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# TRANSPORT PROPERTIES OF RIGID, SYMMETRICAL OLIGOMERIC STRUCTURES COMPOSED OF PROLATE, ELLIPSOIDAL SUBUNITS

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We have calculated translational and rotational diffusion coefficients and intrinsic viscosities of oligomeric structures composed of $n$ identical subunits having a prolate ellipsoidal shape with axial ratio $p$. Results are presented for $p = 1$–$6$ for a variety of structures with $n = 1$–$6$. We compare our results with those obtained by a different modeling procedure, proposed by other workers, in which the monomeric subunit is represented as a string of touching, colinear spheres. If $n$ and an estimate of $p$ are known, the structure of the oligomer can be, in most cases, unambiguously determined by comparison of the experimental oligomer-to-monomer ratios of a given property with the numerical results of this work. As examples of the applicability of our results, we examine the relationship between structure and properties for neurophysin, bovine serum albumin, hemoglobin and phycocyanin.

## 1. Introduction

An important problem in biophysical chemistry is the determination of the structure of macromolecular complexes composed of a few subunits. Oligomeric proteins [1,2], in which a moderate number of subunits are spatially arranged in a quite specific manner, are good examples of these complexes. Hydrodynamic properties, such as the translational and rotational diffusion coefficients and the intrinsic viscosity, are sensitive to a lesser or greater extent, to the details of macromolecular size and shape, and therefore they are potentially useful in the structural determination of oligomeric complexes. An additional advantage of hydrodynamic properties is that, unlike electron microscopy or X-ray diffraction, they can be experimentally measured under conditions close to the physiological ones.

The relationship between the geometry of oligomeric structures and their hydrodynamic properties has been studied by several workers. The particular case of structures composed of identical, spherical subunits has been treated extensively by Bloomfield, Garcia de la Torre and their co-workers [3–5] and also by Swanson et al. [6]. The most recent work [5] presents results obtained using the latest advances in hydrodynamic theory [7]. We believe that those results are rather accurate and can be reliably applied in structural determination insofar as the subunits are very nearly spherical. Unfortunately, in many cases, the subunits composing typical oligomeric proteins deviate appreciably from spherical shape. This complicates the problem because one has to consider one or more additional geometric magnitudes (for instance, the axial ratio of the subunits) and a greater variety of possible conformations.

Anyhow, the analysis of hydrodynamic properties can still cope with such complex situations. As

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an illustration, let us comment on the simplest cases of the translational friction coefficients, $f_t$, of dimers. For spherical subunits, the ratio $f_t$(dimer)/$f_t$(monomer) = 1.39 [5]. On the other hand, for rather elongated, approximately rod-like subunits the ratio must be close to 2 for an end-to-end dimer and close to 1 for a side-by-side dimer. (This is so because $f_t$ for elongated particles has an approximately linear dependence on total length while the dependence on the size of the cross-section is much less pronounced.) The sensitivity of the rotational coefficients and intrinsic viscosities to changes in the geometry of the complex and its subunits is even greater.

According to the above paragraphs it seems worthwhile to undertake the calculation of hydrodynamic properties of oligomeric structures composed of nonspherical subunits. Andrews and Jeffrey [8–10] have already evaluated a single property, namely, the translational coefficient, of such structures. In their work, they model the monomeric subunit as an array of spherical beads. Thus, a prolate subunit of axial ratio 2 is represented by two touching beads. However, a truly ellipsoidal shape for the subunits would be more realistic. Furthermore, if the geometry of the monomer is obtained from hydrodynamic properties in terms of ellipsoidal models, using the equations of Perrin and Shima, then for consistency the monomer should be ellipsoidal in the model calculations for the plausible oligomers.

In this paper we present the results of calculations of three types of hydrodynamic properties of oligomers composed of identical, prolate ellipsoidal subunits of varying axial ratio. We utilize the experience gained in previous works [11–13] on the hydrodynamic modeling of prolate ellipsoids. A similar study for oblate subunits would pose some modeling difficulties and is postponed for a later work.

## 2. Methods

### 2.1. Model for a prolate ellipsoid

For the hydrodynamic calculations, a prolate ellipsoid with semimajor and semiminor axes $a$ and $b$ is modeled as a colinear string of touching spherical beads using the method of Garcia de la Torre and Bloomfield [12] that includes volume correction. Following the suggestion of Wilson and Bloomfield [13], the central, largest bead of radius $b'$ is in turn substituted by a cubic array of eight smaller beads with radius $0.4668b'$ [5]. The details of this modeling method are displayed in fig. 1.

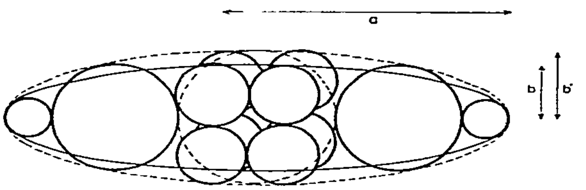

Fig. 1. Bead model for a prolate ellipsoid (example for $p = a/b = 4$). Beads (circles) are placed along the major axis so that each bead is tangent to its neighbors and to an ellipsoidal envelope (dashed ellipse) with semiaxes $a$ and $b'$. The value of $b'$ is determined by the condition that the volume of the model (sum of the volumes of the spheres) is the same as the volume of the modeled ellipsoid, $4\pi ab^3/3$. Then, the central sphere (dashed circle) of radius $b'$ is substituted by eight smaller beads of radius $0.4668b'$ placed at the corners of a cube.

This modeling strategy has been shown [13,14] to give values of the ellipsoid's transport properties that are rather close to the exact values obtained from the equations of Perrin and Shima. In table 1 we have listed the percent differences with respect to the exact values for three properties and several values of the axial ratio, $p = a/b$. The differences approach zero for large values of $p$, but we have only presented in table 1 results for the moderate $p$ values considered in this work.

Table 1

Percent deviation of the properties of the bead model with respect to the exact values for the ellipsoid

Percent deviation = 100 × [value(model)/value(exact) − 1]. $T$, translation: value $\equiv f_t = k_B T/D_t$. $R$, rotation around minor axis: value $\equiv f_r = k_B T/D_r$. $V$, intrinsic viscosity: value $\equiv [\eta]$.

| $p$ | $T$ | $R$ | $V$ |
|---|---|---|---|
| 1.0 | 3.1 | 1.0 | −3.8 |
| 1.5 | 1.1 | −8.7 | −3.1 |
| 2.0 | 0.8 | −12.1 | −4.0 |
| 3.0 | 0.9 | −5.7 | −7.3 |
| 4.0 | 0.2 | −6.4 | −12.8 |
| 6.0 | 0.3 | −3.0 | −11.4 |

### 2.2. Models for oligomers

We have restricted our study to oligomeric structures in which the subunits are identical and, in most cases, geometrically equivalent. We also impose the condition that each subunit is tangent, at least, to another subunit. Even with these restrictions, the number of possible structures is countless. Thus, in the case of dimers, there are infinite possible V-shaped geometries with different angles between the long axes, ranging from the end-to-end dimer (angle of 180°) to the side-by-side dimer (0°). Therefore, we have selected shapes having a high symmetry. The number of plausible structures increases rapidly with the number of monomers, so that we have only considered dimers, trimers, tetramers, one pentamer and one hexamer. The geometry of the various oligomeric structures studied in this work is displayed in fig. 2.

In the calculations, each ellipsoidal subunit was replaced by spheres as described in the previous paragraphs (see again fig. 1). For instance, the models for $n$-mers with $p = 4$ were composed for $12b$ spheres of varying radius. The positions of the centers and radii of the spheres were the input data for the hydrodynamic computations, which were carried out using the rigorous form of the Kirkwood-Riseman theory, including the modified interaction tensor and numerical matrix inversion. The computational details have been presented in previous papers [4,12] and two reviews [7,14].

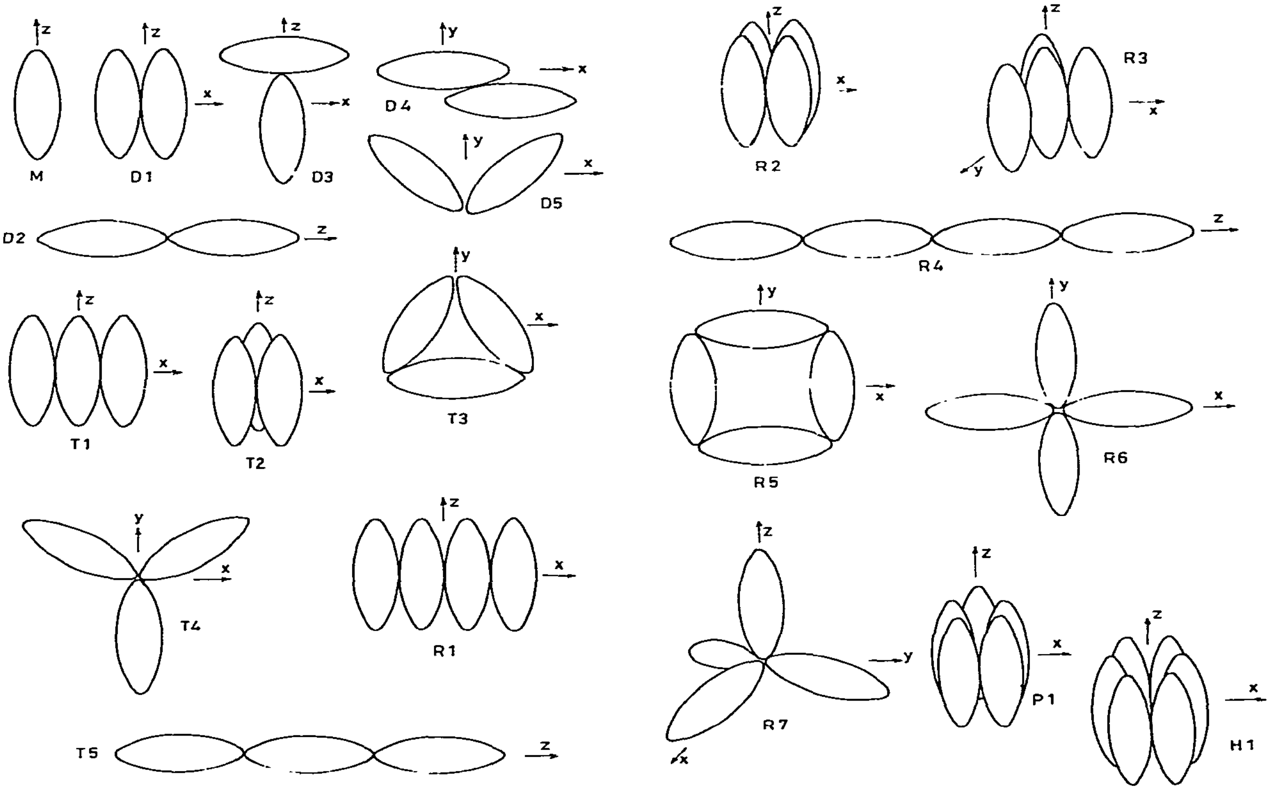

Fig. 2. Oligomeric structures with up to $n = 6$ for which the transport properties have been calculated. The directions of the Cartesian axes of reference, to which the rotational coefficientes are referred, are shown.

## 3. Theoretical results and discussion

The translational and rotational coefficients and the intrinsic viscosities of the oligomeric structures are presented in tables 2–4 in the following dimensionless forms:

$$D_t^* = D_t 6\pi\eta_0 b/k_B T \tag{1}$$

$$D_r^{\alpha *} = D_r^\alpha 6\pi\eta_0 b^3/k_B T, \qquad \alpha = x, y, z \tag{2}$$

$$[\eta]^* = [\eta] M/N_A b^3 \tag{3}$$

The values of $D_t$, $D_r$ and $[\eta]$ can be obtained from those in tables 2–4, using the semiminor axis, $b$, along with solvent viscosity, $\eta_0$, the temperature, $T$, and molecular weight of the oligomer, $M$. $N_A$ in eq. 3 is Avogadro's number. We note that since all the structures are highly symmetrical, and we have chosen the symmetry axes for the Cartesian system of reference, the complete description of the rotational behavior can be made with the three $D_r^{\alpha *}$ values reported in table 3 by means of the expressions [15] that relate then to the five relaxation times of a general body. It may be valid in some cases to assume that the directions or principal axes of the vectorial or tensorial electro-optical quantities coincide with the principal axes of rotational diffusion (as a consequence of symmetry, this can be true for symmetric oligomers even if it is false for the monomer). Then, only two relaxation times will be observed:

$$\tau_1 = 1/(6D - 2\Delta) \tag{4}$$

$$\tau_2 = 1/(6D + 2\Delta) \tag{5}$$

where

$$D = 1/3(D_r^x + D_r^y + D_r^z) \tag{6}$$

and

$$\Delta = \left(D_r^{x^2} + D_r^{y^2} + D_r^{z^2} - D_r^x \cdot D_r^y - D_r^x D_r^z - D_r^y D_r^z\right)^{1/2} \tag{7}$$

Furthermore, if the oligomer has a $C_\nu$ symmetry axis ($\nu > 3$), taken as $z$, the decay or relaxation will be a single exponential with

$$\tau = 1/6D_r^x \tag{8}$$

Table 3 contains all the data needed to calculate

Table 2
Reduced translational diffusion coefficients, $D_t^*$ (eq. 1), for oligomeric structures composed of ellipsoidal subunits

| | $p$ | | | | | |
|---|---|---|---|---|---|---|
| | 1.0 | 1.5 | 2.0 | 3.0 | 4.0 | 6.0 |
| M | 0.9903 | 0.8516 | 0.7539 | 0.6177 | 0.5316 | 0.4173 |
| D1 | 0.7185 | 0.6366 | 0.5724 | 0.4807 | 0.4225 | 0.3402 |
| D2 | 0.7185 | 0.5847 | 0.5020 | 0.4007 | 0.3370 | 0.2583 |
| D3 | 0.7185 | 0.6071 | 0.5291 | 0.4236 | 0.3580 | 0.2745 |
| D4 | 0.7185 | 0.6171 | 0.5463 | 0.4486 | 0.3851 | 0.3012 |
| D5 | 0.7185 | 0.6089 | 0.5294 | 0.4181 | 0.3510 | 0.2679 |
| T1 | 0.5811 | 0.5226 | 0.4723 | 0.4030 | 0.3567 | 0.2910 |
| T2 | 0.6158 | 0.5513 | 0.4991 | 0.4257 | 0.3784 | 0.3091 |
| T3 | 0.6158 | 0.5352 | 0.4746 | 0.3883 | 0.3265 | 0.2464 |
| T4 | 0.6158 | 0.5001 | 0.4235 | 0.3265 | 0.2697 | 0.2023 |
| T5 | 0.5811 | 0.4604 | 0.3893 | 0.3062 | 0.2546 | 0.1928 |
| R1 | 0.4945 | 0.4489 | 0.4100 | 0.3515 | 0.3132 | 0.2581 |
| R2 | 0.5447 | 0.4934 | 0.4493 | 0.3855 | 0.3428 | 0.2829 |
| R3 | 0.5032 | 0.4555 | 0.4161 | 0.3583 | 0.3196 | 0.2639 |
| R4 | 0.4945 | 0.3854 | 0.3227 | 0.2512 | 0.2075 | 0.1558 |
| R5 | 0.5421 | 0.4524 | 0.3905 | 0.3100 | 0.2550 | 0.1890 |
| R6 | 0.5421 | 0.4486 | 0.3807 | 0.2929 | 0.2411 | 0.1790 |
| R7 | 0.5585 | 0.4538 | 0.3806 | 0.2871 | 0.2352 | 0.1740 |
| P1 | 0.4864 | 0.4429 | 0.4055 | 0.3515 | 0.3150 | |
| H1 | 0.4409 | 0.4040 | 0.3716 | 0.3221 [a] | | |

[a] Extrapolated.

Table 3

Reduced rotational diffusion coefficients, $D_r^*$ (eq. 2), for oligomeric structures composed of ellipsoidal subunits

When three values are given for a structure, they correspond to $\alpha = x, y, z$, in this order. When we give only two numbers, the first one corresponds to the $x$ and $y$ coefficients, which are identical, and the second one is $D^{*z}_r$. For the linear structures we report only $D^{*x}_r = D^{*y}_r$, because the values that we obtain for $D^{*z}_r$ may be nonphysical for larger $p$ values, since only the central bead is substituted. For structure R7 the three coefficients are identical owing to its tetrahedral symmetry.

| | $p$ | | | | | |
|---|---|---|---|---|---|---|
| | 1.0 | 1.5 | 2.0 | 3.0 | 4.0 | 6.0 |
| M | 0.7426 | 0.4591 | 0.2832 | 0.1132 | 0.0590 | 0.0213 |
| D1 | 0.4233 | 0.2714 | 0.1681 | 0.0706 | 0.0376 | 0.0141 |
| | 0.1978 | 0.1466 | 0.1105 | 0.0607 | 0.0357 | 0.0148 |
| | 0.1978 | 0.1491 | 0.1216 | 0.0988 | 0.0833 | 0.0625 |
| D2 | 0.1978 | 0.0925 | 0.0493 | 0.0182 | 0.00905 | 0.00319 |
| D3 | 0.1978 | 0.1160 | 0.0736 | 0.0348 | 0.0196 | 0.00784 |
| | 0.1978 | 0.1155 | 0.0721 | 0.0317 | 0.0171 | 0.00658 |
| | 0.4233 | 0.2642 | 0.1772 | 0.0907 | 0.0522 | 0.0232 |
| D4 | 0.3604 | 0.2016 | 0.1173 | 0.0467 | 0.0240 | 0.00868 |
| | 0.1978 | 0.1280 | 0.0824 | 0.0355 | 0.0187 | 0.00684 |
| | 0.2511 | 0.1919 | 0.1590 | 0.1409 | 0.1260 | 0.1030 |
| D5 | 0.4233 | 0.2766 | 0.1865 | 0.0994 | 0.0583 | 0.0231 |
| | 0.1978 | 0.1140 | 0.0689 | 0.0288 | 0.0151 | 0.00560 |
| | 0.1978 | 0.1155 | 0.0708 | 0.0301 | 0.0159 | 0.00591 |
| T1 | 0.2975 | 0.1940 | 0.1206 | 0.0515 | 0.0276 | 0.0106 |
| | 0.0812 | 0.0649 | 0.0532 | 0.0344 | 0.0226 | 0.0107 |
| | 0.0812 | 0.0646 | 0.0537 | 0.0423 | 0.0356 | 0.0265 |
| T2 | 0.1758 | 0.1291 | 0.0947 | 0.0506 | 0.0293 | 0.0121 |
| | 0.1316 | 0.1016 | 0.0838 | 0.0672 | 0.0559 | 0.0412 |
| T3 | 0.1758 | 0.1150 | 0.0783 | 0.0411 | 0.0234 | 0.00920 |
| | 0.1316 | 0.0874 | 0.0611 | 0.0314 | 0.0177 | 0.00691 |
| T4 | 0.1758 | 0.0922 | 0.0532 | 0.0317 | 0.0112 | 0.00410 |
| | 0.1316 | 0.0648 | 0.0348 | 0.0127 | 0.00627 | 0.00220 |
| T5 | 0.0812 | 0.0343 | 0.0175 | 0.00630 | 0.00306 | 0.00106 |
| R1 | 0.2296 | 0.1512 | 0.0943 | 0.0407 | 0.0219 | 0.00858 |
| | 0.0417 | 0.0348 | 0.0297 | 0.0212 | 0.0150 | 0.00787 |
| | 0.0417 | 0.0342 | 0.0288 | 0.0225 | 0.0189 | 0.0141 |
| R2 | 0.1197 | 0.0919 | 0.0702 | 0.0398 | 0.0238 | 0.0102 |
| | 0.0898 | 0.0709 | 0.0509 | 0.0476 | 0.0400 | 0.0298 |
| R3 | 0.0907 | 0.0704 | 0.0551 | 0.0327 | 0.0203 | 0.00905 |
| | 0.0602 | 0.0484 | 0.0405 | 0.0320 | 0.0270 | 0.0201 |
| R4 | 0.0417 | 0.0167 | 0.00833 | 0.00296 | 0.00142 | 0.00049 |
| R5 | 0.1182 | 0.0676 | 0.0419 | 0.0194 | 0.0102 | 0.00376 |
| | 0.0893 | 0.0509 | 0.0323 | 0.0154 | 0.00815 | 0.00301 |
| R6 | 0.1182 | 0.0662 | 0.0391 | 0.0163 | 0.00848 | 0.00310 |
| | 0.0893 | 0.0480 | 0.0268 | 0.0100 | 0.00500 | 0.00176 |
| R7 | 0.1239 | 0.0644 | 0.0358 | 0.0135 | 0.00684 | 0.00244 |
| P1 | 0.0836 | 0.0658 | 0.0518 | 0.0314 | | |
| | 0.0631 | 0.0505 | 0.0426 | 0.0347 | | |
| H1 | 0.0608 | 0.0490 | 0.0397 | | | |
| | 0.0402 | 0.0377 | 0.0320 | | | |

the relaxation times of most of the structures in fig. 2.

To determine the structure of an oligomer one needs firstly the monomer's value of $p$. X-ray diffraction can furnish an estimate of $p$, but with regard to the consistency of the procedure it would be preferable to obtain $p$ from hydrodynamic properties. A single property plus the degree of hydration, or two properties independently of hydration suffice for the calculation of $p$ by means of well known methods [16]. An important point is the actual prolateness of the monomer. It is usually possible to distinguish between prolate and oblate shapes, either from previous knowledge of the structure of the monomer or by combination of its hydrodynamic properties. In fact, recently developed methods [17,23] allow the determination of the three semiaxes of a general ellipsoid, $\alpha$, $\beta$ and $\gamma$. These will usually be different, but cases with $\alpha/\gamma$ appreciably larger than $\beta/\gamma$ can be considered as prolate ellipsoids with $a = \alpha$, $b = (\beta + \gamma)/2$.

From tables 2–4 one can compute oligomer-to-monomer ratios of hydrodynamic properties:

$$\frac{D_t(n)}{D_t(1)} = \frac{D_t^*(n)}{D_t^*(1)} \tag{9}$$

$$\frac{s(n)}{s(1)} = n\frac{D_t(n)}{D_t(1)} \tag{10}$$

and

$$\frac{[\eta](n)}{[\eta](1)} = \frac{1}{n}\frac{[\eta]^*(n)}{[\eta]^*(1)} \tag{11}$$

where $n$ is the number of subunits and $s$ the sedimentation coefficient. Several similar ratios can be formulated for rotational diffusion coefficients or relaxation times.

As examples, we present in figs. 3 and 4 the $D_t(n)/D_t(1)$ and $[\eta](n)/[\eta](1)$ ratios for dimers and trimers. The variation of these ratios with $p$ is more or less strong, depending on the range of $p$ and the geometry of the structure. Nonetheless, the dependency of the ratios on oligomer shape is usually much stronger. This means that our results can be reliably used for structural determination even if the value of $p$ is not perfectly established.

Table 4
Reduced intrinsic viscosities $[\eta]^*$ (eq. 3) of oligomeric structures composed of ellipsoidal subunits

| | $p$ | | | | | |
|---|---|---|---|---|---|---|
| | 1.0 | 1.5 | 2.0 | 3.0 | 4.0 | 6.0 |
| M | 10.07 | 16.00 | 23.38 | 42.90 | 68.12 | 158.04 |
| D1 | 28.13 | 40.03 | 54.63 | 91.92 | 137.19 | 282.91 |
| D2 | 28.13 | 54.30 | 90.18 | 198.00 | 362.72 | 948.86 |
| D3 | 28.13 | 47.13 | 71.76 | 140.48 | 235.32 | 557.22 |
| D4 | 28.13 | 44.34 | 64.97 | 123.40 | 205.20 | 486.20 |
| D5 | 28.13 | 46.82 | 72.42 | 151.30 | 264.80 | 660.76 |
| T1 | 57.18 | 76.61 | 100.02 | 157.69 | 224.38 | 427.44 |
| T2 | 43.74 | 60.61 | 81.31 | 131.59 | 191.64 | 376.92 |
| T3 | 43.74 | 67.12 | 97.39 | 178.28 | 297.28 | 701.53 |
| T4 | 43.74 | 82.48 | 137.62 | 311.25 | 571.69 | 1486.0 |
| T5 | 57.18 | 122.35 | 217.58 | 519.50 | 1004.5 | 2745.2 |
| R1 | 99.39 | 128.00 | 161.96 | 243.84 | 335.50 | 602.86 |
| R2 | 63.63 | 84.83 | 111.56 | 177.24 | 253.32 | 480.30 |
| R3 | 81.93 | 108.97 | 141.24 | 218.59 | 306.69 | 563.85 |
| R4 | 99.39 | 227.19 | 421.30 | 1055.6 | 2100.6 | 5877.8 |
| R5 | 64.85 | 112.35 | 176.85 | 358.18 | 647.11 | 1661.4 |
| R6 | 64.85 | 114.92 | 188.50 | 420.43 | 769.06 | 1993.2 |
| R7 | 57.78 | 107.70 | 181.58 | 423.56 | 782.08 | 2033.6 |
| P1 | 90.33 | 118.70 | 153.70 | 236.14 | 329.31 | |
| H1 | 122.13 | 157.37 | 200.76 | 299.8 [a] | | |

[a] Extrapolated.

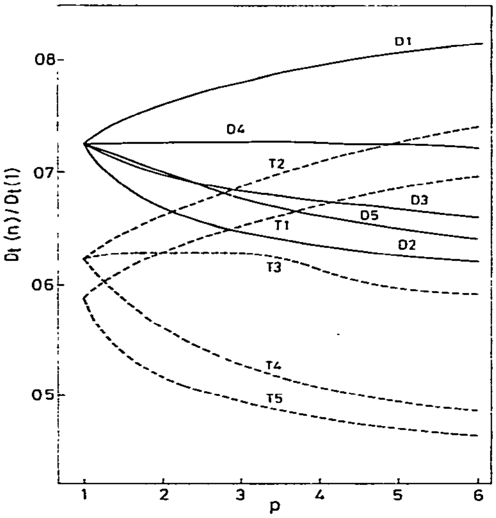

Fig. 3. Variation of the ratio $D_t(n)/D_t(1)$ with $p$ for some oligomers. Solid curves are for dimers and dashed curves for trimers.

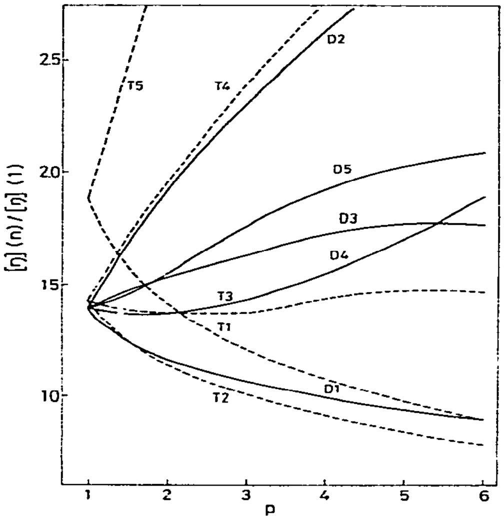

Fig. 4. Variation of the ratio $[\eta](n)/[\eta](1)$ with $p$ for some oligomers. Solid curves are for dimers and dashed curves are for trimers.

When comparing our theoretical results with experimental data one should bear in mind some factors that can bring about some discrepancy. One of them is that the theoretical values are not entirely exact owing to the remaining imperfections in modeling procedure and theory. This has been already shown in table 1 for the monomer, for which typical errors are of a few percent. For oligomers these type of theoretical errors must be even smaller, since the exact description of the ellipsoidal shape becomes less important than the good description of the oligomer's geometry.

Another source of possible discrepancy is hydration. It is very likely that when the oligomer is formed from the free monomeric subunits, some water will be squeezed out, so that the hydrody-

Table 5
Translational diffusion ratios and intrinsic viscosity ratios for some oligomeric structures obtained by different methods (A, B C) Methods: (A) monomer of $p$ spheres; Kirkwood formula for $D_t$ and Tsuda formula for $[\eta]$. (B) monomer of $p$ spheres; values calculated with substitution, obtained from ref. 5; (C) this work.

| Structure | $p$ | $D_t(n)/D_t(1)$ | | | $[\eta](n)/[\eta](1)$ | | |
|---|---|---|---|---|---|---|---|
| | | A | B | C | A | B | C |
| D1 | 2 | 0.784 | 0.758 | 0.759 | 1.091 | 1.130 | 1.168 |
| D2 | 2 | 0.694 | 0.688 | 0.666 | 1.581 | 1.766 | 1.928 |
| T2 | 2 | 0.712 | 0.664 | 0.662 | 1.042 | 1.097 | 1.148 |
| T3 | 2 | 0.628 | 0.613 | 0.629 | 1.301 | 1.445 | 1.374 |
| T5 | 2 | 0.544 | 0.540 | 0.516 | 2.430 | 2.742 | 3.071 |
| R2 | 2 | 0.642 | 0.597 | 0.596 | 1.067 | 1.130 | 1.192 |
| R5 | 2 | 0.524 | – | 0.518 | 1.616 | – | 1.890 |
| P1 | 2 | 0.580 | – | | 1.114 | – | |
| D1 | 3 | 0.806 | – | 0.778 | 0.972 | – | 1.071 |
| D2 | 3 | 0.668 | 0.668 | 0.649 | 1.949 | 2.023 | 2.307 |

namic volume of the oligomer would be smaller than the sum of the hydrodynamic volumes of the subunits. This situation is not accounted for in our models. As a consequence, we can expect that the experimental values of the ratios of sedimentation and diffusion coefficients may be slightly larger than the theoretical results. Conversely, the experimental ratios for $[\eta]$ may be smaller. It is difficult to determine quantitatively the magnitude of this effect, which should depend on the degree of hydration of the monomer and also on the geometry of the oligomer (for instance, this effect must be smaller for D2 than for D1, since the contact area is smaller for the former structure). Thus, the combined influence of the circumstances described in the two preceding paragraphs cannot be estimated precisely. Anyhow, and without excessive optimism, we feel that the global discrepancy can be about 10%, reaching perhaps 20% in the worst cases. Fortunately, in most instances, the difference between the properties of two different oligomers is greater than these estimates, so that we conclude that our theoretical calculations are still useful for structural elucidation.

As mentioned in section 1, the difficulty of modeling an ellipsoidal shape was circumvented in earlier works representing the monomer of axial ratio $p$ by a string of $p$ identical, touching spheres [8–10, 18–21]. Moreover, in those works the transport properties were calculated using the approximate double-sum formulas of Kirkwood [22] and Tsuda [18,19]. We have used previous values [4,5] obtained for polygonal or polyhedral structures to calculate the diffusion and viscosity ratios of oligomeric structures according to the simpler modeling method. If, for instance, a structure composed of two spheres is regarded as the monomer with $p = 2$, then a square structure would correspond to D1, a trigonal prism to T2, a cube to R2, and so on. The results for some structures are presented in table 5. Method A uses monomers of $p$ spheres and the double-sum formulas of Kirkwood and Tsuda for translation and viscosity, respectively. In method B the calculations were made using the substitution strategy for all the spheres in the model; actually, the results have been directly obtained from those in tables 2 and 3 of ref. 5. method C is that proposed in this paper.

Taking the results of method C as reference, we see that the deviations in the case of $D_t$ are at most 8% for method A and 4% for method B. In the case of $[\eta]$ the deviations are larger, reaching 20% for method A and 11% for method B.

## 4. Applications

To illustrate the applicability of the results of this work, we finally present some application to biologically relevant oligomeric structures.

### *4.1. Neurophysin II*

The hydrodynamic properties of neurophysin II have been recently analysed by Harding and Rowe [23]. Using a procedure developed by them for general ellipsoids, these authors found that the monomer is actually a prolate ellipsoid of revolution with $p = 4$. According to Nicolas and coworkers [24,25], for the monomer $s(1) = 1.25$ S and $[\eta](1) = 5.5$ ml g$^{-1}$. They also found that the properties of the dimer are $s(2) = 2.20$ S and $[\eta](2) = 4.6$ ml g$^{-1}$. Harding and Rowe have revised the dimer values on grounds that we do not wish to discuss here, claiming that the more correct values are $[\eta](2) = 4.32$ ml g$^{-1}$ and $s(2) = 2.40$ S. By combining the two sets of dimer properties with those of the monomer, we obtain the property ratios listed in table 6. Interpolating any of the four ratios in figs. 3 and 4, we see that the structure that gives the closest theoretical results is D1. Thus, the neurophysin dimer is undoubtedly side-by-side, in agreement with the conclusion of Hardin and Rowe [23]. We also see that the experimental viscosity ratio and diffusion ratio are somewhat higher and lower, respectively, than the values calculated for D1, as expected from the discussion presented above on hydration effects.

### *4.2. Bovine serum albumin*

From a variety or properties Squire et al. [21] have shown that the most reliable value of the axial ratio of monomeric bovine serum albumin is $p = 3.5$. There have been a number of experimental determinations of the translational properties

Table 6
Ratios of hydrodynamic properties and structures for some proteins

| Protein | $n$ | $p$ | Experimental | | | Structure | Calculated | |
|---|---|---|---|---|---|---|---|---|
| | | | $s(n)/s(1)$ | $D_t(n)/D_t(1)$ | $[\eta](n)/[\eta](1)$ | | $D_t(n)/D_t(1)$ | $[\eta](n)/[\eta](1)$ |
| Neurophysin II [a] | 2 | 4 | 1.76 | 0.88 | 0.84 | D1 | 0.80 | 1.00 |
| Neurophysin II [b] | | | 1.92 | 0.96 | 0.79 | | | |
| Bovine serum albumin [c] | 2 | 3.5 | | 0.73 | | D4 | 0.726 | 1.50 |
| Bovine serum albumin [d] | | | 1.52 | 0.76 | | D1–D4 | 0.726–0.788 | 1.50–1.03 |
| Hemoglobin | 4 | 1 | 2.51 | 0.63 | | R7 | 0.564 | |
| Hemoglobin-myoglobin [e] | | | | | 1.2 | R7 | | 1.433 |
| Phycocyanin | 5 | 3 | 3.27–3.37 | 0.56 | | H1 | 0.52 | |
| | 3 | | 1.83 | 0.61 | 0.91 [f] | T3 | 0.63 | 0.84 [f] |
| | | | | | | T1 | 0.65 | 0.95 [f] |

[a] Data of Nicolas and co-workers [24,25] for the dimer.
[b] Dimer values as corrected by Harding and Rowe [23].
[c] Diffusion data of Harvey et al. [26].
[d] Sedimentation data of Squire et al. [21].
[e] Myoglobin data assumed for hemoglobin monomer.
[f] Value of $[\eta](6)/[\eta](3)$.

(diffusion and sedimentation). To our knowledge, the most recent study is the one carried out by dynamic light scattering by Harvey et al. [26] who found $D_t(2)/D_t(1) = 0.73 \pm 0.02$. From fig. 3 we see that this corresponds very precisely to structure D4, i.e., a side-by-side dimer with 50% overlap. This model was also proposed in previous works [21,27]. We also have the older measurements of sedimentation coefficients at finite concentration of Squire et al. [21] who gave $s(2)/s(1) = 1.52$, from which $D_t(2)/D_t(1) = 0.76$. This value is halfway between the theoretical results for models D1 and D4, as seen in fig. 3, and could be interpreted as due to a D1 structure somehow altered by the dehydration effect. We are unfortunately unable to compare viscosity ratios. We are not aware of the measurement of $[\eta](2)$, and the reported $[\eta](1)$ data show a noticeable scattering (see the values quoted in refs. 20 and 27). Then, the structural determination from $s$ and $D_t$ cannot be contrasted. At any rate, it seems that the staggered dimer with a most 50% side-by-side overlap is the correct structure.

### *4.3. Hemoglobin*

It is now known that the four subunits in hemoglobin are arranged tetrahedrally. In the recent book of Cantor and Schimmel [16] this oligomeric protein is presented as an example of the use of sedimentation ratios for structural determination. These authors quote (p. 610) the following values: $s(1) = 1.77 \pm 0.55$ S and $s(4) = 4.45$ S, from which $s(4)/s(1) = 2.51$ and $D_t(4)/D_t(1) = 0.63$. Assuming $p = 1$ (spherical shape) for the subunits, the comparison of this ratios with values obtained from the approximate Kirkwood formula for linear, square and tetraheral arrays (p. 567), they arive at the conclusion that hemoglobin has, as expected, a tetrahedral geometry.

Although their final conclusion should be true, the good agreement that they found must be somewhat fortuitous. For $p = 1$, more rigorous values of $D_t(2)/D_t(1)$ can be calculated from our table 2 or taken directly from table 3 in ref. 7. The ratios for linear, square and tetrahedral structures are 0.500, 0.550 and 0.564, respectively. The experimental result is closest to the value for the tetrahedron, but the discrepancy is large.

With regard to the intrinsic viscosity, no data are available for the subunits. Following Tsuda [17], we invoke the morphological similarity of the hemoglobin subunits and myoglobin, and consider myoglobin as the monomer. From the tabulation of Tanford, $[\eta](4)/[\eta](1) = [\eta]$(hemoglobin)/

$[\eta]$(t oglobin) = 1.2, while the respective results for the linear, square and tetrahedral structure are 1.892, 1.579 and 1.433. The best agreement, although not entirely satisfactory, is found for the tetrahedron.

### 4.4. Phycocyanin

Phycocyanin is know to be composed of subunits of 35 Å in diameter and 105 Å in length, so that $p = 3$. Under certain conditions, this protein exists as a trimer or a hexamer. The following data have been taking from the paper by Bloomfield and Jennings [29] for the monomer and hexamer: $D(6)/D(1) = 4.2/7.5 = 0.56$ and $s(6)/s(1) = 11.8/3.6 = 3.27$, from which we calculate $D(6)/D(1) = 0.54$. Kotera et al. [20] have reported $s(6)/s(1) = 10.8/3.2 = 3.37$, from which $D(6)/D(1) = 0.56$. The ratios of translational diffusion coefficients are then in good agreement. The latter authors measured $[\eta]$ for phycocyanin, finding that under the conditions in which the hexamer is present, $[\eta](6) = 3.92$ ml g$^{-1}$, but $[\eta](1)$ has not yet been reported.

Another oligomeric form of phycocyanin is the trimer, with $s(3) = 6.4$ S [20] or 6 S [30], with an average of 6.2 S. This leads to $s(3)/s(1) = 1.83$, or $D_t(3)/D_t(1) = 0.61$. Kotera has measured also $[\eta]$ under conditions which would favor the trimeric form, obtaining $[\eta](3) = 4.32$, so that $[\eta](6)/[\eta](3) = 0.91$.

The presumed structure of hexameric phycocyanin is a prism, like H1, for which the theoretical ratios are $D_t(6)/D_t(1) = 0.52$, in good agreement with the experimental value, 0.54–0.56. The closest fitting structure for the trimer is T3, with $D_t(3)/D_t(1) = 0.63$. For this structure, $[\eta](6)/[\eta](3) = 0.84$, which compares quite well with the experimental value, 0.91. If one accepts H1 for the hexamer, structure T3 for the trimer does not look very plausible, since the T3 → H1 process would require a large rearrangement of the subunits. A more likely structure of the trimer is T1, for which the calculated $D_t$ ratio is slightly higher than the experimental value, while the viscosity ratio is somewhat better than for T3.

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