Precise Redox-dependent Structural Change of the plant-type Ferredoxin revealed by X-ray structures at 0.77 Å resolution, originated and propagating from the [2Fe-2S] cluster

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Ferredoxin (Fd) is a redox protein containing the iron-sulfur cluster as the active site, and distributed in various organisms, archaea, bacteria, plants and animals. Specific Fd located in the stroma of chloroplast or cyanobacteria is called "plant-type" and possesses a [2Fe-2S] cluster ligated by four conserved cysteine residues. It carries one electron from Photosystem I reaction centre on the thylakoid membrane to several Fd-dependent enzymes. Unique feature of this plant-type Fd is its low redox-potential around -400 mV, which can reduce NADP+ to NADPH (E_m = -350 mV) *in vivo*[1]. However *in vitro*, it means that the chemically reduced samples are easily oxidized by air and, on the other hand, the modern strong X-ray beam could reduce the oxidized form of crystallized sample[2]. Previous structural analyses of Ser46, Phe64 and Glu93 mutants showed the structural basis for the redox potential increase of mutants by 50~90 mV[3], while these mutated residues were completely conserved among the plant-type Fds[3]. Furthermore, X-ray crystallography on oxidized and reduced Fd from *Anabaena* showed that the peptide bond next to S46 flipped upon partial reduction[4]. Although several X-ray structures of plant-type Fds including above were available in the PDB, their redox states were not precisely controlled and probably in the mixed states. Consequently, it is not clear how dissociation/association between the plant-type Fd and partner proteins is controlled by one electron redox on the [2Fe-2S] cluster. Here, we solved the X-ray structures of oxidized Fd with minimum X-ray dose and fully reduced Fd from cyanobacterium *Thermosynechococcus elongatus* (*Te*Fd) at 0.78 and 0.77 Å resolution, respectively. Both oxidized/reduced crystals had a space group of *C*2 and all used crystals were isomorphous. Our high-resolution structures newly reveal the redox-linked repositioning of Ser46, Phe64 and Glu93 (**Fig 1**). To investigate the reason how these structural changes are originated from the small but significant structural change in the [2Fe-2S] cluster, we solved the crystal structures of the oxidized/reduced S46A or F64A mutants of *Te*Fd at 0.95~1.05 Å resolution, independently (**Fig 2**). All our obtained high-resolution structures imply how the small structural changes of the cluster are spatially amplified and propagated through the peptide chain. The detail of our discussion will be presented in our presentation.

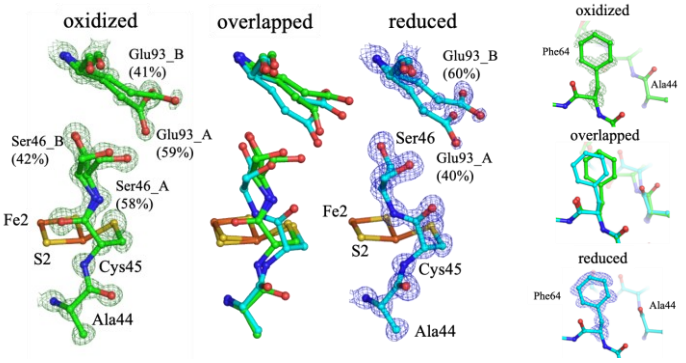

Fig 1. Crystal structures and density maps (1.5 σ) of oxidized and reduced TeFd focused on around the cluster binding Cys45. Numbers in the parentheses show the occupancies of each alternative conformer.

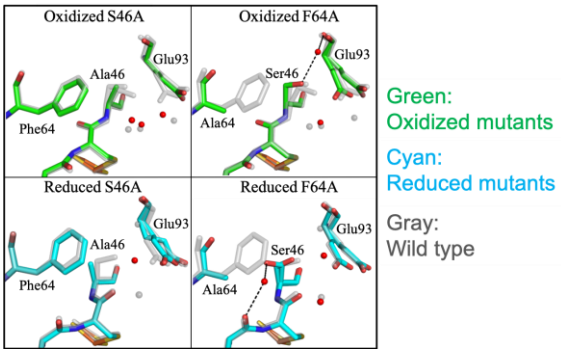

Fig 2. Comparison of the structure between the native and S46A or F64A TeFd.

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