

3D Structures of Reduced State NADH-Ferredoxin Reductase (BphA4) Solved in X-Ray Crystallography and Cryo-EM

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NADH-ferredoxin reductase (BphA4), an electron transport protein derived from *Acidovorax* sp. strain KKS102, has three redox states: (two-electron) reduced, one-electron reduced, and oxidized. Our group has determined the crystal structures of all three states of BphA4 in the past. However, we believed that the reduced state structure may have been an artifact due to the crystal packing effect [1, 2]. To remove the crystal packing effects from the reduced state structure, we attempted cryo-EM. However, freezing a grid inside an anaerobic chamber to avoid the oxidation of reduced BphA4 was challenging since widely used commercial grid freezing machines were too big to fit in the chamber. Here, we present our method for freezing a grid in an anaerobic chamber and obtaining a reduced state structure of BphA4 through cryo-EM single particle analysis.

The electron transfer system of the biphenyl dioxygenase BphA consists of an FAD-containing NADH-ferredoxin reductase (BphA4) and a Rieske-type [2Fe-2S] ferredoxin (BphA3). Crystal structures of the productive BphA3-BphA4 complex, and of free BphA3 and BphA4 have been determined in the past [1]. These structures demonstrated that each elementary electron transfer induces a series of redox-dependent conformational changes in BphA3 and BphA4, which seem to regulate the interaction between them. The preceding electron transfer appears to induce the next electron transfer through the conformational changes induced. The interplay between electron transfer and induced conformational changes is critical to the sequential electron-transfer reaction from NADH to BphA3 through BphA4.

Cryo-EM measurements require protein samples to be frozen at a thickness of a few tens to few hundred of nanometers on a grid and measured by an electron beam. Cryo-EM does not require crystallization, which can avoid the crystal packing effect causing structural artifacts, but the reduced state structure by cryo-EM is difficult to solve due to the difficulty of freezing grids in an anaerobic environment.

To freeze a grid in an anaerobic environment, all samples and devices for grid freezing were kept inside an anaerobic chamber. The experiment was performed with gloves of an anaerobic chamber. We created a small-size grid freezing robot, mini-GFM (mini Grid Freezing Machine), to establish our experiment inside the anaerobic chamber. A small grid freezing device with dimensions of 70mm x 70mm x 300mm can fit through the door of the anaerobic chamber. With this device, we established a method to plunge freeze grids in an anaerobic chamber and solved the oxidized and reduced state structure of BphA4. The obtained structures of the oxidized and reduced states showed clear structural differences.

The intermolecular electron transfer system is a fundamental process in many biological pathways, including photosynthesis and multi-component oxygenase systems. Structural information on the oxidized and reduced states of proteins or protein complexes helps to understand how the intermolecular electron transfer system works. Our method to freeze cryo-EM grids in an anaerobic chamber will open an opportunity to access those reduced state structures.

References

[1] *Molecular Mechanism of the Redox-dependent Interaction between NADH-dependent Ferredoxin Reductase and Rieske-type [2Fe-2S] Ferredoxin*

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[2] *Redox control of protein conformation in flavoproteins. Toshiya Senda, Miki Senda, Shigenobu Kimura, Tetsuo Ishida, Antioxid. Redox Signal.* 11, 1741-1766. (2009)