Poster Presentation

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Structural analyses of the two cysteine desulfurases from Aquifex aeolicus

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The cysteine desulfurase IscS is a highly conserved master enzyme initiating sulfur transfer to a wide range of acceptor proteins. IscS degrades L-cysteine into L-alanine and a sulfur atom in a pyridoxal 5'-phosphate (PLP) dependent manner. In this reaction, it is essential for a conserved Lys residue of IscS to form Schiff base (the covalent bonding interaction) with PLP. Recent accumulations of genomic information have revealed that some IscS homologues in archaea and thermophilic bacteria lack this invariant Lys. Here we report the crystal structures of two paralogous cysteine desulfurases, the canonical Aa IscS1 and the invariant Lys lacking Aa IscS2, from Aquifex aeolicus. Aa IscS1/Aa IscS2 were overproduced in E. coli, and purified by heat-treatment and several column chromatography, and crystallized. The structure of Aa IscS1 was determined at 2.00 Å (Rcryst= 19.4% and Rfree = 22.0%), and Aa IscS2 at 2.55 Å (Rcryst= 21.8% and Rfree = 27.0%). Overall structures as well as orientations of the residues in the active site were quite similar to each other. In Aa IscS1 the PLP adduct was anchored in the catalytic pocket of Aa IscS1 by the formation of the aldimine Schiff base with the invariant Lys. Whereas in Aa IscS2 the PLP was not seen in the active pocket, since the catalytic Lys was substituted by Leu. Alternatively, an electron density derived from unknown-small molecule was located in the catalytic site of Aa IscS2. The shape of this electron density was completely different from that of PLP. The Bijvoet difference map calculated from data collected at λ=1.7 Å overlapped with the electron density observed in the active site; the unknown-small molecule probably contains such metals as iron atoms. Furthermore, the ICP-MS analysis demonstrated that as-isolated Aa IscS2 harbored the iron atom in the solution state. More recently we obtained the experimental evidences that non-canonical Aa IscS2 was able to form the binary complex with Aa IscU, which is responsible for a scaffold for the assembly of a nascent Fe-S cluster. Base on the structural/biochemcal results, possible physiological functions of two cysteine desulfrurases will be discussed.

Keywords: cysteine desulfurase, iron-sulfur clusters, PLP