

### Structure of the $\beta$ -Lactoglobulin Tetramer

DETAILED investigations of the association behaviour of the genetic variants of  $\beta$ -lactoglobulin have shown that the A variant aggregates strongly and reversibly to a tetramer<sup>1,2</sup> of 144,000 molecular weight in the cold between pH 3.7 and 5.2. The thermodynamics of this association have established the tetramer structure to be cyclic, involving the closing of four bonds<sup>1,2</sup>, and its hydrodynamic properties<sup>2,3</sup> show it to be compact. A small-angle X-ray scattering examination of this association<sup>4</sup> has led to the conclusion that, of the various general types of possible tetramer structures, a cubic array of eight spheres was the most probable. This conclusion was based on comparison of the measured radius of gyration of the tetramer (34.4 Å) with values calculated for various models formed of four of the monomeric units deduced by Green and Aschaffenburg<sup>5</sup> from X-ray diffraction examination. These monomeric units have been shown to persist in solution<sup>4</sup>. The monomer unit consists of a combination of two identical spheres (of molecular weight 18,000) 17.9 Å in radius, impinging by 2.3 Å along their centre-to-centre axis; these two spheres are, furthermore, related by a dyad axis of symmetry through the plane of contact formed between them. Recently, we have carried out a detailed examination of compact models in the light of known structural requirements, the results of which we wish to report in this communication. The basic requirements are that all four monomer molecules must be identical with each other<sup>2</sup>, the monomer structure must be maintained intact in the tetramer<sup>4</sup>, and the structure must fit all known experimental information and be symmetrical in nature<sup>6</sup>.

First, let us examine the previously proposed structure<sup>4</sup>, that is, the cubic array of eight spheres, shown in Fig. 1A. In this model the four 36,000 molecular weight monomer units are aligned with polar axes vertical, and with the surfaces of contact between the spherical sub-units lying in one plane (Fig. 1B); the four dyad axes of symmetry are in the same plane along the diagonals of the cross-section square. Careful examination of this structure reveals that the closing of four bonds between the monomers in such manner that each spherical sub-unit is linked to one belonging to another monomer molecule, without at the same time violating the dyad axes of symmetry, requires the presence of two pairs of monomers in which the tetramerization sites are disposed as shown in Fig. 1C, that is, each type is the mirror image of the

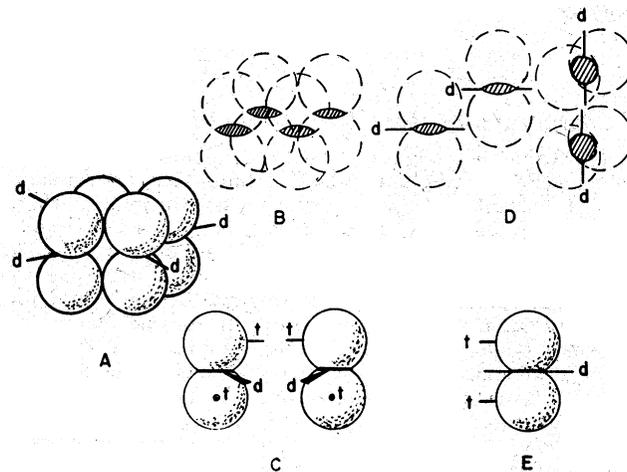


Fig. 1. Cubic models of lactoglobulin tetramer. (A) All four double spheres aligned vertically; (B) same, showing planes of sub-unit contact; (C) monomer structures required to close tetramer bonds in model (A); (D) monomers aligned in two pairs, one vertical, the other horizontal; (E) tetramer bond location on monomers in structure (D). In this and Fig. 2 the dyad axes of symmetry are designated by  $d$ , the tetramer bonds by  $t$ .

other<sup>7</sup>. Since this structure violates the requirement of identity between the four monomers, it must be ruled out. This requirement can be satisfied, however, by the second cubic structure shown in Fig. 1D. In this, one pair of monomers is rotated  $90^\circ$  with respect to the other, the surfaces of sub-unit contact (shown shaded) now lying in pairs in two mutually perpendicular planes; the dyad axes are located in pairs, one pair remaining in the horizontal intersphere plane, the other pair on a vertical line going through the sub-unit contact planes of the monomers the polar axes of which are in the horizontal position. In this arrangement, the two tetramer bonds of a monomer must be located in one plane and directed at a right angle to the polar axis of the two-sphere structure, as shown in Fig. 1E. While this structure satisfies the requirements of structural identity, it lacks overall symmetry and, as such, is probably less likely. Furthermore, this model requires a  $90^\circ$  rotation of each sub-unit along the tetramer bond with respect to its tetramer partner: complementarity, if necessary for this type of bond, would not be compatible with the dyad axis of symmetry.

A symmetrical tetramer structure, satisfying all the requirements, can be constructed by arranging the monomers in an inclined array, which results in the decahedron shown in two views in Fig. 2A and B. Here, the four dyad axes of symmetry,  $d$ , and the four points of tetramer contact,  $t$ , are all in one single plane perpend-

icular to a central tetramer axis,  $T$ . The dyad axes are normal to this central axis. The tetramer bonds lie on the corners of a square; the two bonds on each monomer are located symmetrically on the sub-units and their projection on the plane of sub-unit contact forms a  $90^\circ$  angle. In the tetramer, they are inclined at an angle of  $35^\circ$  with respect to the vertical, the bond from the upper sphere pointing downward, that from the lower sphere upward. Thus, the upper sphere of each monomer forms a tetramer bond with the lower sphere of another monomer. Since the monomeric units are inclined at an angle of  $31^\circ$  with respect to the vertical, the inclination of the tetramer bonds with respect to the polar axis of the monomer is  $66^\circ$ . When viewed along the tetramer axis,  $T$  (Fig. 2A), the centre of each sphere in the upper layer is located asymmetrically between those of the two lower ones with which it is in contact. This arises because of the difference in centre to centre distances:  $33.5 \text{ \AA}$  within the monomer,  $35.8 \text{ \AA}$  along the tetramer bond. The locations of the tetramer bonds and dyad axis in the monomer are shown in Fig. 2C. The three-sphere relationship in the tetramer structure of Fig. 2A-B is identical to the one shown by triangle  $ABC$  in Fig. 2D (in which another possible structure, to be discussed as follows, is depicted); angles  $a$  are  $62^\circ 8'$ , angle  $b$  is  $55^\circ 44'$ . The structure of Fig. 2A-B is characterized by a principal four-fold axis of symmetry,  $T$ , which coincides with the vertical axis through the centre of the structure. Fig. 2A-B, then, has  $422$  symmetry. Furthermore, in this model, intermolecular contacts are limited to the tetramer bond sites plus two further points of contact with neighbouring spheres. A cavity is present along the vertical tetrad axis, with an average diameter of about  $18 \text{ \AA}$ ; this permits access of water molecules, leaving some interior molecular surface in contact with solvent. Since it is quite probable that the surface of the protein molecule contains a high density of polar residues<sup>8</sup>, this structure entails the burial of a minimal amount of polar side-chains in the low dielectric constant interior of the tetramer and, thus, the expenditure of least energy for this purpose<sup>8-11</sup>.

One more structure which should be considered is shown in Fig. 2D. This is derived from the previous one by rotating alternate monomers through  $45^\circ$  in opposite directions about the tetramer bonds. In this structure the monomers are located in two lozenge-shaped arrays of four spheres each, the short diagonals of the two lozenges being in parallel planes and inclined in opposite directions. The lozenges are slightly folded inward along the short diagonal. The four dyad axes of symmetry,  $d$ , remain, as before, in one plane which also passes through the points of tetramer contact. The tetramer bonds,  $t$ , are present here in two conformations, two bonds lying

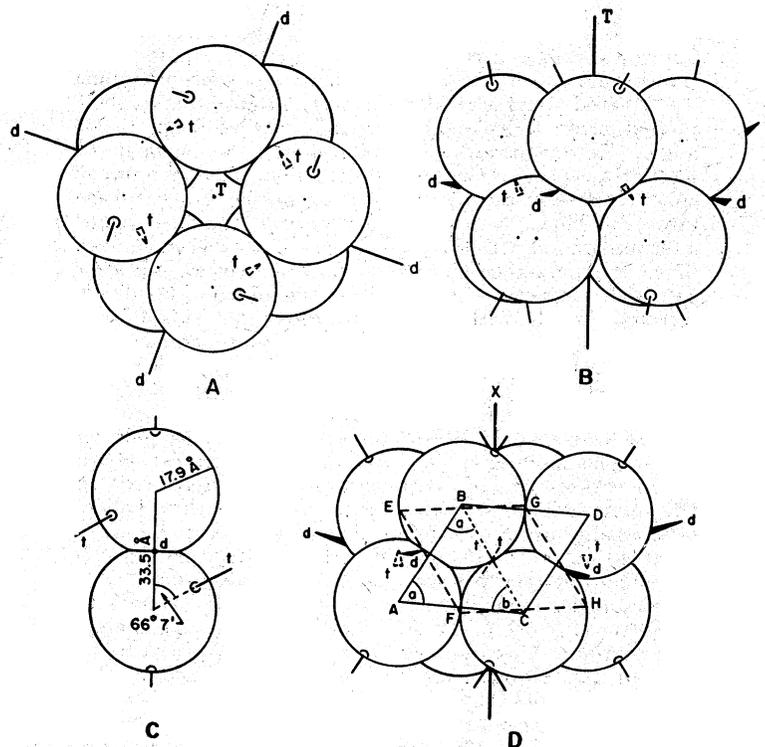


Fig. 2. Staggered tetramer structures. 422 symmetry: (A) top view; (B) side view.  $T$  indicates the tetrad axis of symmetry; (C) monomer structure required by structure (A, B); (D) 222 symmetry:  $X$  indicates overall dyad axis of symmetry; distances  $AB$ ,  $CD$ ,  $EF$ ,  $GH$  = 33.5 Å;  $AE$ ,  $BC$ ,  $FG$ ,  $DH$  (along tetramer bonds) = 35.8 Å. Angles  $a = 62^{\circ} 8'$ ;  $b = 55^{\circ} 44'$ .

along the short diagonals and two perpendicular. The presence of a principal dyad axis of symmetry,  $X$ , gives this structure 222 symmetry. In this structure, however, there is less symmetry than in the previous one. The tetramer bonds exist in two different conformations making structural complementarity across these bonds impossible. Also, the spherical sub-units are in two groups, differing by their environment: four, units  $B$ ,  $C$ ,  $F$  and  $G$ , make contact with five other spheres each; the others,  $A$ ,  $D$ ,  $E$  and  $H$ , make contact with four other sub-units each. Thus, the amount of monomer molecular surface which must be buried in the hydrophobic interior of the tetramer is considerably larger than in structure 2A. As a result, it is considered that structure 2D is less likely to occur, even though it is an isomer of the previous one formed only by rotation about the tetramer bonds. It is of interest to note that the model of Fig. 2D

Table 1. RADII ( $R$ ) OF GYRATION OF TETRAMER MODELS

Model	$R$ (Å)
Cube	33.4
422	33.4
222	33.1
Experimenta	34.4 ± 4

is one of the extremes allowed by the rotation of Fig. 2A about the tetramer bonds (assuming no complementarity); the other extreme (a mirror image) is produced by 90° rotation of 2D in the opposite direction.

The radii of gyration of the three structures have been calculated using the monomer molecular dimensions of Green and Aschaffenburg<sup>5</sup>, with the results shown in Table 1. As can be seen, all three fit the small-angle X-ray scattering data<sup>4</sup> equally well.

On the basis of the foregoing analysis, and in particular the element of symmetry present in each model considered and the energy required to bring polar residues into the low dielectric medium in the interior of the tetramer, the structure of Fig. 2A-B (422 symmetry) is regarded as the favoured one. (The identical tetramer structure has been deduced independently by Dr. D. W. Green (private communication).) Structural fluctuations between it and the two 222 structures (Fig. 2D and its mirror image) are not excluded, although unlikely, since they would destroy complementarity about the tetramer bonds and would involve the expenditure of extra energy in the burial of additional polar groups.

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<sup>1</sup> Townend, R., and Timasheff, S. N., *J. Amer. Chem. Soc.*, **82**, 3168 (1960).

<sup>2</sup> Timasheff, S. N., and Townend, R., *J. Amer. Chem. Soc.*, **83**, 464 (1961).

<sup>3</sup> Townend, R., Winterbottom, R. J., and Timasheff, S. N., *J. Amer. Chem. Soc.*, **82**, 3161 (1960).

<sup>4</sup> Witz, J., Timasheff, S. N., and Luzzati, V., *J. Amer. Chem. Soc.*, **86**, 168 (1964).

<sup>5</sup> Green, D. W., and Aschaffenburg, R., *J. Mol. Biol.*, **1**, 54 (1959).

<sup>6</sup> Herskovits, T. T., Townend, R., and Timasheff, S. N., *J. Amer. Chem. Soc.* (in the press).

<sup>7</sup> The cubic structure (Fig. 1B) could be satisfied also if each monomer sub-unit formed bonds with two sub-units of other monomers on tetramerization. This would require the closing of eight bonds on tetramerization and is thus rather unlikely. In this case, the symmetry requirements would be satisfied only if the eight bonds were of a heterologous, rather than homologous, nature.

<sup>8</sup> Tanford, C., *J. Amer. Chem. Soc.*, **84**, 4240 (1962).

<sup>9</sup> Kauzmann, W., *Adv. in Protein Chem.*, **14**, 1 (1959).

<sup>10</sup> Némethy, G., Steinberg, I. Z., and Scheraga, H. A., *Biopolymers*, **1**, 43 (1963).

<sup>11</sup> Timasheff, S. N., in *Proteins and Their Interactions* (Avi Publishing Co., Westport, Conn., in the press).