Crystal Structure of a NifS Homologue CsdB from *Escherichia coli*

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Escherichia coli CsdB is a dimeric NifS-homologue belonging to the fold-type I family of PLP-dependent enzymes, and catalyzes the decomposition of L-selenocysteine into selenium and L-alanine with specificity higher than that for a substrate of cysteine. The structure of the enzyme has been determined at 2.8 Å resolution by an X-ray crystallographic method. The subunit of CsdB comprises a large domain, a small domain, and an N-terminal segment. A remarkable structural feature of CsdB is that an α-helix in the lobe extending from the small domain to the large domain in one subunit of the dimer interacts with a β-hairpin loop protruding from the large domain of the other subunit. Cys364, which is essential for the activity toward cysteine but not toward selenocysteine, is clearly seen on the loop of the extended lobe (Thr362–Arg375) although the corresponding loop (Ser321–Arg332) is disordered in the *Thermotoga maritima* NifS-like protein, which is closely related to the cysteine-specific NifS and whose crystal structure has recently been determined as the second example.

Key words: X-ray crystallography / CsdB / NifS-like protein / iron-sulfur cluster / PLP

CsdB, the *csdB* gene product from *Escherichia coli*, is a PLP-dependent protein, which decomposes L-selenocysteine, L-cysteine sulfinic acid and L-cysteine into selenium, sulfur dioxide and sulfur, respectively, and L-alanine [1]. This enzyme shares 23% sequence identity with the NifS protein, that catalyzes the decomposition of L-cysteine and functions in the nitrogen fixation system of *Azotobacter vinelandii* by supplying sulfur to stabilize or repair the iron-sulfur cluster of the nitrogenase component protein. In order to clarify structure-function relationships of a family of NifS-like proteins, three-dimensional structure of the *E. coli* CsdB has been determined by X-ray crystallographic analysis [2].

The crystals of CsdB were grown by a hanging drop vapor diffusion method using sodium acetate as a precipitant. Two heavy-atom derivatives were prepared by soaking the crystals into phenyl mercury acetate and potassium tetracyanoplatinate. Diffraction data were collected on an R-AXIS IIc imaging-plate detector system using CuKα radiation produced by a RU-300 generator. The structure of CsdB was solved by the multiple isomorphous replacement with anomalous scattering and density modification techniques. The polypeptide chain was traced based on the bones generated by truncating the map. The structure was refined at 2.8 Å resolution. The subunit structure of CsdB is composed of a polypeptide chain of Phe3–Gly406, one PLP, and one acetate.

The overall fold of the CsdB subunit (Figure 1) is similar to those of the fold-type I family of PLP-dependent enzymes, represented by an aspartic acid

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Scope of research

Our research aims are to elucidate structure-function relationships of biological macromolecules, mainly proteins, by using physicochemical methods such as spectroscopic and X-ray diffraction methods. The following attempts have been mainly made in our laboratory for that purpose. (1) X-ray diffraction studies on protein structures in crystal and in solution are carried out by crystallographic and/or small-angle X-ray scattering techniques to elucidate structure-function relationships of proteins. (2) Molecular mechanism for myosin assembly is studied by proteolytic method, electron microscopy, and computer analysis of the amino acid sequence.

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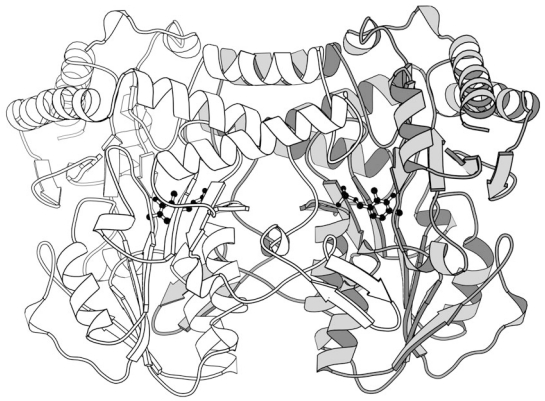

Figure 1. The dimeric molecule of CsdB viewed perpendicularly to the molecular two-fold axis. The figure, showing a pair of subunits shaded differently, was drawn with program MOLSCRIPT [5].

aminotransferase (AAT), especially to that of a phosphoserine aminotransferase (PSAT) which belongs to the class V of fold-type I enzymes. The subunit consists of three parts: the large domain of an α/β fold containing a seven-stranded β-sheet flanked by helices, the small domain containing a four-stranded β-sheet with three α-helices, and the N-terminal segment.

The circumstances of active site in CsdB, AAT, and PSAT are almost similar among one another. PLP has the C4' atom connected with the Nζ atom of Lys226, providing the Schiff base to form an internal aldimine. The O3' and N1 atoms of PLP hydrogenbond with the Nε2 atom of Gln203 and with Asp200, respectively. The most striking difference in the interactions between the protein and PLP lies in the stacking mode of the aromatic residue and the pyridine ring of PLP. In CsdB, the imidazole ring of His123 is stacked against the pyridine ring of PLP, whereas the indole ring of the Trp residue in AAT and PSAT. The expected binding mode of a substrate selenocysteine to the active site of CsdB was considered based on the crystal structures of complexes of AAT or PSAT with their substrate analogues. In the model of the CsdB complex, the α-carboxyl group of the substrate is recognized with Arg379 and Asn175. The selenium at the γ-position of the substrate is close to the side chain of Cys364.

CsdB has several structural features which are different from those of other families of enzymes. A β-hairpin loop (residues 256 – 267) protruding from one subunit of the dimer interacts with an α-helix in a lobe (residues 366 – 371) extending from the small domain to the large domain of the other subunit. These two portions of the β-hairpin loop and the lobe form one side of a limb of an active site in the enzyme. Cys364, which is essential for the activity toward L-cysteine but not toward L-selenocysteine [3], lies on the extended lobe which has an ordered conformation. This structure is not found in other 'fold-type I' PLP dependent enzymes.

The CsdB structure in Figure 2 shows the regions (A – D) markedly different in sequence between CsdB and NifS. The β-hairpin loop (region C) in CsdB is deleted in NifS. In contrast to this deletion, 10 residues are inserted in NifS between the residues corresponding to Met366

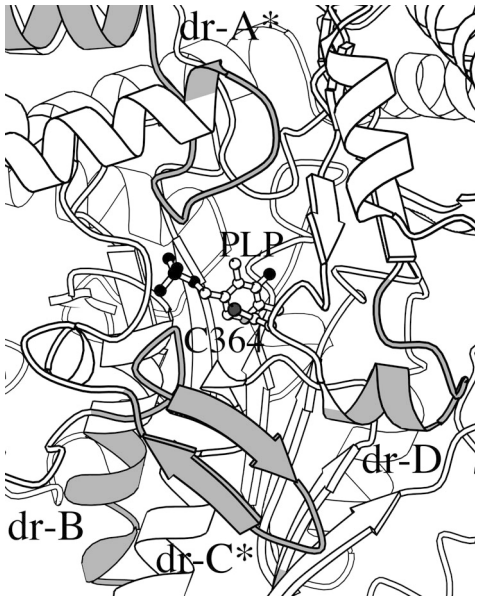

Figure 2. Schematic representation of CsdB showing differences in sequence between CsdB and NifS. These regions are shaded and labeled "dr-B" for residues 99 – 115 and "dr-D" for residues 366 – 376 in one subunit, and "dr-A*" for residues 34 – 60 and "dr-C*" for residues 251 – 268 from the other subunit. PLP and Cys364 are depicted as ball-and-stick models. Helices and β-strands are represented by spirals and arrows. The figure was drawn with program BOBSCRIPT [6].

and Pro367 on α-helix of the extended lobe in CsdB (region D). The region just follows the putative catalytic residue Cys364. Therefore, the extended lobe anchoring the catalytic cysteine residue seems to be much larger in NifS than in CsdB and to have a different structure as well as a different location of the catalytic cysteine residue. Recently, the crystal structure of *Thermotoga maritima* NifS-like protein (tmNifS), which is closely related to the cysteine-specific NifS, has been determined as the second example [4]. However, the loop of tmNifS corresponding to the extended lobe of CsdB is disordered. The extending lobe is the most remarkable structural feature common to the NifS homologues and probably a prerequisite for their function.

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References

1. Mihara H, Maeda M, Fujii T, Kurihara T, Hata Y, and Esaki N, *J. Biol. Chem.*, **274**, 14768-14772 (1999).
2. Fujii T, Maeda M, Mihara H, Kurihara T, Esaki N, and Hata Y, *Biochemistry*, **39**, 1263-1273 (2000).
3. Mihara H, Kurihara T, Yoshimura T, and Esaki N, *J. Biochem.*, **127**, 559-567 (2000).
4. Kaiser JT, Clausen T, Bourenkow GP, Bartunik H-D, Steinbacher S, and Huber R, *J. Mol. Biol.*, **297**, 451-464 (2000).
5. Kraulis PJ, *J. Appl. Crystallogr.*, **24**, 946-950 (1997).
6. Esnouf RM, *J. Mol. Graphics Modell.*, **15**, 132-134 (1997).