Acta Cryst. (1954). **7**, 409

X-ray Diffraction by Imidazole Methaemoglobin

By W. L. Bragg* and E. R. Howells†
Cavendish Laboratory, Cambridge, England

(*Received* 11 *February* 1954)

The precession X-ray photographs of imidazole methaemoglobin appear to indicate a structure with orthorhombic symmetry, but it is actually only 'statistically orthorhombic'. The sheets of molecules in the c planes have a definite structure identical with that in monoclinic horse methaemoglobin. Successive sheets, however, are stacked together with a displacement in the a direction which is definite in amount, but of plus or minus sign in a completely random way. The diffraction picture shows an interesting sequence of diffuse and sharp layer lines and also relationships between the intensities of its reflexions and those of the monoclinic form.

1. The X-ray data

Howells & Perutz (1954) have obtained precession X-ray photographs of crystals of horse methaemoglobin in which an imidazole group has been attached to each of the four haem groups in the molecule. These crystals show interesting diffraction effects which indicate a structure of a somewhat novel type. This structure will be described here, and a simple physical explanation will be given of the way the diffraction effects arise; a full analytical treatment and a quantitative comparison between theory and experiment are given in the following paper by Cochran & Howells (1954).

The symmetry of the precession photographs about the three principal axes and the positions of the spots indicate an orthogonal cell whose dimensions are closely related to those of the monoclinic forms of horse methaemoglobin:

	Monoclinic methaemoglobin (E)	Imidazole methaemoglobin
a (Å)	109·2	109
b (Å)	63·2	63
c (Å)	57·4	104
β (°)	110·7	
$c \sin \beta$ (Å)	51·2	

The absences are: general reflexions, $h+k = 2n+1$; $(00l)$ reflexions, $l = 2n+1$. Further, the $00l$ spectra of imidazole methaemoglobin are identical in intensity with those of the monoclinic form at corresponding angles. This correspondence, and the correspondence of the a and b axes, prove that the sheets of molecules in the c planes have an identical arrangement in both crystals.

The orthogonal symmetry of the diffraction pattern shows, however, that molecules in successive c sheets of the crystal cannot be all inclined the same way as in Fig. 1(a), but must be inclined alternately right and left as in Fig. 1(b) and (c). The apparent c axis of 104 Å between similar R molecules, or L molecules, is therefore about twice as great as $c \sin \beta$ for a monoclinic

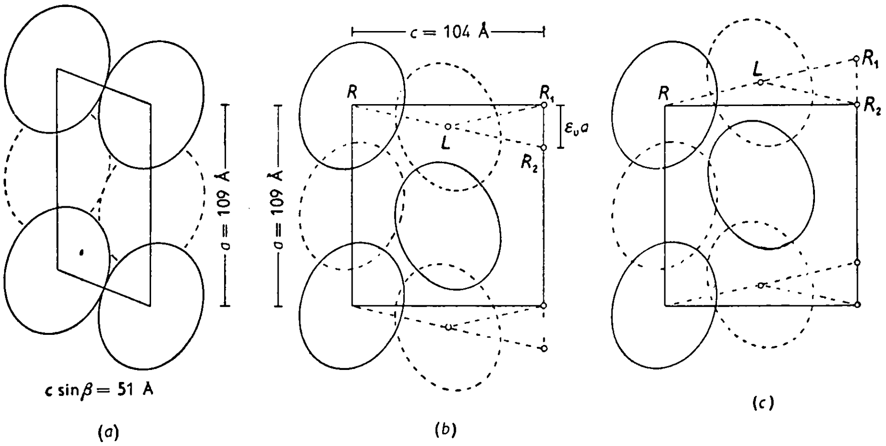

Fig. 1. (a) Monoclinic horse methaemoglobin. (b, c) Relative displacements of c sheets of molecules in imidazole methaemoglobin.

---

* Now at Royal Institution, Albemarle Street, London W. 1, England.

† Now at Imperial Chemical Industries Ltd, Black Fan Road, Welwyn Garden City, Herts., England.

form. It will be shown that successive sheets are displaced relatively to each other in the a direction, and it will be clear from the figure that the contacts between them are the same whether this displacement is downwards, as in Fig. 1(b), or upwards, as in Fig. 1(c), since these two figures are related to each other by a rotation axis parallel to a.

2. Sharpness of the reflexions

The reflexions vary greatly in sharpness from one layer line to another. Fig. 2 is a conventional representation of both the spread of the reflexions along the layer lines and their intensity in the $h0l$ precession picture.

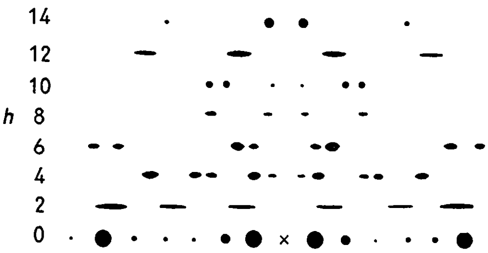

Fig. 2. Diagrammatic representation of the strength and diffuseness of the ($h0l$) reflexions, imidazole methaemoglobin.

Only layer lines with even values of h are present, owing to the centring of the C face.

The extent of the diffuseness follows a regular sequence which can be observed up to the 38th layer line. The sequence repeats as if there were a centre of inversion at layer line 12, and again at layer lines 24 and 36.

The variation of diffuseness up to the 16th layer line is shown in Table 1. Reflexions with odd values of h can be observed as ($h1l$) spectra, and fall into the same sharp and diffuse scheme, as shown in Fig. 3.

Table 1. ($h0l$) *reflexions*

h	Reflexion	l even	l odd	$h\varepsilon_0$	ε
0	Sharp	$2F$	Zero	0	0
2	Very diffuse	—	—	0·42	0·42
4	Moderately sharp	F_R-F_L	F_R+F_L	0·83	0·17
6	Moderately sharp	F_R-F_L	F_R+F_L	1·25	0·25
8	Diffuse	—	—	1·67	0·33
10	Sharp	F_R+F_L	F_R-F_L	2·08	0·08
12	Very diffuse	—	—	2·50	0·50
14	Sharp	F_R-F_L	F_R+F_L	2·92	0·08
16	Diffuse	—	—	3·33	0·33

Perutz has further shown that there is a regular relationship between the intensities of the sharp reflexions and those given by monoclinic methaemoglobin. In the latter case all the molecules are in the same orientation R. The values and signs of F_R along the layer lines of the transform have been completely determined (Howells & Perutz, 1954). The transform of an L molecule is of course identical with the transform of an R molecule, but with its left- and right-hand sides interchanged. Perutz has shown that the values of F for imidazole haemoglobin are either got by adding or by subtracting the two values of F at corresponding points on either side of the monoclinic transform, due regard being paid to their signs, according to the rule shown in Table 1. The values of F (imidazole) are either F_R+F_L for l even and F_R-F_L for l odd, or vice versa, the relation changing sign each time one passes through a diffuse layer line.

3. Nature of the structure

These relations can be explained if it is supposed that the up or down displacement of successive sheets in the crystal, in proceeding in the c direction, occurs in a completely random way. Starting on the left in Fig. 1(b) and (c), the relation of L to R may be up or down. In the next sheet R_1 may be level with R, above it, or below it, with relative probabilities 2 : 1 : 1.

Let L be displaced in the a direction an amount $\varepsilon_0 a/2$ compared with R. The possibilities for R_1 are $\pm\varepsilon_0 a$ or zero. The corresponding phase relationships between the centres of the similarly oriented molecules R and R_1 are

$$2\pi(h+h\varepsilon_0+l),\ 2\pi(h-h\varepsilon_0+l),\ 2\pi(h+l)\,.$$

When $h\varepsilon_0$ is nearly a whole number m, the displacement will have a small effect on the sharpness of the spots, since it brings the molecule nearly back into position in the reflecting planes. On the other hand the phase relationships are very irregular when $h\varepsilon_0$ is near to $m+\frac{1}{2}$. The last column in Table 1 gives the difference ε between $h\varepsilon_0$ and the nearest whole number; a small value of ε corresponds to a sharp spot and vice versa. The sequence of the diffuseness fixes the value of ε_0, since it shows that $h\varepsilon_0$ must be 1 near the fifth layer line, 2 near the tenth, and 2·5 at the twelfth:

$$\varepsilon_0 = 2{\cdot}5/12 = 0{\cdot}208\,.$$

The rule for adding or subtracting F_R and F_L in the case of the sharp reflexions is also explained by this shift. The phase difference between the centres of R and L is

$$2\pi(h\varepsilon_0/2+l/2)\,.$$

Thus when $h\varepsilon_0$ is near an odd number, F_R and F_L must be added for l odd and subtracted for l even, and vice versa when $h\varepsilon_0$ is near an even number.

4. The extent of diffuseness

The complete theory is given in the subsequent paper, but a simple though approximate derivation is outlined here and may help towards an understanding of the way the diffraction effects arise.

The crystal in effect diffracts as if it were a mass of crystallites of limited extent in the c direction, because the phase relationship between two molecules becomes increasingly uncertain as the distance between them increases. The effect on a reflexion ($h0l$) obviously depends only on the value of ε by which $h\varepsilon_0$ differs from an integer. Starting from a given molecule R and going a distance nc in the c direction, there are $2n$ displacements of amount $a\varepsilon/2$ with random sign. The average displacement of the last molecule as compared with the first will be $(2n)^{\frac{1}{2}}.a\varepsilon/2$. As a first approximation we may consider that coherence is lost when this average displacement amounts to $a/2$, since a plane-to-plane relationship at points so far apart ceases to be unique. This occurs for a value n_0 given by

$$(2n_0)^{\frac{1}{2}}.\varepsilon a/2 = a/2\ ,$$
$$n_0 = 1/2\varepsilon^2\ .$$

It will be assumed therefore that the average width of the crystallites in the c direction is $c/2\varepsilon^2$.

A crystal of limited width gives a broadened reflexion which falls to zero on either side when the phase difference between waves from its edges is 2π. In going a distance c^* from one reflexion to the next along a layer line, the phase difference for two molecules separated by a distance c alters by 2π. The distance between centre and edge of a reflexion on a layer line is thus $2\varepsilon^2.c^*$ and the whole width is $4\varepsilon^2.c^*$. This width is plotted against $h\varepsilon_0$ in Fig. 3.

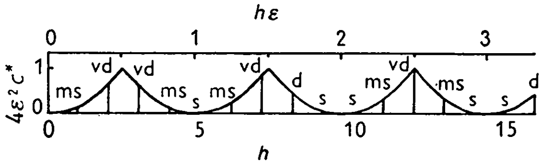

Fig. 3. Calculated width of reflexions, plotted against values of $h\varepsilon_0$.
s = sharp; ms = moderately sharp; d = diffuse; vd = very diffuse.

This calculation is of course only approximate, but it serves to show that the reflexions on a majority of the layer lines will be quite sharp, even in such a case of completely random displacement. On the layer line for which ε is nearly 0·5, the calculation would indicate that the spots run into each other, but the approximation of regarding the irregular crystal as a mass of crystallites cannot be pushed so far. In any event, the 'width of a reflexion' would have a meaning only for point molecules. The values of F_R and F_L change in magnitude and often in sign between one reflexion and the next, and this variation will be a major factor in the distribution of intensity along one of the very diffuse layer lines.

5. Discussion

The photograph was at first thought to indicate multiple twinning on the c face at intervals a few atomic planes apart. It seems clear, however, that the displacement between successive planes is up or down in a completely random way. Multiple twinning would imply neighbouring regions of the crystal in each of which there is a definite symmetry scheme. In the present case there are no symmetry elements relating one sheet of molecules to the next, and no space-group to which the crystal can be referred. It is 'statistically orthorhombic'.

It is by no means uncommon for 'mistakes' to occur in layer structures. Examples are the micas, graphite and hexagonal cobalt. The case dealt with here is very similar, except that we are concerned with molecules and not with an atomic lattice, and the displacement is not a sub-multiple of a cell dimension. It is interesting in that the completely random sign of the displacement makes it possible to specify the character of the structure precisely, and so to obtain an accurate quantitative answer to the problem of its diffraction. This analysis forms the subject af the following paper.

References

Cochran, W. & Howells, E. R. (1954). *Acta Cryst.* **7**, 412.

Howells, E. R. & Perutz, M. F. (1954). *Proc. Roy. Soc.* A. Submitted for publication.