IscS Functions as a Primary Sulfur-donating Enzyme by Interacting Specifically with MoeB and MoaD in the Biosynthesis of Molybdopterin in *Escherichia coli*<sup>***</sup><sup>‡</sup><sup>†</sup><sup>¶</sup><sup>§</sup>

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The persulfide sulfur formed on an active site cysteine residue of pyridoxal 5′-phosphate-dependent cysteine desulfurases is subsequently incorporated into the biosynthetic pathways of a variety of sulfur-containing cofactors and thionucleosides. In molybdenum cofactor biosynthesis, MoeB activates the C terminus of the MoaD subunit of molybdopterin (MPT) synthase to form MoaD-adenylate, which is subsequently converted to a thio酯酰基 group for the generation of the dithiolene group of MPT. It has been shown that three cysteine desulfurases (CsdA, SufS, and IscS) of *Escherichia coli* can transfer sulfur from l-cysteine to the thio酯酰基 group of MoaD in vitro. Here, we demonstrate by surface plasmon resonance analyses that IscS, but not CsdA or SufS, interacts with MoeB and MoaD. MoeB and MoaD can stimulate the IscS activity up to 1.6-fold. Analysis of the sulfuration level of MoaD isolated from strains defective in cysteine desulfurases shows a largely decreased sulfuration level of the protein in an iscS deletion strain but not in a csdA/sufS deletion strain. We also show that another iscS deletion strain of *E. coli* accumulates compound Z, a direct oxidation product of the immediate precursor of MPT, to the same extent as an MPT synthase-deficient strain. In contrast, analysis of the content of compound Z in ΔcsdA and ΔsufS strains revealed no such accumulation. These findings indicate that IscS is the primary physiological sulfur-donating enzyme for the generation of the thio酯酰基 group of MPT synthase in MPT biosynthesis.

The presence of sulfur in cofactors and RNA thionucleoside confers significant functionality to these biomolecules because of its unique chemical properties. The sulfur atoms of thiamin, lipoic acid, biotin, iron-sulfur (FeS)<sup>2</sup> clusters, and molybdenum cofactors participate in stabilizing reaction intermediates (1, 2), alternating between the reduced dithiol and oxidized disulfide, facilitating electron transfer, or modulating the redox potential of the cofactor (3). The thionucleosides in tRNAs fine-tune the efficiency or accuracy of translation (4, 5), serve as recognition sites for aminoc酰基-tRNA synthetase (6), or act as photosensors (7). Despite the diverse chemical environments in which the sulfur atoms reside in cofactors and thionucleosides, it has been found that the incorporation of sulfur into them involves a few enzymes with the persulfide (R-S-SH) groups on the cysteine residues of these enzymes; thus, these enzymes act as the key agents of sulfur transfer (8, 9).

Protein persulfide groups are generated by a family of cysteine desulfurases; these use the cofactor pyridoxal 5′-phosphate to form a persulfide group on an active site cysteine residue by using the free amino acid l-cysteine and generate l-alanine as the second product (10). The terminal sulfur (formally S)<sup>6</sup> is transferred to the cysteine residues in acceptor proteins to form new persulfide groups that are subsequently used directly in the biosynthesis of cofactors or thionucleosides or transferred to other protein acceptors for the eventual incorporation of sulfur into an end product (8–10).

*Escherichia coli* contains the following three cysteine desulfurases: CsdA (also termed CSD), SufS (also termed CsdB), and IscS (10, 11). These are classified into 2 groups, I and II, based on differences in four sequence regions and the consensus sequence motif (12). Unlike IscS, which has the consensus group I sequence GSGSACTS around the active site Cys-328, CsdA and SufS possess consensus group II sequences. CsdA is encoded by the *csdAE* operon, which is presumed to function in the maturation of an FeS protein (13). The activity of CsdA is enhanced by CsdE via sulfur transfer from the persulfide group of the former to the cysteine residue of the latter (13). The *sufABCDSEL* operon containing the gene encoding SufS is specifically adapted to synthesize FeS clusters under conditions of iron starvation or oxidative stress (14, 15). The catalytic activity of SufS is significantly lower than that of CsdA and IscS (16), but it is enhanced by SufE through persulfide sulfur transfer; its catalytic activity is further enhanced by the SufBCD complex.

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Three cysteine desulfurases (CsdA, SufS, and IscS) transfer the sulfur atom from L-cysteine to the C-terminal thiocarboxylate of MoaD oxidation product of precursor Z, i.e. compound Z, are shown. The incorporation of sulfur atoms into precursor Z is catalyzed by MPT synthase (MoaE/MoaD). Three cysteine desulfurases (CsdA, SufS, and IscS) transfer the sulfur atom from L-cysteine to the C-terminal thiocarboxylate of MoaD in vitro (36, 41).

In the present study, we have investigated the functional and physical interactions of three cysteine desulfurases with MoeB and MoaD. Each of three cysteine desulfurases could catalyze the transfer of $^{35}$S from [35S]cysteine to MoaD in the presence of MoeB and Mg-ATP. However, only IscS was activated by MoeB. MoaD further enhanced the IscS activity in the presence of MoeB. Surface plasmon resonance studies further revealed that IscS specifically binds to MoeB and MoaD. In contrast, neither CsdA nor SufS interacted with MoeB or MoaD. We also show that the sulfuration level of MoaD isolated from strains defective in IscS exhibits a large decrease in contrast to that of MoaD expressed in a csdA/sufS deletion strain. In accord with this result, a genetic study exhibits that an $\Delta$iscS strain, but not the $\Delta$csdA and $\Delta$sufS strains, accumulates compound Z. Based on the results presented here, we propose that IscS is the primary physiological sulfur-donating enzyme in the conversion of precursor Z to MPT.

EXPERIMENTAL PROCEDURES

Bacteria, Plasmids, and Growth Conditions—The bacterial strains and plasmids used in this study are listed in Table 1. E. coli cells were grown aerobically at 37 °C in a Luria-Bertani (LB) medium unless otherwise noted.

Construction of Plasmids—A DNA fragment containing the iscS gene and a Shine-Dalgarno sequence was cleaved from the XbaI and HindIII sites of pEF1 (11) and inserted into the XbaI and HindIII sites of pUC118 to yield plscS. The DNA fragment containing moaD was PCR-amplified from the chromosomal DNA of E. coli K-12 MG1655 with specific primers (5'-GGGAATTCCATATGCGGAACTCAGCG-3' and 5'-CGCGGATCTTTAACCCTCGGTTACCGGCG-5') and cloned into the Ndel and BamHI sites of pET14b to give a plasmid, pMOAD. For the cloning of moeb, a PCR product obtained with primers 5'-GGGAATTCCATATGGCGGAACTCAGCGATCACG-3' and 5'-GGCGGATCTTTAACCCTCGGTTACCGGCG-5' was ligated into pET14b to yield a plasmid, pMOEB.
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**TABLE 1**

| Strains       | Genotype         | Source               |
|---------------|------------------|----------------------|
| BW25113       | lacI, rnmBT14, ΔlacZWJ16, hsdR514, ΔsarABADAH33, ΔsarBADLD78 | NBRP* collection    |
| ΔcsdA         | BW25113 ΔcsdA    | NBRP* collection     |
| ΔsusF         | BW25113 ΔsusF    | NBRP* collection     |
| ΔiscS         | BW25113 ΔiscS    | NBRP* collection     |
| ΔmoaD         | BW25113 ΔmoaD    | NBRP* collection     |
| MC1061        | FecA-D139 Δ(araI-leu17696 Δ(lacY74) galll galk hsdR hsdM + strA | (23)                 |
| CL100         | MC1061 ΔiscS    | (23)                 |
| CL102         | MC1061 ΔcsdA/ΔsusF | (26)               |
| Plasmids      | Property         | Source               |
| pUC118        | lac promoter-based expression vector | Takara Shuzo        |
| pET1Hb        | T7 RNA polymerase-based expression vector | Novagen             |
| pCSD1         | csaA gene cloned into EcoRI and PstI sites of pUC118 | (12)                |
| pCSDB         | susF gene cloned into EcoRI and HindIII sites of pUC118 | (16)               |
| pEF1          | iscs gene cloned into Ndel and HindIII sites of pET21a | (11)                |
| psc5          | An Xbal-HindIII iscs fragment of pEF1 was ligated into pUC118 | This work         |
| pMoaD         | moaD gene cloned into Ndel and BamHI sites of pET14b | This work         |
| pMoeB         | moeb gene cloned into Ndel and BamHI sites of pET14b | This work         |
| pMW15aD       | moaD gene cloned into Ncol and BamHI sites of pET15b | (35)               |

*National BioResource Project (NIG, Japan): E. coli.

Expression and Purification of Proteins—pCSD1 (12) and pCSDB (16) were introduced into E. coli JM109 to give CsdA- and SusF-overexpressing strains, respectively. E. coli BL21(DE3)/pLysS carrying pEF1 and E. coli BL21(DE3) carrying pMOAD and pMOEB were used for the expression of IscS, MoaD, and MoeB, respectively. Cells were cultured in LB with 100 mg/liter of ampicillin at 26 °C for 5 h. After induction by 1 mM isopropyl-β-d-thiogalactopyranoside for 6 h, the cells were harvested by centrifugation, disrupted by sonication, and purified by column chromatography as detailed in supplemental Experimental Procedures. For the quantification of thiocarboxylated MoaD in different E. coli strains, MoaD was expressed from pMW15aD (35) in the DE3 lysogens of E. coli strains MC1061 (23), CL100 (iscS deletion mutant) (23), and CL102 (csdA/ΔsusF deletion double mutant) (26). Strains were grown in an LB medium supplemented with 100 mM isopropyl-β-d-thiogalactopyranoside and 150 mg/liter ampicillin at 30 °C to an A460 = 0.8 and harvested by centrifugation at 5,000 × g. MoaD was purified as described previously (34).

35S Transfer Assay—All the experiments were performed as described by Loiseau et al. (13) with modifications. Under anaerobic conditions, purified proteins were pretreated with 10 mM dithiothreitol and then repurified by gel filtration before anaerobic conditions, purified proteins were pretreated with 10 mM dithiothreitol and then repurified by gel filtration before 35S transfer from cysteine as described previously (22). A reaction mixture containing 1 mM l-cysteine, 10 mM PLP, 10 mM MgCl₂, 1 mM ATP, 5 mM dithiothreitol, and the enzyme in a 50 mM Tris-HCl buffer, pH 8.0, was incubated at 30 °C.

Surface Plasmon Resonance Analysis—Surface plasmon resonance analysis was carried out with BIACORE X (Biacore AB, Uppsala, Sweden). MoaD or MoeB in a 10 mM acetate buffer (pH 6.0) was immobilized on a sensor chip NTA (Biacore AB). The chip was blocked with a purified N-terminal His₆-tagged protein 2,4-diaminopentanoate dehydrogenase (37) from Thermoanaerobacter ethanolicus. The flow rate was 20 μl/min, and the sensor chip was regenerated with 350 mM EDTA and 500 mM NiCl₂.

Analysis of the Sulfuration Level of MoaD from Different E. coli Strains—75 μM purified MoaD from different E. coli strains, MC1061, CL100, and CL102, 75 μM MoeB (34), and precursor Z (38) in excess amounts were incubated for 30 min in 400 μl of 100 mM Tris, pH 7.2. The MPT produced was oxidized to Form A and quantified following published procedures (36, 39, 40).

Preparation of Acid Extracts of E. coli Cells—E. coli cells were cultured in an LB medium for 18 h at 37 °C with vigorous shaking and harvested by centrifugation. The cell pellets were washed twice with 30 ml of water (H₂O), suspended in 3 ml of H₂O/g wet weight, and frozen at −30 °C. Acid extracts of the cells were prepared by essentially following the procedure proposed by Wuebbens and Rajagopalan (38). The extracts were concentrated to 900 μl by rotary evaporation and centrifuged to remove the precipitated protein. A portion of the supernatant (12.5–25 μl) was analyzed using high-performance liquid chromatography (HPLC) following air oxidation, which enabled the conversion of precursor Z to compound Z.

HPLC Analysis—All HPLC analyses were performed at 25 °C using a Capcell Pak C₁₈ SG120 column (4.6 × 250 mm, Shiseido, Japan) and a Shimadzu HPLC system (Shimadzu, Japan). The column was equilibrated with 5 mM ammonium acetate (pH 5.0) with a flow rate of 1.0 ml/min. Eluents were monitored for absorption at 300 nm.

RESULTS AND DISCUSSION

35S Transfer from l-Cysteine to the MoaD Thiocarboxylate Catalyzed by Cysteine Desulfurases—We examined the relative abilities of CsdA, SusF, and IscS to transfer sulfur from l-cys-
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Evidence for the Specific Interaction of IscS with MoeB and MoaD—An intrinsic function of a protein \textit{in vivo} is highly dependent on its specific interactions with other proteins. In fact, the activation of a cysteine desulfurase by a partner protein is coincident with the specific interaction between them (13, 18, 42, 46, 47). To investigate whether the observed activation of IscS by MoeB and MoaD involves specific physical protein-protein interactions, we conducted surface plasmon resonance measurements. MoeB or MoaD was immobilized on a sensor chip, while CsdA, SufS, or IscS was added to a solution as the analyte. For both MoeB and MoaD, the response unit was the amount of analyte that bound to the protein interactions, we conducted surface plasmon resonance measurements. The specific activities of purified CsdA, SufS, and IscS were 0.2 units/mg, 0.01 units/mg, and 0.24 units/mg, respectively. The addition of MoeB increased the desulfurase activity of IscS (1.6-fold in the presence of 10-fold molar excess of MoeB) but had no significant effect on the activity of CsdA and SufS (Fig. 3). However, the addition of MoaD to CsdA, SufS, and IscS had little effect on the desulfurase activity. Therefore, the addition of MoaD to a mixture of IscS and MoeB detectably enhanced the desulfurase activity to levels much higher than those observed for the addition of MoeB alone to IscS (Fig. 3C). The enhancement of desulfurase activity by MoaD in the presence of MoeB is specific for IscS, as the addition of a C142A/C187A mutant of MoeB had only a slight effect on their activities (Fig. 3, A and B). The activation of IscS by MoeB and further enhancement of the activity by MoaD resembles the activation of SufS by SufE and the additional enhancement of the Sufs-SufE complex by sulfur transfer reaction to active MPT synthase (41).

Activation of IscS by MoeB and MoaD—Previous studies have shown that the activity of cysteine desulfurases is enhanced by their physiological interacting partners, such as CsdE for CsdA (13), SufE-SufBCD for SufS (17, 18), and IscU (42), ThiI (43), and TusA (29) for IscS. The mechanism of the activation is not completely understood, but it is proposed that the binding by partner proteins probably induces a conformational change of the flexible loop carrying the persulfide-forming cysteine residue in cysteine desulfurase to facilitate persulfide formation and/or its subsequent cleavage (44, 45). Therefore, it seemed possible that MoeB and/or MoaD could alter the enzyme activity of a physiologically relevant cysteine desulfurase. To determine whether MoeB and MoaD affect the activity of three cysteine desulfurases, we measured the activity of CsdA, SufS, and IscS in the presence of MoeB and MoaD. The specific activities of purified CsdA, SufS, and IscS were 0.2 units/mg, 0.01 units/mg, and 0.24 units/mg, respectively. The addition of MoeB increased the desulfurase activity of IscS (1.6-fold in the presence of 10-fold molar excess of MoeB) but had no significant effect on the activity of CsdA and SufS (Fig. 3). However, the addition of MoaD to CsdA, SufS, and IscS had little effect on the desulfurase activity. Therefore, the addition of MoaD to a mixture of IscS and MoeB detectably enhanced the desulfurase activity to levels much higher than those observed for the addition of MoeB alone to IscS (Fig. 3C). The enhancement of desulfurase activity by MoaD in the presence of MoeB is specific for IscS, as the addition of MoaD together with MoeB to CsdA and SufS had only a slight effect on their activities (Fig. 3, A and B). The activation of IscS by MoeB and further enhancement of the activity by MoaD resembles the activation of SufS by SufE and the additional enhancement of the Sufs-SufE complex by sulfur transfer reaction to active MPT synthase (41).

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and CL102 strains was incubated with purified MoaE and precursor Z. Produced MPT was quantified by a conversion to Form A. As shown in Fig. 5, a largely reduced level of MPT formation was detected (quantified as Form A) when MoaD was purified from the \textit{iscS}-deficient strain CL100, whereas MoaD isolated from the \textit{csdA/sufS} deletion mutant strain CL102 showed the same activity as the corresponding wild-type strain. The amount of about 1% of activity of MoaD purified...
from the CL100 strain is consistent with previous results, which show that the CL100 strain contained only a level of 10% Moco of wild-type strain (36). This result strongly suggests that IscS is involved in the sulfuration of MoaD. Because no effect on the sulfuration level of MoaD was obtained using the CL102 strain, CsdA and SufS are not involved in Moco biosynthesis in vivo.

Accumulation of Compound Z in the ΔiscS Strain—Previously, Leimkühler and Rajagopalan (36) showed that precursor Z is not accumulated in the ΔiscS strain; however, the crude extract of the ΔiscS strain was able to convert externally added precursor Z to MPT to the same level as the parental strain. Accordingly, they suggested that the low level of MPT in an ΔiscS strain is due to the impaired activity of the Fe-S protein MoaA involved in the synthesis of precursor Z, and, therefore, it could not be clearly determined whether IscS is required for the in vivo sulfuration of MPT synthase. Because the amount of Moco in the ΔiscS mutant was determined to be 10% of that in the wild-type strain, it was concluded that CsdA, SufS, or other sulfur transferases in the cells are able to compensate to some extent for the function of IscS in the Fe-S cluster formation of MoaA and the conversion of precursor Z to MPT. Therefore, the amount of precursor Z in an ΔiscS strain seemed to differ depending on the balance of CsdA, SufS, or other sulfur transferases available for Fe-S cluster synthesis and for the sulfuration of MoaD.

To test this possibility, we examined whether compound Z is accumulated in E. coli strains deficient in csdA, sufS, or iscS. An earlier study demonstrated that the accumulated compound Z could be released by direct acidification of whole-cell suspensions of appropriate E. coli MPT-deficient mutants, such as the ΔmoaD and ΔmoeB strains (33). HPLC analysis of the acid extracts of E. coli cells revealed that the peak absorbing at 300 nm, which eluted at 5.75 min, was observed in the ΔiscS strain (Fig. 6A); the ΔmoaD strain also accumulated compounds absorbing at 300 nm with the same retention time as the ΔiscS strain (Fig. 6B). In contrast, such accumulation was not observed in the ΔcsdA and ΔsufS strains as well as in the wild-type strain (Fig. 6A). HPLC/ESI-MS, ESI-MS/MS, and UV-Vis absorption analyses clearly identified the accumulated 300 nm absorbing material to be compound Z (supplemental Fig. S2, S3, and S4). A genetic complementation analysis of the ΔiscS strain by the expression of IscS further confirmed that the accumulation of compound Z is specifically caused by the deletion of the iscS gene (supplemental Fig. S5). These results indicate that MoaA is active in the strain. CsdA, SufS, or other sulfur transferases presumably support the assembly of the FeS cluster in MoaA present in the ΔiscS strain. However, the contributions of the three cysteine desulfurases for it are still unclear and will be interesting to study in the future.

The studies presented here identify the protein involved in the sulfur transfer for the formation of the diithiolene moiety of Moco in E. coli. Earlier work demonstrated that any of the three NifS-like sulfur transferases, namely IscS, CsdA, or SufS, is capable of mobilizing and transferring sulfur from l-cysteine to MoaD in vitro (36). The minimal requirements for the activation of inactive MPT synthase in vitro were shown to be MoeB, Mg-ATP, l-cysteine, and a sulfur transferase. Thus, the specific protein involved in sulfur transfer to MoaD remained unknown in E. coli.

Using different approaches, we show the IscS is the specific cysteine desulfurase transferring the sulfur to the MoaD protein.

It was recently shown that, in humans, the cysteine desulfurase Nfs1 acts as direct sulfur donor for MOCS3 in the cytosol (49). Thus, in humans and E. coli, the same protein components participate in the sulfur transfer pathway to MPT synthase during Moco biosynthesis: l-cysteine is the direct sulfur donor for a cysteine desulfurase, which transfers the sulfur to the adenylated small subunit of MPT synthase. In humans, this sulfur transfer occurs with the aid of the C-terminal rhodanese-like domain of MOCS3. For this additional sulfur transfer step, there must have been an evolutionary advantage for eukaryotes, which, likely, made this reaction more specific.

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REFERENCES
1. Kluger, R. (1987) Chem. Rev. 87, 863–876
2. Knowles, J. R. (1989) Annu. Rev. Biochem. 58, 195–221
3. Hille, R., Rétyé, J., Bartlewski-Hof, U., and Reichenbecher, W. (1998)
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