Crystal structure of the electron transfer flavoprotein -ubiquinone oxidoreductase

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Electron transfer flavoprotein -ubiquinone oxidoreductase (ETF-QO) is bound to the inner mitochondrial membrane. It accepts electrons from electron transfer flavoprotein (ETF) located in the mitochondrial matrix, and transfers them to ubiquinone in the inner mitochondrial membrane [1], thus linking the primary flavoprotein dehydrogenases with ubiquinol-cytochrome c reductase (cytochrome bc1 complex or complex III). ETF-QO, together with ETF, is an essential component of fatty acid metabolism and the catabolism of some amino acids. The protein is a 66 kDa monomer and contains two redox cofactors, an FAD and a [4Fe-4S] cluster. Deficiency of either ETF or ETF-QO results in glutaric acidemia type II, an often fatal human metabolic disease. Little is known about the functional domains of the ETF-QO, although primary sequence analysis suggests a putative AMP-binding sequence and reveals a motif characteristic of a 4Fe-4S binding site. The present study reports on the complete three-dimensional structure of porcine ETF-QO determined by x-ray diffraction methods.

The protein was purified from porcine liver mitochondria and crystallized in the presence of β-hexyl-D-glucopyranoside, using the hanging-drop vapor-diffusion method. The crystals belong to the tetragonal space group P4212, with unit cell parameters a = b = 154.3 Å, c = 128.5 Å. The structure was solved by multiple isomorphous replacement with anomalous diffraction (MIRAS)/multi-wavelength anomalous diffraction (MAD) methods. A native data set, four heavy-atom derivative data sets (thimerosal, trimethyl lead acetate, KAu(CN)2, and PIP), as well as a native MAD data set exploring the 4Fe-4S cluster in the native protein, were collected at the BioCARS 14-BM beamline of the Advanced Photon Source (APS). The structure was refined to 2.5 Å resolution with R_{free} and $R_{crystal}$ values of 25.4 % and 22.6 %, respectively.

The overall structure (Figure 1) shows a single large domain composed of 21 β-strands and 10 α-helices. The noncovalently bound FAD is completely buried in the center of the molecule with the pyrophosphate moiety at the N-terminus of an α -helix, which is a part of the β α β Rossman fold motif. The 4Fe-4S cluster is located about 13 Å away from the FAD isoalloxazine ring and about 20 Å away from the bound ubiquinone (UQ) polar head. The FAD isoalloxazine ring and the quinone head are situated in relatively close proximity (about 8.7 Å apart). All three redox centers (FAD, 4Fe-4S cluster, and the UQ head) are situated in the interior of the molecule. Residues I146–L154 and G450–F468 (β -strand and α -helix at the bottom of the Figure 1) are hydrophobic, presumably forming the membrane-binding surface of the molecule. The long polyisoprene tail of UQ is surrounded by these hydrophobic residues which act as an anchor to the mitochondrial lipid bilayer. As expected, the UQ binding pocket is also very hydrophobic.

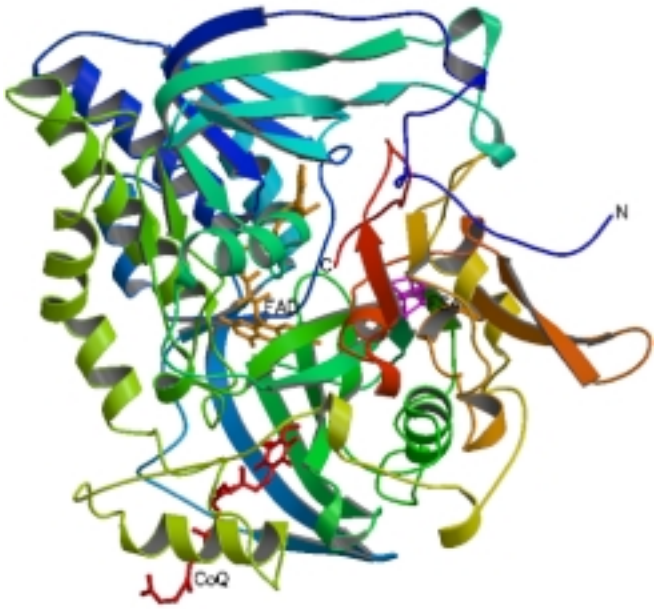

Figure 1: A ribbon diagram showing the overall polypeptide folding of ETF-QO. The color scheme is from blue (N-terminus) to red (C-terminal end). The redox centers are shown as stick models; FAD in gold, 4Fe-4S in pink, and UQ in red. The putative membrane-binding site is located at the bottom of the molecule where the QO molecule binds.

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Reference

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