Crystal Structure of Glutathione-dependent Dehydroascorbate Reductase from Spinach Chloroplast

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Glutathione-dependent dehydroascorbate reductase (GSH-DHAR) catalyzes the reduction of dehydroascorbate (DHA) to ascorbate using reduced glutathione as the electron donor. GSH-DHAR existing in chloroplast plays a pivotal role in the regeneration of ascorbate, which is oxidized to scavenge active oxygen species in the process of photosynthesis. The catalytic mechanism of the GSH-DHAR from spinach chloroplast is intriguing, because the specific constants for DHA and GSH are much higher than those of the other characterized DHARs.

Here, we report the three-dimensional structure of GSH-DHAR from spinach chloroplasts at 1.65 Å resolution, which has been determined by the multiwavelength anomalous dispersion (MAD) phasing method. The crystal structure reveals a monomeric form, which corresponds to the results observed in the analyses of gel filtration and dynamic light scattering. The structure is mainly composed of the similar domain to that of previously solved thioltransferase [1] and an extra alpha-helical domain. The catalytically essential cystein was completely reduced, because it was crystallized in the solution including high concentration of DTT. The model study using the coordinates of glutathione transferases suggested that the putative glutathione binding site was formed by the amino residues corresponding to those of the other glutathione transferases. These observations support that glutathione could be bound near the catalytically essential cystein. We will show the detailed reaction mechanism to describe how it establishes its high specificities.

[1] Katti S. K., *et al*., *Protein Sci.,* 1995, **4**, 1998.

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