Description of the Quaternary Structure of Tetrameric Proteins

FORMS THAT SHOW EITHER RIGHT-HANDED OR LEFT-HANDED SYMMETRY AT THE SUBUNIT LEVEL

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A method is described for comparing the shapes of tetrameric proteins whose three-dimensional structure is known. The centres of mass of single subunits are calculated as Cartesian co-ordinates with respect to their three dyad axes. The axes are allocated on the basis of the extent of the intersubunit contacts that they relate. This results in the division of proteins into two classes called right-handed and left-handed. A second division, which also contains right-handed and left-handed forms, is made according to the distances between the centres of mass of the subunits measured across the two axes with the most extensive contacts. Two other parameters have been calculated from the coordinates; they are named 'aplanarity' and 'twist'. The eight tetramers so far investigated are discussed. One, lactate dehydrogenase, cannot be treated in this way. Among the others, right-handed structures (according to both definitions) are found to be commoner; most have low twist; all are of fairly high aplanarity except phosphoglycerate mutase. Prealbumin is exceptional, being left-handed in both ways and of high twist; it has a figure-of-eight structure with the centres of mass lying in one plane. The changes in the quaternary structure of haemoglobin are also presented by using this approach; on deoxygenation the aplanarity and the twist decrease.

The majority of soluble proteins exist as dimers or tetramers (Klotz *et al.*, 1975). Now that the study of the three-dimensional structure of several tetrameric proteins is complete [reviewed by Matthews (1977) and by Blake (1975)], comparisons of their quaternary structure may be made. For example, all known tetramers with identical subunits have been found to have dihedral rather than cyclic symmetry (Klug, 1967); also it may be stated that the tetramers of haemoglobin (Perutz *et al.*, 1968; Ladner *et al.*, 1977), lactate dehydrogenase (Rossmann *et al.*, 1973), concanavalin A (Reeke *et al.*, 1975; Hardman & Ainsworth, 1972) and glyceraldehyde 3-phosphate dehydrogenase (Buehner *et al.*, 1974; Moras *et al.*, 1975) have an approximately tetrahedral shape, whereas yeast phosphoglycerate mutase (Campbell *et al.*, 1972, 1974) and prealbumin (Blake *et al.*, 1978) are almost planar in structure; the tetramers of pyruvate kinase (Stammers & Muirhead, 1975, 1977) and phosphofructokinase (Evans & Hudson, 1979) have shapes that lie between these two extremes. It is the purpose of the present paper to put such comparisons on a more quantitative basis. As part of some brief

suggestions [Milner-White, 1971; 1972 (correction: line 25, change 'bisecting ϕ_A ' to 'bisecting θ_A '); Milner-White & Spain, 1972], it was proposed that the description of quaternary structure is facilitated by designating one point in each protomer as its centre. The centre of mass can best act as this point, since it corresponds to the position of the bulk of the subunit. Where possible the co-ordinates of the centre of mass of one of the protomers has been calculated from the atomic co-ordinates taken from the Protein Data Bank (Bernstein *et al.*, 1977).

Methods

The right-handed Cartesian co-ordinates of the centres of mass of single subunits are given in Table 2 (below); their origin lies at the intersection of the dyad axes of the tetramer. The SPSS (Statistical Package for the Social Sciences) package was used on an I.C.L. 2976 computer to calculate the centres of mass from the atomic co-ordinates. For pyruvate kinase, complete data were not available; therefore the positions of the centres of mass were estimated by visual inspection of the Kendrew model at the

Table 1. Assignment of axes according to the extent of the intersubunit interactions that they relate

For a given protein each of the three axes of symmetry is designated x' , y' or z' . The symbols or letters used for these axes are those of the authors indicated at the right. For foetal deoxyhaemoglobin (Frier & Perutz, 1977) the γ -subunit corresponds to the β -subunit in the other haemoglobins. The axes for lactate dehydrogenase were chosen by the supposed analogy with glyceraldehyde 3-phosphate dehydrogenase according to the labelling scheme of Rossmann *et al.* (1973); otherwise assignment is difficult. The x' - and y' -axes of pyruvate kinase, yeast phosphoglycerate mutase and phosphofructokinase are assigned arbitrarily because the extent of the contacts is uncertain at present. Although the assignment is on the basis of the number of atoms in contact, some chemical features of these interactions are indicated in parentheses thus: (G), hydrophobic interactions with some hydrogen bonds; (F), similar interactions between two faces of β -sheet domains; (H), hydrogen bonds between antiparallel strands at the edges of β -sheet domains. The interaction across the R-axis of lactate dehydrogenase consists mainly of an arm formed by residues 1–22; that across the R-axis of glyceraldehyde 3-phosphate dehydrogenase involves a loop formed by residues 179–205; in both cases the arm or loop exists as a projection that is deeply embedded in the symmetry-related subunit.

Axes	x'		y'		z'	
Concanavalin A	z	(H)	y	(F)	x	Reeke <i>et al.</i> (1975)
	X		Y		Z	Hardman & Ainsworth (1972)
Glyceraldehyde 3-phosphate dehydrogenase	P	(F)	R	(G loop)	Q	(G) Buehner <i>et al.</i> (1974)
Haemoglobin	a	(G)	c	(G)	b	Ladner <i>et al.</i> (1977)
	X		Z		Y	Chothia <i>et al.</i> (1976)
(axes or pseudo-axes between subunits)	$\left\{ \begin{matrix} \alpha_1\beta_1 \\ \alpha_2\beta_2 \end{matrix} \right\}$		$\left\{ \begin{matrix} \alpha_1\beta_2 \\ \alpha_2\beta_1 \end{matrix} \right\}$		$\left\{ \begin{matrix} \alpha\alpha \\ \beta\beta \end{matrix} \right\}$	Perutz (1969)
Lactate dehydrogenase	P	(F)	R	(G arm)	Q	(G) Rossmann <i>et al.</i> (1973)
Phosphofructokinase	x	(G)	z	(H and G)	y	Evans & Hudson (1979)
Phosphoglycerate mutase	Z	(H and G)	Y	(G)	X	Campbell <i>et al.</i> (1974)
(axes between subunits)	$\left\{ \begin{matrix} \text{I-II} \\ \text{Ia-IIa} \end{matrix} \right\}$		$\left\{ \begin{matrix} \text{I-Ia} \\ \text{II-IIa} \end{matrix} \right\}$		$\left\{ \begin{matrix} \text{Ia-II} \\ \text{I-IIa} \end{matrix} \right\}$	
Prealbumin	y	(H)	x	(G)	z	Blake <i>et al.</i> (1978)
Pyruvate kinase	c	(G)	b	(H and G)	a	Stammers & Muirhead (1977)

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The x' -axis is defined as that which relates the subunit interaction with the largest number of atoms in contact; the y' -axis is that passing through the next most extensive interaction and the z' -axis corresponds to the weakest subunit interaction. The allocations of the axes according to this system are given in Table 1. The x' and y' co-ordinates are taken as positive so that the value of the z' co-ordinate may be either positive or negative (see below). Further details are considered in the Legend to Table 2 (below).

Results and Discussion

Right-handed and left-handed forms of one kind

Eight tetrameric proteins have been studied by X-ray crystallography and are considered in the present paper. Proteins with dihedral symmetry may have either two or three types of intersubunit contact corresponding to the three dyad axes. In lactate dehydrogenase the three contacts present all involve similar numbers of atoms in contact (Adams *et al.*, 1972); in this case it is not possible to ascribe any hand to it, although it has been included in Table 2 by arbitrarily assigning the axes. In all the other tetramers investigated in detail, one of the three

Fig. 1. Shapes of tetramers, showing R_A and L_A forms. Straight lines have been drawn to connect the centres of mass of the subunits. The thick lines represent the more extensive interactions (across the x' -axis); the thin lines mark the somewhat less extensive contacts (across the y' -axis). The tetramers are viewed along their z' -axes, defined as that across which there is least subunit-subunit interaction. To distinguish between right- and left-handed forms, tetramers are positioned in the diagram so that the thick and thin lines are orientated vertically and horizontally respectively.

possible interactions is either lacking or involves only a few residues. The corresponding axis of symmetry is designated as the z' -axis.

The x' -axis and y' -axis are likewise distinguished by choosing the more extensive subunit interaction for the x' -axis. In four of the seven remaining proteins the choice is a clear one, but, for pyruvate

kinase, yeast phosphoglycerate mutase and phosphofructokinase, structure determination is insufficiently advanced to permit such a judgement. Under suitable dissociating conditions, concanavalin A

Table 2. Co-ordinates of the centres of mass of the identical subunits of various tetramers, giving right-handed and left-handed forms

These co-ordinates are expressed relative to the dyad axes of the tetramer. The axes were chosen on the basis of the number of atoms of different subunits in contact across them; the assignments are given in Table 1. The significance of the 'twist' and 'flatness' parameters are discussed in the text. In calculating the centres of mass, a program was written in order to allow for the atomic weight of each atom and for the hydrogen atoms covalently linked to each of the heavier atoms at pH 7. Prosthetic groups such as haem and tightly bound metal ions were included, but substrates and coenzymes, even when bound, were not. The centres of mass for pyruvate kinase were obtained by visual inspection of models of the protein and are therefore only approximate. Those for yeast phosphoglycerate mutase and phosphofructokinase were computed from the main chain α -carbon co-ordinates, these being the only ones available; the co-ordinates for phosphofructokinase were kindly given by Dr. P. R. Evans. For glyceraldehyde 3-phosphate dehydrogenase and haemoglobin, where two out of three axes are not true axes, the positions of the pseudo-axes were estimated by assuming that they pass through a point midway between the centres of mass of adjacent subunits. This involves a rotation of the axes corresponding to x' and y' about the z' -axis (the true axis in each case); since the rotations are somewhat different for the two axes, the average rotation was calculated and used to generate the optimum position of the new axes. For glyceraldehyde 3-phosphate dehydrogenase the colour code of Moras *et al.* (1975) is used to refer to the different subunits. About 10% of the sequence of prealbumin cannot be detected by crystallography and has been omitted; some sequences are also missing in other proteins. The co-ordinates of the bound form of lactate dehydrogenase are based on an earlier and substantially incorrect sequence of the protein; therefore the two sets of co-ordinates cannot usefully be compared. The two sets of co-ordinates given for concanavalin are those of the two groups that have worked on this protein (see Table 1). The hand of the co-ordinates of concanavalin does not agree with Fig. 5 of the paper by Reeke *et al.* (1975). However, it appears (Dr. G. N. Reeke, personal communication) that the numerals III and IV in this Figure should be reversed.

	Co-ordinates ($\text{\AA}$)*			Hand [right (R) or left (L)] [A-form]	Aplanarity parameter $\frac{z'}{\sqrt{(x')^2 + (y')^2}}$	Hand [right (R) or left (L)] [B-form]	'Twist' parameter $\frac{z'(x' - y')}{(x')^2 + (y')^2}$
	x'	y'	z'				
Concanavalin A (jack bean) (Reeke <i>et al.</i> , 1975)	13.7	13.5	+11.5	R	+0.60	(R)	+0.006
(Hardman & Ainsworth, 1972)	14.0	13.7	+11.5	R	+0.59	(R)	+0.009
Glyceraldehyde 3-phosphate dehydrogenase (lobster)							
(Green subunit)	15.4	13.3	+15.1	R	+0.74	R	+0.08
(Red subunit)	14.9	12.7	+15.1	R	+0.77	R	+0.09
Haemoglobin (horse met-)							
α	13.3	8.0	+ 8.8	R	+0.57	R	+0.19
β	13.4	9.1	+ 8.8	R	+0.55	R	+0.14
Haemoglobin (horse deoxy-)							
α	13.1	8.8	+ 8.2	R	+0.52	R	+0.14
β	13.8	10.0	+ 8.2	R	+0.48	R	+0.10
Haemoglobin (human deoxy-)							
α	13.0	8.7	+ 8.0	R	+0.51	R	+0.14
β	13.7	10.4	+ 8.0	R	+0.47	R	+0.09
Haemoglobin (human foetal deoxy-)							
α	13.0	8.7	+ 8.2	R	+0.52	R	+0.14
γ	13.9	10.3	+ 8.2	R	+0.47	R	+0.10
Lactate dehydrogenase (dogfish)							
(No substrates)	12.8	10.8	-15.0	—	-0.90	—	-0.11
(Plus NAD-pyruvate complex)	12.7	10.6	-15.6	—	-0.94	—	-0.12
Phosphofructokinase (<i>Bacillus stearothermophilus</i>)	16.0	11.5	+ 7.7	(R)	+0.13	R	+0.09
Phosphoglycerate mutase (yeast)	16.7	16.8	+ 3.2	(R)	+0.14	(L)	-0.001
Prealbumin (human)	13.1	1.65	- 9.8	L	-0.73	L	-0.64
Pyruvate kinase (cat muscle)	20	9	+ 8	(R)	+0.36	R	+0.18
A perfect tetrahedron	1	1	1	—	0.71	—	0

* Previous papers have tended to give values in angströms ($\text{\AA}$), and this practice is adopted here. To convert values into S.I. units (nm): $1 \text{ \AA} = 0.1 \text{ nm}$.

(McKenzie *et al.*, 1972), oxyhaemoglobin (Kellett, 1971), glyceraldehyde 3-phosphate dehydrogenase (Osborne & Holloway, 1974) and pyruvate kinase (Cardenas *et al.*, 1977; Cottam *et al.*, 1969) exist as stable dimers; the two subunits are considered to be held together by the more extensive subunit interactions where this is known. With lactate dehydrogenase (Hermann *et al.*, 1979), dimers can only be detected as short-lived intermediates during the reassociation process; for prealbumin (Nilsson *et al.*, 1975) and yeast phosphoglycerate mutase (Spragg *et al.*, 1977) dimers were not detected during dissociation.

Fig. 1 shows that a tetramer for which the x' -, y' - and z' -axes can be defined may appear as one of two forms. These are arbitrarily called right-handed and left-handed (R_A and L_A); R_A forms have positive z' -co-ordinates, whereas those for L_A forms are neg-

ative. It is found that three of the proteins are right-handed, whereas only prealbumin is left-handed. The numerical value of z' is of interest; if the centres of mass all lie in the plane of the axes x' and y' as in Figs. 2(a) and 2(c), the value of z' is zero. The values of $z'/\sqrt{(x')^2 + (y')^2}$ given in Table 2 may be used to compare the non-planarity of molecules of different shapes; it is therefore called 'aplanarity'. Pyruvate kinase and phosphofructokinase have low values for this parameter, whereas that of yeast phosphoglycerate mutase is so low that it is almost planar.

Right-handed and left-handed forms of another kind

Another way to consider the structure of a given tetramer is to compare the distances between the centres of adjacent subunits measured across the

Fig. 2. Shapes of tetramers, showing R_B and L_B forms

As in Fig. 1, tetramers are viewed along their z' -axes and straight lines have been drawn to join the centres of mass of the subunits. To distinguish between right- and left-handed forms, the distances between the centres are measured. The diagram of each tetramer is then positioned so that the longest lines joining the centres lie horizontally and the shortest lines lie vertically. [In (e) and (g) the shorter lines are not seen in the diagram because they lie parallel to the z' -axis, but positioning of the longest lines will suffice.]

two axes that relate the most extensive subunit interactions. This is illustrated in Fig. 2. It is evident that a new set of right- and left-handed forms can be defined. They are designated R_B and L_B . It should be noted that, even when the interactions related by the x' - and y' -axes are of similar extent, the hand can still readily be determined.

A measure of the degree of this right-handed or left-handed effect is given by the values of the parameter z' $(x' - y') / [(x')^2 + (y')^2]$ presented in Table 2. It has been given the name 'twist' because its absolute value increases as structures become more twisted, as in Figs. 2(d) and 2(f) and especially Figs. 2(e) and 2(g). The sign of the parameter is governed by the hand. Haemoglobin, glyceraldehyde 3-phosphate dehydrogenase, phosphofructokinase and pyruvate kinase are right-handed, whereas prealbumin is left-handed. For concanavalin and yeast phosphoglycerate mutase, x' is about equal to y' , so that the right-handed or left-handed effect is very slight. For the other proteins the twist increases in the order: glyceraldehyde 3-phosphate dehydrogenase and phosphofructokinase < haemoglobin < pyruvate kinase < prealbumin.

Prealbumin

Prealbumin is the most extreme example of the structures described above; the centres of mass of its subunits are roughly oriented in a rectangular configuration; they lie on or near the plane encompassing the x' - and z' -axes. The most extensive contacts are therefore not all around the periphery of this rectangle as in Fig. 2(c), but along two sides and the diagonals. This gives rise to what may be called a 'figure-of-eight' shape, with the same configuration as in Fig. 2(g). It may be added that Blake & Oatley (1977) have evidence for two double-helical DNA-binding sites on the surface of this protein that lie across the x' -axis and parallel to the y' -axis. It is noteworthy that a protein with such a large and unique binding site also possesses a most distinctive quaternary structure.

Haemoglobin

Table 2 gives the co-ordinates of the centres of mass of the α - and β -subunits of methaemoglobin [which is assumed to have the same structure as oxyhaemoglobin (Perutz, 1976)], as well as those for deoxyhaemoglobin. On deoxygenation there is found to be an increase in the y' -co-ordinate and a decrease in the z' -co-ordinate as well as smaller changes in the x' -co-ordinates. It should be noted that Muirhead *et al.* (1967) give the co-ordinates of the 'centres of mass of the α and β subunits' in Table 7 of their paper. However, these values refer to the centres of mass of the haem groups, not of the whole subunits. It appears unlikely that any of the data in Table 2

Table 3. Distances between centres of gravity and between haem Fe atoms of different subunits of horse haemoglobins in Å*

	$\alpha_2\beta_2$	$\alpha_2\beta_1$	$\alpha\alpha$	$\beta\beta$
	$\alpha_1\beta_1$	$\alpha_1\beta_2$		
Deoxyhaemoglobin centres	25.1	31.5	31.5	34.1
Methaemoglobin centres	24.6	32.0	31.0	32.4
Deoxyhaemoglobin Fe atoms	36.4	24.6	34.3	39.1
Methaemoglobin Fe atoms	35.0	25.7	35.5	34.2

* See the footnote to Table 2.

have been tabulated previously. Table 3 shows that the changes in the distances between the centres of mass of the subunits on deoxygenation are mostly small (except for that for the β - β contact) compared with the changes in co-ordinates measured along the axes of symmetry (the values in Table 2 should be doubled for purposes of comparison with those in Table 3). Also included in Table 3 are the distances between the haem Fe atoms of met- and deoxy-haemoglobin; the changes on deoxygenation are much greater than for the centres of mass of the subunits.

Muirhead *et al.* (1967), Chothia *et al.* (1976) and Baldwin & Chothia (1979) have demonstrated that changes in the tertiary structure of the subunits are minor compared with changes in the quaternary structure; these can be described by rotations of each $\alpha\beta$ dimer by 13° with respect to the other about an axis that passes through the α -subunits. The changes observed in the positions of the centres of mass of the subunits are consistent with the geometry of these rotations.

The results of Tables 2 and 3 may be summarized as follows: on deoxygenation there is an expansion of haemoglobin by 0.17 nm (1.7 Å) along the y' -axis and a contraction of 0.12 nm (1.2 Å) along the z' -axis; the β -subunits move apart by 0.17 nm (1.7 Å), but other changes are quite small. Resulting changes in shape on deoxygenation are that the aplanarity decreases and the twist decreases to an even greater extent; thus the molecule becomes less strikingly right-handed, both as the A-form and especially as the B-form.

Biochemical significance

Much work has been done to compare the different patterns of secondary and tertiary structure of proteins (e.g. Levitt & Chothia, 1976; Richardson, 1977; Chothia & Janin, 1978). In the present study, interest is focused on the quaternary structure. The handedness that has been described is that resulting from the arrangement of the subunits; it does not apply at the atomic level, where all the constituent amino acids are of the L-type. Whether the predominance of right-handed tetramers that has

been found is merely a coincidence due to the small sample size remains to be shown. However it should prove useful to have assembled together the available information about the shape of these proteins as given in Tables 1 and 2, for example as a means of making evolutionary comparisons between tetramers; the present work suggests that prealbumin is quite different from the other proteins considered. It is also evident that changes in quaternary structure can be analysed by the procedures described in the present paper, as shown for haemoglobin; changes of this sort are thought to form the structural basis of the allosteric mechanism of other regulatory proteins such as phosphofructokinase (Evans & Hudson, 1979).

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