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New crystal forms of the small subunit of ribonucleotide reductase from *Escherichia coli*

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The small subunit of ribonucleotide reductase from *Escherichia coli* has been crystallized in two new crystal forms The form most suitable for X-ray analysis belongs to the orthorhombic space group $P2_12_12_1$ It has the cell dimensions 74 3 Å, 85 5 Å, 115 7 Å and diffracts to about 2 1 Å resolution The asymmetric unit most probably contains one dimer Absorption spectra of single crystals confirm that the crystals contain a binuclear iron center Crystals of the iron-depleted apoenzyme have also been obtained

Crystallization, Ribonucleotide reductase, Microspectrophotometry

1. INTRODUCTION

Deoxyribonucleotides for DNA synthesis are synthesized by direct reduction of the corresponding ribonucleotides. This essential reaction is catalyzed by the enzyme ribonucleotide reductase. The ribonucleotide reductase in *Escherichia coli* consists of two non-identical homodimeric proteins, denoted B1 and B2 (for reviews see [1–3]). The catalytic unit consists of a Mg^{2+}-dependent one-to-one complex of proteins B1 and B2 with the active site located at the B1-B2 interface. The larger protein, B1, contributes redox-active dithiols, which are oxidized during the reduction of substrates, and binding sites for allosteric effectors. The smaller protein, B2, is a dimer of two identical polypeptide chains, each containing 375 residues with a polypeptide molecular mass of 43.4 kDa [4]. B2 contains a binuclear iron center bridged by a μ-oxo group. The ligands are suggested to be histidine and carboxylic acid residues [5]. B2 was the first protein shown to contain a stable free radical [6]. Close to the iron center is a tyrosine residue, Tyr-122, which harbours a stable free radical that is essential for the activity of the enzyme [7]. The structure determination of the small subunit of ribonucleotide reductase will undoubtedly shed light on the structural basis for this unique tyrosyl radical, which has been conserved throughout the iron-containing ribonucleotide reductases.

Crystals of B2 formed from ammonium sulphate solutions were described by Joelson et al. [8]. These crystals will be referred to below as crystal form A. However, these crystals proved not to be useful for crystallographic investigations since their diffraction pattern on oscillation photographs shows streaks in some of the lines. This is probably due to a serious disorder in the crystals arising from more than one packing distance along the long axis. For crystals of spinach ribulose-1,5-bisphosphate carboxylase, such phenomena have been analyzed in terms of stacking faults between layers of molecules [9].

The two new crystal forms described in this communication were obtained using very different conditions than for form A. One crystal form (called crystal form B) was obtained at 37°C using PEG. Another new improved crystal form of B2 (called crystal form C) was obtained with addition of a mercury compound. Finally, we have succeeded in crystallizing the apoB2 form of the protein which lacks bound iron ions.

2. MATERIALS AND METHODS

Protein B2 was purified from an overproducing strain as described before [10,11] ApoB2 was prepared as described by Atkin et al. [12] Crystals were obtained using the hanging drop method. A 5-μl drop of protein solution (20 mg/ml) was mixed with 5 μl of the buffered precipitation mixture. Crystal form B was grown from 20% PEG 4000 (Merck), 0 2 M NaCl and 0 3% dioxane (Merck) in 0 05 M Mes

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Abbreviations PEG, polyethylene glycol, EMTS, ethylmercurythiosalicylate; Mes, 2-(*N*-morpholino)ethanesulfonic acid

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Table 1

Crystallization conditions and preliminary crystallographic data for the three different crystal forms of the small subunit of ribonucleotide reductase from *Escherichia coli*

	Space group	Cell dimensions (Å)	Resolution (Å)	Dimers/ asymmetric unit	V_m (Å^3/Da)	Crystallization conditions (in 50 mM Mes, pH 6.0)		
						Precipitant	Temp.(°C)	Additions
A	$P2_12_12_1$	58, 73, 205	2.5	1	2.50	$(NH_4)_2SO_4$	20°	–
B	$P2_12_12_1$	58, 74, 355	2.9	2	2.23	PEG 4000	37°	dioxane NaCl
C	$P2_12_12_1$	74.3, 85.5, 115.7	2.1	1	2.12	PEG 4000	20°	dioxane NaCl EMTS

(Sigma) at pH 6.0 in small hanging drop chambers. To prevent twinning, the crystals had to be grown at 37°C.

Crystal form C was grown under the same conditions as B, but EMTS (Sigma) was also added. The quality of these crystals was improved further by crystallization at 20°C using Petri dishes instead of small hanging drop chambers.

ApoB2 crystals grew under the same conditions as C, but crystal growth had to be initiated by microseeding from crystal form C's hanging drops, using a standard dilution protocol.

Absorption spectra of crystalline material were recorded with a Zeiss microspectrophotometer 03 linked to an HP 9845 B microcomputer using polarized light. Small crystals with an approximate size of 0.5 × 0.10 × 0.10 mm, were mounted between two quartz slides separated by glass slips of 0.12 mm thickness. The mounts were immersed in mother liquor.

Measurements of X-ray reflections on crystal form C were made to 5 Å resolution on a Stoe diffractometer. Data sets to 2.4 Å were collected on a Nicolet multiwire area detector and were evaluated using the package Buddha [13]. A partial data set of crystal form B and data sets on crystal form C were collected on film (CEA Reflex) using a conventional source (Elliot GX10) and the Daresbury synchrotron.

3. RESULTS AND DISCUSSION

Two new crystal forms B and C have been obtained. Crystal form B was grown at 37°C with PEG, NaCl and dioxane (table 1). These crystals have two crystal axes common to the previous crystal form A, but the third axis is considerably longer. Packing problems in the z-direction in crystal form A resulted in disorder in the diffraction pattern. In crystal form B, there is no packing problem in the long direction, but the unit cell is larger and probably contains two dimers per asymmetric unit along the z-axis. However, even these crystals were problematic for high-resolution crystallographic studies, since a majority of the crystals were twinned, small and diffracted only slightly better than 3 Å. Only a partial native data set has been obtained from this crystal form.

Crystal form C was obtained by using similar conditions as for crystal form B, but with EMTS present. The crystals grow to a maximal size of 1.0 × 0.5 × 0.4 mm (fig.1). The crystals are generally not compact but have a central cavity of variable depth lengthwise. These crystals diffract to about 2.1 Å resolution (fig.2). The lattice is orthorhombic with systematic absences on

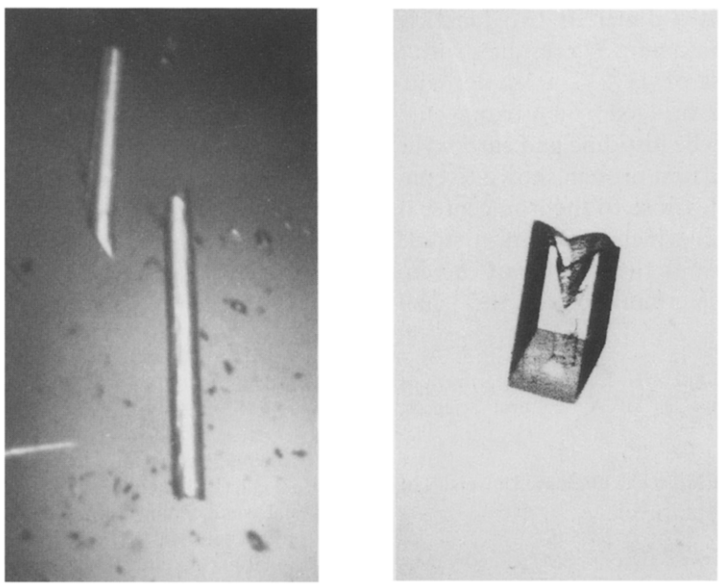

Fig.1. The two new crystal forms B (left) and C (right) of the small subunit of ribonucleotide reductase from *E. coli*.

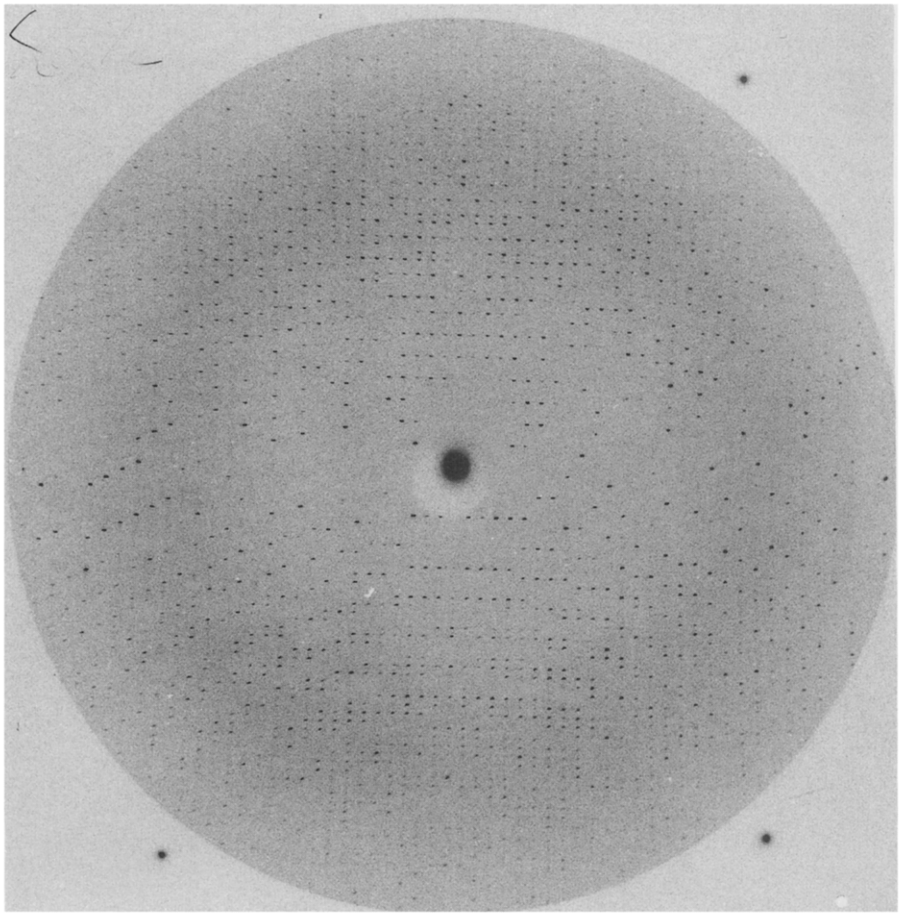

Fig 2 Diffraction pattern to about 2 2 Å resolution obtained from crystal form C at the Daresbury synchrotron The picture shows a 2° oscillation with the *a*-axis along the spindle (horizontal)

all the three axes compatible with the space group $P2_12_12_1$. The cell dimensions measured on diffractometer and a multiwire area detector were 74.3, 85.5 and 115.7 Å. The V_m value calculated from these cell dimensions assuming a dimeric molecule of molecular mass 86800 in the asymmetric unit is 2.12 $Å^3$/Da, which is in the normal range for protein crystals [14]. The crystals are stable in the X-ray beam and a full data set can usually be collected on one crystal.

Crystallization of B2 in the presence of mercury merits some caution. The mercury may in the worst case substitute the iron atoms of B2. However, it has not been possible to substitute the iron ions of B2 by other metals [15]. The probable iron center ligands, histidines and carboxylates, are not expected to bind the mercury to any significant extent since EMTS is known to have a strong preference for cysteine ligands (for example, see [16]). Microspectrometry of single crystals of B2 of form C show a characteristic spectrum of binuclear iron center metB2 (fig.3). MetB2 contains μ-oxy bridged ferric ions but no tyrosyl radical. It has recently been shown that crystals of type C can be reduced and reoxidized giving a tyrosyl radical (Nordlund, unpublished).

Measurements of reflections on different crystals from several crystallization batches showed large variations, which may be due to different occupancies of mercury in different crystals. The use of one specific enzyme preparation, carefully controlled for all

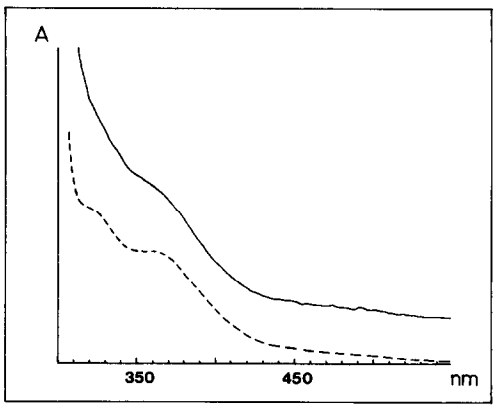

Fig.3 Absorption spectrum of a single crystal of protein B2 using polarized light normal to the crystal plane (100) The spectrum shows the 325 nm and 370 nm absorption features characteristic for metB2 The dashed line shows the absorption spectrum of a 35 μM solution of metB2 ($E_{280-310}$ = 120000 M^{-1} cm^{-1}) We are indebted to Dr Margareta Sahlin for providing the spectrum of metB2 in solution

crystallization conditions and a standardized procedure for mounting and soaking has now resulted in data sets which scale to each other within 5% ($\Delta F/F$).

The iron ions can be removed from the B2 protein by dialysis [12] against a chelating solution. Several attempts to crystallize the apoB2 form, using the same conditions as for the native enzyme, were finally successful after seeding. The crystals are almost isomorphous to crystal type C, with a 2% longer *b*-axis than for native protein.

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