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Crystallization and preliminary X-ray diffraction study of the flavoenzyme 2,4-pentadienoyl-CoA reductase from *Clostridium aminovalericum*. By U. EIKMANNS, *Departments of Biochemistry and Molecular and Medical Genetics, University of Toronto, Medical Sciences Building, 1 King's College Circle, Toronto, Ontario M5S 1A8, Canada, and Laboratorium für Mikrobiologie, Karl-von-Frisch-Strasse, Fachbereich Biologie der Philipps-Universität Marburg, D35043 Marburg, Germany*, S. CHIU, *Department of Biochemistry, University of Toronto, Medical Sciences Building, 1 King's College Circle, Toronto, Ontario M5S 1A8, Canada*, W. BUCKEL, *Laboratorium für Mikrobiologie, Karl-von-Frisch-Strasse, Fachbereich Biologie der Philipps-Universität Marburg, D35043 Marburg, Germany*, and E. F. PAI,* *Departments of Biochemistry and Molecular and Medical Genetics, University of Toronto, Medical Sciences Building, 1 King's College Circle, Toronto, Ontario M5S 1A8, Canada*

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Abstract

The tetrameric flavoenzyme 2,4-pentadienoyl-CoA reductase has been crystallized from solutions containing polyethylene glycol as precipitant. The crystals grow in the monoclinic space group $C2$ with unit-cell dimensions a = 160.2, b = 120.2, c = 95.3 Å, β = 99.0°. The packing parameter V_M is 2.3 $Å^3\ Da^{-1}$ (Matthews parameter) for four monomers per asymmetric unit. Complete data sets to about 2.9 Å resolution have been collected.

Introduction

Several *Clostridia* species are able to reduce proline to 5-aminovalerate. *Clostridium aminovalericum* (strain T2-7) ferments this compound further to ammonia, *n*-valerate, propionate and acetate (Hardman & Stadtman, 1960). Barker, d'Ari & Kahn (1987) proposed a pathway for these reactions. Initially, the amino group of 5-aminovalerate is exchanged for a hydroxyl group in two steps, transamination with 2-oxoglutarate followed by reduction with NADH. The resulting 5-hydroxyvalerate is activated to 5-hydroxyvaleryl-CoA by the enzyme CoA transferase, which has been purified (Eikmanns & Buckel, 1991*a*). The next three reaction steps are catalyzed by two green enzymes, the bifunctional 5-hydroxyvaleryl-CoA dehydrogenase/dehydratase, which has recently been crystallized (Eikmanns & Buckel, 1991*b*; Eikmanns, Buta, Buckel & Pai, 1994), and 2,4-pentadienyl-CoA reductase (Eikmanns & Buckel, 1991*c*). The product, 3-pentenoyl-CoA, isomerizes to 2-pentenoyl-CoA which then disproportionates to valerate as well as to acetate and propionate. Although the pathway has been firmly established there is nothing known yet about its regulation.

5-Hydroxyvaleryl-CoA dehydrogenase/dehydratase catalyses the oxidation of 5-hydroxyvaleryl-CoA to (*E*)-5-hydroxy-2–pentenoyl-CoA and its consecutive dehydration to 2,4–pentenoyl-CoA. Reversible 2,5-addition of reducing equivalents to the latter compound catalysed by 2,4–pentadienoyl-CoA reductase yields 3-pentenoyl-CoA. *In vitro*, methylviologen serves as electron donor, whereas ferricenium hexafluorophosphate (Lehman, Hale, Bhala & Thorpe, 1990) can be applied as electron acceptor in discontinuous assays (k_{cat} = 840 s^{-1}). To date, the natural electron carrier transferring the electrons between the two green enzymes has not been identified; however, it is not NAD(P)H as published for other 2,4-dienoyl-CoA reductases (Dommes & Kunau, 1984).

As purified to homogeneity from cell-free extracts of *C. aminovalericum*, 2,4-pentadienoyl-CoA reductase consists of four apparently identical subunits, with 0.4 mol FAD per 47.5 kDa monomer. Incubation of the enzyme with an excess of FAD results in 0.9 mol FAD/subunit and the specific activity increases twofold. The unusual UV–visible spectrum of this greenish enzyme (absorption maxima at 404 and 707 nm, shoulder at 371 and 460 nm) can be converted to that of a normal yellow flavoprotein (absorbance maxima at 370 and 450 nm) by removal of the prosthetic group followed by reconstitution with FAD, but not by reduction with dithionite followed by reoxidation by air (Eikmanns & Buckel, 1991*c*). Neither of these treatments abolished enzymic activity, indicating that the green charge-transfer complex was not an intermediate in the reaction pathway. The 'greening factor' has not yet been analyzed in detail, but may well be a CoA-persulfide as reported for other acyl-CoA dehydrogenases (Williamson, Engel, Mizzer, Thorpe & Massey, 1982).

Experimental

2,4-Pentadienoyl-CoA reductase was purified to homogeneity from *C. aminovalericum* strain T2-7 (DSM 6836) as described previously with some minor modifications (Eikmanns & Buckel, 1991*b*). 1 g of lyophilized cells were suspended in 10 ml of 20 m*M* Tris, pH 7.5, and the cells were broken by passing them three times through a French press at 1200 psi (8 MPa). The extract was cleared by centrifugation at 27 000*g* for 1 h and the resulting green supernatant was brought to 50% saturation in ammonium sulfate. The precipitate was discarded and the remaining solution applied to a phenyl-sepharose HP column. For continuous routine measurements 3-pentenoyl-CoA served as substrate and Meldola blue (8-dimethylamino-2,3–benzophenoxazine) together with INT [2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride] was used as electron acceptor (k_{cat} = 16 s^{-1}) (Eikmanns & Buckel, 1991*c*). The most active colored fractions were pooled and purified further by size-exclusion chromatography on Superdex 200 followed by anion-exchange chromatography. After each purification step FAD was added to the active fractions to a final concentration of 25 μ*M*.

The concentration of the protein solution used for the crystallization set-ups was about 10 mg ml^{-1}. Crystals were grown using the hanging-drop method (McPherson, 1985). Different buffers, pH values, and precipitants were tested.

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Hanging drops were prepared by mixing 2 µl of the enzyme solution with 2 µl of the corresponding reservoir solution.

X-ray data were collected at room temperature with the crystals mounted in thin-walled glass capillaries (Supper, Natick, MA, USA). Precession photographs were taken with Ni-filtered collimated Cu $K\alpha$ radiation produced by a fine-focus rotating-anode generator (RU200H, Rigaku, Japan) operating at 37 kV and 70 mA. Native data sets have been collected on an X-1000 area detector (Siemens; Madison, WI, USA) mounted on the same source but using Franks double-mirror optics for focussing. Data were reduced using an updated version of the program package *XDS* (Kabsch, 1988) which allowed assignment of the space group and the unit-cell parameters. In addition, the assignments were checked by precession photographs.

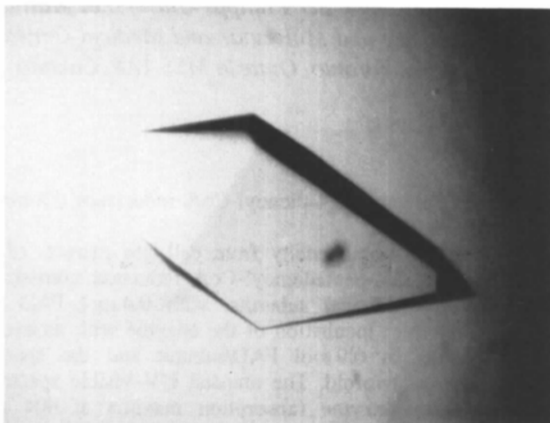

Fig. 1. Crystal of *C. aminovalericum* 2,4-pentadienoyl-CoA reductase. The green crystal was grown using the hanging-drop method. The reservoir conditions were: 0.2 *M* ammonium acetate, 100 m*M* citrate, pH 6.0, 25% PEG 4000 and 3 m*M* sodium azide. It took about 7 d for the crystal to reach its final size of 0.4 × 0.2 × 0.1 mm.

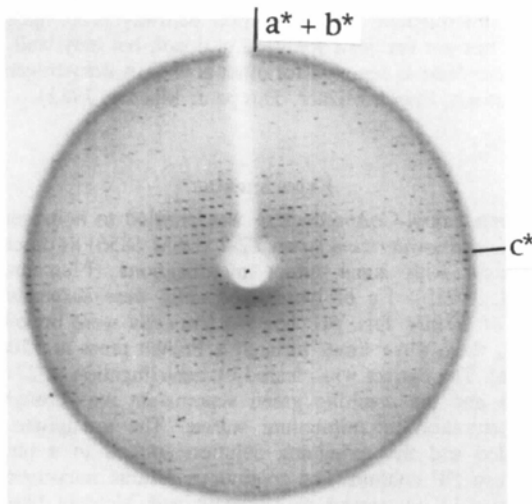

Fig. 2. X-ray precession photograph of a crystal of *C. aminovalericum* 2,4-pentadienoyl-CoA reductase. The photograph of the *hhl* plane was taken with a precession angle of μ = 12° and a crystal-to-film distance of 100 mm. The exposure time was 20 h on a 200 µm focus rotating-anode X-ray generator (Cu $K\alpha$) operated at 37 kV and 70 mA.

Results and discussion

The best crystals were obtained at room temperature using protein concentrations of 10 mg ml^{-1} and reservoir solutions of 100 m*M* citrate pH 6.0, 0.2 *M* ammonium acetate, 3 m*M* NaN_3 and 25%(*w*/*v*) polyethylene glycol (PEG) 4000. Crystals grew up to a size of 0.5 × 0.4 × 0.15 mm within one week (Fig. 1).

Precession photographs (Fig. 2) and the results of the autoindexing routine of *XDS* (discrimination by a factor of 25) are consistent with the crystals adopting the monoclinic space group *C*2. The unit-cell parameters are a = 160.2, b = 120.2, c = 95.3 Å, β = 99.0°. Assuming a homotetramer per asymmetric unit, one calculates a Matthews parameter V_M of 2.3 $Å^3$ Da^{-1} (equivalent to a solvent content of about 55%), well within the range of previously observed values for crystals of globular proteins (Matthews, 1968).

Still photographs show reflections to *ca* 2.5 Å resolution. The crystals are stable in the X-ray beam for a few days at room temperature. Native data sets have been collected on an area detector to a resolution of 2.8 Å with symmetry *R* factors of 6% overall. In the resolution shell from 3.2 to 2.8 Å, 66% of the reflection intensities are over 1σ, 19% are over 3σ. Further data collection, especially a search for heavy-atom derivatives is in progress.

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References

Barker, H. A., d'Ari, L. & Kahn, J. (1987). *J. Biol. Chem.* **262**, 8994–9003.

Dommes, V. & Kunau, W.-H. (1984). *J. Biol. Chem.* **259**, 1781–1788.

Eikmanns, U. & Buckel, W. (1991*a*). *Biol. Chem. Hoppe-Seyler*, **371**, 1077–1082.

Eikmanns, U. & Buckel, W. (1991*b*). *Eur. J. Biochem.* **197**, 661–668.

Eikmanns, U. & Buckel, W. (1991*c*). *Eur. J. Biochem.* **198**, 263–266.

Eikmanns, U. Buta, C., Buckel, W. & Pai, E. F. (1994). *Proteins*. **19**, 269–271.

Hardman, J. K. & Stadtman, T. C. (1960). *J. Bacteriol.* **79**, 549–552.

Kabsch, W. (1988). *J. Appl. Cryst.* **21**, 916–924.

Lehman, T. C., Hale, D. E., Bhala, A. & Thorpe, C. (1990). *Anal. Biochem.* **186**, 280–284.

Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.

McPherson, A. (1985). *Methods Enzymol.* **114**, 112–128.

Williamson, G., Engel, P. C., Mizzer, J. P., Thorpe, C. & Massey, V. (1982). *J. Biol. Chem.* **193**, 265–275.