Use of Molecular Replacement in the Structure Determination of the P21212 and the P21 (pseudo P21212) crystal forms of Oxidized Uteroglobin

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LETTER TO THE EDITOR

Use of Molecular Replacement in the Structure Determination of the $P2_12_12$ and the $P2_1$ (Pseudo $P2_12_12$) Crystal Forms of Oxidized Uteroglobin

The structure of the symmetrical dimer of oxidized rabbit uteroglobin, as determined from the crystal form in space group $C222_1$, has been used as a model to determine the general parameters of this protein in two other crystal forms; namely, a symmetrical dimer in $P2_12_12$ and an asymmetrical dimer in $P2_1$ with non-crystallographic symmetry approaching $P2_12_12$. Independently, the structure in $P2_12_12$ was solved by multiple isomorphous replacement.

After exchanging data, the analysis was carried out in two different laboratories with different methods of molecular replacement. The result was the same for both approaches, and it could be shown further that the packing of molecules in both crystal forms analysed is so similar that they can be considered pseudo-isomorphous, i.e. distinguished only by the fact that two out of three symmetry operators are crystallographically perfect in one case and molecular and approximate only in the other.

The principal fold of the polypeptide chain is the same in all crystal forms considered so far, but there is evidence for differences in the detail, which will be worked out later with progressing refinement.

Six crystal forms of oxidized rabbit uteroglobin have been characterized (Buehner & Beato, 1978; Mornon *et al.*, 1978,1979,1980). Of these, the orthorhombic $P2_12_12$ form (one monomer in the asymmetric unit, $a = 44{\cdot}5$ Å, $b = 36{\cdot}9$ Å, $c = 32{\cdot}3$ Å) and the monoclinic $P2_1$ form (pseudo $P2_12_12$, one dimer in the asymmetric unit, $a = 43{\cdot}3$ Å, $b = 38{\cdot}1$ Å, $c = 34{\cdot}5$ Å, $\beta = 90{\cdot}7°$) constitute a sub-group/super-group pair whose near-identity of cell dimensions suggests great similarity in the molecular arrangement.

Both of these crystal forms were analysed by molecular replacement, using the high-resolution structure of the $C222_1$ crystal form (Mornon *et al.*, 1980) as a model. The $P2_1$ (pseudo $P2_12_12$) form was studied in Paris and in Würzburg, whereas the $P2_12_12$ form was analysed in the Paris laboratory only, by m.r.† as well as independently by multiple isomorphous replacement.

(a) *The* $P2_12_12$ *form*

(i) *Molecular replacement*

Since a uteroglobin dimer sits on a crystallographic 2-fold axis in both the $P2_12_12$ as well as the $C222_1$ crystals, the number of degrees of freedom of the model is

† Abbreviations used: m.r., molecular replacement; m.i.r., multiple isomorphous replacement; R, residual difference, given in %

$$R = 100 \times \Sigma||F_{obs}|-|F_{calc}||/\Sigma|F_{obs}|.$$

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reduced to two when we assume that the assembly of the dimer is identical in both. To fit the $C222_1$ monomer into the $P2_12_12$ unit cell, only the rotation Φ about the c-dyad and the translation along this axis had to be determined.

The calculations have been done in two steps. First, a rough estimation of possible values of Φ and Δz, using systematic search for minimum residual difference (R) in two and one dimensions on $hk0$ and $00l$ data, respectively; second, a "heuristic" three-dimensional search using the rigid body refinement program ARTÉMIS (adapted version of RAFMLC (Vallino, 1969) written in FORTRAN for IBM 370/165, available from A.L.) with 133 reflexions in the resolution range 6 to 10 Å. The starting position for the dimer centre of gravity (0, 0, 0·25) was deduced from packing considerations based on spheroidal shapes approximating the uteroglobin dimer (Fig. 1(a)).

Both calculations converged to the same solution with $R = 47\%$ (3 dimension data): $z = 0{\cdot}253$, $\Phi = 33°$. This solution puts the centre of gravity of the monomer into position x, y, $z = -0{\cdot}141$, 0·072, 0·253. The resulting R-value has to be

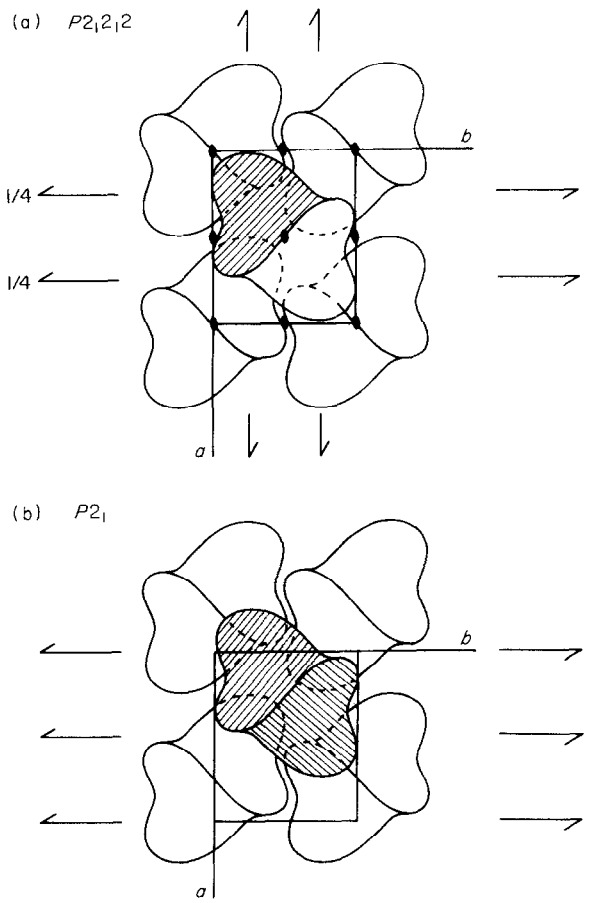

FIG. 1. Molecular packing of uteroglobin dimers (a) in the $P2_12_12$ and (b) in the $P2_1$ (pseudo $P2_12_12$) crystal forms. Asymmetric units are shaded.

compared to that of the $C222_1$ structure itself, which is 43% at the same range of resolution (146 reflexions). This comparison shows the reliability of the molecular replacement results. The second lowest minimum in the R-search led to $R = 48{\cdot}5\%$ at $\Phi = 43°$. In the next step, all restraints were relaxed: R did not further decrease significantly.

(ii) *Multiple isomorphous replacement*

This crystal form was also studied by m.i.r. methods (R. Bally & J. P. Mornon, unpublished results). The structure was solved with three derivatives and led to a figure of merit of 0·72 at 3·0 Å resolution. The electron density showed all of the monomer clearly.

In Figure 2 a difference map section of the platinum site of the $PtCl_4^{2-}$ derivative is shown, calculated with m.r. phases that yield the same Pt position as m.i.r. This clearcut answer proves the reliability of the m.r. results. Furthermore, comparison of the experimentally determined α-carbon positions (m.i.r.) with the theoretical positions (m.r.) shows good agreement.

(b) *The* $P2_1$ *(pseudo* $P2_12_12$*) form*

(i) *Work done in the Paris laboratory*

If the molecular packing is indeed roughly the same in the $P2_12_12$ and the $P2_1$ (pseudo $P2_12_12$) crystals, only two aspects have to be considered for the transformation of one lattice into the other (Fig. 1). (1) Due to a different origin definition in both space groups, there is a translation by 1/4 a; (2) one axis and one screw axis are regular symmetry elements in one case and only approximate ones in the other, thus somewhat relaxing the parameters controlled by them.

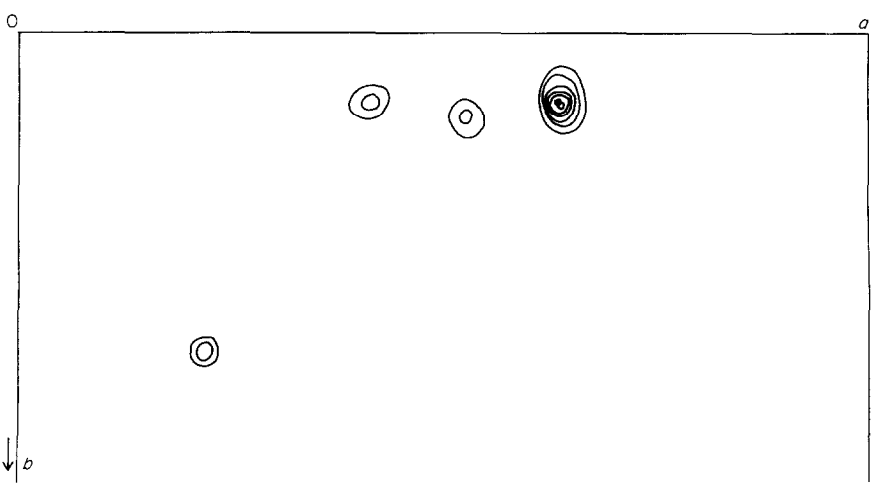

FIG. 2. Section of $PtCl_4$ difference electron density map of $P2_12_12$ uteroglobin with molecular replacement phases. The black dot indicates the Pt position as derived from multiple isomorphous refinement.

TABLE 1

Orientational and positional parameters of the asymmetric uteroglobin dimer in the crystal form $P2_1$ *(pseudo* $P2_12_12$*)*

	Subunit	Rotation (Eulerian angles) Θ_1 (°)	Θ_2 (°)	Θ_3 (°)	Translation (centre of gravity) x	y	z
Paris	1	179·3	88·1	39·5	0·366	−0·025	0·221
	2	182·1	91·6	216·9	0·131	0·020	0·222
	1+2(1)	178·8	88·5	38·0	0·248	−0·002	0·221
Würzburg	1	176·0	89·0	38·0	0.387	0·006	0·266
	2	174·0	91·5	225·2	0·142	−0·028	0·236
	1+2(2)	178·5	90·0	37·0	0·264	−0·011	0·251

(1) Orientation found by rigid body refinement using a rigid dimer $C222_1$ against 6 to 10 Å data.
(2) Main peak of the rotation function $(C222_1)\times(P2_1)$ using 4 to 10 Å data.

One starting position (0·25, 0, 0·25) and two starting orientations were deduced directly from the $P2_12_12$ results. Three-dimensional searches done by ARTÉMIS, with the rigid $C222_1$ dimer as a model, permitted selection of the best orientation, using 169 reflexions in the resolution range 6 to 10 Å: $\Phi_1 = -32{\cdot}8°$, $R_1 = 55\%$; $\Phi_2 = 32{\cdot}7°$, $R_2 = 43\%$. R_2 is comparable with R for the $C222_1$ model itself. We have to consider, however, that the $P2_1$ F_{obs} terms are film data as compared to diffractometer data for the other crystal forms. Therefore, R-values might not be comparable directly.

The molecular 2-fold axis refined further to the orientation given in Table 1 (1+2(1)) (Eulerian angles, Θ_3 roughly corresponds to Φ above) and thus is tilted to the crystallographic c-axis by 2°.

When the model is split into two identical, independent rigid monomers, the model becomes asymmetric and R decreases to 39% at the same resolution. The position and orientation of the two monomers are given in Table 1 (top), for 1589 reflexions in the resolution range 2·6 to 10 Å, leading to $R = 52\%$. This refinement permits direct access to an important aspect of the asymmetric dimer: a relative deviation of $\delta = 3{\cdot}7°$ of the two monomers from perfect dimer symmetry. In this particular case, δ can be evaluated independently from the two sets of F_{obs} terms by the cross-rotation function $(C222_1)\times(P2_1)$ (cf. subsection (ii), below) with good agreement (A. Lifchitz, unpublished results).

(ii) *Work done in the Würzburg laboratory*

Data were collected from $P2_1$ uteroglobin crystals by precession and rotation photography. The data set has a low-intensity cutoff and thus is only 79% complete within the 3 to 10 Å resolution range.

The solution was approached by general search to confirm the pseudo space group independently rather than relying on it for constraints. In the rotation function (Rossmann & Blow, 1962), using 5 to 10 Å data and a radius of integration of 20 Å in a dyad search ($\kappa = 180°$), the highest peak (79% of origin) was found

numerically identical in position $\psi = 90°$, $\varphi = 0°$ and in $\psi = 90°$, $\varphi = 90°$, i.e. straight on the a^*- and c-axes.

Further refinement using 4 to 10 Å data led to $\psi = 90{\cdot}0°$, $\varphi = 90{\cdot}4°$. The general model based on precession photographs (Buehner & Beato, 1978) has thus been corroborated by the rotation function.

The next step was a general search of the asymmetric unit of the cross-rotation function $(C222_1) \times (P2_1)$ from the two sets of F_{obs} terms in the 5 to 12 Å resolution range. The main peak, after rotation function refinement using 4 to 10 Å data, is given in Table 1 (1 + 2 (2)). The molecular axis is only 1·5° from the c-axis (which is in good agreement with the 2° found in subsection (i), above) and 1·2° from the position predicted by the self-rotation function.

All further steps of the procedure were carried out with F_{calc} rather than F_{obs} (model). R-model was 49·2% with 2940 reflexions in the resolution range 2·2 to 30 Å. The orientation of the individual subunits was determined by the cross-rotation function $(P1) \times (P2_1)$, using a monomer in a large artificial unit cell.

The translation problem was tackled by three independent methods: a systematic R-search, the translation function (Crowther & Blow, 1967), and packing calculations. The former two reciprocal space methods performed rather poorly and converged only after both subunits were allowed to move independently instead of locked in a dimer. Convergence improved when the orientation was adjusted concurrently.

The final solution is given in Table 1 (bottom), and agrees reasonably well with that of crystal form $P2_12_12$ (cf. subsection (a) (i) above, y is arbitrary in $P2_1$). The corresponding R value was 53·8% at 2·5 to 30 Å resolution. At this resolution, an F_{obs}/α_{calc} map was only twice as high as the F_{obs}-F_{calc}/α_{calc} difference map, indicating significant differences between model and structure. A $2F_{obs}$-F_{calc} electron density map was interpreted by model building in a Richards' box.

The map is not equally well-defined in all parts of the molecule, again indicating significant structural changes. Further improvement of the phases is required before detailed structural interpretations can be given with confidence.

Calculations were done on the TR440 computer of the Würzburg University computer centre.

(c) *Further development*

The crystal structure of the other $P2_1$ form ($a = 44{\cdot}6$ Å, $b = 46{\cdot}1$ Å, $c = 37{\cdot}4$ Å, $\beta = 120{\cdot}9°$) with one dimer in the asymmetric unit was solved by isomorphous replacement (R. Bally & J. P. Mornon, unpublished results). It can be expected that the analysis of (up to now) two symmetrical (likely disordered in the central part of the binding site) and two asymmetrical dimers (with the protein coming from two different biochemical laboratories) will bring us improved insight into the variability of this relatively small protein.

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