A Soluble Domain of Na^+ Pumping Cytochrome *bo* Quinol Oxidase from *Vitreoscilla*

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Cytochrome *bo* is one of the most widespread terminal oxidases in bacteria and belongs to a superfamily of respiratory terminal oxidases with a heme/copper binuclear center. A primary function of these terminal oxidases is to conserve the energy generated in terminal respiration during the transfer of electrons to oxygen by pumping cations (usually H^+) across the membrane to establish a proton electrochemical gradient. Some microorganisms substitute Na^+ for H^+ as a direct coupling cation for ATP synthesis. *Vitreoscilla* cytochrome *bo* quinol oxidase was known to have a function of pumping Na^+, generating a Na^+ electrochemical gradient during terminal respiration.[1] Proteoliposomes containing cytochrome *b*o purified from *Vitreoscilla* translocate Na^+ when energized with ubiquinol. The soluble domain of CyoA (subunit II of the cytochrome complex) is believed to act as an entry point of an electron from ubiquinol. The structure of the *Vitreoscilla* CyoA soluble domain would be of interest, as it explains the binding mechanism of quinol to the complex and the structural differences of a Na^+ pumping cytochrome.

The gene encoding the 24 kDa CyoA soluble domain of *Vitreoscilla* cytochrome *bo* quinol oxidase has been cloned into pQE expression system, expressed in *E. coli*, and purified by affinity chromatography to >95% homogeneity. The enzyme has been crystallized from 2 M ammonium sulfate and 5% isopropanol. The crystal diffracts to 3.3 Å. An x-ray data set has been collected from frozen crystals at the 17-ID beamline of the Advanced Photon Source. The 52,232 observations were averaged to give 5,077 unique reflections with a completeness of 99.9% to 3.3 Å. The crystals belong to space group $P4_132$ or $P4_332$ with unit cell dimensions $a = b = c = 122.20$ Å and $\alpha = \beta = \gamma = 90^o$, and there is a single molecule (24 kDa) in the asymmetric unit. The high symmetry of this space group enabled us to obtain ninefold-redundant data in only 90º of data collection. We are attempting to solve the structure of CyoA soluble domain by molecular replacement using a model derived from the homologous protein from *Escherichia coli*.[2] We also plan to attempt the structure determination by the multiple-wavelength anomalous diffraction (MAD) with selenomethionine as the anomalously scattering species, because the protein contains sufficient methionine—6 methionines out of 208 amino acids.

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References

[1] B.J.S. Efiok and D.A. Webster, Biochem. **29**, 4734-4739 (1990).

[2] M. Wilmanns, P. Lappalainen, M. Kelly, E. Sauer-Eriksson, and M. Saraste. Proc. Natl. Acad. Sci. USA **92**, 11955-11959 (1995).