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Crystallization and preliminary X-ray analysis of the full-size cubic core of pig 2-oxoglutarate dehydrogenase complex

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The full-length (untruncated) dihydrolipoamide succinyltransferase from pig heart was crystallized by the hanging-drop vapour-diffusion method. X-ray diffraction patterns indicate that the crystal belongs to space group *I*432, with unit-cell parameter a = 189.9 Å. The crystal structure has been preliminarily solved at 7 Å resolution by the molecular-replacement method. The unit cell contains two cubic cores, in each of which 24 subunits of E2 are associated according to crystallographic 432 symmetry. At the corners of each cubic core, the catalytic domains of E2s form a trimer through tight interactions around the crystallographic threefold axes. In the electron-density maps, many small broad peaks are observed in regions expected to contain the remaining N-terminal domains (the E1/E3-binding domain and the lipoyl domain), suggesting flexibility of these domains relative to the core. The architecture of the cubic core is similar to that of the other truncated E2s. In the unit cell, however, the core–core contact occurs in a different direction from that found for the truncated proteins.

1. Introduction

Pyruvate dehydrogenase complex (PDC), 2-oxoglutarate dehydrogenase complex (OGDC) and branched chain 2-oxoacid dehydrogenase complex (BCDC) are classified into a family of 2-oxoacid dehydrogenase multienzyme complexes with similar architectures and molecular weights of millions of daltons (Yeaman, 1989). These complexes consist of multiple copies of three different component enzymes, E1, E2 and E3, in which E2s form the central core to which E1s and E3s are bound through non-covalent bonds (Reed, 1974; Koike & Koike, 1976). Two types of central core are observed by electron microscopy (Hayakawa *et al.*, 1969; Tanaka *et al.*, 1972): a cube of 24 E2s with 432 symmetry and an icosahedron of 60 E2s with 532 symmetry. Cubic cores occur in the OGDC of all organisms and in the PDC of Gram-negative bacteria, while icosahedral cores occur only in the PDC of eukaryotes and some Gram-positive bacteria.

Among the three different complexes, E3 (dihydrolipoamide dehydrogenase) is a common component, while E1 and E2 are changed depending on the substrates (E1o: 2-oxoglutarate dehydrogenase and E2o: dihydrolipoamide succinyltransferase in OGDC). The E2 subunit is generally composed of three domains: (i) one or more lipoyl domains which are located at the N-terminus, (ii) a peripheral domain with E1- and/or E3-binding sites in the middle and (iii) the C-terminal catalytic domain (CD) which contains the catalytic site and the inter-subunit association sites (Reed & Hackert, 1990; Perham, 1991). These domains are linked linearly through flexible linker segments.

Several X-ray structures of truncated E2s have been reported: dihydrolipoamide succinyltransferase (E2o) from *Escherichia coli* (De Rosier *et al.*, 1971; Knapp *et al.*, 1998) and dihydrolipoamide acetyltransferases (E2p) from *E. coli* (Fuller *et al.*, 1979), *Azotobacter vinelandii* (Mattevi *et al.*, 1992; Mattevi, Obmolova, Kalk, Teplyakov *et al.*, 1993; Mattevi, Obmolova, Kalk, Westphal *et al.*, 1993; Hendle *et al.*, 1995), *Bacillus stearothermophilus* (Mande *et al.*, 1996; Izard *et al.*, 1999) and *Enterococcus faecalis* (Izard *et al.*, 1999). However, they lack the lipoyl and peripheral domains.

To investigate the structural architecture and reaction mechanisms of such highly organized complexes, we have initiated X-ray analyses of E3 components from different sources (Takenaka *et al.*, 1988; Toyoda *et al.*, 1997; Toyoda, Kobayashi *et al.*, 1998; Toyoda, Suzuki *et al.*, 1998). To reveal the detailed structure of such a large complex, it is necessary to analyze the binding geometry of each component with the E2 core. In the present study, we have succeeded in crystallizing the full-length E2o of pig heart OGDC, which is the first example of this.

2. Materials and methods

Recombinant pig E2o was produced in *E. coli* BL21(DE3)pLysS containing expression plasmid pET-11d-PE2o (Koike *et al.*, 2000). The cultured cells were disrupted by ultrasonication. Cell debris and unbroken cells were removed by centrifugation. The supernatant was fractionated with ammonium sulfate between 29 and 36% saturation. E2o was highly purified by column chromatography on Q-Sepharose fast-flow, PBE 94 Polybuffer exchanger and Superdex 200 (Amersham Pharmacia Biotech AB, Uppsala, Sweden). The purity was evaluated by SDS–PAGE and the activity was assayed by detecting succinylthioester formation. The molecular mass of the oligomeric state was estimated by gel filtration on a Superdex 200 column.

Figure 1
Crystals of pig E2o (dimensions 0.1 × 0.1 × 0.1 mm).

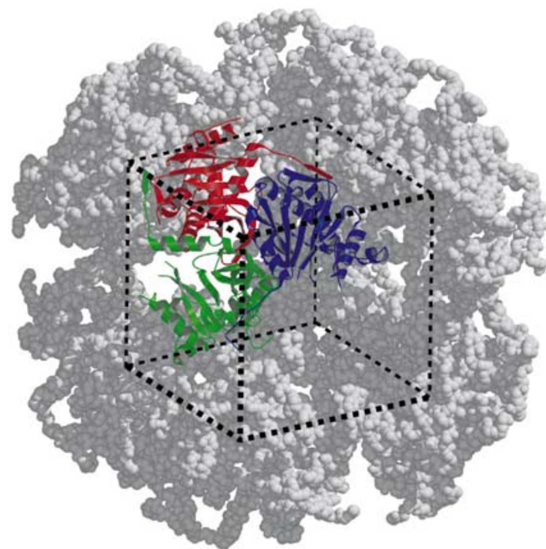

Figure 2
The architecture of the pig E2o cubic core. The catalytic domains of 24 E2os are associated according to the 432 symmetry. The trimer at a corner is shown as a ribbon drawing with a different colour for each subunit. The diagram was produced with the programs *RasMol* from the *CCP*4 suite (Collaborative Computational Project, Number 4, 1994), *MOLSCRIPT* (Kraulis, 1991) and *Raster3D* (Merritt & Bacon, 1997).

For crystallization, fresh protein was concentrated to 20 mg ml^{-1} to avoid precipitation in 20 m*M* Tris–HCl buffer pH 7.5. Crystallization conditions were surveyed by the hanging-drop vapour-diffusion method, each droplet being equilibrated with 1 ml of reservoir solution. Crystal Screen kits (Hampton Research, Laguna Niguel, USA) were used as the reservoir solutions. Each droplet was prepared by mixing 2.5 µl protein solution and 2.5 µl reservoir solution.

The X-ray diffraction experiment was carried out at 100 K with synchrotron radiation (λ = 0.900 Å) at BL44XU, SPring-8 (Nishiharima, Japan). Intensity data were recorded on a 3 × 3 CCD detector array (PX210, Oxford Instrument Co.) set at 700 mm from the crystal. Each frame was taken with a 1° oscillation and 10 s exposure in the ω range −135 to +135°. A total of 270 frames were processed at 7 Å resolution with the program *d*TREK* (Rigaku/MSC Inc., The Woodlands, USA). It is plausible that the catalytic domain of pig E2o (residues 154–387) has a similar peptide folding to that of *E. coli* E2o (residues 172–404), as they have a high sequence identity of 71% (Koike *et al.*, 2000). The atomic coordinates of *E. coli* E2o (Knapp *et al.*, 1998; PDB code 1e2o) were employed as a probe for molecular replacement. The initial phases were derived with the program *AMoRe* (Navaza, 1994) as a unique solution at a high significance level (CC = 0.73 and *R* = 0.42). A solvent-flattening technique from the program *CNS* (Brünger *et al.*, 1998) was applied to improve the electron-density map, on which the structural model was constructed with the X-ray option of the program *QUANTA* (Molecular Simulations Inc., San Diego, USA).

3. Results and discussion

SDS–PAGE showed that pig E2o was highly homogenized, the molecular weight of the subunit being estimated as 48 kDa. A single gel-filtration peak appeared between thyroglobulin (MW = 669 kDa) and Blue Dextran (MW = 2000 kDa) consistent with an apparent molecular mass of 1000 kDa for the E2o 24-mer (Koike *et al.*, 2000). When 0.1 *M* MES buffer solution pH 6.5 containing 30%(*v*/*v*) Jeffamine M-600 and 0.05 *M* CsCl was used as a reservoir solution, cubic crystals of E2o appeared and grew to dimensions of 0.1 × 0.1 × 0.1 mm in two months, as shown in Fig. 1. Jeffamine (McPherson, 1992) may be effective for crystallization of proteins containing flexible domains. Crystals were only obtained from buffer solutions containing Jeffamine M-600; changing the Jeffamine concentration from 20 to 35% varied the crystal size. Using polyethylene glycol as a similar polyalkylene glycol gave only precipitation.

The crystallographic data were determined from the diffraction patterns: the space group was *I*432, with unit-cell parameter *a* = 189.9 Å. The intensity data of 27 203 reflections were observed in the resolution range 100–7.0 Å. The $R_{\rm merge}$ value was 10.7% for the 1058 independent reflections, with a completeness of 100%. In spite of the high multiplicity, $R_{\rm merge}$ was slightly high in the outer shell, with low $I/\sigma(I)$ values. For this reason, a few diffraction spots detected beyond 7.0 Å resolution were truncated.

The number of E2o subunits in the asymmetric unit was estimated to be one from the crystallographic data. It indicates that there are two cubic cores in the body-centered unit cell. The catalytic domain is located in the unit cell by molecular replacement as a unique solution at the highest significance level.

The 24 catalytic domains (related by the crystallographic 432 symmetry) are associated to form a cubic core. At each of the eight corners of the cube, three catalytic domains are tightly bound to each other to form a pyramid-shaped trimer with a diameter of 70 Å and a thickness of 50 Å around the crystallographic threefold axis (Fig. 2). Furthermore, two subunits from different trimers related by crystallographic twofold symmetry make a contact on each edge (110 Å length) of the cube where the C-terminal eight residues interact with the other subunit. On the six faces of the cube there are large pores of 40 Å diameter around the fourfold axes (Fig. 2). The core size (125 Å) is consistent with the electron-microscopic data (Koike *et al.*, 2000). The architecture of the CD cubic core looks very similar to those of other E2 CDs.

In the unit cell of the full-length E2 crystal, the cubic cores make contact with each other at their corners in the body-diagonal direction of the cube according to the space group *I*432. In the truncated E2 crystals (space group *F*432), however, the

core–core contacts occur in the face-diagonal direction, two cubes being associated at two corners or on one edge. Therefore, the interacting surfaces are different in the two crystal forms.

In the modified electron-density map, many small broad peaks were observed in a region expected to contain the remaining N-terminal domains (the E1/E3-binding domain and the lipoyl domain). These domains may be flexible relative to the core and disordered in the crystal. It is difficult to assign these parts in the map derived from the molecular-replacement technique at the present resolution. *Ab initio* phasing such as MAD will be applicable to improve this situation. In addition, visualization of the detailed structure requires higher resolution data from large high-quality crystals, which will be obtained by further optimizing concentration of Jeffamine or other additives.

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