Acta Cryst. (1954). 7, 347

A Pseudo-Orthorhombic Crystal Form of Horse Myoglobin

By J. C. Kendrew and I. F. Trotter*

Medical Research Council Unit for the Study of the Molecular Structure of Biological Systems, Cavendish Laboratory, Cambridge, England

(*Received* 9 *December* 1953)

The crystal symmetry and Patterson projections of a monoclinic, pseudo-orthorhombic form of horse myoglobin crystal are described (true symmetry $P2_1$, pseudo-symmetry $P22_12_1$). This unit cell is very closely related to the monoclinic form of the same protein previously described: their a and b dimensions are virtually the same, while c in the new form has double the value of $c \sin\beta$ in the monoclinic form. The Patterson projections are also closely related to those of the monoclinic form.

These results make it possible to obtain a general idea of the relationships between the two forms and to make plausible suggestions about the mode of packing of the molecules.

1. Preparation of crystals, morphology and optics

The preparation of monoclinic crystals of met-myoglobin by salting-out from 3–3·5 M phosphate buffer (pH 6·4) has been described in an earlier publication (Kendrew, 1950), which also gave an account of their X-ray analysis.

A particular batch of crystals prepared by an almost identical method, although at first glance similar to the original ones, proved to give different X-ray patterns. Closer examination of the crystals showed that they were in fact appreciably different, morphologically and optically. As before, they were flattened four-sided prisms and exhibited straight extinction when viewed along either the a or c axes (perpendicular to the needle axis, and respectively parallel and perpendicular to the flattened face); however, their cross-section was apparently a rectangle instead of a parallelogram and when viewed in polarized light parallel to the needle axis (the b axis) they exhibited straight instead of oblique extinction. These deviations suggested that the symmetry might be orthorhombic instead of monoclinic. Other recent attempts to prepare horse myoglobin crystals have always given similar results.

2. Crystal symmetry and cell dimensions

A preliminary examination of the ac reciprocal-lattice plane, using as before the Buerger precession camera, supported the idea of orthorhombic symmetry, since at first sight the reciprocal net was rectangular and $F^2(h0l) = F^2(h0\bar{l})$. Closer examination showed, however, that the symmetry was in fact only pseudo-orthorhombic with $\beta = 91°$; slight differences between $F^2(h0l)$ and $F^2(h0\bar{l})$ were also apparent in the outer regions of the reciprocal lattice.

The cell dimensions proved to have the following values, corresponding figures for the monoclinic form (lattice A) being given for comparison:

	Pseudo-orthorhombic	Monoclinic (lattice A)
a (Å)	57·0	57·3
b (Å)	30·8	30·8
c (Å)	106·0	57·3
β (°)	91	112
$c \sin\beta$(Å)	106·0	52·8

The two forms are thus very closely related; a and b are virtually identical in the two forms, but c in the pseudo-orthorhombic form is almost exactly twice $c \sin\beta$ in the monoclinic, so that the volume of the unit cell of the new form is just double that of the old (see Fig. 1 (a)). Since the latter has two molecules per unit cell, it may be assumed that the former has four.

The only general absences among the reflexions are $0k0$ for k odd and $00l$ for l odd; hence the true space group is $P2_1$ (as in the earlier form) and the pseudo-symmetry is $P22_12_1$.

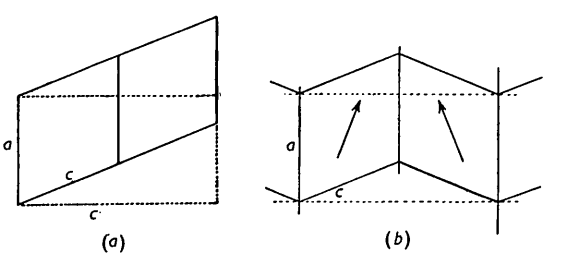

Fig. 1. (*a*) Relation between unit-cell dimensions of monoclinic form (full lines) and pseudo-orthorhombic form (broken lines) of horse myoglobin. (*b*) Suggested mode of 'twinning' of unit cells of monoclinic form (full lines) to give the pseudo-orthorhombic form (broken lines). The rod directions are shown by the arrows.

* On leave from No. 1 Research Laboratory, Courtaulds Ltd, Coventry, England.

No extensive search has been made for shrinkage stages, but when the crystals are placed in saturated phosphate (instead of 3–3·5 M) the cell dimensions remain unchanged, unlike those of the monoclinic form which under these circumstances shrink to a new lattice (lattice *B*). The air-dried crystals give a poor diffraction pattern; their cell dimensions are as follows, with those of the air-dried monoclinic form for comparison:

	Pseudo-orthorhombic, air-dried	Monoclinic, air-dried
a (Å)	53·0	51·5
b (Å)	28·5	28·0
c (Å)	78·0	37·0
β (°)	90	100 or 80
$c \sin\beta$(Å)	78·0	36·6

In many respects the diffraction pattern of the dry crystal resembles that of the dry monoclinic form, in

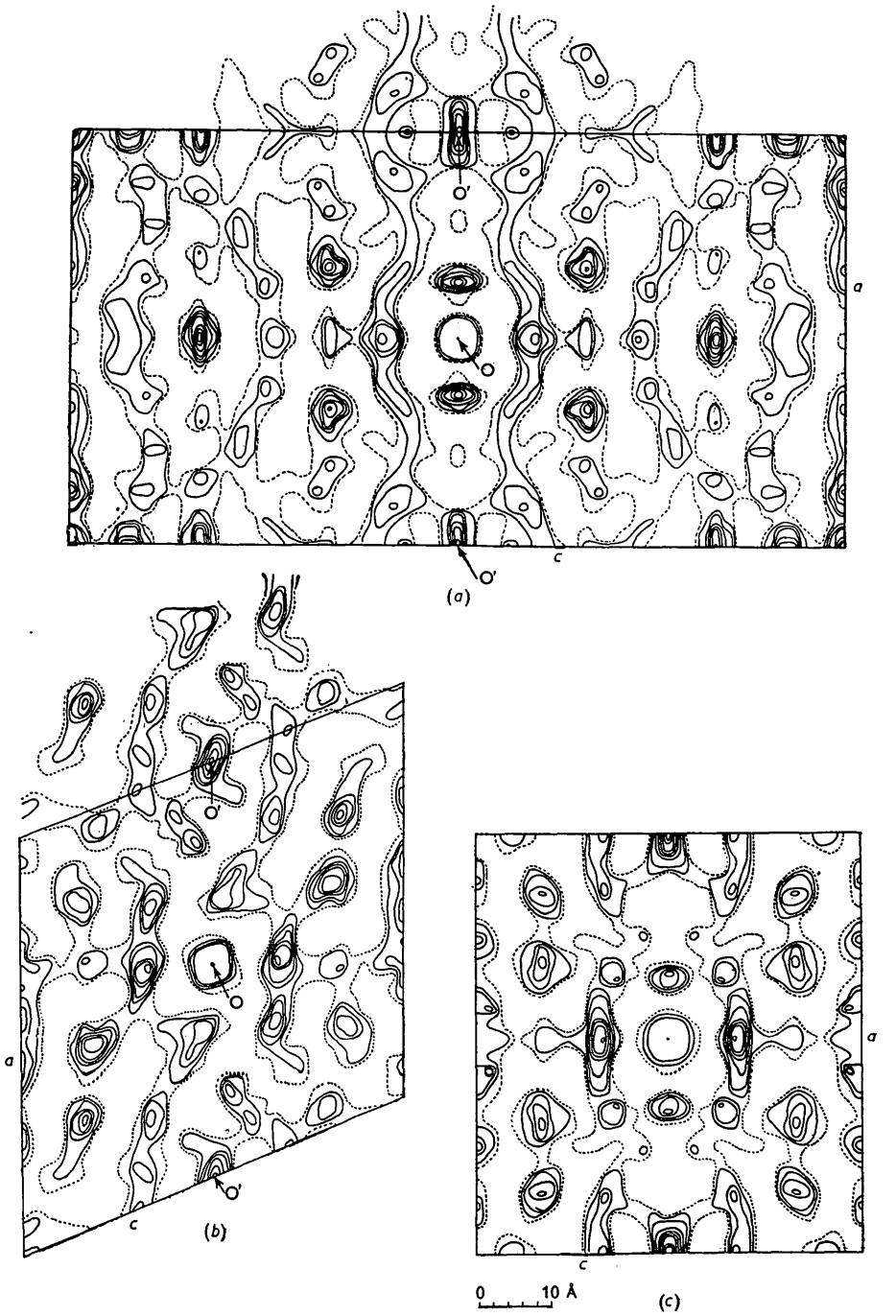

Fig. 2. (*a*) The *b* Patterson projection of pseudo-orthorhombic horse myoglobin. (*b*) The *b* Patterson projection of monoclinic horse myoglobin. (*c*) The monoclinic *b* projection superimposed on its inverse. (Origins at centres.)

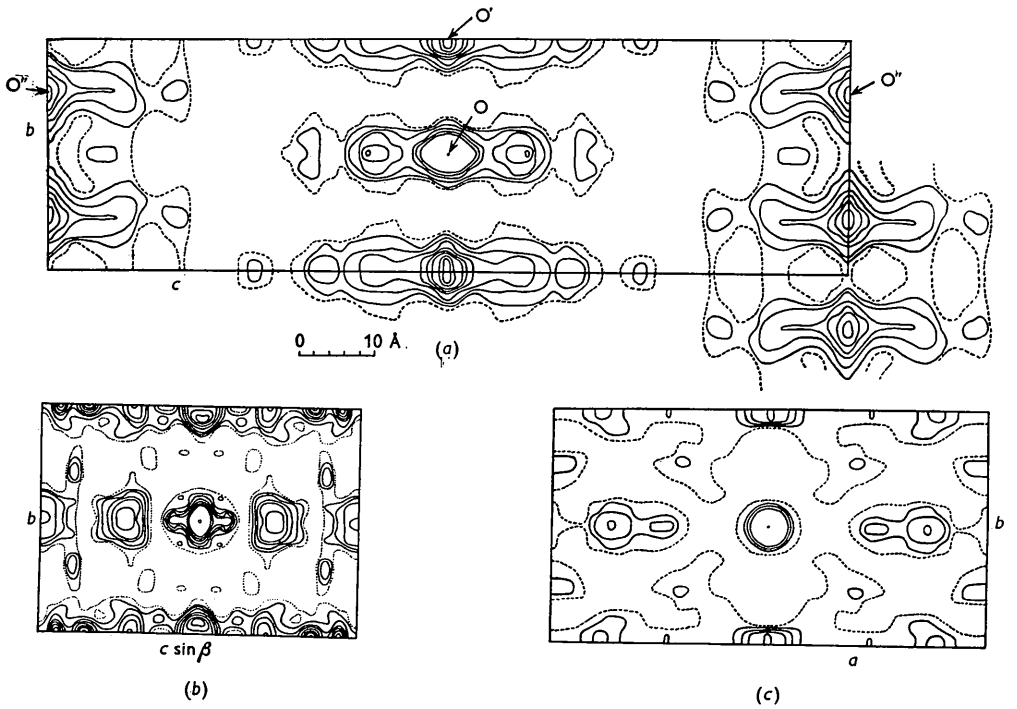

Fig. 3. (*a*) The *a* Patterson projection of pseudo-orthorhombic horse myoglobin. (*b*) The *a* Patterson projection of monoclinic horse myoglobin. (*c*) The *c* Patterson projection of pseudo-orthorhombic horse myoglobin. (Origins at centre.)

which, as was pointed out in the earlier publication, there is considerable disordering corresponding to some kind of statistical twinning, involving translations along *a* and ambiguity in the value of β. The presumption is that this disordering is related to the regular twinning which leads to the pseudo-orthorhombic form. One striking feature common to both dry crystals is the extraordinarily strong reflexion indexed as 001 in the monoclinic and as 002 in the pseudo-orthorhombic form.

3. Patterson projections

Reflexions of the three principal zones were recorded with the Buerger precession camera (precession angle 17°; Cu $K\alpha$ radiation); their intensities were measured on an arbitrary relative scale by visual comparison and corrected by the usual factors. The three sets were used to prepare Patterson projections, the computations being carried out with the aid of Beevers–Lipson strips.

The intervals used were *a*/30, *b*/15, and *c*/60, and the resulting projections are shown in Figs. 2(*a*), 3(*a*) and 3(*c*). In view of the very close approximation to orthorhombic symmetry of the reflexions in the [010] zone, and of the small divergence of β from 90°, the *b* projection was computed and plotted as if the cell were orthorhombic.

The projections show suggestive resemblances to those of the monoclinic form. We proceed to discuss these in turn.

(*a*) *The b projection* (Fig. 2(*a*))

At first sight this projection does not bear any obvious relation to the *b* projection of the monoclinic form (Fig. 2(*b*)), which has been interpreted as containing projected rods parallel to [20$\bar{1}$] and considered to be the vector equivalents of parallel lengths of polypeptide chain in the same orientation. However, the relationship between the cell dimensions of the two forms shown in Fig. 1(*a*) suggested that what has actually happened is a periodic twinning on the *c* faces of each unit cell, the larger unit cell arising as shown in Fig. 1(*b*).

If this hypothesis is correct, one would expect that the region round the origin of the Patterson projections of the larger unit cell would be made up of the self-Pattersons of the two molecules in the left-hand half of the cell superimposed on that of those in the right-hand half. Now the self-Patterson of the former two molecules is just the *b* Patterson projection of the monoclinic form; while that of the latter is the same thing inverted about *a*. Accordingly the monoclinic *b* projection was superimposed on its inverse, producing the double projection shown in Fig. 2(*c*). It will be observed that it is in fact astonishingly similar to the central region of the pseudo-orthorhombic projection (Fig. 2(*a*)): if the two are superimposed it is found that *every* peak in each has a precisely corresponding counterpart in the other.

These results confirm the hypothesis illustrated in Fig. 1(*b*) and, furthermore, they form a striking example of a hidden relation between two apparently

quite dissimilar projections. It is worth noting that if the monoclinic form were unknown it is improbable that in the present state of knowledge the pseudo-orthorhombic b projection could have been interpreted at all.

(*b*) *The a projection* (Fig. 3(*a*))

This projection, too, exhibits striking similarities to the corresponding projection of monoclinic horse myoglobin (Fig. 3(*b*)), and this time the resemblance is obvious from inspection. The central region of the pseudo-orthorhombic projection closely resembles the monoclinic projection; in each case there are ridges of high vector density perpendicular to b and separated by $\frac{1}{2}b$ (15·4 Å), and these ridges contain periodic antinodes about 9–11 Å apart. The outer part of the pseudo-orthorhombic projection also has a simple appearance, and is very similar to the inner part; here again there are ridges $\frac{1}{2}b$ apart, but these are displaced in the b direction a distance approximately $\frac{1}{4}b$, so that they alternate with the inner ridges. Clearly these relations confirm the scheme set out in Fig. 1(*b*), and extend it by indicating that the molecules in the right-hand part of the cell are displaced by $\frac{1}{4}b$ or 7·7 Å relative to those in the left-hand part.

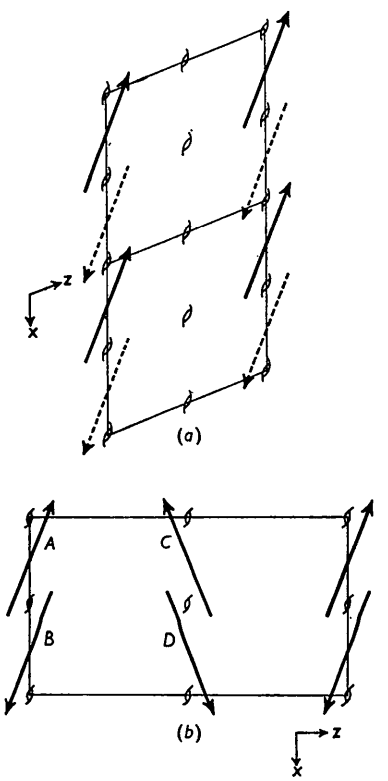

Fig. 4. (*a*) Arrangement of molecules in the monoclinic unit cell (lattice A) seen in b projection. The arrows indicate the rod direction; →, molecule at $y = 0$; ⇢, molecule at $y = \frac{1}{2}$. (*b*) Arrangement of molecules in the pseudo-orthorhombic unit cell, seen in b projection. In each sheet alternate molecules are displaced $\frac{1}{2}b$ relative to one another.

(*c*) *The c projection* (Fig. 3(*c*))

The X-ray photograph from which this projection is derived is less good than the others (this is always true of the [001] zone of reflexions from these crystals), and correspondingly the projection contains few features of interest. So far as they go these features provide confirmation of the scheme derived from the other two projections. The relative displacement of $\frac{1}{4}b$ between the layers in the two parts of the cell means that in the c projection little trace of layering should be visible; this accords with observation (in the c projection of the monoclinic form, on the other hand, the layering is at least as pronounced as in the a projection; see Kendrew (1950), Fig. 4(*a*)). So far as layering is visible at all, there should be *four* layers per repeat in the b direction; traces of these can in fact be seen, though the resolution is inadequate.

4. Discussion

We may enquire whether it is possible to assign co-ordinates to the molecules in this structure which would account both for its relationship to the monoclinic lattice and for the features of its Patterson projections. Now in the monoclinic form it has been concluded (see the earlier paper) that sheets of molecules in the ab plane slide over one another (in the a direction) to produce changes in the monoclinic angle β. Such a conclusion is supported by a close examination of the b Patterson projections of both its A and B lattices; these exhibit fairly definite signs of pseudo-origins at $x = \frac{1}{2}$, $z = 0$ (see for example Fig. 2(*b*), where the pseudo-origin is marked O′ and where it will be noted that the arrangement of peaks in its neighbourhood resembles that near the true origin O). Such pseudo-origins could arise only if the co-ordinates of the molecules were approximately $(\frac{1}{4}, 0, 0)$ and $(-\frac{1}{4}, \frac{1}{2}, 0)$, that is to say if the molecules were close-packed in ab sheets (see Fig. 4(*a*)). It is now proposed that the new unit cell arises by the reversal of alternate sheets of molecules, giving a pseudo-orthorhombic symmetry. But the *true* symmetry of the new cell is monoclinic, $P2_1$, with screw dyad axes having x and z co-ordinates (0, 0), $(0, \frac{1}{2})$, $(\frac{1}{2}, 0)$ etc.; it will be seen from Fig. 4(*b*) that if the integrity of the sheets is maintained (as seems highly probable in view of the relationships between the Patterson projections of the two forms) there is only one way in which they can be inserted in the cell relative to the screw dyad axes, namely, so that the axes lie midway between neighbouring pairs of molecules in each sheet, just as they did in the monoclinic cell. Accordingly the x and z co-ordinates of the molecules must be $(\frac{1}{4}, 0)$, $(-\frac{1}{4}, 0)$; $(\frac{1}{4}, \frac{1}{2})$, $(-\frac{1}{4}, \frac{1}{2})$. We should then expect to find a well-developed pseudo-origin in the b projection corresponding to vectors between pairs of molecules whose chains are parallel (e.g. A and B, C and D in Fig. 4(*b*)), i.e. at $x = \frac{1}{2}$, $z = 0$. This is in fact observed (O′ in Fig. 2(*a*)).

We may now establish the y co-ordinates of the molecules by reference to the a projection (Fig. 3(a)). While confirming the conclusion that the molecules are in pairs at $z = 0$ and $z = \frac{1}{2}$ (pseudo-origins O'), this projection also suggests strongly that they are displaced in neighbouring sheets by $\frac{1}{4}b$, giving the pseudo-origins marked O'' at $y = \frac{1}{4}, z = \frac{1}{2}$. This arrangement is sketched in Fig. 5(a), which includes all the axes of the pseudo-orthorhombic symmetry; it would yield the point-Patterson shown in Fig. 5(b) which may be compared with Fig. 3(a).

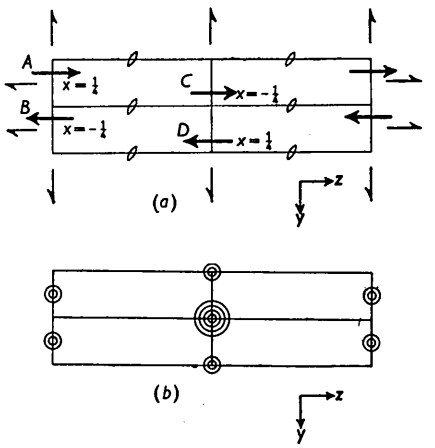

Fig. 5. (a) Arrangement of molecules in the pseudo-orthorhombic unit cell, seen in a projection. (b) Point-Patterson projection derived from (a).

It may thus be concluded that the co-ordinates of the molecules are $(\frac{1}{4}, \frac{1}{8}, 0)$, $(-\frac{1}{4}, -\frac{3}{8}, 0)$, $(\frac{1}{4}, -\frac{1}{8}, \frac{1}{2})$ and $(-\frac{1}{4}, \frac{3}{8}, \frac{1}{2})$, as shown in Figs. 4($b$) and 5($a$). This unit cell may be sheared slightly to give a monoclinic angle $\beta = 91°$; on the other hand the symmetry of the reflexions is very nearly orthorhombic and it is admittedly very difficult to understand why the structure does not exhibit twinning or disordering as a consequence of its slight distortion from orthorhombic symmetry. The fact is, however, that twinning has not been encountered in this structure.

The authors are grateful to Sir Lawrence Bragg, F.R.S., for his interest in the work and in particular for his contributions to the last section of this paper.

Reference

KENDREW, J. C. (1950). *Proc. Roy. Soc.* A, **201**, 62

Acta Cryst. (1954). **7**, 351

'β-Tungsten' as a Tungsten Oxide

BY G. HÄGG AND N. SCHÖNBERG

Institute of Chemistry, University of Uppsala, Uppsala, Sweden

(*Received* 7 *December* 1953)

The existence of 'β-W' as a modification of tungsten is contradicted by the low density of the phase and by its decomposition into W and WO_2 at about 700°C. It is assumed that 'β-W' is a metallic tungsten oxide with the probable ideal formula W_3O. The unit cell would then contain 6 W + 2O atoms distributed at random over the eight positions of the $A15$ structure.

Introduction

The phase, which hitherto has been considered as the β modification of tungsten (β-W), is referred to as the $A15$ type. The space group is $Pm3n$ with 2 W in (a): 0, 0, 0; $\frac{1}{2}, \frac{1}{2}, \frac{1}{2}$, and 6 W in ($c$): $\frac{1}{4}, 0, \frac{1}{2}$; $\frac{1}{2}, \frac{1}{4}, 0$; $0, \frac{1}{2}, \frac{1}{4}$; $\frac{3}{4}, 0, \frac{1}{2}$; $\frac{1}{2}, \frac{3}{4}, 0$; $0, \frac{1}{2}, \frac{3}{4}$. It has been obtained only by electrolysis of fused mixtures of either tungsten trioxide and alkali metal phosphates (Hartmann, Ebert & Bretschneider, 1931) or of alkali metal tungstates (Burgers & van Liempt, 1931) at temperatures below 700°C. Hartmann *et al.* stated that β-W irreversibly transforms into α-W at temperatures above 700°C.

Experimental

The same method of preparation as described by the above mentioned authors was used in the present investigation, and the reaction temperature was 625–650°C.

Powder photographs were taken in focusing cameras of the Guinier type using Cu $K\alpha$ radiation. It was found impossible to prepare β-W in a pure state. Some samples contained alkali tungsten bronzes and others α-W as impurities. One sample of the latter type contained only small amounts of α-W, and this sample was used in the following study.

Results and discussion

A lattice constant of $a = 5{\cdot}036$ Å ($V = 127{\cdot}7$ Å^3) was obtained for β-W, in close agreement with the values given by Hartmann *et al.* (1931) and Neuburger (1933). The $p|F|^2$ values calculated for the $A15$ arrangement of atoms were further in good agreement with