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Crystallization and preliminary X-ray analysis of pig E3, lipoamide dehydrogenase

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Abstract

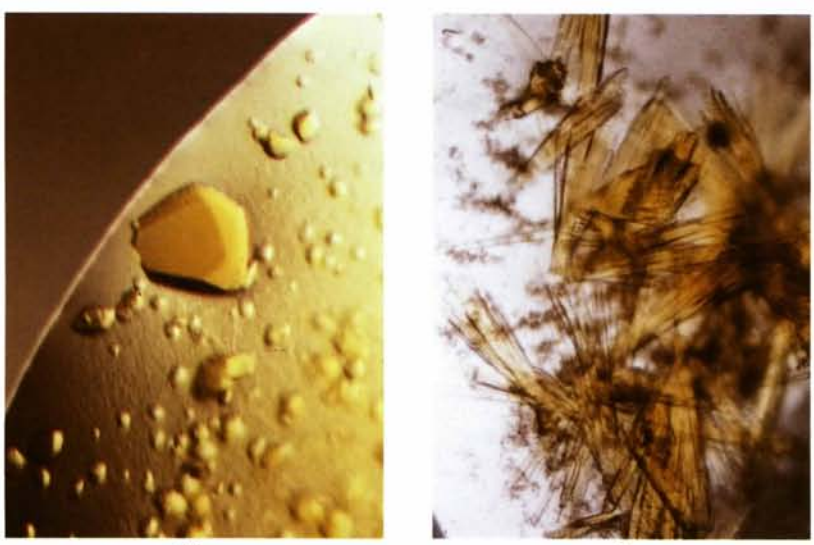
Pig heart lipoamide dehydrogenase was crystallized by the hanging-drop vapor-diffusion method. X-ray diffraction patterns show that the hexagonal crystals have unit-cell dimensions of $a = b = 359.3$, $c = 140.5$ Å. The crystal structure has been preliminarily solved by the molecular-replacement method in the space group $P6_322$. Three dimeric molecules have been found in the asymmetric unit, one of them located on the crystallographic twofold axis.

1. Introduction

Pig heart lipoamide dehydrogenase is a dimeric enzyme composed of two identical subunits with molecular weight of 50 141 for 474 amino-acid residues (Otulakowski & Robinson, 1987). This protein is known as the E3 component of the pyruvate (PDC), 2-oxoglutarate (OGDC) and branched-chain 2-oxoacid (BCODC) dehydrogenase multi-enzymatic complexes (Reed, 1974; Mattevi *et al.*, 1992; Perham, 1991; Mattevi, Obmolova *et al.*, 1992). The architecture of the complexes is composed of multiple copies of, in general, E1, E2 and E3. The former two enzymes, E1 and E2, are substrate specific. For example, in PDC E1 and E2 are pyruvate dehydrogenase and dihydrolipoamide acetyltransferase, respectively, and in OGDC E1 and E2 are 2-oxoglutarate dehydrogenase and dihydrolipoamide succinyltransferase, likewise. A common component among them is E3, which catalyzes the oxidation of dihydrolipoic acid covalently bound to the lysine residue of E2 in the last step (1) of the overall reaction.

$$\mathrm{E2{-}Lip(SH)_2 + NAD^+ \rightarrow E2{-}LipS_2 + NADH + H^+}. \quad (1)$$

The E2 components form the structural core of the complex. OGDC in all organisms and PDC in Gram-negative bacteria have a similar core with 24 copies of E2 packed with 432 symmetry. While the cores of PDC in Gram-positive bacteria and eukaryotes exhibit a symmetry of 532 containing 60 copies of E2. It is interesting that in the latter organisms, E3 is assembled into both cores with 532 and 432 symmetries to form the complex. More strictly speaking, protein X (De Marcucci & Lindsay, 1985) may be required for binding with E3 in the icosahedral cores of yeast PDC (Lawson *et al.*, 1991) and mammalian PDC (Neagle & Lindsay, 1991). Even among eukaryotes, the number of E3's in PDC differs between yeast and mammals, 12 copies in yeast PDC (Maeng *et al.*, 1994) and six copies in mammalian PDC (Wu & Reed, 1984) are identified. Furthermore, the primary structures of E3s from various sources have been reported. However, a few tertiary structures from prokaryotes have been reported (Mattevi *et al.*, 1991, 1993; Mattevi, Obmoleva *et al.*, 1992). To elucidate the evolutional relationship of the architecture among them, it is essential to reveal the structures in different organisms. It is known that mutations of E3 cause human diseases, such as neurological dysfunctions and lactic acidosis (Liu *et al.*, 1993).

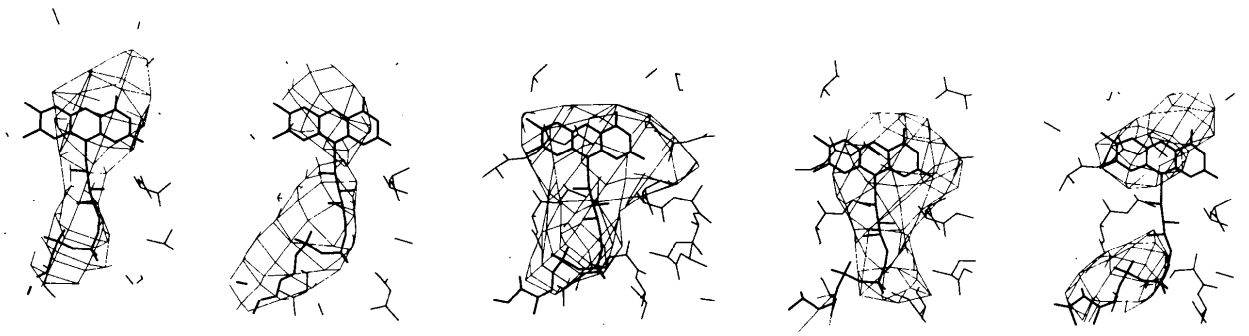


(*a*) (*b*)

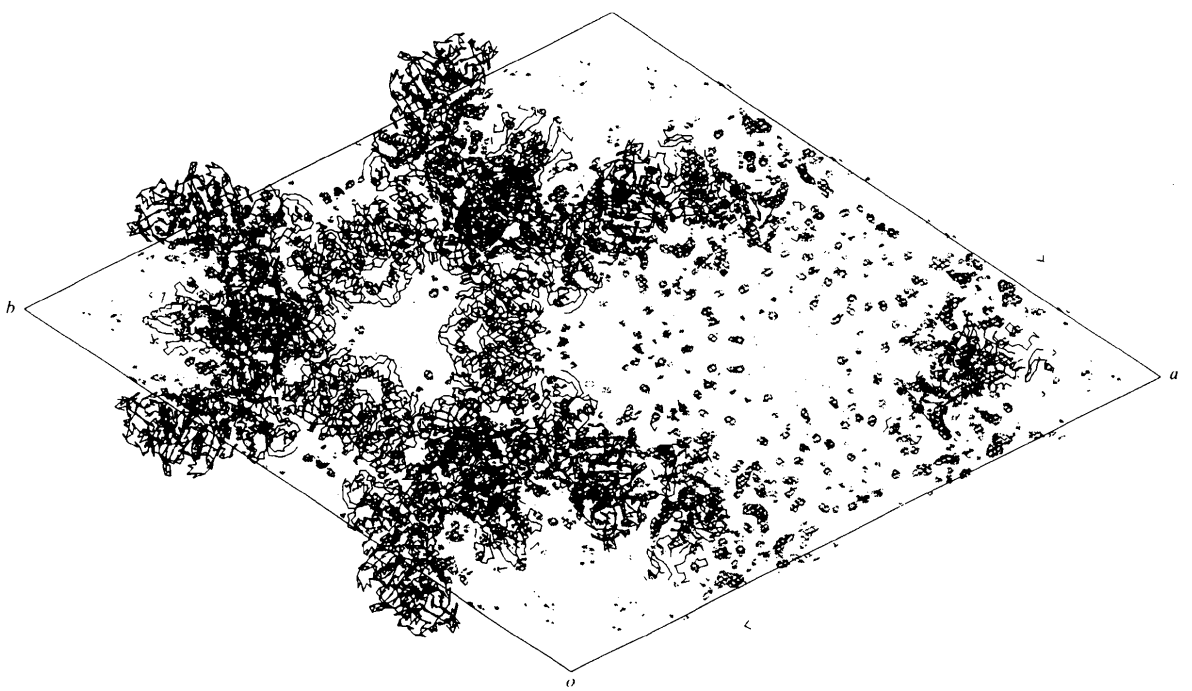
Fig. 1. Crystals of pig E3, lipoamide dehydrogenase obtained under conditions given in Table 1. The hexagonal crystal (*a*) has a size of 0.2 × 0.2 × 0.05 mm and the needle crystals (*b*) have a size of 0.5 × 0.05 × 0.05 mm.

Table 1. *Crystallization conditions of pig lipoamide dehydrogenase*

	Hexagonal crystal	Needle crystal
Drop (5 µl)		
Buffer solution	50 m*M* potassium phosphate (pH 7.0)	50 m*M* potassium phosphate (pH 7.0)
Protein (mg ml^{-1})	6.6	9.1
FAD (mm)	0.25	0.10
Ammonium sulfate (*M*)	0.75	1.0
Isopropanol [%(*v*/*v*)]	—	2.5
Reservoir (1 ml)		
Buffer solution	50 m*M* potassium phosphate (pH 7.0)	50 m*M* potassium phosphate (pH 7.0)
Ammonium sulfate (*M*)	1.5	2.0
Isopropanol [%(*v*/*v*)]	—	5



(*a*)



(*b*)

Fig. 2. (*a*) $|F_o|-|F_c|$ maps around five FAD binding sites, calculated without FAD. Electron densities are drawn at 2.5σ contour level (i–iii), and at 2.0σ level (iv, v). (*b*) An $|F_o|-|F_c|$ map of a lower half of the unit cell. Electron densities are drawn at 2.5σ contour level. All molecules in the region are drawn with Cα model.

As pig E3 has high sequence identity (96%) with human E3, an achievement of the present X-ray analysis of pig E3 will give some structural basis for cause of the deficiencies. In this paper, we describe the crystallization and preliminary structure determination of pig heart E3, which is the first example of a mammalian lipoamide dehydrogenase.

2. Materials and methods

Pig E3, isolated from fresh pig heart (Moriyasu *et al.*, 1986), was further purified by hydroxyapatite column chromatography and nickel-chelating Sepharose FF chromatography. Crystallization conditions were surveyed by the hanging-drop vapour-diffusion method, the microdialysis method and the free-interface diffusion method (Ducruix & Giegé, 1992) with ammonium sulfate or polyethylene glycol as precipitants using 50 m*M* potassium phosphate buffer (pH 7.0). In some cases, isopropanol was added to control nucleation or to prevent aggregation of crystals.

X-ray diffraction data of the hexagonal crystal were collected with synchrotron radiation (λ = 1.04 Å) at the Photon Factory in Tsukuba using the Weissenberg camera for macromolecules (Sakabe, 1991). The intensities recorded on imaging plates (Fuji Film) were digitalized with a Fuji Film BA100. 14 frames with 6° oscillations during 80 s exposure were processed by the program *DENZO* (Otwinowski, 1993). The reflection data obtained from different frames were scaled in one data set by the program *SCALA* in *CCP*4 (Collaborative Computational Project Number 4, 1994).

3. Results and discussion

Two kinds of crystals which were different in shape, as seen in Fig. 1, were obtained by the hanging-drop vapor-diffusion method. Their crystallization conditions are given in Table 1. As the needle-shaped crystals were too small for X-ray work, only the hexagonal crystal 0.2 × 0.2 × 0.05 mm in size was used to collect X-ray data. The crystal gave diffraction at 5.5 Å resolution, and has the Laue symmetry 6/*mmm* with unit-cell dimensions $a = b = 359.3$ and $c = 140.5$ Å. The space group was determined to be $P6_n22$ (n = 0–5), because of lacking (00*l*) reflections. From 13 615 observed reflections in a range of 50–8.0 Å resolution, 5 838 independent reflections were obtained with an $R_{\rm merge}$ of 6.6%, the completeness of the data being 98.0%.

It was expected that pig E3 has a similar structure to yeast E3, because both have a sequence identity of 57% and their diagonal plot showed a similarity in the tertiary structure throughout all 474 residues. We have already solved the crystal structure of yeast E3 at 2.4 Å resolution which showed that the tertiary structure is highly conserved with other procaryotic E3's (Toyoda *et al.*, 1998), so that molecular replacement was applicable to solve the structure of the present crystal. A polyalanine structure was constructed as a probe molecule from the yeast E3 structure. Calculations of molecular replacements were carried out with the program *AMoRe* (Navaza, 1994) in all the possible six space groups. For the first dimeric molecule, a unique solution with the lowest R factor of 47.6% and the highest Cc (correlation coefficient) of 0.294 was obtained for 50–8.0 Å resolution data in the space group of $P6_322$. While all the other solutions including the other space groups had R values higher than 52% and Cc values less than 0.16. For the second dimer, the R factor was reasonably decreased to 43.4% and Cc increased to 0.429 in the space group of $P6_322$. The third dimer, which further dropped the R factor and increased the Cc successively (R = 43.2%, Cc = 0.449), was found on the crystallographic twofold axis. We tried to find another dimer, but failed. In total, 2.5 dimers (five subunits) were successfully found.

The positions and orientations of five subunits were refined using the program *X-PLOR* (Brünger, 1992*a*), each subunit being treated as a rigid polyalanine molecule. The final $R_{\rm working}$ was 37.8% ($R_{\rm free}$ = 37.9 for 10% of the data) for 50–8.0 Å resolution data (Brünger, 1992*b*). Fig. 2(*a*) shows $|F_o| - |F_c|$ difference maps around the five FAD binding sites postulated from the other E3 structures. Electron densities for FAD's clearly appear in all the five FAD binding sites despite the fact that they were omitted in structure-factor calculations.

The dimers are reasonably packed in the unit cell. There are no abnormal molecular contacts among them. The V_m value is calculated to be 5.71 Å^3 Da^{-1}, corresponding to a solvent content of 78.5%. This value is out of the range reported by Matthews (1968) and beyond even the highest value of 75% found in the crystal of glycolate oxidase (Lindqvist & Brändén, 1980). Therefore, it might suggest the presence of other subunits in the asymmetric unit. In fact there are two large spaces found in the unit cell. One is around the 6_3 screw axis and the other is in the upper/lower half space around the threefold axis. It may be possible to put a dimer on the twofold axes across these 6_3 screw and/or threefold axes. It is, however, difficult to find the corresponding densities in these regions of the $|F_o| - |F_c|$ difference map, as seen in Fig. 2(*b*). Higher resolution data will resolve this ambiguity. We are further surveying crystallization conditions to obtain crystals that diffract at much higher resolution.

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