Acta Cryst. (1994). D**50**, 884–888

Crystal Structure of a Monoclinic Form of Dihydropteridine Reductase from Rat Liver

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(*Received* 2 *September* 1993; *accepted* 23 *May* 1994)

Abstract

A binary complex of dihydropteridine reductase and NADH crystallizes in the space group $C2$, with a = 222.2, b = 46.5, c = 95.3 Å and β = 101.1°. There are two dimers in the asymmetric unit. The structure was solved by molecular-replacement techniques and refined with 2.6 Å data to a crystallographic R factor of 16.8%. Each dimer has twofold non-crystallographic symmetry and the four individual monomers in the asymmetric unit have the same overall molecular conformation.

Introduction

Dihydropteridine reductase (DHPR, E.C. 1.6.99.10) catalyzes the reduction of quinonoid dihydrobiopterin (qBH_2) to tetrahydrobiopterin (BH_4). BH_4 is a cofactor for the aromatic amino-acid hydroxylases. These hydroxylation reactions are essential steps in the reactions that lead to the synthesis of the catecholamine neurotransmitters dopamine, epinephrine and serotonin. DHPR uses NADH as a cofactor which donates a hydride for the reduction of qBH_2. The DHPR–NADH binary complex crystallizes in two forms: (i) a monoclinic (Matthews, Webber & Whitely, 1986) and (ii) an orthorhombic form (Varughese, Skinner, Whiteley, Matthews & Xuong, 1992). Although the monoclinic form was obtained first, the structure solution was delayed because of the difficulty in obtaining isomorphous heavy-atom derivatives. However the orthorhombic form provided highly isomorphous heavy-atom derivatives and was, therefore, the first one to be solved. We now report the structure solution of the monoclinic form by molecular replacement (MR) techniques.

DHPR from various mammalian sources are remarkably similar. Fig. 1 lists the sequences of the human and rat liver enzymes. Other than the deletion of three residues and substitution of another at the N-terminus, there are only ten conservative amino-acid differences between the rat enzyme (Weber, Hural & Whiteley, 1987) and human enzyme (Lockyer *et al.*, 1987). Hence the structure of the rat liver enzyme structure can be thought of as a prototype for all mammalian DHPR enzymes. Our recent analysis (Su *et al.*, 1993) of the human enzyme showed that the crystal structures of the rat enzyme and human enzyme are remarkably similar.

Experimental

DHPR was isolated from rat liver and purified by procedures described earlier (Webber & Whiteley,

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Table 1. *Data-collection statistics*

Resolution shell lower limit (Å)	Average $I/\sigma(I)$	No. of reflections	Completeness (%)
5.13	37.2	3955	98.7
4.07	26.3	3919	99.9
3.56	13.2	3781	99.4
3.23	6.3	3873	99.0
3.00	3.8	3568	94.0
2.80	3.1	3354	76.0
2.60	2.3	3187	54.7

Total No. of reflections = 26789
R_{sym}* = 5.1%

* $R_{sym} = \sum_h \sum_i^N {}_1|I(h) - I(h)_{i1}/\sum_h \sum_{i=1} I(h)_i$, where $I(h)_i$ is the ith measurement of reflection h and $I(h)$ is the mean value of the N equivalent reflections.

1978). DHPR exists as a homodimer in solution with each subunit of the rat enzyme consisting of 240 residues. Crystals were grown by a hanging-drop method using polyethylene glycol as a precipitant and Tris buffer at pH 7.8 and 277 K (Matthews, Webber & Whiteley, 1986; Varughese *et al.*, 1992). The crystals used for data collection were approximately 0.8 × 0.3 × 0.3 mm. Diffraction data were measured with graphite-monochromated Cu $K\alpha$ radiation (λ = 1.5418 Å) at 277 K using a multiwire area detector system (Hamlin *et al.*, 1981; Xuong, Sullivan, Nielsen & Hamlin, 1985). The crystals diffracted to 2.6 Å. Diffraction data were collected by scanning ω at seven different orientations. R_{sym} estimated from the multiple observations, was 5.1%. Statistics for this data are given in Table 1. The average $I/\sigma(I)$ at 2.6 Å is 2.3. The data is 96.6% complete to 2.6 Å. The crystals belong to space group $C2$, with a = 222.2, b = 46.5, c = 94.3 Å and β = 101.1°, and two dimers in the asymmetric unit. The two dimers are referred to as dimer A and dimer B with individual subunits $A1$ and $A2$ for dimer A, and as $B1$ and $B2$ for dimer B, respectively. The Matthews coefficient for this form is 2.46 Å^3 Da^{-1} corresponding to a solvent content of 49%, compared to 45.6% for that of the orthorhombic crystal form of DHPR reported previously (Varughese *et al.*, 1992).

Structure solution

The crystal structure was solved by molecular-replacement techniques using the program *MERLOT* (Fitzgerald, 1988). The crystal structure of DHPR (NADH excluded) in the orthorhombic form was used as the search model. The orthorhombic form was refined using 2.3 Å data to an R factor of 15.4%. Although the orthorhombic form has only one monomer per asymmetric unit, the structure is a dimer in the crystal lattice, individual monomeric subunits being related by a crystallographic twofold axis. The search model used for the molecular replacement procedures was this dimer having exact twofold symmetry. The twofold axis of the dimer

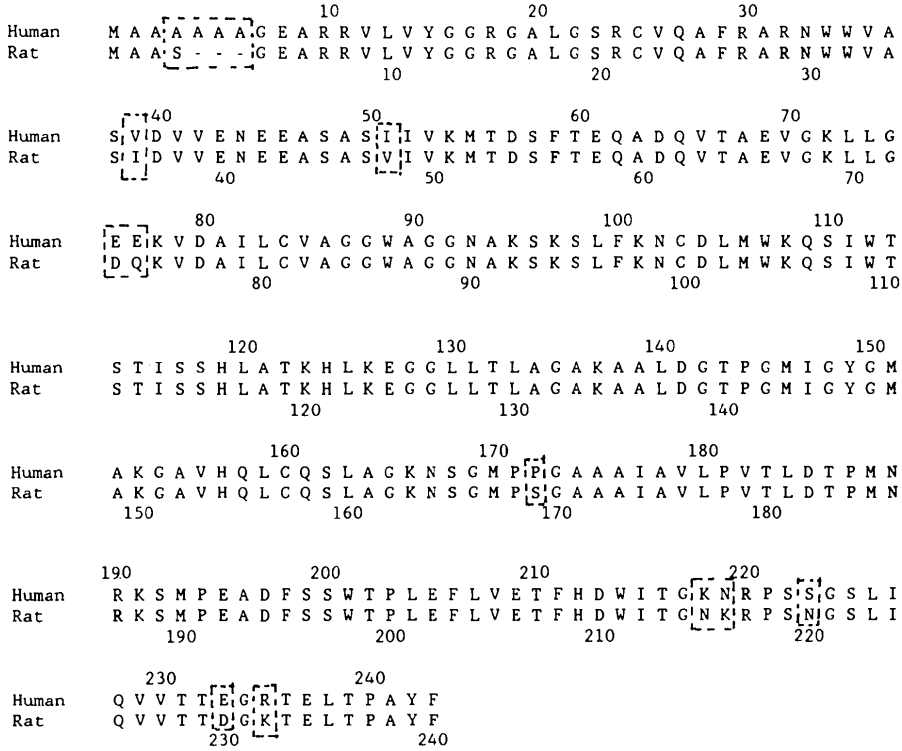

Fig. 1. Sequences of the human and rat dihydropteridine reductases. Differences in sequence are highlighted by dashed boxes.

Table 2. *Rotation-function peaks*

Peak	α (°)	β (°)	γ (°)	σ level	Ratio with the highest level
1	127.5	148.5	45.0	10.3	1.0
2	0.0	52.0	5.0	9.9	0.97
3	122.5	148.0	40.0	9.7	0.95
4	132.5	150.0	50.0	9.3	0.90

was oriented along the z axis to reduce the Patterson space that would be necessary to explore. A Crowther rotation function (Crowther, 1972) was calculated using data from 10.0 to 4.0 Å with a Patterson cut-off radius of 23.0 Å, and a search step of $\Delta\alpha = 2.5$, $\Delta\beta = 2.0$, $\Delta\gamma = 5.0°$. Four peaks were found to have peak values greater than 90% of the highest peak and there were no peaks having values within the range 65–89%. These four peaks were 9.3–10.3σ above the mean value (see Table 2). Lattman's rotation function (Lattman & Love, 1970) was used for performing a fine search around the four solutions with a search grid of $\Delta\alpha = 1.0$, $\Delta\beta = 1.0$, $\Delta\gamma = 1.0°$. Peaks 1, 3 and 4 merged into a single peak (126.0, 149.0, 44.0). The refined peak 2 has $\alpha_2 = 0.0$, $\beta_2 = 53.0$, $\gamma_2 = 5.0°$. These two peaks are related by a pseudo-twofold axis, $\varphi = 48.0$, $\psi = 73.7$, $\chi = 186.7°$.

The translation search for the positions of peak 1, (x_1,y_1,z_1) and peak 2 (x_2,y_2,z_2) was performed using the Crowther & Blow method (Crowther & Blow, 1967). Vectors $(2x_1,0,2z_1)$ and $(2x_2,0,2z_2)$ between two dimers related by the twofold crystallographic axis were searched initially. This was followed by a search for the $(x_1 - x_2,\ y_1 - y_2,\ z_1 - z_2)$ vector between the two dimers in the asymmetric unit. Each vector-search map yielded a unique solution. The results are $x_1 = 0.045$, $y_1 = 0.50$, $z_1 = 0.32$ and $x_2 = 0.02$, $y_2 = -0.14$, $x_2 = 0.54$.

Refinement

When the two dimers were oriented and positioned according to the MR solution, the initial R factor was 36.7% for 8–3.0 Å data. 20 cycles of rigid-body minimization using *X-PLOR* (Brünger, Kuriyan & Karplus, 1987) taking each monomer as a rigid body, reduced the R factor to 31.9%. An $(F_o - F_c)$ map calculated at this stage showed electron density for NADH bound to each individual monomer. The density for the nicotinamide portion was weak in subunits *A*1 and *A*2, but was in agreement with the orientation for NADH observed in the orthorhombic crystal form. Hence, at this stage NADH molecules were added to all the four monomers and 140 cycles of conventional refinement using data between 8.0 and 2.6 Å were performed giving an R factor of 21.6% with an overall temperature factor of 15.0 Å^2. This was followed by 20 cycles of individual B-factor refinement which brought the R factor to 20.2%. At this stage, omit maps (each omitting about 10% of the total residues) were calculated and the program *FRODO* (Jones, 1978) was used to examine the maps and to rebuild the model. The rebuilding consisted of mainly refitting several side chains. This was followed by an additional 120 cycles of conventional refinement and 20 cycles of individual B-factor refinement yielding an R factor of 18.5%. A total of 148 water molecules were then added and further refinement produced a final R-factor of 16.7%. The r.m.s. deviation in the bond lengths and angles are 0.018 Å and 3.5°.*

The starting model for the refinement, as stated earlier, consisted of two dimers each with an exact internal twofold symmetry. During refinements no constraints were imposed to maintain the internal symmetry. However, after the final refinements, the subunits in dimer *A* were found to retain the twofold symmetry and the subunits in dimer *B* were found to have nearly an exact twofold symmetry with a rotation angle of 179.3°. The two dimers are not related to each other by any non-crystallographic twofold or screw axis. The cofactors in this crystal form are found to have higher temperature factors compared to the orthorhombic form. The average B factors for cofactor molecules in the two subunits in dimer *A* are 42 and 55 Å^2. The corresponding values in dimer *B* are 37 and 30 Å^2. The average B factor for the cofactor in the orthorhombic form is 24 Å^2. The cofactors were refined with parameter and topology files constructed using the crystal structure of the Li salt of NAD$^+$ (Reddy, Saenger, Muhlegger & Weimann, 1981). No restrictions were imposed on the sugar pucker. However, given the resolution of the present data and the high B factors of the cofactor, any discussion of the sugar pucker is not meaningful.

Results and discussion

Fig. 2 shows a stereoview of the dimers. The molecular geometry is the same as in the orthorhombic form. The r.m.s. Cα differences between the monomer in the $C222_1$ form and the two subunits in dimer *A* are 0.32 and 0.36 Å. The corresponding r.m.s. differences between the orthorhombic form and the two subunits in dimer *B* are 0.35 and 0.40 Å. Thus, it is evident that the molecular geometry of all the four monomers in the $C2$ form is very close to the

* Atomic coordinates and structure factors have been deposited with the Protein Data Bank, Brookhaven National Laboratory (Reference: 1DHR, R1DHRSF). Free copies may be obtained through The Managing Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England (Reference: GR0327). At the request of the authors the structure factors will remain privileged until 1 July 1996.

Table 3. *Interdimeric interactions*

Interaction distances less than 3.5 Å are listed.

Monomer	Residue	Atom	Monomer	Residue	Atom	Distance (Å)	Symmetry operation
*A*1	Arg21	NH1	*B*1	Tyr239	OH	2.81	$-x+\frac{1}{2}, y+\frac{1}{2}, -z$
*A*1	Ala28	O	*B*1	Phe240	OT2	2.92	$-x+\frac{1}{2}, y+\frac{1}{2}, -z$
*A*1	Asp73*	OD1	*B*2	Glu234	OE2	3.13	$x+\frac{1}{2}, y+\frac{1}{2}, z$
*A*1	Asp73*	OD2	*B*2	Glu234	OE2	3.11	$x+\frac{1}{2}, y+\frac{1}{2}, z$
*A*1	Glu124	OE1	*B*2	Glu202	OE2	2.95	$x+\frac{1}{2}, y+\frac{1}{2}, z$
*A*1	Arg187	NH2	*B*1	Glu234	OE1	3.43	$x+\frac{1}{2}, y+\frac{1}{2}, z$
*A*1	Arg187	NH2	*B*1	Glu234	OE2	2.65	$x+\frac{1}{2}, y+\frac{1}{2}, z$
*A*1	Asp230	O	*B*1	Lys216*	NZ	3.34	$x+\frac{1}{2}, y+\frac{1}{2}, z$
*A*2	Glu5	OE2	*B*1	Asp182	OD2	3.25	x, y, z
*A*2	Glu124	OE1	*B*1	Thr233	N	3.39	x, y, z
*A*2	Lys163	NZ	*B*1	Asp210	OD1	3.12	x, y, z
*A*2	Lys163	NZ	*B*1	Asn215	ND2	3.47	x, y, z
*A*2	Ser169*	OG	*B*1	Glu206	OE1	3.22	x, y, z
*A*2	Gly170	N	*B*1	Glu206	OE1	3.31	x, y, z
*A*2	Gly170	N	*B*1	Glu206	OE2	2.67	x, y, z
*A*2	Pro191	O	*B*1	Lys92	NZ	2.89	$-x+\frac{1}{2}, y+\frac{1}{2}, -z$
*A*2	Glu192	OE1	*B*1	Lys92	NZ	3.11	$-x+\frac{1}{2}, y+\frac{1}{2}, -z$
*A*2	Glu192	OE1	*B*2	Thr119	O	2.75	$-x+\frac{1}{2}, y+\frac{1}{2}, -z$
*A*2	Glu192	OE2	*B*2	Lys75	NZ	3.38	$-x+\frac{1}{2}, y+\frac{1}{2}, -z$
*A*2	Ala193	O	*B*1	Lys92	NZ	3.15	$-x+\frac{1}{2}, y+\frac{1}{2}, -z$
*A*2	Asp194	OD1	*B*1	Lys94	NZ	2.96	$-x+\frac{1}{2}, y+\frac{1}{2}, -z$
*A*2	Asn219	ND2	*B*1	Ala25	O	3.38	x, y, z
*A*2	Asp230*	OD1	*B*1	Ser93	OG	2.73	$-x+\frac{1}{2}, y+\frac{1}{2}, -z$
*A*2	Asp230*	OD1	*B*1	Ser95	OG	2.99	$-x+\frac{1}{2}, y+\frac{1}{2}, -z$
*A*2	Asp230*	OD2	*B*1	Ser95	OG	2.71	$-x+\frac{1}{2}, y+\frac{1}{2}, -z$
*A*1	Asn219	ND2	*A*2	Lys51	NZ	2.83	$x, y+1.0, z$
*A*1	Asn219	OD1	*A*2	Glu58	OE1	2.85	$x, y+1.0, z$
*A*1	Tyr239	OH	*A*2	Gln62	N	3.41	$x, y+1.0, z$
*B*1	Arg8	NH1	*B*1	Asp230	OD1	3.18	$x, y+1.0, z$

* Mutation differences between rat and human DHPRs.

molecular geometry of the orthorhombic form. There is no indication of asymmetry within the individual dimers, as monomers in each dimer are related by nearly an exact twofold rotation. The r.m.s. Cα difference between the two subunits in dimer *A* are 0.43 Å and the corresponding difference in dimer *B* is 0.40 Å. The r.m.s. Cα difference between the two dimers in the asymmetric unit is 0.52 Å. The dimers in the monoclinic crystal lattice pack differently than in the orthorhombic lattice. A stereoview of the packing in the monoclinic crystal form is shown in Fig. 3. This crystal form packs with an extensive solvent channel running parallel to the *b* axis such that individual active sites have access to solvent. In the orthorhombic form access to the active site is more restricted and hence it appears that the monoclinic form is more suited for diffusing inhibitors or substrates into the crystal lattice. Each dimer molecule contacts four neighboring molecules in the crystal. Table 3 lists the interdimeric ionic and hydrogen-bonding interactions in the crystal lattice. It is interesting to note that these packing interactions involve some (see Table 3) of the ten residues which differ between the rat and human DHPR

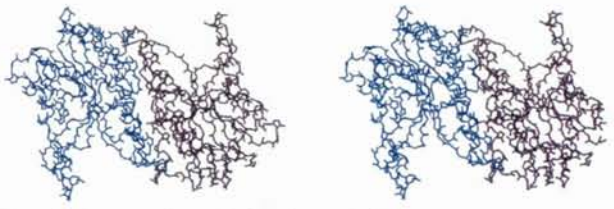

Fig. 2. A stereoview of the dimer *A*. The two monomers are colored yellow and red for easy identification.

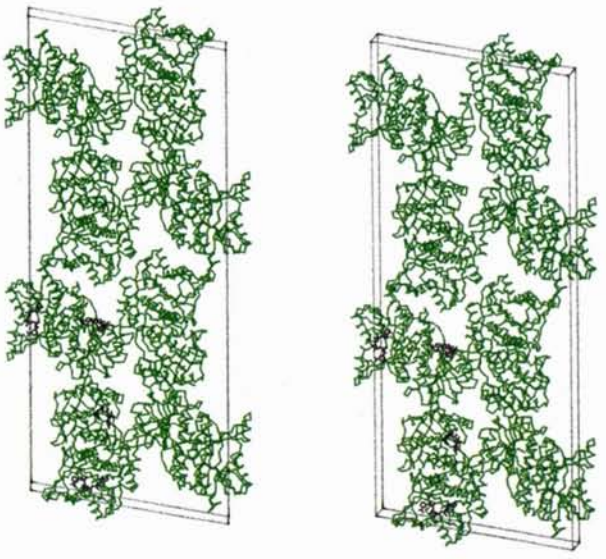

Fig. 3. A stereoview of the packing in the monoclinic crystal form, as viewed along the *b* axis. The location of the cofactor is also seen in the diagram.

sequences. This may be related to the observation that so far all the human DHPR crystals we have grown are in the $C222_1$ space group in contrast to the rat liver enzyme which crystallizes readily in both $C222_1$ and $C2$ forms, often from the same drops.

This investigation was supported by Grant RR01644 (UCSD) and DK44125 from the National Institutes of Health, Grant DIR88-22385 from the National Science Foundation (UCSD), the Lucille P. Markey Foundation (UCSD) and Grant CA11778 from NIH (TSRI).

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