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An X-ray examination of some crystal forms of pig and rabbit haemoglobin.

By D. M. Blow,*
Medical Research Council Unit for the Study of the Molecular Structure of Biological Systems, Cavendish Laboratory, University of Cambridge, England

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In the course of experiments on the crystallization of haemoglobins from different species, the unit cells of three new forms of haemoglobin have been determined by X-ray analysis.

The haemoglobins were obtained from a Large White pig and from various common domestic rabbits. After purifying and recrystallizing according to the usual techniques, crystals large enough for X-ray study were grown in either the oxy- or the met-form. The crystals were grown either from 'solution *A*' (2 vol. saturated ammonium sulphate: 1 vol. 2*M* ammonium phosphate), whose pH was adjusted by adding sulphuric acid, or from phosphate buffer whose pH was controlled by the relative concentrations of the two phosphates (K_2HPO_4:NaH_2PO_4). The habits of the resulting crystals are shown in Fig. 1.

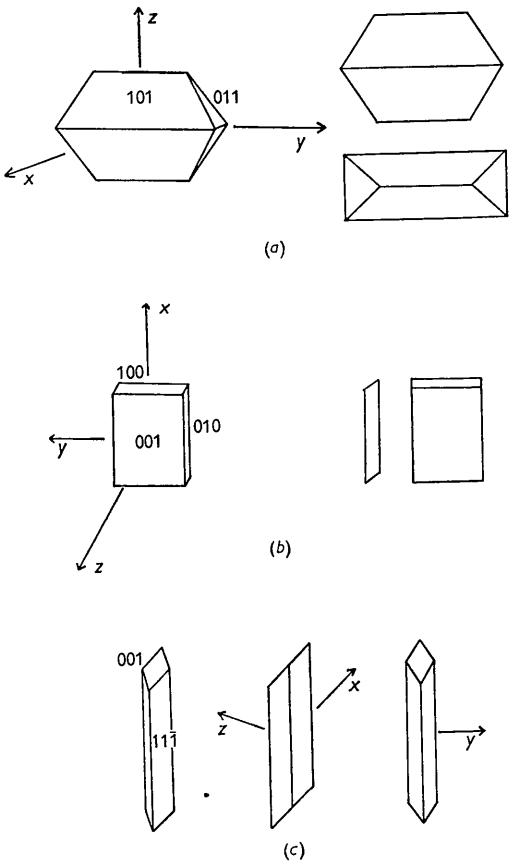

Fig. 1. Habits and choice of axes in pig and rabbit haemoglobins. (*a*) Pig I, (*b*) pig II, (*c*) rabbit I.

The unit-cell dimensions and space groups of the different forms were found from precession photographs taken along the prominent axes. These are given, together with the crystallization conditions, in Table 1. The number of molecules in the unit cell is estimated on the assumption that the molecular volumes of the wet crystalline haemoglobins lie in the range of such volumes found for haemoglobins previously examined, namely, between 135,000 Å^3 and 190,000 Å^3 per molecule.

The number of molecules in the unit cell of the form designated rabbit haemoglobin I is only half the number of equivalent positions in the unit cell. This means that, as in horse haemoglobin I (Bernal, Fankuchen & Perutz, 1938) and human haemoglobin III (Perutz, Liquori & Eirich, 1951), a crystallographic dyad axis passes through the centre of the molecule, which is composed of two identical units.

Bragg & Perutz (1952) and Bragg, Howells & Perutz (1954) have shown that the external shape of the haemoglobin molecule is closely represented by a prolate spheroid whose dimensions are about 54 Å × 54 Å × 71 Å in the wet state. All known unit cells for adult mammalian haemoglobins are consistent with this model. In the three new forms described above, it is possible in each case to deduce a unique way of packing the spheroids into the observed unit cell. (In the case of pig haemoglobin I, the X-ray data indicate that some of the screw dyads parallel to *c* are pseudo screw tetrads, making the unit cell pseudo $P4_12_12$. This fact has been used in deducing the packing of the molecules.)

The types of packing may be briefly described as follows: In pig haemoglobin I, the molecules lie with their centres approximately half way between adjacent pseudo screw tetrads. The long axes of the ellipsoids lie in the (110) plane and make an angle of about 60° with [001]. In pig haemoglobin II the long axes of the ellipsoids lie in the (101) plane and enclose an angle of about 30° with [010]. In rabbit haemoglobin I, the long axes lie in the (001) plane, making an angle of about 15° with the needle axis [101] and being tilted towards [100].

Table 1

Form	Growth conditions: Buffer	pH	Unit cell (wet): Space group	*a* (Å)	*b* (Å)	*c* (Å)	*β* (°)	Probable number of molecules per unit cell
Pig I	85% solution *A* 2·8*M* phosphate	7·8 6·8	$P2_12_12_1$	69·8	72·5	115	—	4
Pig II	3·2*M* phosphate	6·6	$P2_1$	55·3	105·5	54·4	115·1	2
Rabbit I	65% solution *A*	7·4	*C*2	70·4	83·0	54·9	118·0	2

* Present address: National Institute of Mental Health, Bethesda 14, Maryland, U.S.A.

Perutz (1953) has shown that the birefringence and dichroism of crystalline haemoglobin is dominated by the intrinsic birefringence and dichroism of the haemoglobin molecule. In all the forms he studied, the orientation of the haem groups resulted in the strongest absorption being in the plane perpendicular to the length of the molecule. The dichroism of the three new forms has been studied and is in agreement with this result. In pig haemoglobin I dichroism appears to be weak or absent owing to the large tilt of the molecules relative to each other; in rabbit haemoglobin I there is strong dichroism, and the direction of maximum absorption corresponds closely with the orientation of the molecule deduced above. When looking down *b* the optical extinction direction is almost parallel to the direction of maximum absorption. It was possible to observe the thin plates of pig haemoglobin II only perpendicular to (001). In this orientation strong dichroism was observed with maximum absorption when the vibration direction was parallel to *a*.

These results are in agreement with the general hypotheses referred to above about the molecular shape, birefringence and dichroism of the mammalian haemoglobins.

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Calculation of atomic scattering factors using Slater wave functions: sodium to calcium.

By Y. TOMIIE* and C. H. STAM, *School of Chemistry, The University, Leeds 2, England*

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Following McWeeny's suggestion (1951), we have calculated the atomic scattering factors for the spherically averaged atoms from sodium to calcium, inclusive, by his method using Slater wave functions. The formulae used in our calculation were:

$$f(1s) = \frac{1}{(1+x_1^2)^2},$$

$$\bar{f}(2p) = \frac{1-x_2^2}{(1+x_2^2)^4},$$

$$f(2s) = (1-\alpha_{12}^2)^{-1}\left[\frac{1-x_2^2}{(1+x_2^2)^4} - \frac{2\alpha_{12}^2}{3}\frac{3-x_{12}^2}{(1+x_{12}^2)^3} + \frac{\alpha_{12}^2}{(1+x_1^2)^2}\right],$$

$$\bar{f}(3p) = (1-\alpha_{23}^2)^{-1}\left[\frac{3-10x_3^2+3x_3^4}{3(1+x_3^2)^6} - \frac{2\alpha_{23}^2}{5}\frac{5-10x_{23}^2+x_{23}^4}{(1+x_{23}^2)^5} + \alpha_{23}^2\frac{1-x_2^2}{(1+x_2^2)^4}\right],$$

$$f(3s) = \bar{f}(3p),$$

$$x_1 = \frac{4\pi X}{2c_1},\quad x_2 = \frac{4\pi X}{2c_2},\quad x_3 = \frac{4\pi X}{2c_3},$$

$$x_{12} = \frac{4\pi X}{c_1+c_2},\quad x_{23} = \frac{4\pi X}{c_2+c_3},\quad X = \sin\theta/\lambda,$$

$$\alpha_{12} = 8\times 3^{\frac{1}{2}} c_1^{\frac{3}{2}} c_2^{\frac{5}{2}}/(c_1+c_2)^4,$$

$$\alpha_{23} = 160\left(\frac{2}{15}\right)^{\frac{1}{2}} c_2^{\frac{5}{2}} c_3^{\frac{7}{2}}/(c_2+c_3).$$

$\bar{f}(2p)$ and $\bar{f}(3p)$ are the averaged contributions of $2p$ and $3p$ electrons, respectively, to the scattering factor. Although $f(3s) = \bar{f}(3p)$ is not strictly correct†, the influence of this approximation is found to be negligibly small for the final results: for example, the maximum difference due to this approximation for Ca^{2+} is only 0·005. This may not be the case for atoms heavier than Ca. α_{12} and α_{23} are the overlap integrals between $1s$ and $2s$, and $2s$ and $3s$ unorthogonalized Slater wave functions, respectively. c_1 is the effective nuclear charge for the $1s$ electrons, c_2 one-half that for the $2s$ and $2p$ electrons and c_3 one-third that for the $3s$ and $3p$ electrons; the numerical values of these can easily be obtained from Slater's rules (1930). The results are given in Table 1.

For Na, Mg^{2+}, Al^{3+}, Si^{4+}, Cl^-, A, K^+ and Ca, revised atomic scattering factors have been given by Berghuis *et al.* (1955), calculated from self-consistent-field electron-density data. Comparison with our results shows the following points:

(*a*) Ca^{2+}, K^+, A and Cl^-.—Good agreement is obtained in the region of $\sin\theta/\lambda < 0{\cdot}5$. (For calcium, comparison cannot be made in the region of $\sin\theta/\lambda < 0{\cdot}15$ as Berghuis

† The exact formula of $f(3s)$ is

$$f(3s) = N^2\left[\frac{3-10x_3^2+3x_3^4}{3(1+x_3^2)^6} + Q_1^2\frac{1-x_2^2}{(1+x_2^2)^4} + Q_2^2\frac{1}{(1+x_1^2)^2} - \frac{2}{5}Q_1\alpha_{23}\frac{5-10x_{23}^2+x_{23}^4}{(1+x_{23}^2)^5} - 2Q_2\alpha_{13}\frac{1-x_{13}^2}{(1+x_{13}^2)^4} + \frac{2}{3}Q_1Q_2\alpha_{12}\frac{3-x_{12}^2}{(1+x_{12}^2)^3}\right],$$

$$N = (1-\alpha_{12}^2)^{\frac{1}{2}}/(1-\alpha_{12}^2-\alpha_{13}^2-\alpha_{23}^2+2\alpha_{12}\alpha_{13}\alpha_{23})^{\frac{1}{2}},$$

$$Q_1 = (\alpha_{23}-\alpha_{12}\alpha_{13})/(1-\alpha_{12}^2),\quad Q_2 = (\alpha_{13}-\alpha_{12}\alpha_{23})/(1-\alpha_{12}^2),$$

$$\alpha_{13} = 32\left(\frac{2}{5}\right)^{\frac{1}{2}} c_1^{\frac{3}{2}} c_3^{\frac{7}{2}}/(c_1+c_3)^5.$$

* On leave from Department of Chemistry, Osaka University, Nakanoshima, Osaka, Japan.