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High Resolution Structure of a Two-subunit Form of the Membrane Protein Cytochrome *c* Oxidase from *Rhodobacter sphaeroides* . Ling Qin¹, Carrie Hiser¹, Xi Zhang¹, Yasmin Hilmi¹, Anne Mulichak^{1,2}, R. Michael Garavito¹, Shelagh Ferguson-Miller¹, ¹Biochem. Dept., Michigan State Univ., E. Lansing, MI, USA. ²IMCA-CAT, APS, Argonne Nat'l Lab, Argonne, IL, USA.

We have focused on refining methodologies of protein production, purification and crystallization to deal with the inhomogeneity in *Rhodobacter* CcO protein preparations, including alternative processing sites in subunits II and IV, variable content of phospholipids, and proteolysis. Using a strain of *Rhodobacter* that produces CcO with a C-terminal histidine tag on a shortened subunit II, and has a shortened version of subunit IV, we obtained crystals containing only the two catalytic subunits of CcO with isotropic X-ray diffraction to 2.35 Å resolution. The redissolved crystals were active, with native spectrum. The structure was determined by molecular replacement with subunit I and II from the four-subunit holoenzyme (Svensson-Ek *et al.* JMB 321, 329-339. 2002) with $R_{\text{work}} = 20.7\%$ and $R_{\text{free}} = 22.9\%$. The new structure reveals two instances in which the histidine tag chelates a cadmium ion; together these create a strong crystal contact. A number of acyl tails of membrane lipids or detergents are observed in the structure, as well as several maltoside head groups. These observations suggest that the specific binding of native membrane lipids and/or detergent substitutes is important for obtaining well-ordered crystals.

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