Biophysical Chemistry 3 (1975) 74–82
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HYDRODYNAMIC STRUCTURE OF HELIX POMATIA HEMOCYANIN

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Received 21 August 1974
Revised manuscript received 5 December 1974

1. Introduction

The most general theory for the analysis of electric birefringence decay observed with macromolecular solutions was presented by Ridgeway [1,2] for a monodisperse suspension of rigid particles of arbitrary shape. For this general case, the birefringence decay is given as

$$\Gamma = (N/2n_l)(A_+ \exp -6\theta_+ t + A_- \exp -6\theta_- t), \qquad (1)$$

where N is the density of particles in suspension, n_l is the index of refraction of the suspending medium, and $A_\pm$ are functions which depend on the initial orientation of the particles and their dielectric and diffusion properties. However, Ridgeway states that more must be known about the local field within the polymer before it would be useful to evaluate eq. (1) for meaning of the coefficients $A_\pm$. The relaxation times $\tau_\pm = 1/6\theta_\pm$, depend only on the temperature, solvent viscosity and terms of the rotational diffusion tensor of the particle. Ridgeway also points out that since at most two relaxation constants may be observed for any monodisperse suspension of rigid particles, it is not possible to infer the shape class of the suspended particles from the relaxation curve alone. However, it is possible to test proposed structures for those cases where the elements of the diffusion tensor have been calculated in terms of the particle shape parameters. Such structures include the sphere, calculated by Einstein [3], the right circular cylinder of large axial ratio, calculated by Burgers [4], and finally, the asymmetric ellipsoid calculated by Perrin [5].

Ridgeway [6] considers four specializations of the general solution. The first is the suspension of a single type of spheroid (two axes equal) of isotropic material. The decay of birefringence is simple exponential and is in agreement with the earlier treatments of Benoit [7], O'Konski and Zimm [8], and Tinoco [9]. The second case is that of a suspension of two or more types of spheroids of isotropic material. This corresponds to a polydisperse system of particles described by case one. In this case, the motions of individual particles are assumed to be independent of one another so that the superposition of the individual contributions of each particle to the birefringence results in a mixed exponential decay curve of the form

$$\Gamma = \sum_{i=1}^{n} \Gamma_i = \sum_{i=1}^{n} \Gamma_i(0) \exp -6D_i t, \qquad (2)$$

where D_i is the rotational diffusion coefficient for rotational motion about one of the cross-axes of the spheroid. The third case is that of a spheroid of anisotropic material, for which a mixed exponential curve with two terms is observed, of which, that with the smaller exponent is the one which would be observed if the material of the particle were isotropic. The fourth case is the asymmetric ellipsoid of unequal axes of an anisotropic material. Ridgeway points out that it is not possible to distinguish experimentally whether a double exponential decay curve indicates the second or the fourth case described here, because any ratio of the two exponents is acceptable in either case. Ridgeway further states that the third case leads to a specific ratio of the exponents and should be detectable as such. An additional case, which we shall

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refer to as case five, is that of a heterogeneous suspension of anisotropic spheres. Each sphere contributes a simple exponential decay to the birefringence.

Accordingly, a mixed exponential decay of birefringency may be due to a suspension of two anisotropic spheres of different radii; to a suspension of two isotropic spheroids of different axial ratios; to a monodisperse suspension of an anisotropic spheroid, and finally an asymmetric ellipsoid.

Cases one and two have been investigated by several researchers including Benoit [7], Krause and O'konski [10], Pytkowicz and O'Konski [11], and Ingram and Jerrard [12]. Case three has been investigated by Wright and Thompson [13], who measured a mixed exponential decay in birefringence for bovine serum albumin. The two measured relaxation times were in agreement with the predicted values according to eq. (3), for the prolate ellipsoid model with major axis 140 Å and minor axis 40 Å, which has been proposed by Squire et al. [14], and Rosseneu–Motreff et al. [15]. Case four has been investigated by Ridgeway [2] in terms of the birefringence relaxation data for Helix pomatia hemocyanin, which was reported by Pytkowicz and O'Konski and interpreted by them in terms of cases one and two above.

The purpose of this paper is to investigate, for completeness, the application of the remaining models, which may account for the mixed exponential birefringence decay, to the data of Pytkowicz and O'Konski. Specifically, the suspension of two anisotropic spheres and the anisotropic spheroid will be used to interpret particle shape, and the results will be compared to the models calculated by Pytkowicz and O'Konski for case two, and by Ridgeway for case four. The resulting models will be used to predict hydrodynamic parameters obtained from other experimental methods.

2. Theory

The decay constants for a spheroid of anisotropic material are given by Ridgeway [1] as

$$\theta_+ = R_1, \qquad \theta_- = (R_1 + 2R_3)/3, \tag{3}$$

where R_1 denotes the rotational diffusion coefficient for rotation of the symmetry axis about a cross-axis, and R_3 that for rotation about the symmetry axis. Wright et al. [16], have shown that R_1 and R_3 are consistent with the hydrodynamic theory of Scheraga–Mandelkern [17], and therefore contain information for the dimensions of the equivalent ellipsoid of revolution. The iteration procedure developed by Wright et al. to approximate the inversion of the Perrin equations for R_1 and R_3 is based on the ratio R_3/R_1 which is tabulated in their table 1 for several arbitrary eccentricities. For the prolate ellipsoid, this ratio is seen to be a continuous, monotonic increasing function of the eccentricity. This ratio is directly related to that suggested by Ridgeway, since

$$\theta_-/\theta_+ = (R_1 + 2R_3)/3R_1 = (1 + 2R_3/R_1)/3. \tag{4}$$

Although θ_-/θ_+ is indeed a specific ratio for a given ellipsoid of revolution, this by itself is not sufficient to yield estimates of the particle dimensions without a procedure similar to that presented by Wright et al.

It is therefore meaningful to apply case three to birefringence decay data which are obviously a mixed exponential of the form

$$\Gamma = A \exp -t/\tau_f + B \exp -t/\tau_s,$$

where τ_f and τ_s denote the fast and slow relaxation times, respectively. For prolate ellipsoids, $R_1 < R_3$, therefore

$$\tau_s = 1/6R_1, \qquad \tau_f = 1/2(R_1 + 2R_3). \tag{5}$$

Thus, R_1 and R_3 may be estimated from a given pair of relaxation times.

3. Application

The birefringence decay for Helix pomatia hemocyanin dissolved in distilled water at 25°C, reported by Pytkowicz and O'Konski [11], was interpreted as a mixed exponential decay. The linear coefficients of the two exponential terms are obtained from the data to be 0.0069 and 0.023 radians, from which $A_\pm$ may be calculated. However, for the reason discussed in the Introduction, these coefficients will not be evaluated. The observed relaxation times for the mixed exponential decay curve are $\tau_f = 11.5 \times 10^{-6}$ s and $\tau_s = 55 \times 10^{-6}$ s. In the case of the suspension of anisotropic spheres, the relaxation times are evaluated according to eq. (2), where the two exponential parameters correspond to the rotational diffusion coefficients for the respective spheres. The radius of a given sphere, a, is calculated from the rotational diffusion coefficient, R_0, by means of the expression derived by Einstein [3], $R_0 = kT/(8\pi\eta a^3)$. Thus, the solution conditions of temperature and viscosity must be specified. The concentration of the H. pomatia hemocyanin solution reported by Pytkowicz and O'Konski was 5 mg/ml in distilled water at 25°C. Since the relaxation data were not extrapolated to zero protein concentration, the solution viscosity is used to calculate the radius, instead of the solvent viscosity. Konings et al. [18] reported an intrinsic viscosity for native Helix pomatia α-hemocyanin of 7.7 ml/g. This value was determined from viscosity measurements on concentrations varying between 5 and 20 mg/ml in 10^{-2} M phosphate buffer, pH 7. Since the reduced viscosity over this concentration range appeared to be independent of the protein concentration, the intrinsic viscosity was used to calculate the solution viscosity at the concentration specified by Pytkowicz and O'Konski. The viscosity of water, $\eta_w^{25°} = 0.8937$ centipoise, and the specified intrinsic viscosity are used to calculate the solution viscosity, 0.928 centipoise, for H. pomatia hemocyanin. Thus, the two relaxation times stated above yield the values for the radii of two anisotropic spheres of 230 and 387 Å.

In the case of the anisotropic spheroid, the two relaxation times are evaluated by means of eq. (5), yielding the rotational diffusion coefficients $R_1 = 3.03 \times 10^3\ \mathrm{s}^{-1}$ and $R_3 = 20.22 \times 10^3\ \mathrm{s}^{-1}$. The reduced coefficients, $R' = 16\pi\eta R/3kT$ according to Wright et al. [16], for the solution conditions stated above, are calculated to be $R'_1 = 11.453 \times 10^{15}\ \mathrm{cm}^{-3}$ and $R'_3 = 76.432 \times 10^{15}\ \mathrm{cm}^{-3}$. These yield the ratio $R_3/R_1 = 6.6735$. By means of the linear interpolation procedure presented by Wright et al., the coordinates shown in fig. 1 are calculated. The point of intersection of the two straight lines, (681, 135), correspond to the first order estimates of the semi-major and semi-minor axes in angstroms, for the equivalent ellipsoid of revolution for H. pomatia hemocyanin, according to Ridgeway's case three. The values of the semiaxial lengths after 17 iterations, described by Wright et al., are 680.66 Å and 134.76 Å. These values indicate the suitability of the linear estimates, due to the rapid rate of convergence of the estimating procedure. These values correspond to a prolate ellipsoid of length 1362 Å and diameter 270 Å, whose axial ratio is 5.04.

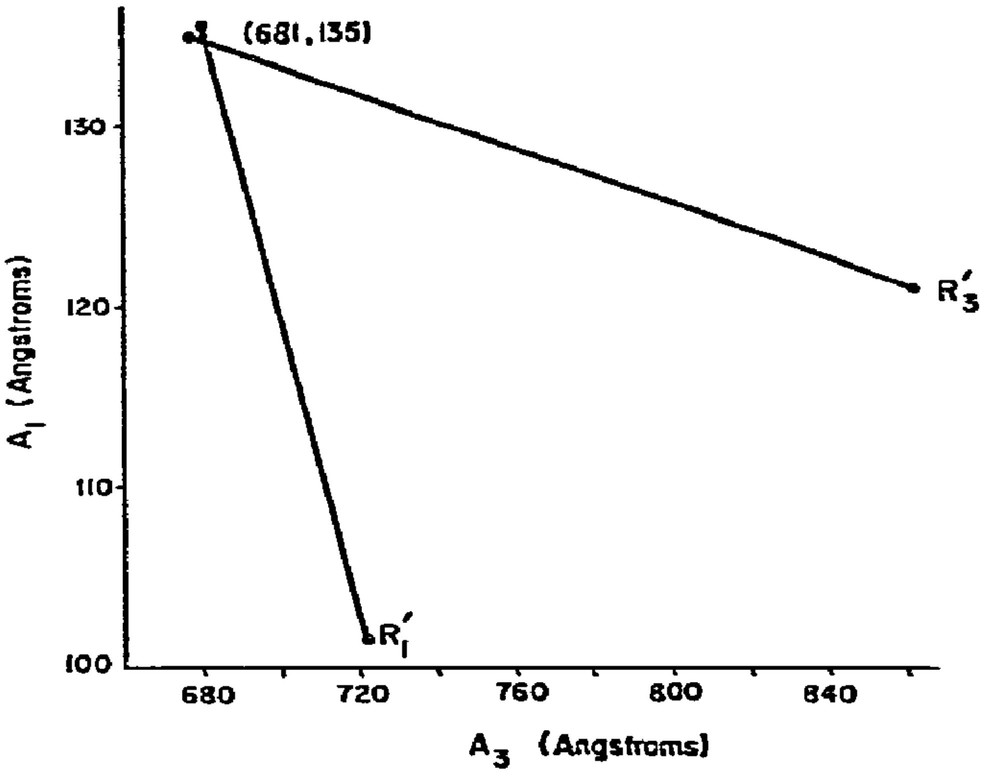

Fig. 1. Graphic analysis of linear estimates of semiaxial length of H. pomatia hemocyanin.

The same data have been analyzed by Ridgeway [2] in terms of case four of his generalized theory. The model calculated by Ridgeway for the hydrated protein is an asymmetric ellipsoid whose axial lengths are 83.4 × 408.3 × 1540 Å.

The final model is that proposed by Pytkowicz and O'Konski who performed the original experiment. Their interpretation is in agreement with Ridgeway's case two, with respect to mixtures of particles contributing to the transient birefringence. The mixture corresponds to whole monomer and its end-to-end dimer of lengths 850 and 1550 Å, respectively. The diameter of these particles, assumed to be right circular cylinders, was given as 105 Å [19].

Table 1 lists the possible structures for H. pomatia hemocyanin which are consistent with the mixed exponential decay observed by Pytkowicz and O'Konski. Based on this information alone, it is impossible to distinguish which case is more representative of the true structure. In order to make such a distinction, we need to compare the cases listed in table 1 with other measured hydrodynamic parameters. For this purpose, we will consider the hydrodynamic parameters of viscosity and sedimentation velocity, in addition to electron micrographs, and small angle X-ray scattering.

Table 1
Structural models for Helix pomatia hemocyanin consistent with mixed exponential birefringence decay

Case [a)]	Model	Dimensions (Å)
five	two anisotropic spheres	radii 230, 387
two	two isotropic cylinders	850 × 105 1550 × 105
three	one anisotropic spheroid	1362 × 270
four	one asymmetric ellipsoid	1540 × 408.3 × 83.4

[a)] The case number refers to those discussed in the Introduction.

4. Viscosity

An independent value of the axial ratio for an ellipsoid of revolution may be calculated from the viscosity data of Konings et al. [18], by means of the equation relating the intrinsic viscosity to the asymmetry factor ν, for rigid ellipsoids, given by equation (23-4) of Tanford [20]

$$[\eta] = \nu(\bar{v}_2 + \delta_1 v_1^0),$$

where $\bar{v}_2$ denotes the partial specific volume of the solute, δ_1 denotes the hydration factor, and v_1^0 denotes the specific volume of pure solvent. Simha [21] calculated the dependence of the asymmetry factor on the axial ratio for rigid ellipsoids of revolution. Substitution of the values for $[\eta]$ and $\bar{v}_2$ reported by Konings et al. into the above equation, and an assumption of a hydration of 0.5 yields a value of 6.4 for the asymmetry factor for H. pomatia hemocyanin. From a graph of ν versus axial ratio for prolate ellipsoids, taken from the tabulated values given by Cohn and Edsall [22], a value of $\nu = 6.4$ corresponds to an axial ratio 5.4. This is in good agreement with the value 5.04 determined above. If a hydration of 0.6 is assumed, then the axial ratio is determined to be 5.04. This is identical to that calculated for the ellipsoid of revolution based on case three of Ridgeway's theory, which is independent of any assumptions concerning hydration.

5. Sedimentation

The sedimentation coefficient corresponding to the derived equivalent prolate ellipsoid of axial ratio 5.04 may be calculated by combining the Svedberg equation [23]

$$s = M(1 - \bar{v}\rho)/N_0 f \tag{6}$$

and the equation for the ratio of the frictional coefficient of a prolate ellipsoid, f, to that which would be observed for the equivalent sphere, f_0, [20]. The radius of the sphere, r_0, is calculated according to eq. (7),

$$r_0 = \left[\frac{3}{4\pi}\frac{M_h \bar{v}_h}{N_0}\right]^{1/3}, \tag{7}$$

where M_h denotes the molecular weight of the *hydrated particle*, and $\bar{v}_h$ denotes its partial specific volume. These quantities are given as $M_h = M(1 + w)$ and $\bar{v}_h = (\bar{v} + w/\rho)/(1 + w)$. A derivation for the latter relation is given in the appendix. The anhydrous molecular weight for H. pomatia hemocyanin was reported by Brohult [19] to be 8.9×10^6 dalton. The values for $\bar{v}$ and w are those given in the previous section.

For the derived axial ratio, 5.04, the ratio f/f_0 is calculated to be 1.25. This is in agreement with the value reported by Cohn and Edsall [22]. The calculated value for f_0 is 29.3×10^{-8} poise cm, and the corresponding value for f is 36.7×10^{-8} poise cm. Substituting this value for f in the Svedberg equation yields a value for the sedimentation coefficient of 106 svedberg. This is in good agreement with the value 103 svedberg experimentally determined by Brohult [19], as well as the values 104.2 S and 105.7 S, reported by Witters and Lontie [24], for α- and β-hemocyanin, respectively. Thus, under the above assumptions, the prolate equivalent ellipsoid derived according to case three is in agreement with sedimentation velocity data for H. pomatia hemocyanin.

To test the hypothesis of Pytkowicz and O'Konski [11] that H. pomatia hemocyanin at low ionic strengths is no longer homogeneous, but rather is composed of a mixture of monomers and dimers, samples were subjected to sedimentation velocity under solution conditions of low ionic strength. At a concentration of 5 mg/ml in 10^{-4} M sodium phosphate buffer, pH 6.1, H. pomatia hemocyanin sedimented as a single, symmetrical boundary of $s_{20,w} = 100$ S. Under solution conditions which others have demonstrated to yield only monomeric hemocyanin, i.e. sodium

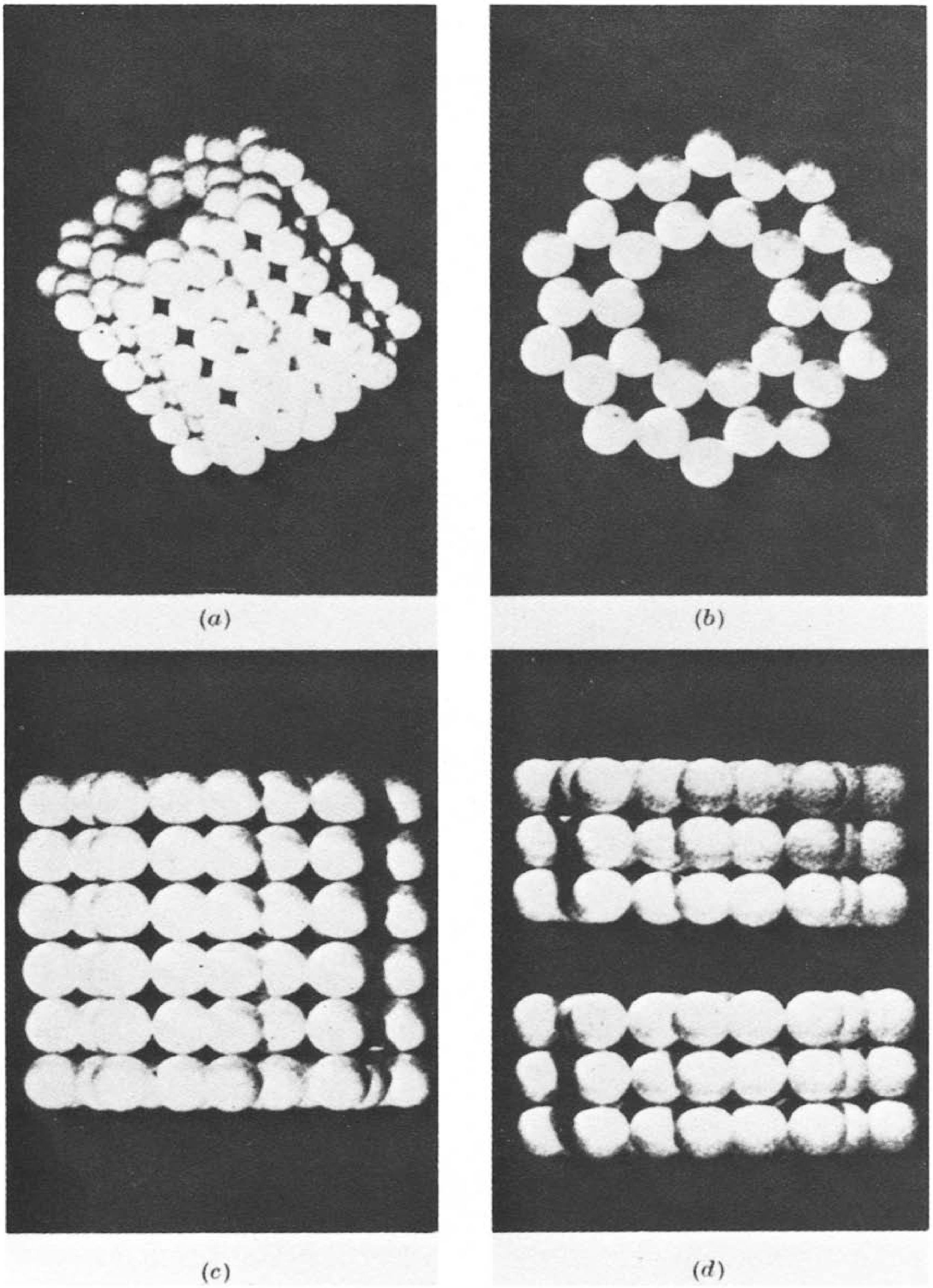

Fig. 2. The Van Bruggen model for Helix pomatia hemocyanin [27] (reprinted with permission of the author and Academic Press, London).

phosphate buffer, pH 6.12, $I = 0.1$, H. pomatia hemocyanin at a concentration of 5 mg/ml again exhibited a single boundary of $s_{20,\mathrm{w}} = 101.3$ S.

These sedimentation velocity results strongly suggest that at low ionic strengths, as were the solution conditions for the birefringence relaxation measurements on H. Pomatia hemocyanin [11], the protein remains as a monodisperse solution of 100 S particles.

This is further substantiated by the low angle X-ray scattering results of Pilz et al. [25] which demonstrated that H. pomatia hemocyanin was a monodisperse solution of particles of molecular weight 8.95×10^6 (100 S) in distilled water.

6. Electron microscopy

Electron micrographs of undissociated H. pomatia hemocyanin of molecular weight 8.9×10^6, have been interpreted as an aggregate of subunits arranged as a right circular cylinder of height 400 Å and diameter ranging from 350 to 400 Å [26]. The cylinder is composed of six layers of circular cross section which show both ten-fold and five-fold symmetry about the axis. Fig. 2 shows a tentative model for H. pomatia hemocyanin, proposed by Van Bruggen [27]. By the symmetry of this model it is possible to specify the mass centers of the individual subunits in terms of cylindrical coordinates. By geometry, the radial coordinates are given by $\rho_1 = 3.236r$, $\rho_2 = 5.236r$, and $\rho_3 = 6.156r$, the angular coordinates increment 18 degrees, and the axial coordinates increment by $2r$, where r denotes the radius of the identical spherical subunits. Given the coordinates of the mass centers, it is possible to calculate the distance between any two subunits, R_{ij}.

According to Kirkwood [28], the translational frictional coefficient for a structure of n subunits is a function of the reciprocal distance between subunits, summed over all values i and j [29]. Thus, according to the model shown in fig. 2, a value for the frictional coefficient of 37.56×10^{-8} poise cm is calculated for subunits of radius 33 Å (a radius of 33 Å would provide the required height of 400 Å for 6 layers). Substituting this value into the Svedberg equation, given by eq. (6), yields a sedimentation coefficient of 104 S. This value is in excellent agreement with the experimentally determined values reported from several laboratories and given in the previous section. The height of the cylinder is 396 Å, and the diameter, given by $2\rho_3$, is 406 Å, for subunits of radius 33 Å. These values also agree with those estimated from electron micrographs.

A recent report by Mellema and Klug [30] using the technique of three-dimensional image reconstruction from electron micrographs reveals a structure for H. pomatia hemocyanin similar to that proposed by Van Bruggen [27]. This report suggests an additional structural element for the molecule, i.e., the subunits of the cylindrical wall are arranged in a helical pattern whose axes coincide with the cylinder axis. The Van Bruggen model can be made to display such helices by the axial rotation through 18° of alternate layers. The calculated sedimentation coefficient for the helical form of the Van Bruggen model is 103.9 S. Obviously, the calculated sedimentation coefficient is insensitive to the differences between the two models.

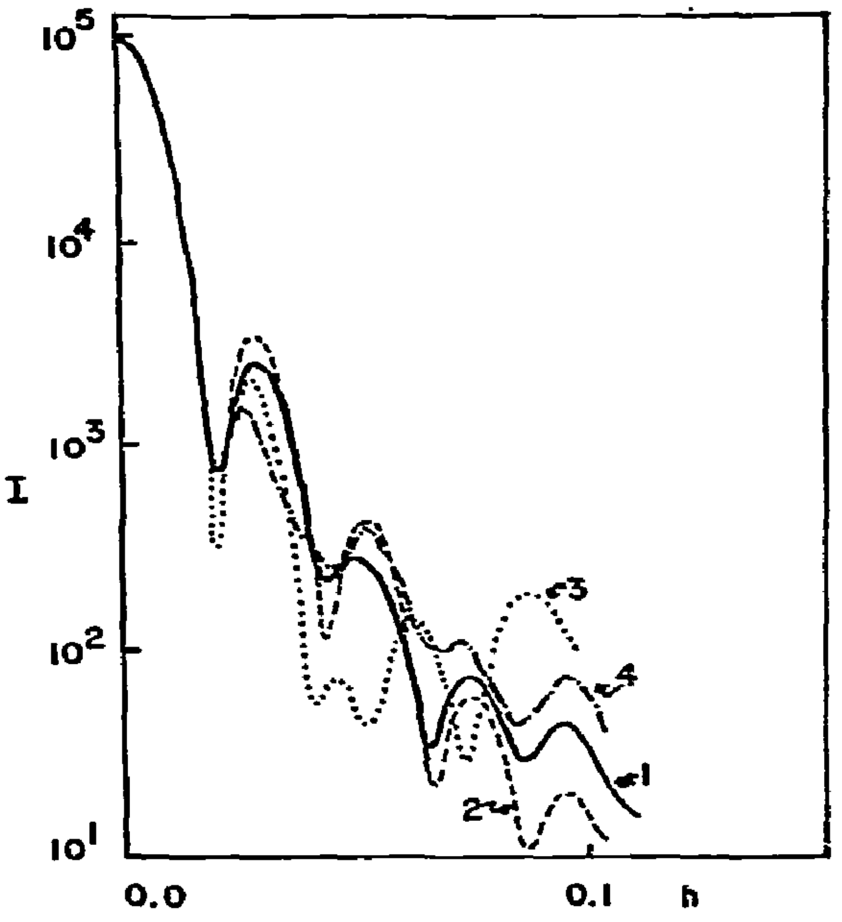

Fig. 3. Experimental scattering curve for H. pomatia hemocyanin (curve 1). Theoretical scattering curves: (2) Pilz et al. model, (3) Van Bruggen model, and (4) helical form of Van Bruggen model. (Curves 1, 2, and 3 reprinted with permission of the authors and Verlag Z. Naturforschung).

7. Small-angle X-ray scattering

Pilz et al. [31] reported small angle X-ray scattering data for monodisperse, 100 S, H. pomatia hemocyanin. Additionally, they calculated the theoretical scattering curve for the Van Bruggen model shown in fig. 2, as well as for a model they propose. The Pilz et al. model is a right circular cylinder composed of 360 subunits, in contrast to the 180 subunits of the Van Bruggen model. The overall dimensions of the Pilz model agree with those of the Van Bruggen model. Their data and calculated curves are shown in fig. 3.

In order to account for the helical form reported by Mellema and Klug [30], we have calculated the theoretical scattering curve for the helical form of the Van Bruggen model discussed in the previous section, according to eq. (8)

$$I = P(h) \sum_i \sum_j (\sin hR_{ij})/hR_{ij}. \qquad (8)$$

$P(h)$ is the scattering function for uniform spheres of fixed radius, and is given by eq. (3) of Anderegg et al. [32]. R_{ij} has been previously defined, and h is the usual function of the Bragg angle. The results of the calculation are shown as curve 4 of fig. 3.

Pilz et al. [31], and Pilz [33] have used the lack of fit of curve 3 to rule out the Van Bruggen model in favor of their double layer model of 360 subunits, which gives a reasonable fit to the data, as seen from curve 2. However, curve 4 shows an equally reasonable fit to the data. Therefore, small angle X-ray scattering also shows agreement with the previously discussed experimental results in support of the helical form of the Van Bruggen model.

8. Discussion

All cases for which theory exists describing transient birefringence for protein solutions in terms of model particles have been applied to the observed relaxation times for Helix pomatia hemocyanin. In the absence of additional information concerning the structure of this protein, the four different models, shown in table 1, are all equally likely. However, given the additional information yielded by sedimentation velocity, viscosity, electrom microscopy, three-dimensional image reconstruction, and small angle X-ray scattering, only one model satisfied all experimental observations. This model is the hydrodynamic equivalent prolate ellipsoid of axial ratio 5.04. This model accounts for the observed intrinsic viscosity, and sedimentation coefficient, as well as the observation that the protein solution is monodisperse under the conditions for which the birefringence relaxation times were observed. The model possesses cylindrical symmetry about the long axis, in agreement with the symmetry observed in electron micrographs of this protein. Such symmetry is not in agreement with the asymmetry intrinsic to the model of case four.

A theoretical calculation of the sedimentation coefficient for a model particle composed of identical spherical subunits is in agreement with the experimentally observed value, which supports the actual structural model of a right circular cylinder. The fact that the length of the hydrodynamic equivalent ellipsoid is four times as great as the height of the actual cylindrical molecule may be a result of the effect of the blunt ends of the cylinder on the hydrodynamic stream lines. The net effect would be to make the particle appear, hydrodynamically, more asymmetric.

Such an effect has been considered by Broersma [34] who states that contributions due to sharp edges tend to be underestimated, and that at points of large curvature the velocity gradient is large so that dissipation can be considerable.

For purposes of comparing the two structures, the translational friction coefficient for the ellipsoid is compared to that for a right circular cylinder of the dimensions given above. The frictional coefficients for the cylinder are calculated according to:
(a) Kirkwood's theory [28], (b) the cylindrical shell model of Bloomfield et al. [35], and (c) the cylindrical model of Broersma [36,37]. The frictional coefficient for Broersma's model is based on the distinct frictional coefficients for a cylinder whose height and diameter are 400 Å (this calculation was done by Broersma [37]). The results are: $f_{\text{ellipsoid}} =$ 36.7×10^{-8} poise cm, $f_{\text{Kirkwood theory}} =$ 37.6×10^{-8} poise cm, $f_{\text{Broersma eq.}} = 42.9 \times$ 10^{-8} poise cm, $f_{\text{cylindrical shell}} = 32.7 \times 10^{-8}$ poise cm. It should be noted that the calculation based on the cylindrical shell model was based on eq. (39) of ref. [35], which involved an approximation of first order in b/a. This approximation may not be valid for $b/a = 1$. The agreement of the calculated frictional coefficient for the equivalent ellipsoid and frictional coefficients for short cylinders calculated by three different theories is taken as support for the equivalent ellipsoid model of the right circular cylinder for H. pomatia hemocyanin.

From the considerations discussed above, two points of interest arise: first, the actual protein configuration cannot be inferred from the dimensions of the hydrodynamic equivalent ellipsoid as was stated by Scheraga and Mandelkern [17], and second, the calculation of the Stokes radius for a dissolved protein is most meaningful when based on the hydrated molecular weight and hydrated partial specific volume (the relation for the hydrated partial specific volume has been rederived in the appendix, according to the scheme used by Yang [28]). Based on these two points, it is possible to state that the dimensions of the equivalent ellipsoid do not represent those of the actual protein configuration when the volume of the equivalent ellipsoid differs from that for the Stokes sphere whose radius is calculated according to eq. (7). If these two volumes agree, then the particles may coincide, but not necessarily so.

In conclusion, we have demonstrated that the transient birefringence results obtained for H. pomatia hemocyanin by Pytkowicz and O'Konski [11] are consistent with an equivalent prolate ellipsoid whose predicted hydrodynamic properties agree with experimentally determined values for both the sedimentation coefficient and intrinsic viscosity. Additionally, the hydrodynamic model is supportive of the model proposed from electron microscopy.

Acknowledgement

The authors express their appreciation to Dr. Marc De Ley, Laboratorium voor Biochemie, Katholieke Universiteit Leuven, for his kind gift of Helix pomatia hemocyanin, to Dr. John W. Anderegg, Physics Department, University of Wisconsin, for helpful discussions concerning calculation of the small angle X-ray scattering curve, and to Bonnie Dumas and Bobby Poole for help with the computer programming.

Appendix: Hydrated partial specific volume

Yang [38] considered the problem of hydration in a protein and water mixture. Mathematically, he considered the volume, V, of the solution as a function of the concentrations of dry solute, m, and solvent, m_0; the latter being the sum of free solvent, m_0', and bound solvent, m_0''. Thus, $V = V(m, m_0) = V(m, m_0' + m_0'')$. Yang considered the differential relation $dm_0'' = w\,dm$ where w is the grams of solvent bound to 1 gm of solute. By partial differentiation Yang observed

$$(\partial V/\partial m)_{m_0'} = \bar{v} + w/\rho,$$

which he identified with the partial specific volume of the hydrated protein. The identification of the above partial derivative with the partial specific volume of the hydrated protein is not rigorous, and forms the basis for the present derivation.

In an analogous manner, consider the solution as a function of the concentrations of hydrated solute, m_h, and free solvent, m_0', where the total solvent is given as the sum of free and bound solvent, $m_0 = m_0' + m_0''$. Thus, $V = V(m_h, m_0') = V(m + m_0'', m_0')$. By partial differentiation,

$$dV = \left(\frac{\partial V}{\partial m_h}\right)_{m_0'} dm_h + \left(\frac{\partial V}{\partial m_0'}\right)_{m_h} dm_0'.$$

Let $dm_0'' = w\,dm$, and since $dm_h = dm + dm_0''$, we have $dm_h = (1+w)\,dm$. Therefore,

$$dV = (1+w)\left(\frac{\partial V}{\partial m_h}\right)_{m_0'} dm + \left(\frac{\partial V}{\partial m_0'}\right)_{m_h} dm_0'.$$

At constant m_0', this equation reduces to:

$$\left(\frac{\partial V}{\partial m}\right)_{m_0'} = (1+w)\left(\frac{\partial V}{\partial m_h}\right)_{m_0'}$$

We note that the partial derivative on the right is by definition the partial specific volume of the hydrated protein. The partial derivative on the left of the equation is identical to that obtained by Yang, and therefore the two derivatives may be equated. Thus, we obtain the value for the hydrated partial specific volume:

$$\bar{v}_h = (\bar{v} + w/\rho)/(1+w).$$

This value for the partial specific volume of the hydrated protein differs from Yang's by the factor in the denominator but is identical to that obtained earlier by a different argument by Lansing and Kraemer [39].

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