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Rotation-Function Study of Flavocytochrome b_2

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Abstract

A rotation-function study of flavocytochrome b_2 has been carried out using X-ray data from crystals which contain one tetramer of 230 000 Dalton per asymmetric unit. The function computed from 10–5·5 Å resolution data clearly shows the orientation of the molecular 222 symmetry axes. The presence of these symmetry elements is consistent with previous structural and biochemical studies of the molecule.

I. Introduction

Bakers yeast flavocytochrome b_2 [L-(+)-lactate cytochrome c oxidoreductase, EC 1.1.2.3) contains four identical subunits and has a molecular weight of 230 000 Dalton (Jacq & Lederer, 1974). It contains one protoheme IX and one flavin mononucleotide prosthetic group per subunit. It catalyzes the oxidation of lactate to pyruvate in mitochondria with the reducing equivalents passing to oxygen *via* the cytochrome c–cytochrome oxidase pathway (Pajot & Claisse, 1974).

When flavocytochrome b_2 is digested exhaustively with trypsin a small trypsin-resistant heme peptide, cytochrome b_2 core, remains intact (Labeyrie, Groudinsky, Jacquot-Armand & Naslin, 1966). The amino-acid sequence of the 96 residues of cytochrome b_2 core is remarkably homologous to that of microsomal cytochrome b_5 (Guiard & Lederer, 1976). Comparison of the two sequences with the atomic model of cytochrome b_5 (Mathews, Argos & Levine, 1971) indicates a possible structural similarity of the two molecules, as supported by other chemical and spectroscopic evidence (Guiard, Groudinsky & Lederer, 1974).

Large single crystals of flavocytochrome b_2 have been prepared which diffract to about 2·5 Å resolution (Mathews & Lederer, 1976). These crystals are trigonal and contain one tetramer in the asymmetric unit cell. The tetramer is expected to possess 222 symmetry (Olive, Barbotin & Risler, 1973). As part of the structural investigation of flavocytochrome b_2 it is

desirable to confirm the 222 symmetry of the molecule and to determine the orientation of the molecular symmetry elements in the unit cell. We report the results of a study of the rotation function (Rossmann & Blow, 1962) of flavocytochrome b_2 at 5·5 Å resolution.

II. Experimental methods

Crystals of flavocytochrome b_2 were grown by dialysis against 30% 2-methyl-2,4-pentanediol, pH 7·0, in the presence of 50 mM sodium DL-lactate as previously described (Mathews & Lederer, 1976). The crystals are trigonal, space group $P3_121$ or its enantiomorph, and contain one molecule per asymmetric unit. The cell parameters are $a = 165·51$, and $c = 113·71$ Å.

X-ray data were collected on a Picker FACS-1 automatic diffractometer using Ni-filtered Cu $K\alpha$ radiation. The Wyckoff step-scan procedure (Wyckoff et al., 1967) was used as modified in this laboratory for the Vanderbilt disk system (Lenhert, 1975). The crystals were cooled to about 258 K by a cold air stream (Marsh & Petsko, 1973) to reduce radiation damage. X-ray data were corrected for background, absorption, radiation-damage and Lorentz–polarization effects as previously described (Czerwinski & Mathews, 1974).

X-ray data were initially collected from 29 to 5·5 Å resolution in four concentric shells of sin θ containing approximately equal numbers of reflections. The shells overlapped slightly to allow intercrystal scaling. Four crystals were used, two of which contributed to two different shells. Crystals were discarded after 10 to 20% decay as monitored by three standard reflections.

Table 1. *Scale and agreement factors for flavocytochrome b_2 crystal data*

Crystal	Range (Å)	Scale factor	R_i factor[a]	Number of overlaps
3	11·3–7·4	1·12	0·100	2931
4A	29–11	3·54[b]	0·053	127
4B	11·3–7·4	2·42[b]	0·085	2139
5	7·5–6·0[c]	1·05	0·099	405
6A	7·5–6·0[c]	1·56	0·087	407
6B	6·0–5·5	1·62	0·095	1633
7	6·0–5·5	0·90	0·095	1636
9	5·5–5·0[d]	0·70	0·113	254
10	5·5–5·0[d]	0·64	0·133	294
11	11·3–7·4	1·00	0·089	2973

(a) $R_i = \Sigma|S_iI_{hi} - I_h|/\Sigma I_h$, where R_i is the agreement factor for the ith crystal, S_i is the scale factor for the ith crystal, I_{hi} is the observed intensity for Miller index h for the ith crystal, and I_h is the mean intensity for Miller index h. The sum is taken over those reflections which were observed at least twice.

(b) Crystal 4 was used for two data sets with different X-ray fluxes.

(c) Crystal 5 was used for only half the 7·6–6·0 Å data set. Crystal 6A was used for the other half.

(d) Crystal 9 was destroyed halfway through the 5·5–5·0 Å data set. Crystal 10 was used to complete the set.

The data set was later augmented and extended to 5·0 Å resolution using four additional crystals from which Friedel equivalent reflections were measured. Scale factors and agreement statistics among the data sets were calculated by the method of Rae (1965), using a program by G. Reeke. The initial and complete data sets were merged and used in the rotation-function study. The scale and agreement factors for individual data sets are given in Table 1. The final overall discrepancy factor between each measured intensity and the average was $R = 0·092$.

A plot of the mean intensity as a function of scattering angle shows a fivefold decrease from a maximum at 12 Å resolution to a minimum at 6 Å. The apparent temperature factor for the structure factors is about 50 Å^2. This value is very approximate because of the non-Gaussian behavior of the intensity distribution in this narrow range of scattering angle.

III. Results

(a) Rotation-function calculations

Two procedures were used to calculate the rotation function for flavocytochrome b_2. In both cases the function calculated was $R(\kappa,\psi,\varphi)$ where R is a measure of the overlap of the Patterson density (Rossmann & Blow, 1962) and κ, ψ and φ are the spherical polar coordinate variables. The first procedure employed the fast rotation function of Crowther (1972) which uses spherical harmonics. This program, executed on the IBM 360/65 computer, is relatively inexpensive, can use a large fraction of the data and computes the rotation function in three dimensions. It is limited to a Patterson radius of 35 Å at 6 Å resolution in its present configuration. The second procedure employed the program originated by Rossmann (Tollin & Rossmann, 1966) and adapted to the PDP 11/34 computer in this laboratory (Czerwinski, Bethge, Mathews & Chung, 1977). This program is much slower and can use only a limited amount of data in practice. However, it allows more flexibility in the calculations and could be executed overnight on the laboratory computer. The Rossmann program was used principally to verify the results of the Crowther program and to test the effects of several variables in the calculations.

(b) The 10–5·5 Å rotation function

The three-dimensional rotation function for flavocytochrome b_2 was evaluated with the Crowther program using data in the range of 10·0 to 5·5 Å resolution. The strongest 75% of the reflections were included and the radius of integration was 32 Å.

The $\kappa = 180°$ section contains the largest non-crystallographic peaks in the map, other than the space-group-related peaks at other values of κ

(Johnson, Argos & Rossmann, 1975). A stereogram of this section is shown in Fig. 1. The six most prominent peaks in the asymmetric units ($30° \leq \varphi \leq 90°, 0 \leq \psi \leq 90°$) are numbered. Their positions are given in Table 2. Peaks 5 and 6 at $\varphi = 30°$ are double-weight peaks lying on a mirror plane. Since these two peaks are 90° away from a crystallographic twofold axis, they generate related peaks 5' and 6' on the same plane at $\varphi = 210°$ (numbered at $\varphi = 90°$). Peaks 3 and 3' at $\psi = 90°$ are related by the crystallographic twofold axis at $\psi = 90°, \varphi = 60°$.

The twofold rotation axes 1, 2 and 3 and their symmetry equivalents form sets of vectors that are nearly at right angles to one another. One such set is indicated in Fig. 1. Their angular separations are also indicated in Fig. 1 and Table 3. These three peaks are the highest single-weight peaks on the map and are nearly as large as peaks 5 and 6 on the mirror planes.

Table 2. *Locations of the six most prominent rotation-function peaks for flavocytochrome b_2 at 10·0–5·5 Å resolution*

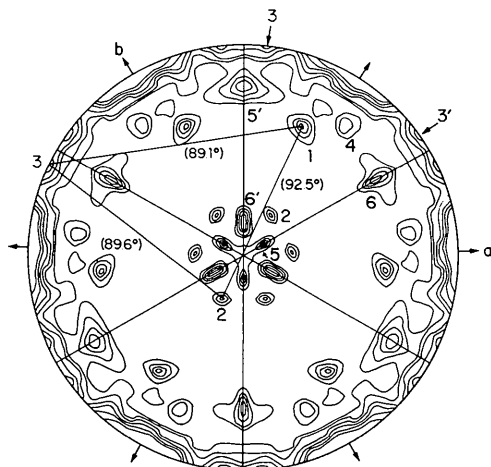| Peak	ψ (°)	φ (°)	Peak height (arbitrary units)
1	67·5	66·0	21
2	25·0	56·0	17
3	90·0	85·0	23
4	76·0	52·5	16
5	12·5	30·0	22
6	70·0	30·0	21
Origin	90·0	60·0	100

Fig. 1. Rotation function for flavocytochrome b_2 using data between 10·0 and 5·5 Å resolution. The stereogram corresponds to $\kappa = 180°$. The c axis is at the center and the a and b axes are indicated at the periphery. The orientations of the mirror planes and twofold axes are indicated. The six most prominent peaks are labeled. The set of peaks corresponding to the 222 molecular symmetry axes, and their relative inclinations, are indicated.

Table 3. *Angular separation between rotation-function peaks which form 222 symmetry sets (see text and Fig. 2 for peak definition)*

First peak	Second peak	Angular separation
1	2	92·5° ⎫
1	3	89·1 ⎬ Symmetry set 1
2	3	89·6 ⎭
2	4	87·9
2	6	91·1
4	6	92·1

A systematic search was carried out for other solutions to the rotation function which would obey the 222 symmetry expected of the molecule. To do this, the inclination angles between all pairs of peaks related by symmetry to the set of six were calculated. Those twofold axes inclined between 85 and 95° with respect to one another were selected and the orientation of a third axis at right angles to the other two was computed. One additional possible solution was found by this search and is listed in Table 2. The latter solution involves peaks which are of lower weight and on a mirror plane and is less likely to represent the true solution. In addition, if one of the molecular symmetry axes is approximately parallel to a crystallographic twofold axis (and therefore not resolvable in the rotation function), peaks 5 and 5' or 6 and 6' could be a solution. This is very unlikely, however, as these peaks would then be quadruple weight and would be expected to be much larger than non-solution peaks. Furthermore, the three-dimensional Patterson function of the native protein at 6·0 Å resolution failed to show the large peak expected for the resulting pure non-crystallographic translation symmetry (Rossmann, Ford, Watson & Banaszak, 1972).

(c) Lower-resolution data

The rotation-function calculation was repeated using data from 15–6 Å resolution. The $\kappa = 180°$ section contained several peaks in addition to those of Fig. 1 and peak 1 was shifted. In an effort to understand the nature and causes of the changes another map was computed using the 15–10 Å range of data only. The $\kappa = 180°$ section, shown in Fig. 2, is completely different from the 10–5·5 Å map. However, the peaks correspond quite well to the additional peaks of the 15–6 Å map. No attempt was made to interpret the 15–10 Å map in terms of the molecular symmetry axes.

The 15–10 Å data contained contributions from several crystals and many strong reflections which might suffer from scaling errors. To eliminate the effect of scaling errors, a rotation function was calculated from just one crystal, B2-11, which appeared to be of good quality. The complete data set, from 11·4–7·3 Å

resolution, gave a map with the expected features of the 10–5·5 Å map plus weak contributions from the set of peaks seen in the 15–10 Å map. However, the same crystal, when limited to the 10–7·3 Å shell, gave a map with very low background density and nearly uniform peak heights for the three major peaks (Fig. 3). Thus the contribution from the low-order reflections must represent real structural features and not poor data.

(d) Studies with the Rossmann program

Several calculations using the Rossmann program were carried out on the PDP 11 computer in order to vary some additional parameters. In most cases about 12% of the data were used and the calculations were carried out at $\kappa = 180°$ only. The calculation using 10–6 Å data was nearly identical to the results of the

Fig. 2. Rotation function, $\kappa = 180°$ section, for flavocytochrome b_2 using 15–10 Å data.

Fig. 3. Rotation function, $\kappa = 180°$ section, for flavocytochrome b_2 using the 10–7·3 Å subset of data taken from crystal 11. The three major peaks corresponding to the molecular 222 symmetry axes are indicated.

Crowther program. When the radius of integration was extended to 70 Å the results were very similar, except that peak 2 was enhanced and peak 3 reduced in size. The areas around peaks 1 and 2 were searched at 1° intervals at $R = 35$ Å and $R = 70$ Å to locate their centers more accurately. The centers of the peaks agreed within 1° using the two radii, but the widths of the peaks decreased from 5 to 3·5° at the larger radius. The results of the fine search were also within 1·5° of the Crowther results.

The map was also calculated with only the ten largest reflections, eight of which are of the $0kl$ type and all of which lie in the 10–15 Å range. The peaks on this map resembled those on the 15–10 Å map shown in Fig. 2. Finally a rotation-function map was calculated from sharpened coefficients between 15 and 6 Å resolution. The Patterson origin was removed and the radius of integration was again set at 35 Å. This map was very similar to that of Fig. 1 except that peak 1 was shifted to $\psi = 70°$ and peak 2 was reduced in magnitude.
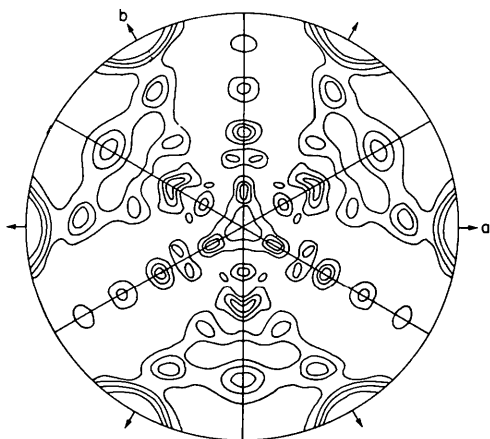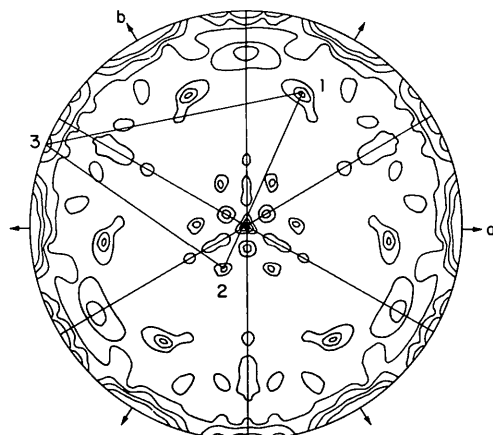
IV. Discussion

The 10–5·5 Å rotation function contains two possible solutions to the orientation of the molecular 222 symmetry axes (Table 3). The first of these solutions, involving peaks 1, 2 and 3 of Fig. 1, is the most likely since it uses the highest single-weight peaks in the map. The validity of this solution is supported by the rotation-function calculation from the single data set of crystal 11 between 10·0 and 7·3 Å. In this map (Fig. 3) peaks 1, 2 and 3 dominate and lead to a nearly unambiguous solution. This crystal was well behaved during data collection, suffered little radiation damage and showed good agreement between Friedel pairs.

The effect of including lower-resolution data (15–10 Å) in the rotation-function calculation is to introduce large peaks, shown in Fig. 2, which dominate the map and obscure or shift the molecular symmetry peaks seen at higher resolution. When the low-resolution limit is reduced progressively from 15 to 10 Å these low-order peaks gradually diminish allowing the true peaks to dominate. The data between 15 and 10 Å is two to four times as intense as the 10–6 Å data so any false peaks produced by the inner data will be very strong. The rotation function using sharpened Patterson coefficients from 15–6 Å does not show the strong low-resolution peaks and agrees quite well with the 10–5·5 Å map.

The causes of the low-resolution peaks seen in Fig. 2 are not readily apparent. They probably do not arise from errors in scaling the large inner reflections since the 11·4–7·3 Å map from crystal 11, which required no scaling, contains many of the low-resolution features. The peaks probably arise from the accidental

alignment of low-resolution structural features such as solvent regions, helices, etc. It is interesting to note that many of the very strong reflections at about 12 Å are of the $0kl$ class. Thus, a 180° rotation about an axis in the $0kl$ photograph (and therefore lying in a reciprocal-lattice mirror plane) will cause a strong reflection to fall on its Friedel mate or on some other strong reflection in the same zone.

The most reasonable interpretation of the rotation function indicates that flavocytochrome b_2 has 222 symmetry. This is in agreement with chemical evidence that the four subunits are identical, or nearly so (Jacq & Lederer, 1974). Furthermore, powder diffraction studies of another crystal form of flavocytochrome b_2 indicate a tetragonal lattice in which the molecule is located on a point of crystallographic 222 symmetry (Monteilhet & Risler, 1970).

The rotation-function result will be useful in the structural analysis of flavocytochrome b_2. First, it can be used to help locate heavy atoms in difference Patterson maps (Argos & Rossmann, 1974). Second, it can aid in the search for a cytochrome b_5-like structure in the flavocytochrome b_2 crystal. If the b_5 and b_2 molecules are sufficiently alike, the three-dimensional cross rotation function should show peaks when the two molecules are aligned. These peaks should obey the same symmetry as the flavocytochrome b_2 molecule itself. We are presently continuing these studies along these lines.

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Topotactical Dehydration of Chloritoid

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Abstract

The dehydration of the silicate mineral chloritoid in air and in vacuo has been investigated by single-crystal X-ray methods and high-resolution electron microscopy. Vacuum dehydration yields an amorphous product, but the reaction in air produces a topotactical transformation to an anhydrous structure with an alteration in the stacking arrangement and symmetry. To be consistent with these observed facts, considerable