Structural Analysis of the Interaction between Plant Sulfite Reductase and Ferredoxin

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Plant type ferredoxin (Fd) is reduced via photosystem I or by Fd:NADPH oxidoreductase and donates reducing equivalents to various Fd-dependent enzymes. Sulfite reductase (SiR) is one of such enzymes, catalyzing six-electron reduction of sulfite to sulfide. SiR contains siroheme and [4Fe-4S] cluster as redox centers and our ongoing x-ray crystallographic analysis of maize SiR has revealed its active site structure consisting of these two prosthetic groups. SiR forms an electron transfer complex with Fd and this inter-molecular interaction is stabilized mainly through electrostatic force between acidic residues of Fd and basic residues of SiR. We have also been investigating the interaction by NMR spectroscopy. When ^{15}N-labeled Fd was titrated with SiR, NMR chemical sift changes were observed on ^{1}H-^{15}N HSQC spectra. The data allowed us to map the interaction sites for SiR on the 3D structure of Fd. Site-specific Fd mutants lacking acidic residues with the large chemical sift perturbation showed lowered affinity to SiR both in the kinetical assay and static interaction analysis, confirming the NMR assignment of the interaction sites. We have introduced a series of mutations on the basic amino acids of SiR and selected mutants with a lowered affinity to Fd. These SiR mutants exhibited little activity in the assay of Fd-dependent sulfite reduction. We will present a detailed interaction mapping of SiR and Fd based on the combined results.

Keywords: iron-sulfur proteins, protein-protein interactions, NMR spectroscopic investigations