Short Communications

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Proposed polypeptide chain configurations and the structure of horse hemoglobin.*

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Proceeding on the basis of the claim by Perutz (1949) that there are in the monoclinic horse methemoglobin crystal parallel sets of rodlike polypeptide chains with a 5 Å repeat, Bragg, Kendrew & Perutz (1950) and Pauling & Corey (1951*b*) have made suggestions regarding the atomic configurations within these chains. In a detailed survey of polypeptide chain configurations in 1950, Bragg *et al.* specially favored their $2_{14}\cdot\frac{1}{3}$ model. In 1951, Pauling & Corey (1951*a*) claimed the α-helix as a 'principal feature' of the horse hemoglobin structure (see also Pauling, Corey & Branson, 1951).

One argument advanced by Pauling & Corey (1951*b*) in favor of the postulated rods being α-helices concerned the well known feature of Perutz's three-dimensional vector map of the horse methemoglobin crystal, namely the high vector density at about 5 Å from the origin. Let us write $h_v(x, y, z)$ for the vector function of an α-helix and $4\pi r^2 h_v(r)$ for the corresponding radial distribution function. Let us also write $g_v(x, y, z)$ for the vector function depicted in the contoured sections of Perutz's vector map some of which are assembled in Fig. 1. From the contoured sections, Pauling & Corey obtained the function $4\pi r^2 g_v(r)$ by numerical integration. Both in the experimental function, as we see from Fig. 1, and in the radial distribution functions obtained by Pauling & Corey for a single α-helix, with the C_β atoms in position 1 and in position 2, there is a pronounced local maximum at about 5 Å from the origin, which is followed by considerably lower values until a distance of about $9\frac{1}{2}$ Å from the origin is reached. It was concluded by Pauling & Corey (1951*b*) that the 'rough agreement' between the curves 'is to be considered as significant'. If we work out the vector function for the $2_{14}\cdot\frac{1}{3}$ chain and deduce the radial distribution function, we again obtain (Fig. 2(*a*)) a curve with a pronounced local maximum at about 5 Å from the origin. Here too some 'rough agreement', at least in regard to the maximum, can be claimed.

The question, however, arises as to the significance of these comparisons. The 'experimental' function $g_v(x, y, z)$ is the transform of relative intensities with the intensity at the origin taken equal to zero: in consequence the actual vector density distribution in the horse methemoglobin crystal is of the form $Ag_v(x, y, z)+B$, where B is the average vector density over the whole crystal. It follows that the actual radial distribution function is not $4\pi r^2 g_v(r)$ but $4\pi r^2 Ag_v(r)+4\pi r^2 B$.

However, there is a second type of comparison, on the same lines, which is less difficult to evaluate. This is the comparison of the mean vector distributions as a function of r, the comparison, that is to say, of $Ag_v(r)+B$ with the spherically smoothed vector functions $h_v(r)$ derived from the α-helix in both forms and from the $2_{14}\cdot\frac{1}{3}$ model.

In Fig. 2(*c*) we depict this function $h_v(r)$ for the α-helix with the C_β atoms in position 1, and in Fig. 2(*b*) for the $2_{14}\cdot\frac{1}{3}$ chain model. These functions are monotonic decreasing over the whole of the ranges shown. This is also the case for the α-helix with the C_β atoms in position 2. However, from the contoured sections of the experimental vector map in Fig. 1, we see that the function $g_v(r)$, and therefore also the function $Ag_v(r)+B$, drop to a minimum at some distance r_0 less than 4 Å, subsequently developing a maximum in the range 4–6 Å. There is therefore a lack of agreement between the experimental function and the functions derived from each of the chain models. Neither the $2_{14}\cdot\frac{1}{3}$ chain model nor the α-helix in either form can account for the large vector density at about 5 Å from the origin in the spherically smoothed experimental vector map.

If, however, we look at the contoured sections of the experimental vector map shown in Fig. 1, it becomes clear that it is beside the point to study the radial distribution function, or even the spherically smoothed vector function, so long as the picture of the horse hemoglobin structure is restricted to a parallel set of rodlike polypeptide chains. Incomparably more information regarding the high vector density at about 5 Å from the origin is contained in the vector function $g_v(x, y, z)$ depicted in the sections than in the function $4\pi r^2 g_v(r)$ or in the function $g_v(r)$. As we see in the sections $60y = 0$–3, $x = 0$ and in the central section normal to the x axis, there is a high vector density at about 4–6 Å from the origin extending over a shell completely surrounding the origin, with five pairs of regions of specially high density within the shell in widely separated directions from the origin. That this feature of the vector map—which Perutz has called the '5 Å shell'—cannot be interpreted in terms of a 5 Å repeat along a set of parallel rodlike polypeptide chains in any direction, no matter how the atomic configurations of the chains are chosen, seems self-evident. The '5 Å shell', the outstanding feature of the experimental vector map, is essentially three-dimensional in character: it follows that its interpretation must be in terms of many pairs of atoms at 4–6 Å apart in a considerable variety of different orientations to one another (Wrinch, 1952*a*, *b*), and cannot be in terms of pairs of atoms restricted to a set of parallel uniaxial (or biaxial) structures.

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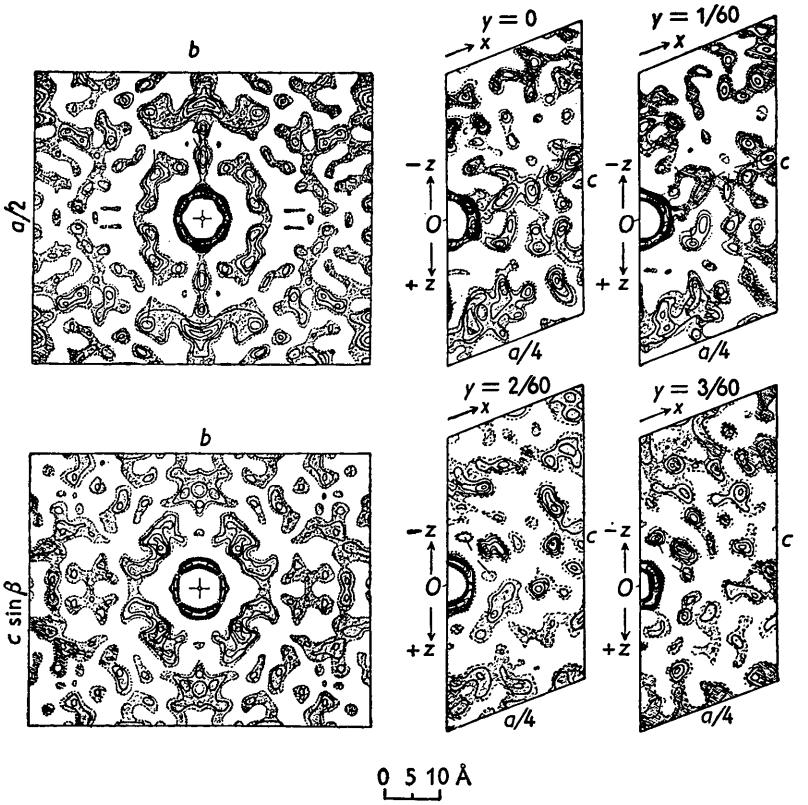

Fig. 1. Sections of the vector map of the normal wet monoclinic horse methemoglobin crystal with space group $C2$, for which $a = 109$, $b = 63{\cdot}2$, $c = 54{\cdot}4$ Å and $\beta = 111°$. The sections, assembled from Perutz's paper (1949), comprise the central sections normal to the a axis and containing the a and b axes, and the sections $60y/b = 0, 1, 2, 3$. (Reproduced by permission of the Royal Society.)

The fact that this statement regarding the rodlike polypeptide chains has been qualified so as to refer specifically to a set of such structures *in parallel* raises the further question as to whether the hypothesis of the presence in the horse hemoglobin structure of either the α-helix or of the $2_{14}\cdot\frac{1}{3}$ model can perhaps be retained by placing them in many or at least several different directions. It is interesting to notice that under these circumstances the spherically smoothed vector functions of the α-helix and of the $2_{14}\cdot\frac{1}{3}$ model shown in Fig. 2 become of considerable interest, since they will also serve, sufficiently near the origin, as the spherically smoothed vector functions for any number of chains in any number of different directions. However, the conclusion to be drawn is exactly as before. No set of chains of either type in any number of different directions can account for the 5 Å maximum in the $g_v(r)$ function obtained from the experimental vector map. That any model of a succession of atoms with atomic numbers as similar as those of carbon, nitrogen and oxygen can achieve this characteristic seems highly questionable. Certainly none of the other models suggested for polypeptide chains in proteins yields this maximum (Bragg *et al.*, 1950; Pauling & Corey, 1951*a*, *b*; Pauling, Corey & Branson, 1951).

In view of the conclusion that no interpretation of the high vector density at about 5 Å from the origin, seen in Perutz's vector map, in terms of 5 Å repeats along rodlike polypeptide chains seems likely to be successful, the claim that there are rodlike polypeptide chains in the horse hemoglobin structures requires careful examination.

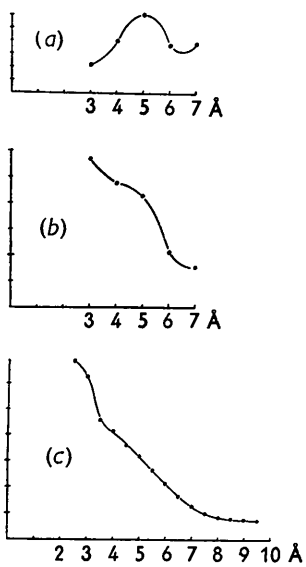

Fig. 2. (*a*) The radial distribution function for the $2_{14}\cdot\frac{1}{3}$ model and (*b*) its spherically smoothed vector function. (*c*) The spherically smoothed vector function for the α-helix with the C_β atoms in position 1.

The study of this claim in the light of the experimental vector map forms the subject of another communication.

References

BRAGG, W. L., KENDREW, J. C. & PERUTZ, M. F. (1950). *Proc. Roy. Soc.* A, **203**, 321.

PAULING, L. & COREY, R. B. (1951*a*). *Proc. Nat. Acad. Sci., Wash.* **37**, 235.

PAULING, L. & COREY, R. B. (1951*b*). *Proc. Nat. Acad. Sci., Wash.* **37**, 282.

PAULING, L., COREY, R. B. & BRANSON, H. R. (1951). *Proc. Nat. Acad. Sci., Wash.* **37**, 205.

PERUTZ, M. F. (1949). *Proc. Roy. Soc.* A, **195**, 474.

WRINCH, D. (1952*a*). *J. Chem. Phys.* **20**, 1051.

WRINCH, D. (1952*b*). *J. Chem. Phys.* **20**, 1332.

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A contribution to the determination of signs in the Fourier analysis of crystals.

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In the preliminary analysis of a crystal structure it may become clear from symmetry or packing that the electron density is low and nearly uniform over certain planes. The writer, in analysing a long-chain fatty acid, was able to conclude from the possible packing that the density was low and nearly uniform over the three bounding planes of a suitably chosen unit cell. A two-dimensional projection of such a cell parallel to one of the axes will have low, uniform density along its bounding lines. The existence of uniform or zero density over certain lines and planes must determine certain relations between the coefficients of the Fourier series giving the density, and this may help to determine their signs.

Consider a two-dimensional projection on the *bc* plane, having a centre of symmetry which is also the origin of coordinates. If (v, w) are the fractional coordinates of a point in the projection in terms of 2π, the density, $\sigma(v, w)$, is given by

$$S\sigma(v, w) = A(0, v)+A(l, v)\cos lw+B(l, v)\sin lw \quad (1)$$

(James, 1948), S being the area of the unit projection, with

$$\left.\begin{aligned} A(0, v) &= F(000)+2\sum_{1}^{k}F(0k0).\cos kv\,, \\ A(l, v) &= 2F(00l)+2\sum_{1}^{k}\{F(0kl)+F(0\bar{k}l)\}\cos kv\,, \\ -B(l, v) &= 2\sum_{1}^{k}\{F(0kl)-F(0\bar{k}l)\}\sin kv\,. \end{aligned}\right\} \quad (2)$$

Suppose $\sigma(v, w)$ to be constant along a line $v =$ const. in the projection throughout the range $w = 0$ to $w = 2\pi$. Then, in the Fourier series (1) $A(l, v)$ and $B(l, v)$ must vanish, while $A(0, v)$ must be constant. If the density is very small or zero, $A(0, v)$ will be small or zero also. We consider the case in which the density is constant along the line $v = 0$. Equations (2) then give

$$\tfrac{1}{2}F(000)+\sum_{1}^{k}F(0k0) = \text{constant}\,, \quad (a)$$

$$F(00l)+\sum_{1}^{k}\{F(0kl)+F(0\bar{k}l)\} = 0 \text{ for any value of } l. \quad (b)$$

We can draw no conclusions from the vanishing of $B(l, v)$ since $\sin kv$ is itself zero.

If the density is uniform or zero along the line $w = 0$, we have the analogous relations

$$\tfrac{1}{2}F(000)+\sum_{1}^{l}F(00l) = \text{constant}\,, \quad (a')$$

$$F(0k0)+\sum_{1}^{l}\{F(0kl)+F(0\bar{k}l)\} = 0, \text{ for any value of } k\,. \quad (b')$$

If the absolute values of F have been determined, conclusions about the signs of the coefficients may be drawn, provided that some of them are already known. In one example many of the signs of $F(00l)$ were fairly certainly known. Equations (*b*) allowed other signs to be determined. One such equation ran

$$\begin{aligned} &F(005)+F(015)+F(0\bar{1}5)+F(025)+F(0\bar{2}5) \\ &\quad -7{\cdot}9 \quad \pm 2{\cdot}3 \quad \pm 2{\cdot}7 \quad 0 \quad 0 \\ &\qquad +F(035)+F(0\bar{3}5)+F(045)+F(0\bar{4}5) = 0\,, \\ &\qquad\quad 0 \quad\quad 0 \quad\quad 0 \quad\quad 0 \end{aligned}$$

from which it is fairly clear that both $F(015)$ and $F(0\bar{1}5)$ are positive.

Prof. R. W. James has pointed out to me that the results can be extended to three dimensions, and to planes of uniform density that do not pass through the origin. For example, if the density is uniform over the plane $w = 0$,

$$F(hk0)+\sum_{1}^{l}\{F(hkl)+F(hk\bar{l})\} = 0$$

for any pair of indices h and k. Each such equation corresponds to the sum of the structure factors along any row of reciprocal-lattice points perpendicular to the plane of constant density. If the density is not zero, the row through the origin must be excluded.

Reference

JAMES, R. W. (1948). *The Optical Principles of the Diffraction of X-rays*, pp. 357, 359. London: Bell.