

Crystallographic Study of an electron-transfer complex between Ferredoxin and Ferredoxin-NADP⁺ reductase

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Ferredoxin (Fd) and Ferredoxin-NADP⁺ reductase (FNR), which catalyzing the reduction of NADP⁺ to NADPH, are well known proteins found in the higher plants, eukaryotic algae, photosynthetic bacteria and other cyanobacteria. They play a particularly important role in the photosynthetic process in the chloroplast. Specifically speaking, electrons ejected from Photosystem I (PSI) passed to Fds. Reduced Fd reduced NADP⁺ in a reaction of FNR, to yield the final product of the chloroplast light reaction, NADPH. A large number of Fds and FNRs from various organisms have been isolated, sequenced and characterized. Fds are small, acidic and soluble proteins, and FNRs are basic FAD-containing flavoproteins. Many of them were structurally analyzed by chemical and physical methods including X-ray crystallography. Based on the previously reported structures of Fds and FNRs, electron transfer pathways from Fd to FNR are predicted. Nevertheless, all three dimensional structures are individually crystallized and analyzed. In order to determine the precise interaction and observe the complex formation between them, we started the crystallographic study of an electron-transfer complex between them.

An electron transfer complex has been crystallized by hanging drop vapor diffusion method and a preliminary structure investigation has been carried out. The complex is composed of FNR, a flavoprotein colored yellow and Fd, a dark-brown [2Fe-2S] protein. The crystals for structural analysis were colored light brown to be a mixture of the two colored proteins. Complex crystals between Fd and FNR are monoclinic, space group C2 with cell dimensions $a = 85.7$, $b = 105.4$, $c = 115.8$ Å and $\beta = 91.5^\circ$. In case of rotating anode X-ray generator, we had collected the diffraction data only up to 4.0 Å resolution. While in case of synchrotron radiation generated by SPring-8, the crystal can diffract up to 3.0 Å resolution at 100K. However, the quality of the diffraction data were not so good, because of the high mosaicity of a crystal. Now we are analyzing the location of the FNR by molecular replacement method and looking for the new crystallization condition to obtain a crystal with high quality at the same time.