as 1.54 from equation (9) $k_s = 6.47$ ml g^{-1}. Combining this with the value of 5.5 ml g^{-1} for the intrinsic viscosity $[\eta]_M$, R_M is found to be 1.18. However, because of the error in the assumed value for $\bar{v}_s/\bar{v}$, R_M can only be accurate to within $\pm 5\%$.

Neurophysin II dimer. From Rholam and Nicolas[19,21], $M_r = 20082$ g mole^{-1}, $\bar{v} = 0.709$ ml g^{-1} and $s_0 = 2.20$ S. Again, taking their value for the hydration of 0.38 g g^{-1} ($v_s/\bar{v} = 1.54$), k_s is then found to be 5.32, and since $[\eta]_D = 4.6$ ml g^{-1}, $R = 1.16$. Again, because of error in $\bar{v}_s/\bar{v}$, R_D can only be accurate to within $\pm 5\%$.

The value for $[\eta]_D = 4.6$ ml g^{-1} given by Rholam and Nicolas[21] is derived from a curve-fit for η_{sp}/c for the system, assuming a Huggins constant $K' = 0.4$. This value, appropriate for extended rigid rods is not very appropriate for the fairly modest degree of asymmetry found for neurophysin, either in its monomer or dimer form (see below). The extrapolation used is likely, however, to give a reasonable estimate for $[\eta]_M$, since the extrapolation to f_D (dimer fraction) = 0 excludes terms in c. A refined estimate for $[\eta]_D$ can be obtained by employing an improved estimate for K', using the theory of Rowe[4]. Since $K' = (v_s/\bar{v})R$, we can take $K' = 1.5$ to an approximation, and by recomputing the partial components of the reduced viscosity at several concentrations and extrapolating these values to $1/c = 0$ (i.e. $f_M = 0$) a value of $[\eta]_D = 4.32$ ml g^{-1} is obtained, slightly lower than that given by Rholam and Nicolas[21].

The value for s_D given by Rholam and Nicolas[21] (= 2.20 S) was obtained by iterative fitting of the s vs. c data set. The assumption that neither s_M nor s_D was c-dependent (i.e. $k_{s,M} = k_{s,D} = 0$) was clearly made, though not explicitly stated. This procedure leads to results which are significantly in error. A much more valid procedure treats $k_{s,M}$ and $k_{s,D}$ as variables to s_M and s_D assumed by equation (9). An interactive computer program[22] has been used to fit the data set of Rholam and Nicolas, and our best fit (*Figure 2*) is obtained with values of $s_D = 2.40$ S, $k_{s,D} = 4.63$ ml g^{-1} [an apparent $k'_{s,M} = 7.18$ ml g^{-1} and $k'_{s,D} = 5.34$ ml g^{-1} was used to allow for the data of Rholam and Nicolas being corrected to *solvent* rather than *solution* density (see Rowe, 1977[4])]. The difference from the fit of Rholam and Nicolas, though not large, is highly significant. If their value of $s_D = 2.20$ S is used, and concentration dependence taken into account, no value of the equilibrium constant, K_a gives other than a very poor fit. Our optimal value for $K_a = 6300$ dm^3 mol^{-1} is also slightly different.

Using the corrected values of $[\eta]$ and k_s for the dimer, values of $\Lambda_D = 2.53$ and $R_D = 1.07$ are obtained.

Results and discussion

From *Figure 3* it is seen that, even with the extra degree of freedom the triaxial ellipsoid allows as compared with the now almost ubiquitously used ellipsoid of revolution, the monomer is prolate of axial ratio ~4:1:1 ($a/b = 4.1 \pm 0.1$). Using the uncorrected data of Rholam and Nicolas[21] for

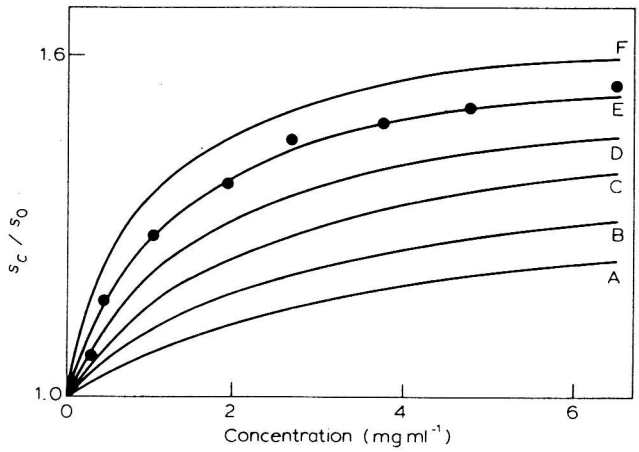

Figure 2 Ratio of the sedimentation coefficient at concentration c to the corresponding value at infinite dilution, as a function of concentration. The experimental points are those of Rholam and Nicolas[21]. The computed curves correspond to $s_M = 1.25$ S, $s_D = 2.40$ S, $k'_{s,M} = 7.18$ ml g^{-1}, $k'_{s,D} = 5.34$ ml g^{-1}, $\bar{v} = 0.709$ ml g^{-1} (monomer and dimer) and monomer–dimer equilibrium constant K_a (ml g^{-1}) of: A, 10^2; B, $10^{2.2}$; C, $10^{2.4}$; D, $10^{2.6}$; E, (best fit) $10^{2.8}$; F, $10^{3.0}$

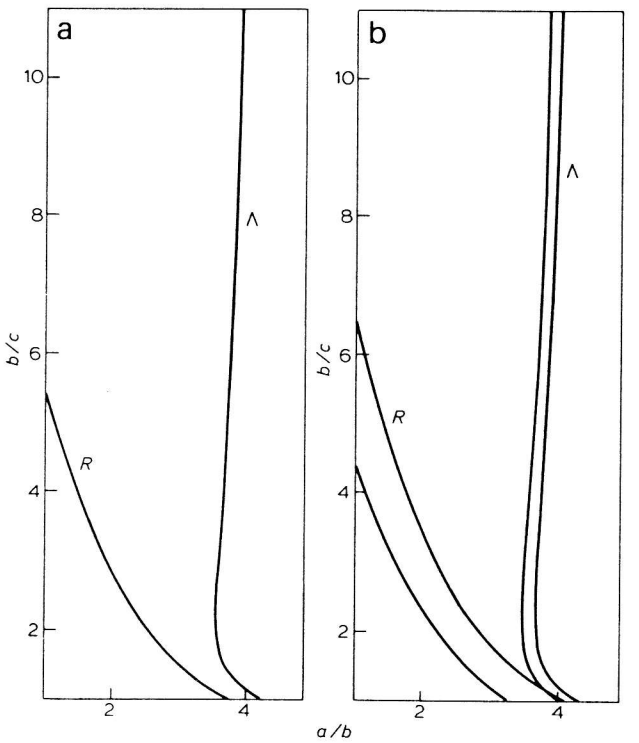

Figure 3 (a) Plots of constant R and Λ in the $(a/b, b/c)$ plane for neurophysin II monomers ($R = 1.18$, $\Lambda = 3.16$). (b) As (a) but allowing for $\pm 5\%$ error in the measured value of R, $\pm 2\%$ in Λ

the dimer ($R = 1.16$; $\Lambda_D = 2.69$) from *Figure 4* an axial ratio of $\sim 2\frac{3}{4}$:2:1 is obtained ($a/b = 2.75 \pm 0.20$, $b/c = 2.0 \pm 0.2$). Using the corrected form of the data ($R = 1.07$, $\Lambda_D = 2.53$) it is seen that an axial ratio of $\sim 2\frac{1}{2}$:$2\frac{3}{4}$:1 is now obtained ($a/b = 2.5 \pm 0.2$, $b/c = 2.85 \pm 0.20$).

It would be expected that if the association of two monomers to form a dimer was a side-by-side process the gross conformation would change from a prolate (axial ratio 4:1:1) to a more triaxial conformation (2:2:1). The results would therefore appear to reinforce the earlier conclusions of Rholam and Nicolas, despite the large error involved in the method used to obtain values for k_s for a dimerizing system.

In the analyses we have followed the assumption of Rholam and Nicolas that the swelling, or equivalently

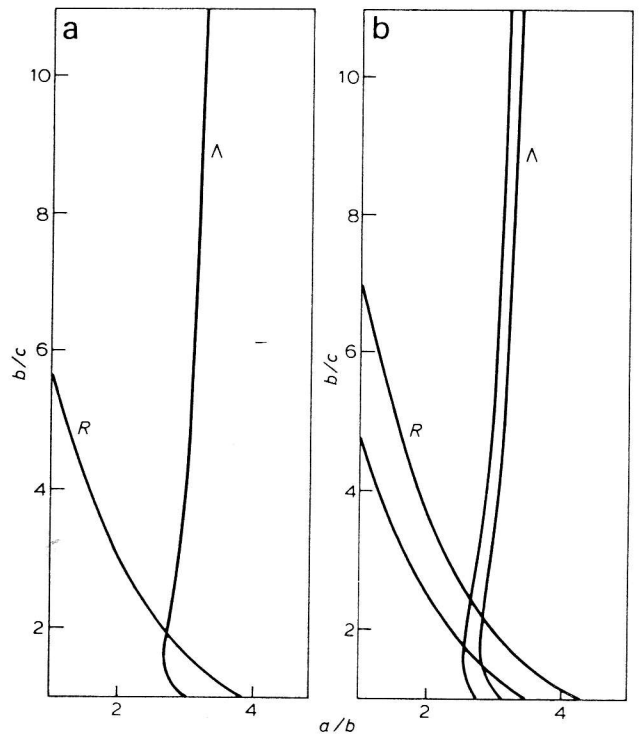

Figure 4 (a) Plot of constant R and Λ in the $(a/b, b/c)$ plane for neurophysin II dimers (uncorrected Rholam and Nicholas data: $R = 1.16$, $\Lambda = 2.69$). (b) As (a) but allowing for $\pm 5\%$ error in the measured value of R, $\pm 2\%$ in Λ

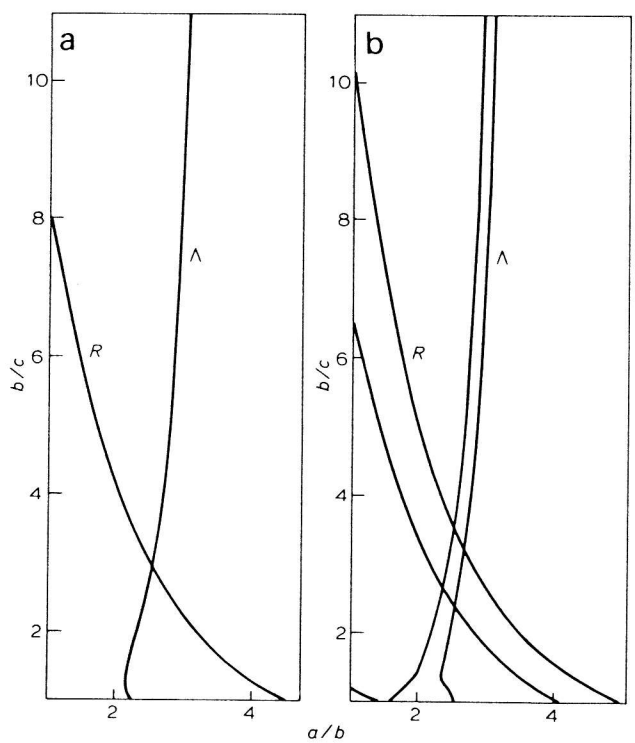

Figure 5 (a) and (b) as *Figure 4* (a) and (b) but using corrected Rholam and Nicolas data: $R = 1.07$, $\Lambda = 2.53$

the hydration, was the same for both monomer and dimer ($v_s/\bar{v}=1.54$). It is difficult to say how accurate this assumption was. On dimerization one would naturally expect some solvent to be excluded from the binding surfaces, but the net hydration shell could well be larger, particularly if the monomer is a prolate ellipsoid. Not only would this contribute another error in the analyses to extract k_s, but the overall shape itself may be appreciably different. The results presented here should, therefore, not be taken as *conclusive* proof of Rholam and Nicholas's proposed mode of association.

Acknowledgement

One of the authors (SEH) would like to acknowledge financial assistance from the Lister Institute of Preventative Medicine.

References

1 Harding, S. E. and Rowe, A. J. *Int. J. Biol. Macromol.* 1982, **4**, 160
2 Harding, S. E. and Rowe, A. J. *Biopolymers* 1982 (in press)
3 Williams, R. C., Ham, W. T. and Wright, A. V. *Anal. Biochem.* 1976, **73**, 52
4 Rowe, A. J. *Biopolymers* 1977, **16**, 2595
5 Harding, S. E., Dampier, M. and Rowe, A. J. *IRCS Med. Sci.* 1979, **7**, 33
6 Harding, S. E., Dampier, M. and Rowe, A. J. *J. Coll. Int. Sci.* 1981, **79**, 7
7 Harding, S. E. *Ph.D. Thesis*, University of Leicester, 1980
8 Rallison, J. M. and Brenner, H. (private communications)
9 Perrin, F. *J. Phys. Radium* 1936, **7**, 1
10 Jeffrey, G. B. *Proc. R. Soc. (London)* 1922, **A102**, 161
11 Harding, S. E. *Biochem. J.* 1980, **189**, 359
12 Weber, G. *Adv. Prot. Chem.* 1953, **8**, 415
13 Perrin, F. *J. Phys. Radium* 1934, **5**, 497
14 Harding, S. E. *Comput. Biol. Med.* (in press)
15 Harding, S. E. *Comput. Biol. Med.* 1982, **12**, 75
16 Johnson, P. and McIntosh, R. P. *Biochim. Biophys. Acta* 1976, **453**, 521
17 McIntosh, R. P. and Johnson, P. *Biopolymers* 1978, **17**, 2373
18 Johnson, P. and Mihalyi, E. *Biochim. Biophys. Acta* 1965, **102**, 476
19 Nicolas, P., Batelier, G., Rholam, M. and Cohen, P. *Biochemistry* 1980, **19**, 3565
20 Nicolas, P., Camier, M., Lauber, M., Masse, M. J., Möhring, J. and Cohen, P. *Proc. Natl. Acad. Sci. USA* 1980, **77**, 2587
21 Rholam, P. and Nicolas, P. *Biochemistry* 1981, **20**, 5837
22 Simon, P. and Rowe, A. J. (to be published)
23 Steiner, R. F. and McAlister, A. J. *J. Polymer Sci.* 1957, **24**, 105