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CALCULATED SEDIMENTATION RATIOS FOR ASSEMBLIES OF TWO, THREE, FOUR, AND FIVE SPATIALLY EQUIVALENT PROTOMERS

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Sedimentation ratios of oligomeric structures containing from two to five protomers in spatially equivalent positions are calculated on the basis of equations derived by Kirkwood. A range of oblate and prolate protomers and a systematic set of assembly modes is considered for each type of oligomer. The results are presented graphically. Dimensions in three mutually perpendicular directions are also given for each structure considered, in a format which allows calculation in Å of the dimensions of appropriate models for actual molecules. Together with the results for hexameric aggregates presented previously these data allow sedimentation velocity measurements with any oligomer, up to and including the hexamer, to be analysed readily and systematically in terms of quaternary structure.

1. Introduction

The sedimentation ratio of an oligomeric structure is defined as the ratio of the sedimentation coefficient of the aggregated structure to that of its subunit. In a previous paper [1], we described how comparison of an experimentally measured sedimentation ratio with values calculated for a systematically generated set of hexameric assemblies of oblate and prolate subunits might be utilized in specifying a structure, or limited set of structures, consistent with the observed hydrodynamic behaviour. The approach allows a more realistic assessment of the range of possibilities than one restricted to spherical protomers, particularly in the context of comparison with structures deduced by other methods.

In the previous work only six-membered oligomers were dealt with, but while these are not uncommon in proteins, those containing two or four subunits are far more numerous, and even three and five subunit proteins are not unknown [2]. For this reason the treatment has been extended to include aggregated structures containing from two to five protomers, thus encompassing almost the whole spectrum of oligomeric proteins likely to be encountered in practice. The availability of calculated sedimentation ratios for all of the oligomers up to the hexamer also allows for inclusion of structures formed via stable intermediates. For example, formation of a hexamer may require consideration in terms of first, trimer formation, and then a dimerization. Larger aggregates can also be treated by combinations of appropriate sedimentation ratio values as, for example, in the formation of a dodecamer, which might be analyzable as a hexamerization followed by a dimerization.

2. Theory

2.1. Definitions

The terms oligomer, protomer and monomer are used in the sense defined by Monod et al. [3]. A range of oblate and prolate shapes is considered for protomers. The approximately disc-shaped oblate protomer, designated O, and the cylindrical prolate protomer, designated P, are planar and linear assemblies, respectively, of identical spheres. Thus, the oblate extreme, with an “axial ratio” of 3 : 1, denoted O_3, is a three by three matrix of identical spheres; the prolate extreme, of "axial ratio" 5 : 1, denoted P_5, consists of five identical spheres with their centres on a common axis. The use of assemblies of identical spheres, rather than the traditional oblate and prolate ellipsoids of revolution, facilitates the use of Kirkwood's equations [4] and does not seem significantly less realistic as an approximation to the actual shape of a protein in solution. The set of seven protomers, O_3 through spherical, S, to P_5 is the same as that used in the sedimentation ratio calculations for hexameric aggregates [1] and was chosen to provide a reasonable range of non-spherical protomers without making subsequent calculations unwieldy.

Assemblies of these protomers for which sedimentation ratios are calculated include only those in which the protomers are spatially equivalent. There are both theoretical and practical arguments which suggest that this is a reasonable practice for oligomeric proteins composed of identical subunits [2]. The way in which sets of aggregated structures were generated and their labelling is explained below in detail, but it follows the same convention as that used before [1]. Again, the point group notation is not used, partly because the sedimentation method is quite incapable of fine distinctions, e.g . between a planar structure with true cyclic symmetry and one with dihedral symmetry; partly to keep the terminology as simple and descriptive as possible. Thus, structures generated from an originally cyclic arrangement of spherical protomers are labelled "C" and those generated from an arrangement with spherical symmetry are labelled "S". Dimers do not lend themselves to this terminology and are treated in terms of translations or rotations of the protomers in a vertical or a horizontal plane.

2.2. *Modes of aggregation*

2.2.1. *Trimers, tetramers and pentamers*

Since aggregates containing odd numbers of identical subunits can only show cyclic symmetry, it is convenient to discuss first the one case, the tetramer, where spherical symmetry is possible. The basic spherically symmetrical tetramer has four spherical protomers arranged in a tetrahedral configuration. From this, generation of structures with prolate protomers may be visualized as a process of stretching each sphere in a radial direction, R, to the required extent. Similarly, to form the structures with oblate protomers, each sphere is imagined to be compressed radially. The resulting cylinders or discs are approximated by assemblages of spheres, as described above, and the resulting structures are denoted $SRRP_n$ or $SRRO_n$, where n is the axial ratio of the protomer. The structures are shown in fig. 1 for an axial ratio of 3 : 1 for each kind of protomer.

All of the remaining structures for trimers, tetramers and pentamers have cyclic symmetry and are formed from the appropriate cyclic arrangement of

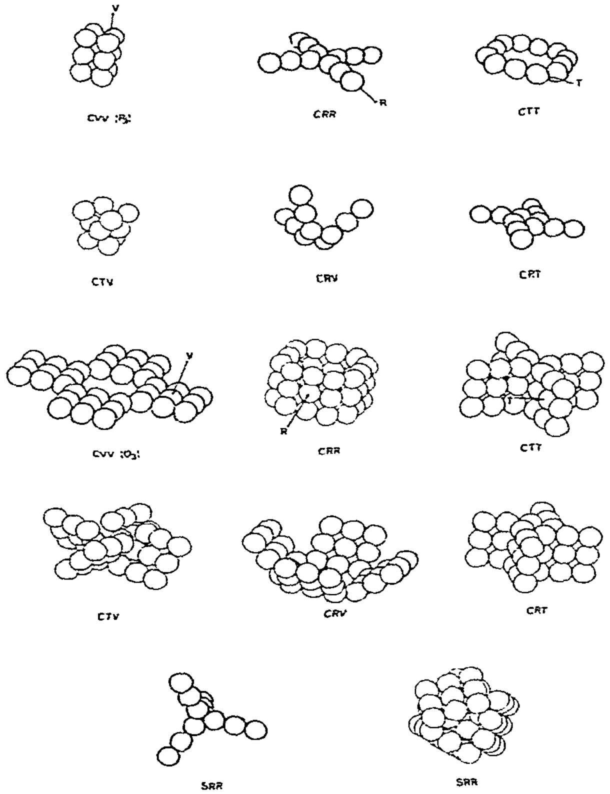

Fig. 1. Aggregated structures for trimers, tetramers and pentamers for which sedimentation ratios were calculated. Tetramers constructed with P_3 and O_3 protomers are illustrated but the same forms and nomenclature (except for the P_3 and O_3 structures with spherical symmetry shown at the bottom of the diagram) apply to trimers and pentamers and to the other protomer shapes. The axes along which spherical protomers are imagined to be compressed or stretched to give oblate or prolate shapes respectively are indicated as "V", "R", and "T". More details of the convention used to generate the structures are given in the text and in [1].

spherical protomers by stretching, for prolate protomers, or compressing, for oblate protomers, along vertical, V, tangential, T, or radial, R axes. Six cyclic structures for which sedimentation ratios have been calculated, for each of the oligomers and for each protomer,type, are illustrated in fig. 1 with tetramers formed of O_3 and P_3 protomers. The same set of cyclic structures was used in the previous work [1] which referred to hexameric aggregates. The set includes the intermediate structures CTV, CRV, and CRT, both to provide extra data points for plotting and to emphasize that the models for which the calculations are done should be viewed as reference points for a continuum of structures, rather than as exclusive examples.

2.2.2. Dimers

The basic structure is two touching spheres; stretching produces two cylinders lying side by side, compressing the spheres, two discs side by side. The prolate and oblate protomers are then approximated in the usual way by suitable assemblages of spheres as illustrated by $HrOP_3$ and $HrOO_3$ in fig. 2. Dimeric structures involving translations are produced by moving, in increments of one sphere diameter, one of the protomers relative to the other in the horizontal plane, which is taken to be the plane of the page. Typical arrangements are illustrated in fig. 2 as $Ht2O_3$ (a horizontal translation by two sphere diameters of O_3 protomers) and $Ht2P_3$. Alternatively, one protomer can be rotated with respect to the other in the horizontal plane. Cases for which calculations have been performed are rotation through angles of 45°, 90°, 135° and 180°. Examples with both O_3 and P_3 protomers are illustrated for rotations of 45° and 180° in fig. 2 where $Hr45O_3$ for example, denotes a 45° rotation of one O_3 protomer with respect to the other, in the horizontal plane.

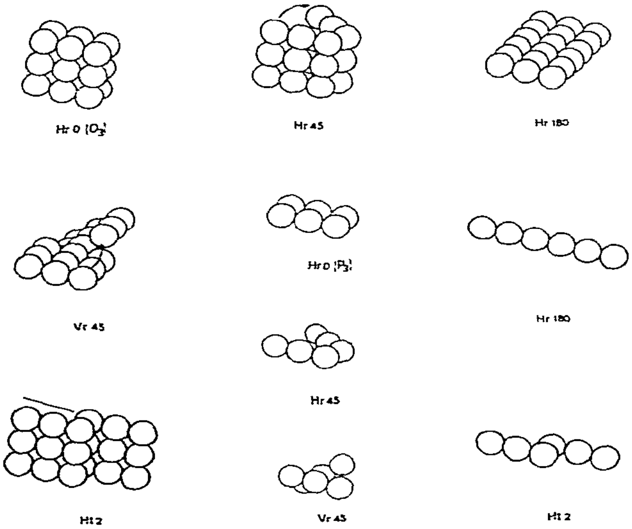

Fig. 2. Dimeric structures for which sedimentation ratios were calculated. Cases with O_3 and P_3 protomers are illustrated but the same nomenclature applies to the other protomer shapes considered. The so-called "horizontal" rotations and translations, Hr and Ht, respectively, and "vertical" rotations, Vr, visualized to generate the structure, are indicated. More details are given in the text.

For structures generated by vertical rotations of one prolate protomer with respect to the other the original structure is the two protomers side by side, and the operation is a rotation, in the vertical plane, about the centre of the structure. Vertical rotation through 45° of P_3 protomers $Vr45P_3$ is illustrated in fig. 2. When vertical rotations of oblate protomers are to be made, the starting configuration is with the two protomers in contact end to end (as in $Hr180O_3$). Rotations are then made about the long axis of the structure as shown for a 45° rotation in $Vr45O_3$ in fig. 2.

2.3. Calculations

Using Kirkwood's equation [4] as a starting point, we have shown [1] that the ratio of the sedimentation coefficient of an oligomer to that of its protomer, where both are composed of arrays of identical spherical subunits, can be written as

$$\frac{s(\mathrm{O})}{s(\mathrm{P})} = \left(1 + \frac{1}{n_{\mathrm{O}}} \sum_{\substack{l=1\\ l\neq s}}^{n_{\mathrm{O}}} \sum_{s=1}^{n_{\mathrm{O}}} (R'_{ls})^{-1}\right) \times \left(1 + \frac{1}{n_{\mathrm{P}}} \sum_{\substack{l=1\\ l\neq s}}^{n_{\mathrm{P}}} \sum_{s=1}^{n_{\mathrm{P}}} (R'_{ls})^{-1}\right)^{-1}, \qquad (1)$$

where n_{O} and n_{P} are the numbers of spheres in oligomer and protomer, respectively, and R'_{ls} is the distance between the centres of the spherical subunits, expressed in units of sphere radius. This ratio is independent of solvent viscosity, solute and solvent density, and the absolute sizes of the components: it depends solely on the shape of the protomer, represented by the summation over n_p in eq. (1), and its mode of aggregation, represented by the summation over n_0. Experimental values of sedimentation coefficients of oligomer and protomer, expressed as a sedimentation ratio, may thus be compared directly with values calculated for various structures to assist in the delineation of the assembly pattern.

Eq. (1) has been used to calculate sedimentation ratios for the range of protomer shapes and oligomeric structures discussed in this paper. The magnitudes of the R'_{ls} values were determined directly from the Cartesian coordinates of the centres of the individual spherical subunits; the coordinates themselves were trigonometrically defined by the choice of protomer shapes and modes of aggregation described above and in figs. 1 and 2. Because of the large number of intersubstituent distances requiring evaluation the calculations were carried out using a Univac 1100/42 computer which was programmed to produce perspective and stereoscopic diagrams of each structure. As well as being useful for illustrative purposes, these diagrams provided an extra check that correct coordinates for the centres of the spherical subunits had been employed. The results of the sedimentation ratio calculations are presented in figs. 3 to 7 and reduced dimensions for each structure in tables 1 to 5. The latter are useful in checking the sizes of models with appropriat hydrodynamic characteristics against those deduced from other methods.

3. Discussion

It is common practice, especially in initial investigations of oligomeric proteins, to speculate about their modes of assembly on the basis of comparisons of sedimentation coefficients of dissociated and aggregated forms. Inasmuch as this is usually done with respect only to the very limited number of possibilitie resulting from the assumption of spherical protomers, it seemed worthwhile to provide for a more systemati procedure, which takes into account the larger numbe of alternatives seen to exist when asymmetric protomer shapes are included in the analysis. It is evident from figs. 3–7 that a given value of the sedimentation ratio is usually compatible with a number of different structures. This emphasizes the importance of a knowledge of the protomer shape in making any predictions about oligomer geometry from experimentally mea-

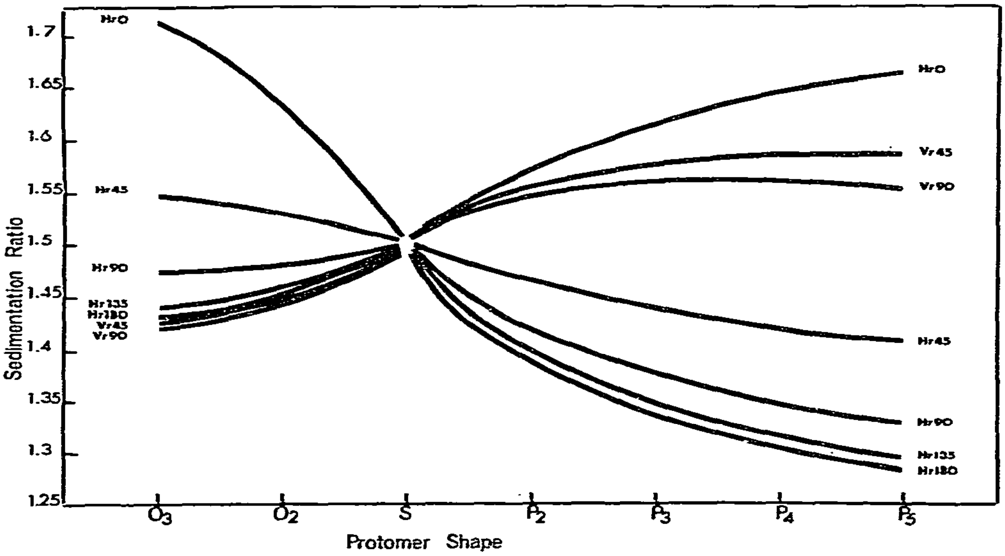

Fig. 3. Calculated sedimentation ratio versus protomer shape for dimeric structures generated by rotation. The structures and nomenclature are explained in the text and illustrated in fig. 2.

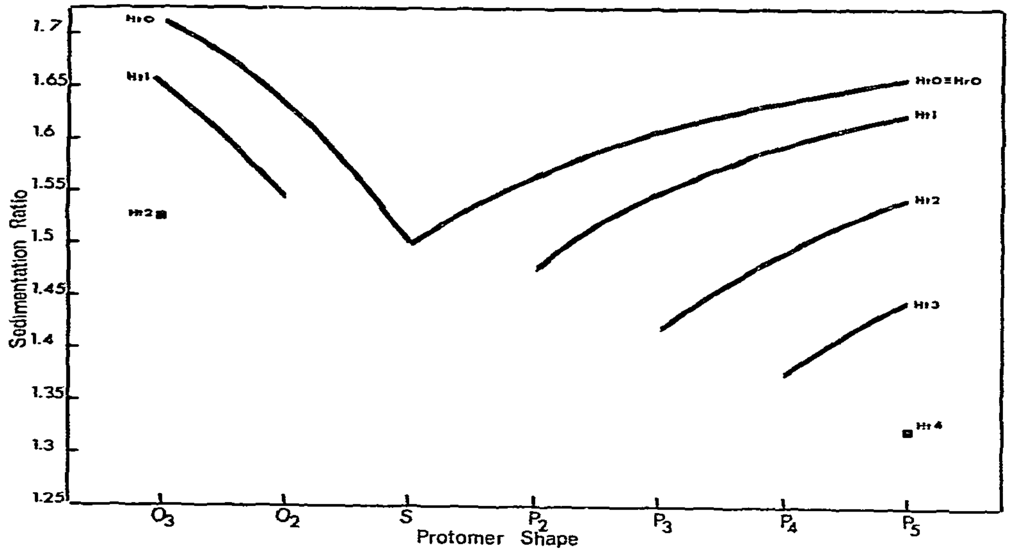

Fig. 4. Calculated sedimentation ratio versus protomer shape for dimeric structures generated by translation. The structures and nomenclature are explained in the text and illustrated in fig. 2.

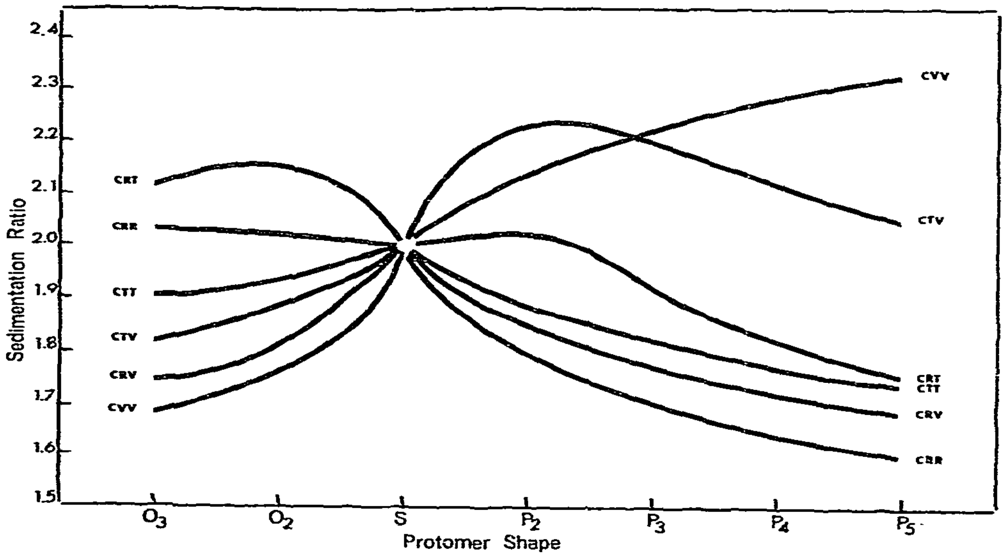

Fig. 5. Calculated sedimentation ratio versus protomer shape for trimeric structures. The structure and nomenclature are explained in the text and illustrated in fig. 1.

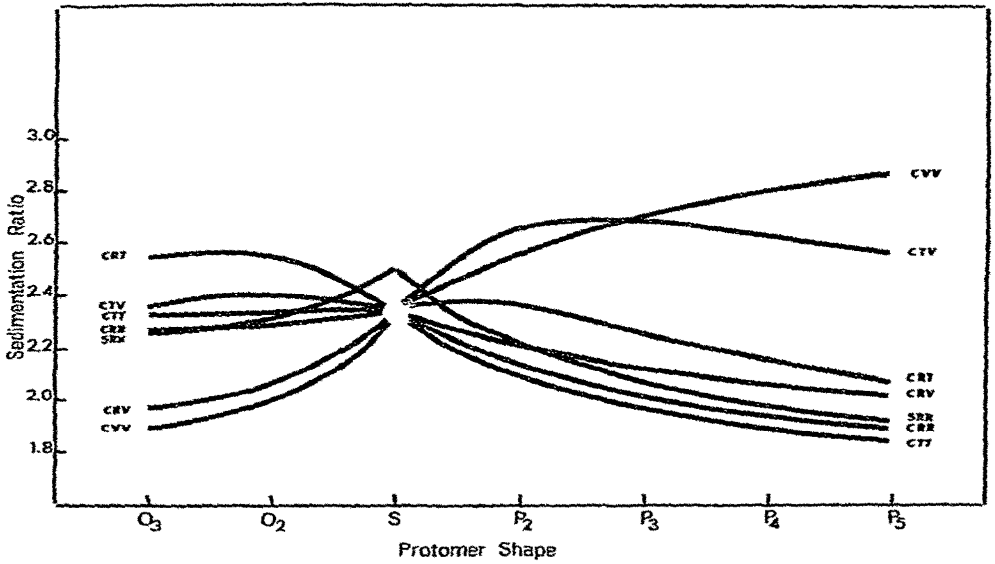

Fig. 6. Calculated sedimentation ratio versus protomer shape for tetrameric structures. The structures and nomenclature are plained in the text and illustrated in fig. 1.

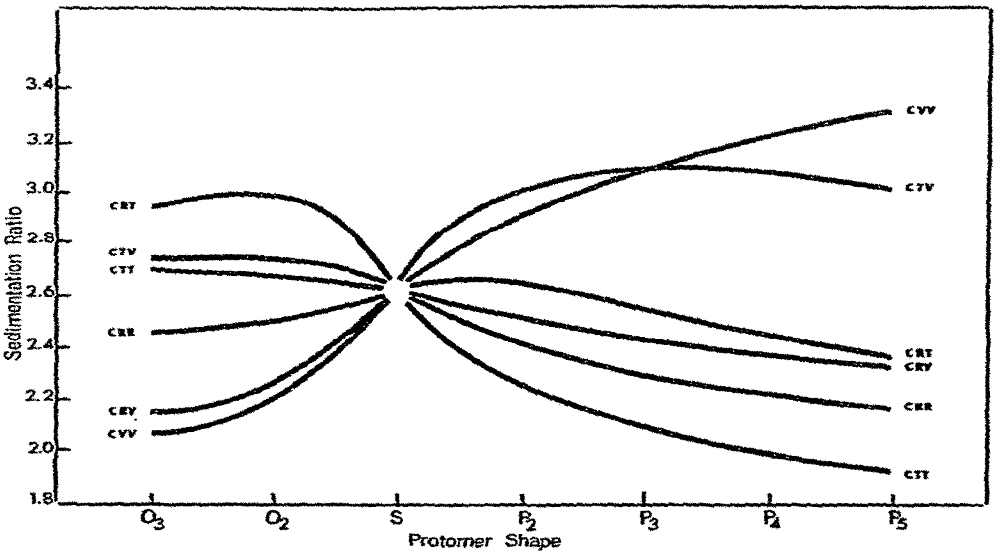

Fig. 7. Calculated sedimentation ratio versus protomer shape for pentameric structures. The structures and nomencl plained in the text and illustrated in fig. 1.

Table 1
Reduced dimensions for dimeric structures generated by rotation a)

Structure a)	Protomer type						
	O_3	O_2	S	P_2	P_3	P_4	P_5
Hr0	1.789	1.563	1.241	1.970	2.581	3.126	3.628
	1.193	1.563	2.481	1.970	1.721	1.563	1.451
	1.789	1.563	1.241	0.985	0.860	0.782	0.726
Hr45	2.018	1.862		2.346	2.910	3.426	3.906
	1.991	2.056		2.591	2.872	3.162	3.448
	1.789	1.563		0.985	0.860	0.782	0.726
Hr90	2.211	2.116		2.666	3.189	3.679	4.141
	2.211	2.116		2.666	3.189	3.679	4.141
	1.789	1.563		0.985	0.860	0.782	0.726
Hr135	3.184	2.838		3.576	4.592	5.507	6.351
	1.668	1.633		2.058	2.406	2.739	3.056
	1.789	1.563		0.985	0.860	0.782	0.726
Hr180	1.789	3.126		3.939	5.162	6.253	7.256
	3.579	1.563		0.985	0.860	0.782	0.726
	0.597	0.782		0.985	0.860	0.782	0.726
Vr45	1.789	3.126		1.970	2.581	1.563	3.628
	3.579	1.563		1.970	1.721	3.126	1.451
	1.440	1.334		1.681	2.077	2.440	2.778
Vr90	1.789	3.126		1.970	2.581	1.563	3.628
	3.579	1.563		1.970	1.721	3.126	1.451
	1.789	1.563		1.970	2.581	3.126	3.628

a) The calculation of real dimensions from reduced dimensions is explained in the text, the forms of the structures and the nomenclature in the text and in fig. 2. It may be noted that Vr0 ≡ Vr180 ≡ Hr0 and Vr135 ≡ Vr45.

sured sedimentation coefficients. The usual method for deducing the likely protomer shape is that devised by Oncley [5] which is based on a calculation of the frictional ratio of the protomer, defined as the ratio of the experimentally determined translational frictional coefficient to that of a sphere of the same molecular weight and anhydrous volume as the particle of interest. A knowledge of the degree of hydration, or a reasonable assumption as to its value, then allows expression of the asymmetry of the particle in terms of an appropriate prolate or oblate ellipsoid of revolution, conveniently by the use of the contour diagram provided by Oncley [5]. The same procedure, and indeed the same diagram, can be employed in approximating real particles by the protomer shapes employed in the present work. Calculation of a contour diagram for the prolate and oblate assemblies of spheres shows that, for the same frictional ratio and degree of hydration, the axial ratios derived from the two diagrams do not differ significantly. An alternative method for determining protomer shapes in terms of ellipsoids of revolution by a combination of the translational frictional coefficient with the co-volume of the particle, measured by sedimentation equilibrium, has recently been published [6]. This has the advantage of not requiring a measurement of the hydration and of distinguishing between oblate and prolate shapes.

The availability of reduced dimensions for model structures (tables 1–5) allows ready comparison of their sizes with those obtained by other methods for a molecule under study, and, thereby, reduces the number of possible assembly patterns. The numbers quoted for each structure in the tables refer to the dimensions, in three mutually perpendicular directions, of an assembly of protomers each of volume 1 cubic Ångstrom. To obtain the actual dimensions relevant

Table 2
Reduced dimensions for dimeric structures generated by translation [a)]

Structure [a)]	Protomer type						
	O_3	O_2	S	P_2	P_3	P_4	P_5
Ht1	2.386	2.345		2.954	3.441	3.908	4.353
	1.193	1.563		1.970	1.721	1.563	1.451
	1.789	1.563		0.985	0.860	0.782	0.726
Ht2	2.982			–	4.301	4.690	5.079
	1.193			–	1.721	1.563	1.451
	1.789			–	0.860	0.782	0.726
Ht3	–			–	–	5.471	5.805
	–			–	–	1.563	1.451
	–			–	–	0.782	0.726
Ht4	–			–	–	–	6.530
	–			–	–	–	1.451
	–			–	–	–	0.726

[a)] The calculation of real dimensions from reduced dimensions is explained in the text. The forms of the structures and the nomenclature, in the text and in fig. 2. The dashes signify structures which do not exist because the translation is equal to or greater than the axial ratio of the protomer. It may be noted that Ht0 ≡ Hr0, which is included in table 1.

Table 3
Reduced dimensions for trimeric structures [a)]

Structure [a)]	Protomer type						
	O_3	O_2	S	P_2	P_3	P_4	P_5
CVV	4.452	3.699	2.481	1.970	1.721	1.563	1.451
	3.936	3.308	2.315	1.838	1.605	1.459	1.354
	0.597	0.782	1.240	1.970	2.581	3.126	3.628
CRR	2.386	2.345		3.675	4.701	5.625	6.478
	2.146	2.135		3.315	4.186	4.976	5.707
	1.789	1.563		0.985	0.860	0.782	0.726
CTT	3.259	2.917		2.954	3.441	3.908	4.354
	2.903	2.631		2.690	3.095	3.489	3.867
	1.789	1.563		0.985	0.860	0.782	0.726
CTV	3.681	3.183		1.970	2.151	2.736	3.265
	3.633	3.110		1.970	2.581	3.126	3.628
	1.440	1.334		2.378	2.685	2.992	3.291
CRV	3.847	3.302		3.176	3.828	4.435	5.006
	3.412	2.964		2.882	3.430	3.946	4.432
	1.440	1.334		1.681	2.077	2.440	2.778
CRT	2.684	2.345		2.954	3.871	4.689	5.442
	2.663	2.135		2.690	2.840	4.843	5.753
	1.789	1.563		0.985	0.860	0.782	0.726

[a)] The calculation of real dimensions from reduced dimensions is explainedin the text, the forms of the structures and the nomenclature, in the text and in fig. 1.

to a molecule being studied the reduced dimensions are multiplied by the cube root, in Ångstrom units, of its protomer volume, as calculated from the molecular weight and partial specific volume.

Fig. 8 summarizes the results of applying eq. (1) to the entire set of structures considered for assemblies of two, three, four, five, and six oblate, spherical, and prolate protomers. The sedimentation ratios of linear assemblies of spherical protomers are included for comparison. Although, of course, these aggregates do not qualify as structures with protomers in spatially equivalent positions, the sedimentation ratio values may be useful in other contexts, for example, they allow calculations of the sedimentation ratio with respect to a spherical protomer, of any of the assemblies built from prolate (linear) protomers. For this reason their numerical values are listed in table 6 along with the sedimentation ratios of O_3 and O_2 oblate structures which allow calculation of the sedimentation ratios, with respect to the individual spheres, of all of the structures built up from oblate protomers. It is clear from fig. 8 that while upper and lower limits for the sedimentation coefficient of an oligomer may be predicted if the protomers are known to be spherical, these limits do not apply for other protomer shapes. The overlap in the ranges of values for the sedimentation ratios of different oligomers indicates that though, in certain cases, it might be possible to use experimental sedimentation ratios as evidence for the number of protomers in an oligomer, it generally is not safe to do so in the absence of other information. Similarly, prediction of the sedimentation coefficient of an oligomer from that of a protomer (or vice versa) is hazardous even when the degree of polymerization is known, unless the mode of assembly is known also. This should be taken into account in applying the relationship $S(\text{oligomer}) = n^{2/3}S(\text{protomer})$ where n is the number of protomers in the oligomer. The dashed line in fig. 8 indicates that sedimentation coefficients calculated from this relationship are placed relatively high in the ranges obtained for the models considered here. Thus, if anything, sedimentation coefficients estimated for

Table 4
Reduced dimensions for tetrameric structures [a)]

Structure [a)]	Protomer type						
	O_3	O_2	S	P_2	P_3	P_4	P_5
CVV	5.019	4.232	2.481	1.970	1.721	1.563	1.451
	5.019	4.232	2.481	1.970	1.721	1.563	1.451
	0.597	0.782	1.241	1.970	2.581	3.126	3.628
CRR	2.633	2.669		3.362	4.154	4.879	5.556
	2.633	2.669		3.362	4.154	4.879	5.556
	1.789	1.563		0.985	0.860	0.782	0.726
CTT	2.880	2.669		3.362	3.797	4.232	4.654
	2.880	2.669		3.362	3.797	4.232	4.654
	1.789	1.563		0.985	0.860	0.782	0.726
CTV	3.891	3.331		2.376	2.431	2.532	2.902
	3.891	3.331		2.376	2.431	2.532	2.902
	1.600	1.439		1.813	2.307	2.754	3.167
CRV	4.320	3.774		2.954	3.441	3.908	4.354
	4.320	3.774		2.954	3.441	3.908	4.354
	1.440	1.334		1.681	2.077	2.440	2.778
CRT	3.259	2.917		3.675	4.701	5.625	6.478
	3.259	2.917		3.675	4.701	5.625	6.478
	1.789	1.563		0.985	0.860	0.782	0.726
SRR	2.656	2.399	2.254	3.103	3.858	4.548	5.190
	2.907	2.638	2.315	3.230	4.038	4.774	5.458
	3.118	2.762	2.481	3.577	4.530	5.392	6.190

[a)] The calculation of real dimensions from reduced dimensions is explained in the text, the forms of the structures and the nomenclature, in the text and in fig. 1.

Table 5
Reduced dimensions for pentameric structures [a)]

Structure [a)]	Protomer type						
	O_3	O_2	S	P_2	P_3	P_4	P_5
CVV	5.761	4.798	3.248	2.578	2.252	2.046	1.900
	5.508	4.601	3.150	2.500	2.184	1.984	1.842
	0.597	0.782	1.241	1.970	2.581	3.126	3.628
CRR	3.492	3.311		4.451	5.525	6.506	7.420
	3.350	3.187		4.282	5.297	6.226	7.093
	1.789	1.563		0.985	0.860	0.782	0.726
CTT	3.831	3.533		4.172	5.036	5.840	6.596
	3.673	3.398		4.016	4.832	5.593	6.308
	1.789	1.563		0.985	0.860	0.782	0.726
CTV	3.904	3.460		2.578	2.682	3.069	3.436
	3.977	3.598		2.660	2.690	3.012	3.486
	1.611	1.447		1.822	2.324	2.776	3.194
CRV	5.096	4.362		3.903	4.566	5.200	5.803
	4.876	4.187		3.760	4.385	4.984	5.555
	1.440	1.334		1.681	2.077	2.440	2.778
CRT	3.342	3.092		3.896	4.821	5.667	6.579
	3.383	2.979		3.754	4.880	5.888	6.816
	1.789	1.563		0.985	0.860	0.782	0.726

[a)] The calculation of real dimensions from reduced dimensions is explained in the text, the forms of the structures and the nomenclature, in the text and in fig. 1.

Table 6
Sedimentation ratios of prolate and oblate assemblies of spheres

Protomer shape	Number of spheres	Sedimentation ratio [a)]
O_3	9	3.771
O_2	4	2.353
P_2	2	1.500
P_3	3	1.833
P_4	4	2.083
P_5	5	2.283
P_6	6	2.450

[a)] Since values are calculated with respect to a spherical subunit they may be used to calculate the sedimentation ratio with respect to a spherical subunit of any of the aggregated structures considered.

real protein oligomers on this basis are likely to be high. Conversely, use of the relationship to estimate n from the sedimentation coefficients of oligomer and protomer may tend to give low values.

It may readily be shown that the sedimentation ratio, SR, is given by the expression

$$\mathrm{SR} = \frac{n^{2/3}(f/f^0)_{\mathrm{prot}}}{(f/f^0)_{\mathrm{olig}}}\,, \qquad (2)$$

where $(f/f^0)_{\mathrm{prot}}$ and $(f/f^0)_{\mathrm{olig}}$ are the frictional ratios of protomer and oligomer respectively. The sedimentation coefficients of oligomer and protomer are thus related by $n^{2/3}$ only when their frictional ratios are identical. This implies that the relationship with generally hold good only when the conformations of the protomer and oligomer are the same, although it could be noted by reference to figs. 3–7 that some structures may fortuitously obey the relationship even though protomer and oligomer differ in their conformations. It is sometimes said that use of the $n^{2/3}$ re-

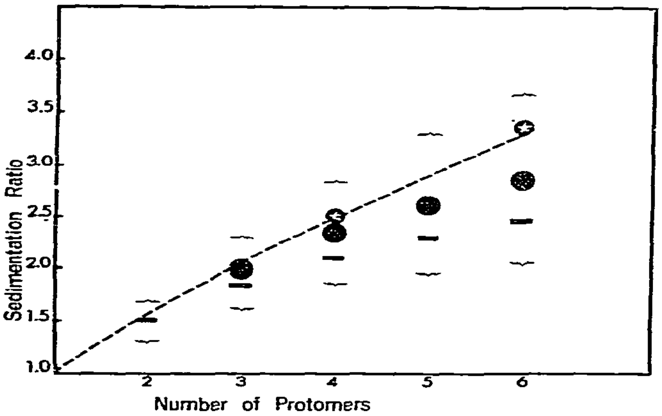

Fig. 8. Calculated sedimentation ratios for oligomers composed of 2, 3, 4, 5 or 6 identical protomers. The braces indicate the upper and lower extremes for the structures considered in the present work and in [1]. The values for spherical, cyclic and linear aggregates of spherical protomers are denoted by the symbols *, o, and ——, respectively. The dashed line shows the sedimentation ratio for the relationship $S(\text{oligomer}) = n^{2/3}S(\text{protomer})$.

lationship assumes no conformational change accompanies aggregation. This is clearly misleading since the aggregation of identical protomers to produce an oligomer of the same shape and axial ratio could generally be accomplished only by a considerable conformational change of the subunits in the aggregated form.

After minor rearrangement, eq. (2) may be used to calculate the frictional ratio of any structure considered in this paper since the value of SR can be read from the appropriate graph and the frictional ratio of the relevant protomer is available from fig. 1 of Oncley [5]. These frictional ratios, while of some interest in relation to those documented by Oncley [5] for ellipsoids of revolution, are of limited practical use and for that reason have not been tabulated here. Thus, for example, a CTT tetramer assembled from P_3 protomers is found to have $f/f^0 = 1.75$ and, as fig. 1 shows, the structure resembles an oblate ellipsoid of "axial ratio" about 4 : 1. However, an *experimental* value of $f/f^0 = 1.75$, even considered only in terms of ellipsoids of revolution, is consistent with structures ranging from spheres through to prolate ellipsoids of axial ratio about 15 : 1 and oblate ellipsoids of axial ratio in excess of 17 : 1 depending on the hydration (Oncley [5]).

Obviously there are limitations in the application of the sedimentation ratio approach to the description of oligomer geometry. Perhaps the most significan may be listed:

(1) The approximation of the shapes of biological molecules by ellipsoids of revolution or assemblages of spheres.

(2) The necessity for measuring sedimentation coefficients of oligomer and protomer under identical conditions.

(3) The requirement that no significant change in conformation of the protomers accompanies their aggregation.

(4) Experimental error leading to uncertainties in axial ratio and sedimentation ratio.

(5) Uncertainty in how well the equations on whic eq. (1) is based reflect the true hydrodynamic behaviour of the models to which it is applied [7]. It is known for example, that use of the pre-averaged Oseen tensor to calculate frictional coefficients, as in the present work, does give incorrect results if the spherical beads are too close together [8]. This is likely to be a relatively more serious effect in the smaller protomers since long range interactions dominate the values of the frictional coefficients calculated for mor extended structures, as exemplified by the findings that random removal of even quite large number of spherical subunits from spherical and cylindrical models has very little effect on the calculated frictional coefficients [7]. Thus, errors might not cancel out in ratios of calculated sedimentation coefficients. Fortunately, what evidence is available suggests that the errors are not large.

In the simplest possible example, that of two identical spheres in contact, the sedimentation ratio obtained by the present method is only 3% lower than the accurate value given by Happel and Brenner [9]. At the other end of the scale it is more difficult to be definitive about the possible error since exact data are not available for complex models. However McCamm and Deutch [7] have calculated averaged translational frictional coefficients by three methods for a linear trimer composed of identical spheres, and for a three-layered hexagonal 18-mer composed of the same spherical subunits. Since this latter is the equivalent of our hexameric CVV structure formed from P_3 protomers (the linear trimers) and, moreover, since one of McCammon and Deutch's computational methods is identical with ours (pre-averaged Oseen tensor, shell model with identical spherical subunits) we can express their answers as sedimentation ratios and compare them. The results are that the sedimentation ratio calculated for this hexameric structure are 3.40 using the pre-averaged Oseen tensor, and 3.28 using either the Oseen or modified Oseen approach. That is to say the present approach gives a result 3% higher than the others. On the basis of these two examples we conclude that the errors in our calculated sedimentation ratios is likely to be of the order of only about ±3%, similar in magnitude to the experimental error in conventionally measured sedimentation coefficients (about 0.05—0.1 Svedbergs).

The sedimentation ratio information embodied in the figures and tables may be useful in two broad applications. First, in the absence of any other information about the structure of an oligomer, it can be applied to eliminate all but a limited number of possibilities. Secondly, in conjunction with data from other techniques it may allow specification of just one structure consistent with all the data, or suggest differences in solution and other structures warranting further investigation. The sedimentation ratios supplied in the figures should also be useful for assigning sedimentation coefficients to polymeric forms in the analysis of weight average sedimentation coefficient data from rapidly interacting protein systems. In the following paper the utility of the method, including the kind of information about aggregated structure which one may reasonably expect from it in practice, is explored with the aid of data for specific oligomeric proteins.

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