On the Estimation of Molecular Dimensions and Shape of Rigid, Asymmetric Macromolecules from Hydrodynamic Measurements*

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ABSTRACT: New equations are presented relating the major axis lengths of both the rigid, prolate ellipsoid and rigid, linear spherical-bead models to the product of the intrinsic viscosity and intrinsic sedimentation coefficients (for axial ratios greater than 20). These hydrodynamically estimated model lengths are compared with the lengths of the same models determined from the light-scattering radius of gyration. It is shown that such a comparison is capable of distinguishing clearly between the two models. Comparison of the light-scattering and hydrodynamically estimated lengths and molecular weights of paramyosin, light meromyosin, tropomyosin B, and tobacco mosaic virus (TMV) shows agreement for the spherical-bead model estimates. Thus, the spherical-bead model hydrodynamic equations apparently g'ves valid results when used to estimate the lengths and molecular weights of rigid, helical macromolecules.

The utility of hydrodynamic methods for the determination of molecular weight and dimension of macromolecules needs no extended comment. Such methods are, of course, of particular importance in the macromolecular size range where other methods are either lacking or relatively unavailable. For example, in the case of rigid, asymmetric particles greater than 3000 A in size, the extrapolation of light-scattering data to zero angle from measurements in the angular range 25–140° cannot be expected to determine a meaningful molecular weight or size (Casassa, 1956; Rice *et al.*, 1964). The alternatives to hydrodynamic methods in such cases are, depending upon the size range, either low-angle light scattering or low-angle X-ray scattering. In either case, neither the equipment nor methodology are, as yet, widely disseminated.

In assaying the general applicability of the hydrodynamic methods for the determination of molecular size and weight of rigid, asymmetric macromolecules, combinations of intrinsic viscosity and sedimentation coefficient or of intrinsic viscosity and rotary diffusion coefficient are of particular interest. We shall be concerned, in this paper, with the former pair of hydrodynamic variables.

Thus far, two models of rigid, asymmetric macromolecules have been used for molecular weight estimates. The unsolvated molecular weight of the ellipsoidal model has been estimated through the use of the relationship proposed by Scheraga and Mandelkern (1953). The molecular weight of the rigid, linear array of spherical-bead models has been estimated by means of a relationship, analogous to that of Scheraga and Mandelkern (1953), proposed by Holtzer and Lowey (1963). The molecular weights of the two hydrodynamic models differ significantly for given values of $[\eta]$ and s. The comparison of the ellipsoidal and spherical-bead model molecular weights of several rigid, asymmetric macromolecules with molecular weights obtained from absolute methods such as light scattering led to the conclusion that misleading molecular weights might result if the model shape does not conform closely to that of the molecule itself (Holtzer and Lowey, 1963). These results gave some indication that the spherical-bead model might serve as a reasonable hydrodynamic model for rigid, helical macromolecules.

It is the particular purpose of this paper to compare the hydrodynamically estimated dimensions of rigid, asymmetric macromolecules based on both the spherical-bead and prolate ellipsoid models with the dimensions for the same models obtained from light scattering. The prolate ellipsoid length obtained from a given value of the light-scattering radius of gyration differs significantly from the spherical-bead or rigid rod length. New relationships among $[\eta]$, s, and the lengths of the spherical-bead and ellipsoidal models are derived which permit this comparison to be made. The correspondence of the hydrodynamically estimated lengths and molecular weights with the light-scattering values for a given model would lend confidence in the general validity of the hydrodynamic relationships for that model. Thus the more general purpose of this paper would be served.

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Comparison of the Hydrodynamically Estimated Lengths of the Major Axes of the Prolate Ellipsoid and Spherical Bead Models with Corresponding Light-Scattering Lengths

The Kirkwood–Auer (1951) equation for the intrinsic viscosity of a rigid, spherical-bead model of a macromolecule of axial ratio J_{SB} is

$$[\eta] = \frac{24}{9000\rho}\left(\frac{J_{SB}^2}{\ln J_{SB}}\right) \tag{1}$$

where ρ is the density (g/cc) of the macromolecule and $[\eta]$ is in dl/g. The Kirkwood–Riseman (1950) equation for the intrinsic sedimentation coefficient for the same case is

$$[s] = \frac{\rho(1 - \bar{v}\rho_0)d_{SB}^2 \ln J_{SB}}{18\eta_0} \tag{2}$$

where η_0 and ρ_0 are, respectively, the viscosity and density of the medium, d_{SB} is the diameter of a bead, and $\bar{v}$ is the partial specific volume. Combining eq 1 and 2 gives

$$L_{SB}^2 = \frac{6750\eta_0[\eta][s]}{(1 - \bar{v}\rho_0)} \tag{3}$$

It must be emphasized at this point that eq 1 and 2 were originally derived for the case where the axial ratios are large (*i.e.*, greater than 20). Therefore, it would not be expected that eq 3 would hold at axial ratios smaller than 20.

The analogous equations for the rigid, prolate ellipsoid model are

$$[\eta] = \frac{\nu_E}{100\rho} \tag{4}$$

where ν_E is Simha's (1940) viscosity increment, a function of axial ratio only, and

$$[s] = \frac{\rho(1 - \bar{v}\rho_0)d_E^2 \ln (2J_E)}{18\eta_0} \tag{5}$$

All the symbols in eq 5 have the corresponding significance as those given in eq 2, d_E being the minor axis. Equation 5 was derived by Peacocke and Schachman (1954) for prolate ellipsoids of large axial ratio. Combining eq 4 and 5 gives

$$\frac{1800[\eta][s]\eta_0}{(1 - \bar{v}\rho_0)} = L_E^2\left[\frac{\ln (2J_E)\nu_E}{J_E^2}\right] \tag{6}$$

The function in brackets on the right-hand side of eq 6 is a function of axial ratio only, and therefore could be used to define a parameter, ϵ_E, in the usual way. However, since $(\ln (2J_E))/(J_E^{2/3}) = F_E$, the reciprocal of the translational friction ratio (Perrin, 1936) for the case where the axial ratios of prolate ellipsoids are large, ϵ_E may be defined more generally for prolate ellipsoids of any axial ratio by

$$\epsilon_E = \frac{F_E\nu_E}{J_E^{4/3}} \tag{7}$$

which reduces appropriately for very asymmetric ellipsoids. Thus, for a prolate ellipsoid of any axial ratio

$$L_E^2 = \frac{1800[\eta][s]\eta_0}{\epsilon_E(1 - \bar{v}\rho_0)} \tag{8}$$

Values of ϵ_E *vs.* J_E are tabulated in Table I. Equations 3 and 8 are new relationships for the estimation of length from hydrodynamic variables.

TABLE I: ϵ_E as a Function of J_E for Prolate Ellipsoids of Revolution.

J_E	ϵ_E	J_E	ϵ_E
1	2.500	20	0.3556
2	1.105	25	0.3458
3	0.7661	30	0.3393
4	0.6215	40	0.3308
5	0.5433	50	0.3258
6	0.4956	60	0.3222
8	0.4406	80	0.3176
10	0.4120	100	0.3145
12	0.3910	200	0.3075
15	0.3736	300	0.3042

The estimation of the length of a macromolecule in terms of the spherical-bead model using eq 3 requires no prior estimates of the axial ratio (other than that it is larger than 20). To estimate the length of a macromolecule in terms of the prolate ellipsoid model, it is necessary to have a prior estimate of the axial ratio in order to obtain a value for ϵ_E in eq 8. Yang (1961a,b) has shown the magnitude of the errors in length produced as a result of errors in axial ratio over a wide range of axial ratios. The axial ratios are frequently obtained from values of the intrinsic viscosity and density for the macromolecule. In many instances, $\bar{v}$, the apparent partial specific volume in the medium, has been used in place of the reciprocal of the anhydrous density. Scheraga (1961) has reviewed the dangers in using $\bar{v}$ in this way. In the case of proteins, the reported values of $\bar{v}$ have often been found to agree with estimates obtained from amino acid composition and the apparent specific volumes of amino acid residues (Schachman 1957). If one assumes an error in the particle density of 50%, and computes the error in the length, L_E, of the ellipsoidal model using Table I, the error is 5% at axial ratio 15 and 0.8% at axial ratio 100.

TABLE II: Comparison of Light-Scattering and Hydrodynamically Estimated Lengths of Various Asymmetric Macromolecules.

Macromolecule	$(L_{SB})_H$ (A)	$(L)_{LS}$ (A)	$(L_E)_H$ (A)	Axial Ratio
Tropocollagen	3070	L_R = 3000[a]		180
		L_E = 3870	2860	
Light meromyosin (Fr. I)	820	L_R = 769[b]		
		L_E = 993	735	40
Paramyosin	1220	L_R = 1330[c]		65
		L_E = 1715	1110	
Tropomyosin B	483	L_R = 490[d]		25
		L_E = 632	424	
Heavy meromyosin	940	L_R = 575[b]		28
		L_E = 740	830	
TMV I	4150		3630	24
		L_R = 3200[e]		
TMV II	4020		3515	24
		L_E = 4130		
TMV III	3450		2980	20

[a] Nishihara and Doty (1958). [b] Lowey and Cohen (1962). [c] Lowey *et al.* (1963). [d] Holtzer *et al.* (1965). [e] Boedtker and Simmons (1958).

In the appendix, the length of the spherical-bead model, in terms of the light-scattering radius of gyration $[R_g]_{SB}$, is derived.

$$[R]_{SB} = \frac{L_{SB}}{\sqrt{12}}\left[1 + \frac{4}{5J_{SB}^2}\right]^{1/2}$$

The radius of gyration of the prolate ellipsoid is given by

$$[R_g]_E = \frac{L_E}{\sqrt{20}}\left(1 + \frac{2}{J_E^2}\right)^{1/2}$$

(Geiduschek and Holtzer, 1958). At axial ratio 20, these radii of gyration are within less than 0.3% of their asymptotic values at high axial ratios.

Comparing the spherical-bead and ellipsoidal lengths derived from a light-scattering radius of gyration, we see that $(L_{SB}/L_E)_{LS} = (3/5)^{1/2} = 0.775$. Comparing the same dimensions using eq 3 and 8, $(L_{SB}/L_E)_H = (3.75\epsilon_E)^{1/2}$. Referring to Table I, it is clear that $(L_{SB}/L_E)_H = 1.18$ at axial ratio 15, and 1.07 at axial ratio 300. The subscripts LS and H stand for the terms light scattering and hydrodynamic, respectively. Thus, if a hydrodynamically estimated spherical-bead model dimension is equal to the light-scattering rigid rod dimension, for example, then the ratio of the corresponding light-scattering ellipsoid length, $(L_E)_{LS}$, to the hydrodynamically estimated ellipsoid length, $(L_E)_H$, will be $(L_E)_{LS}/(L_E)_H = 1.52$ at axial ratio 15, and 1.38 at axial ratio 300. In view of the above discussion of the magnitude of the errors to be expected as a result of an error in density (or axial ratio), it is clear that a comparison of light-scattering and hydrodynamically estimated dimensions can adequately serve to distinguish the prolate ellipsoid from the spherical-bead model. In Table II, the light-scattering and hydrodynamically estimated lengths of various asymmetric macromolecules are compared. The results of Table II indicate that the spherical-bead model lengths are in reasonable accord with the light-scattering rod lengths in the case of tropocollagen, tropomyosin B, paramyosin, light meromyosin (Fr. I), and tobacco mosaic virus (TMV) III data. In the case of heavy meromyosin, the hydrodynamically estimated ellipsoid model length appears to be in accord with the light-scattering ellipsoid length. The TMV I data are based upon $[\eta]$ = 0.367 dl/g and $s_{20,w}$ = 188 S (Boedtker and Simmons, 1958); the TMV II data are based upon $[\eta]$ = 0.367 dl/g and $s_{20,w}$ = 176 S (Hearst and Vinograd, 1961); the TMV III data are based upon the set of hydrodynamic values, $[\eta]$ = 0.27 dl/g (Schachman and Kauzmann, 1949) and $s_{20,w}$ = 176 S. The reasons for bringing in the various sets of hydrodynamic data for TMV will be given in the discussion section. The values of $[\eta]$ and s for tropocollagen, light meromyosin (Fr. I), heavy meromyosin, and paramyosin are the same as those used by Holtzer and Lowey (1963). The references to the original papers from which those values were taken are given in Holtzer and Lowey's (1963) Table II. The values $[\eta]$ = 0.35 dl/g, $s_{20,w}$ = 2.59 S, and $\bar{v}$ = 0.739 used for the estimation of the spherical-bead and ellipsoid model lengths of tropomyosin B are from Holtzer *et al.* (1965).

Discussion

When the results of our Table II are compared with the molecular weight results of Holtzer and Lowey (1963) (see their Table II), the agreement between hydrodynamic and light-scattering length results for the spherical-bead model parallels their hydrodynamic and light-scattering molecular weight results in the case of light meromyosin and paramyosin. Their hydrodynamic estimate of the prolate ellipsoid molecular weight for heavy meromyosin is in accord with the absolute molecular weight. This parallels our length results for heavy meromyosin. In the case of tropomyosin B, both the length and molecular weight results favor the spherical bead model for this apparently rigid, α-helical macromolecule.

The experimental findings of Rice *et al.* (1964) on tropocollagen and their discussion of the comparison of the light-scattering, electron microscope, and hydrodynamic data makes further comment unnecessary here. Their results do not lend confidence to any attempt to use tropocollagen as a rigid, asymmetric macromolecular species with which to test the validity of either the rigid spherical bead or ellipsoid model hydrodynamic equations.

The TMV results of Table II must be examined in the context of an explanation for the apparent dependence of the sedimentation coefficient of TMV on rotor speed (Hearst and Vinograd, 1961). Rosenbloom and Schumaker (1963, 1965) have recently provided an explanation for the apparent dependence of the sedimentation coefficient of high molecular weight deoxyribonucleic acid (DNA) on rotor speed in terms of a speed-dependent aggregation process. They find aggregation to be sharply dependent upon molecular weight from 25,000,000 to 130,000,000 daltons. In view of the known tendency of TMV to aggregate, it would appear that Rosenbloom and Schumaker's conclusions with regard to DNA might be extended to TMV as an explanation for the observations of Hearst and Vinograd (1961).

If this is accepted, it would appear that the TMV II and TMV III data are the relevant sets for further discussion. In these two sets, the 176S sedimentation coefficient, obtained from experiments at lower rotor speeds than the 188S value of the TMV I data, was used to compute the results reported in Table II.

The hydrodynamically estimated ellipsoid length from the TMV III data in Table II is, at first glance, in excellent agreement with the number-average length from the electron microscope results of Hall (1958) and Williams and Steere (1951). It is in equally good agreement with the length computed from the rotary diffusion coefficient of TMV (Haltner and O'Konski, 1956) on the assumption of a cylindrical shape for TMV (Haltner and Zimm, 1959). The comparison of the hydrodynamic ellipsoid length for TMV with the light-scattering ellipsoid length does not permit the easy conclusion that the assumption of a correct shape is basically irrelevant so long as the lengths computed from different experiments are in agreement. The spherical-bead length, on the other hand, is seen to be in reasonable agreement with the light-scattering rod length. The ellipsoid and spherical-bead molecular weights are 29.9 and 42.2 $\times$ 10^6, respectively, compared with the 39 $\times$ 10^6 light-scattering molecular weight of Boedtker and Simmons (1958).

Neither the lengths nor the molecular weights of either model are in as good agreement when the TMV II data are used for the calculations. If one uses the TMV I data (ignoring the findings with regard to the rotor speed dependence of the sedimentation coefficient), the molecular weights for the spherical-bead and ellipsoid models are 51.4 and 37.1 $\times$ 10^6, respectively. In the absence of the Haltner–Zimm finding that a cylindrical model was the preferred shape for TMV, one might be tempted to draw the conclusion that a prolate ellipsoid might be suitable shape for TMV if one considered only the molecular weight results from the TMV I data. The comparison of the hydrodynamic and light-scattering ellipsoid lengths would not lend assurance to such a conclusion. It becomes apparent from the consideration of the TMV data that both length and molecular weight comparisons should be made before drawing conclusions with regard to shape.

It is clear that the degree of correspondence between light-scattering data and hydrodynamic estimates will depend on considerations with regard to polydispersity of length and molecular weight, charge, and solvent interactions. Yang (1961a) has reviewed the effect of polydispersity of molecular weight and length and the effect of charge on the intrinsic viscosity. The effect of polydispersity of molecular weight and dimension on sedimentation velocity has been reviewed by Schachman (1959). The effect of charge on the sedimentation velocity of macromolecules has been considered by Pedersen (1958), Schachman (1959), and Alexandrowicz and Daniel (1963). The different values for the intrinsic viscosity of TMV reported by Schachman and Kauzmann (1949) and Boedtker and Simmons (1958) as expressed in the differences between the TMV II and III results must be couched in terms of the above mentioned considerations.

The results of Table II of this paper taken in conjunction with those of Holtzer and Lowey (1963) and Holtzer *et al.* (1965) suggest that the prolate ellipsoid and spherical-bead models of large axial ratio are not hydrodynamically equivalent. They also suggest that the spherical-bead model hydrodynamic relationships provide valid estimates of length and molecular weight for rigid, helical macromolecules of large axial ratio.

Appendix

The Light-Scattering Radius of Gyration of a Rigid String of Beads

The light-scattering radius of gyration is given by

$$R_g^2 = \frac{1}{NV} \sum_i [\int R^2 \mathrm{d}v]_i \qquad (1)$$

where R is the distance from volume element, $\mathrm{d}v$, of the ith bead to the centroid of the molecule; V is the

total volume of a bead; N is the total number of beads; the integration is carried over the volume of each bead, and the summation is over all the beads.

First consider the problem for a molecule containing an odd number of beads. The molecular center of gravity is at the center of the $i = 0$ bead. Placing our origin of coordinates at the center of bead number i, a semicircle of radius Y ($<r$, where r is the bead radius) is drawn on a plane parallel to the yz plane from the point $y = 0$, $z = y$ to the point $y = 0$, $z = -Y$. Another semicircle, drawn with radius $Y + dY$ then defines a half-ring of area $\pi Y dY$; if the half-ring is extended in the x direction by an amount dx, the result is an "half-washer" of volume $dv = \pi Y dY dx$. This volume element is at a distance $R = [(id + x)^2 + Y^2]^{1/2}$ from the centroid, where d is the bead diameter.

Equation 1 can be simplified by recognizing that the contribution to the sum of the $(N - 1)/2$ beads to the left of the central one must equal that of those to the right, and that the central bead must contribute an amount equal to the well-known radius of gyration of a sphere ($3r^2/5$). Thus

$$NR_g^2 = \frac{\int_{\text{central bead}} R^2 dv}{V} + 2\sum_{i=1}^{(N-1)/2}\left[\frac{\int R^2 dv}{V}\right]_i$$

or

$$NR_g^2 = \frac{3r^2}{5} + 2\sum_{i=1}^{(N-1)/2}\left[\frac{\int R^2 dv}{V}\right]_i \qquad (2)$$

The integral in (2) is evaluated as

$$\left[\frac{\int R^2 dv}{V}\right]_i = \frac{2\pi \int_x^r = -r\left[\int_{y=0}^{\sqrt{r^2-x^2}} [(id + x)^2 + Y^2] Y dY\right] dx}{(4\pi r^3/3)}$$

the factor 2 converting integration over the half-washer to integration over the full washer.

The double integration is readily carried out, the result being

$$\left[\frac{\int R^2 dv}{V}\right]_i = i^2 d^2 + \left(\frac{3r^2}{5}\right) \qquad (3)$$

Substituting (3) into (2) gives

$$NR_g^2 = (3r^2/5) + (N - 1)(3r^2/5) + 2d^2 \sum_{i=1}^{(N-1)/2} i^2 \qquad (4)$$

$$\sum_{i=1}^{(N-1)/2} i^2 = \frac{N(N - 1)(N + 1)}{24} \qquad (5)$$

so eq 4 becomes

$$NR_g^2 = N(3d^2/20) + N(N - 1)(N + 1)d^2/12) \qquad (6)$$

Since the length of the rigid array of beads, L, $= Nd$ and since the axial ratio is given by $J = L/d = N$ one obtains, finally

$$R_g^2 = L^2\left[\frac{1}{12} + \frac{1}{15J^2}\right] \qquad (7)$$

The result for an even number of beads is the same. When $J = 1$, eq 7 reduces to ($3r^2/5$), the value expected for a sphere; when $J \rightarrow \infty$, $R_g^2 = L^2/12$, the value for an infinitely thin rod, as expected. The rapidity with which eq 7 approaches its asymptotic value as J increases is of interest. It is seen from eq 7 that at axial ratio 20 and more, the "J^2" term within the brackets makes a contribution of 0.2% or less to the total value of the sum of the terms in the brackets. The hydrodynamic relationships for the spherical-bead model are valid only when $J > 20$. Therefore, the radius of gyration of the infinitely thin rod may be used for the spherical-bead model in all instances where the hydrodynamic relationships are valid.

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On the Similarity of Plant and Animal Histones*

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ABSTRACT: Histones prepared by acid extraction of purified chromatin from buds of pea seedlings and from calf thymus were fractionated by column chromatography on Amberlite CG-50, using a gradient of guanidinium chloride. Resulting histone fractions were further characterized by electrophoresis in polyacrylamide gel and by determination of amino acid composition and N-terminal amino acids. Striking similarities were noted between pea bud and calf thymus histones by every criterion of characterization employed.

Histones are basic proteins associated with deoxyribonucleic acid (DNA). They have been found in a wide variety of plant and animal phyla. Structural organization and stabilization of the genetic material (Zubay, 1964), regulation of DNA synthesis (Irvin *et al.*, 1963), and control of gene expression (Stedman and Stedman, 1950; Huang and Bonner, 1962) are among the many functions which have been suggested for histones. Although there have been numerous chemical studies of the histones of vertebrates (Murray, 1965; Phillips, 1962), histones of other organisms have been studied almost exclusively by histological methods (Das *et al.*, 1964; Bloch, 1962; Rasch and Woodward, 1959). Thus little is known about the properties of histones from organisms other than vertebrates. The fractionation and further characterization of pea bud histones, reported in this paper, make possible for the first time a comparison of the histones of very distantly related organisms. Such a comparison suggests answers to the questions: (a) Do the histones of all organisms have a common origin in evolution? (b) Do the histones of all organisms perform the same functions? (c) What chemical and physical properties of histones are essential to histone function?

Methods

Preparation of Chromatin. For the preparation of histones minimally contaminated by nonchromosomal protein it is necessary to use purified chromatin as the starting material for histone extraction. For the preparation of pea bud chromatin approximately 5 kg of pea seeds was soaked overnight in water, planted in vermiculite, and germinated in the dark for 4 days at 25°. The apical buds (approximately 1 cm of stem plus bud) were than harvested to yield about 600 g of fresh weight of buds. The buds were homogenized with approximately 1 l. of grinding medium (0.25 M sucrose–0.05 M Tris buffer, pH 8.0–0.001 M $MgCl_2$) for 1.5 min at 100 v in a Waring blender. This and all subsequent steps were performed at 0–5°. The homogenate was filtered through four layers of cheesecloth and then through two layers of Miracloth (Chicopee Manufg. Co., Miltown, N. J.). The filtrate was next centrifuged at 4000*g* for 30 min. The soft pellets were scraped from the underlying layers of starch, suspended in 300 ml of grinding medium, and centrifuged at 10,000·*g* for 20 min. The pellets were again separated from the starch, suspended in 300 ml of 0.05 M Tris buffer (pH 8.0), and centrifuged at 10,000*g* for 20 min. This step was twice repeated. The resulting pellets of crude chromatin were suspended in a total of 30 ml of 0.01 M Tris buffer (pH 8.0), homogenized with a Potter–Elvehjem homogenizer (about 20 strokes), and layered in 5-ml portions on 25-ml aliquots of 1.7 M sucrose in cellulose nitrate tubes. The upper two-thirds of the contents of each tube was stirred to form a rough gradient. The tubes were then centrifuged at 22,000 rpm for 3 hr in the SW-25 Spinco rotor. The resulting gelatinous pellets (purified chromatin) were suspended in 0.01 M Tris buffer and dialyzed against 100 volumes of the same buffer overnight. Recovery of DNA from the tissue homogenate was 70–80%.

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