Structure of the Electron Transfer Complex Between Ferredoxin and Ferredoxin-NADP$^+$ Reductase

All oxygenic photosynthetically derived reducing equivalents are utilized by combinations of a single multifuctional electron carrier protein, ferredoxin (Fd), and several Fd-dependent oxidoreductases. Plant-type Fd is a small, soluble, acidic protein distributed in plants, algae and cyanobacteria. Each Fd-dependent enzyme is also distributed in the same organism and functions in photosynthetic metabolism, such as Fd-NADP$^+$ reductase (FNR), which is involved in the process of carbon assimilation; nitrite reductase and glutamate synthase, which are involved in nitrogen assimilation; sulfite reductase, which is involved in sulfur assimilation; and ferredoxin-thioredoxin reductase, which is involved in the redox regulation of several enzymes.

About 20 years ago, the first structure of a plant-type Fd was reported [1]. Many biochemists have been studying the interaction sites of Fd based on this 3-D structure. After the crystal structure of FNR as a representative of Fd-dependent enzymes was reported in 1991 [2], further experiments including computer modeling and continuous mutational experiments of this protein-protein interaction have been carried out extensively.

We determined the first crystal structure of the complex of Fd and FNR from maize leaf at 2.59 Å resolution [3] (Fig. 1). The diffraction data was collected at beamline **BL41XU**. The redox partners are in close contact at the prosthetic groups, the 2Fe-2S cluster of Fd and FAD of FNR, the shortest distance being 6.0 Å. Interaction mainly occurs by electrostatic force through salt bridges, and the interface near the prosthetic groups is hydrophobic (Fig. 2). Interestingly, the structures of Fd and FNR in the complex and in the free state alter in a number of ways. Consistent with this, we confirmed FNR recognition sites on the Fd protein by NMR spectroscopy of the complex in solution. Such structural alteration is found at Glu 312 in the active site of FNR (Fig. 3). We propose that this type of molecular communication not only determines optimal orientation of the two proteins for electron

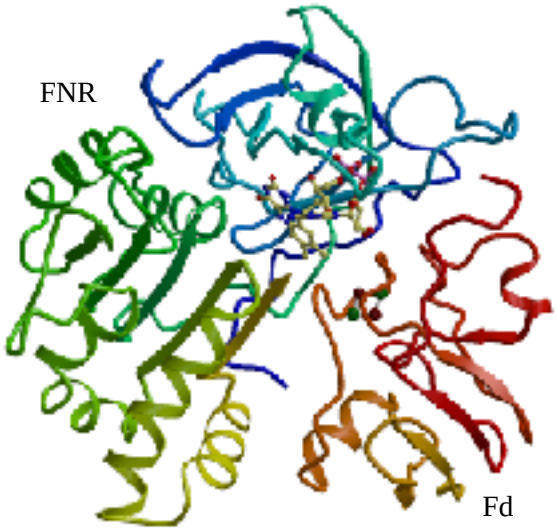

Fig. 1. Whole structure of the electron transfer complex between Fd (lower right) and FNR (upper left). Two prosthetic groups, FAD and [2Fe-2S] cluster, are located at the interface of two proteins and shown in ball-and-stick models.

transfer, but also contributes to the modulation of the enzymatic properties of FNR. These structural alterations of two proteins are consistent with previous biochemical and biophysical reports and thought to be important for efficient electron transfer between them. The 3-D structure of the photosynthetic electron transfer complex is important for further understanding of assimilatory reduction and molecular recognition mechanism closely related to the physiological conditions of higher plants.

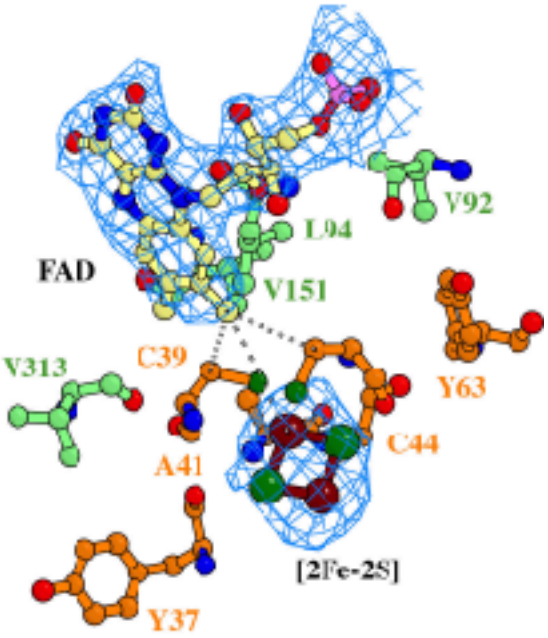

Fig. 2. Structure of the interface of Fd and FNR. The final 2Fo-Fc map was drawn around the prosthetic groups. The vicinity of the redox active center is hydrophobic and seems to be suitable for the direct electron transfer between two prosthetic groups.

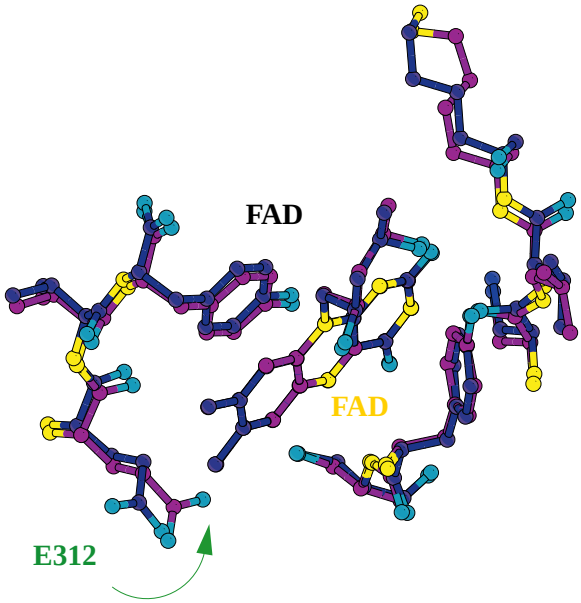

Fig. 3. Structural alteration of FNR induced by the complex formation with Fd. The structure around the FAD from single FNR is colored in yellow and that of the complex is in green. The side chain of E312 was moved into the active site upon the complex formation with Fd.

Genji Kurisu and Masami Kusunoki

Osaka University

E-mail: kurisu@protein.osaka-u.ac.jp

References

[1] K. Fukuyama *et al.*, Nature **286** (1980) 522.
[2] P.A. Karplus *et al.*, Science **251** (1991) 60.
[3] Genji Kurisu, Masami Kusunoki, Etsuko Katoh, Toshimasa Yamazaki, Keizo Teshima, Yayoi Onda, Yoko Kitama-Ariga and Toshiharu Hase, Nature Struct. Biol. **8** (2001) 117.