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The crystal structure of *Escherichia coli* CsdE

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ABSTRACT

Sulfur incorporations both in the biosynthesis of sulfur-containing cofactors and in the sulfur-modifications of certain tRNAs are all mediated by the sulfur initially delivered from the cysteine desulfurases. Sulfur generated as persulfide from cysteine is transferred to the sulfur acceptor protein to further allow delivery to the required steps within an enzymatic process. CsdA which is one of the three cysteine desulfurases identified in *Escherichia coli* transfers sulfur to the non Fe–S sulfur-acceptor CsdE, however, the consequence of CsdE accepted sulfur is mostly unknown. In this study, we report the 2.4 Å structure of free CsdE determined using X-ray crystallography, and compare the structure with the CsdE structure determined using NMR and also CsdE within the crystal CsdA–CsdE complex. Further analysis suggests that the positive electrostatic potential surfaces of CsdE may mediate interaction with a yet unidentified protein or possibly tRNA to deliver sulfur.

1. Introduction

Sulfur incorporations both in the biosynthesis of sulfur-containing cofactors (*e.g.* iron–sulfur clusters, thiamine, molybdopterin, *S*-adenosylmethionine and biotin) and in the sulfur-modifications of certain tRNAs (*eg.* 2-methylthiolation at A37 and 2-thiolations at U34 and C32) are mediated by the sulfur initially delivered from the cysteine desulfurases that use cysteine as the substrate [1–3]. In *Escherichia coli*, three cysteine desulfurases, namely IscS, SufS, and CsdA, respectively from the operons of iron–sulfur cluster formation (ISC), sulfur mobilization (SUF) and cysteine sulfinate desulfinase (CSD) exist, and the sulfurs generated in each case as persulfides are transferred to the sulfur acceptor proteins of the respective operons that allow further sulfur deliveries to the required steps within an enzymatic process. In the most recently characterized CSD operon, the cysteine desulfurase CsdA transfers sulfur to the non Fe–S sulfur-acceptor CsdE [4–7]. CsdE shares 35% sequence identity with the *E. coli* SufE which is the non Fe–S sulfur acceptor that receives sulfur from the cysteine desulfurase SufS of the SUF operon. However, unlike in the case of SufE, the consequence of CsdE accepted sulfur is mostly unknown. In the CSD operon, only *csdA* and *csdE* exist along the same direction, which is unique compared to the other ISC and SUF operons. However, another *csdL* gene is located downstream of *csdE* in an opposite direction. The gene product CsdL (also known as TcdA) with an ATP-binding fold functions as an N^6-threonylcarbamoyl dehydratase on tRNA A37 [8–10].

The structure of CsdE was first uncovered by NMR [11]. The NMR structure of CsdE revealed CsdE with nearly identical fold to the *E. coli* SufE (C^{α} rmsd = 2.5 Å) [11,12]. The free CsdE in solution exhibits a compact two-layered α/β-sandwich composed of three-stranded anti-parallel β-sheet (β-strands noted as β_A, β_B, and β_C) flanked by seven α-helices [11]. The similar globular fold of CsdE was later confirmed within the CsdA–CsdE complex using X-ray crystallography [13]. The CsdE within the CsdA–CsdE complex revealed most of the α-helical and β-sheet elements identical to the CsdE from NMR with C^{α} rmsd = 2.4 Å. However, the structural difference between the two took place around the active Cys61 residue that accepts sulfur as a persulfide from CsdA *via* the trans-persulfuration reaction [13]. In the free CsdE in solution, Cys61 which is located in the loop connecting the two anti-parallel β-strands β_A and β_B lay internally buried in a low solvent accessible hydrophobic cavity. However, upon CsdA interaction, Cys61 shifted ~11 Å along with the loop to expose itself toward CsdA at a mode positioned to accept the sulfur [13]. This conformational oscillation of Cys61 in CsdE was unique when compared to other sulfur acceptors such as TusA and IscU upon interactions with the cysteine desulfurase IscS, where no significant conformational changes on the sulfur accepting regions were observed [14].

Abbreviations: PDB, Protein Data Bank; AU, asymmetric unit; rmsd, root-mean-square deviation.

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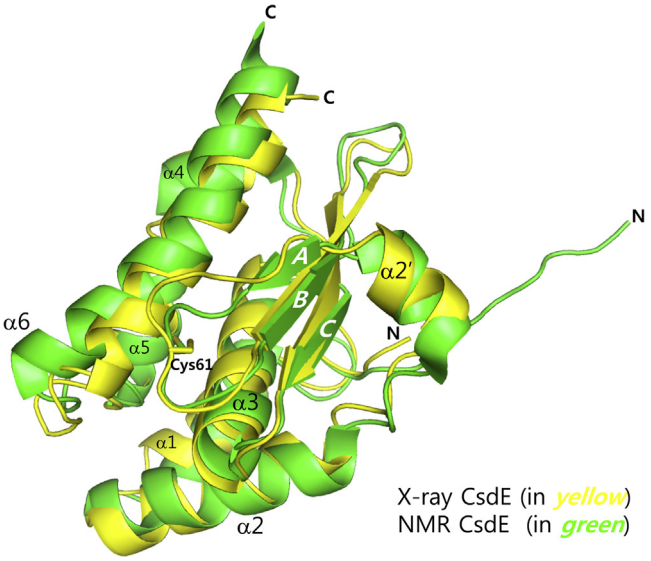

Fig. 1. Comparison of CsdE structures from X-ray and NMR determinations. Superimposed structures of CsdE from X-ray (in *yellow*) and NMR (in *green*) determinations are shown with the sulfur-accepting Cys61. The structure of NMR CsdE is from PDB accession code 1NI7. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

In this study, we report the structure of free CsdE determined by using X-ray crystallography (X-ray CsdE), and compare the structure with the CsdE from NMR (NMR CsdE) and CsdE within the CsdA–CsdE complex. Further analysis on X-ray CsdE shows that the positive electrostatic potential surfaces of CsdE may mediate interaction with a yet unidentified protein or possibly tRNA to deliver sulfur.

2. Results and discussion

2.1. Overall structure

The fold of free CsdE revealed in the X-ray crystal structure is a two-layered α/β-sandwich composed of two-stranded anti-parallel β-sheet flanked by seven α-helices (α-helices and β-strands noted respectively as α1-α6 and α2′, and β_B and β_C in accordance with previous studies) (Fig. 1). Discernible electron density only begins with Gly8 of X-ray CsdE despite the fact that the full-length CsdE was used for crystallization. This disorder was similarly observed for the ten flexible N-terminal residues having multiple conformations in the NMR CsdE. When compared to the CsdE structure uncovered by NMR, the region identified as β_A (3-residue β-strand) in NMR CsdE does not fold into a β-strand element, and the β-strand elements of β_B and β_C are elongated in the X-ray CsdE (Fig. 1). In X-ray CsdE, β_B extends three residues more to the C-terminus and β_C begins to form a β-strand three-residue earlier when compared to that of the NMR CsdE. These discrepancies in the central β-sheet of CsdE result in a C^α rmsd of 1.6 Å between X-ray CsdE and NMR CsdE. The differences between a structure determined on an identical protein either by NMR or X-ray crystallography can be attributed to the crystal packing environment in the latter method where the protein molecules are arranged into a periodic crystal lattice specific to the crystal space group [15]. In such a state, flexible regions sometimes can be locked into a non-native conformation in order to minimize possible steric clash. To see whether certain conformation results from this crystal artifact, symmetry operations specific to the crystal space group can be performed to generate symmetry mates to analyze the contacts made. Hence, symmetry mates near the central β-sheet of X-ray CsdE was analyzed for the nearby crystal contacts (Fig. 2A). No significant contact made between the symmetry mates to β-strand elements of CsdE suggests that the discrepancies between NMR CsdE and X-ray CsdE result from other unknown factors, perhaps the water environment within the crystal.

Also in X-ray CsdE, the sulfur-accepting Cys61 which was shown to shift significantly upon CsdA interaction in the CsdA–CsdE complex is located at the internally positioned hydrophobic cavity as in the NMR CsdE. Similar symmetry mate analysis near the Cys61-containing loop was performed to see whether this internalization of Cys61 in X-ray CsdE results from a crystal artifact (Fig. 2B). The analysis indicates that substantial space exists near the Cys61-containing loop between CsdE (x,y,z) and the closest symmetry mate $[(-y,-x,-z+½)+(1,1,0)]$. Superposition of CsdE from the CsdA–CsdE complex to these two symmetry pairs further demonstrates that this space can accommodate the two externally positioned Cys61-containing loop with the cysteine sulfur-to-sulfur distance measured up to ~8 Å (Fig. 2B). Hence, the internalization of Cys61-containing loop observed in X-ray CsdE seems biologically relevant as in the NMR CsdE.

The lack of β-strand element β_A, and the elongated boundaries of β_B and β_C in X-ray CsdE compared to the NMR CsdE are also observed in the CsdE of the CsdA–CsdE complex (Fig. 3). However, the external exposure of Cys61 along with the conformational shift of Cys61-containing loop upon CsdA interaction results in a C^α rmsd of 1.6 Å between the X-ray CsdE and the CsdE within CsdA–CsdE complex. The two free CsdE structures (X-ray and NMR) in comparison to the CsdE within the CsdA–CsdE complex indicate that CsdA interaction to CsdE induces the conformational change in CsdE.

Additional differences between the X-ray CsdE and CsdE within the CsdA–CsdE complex were analyzed to identify the factors that trigger the movement of Cys61 and the Cys61-containing loop (Fig. 3). As a result, the conformational changes happening at the N-terminal region near α6 (Ser^{126}-Ala^{127}-Ser^{128}-Arg^{129}-Ser^{130}) in the free CsdE upon CsdA interaction seem to initiate the process. In the free CsdE (X-ray), Ser^{126}-Ala^{127} is part of a loop connecting α5 and α6, and Ser^{128}-Arg^{129}-Ser^{130} folds into α6. However, the CsdE interaction with the α16 of CsdA modifies this Ser^{126}-Ala^{127}-Ser^{128}-Arg^{129}-Ser^{130} region of CsdE into a 3_{10}-helix to avoid unfavorable contact. This movement likely acts as a switch to allow the opening of the Cys61-containing loop toward the active CsdA Cys358 for trans-persulfuration. These series of event mark a good example of protein–protein interaction inducing the necessary conformational changes for the subsequent reaction.

2.2. The positive electrostatic potential surface of CsdE

Since the first functional characterization of CsdE in 2005 [5], the subsequent sulfur-receiving partner of CsdE and the detailed sulfur utilization pathway of CsdE still remain unknown. Since thiolations happen frequently in tRNA, there is a possibility that CsdE may not provide sulfur to proteins but rather to certain tRNAs. In such case, the positively patched electrostatic potential surfaces of CsdE can be crucial in mediating the complimentary interactions. The electrostatic potential analysis of the X-ray CsdE revealed three surfaces clustered with solvent-exposed positive Arg or Lys residues (Surfaces 1–3, Fig. 4). Surface 1 (R35/K42/R86/R129) is formed by Arg35/Lys42 of α2, Arg86 of α3, and Arg129 of the N-terminal α6 (Fig. 4 left). All four residues in Surface 1 were shown to mediate interactions with CsdA in the CsdA–CsdE complex [13]. Surface 2 (K56/K145) is formed by Lys56 of α2′-β_B loop and Lys145 of the C-terminal α6 (Fig. 4 right). These two residues were also shown mediate CsdA interaction [13]. Surface 3 (K76/K102) is formed by Lys76 of the β_B–β_C loop and Lys102 of the α3-α4 loop. Unlike the residues of Surfaces 1 and 2, these two residues of CsdE do not mediate CsdA interaction. It is interesting to note that except for