Acta Cryst. (1955). 8, 241

An X-ray Study of Reduced Human Haemoglobin

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(*Received* 1 *November* 1954)

Reduced human haemoglobin exists in three polymorphous modifications which all have the same space group ($P2_1$), but different molecular arrangements. Patterson projections of the simplest of the three forms indicate that the two molecules in the unit cell are nearly parallel to each other and centred approximately at $\pm(x = 0{\cdot}225,\ y = 0{\cdot}25,\ z = 0{\cdot}20)$. The peaks near the origin in two of the Patterson projections of this form resemble those in corresponding projections of horse methaemoglobin, and suggest similarities in the molecular structure of the two proteins.

1. Introduction

Haemoglobin of normal adult man crystallizes in six different forms. There are three forms in which oxy-, carboxy- or methaemoglobin have been observed to crystallize and three others characteristic of reduced haemoglobin. Five of the six forms are listed in Table 1 of Bragg & Perutz (1952*b*), which gives the cell dimensions, optics and habits of growth. The present paper describes Patterson projections of a form of reduced haemoglobin listed as No. II in that table and also of a new form (III) not previously investigated.

X-ray analysis of horse haemoglobin led Bragg & Perutz (1952*a*) to propose molecular dimensions approximating to a spheroid of $54\times54\times71$ Å. The same spheroidal model also fitted the unit-cell dimensions and space groups of several other forms of haemoglobin, suggesting the *external* shape of the haemoglobin molecule to be the same in different animal species (Bragg & Perutz, 1952*b*; Bragg, Howells & Perutz, 1952). It has now been found that Patterson projections of horse and human haemoglobin possess similarities which imply a close relationship between the *internal* architecture of these two proteins.

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2. Experimental

Crystalline human oxyhaemoglobin was prepared by the method of Drabkin (1946). The crystals were dissolved in a minimum of water, and a few drops of the concentrated haemoglobin solution was added to a series of test tubes each containing 10 ml. phosphate buffer of pH 6·7 and a small quantity of ferrous citrate. The total buffer concentration in the different tubes was varied between 2·0 and 2·8*M*. The tubes were filled with nitrogen before being sealed. After

Table 1. *Crystallographic data*

Form	Space group	State	*a* (Å)	*b* (Å)	*c* (Å)	β (°)	Unit-cell volume (Å$^3\times10^{-3}$)	*n*	Pleochroism	Direction of low absorption	Crystal habit (dominant habit bold)
I	$P2_1$	Wet	66	98	110	98	700	4	Strong	[100]	{**001**}
II	$P2_1$	Wet	62·5	83·2	52·8	98	272	2	Strong	[100]	{**011**} {110} {010}
		Dry	59	70	47·5	97·5	194				
III	$P2_1$	Wet	64·8	97·5	100	101·5	619	4	Strong	[100]	{**100**} {011}

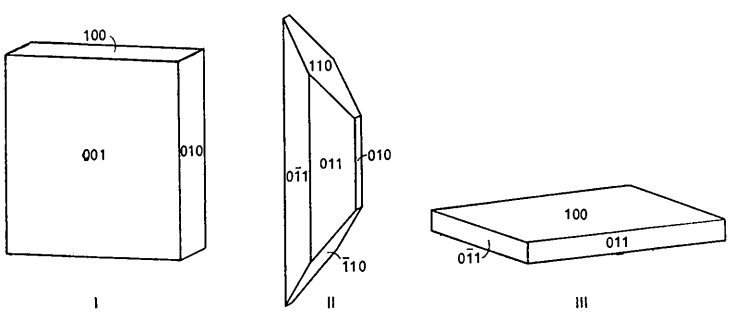

Fig. 1. Clinographic projections of the three crystal forms of human reduced haemoglobin.

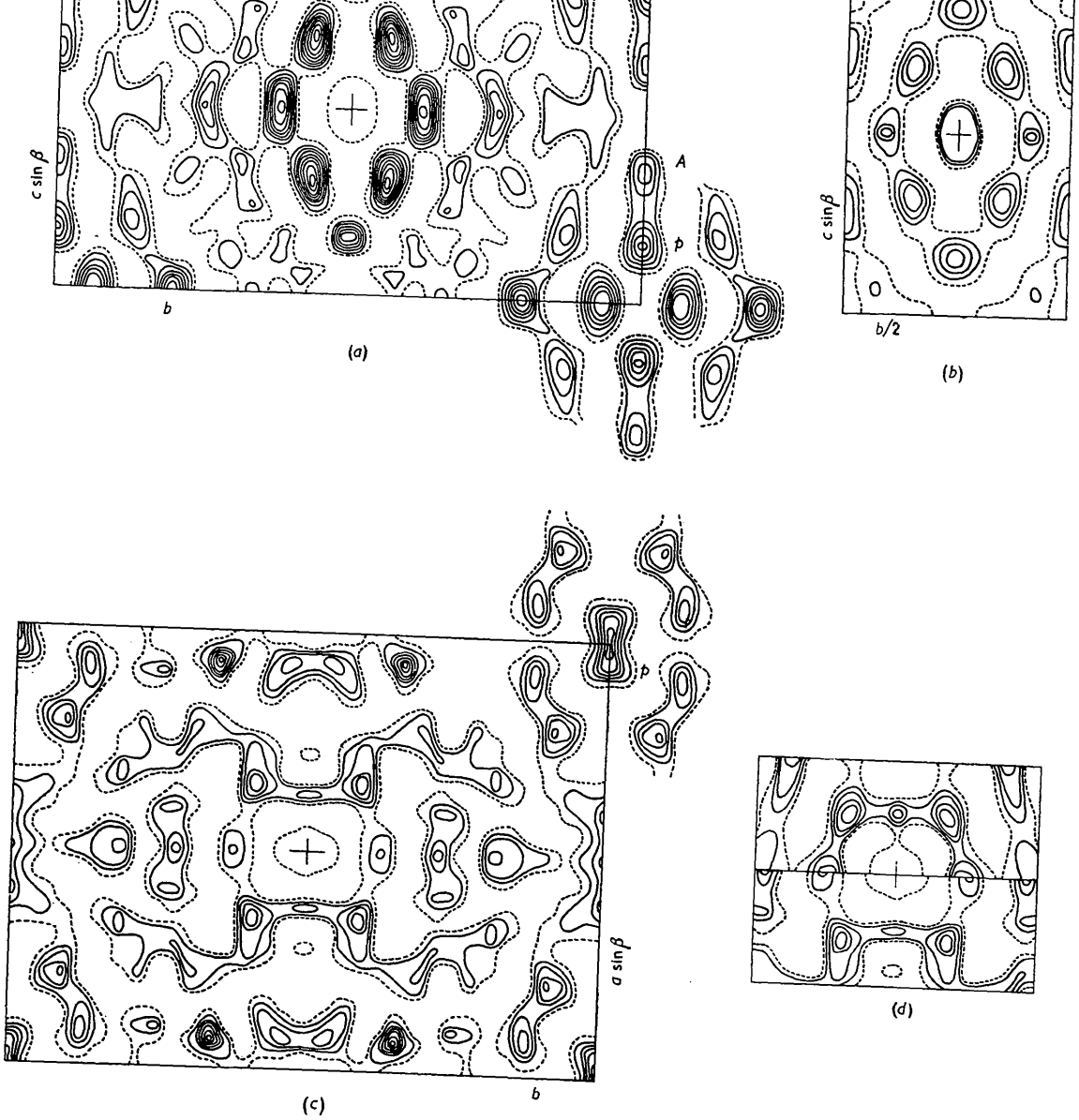

Fig. 2. (*a*) $P(y, z)$ of human reduced haemoglobin II. The pseudo-origin p is shown in the bottom right-hand corner. Scale: 1 cm. = 10 Å.
(*b*) $P(y, z)$ of horse methaemoglobin. Terms of $d < 7$ Å are omitted. Scale: 1 cm. = 10 Å.
(*c*) $P(x, y)$ of human reduced haemoglobin II. Scale: 1 cm. = 10 Å.
(*d*) Comparison of peaks surrounding the origin in $P(x, y)$ of horse methaemoglobin (top) and human haemoglobin (bottom).

some days good crops of well formed crystals were found in some of the tubes. When exposed to air, crystals of reduced haemoglobin become oxygenated and are liable to break up. To prevent this the test tubes were opened only under nitrogen, and the entire manipulation needed for mounting wet single crystals in thin-walled glass capillaries was carried out in a

crystals could be kept for long periods without becoming oxygenated. Precession pictures of the three principal zones of the wet crystals of form II were taken with precession angles of 8° and 17°; 8° precession pictures were also taken of the dried crystals. Of the new form of reduced haemoglobin (III) only

dried crystals of this form were not investigated. All Patterson projections were calculated from 8° precession pictures, omitting terms of less than 7 Å spacing.

3. Crystallographic data

The unit-cell dimensions, space group, optics and growth habits of the three forms of human reduced haemoglobin are given in Table 1. Clinographic drawings from which the crystals may be identified are shown in Fig. 1. The habits of all three reduced forms are easily distinguishable from those of oxy-, carboxy- and methaemoglobin, which commonly crystallize either in tetragonal bipyramids (Drabkin, 1946), tetrahedra (Jope & O'Brien, 1949), or in pseudo-tetragonal prisms terminated by bipyramids (Perutz & Weisz, 1947).

4. Patterson projections of form II

Bragg & Perutz (1952*b*) suggested the two molecules in the unit cell to be in a body-centred arrangement and parallel to each other with the long axis of the spheroids inclined at a small angle to the *a* axis. Perutz (1953) later showed the polarization dichroism of the crystals to be consistent with this orientation of the molecules. This deduction is now confirmed by the Patterson projections. The most interesting looking projection is that on (100), which shows a pattern of peaks at a distance of 10–11 Å from the origin (Fig. 2(*a*)). This pattern is strikingly similar to that found in the Patterson projection on (100) of horse haemoglobin, which has been the subject of previous investigations (Fig. 2(*b*)). It was shown by Perutz (1949) that the peaks are the projections of rod-like features in the three-dimensional Patterson, tentatively interpreted as the vector equivalents of parallel polypeptide chains of the α-form. The occurrence of of a similar pattern of vector peaks in human haemoglobin suggests a corresponding similarity in the general lay-out of the polypeptide chains.

In horse haemoglobin the molecules lie on lattice points in a *C*-face-centred unit cell; hence the vector peaks in the Patterson projection of Fig. 2(*b*) repeat at intervals of $b/2$. In human haemoglobin the two molecules lie in general positions related by the screw dyad along [010], but if the two molecules are parallel a 'pseudo-origin' ought to appear in the Patterson projection at $y = \frac{1}{2}$, with surroundings similar to those of the origin peak. Such a peak appears at $z = 0{\cdot}33$ (marked *p* in Fig. 2(*a*)). Its surroundings are similar to those of the origin peak, except for the peak *A* which should be absent on this simple interpretation.

We may now consider the Patterson projection on (001) (Fig. 2(*c*)). This again shows an arrangement of peaks around the origin which is similar to that on the (001) projection of horse haemoglobin (Fig. 2(*d*)). The other marked feature of Fig. 2(*c*) is a pseudo-origin at $x = 0{\cdot}45$, $y = \frac{1}{2}$, where the system of peaks surrounding the origin itself is repeated. The two Patterson projections on (100) and (001) thus suggest the molecular co-ordinates to be $\pm(x = 0{\cdot}225,\ y = 0{\cdot}25,\ z = 0{\cdot}20)$ in good agreement with the body-centred arrangement deduced from packing arguments.

The Patterson projection on (010) (Fig. 3(*a*)) is the least interesting. Owing to the great length of *b* the molecules overlap considerably; in consequence neither the similarity with horse haemoglobin, nor the parallelism of the two molecules in the unit cell is apparent. There is a suggestion of a pseudo-origin near $x = \frac{1}{2}$, $z = \frac{1}{2}$, but without the aid of the other two projections it would hardly be recognized as such.

X-ray pictures of the dry form were also taken. As usual, these fail to show reflexions of less than 7 Å spacing, but a Patterson projection on (100) nevertheless shows some features of interest (Fig. 3(*b*)). At this low resolution one would expect the vectors between molecules to predominate; accordingly a

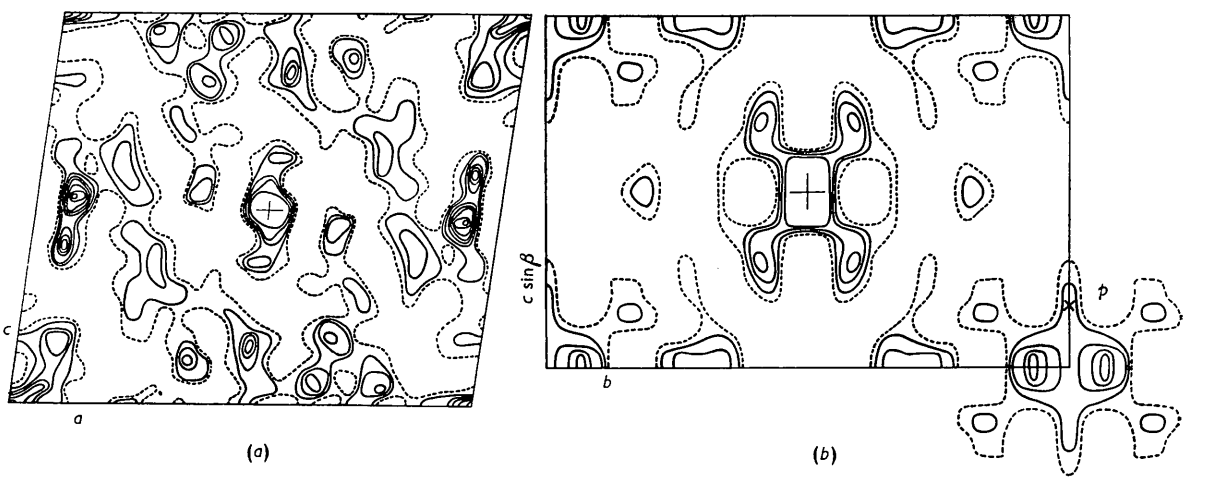

Fig. 3. (*a*) $P(x, z)$ of human reduced haemoglobin II. Scale: 1 cm. = 10 Å.
(*b*) $P(y, z)$ of human reduced haemoglobin II, air dried crystal. Scale: 1 cm. = 10 Å.

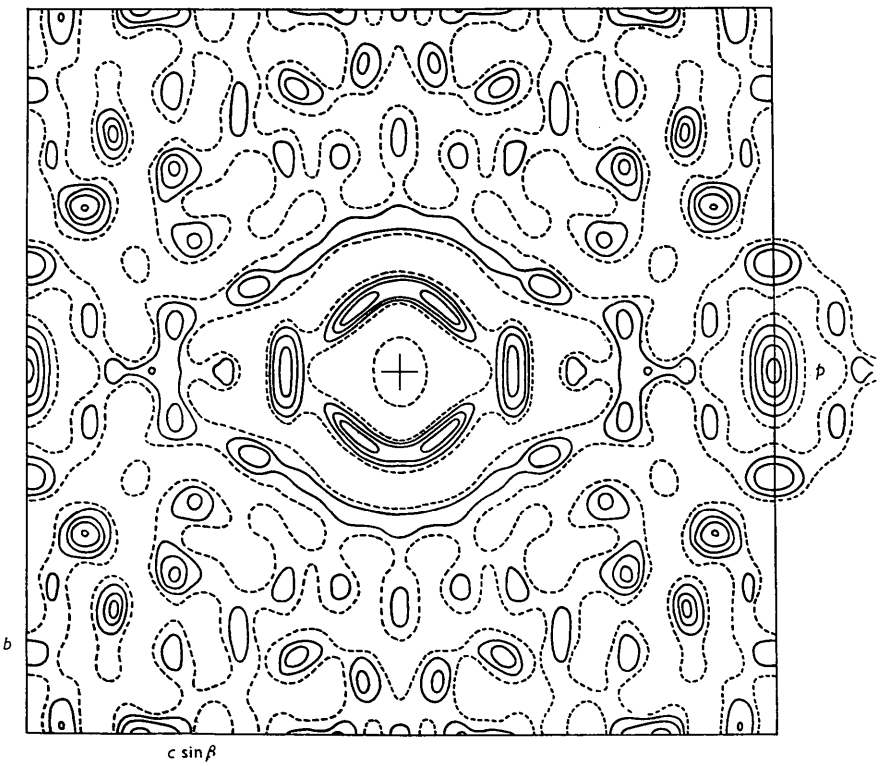

Fig. 4. $P(y, z)$ of human reduced haemoglobin III.

striking system of peaks is found near the pseudo-origin at $y = \frac{1}{2}$, $z = 0{\cdot}4$, providing further evidence in favour of the molecular arrangement deduced above.

It is interesting to see if the correspondence between the two Patterson projections on (100) of horse and human haemoglobin (Figs. 2(*a*) and 2(*b*)), can be subjected to a more quantitative analysis, based on the electron-density distribution tentatively proposed by Bragg, Howells & Perutz (1952). This showed a system of 17 peaks in roughly hexagonal array, representing polypeptide chains in the haemoglobin molecule in end-on projection. The question may now be put whether two such systems of electron-density peaks displaced by the vector p (Fig. 2(*a*)) would explain the observed Patterson, not only as far as the positions but also as far as the heights of the vector peaks are concerned.

The procedure was as follows. Sets of points corresponding to the peak positions in the Fourier projection of Bragg, Howells & Perutz (1952) were placed at positions corresponding to various molecular arrangements. Their optical diffraction patterns were then recorded with a machine similar to that of Hanson, Lipson & Taylor (1953) and the intensities of the diffraction spectra were used as the terms of Patterson syntheses. It was found that a calculated Patterson giving peaks of approximately the right shape and relative height could be obtained with molecules at $\pm(y = 0{\cdot}25, z = 0{\cdot}20)$, but it was not possible in any of the calculated Pattersons to reproduce the anomalous peak A. Its appearance suggests that the projected electron-density distribution in the haemoglobin molecule is more complex than the simple system of intra-molecular Patterson peaks would lead one to expect.

5. The crystal structure of form III

This form has the same space group as form II ($P2_1$), but it has four molecules in the unit cell, and a more complex structure. [100] is again the axis of minimum pleochroic absorption, which is generally found to coincide approximately with the long axis of the molecular spheroids. This molecular orientation is also suggested by the Patterson projection on (100) (Fig. 4). The system of peaks around the origin resembles the one in Fig. 2(*a*), except that the peaks appear to be smeared out by rotation around [100]. There is also a peak which might be a pseudo-origin (p) at $z = \frac{1}{2}$, $y = 0$, but none that could be definitely identified as such on the line of $y = \frac{1}{2}$, where it would be expected if the molecules related by the screw dyad were strictly parallel. Absences among the low-order reflexions suggest that the molecules are in approximately face-centred positions; this arrangement is also suggested by considerations of packing, but the structure of this form is evidently too complex for more detailed analysis.

6. Conclusions and discussion

Only one of the three crystal forms of human haemoglobin is suitable for detailed analysis. This has two molecules related by a screw dyad in a monoclinic unit cell. The two molecules appear to be very nearly parallel to each other and centred approximately at $\pm(x = 0{\cdot}225, y = 0{\cdot}25, z = 0{\cdot}20)$. The peaks near the origin in two of the Patterson projections closely resemble those in corresponding projections of horse haemoglobin and suggest similarities in the molecular structure of the two proteins. The resemblance is particularly striking in the projections on (100), which correspond to the end-on view of the vector chains in the three-dimensional Patterson. An attempt was made to interpret this projection as being due to two identical sets of electron-density peaks, representing close-packed chains viewed end-on, displaced by the intermolecular vector p. Success was only partial: our assumed structure accounted for the positions and relative heights of all the Patterson peaks except one, which remained entirely unexplained. It seems difficult to make further progress with this projection until the electron density is determined directly by the isomorphous-replacement method (Green, Ingram & Perutz, 1954).

Even the very limited interpretation of the Patterson projections presented here involves difficulties which are hard to resolve at this stage. For instance, if the haemoglobin molecule were a completely asymmetric object, parallelism of two such molecules related by screw dyads would have no meaning. In that case the similarity of self-Patterson and cross-Patterson, which appears in two projections of form II, would have a more complex interpretation than the one given. On the other hand, if human reduced haemoglobin had dyad symmetry, as human oxy- and methaemoglobin are known to have, and if the molecular dyads were parallel to the b axes of the crystal, as the similarities of the Patterson projections on (100) of human and horse haemoglobin seem to indicate, then the self- and cross-Pattersons would have to be identical and peak p would be considerably higher than is observed. The fact that this is not so suggests either that in the reduced form the dyad symmetry of the molecule is only approximate, or else that the dyads of the two molecules in the unit cell are not strictly parallel. None of these points can as yet be decided.

So far there is nothing in the results which would explain why reduced haemoglobin crystallizes differently from oxy-, carboxy- and methaemoglobin.

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Acta Cryst. (1955). **8**, 245

The lattice of rhombohedral sulfur. By J. D. H. Donnay, *The Johns Hopkins University, Baltimore* 18, *Maryland, U.S.A.*

(*Received* 21 *January* 1955)

Frondel & Whitfield (1950) established Laue class $\bar{3}$ from a Weissenberg [0001] zero layer. They used the morphology (hexagonal prism $11\bar{2}0$ and rhombohedron $10\bar{1}1$) to rule out point group 3 and establish point group $\bar{3}$ (Groth's 'rhombohedral' crystal class). The lattice mode could not be deduced from the X-ray photographs available to them.

The indices of the crystal forms obey the criterion '$(2h+k+l)$ divisible by 3', so that on purely morphological grounds the lattice is rhombohedral, the space group is uniquely determined as $R\bar{3}$, and the cell given in hexagonal co-ordinates ($a = 10{\cdot}9$, $c = 4{\cdot}26$ kX., $Z = 18$) is a triple cell. The smallest cell is given by $a_{\text{rh.}} = 6{\cdot}45$ kX., $\alpha = 115°\ 18'$, $Z = 6$. In the name 'rhombohedral sulfur', the adjective rhombohedral may well be taken to refer to the lattice mode.

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