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The SufE Protein and the SufBCD Complex Enhance SufS Cysteine Desulfurase Activity as Part of a Sulfur Transfer Pathway for Fe-S Cluster Assembly in *Escherichia coli**

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F. Wayne Outten, Matthew J. Wood, F. Michael Muñoz, and Gisela Storz‡

From the Cell Biology and Metabolism Branch, NICHD, National Institutes of Health, Bethesda, Maryland 20892

The *sufABCDSE* operon of the Gram-negative bacterium *Escherichia coli* is induced by oxidative stress and iron deprivation. To examine the biochemical roles of the Suf proteins, we purified all of the proteins and assayed their effect on SufS cysteine desulfurase activity. Here we report that the SufE protein can stimulate the cysteine desulfurase activity of the SufS enzyme up to 8-fold and accepts sulfane sulfur from SufS. This sulfur transfer process from SufS to SufE is sheltered from the environment based on its resistance to added reductants and on the analysis of available crystal structures of the proteins. We also found that the SufB, SufC, and SufD proteins associate in a stable complex and that, in the presence of SufE, the SufBCD complex further stimulates SufS activity up to 32-fold. Thus, the SufE protein and the SufBCD complex act synergistically to modulate the cysteine desulfurase activity of SufS. We propose that this sulfur transfer mechanism may be important for limiting sulfide release during oxidative stress conditions *in vivo*.

Numerous processes within the cell require the mobilization of elemental sulfur from L-cysteine. These processes include Fe-S cluster assembly as well as the synthesis of molybdopterin, thiamine, biotin, and thionucleosides in tRNA (1). Often sulfur mobilization occurs via a cysteine desulfurase enzyme that converts L-cysteine to sulfane sulfur and L-alanine in a process that requires pyridoxal 5′-phosphate as a cofactor (2). The sulfane sulfur, present as a persulfide intermediate on the active site cysteine of the desulfurase, is then transferred to various sulfur acceptors depending on the physiological pathway. One of the earliest identified cysteine desulfurases is the NifS protein of *Azotobacter vinelandii*, which is involved in Fe-S assembly in the nitrogenase enzyme (2). *A. vinelandii* also contains another NifS homologue, IscS, which is involved in Fe-S cluster assembly in enzymes other than nitrogenase (3).

The Gram-negative bacterium *Escherichia coli* contains three NifS homologues, IscS, CsdA (also known as CSD), and SufS (also known as CsdB). All three *E. coli* enzymes have been purified and shown to exhibit cysteine desulfurase activity (4–7). The three NifS homologues are present at separate loci and are co-expressed with different accessory proteins. The question arises as to why three NifS homologues are present in *E. coli*. Possibly they carry out divergent functions within the cell or are regulated differentially to provide similar functions under different conditions. One way to examine the functions of the three NifS homologues is to characterize the activities of the accessory proteins co-expressed with each homologue.

In *E. coli*, IscS is part of an operon that includes IscR, IscU, and IscA. The IscR transcriptional repressor regulates the *isc* operon by sensing changes in Fe-S cluster assembly status (8). IscU is a Fe-S assembly scaffold protein used to construct nascent Fe-S clusters (9). A conserved cysteine residue on IscU accepts sulfur from IscS during Fe-S cluster building (10–12). IscA also can form Fe-S clusters and may function as an alternate scaffold (13, 14). Downstream of the *isc* operon is the *hscB-hscA-fdx* operon. Both *hscA* and *hscB* encode molecular chaperone proteins while *fdx* encodes a ferredoxin, and all three proteins play a part in *isc*-mediated Fe-S cluster assembly (15–18). In some organisms, the *hscB-hscA-fdx* operon is co-expressed with *isc* but in *E. coli* the two operons are regulated separately (3, 19, 20). *In vivo* the *isc* operon together with the *hscB-hscA-fdx* genes have been shown to be important for the assembly of a variety of Fe-S enzymes (18, 21). The second *E. coli* NifS homologue, CsdA, is encoded adjacent to the *ygdK* gene, which encodes a homologue of SufE. Little is known about the *in vivo* role of CsdA. CsdA is the most efficient of the three *E. coli* NifS homologues at providing sulfur to MoaD for synthesis of molybdopterin *in vitro* (22) and may be involved in molybdopterin synthesis *in vivo*.

The SufS cysteine desulfurase is co-expressed with five additional gene products, SufA, SufB, SufC, SufD, and SufE (23). Besides its cysteine desulfurase activity, SufS has also been shown to exhibit a strong selenocysteine lyase activity *in vitro* (5), although SufS does not appear to be important for selenium metabolism *in vivo* (24). SufA is a homologue of IscA and can form Fe-S clusters *in vitro* (25). SufC has the sequence hallmarks of the ATPase subunit of ABC transporters and has been shown to exhibit ATPase activity (26). The biochemical functions of SufB, SufD, and SufE are unknown, although fluorescence anisotropy and yeast two-hybrid assays indicate that SufB and SufD interact with SufC (26, 27). Most of the *suf* genes are well conserved in a variety of microorganisms, including cyanobacteria, as well as in higher plants (28, 29).

Previous experiments are consistent with a role for the Suf proteins in Fe-S assembly. In *E. coli*, mutations in *sufS* or *sufD* result in the loss of a Fe-S cluster in the FhuF iron reductase (30), thereby preventing the use of ferrioxamine B as a sole iron source. Similarly, mutations in SufB, SufC, or SufD impair

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[S] The on-line version of this article (available at http://www.jbc.org) contains Supplemental Figs. 1–3.

‡ To whom correspondence should be addressed. Tel.: 301-402-0968; Fax: 301-402-0078; E-mail: storz@helix.nih.gov.

Erwinia chrysanthemi growth on ferric chrysobactin as a sole iron source, a phenotype that has been attributed to loss of an unidentified Fe-S enzyme (27). In *Arabidopsis thaliana*, disruption of a SufB homologue leads to accumulation of protoporphyrin IX, indicating an undefined role for SufB in chlorophyll biosynthesis in that organism (31). Most *suf* mutants are synthetically lethal with *isc* mutants in *E. coli*, indicating overlap in the roles of the two operons (29). *E. coli* deletion mutants of *suf* also show decreased growth and loss of some Fe-S enzyme activity under oxidative stress conditions (23, 27). In addition, transcription of the *E. coli suf* operon is induced by oxidative stress through the OxyR hydrogen peroxide sensor (19) and by iron starvation through loss of repression by Fur (30, 32).

To learn more about the roles of the individual Suf proteins, we have purified the *suf* gene products and determined that SufE stimulates the activity of the SufS cysteine desulfurase. In addition, the SufB, SufC, and SufD proteins interact in a stable complex and can further increase the desulfurase activity of SufS in a SufE-dependent manner. These results indicate that interactions with accessory proteins can enhance the activity of NifS homologues above that observed with the cysteine desulfurase alone. We propose that regulated sulfur transfer conferred by SufE and SufBCD may be important under iron-limited and oxidative stress conditions.

EXPERIMENTAL PROCEDURES

Strains and Media—$(His)_6$-SufE and SufABCDSE were expressed in TOP10 (Invitrogen), and $(His)_6$-SufA and $(His)_6$-SufS were expressed in BL21(DE3)plysS (Invitrogen). All of the bacterial growth was in Lennox Broth (10 g of tryptone, 5 g of yeast extract, 5 g of NaCl/liter). Ampicillin was used at 100 mg/liter, and chloramphenicol was used at 30 mg/liter. L-Arabinose was added to 0.2% final concentration by weight, and isopropyl-1-thio-β-D-galactopyranoside was added to 1 mM final concentration. All of the chemicals used were obtained from Sigma unless indicated otherwise.

Plasmid Construction—All of the PCR reactions were carried out using MG1655 chromosomal DNA as template. SufABCDSE was PCR-amplified using two primers (5′-GAGGTAAATCGATGGATCCGCATTCAGGAAC-3′ and 5′-GTTCACCTGAATTCAAAACACTCCTGTGC-3′). The *Eco*RI-digested PCR fragment was ligated into the *Nco*I (blunt-ended with Klenow fragment) and *Eco*RI sites of pBADmychisC (Invitrogen) to generate pGSO164. SufE was amplified using two primers (5′-GAGGCACCATGGCTTTATTGCCGGATAA-3′ and 5′-CCTTTTAGTTTAGCTGAATTCAGCGGCTTTG-3′), digested with *Nco*I and *Eco*RI, and cloned into the corresponding sites of pBADmychisC to generate pGSO165. SufS was amplified using two primers (5′-GGAGGTGCAAGATGAGATCTTCCGTCGACAAAGT-3′ and 5′-CCATAGTGAATTCCTGTTATCCCAGCAAACGG-3′), digested with *Bgl*II and *Eco*RI, and cloned into the corresponding sites of pRSETB (Invitrogen) to generate pGSO166. SufA was PCR-amplified using two primers (5′-GTTGCTTCAGAATTCCGAGACATAGTACCGCC-3′ and 5′-GAGGTAAATCGATGGATCCGCATTCAGGAAC-3′), digested with *Eco*RI and *Bam*HI, and cloned into the corresponding sites of pRSETB to generate pGSO167. Plasmids were designed such that $(His)_6$-SufA and $(His)_6$-SufS were fused with a $(His)_6$-tag at their N termini while $(His)_6$-SufE was fused with a Myc and $(His)_6$-tag at the C terminus. Mutation of SufE Cys^{51} to Ser was performed using the pGSO165 construct, two mutagenic primers (5′-CAAAATAGCATTCAGGGCAGCCAGAGTCAGGTGTGG-3′ and 5′-CCACACCTGACTCTGGCTGCCCTGAATGCTATTTTG-3′), and the QuikChange site-directed Mutagenesis kit (Stratagene) according to the manufacturer's protocol to generate pGSO168. The nucleotide sequences of all of the plasmid inserts were confirmed.

Protein Expression and Purification—Strains carrying the $(His)_6$ expression constructs were induced by isopropyl-1-thio-β-D-galactopyranoside ($(His)_6$-SufA and $(His)_6$-SufS) or L-arabinose ($(His)_6$-SufE) when the cultures obtained an A_{600} of 0.4–0.6. After 3 h of expression at 37 °C, cells were harvested by centrifugation. All of the cells were lysed in 50 mM NaH_2PO_4, pH 8.0, 300 mM NaCl, 5 mM imidazole, 1 mM phenylmethylsulfonyl fluoride, 1× EDTA-free protease inhibitor tablet (Roche Applied Science) via sonication ($(His)_6$-SufA and $(His)_6$-SufS)) or with a high pressure cell disrupter (Constant Systems LTD) at 10,000 p.s.i. ($(His)_6$-SufE). Following centrifugation at 20,000 × *g* for 30 min, cleared lysate was loaded on a nickel-nitrilotriacetic acid Superflow (Qiagen) column on a fast protein liquid chromatography system (Amersham Biosciences) and eluted with a step gradient of 50 mM NaH_2PO_4, pH 8.0, 300 mM NaCl, 250 mM imidazole. $(His)_6$-SufA and $(His)_6$-SufS were dialyzed against 25 mM Tris-Cl, 100 mM NaCl, pH 7.4, concentrated, and stored at −70 °C. Purified $(His)_6$-SufE was dialyzed against 25 mM Tris-Cl, 100 mM NaCl, pH 7.4, and then was digested with trypsin (1:400 ratio by weight of trypsin to $(His)_6$-SufE). The digested SufE lacked the Myc and $(His)_6$ tag but contained the full sequence of the native protein, as confirmed by matrix-assisted laser desorption ionization time-of-flight mass spectrometry and N-terminal amino acid sequencing. The digested SufE was treated with 10 mM DTT,[1] further purified on a Mono Q column (Amersham Biosciences), concentrated, and stored at −70 °C. $SufE^{C51S}$ was purified as described for SufE. Similar yields were obtained for the $SufE^{C51S}$ mutant protein as compared with native SufE, and it was as resistant to proteolysis as the native SufE protein, indicating that it was correctly folded.

The entire *suf* operon was expressed simultaneously to purify SufBCD. Cells expressing the entire *suf* operon induced by the addition of L-arabinose at A_{600} of 0.4–0.6 turned grayish-black after the 3-h expression period, possibly because of the accumulation of iron sulfides within the cell. Cell pellets from the SufABCDSE expression were lysed in 25 mM Tris-Cl, pH 8.0, 50 mM NaCl, 10 mM β-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride, 1× EDTA-free protease inhibitor tablet using a high pressure cell disrupter at 10,000 p.s.i. Following centrifugation at 20,000 × *g* for 30 min, cleared lysate was loaded onto a Mono Q column and eluted with a linear gradient of 1 M NaCl. Fractions containing SufBCD were pooled, dialyzed overnight in 25 mM Tris-Cl, pH 7.4, 150 mM NaCl, and concentrated. The concentrated fractions then were separated on a Superdex 200 gel filtration column (Amersham Biosciences). Purified SufBCD was concentrated and stored at −70 °C. Purified IscS and NifS from *A. vinelandii* were kindly provided by D. R. Dean.

Size Determination—A Superdex 200 gel-filtration column was used to determine size by gel-filtration chromatography. Concentrated protein samples in 50 mM NaH_2PO_4, pH 7.0, 150 mM NaCl were loaded onto the column in 1-ml volumes to minimize dilution effects. Molecular weights were calculated by plotting log molecular weight *versus* the ratio of the elution volume/void volume for the standard proteins. Sizes were also determined by dynamic light scattering (DLS) using a DynaPro instrument (Protein Solutions) and Dynamics software.

Cysteine Desulfurase Activity Assays—Total sulfide was measured by a previously published protocol (11). Reactions were carried out anaerobically in 25 mM Tris-Cl, pH 7.4, 100 mM NaCl using 500 nM cysteine desulfurase ($(His)_6$-SufS, $IscS_{A.\ vinelandii}$, or $NifS_{A.\ vinelandii}$) and varying ratios of $(His)_6$-SufA, SufBCD, SufE, and/or BSA. A molecular mass of 165 kDa (as measured by DLS) was used to calculate molar concentrations of the SufBCD complex. Pyridoxal 5′-phosphate was added to 10 μM, and reactions were initiated by dilution of a 10 mM L-cysteine/10 mM DTT stock to a 100 μM final concentration of each in a total reaction volume of 800 μl. Reactions were allowed to proceed for 20 min at 27 °C and then were quenched by the addition of 100 μl of 20 mM *N*,*N*-dimethyl-*p*-phenylenediamine in 7.2 M HCl. The addition of 100 μl of 30 mM $FeCl_3$ in 1.2 M HCl and incubation for 20 min led to the formation of methylene blue. Precipitated protein was removed by 30-s centrifugation at 20,000 × *g*, and methylene blue was measured at 670 nm. Na_2S was used as a standard for calibration.

Sulfur Transfer Assays—Reactions consisted of 1 μM $(His)_6$-SufS mixed with 20 μM $(His)_6$-SufA, SufE, SufBCD, and/or BSA in 25 mM Tris-Cl, pH 7.4, 100 mM NaCl, 100 μM DTT. Reactions were initiated by the addition of L-[^{35}S]cysteine (150 mCi/mmol, Amersham Biosciences) to a final concentration of 30 μM. The final reaction volume was 35 μl. After a 30-s incubation, samples were loaded onto preequilibrated G50 ProbeQuant columns (Amersham Biosciences) and spun for 2 min at 1700 × *g*. Samples were separated on a non-reducing 10–20% Tris-glycine gel, dried onto filter paper, and exposed overnight to a phosphorimaging screen or film. Control transfer reactions without DTT showed no increase in labeling as compared with samples with DTT.

RESULTS

Purification of the Suf Proteins—The SufS cysteine desulfurase and its accessory proteins, SufA, SufB, SufC, SufD, and SufE, were purified to characterize their biochemical functions

[1] The abbreviations used are: DTT, dithiothreitol; BSA, bovine serum albumin; CD, circular dichroism; DLS, dynamic light scattering.

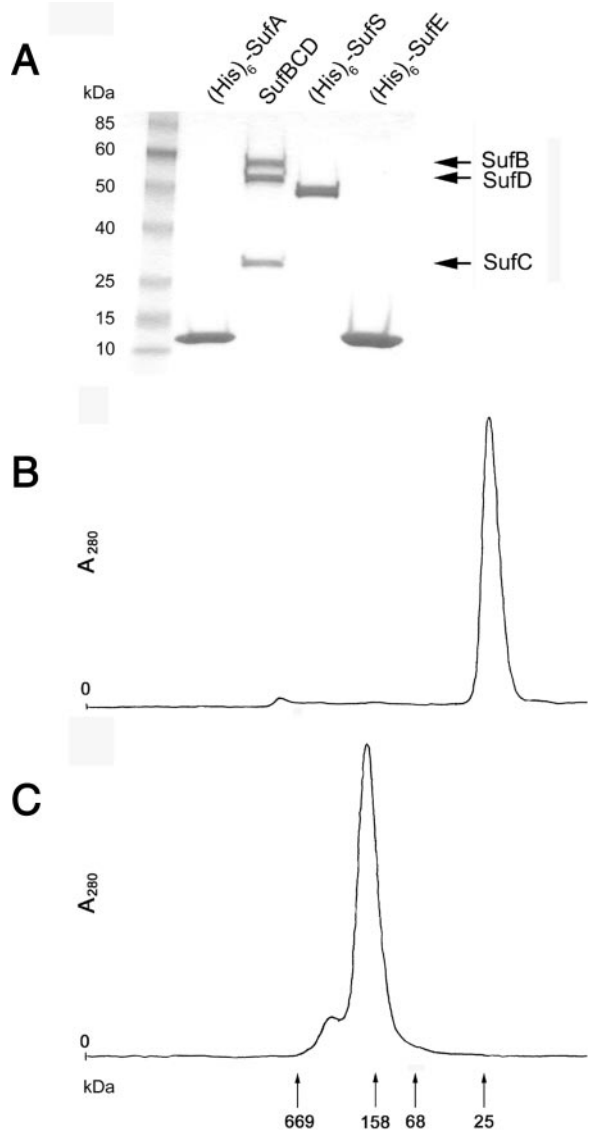

FIG. 1. **Purification of the Suf proteins.** *A*, purified $(His)_6$-SufA, SufB, SufC, SufD, $(His)_6$-SufS, and $(His)_6$-SufE proteins. Samples were separated on a 10–20% Tris-glycine gel. SufBCD are shown in the same lane based on their purification as a stable complex from a construct that expresses the entire *suf* operon. *B*, elution profile of SufE on a Superdex 200 gel-filtration column. *C*, elution profile of the SufBCD complex on a Superdex 200 gel-filtration column. Molecular masses were determined based on calibration with the following standards: thyroglobulin (670 kDa), aldolase (158 kDa), BSA (67 kDa), and carbonic anhydrase (25 kDa).

(Fig. 1*A*). $(His)_6$-SufA, $(His)_6$-SufS, and SufE were expressed and purified separately. $(His)_6$-SufS was purified as a dimer with an absorption maximum at 420 nm as has been reported for native SufS (5). SufE was expressed as a Myc and $(His)_6$ fusion, but the tag was later removed by limited proteolysis. The molecular weight of SufE was analyzed using two different techniques. SufE eluted from a gel-filtration column as a single peak at a molecular mass of 19.8 kDa (Fig. 1*B*). This molecular mass is between the predicted molecular masses for a SufE monomer (15.8 kDa) and dimer (31.6 kDa). However, a monodispersed species with a molecular mass of 32 kDa was detected using DLS, indicating that SufE can exist in a homodimeric form. These differences in SufE molecular mass and oligomerization probably reflect differences between the two techniques. The SufE protein eluted from the gel-filtration column in a very dilute peak; therefore, its molecular weight was measured at a concentration of ~0.1 mg/ml. In contrast, the molecular mass was measured by DLS using a SufE concentration of 4 mg/ml. These results suggest that SufE can form monomers at low protein concentrations and homodimers at higher protein concentrations.

The SufB, SufC, and SufD proteins were expressed simultaneously with all of the *suf* genes from a single expression construct. During isolation of Suf proteins from cells expressing the SufABCDSE construct, we found that SufB, SufC, and SufD always co-purified on both anion-exchange and gel-filtration resins (Fig. 1*A*). In addition, the SufBCD proteins co-purified during ammonium sulfate precipitation and on a variety of hydrophobic interaction resins. In fact, the individual SufB, SufC, and SufD proteins could only be completely separated from the complex using reverse phase high performance liquid chromatography, indicating tight association. This result is consistent with previous yeast two-hybrid (27) and fluorescence anisotropy (26) studies that suggest interactions between SufC and the SufB and SufD proteins. The SufBCD complex was in a folded conformation based on its CD spectra and also possessed ATPase activity (data not shown).

Analysis of the SufBCD complex using gel filtration resulted in a major species of 172 kDa (Fig. 1*C*). A similar monodispersed molecular mass of 165 kDa was obtained for the SufBCD complex by DLS. A small amount of SufBCD was also seen in a higher molecular mass peak of 324 kDa during gel filtration, which corresponds to a multimer of the complex. Several protein stoichiometries could match the 172-kDa molecular mass measured for the SufBCD complex, but on a denaturing gel the proteins appear to be present in equimolar amounts (Fig. 1*A*). Further experiments are necessary to elucidate the exact stoichiometry of the SufBCD complex. In this study, we simply refer to the 172-kDa complex of the SufB, SufC, and SufD proteins as SufBCD.

SufE Enhances SufS Cysteine Desulfurase Activity—Our purified $(His)_6$-SufS has a specific activity of 0.008 units/mg toward L-cysteine at 37 °C. This activity is slightly less (2–3-fold) than the 0.019 units/mg activity obtained for native SufS using a different assay that contained 500-fold higher levels of reductant and 120-fold higher levels of L-cysteine (5). In general, the *in vitro* cysteine desulfurase activity of isolated SufS is low compared with the activities of the isolated IscS or CsdA enzymes (6). However, all of the previous experiments were carried out with purified SufS in the absence of the other *suf* gene products. Because the addition of IscU to IscS enhances the desulfurase activity of IscS (12), it seemed possible that accessory proteins could alter the enzyme activity of SufS. To determine whether the SufA, SufBCD, or SufE proteins affect the cysteine desulfurase activity of SufS, we measured $(His)_6$-SufS activity in the presence of the accessory proteins. The addition of SufE increased the desulfurase activity of $(His)_6$-SufS by nearly an order of magnitude (8-fold) while the addition of $(His)_6$-SufA, SufBCD, or a nonspecific BSA control had little effect (Fig. 2*A*). Maximum enhancement was observed at a 6-fold molar excess of SufE (Fig. 2*A*), possibly reflecting a need for SufE in excess to fully promote $(His)_6$-SufS activity or indicating that some fraction of the SufE protein is not fully active. $(His)_6$-SufA, SufBCD, and SufE did not exhibit any measurable desulfurase activity in the absence of $(His)_6$-SufS (data not shown).

The SufBCD Complex Magnifies the SufE Enhancement of SufS Activity—To determine whether the other Suf proteins affect the SufE-dependent enhancement of $(His)_6$-SufS activity, we also measured desulfurase activity in the presence of various Suf protein combinations (Fig. 2*B*). Although the addition of $(His)_6$-SufA or BSA to a mixture of SufE and $(His)_6$-SufS had

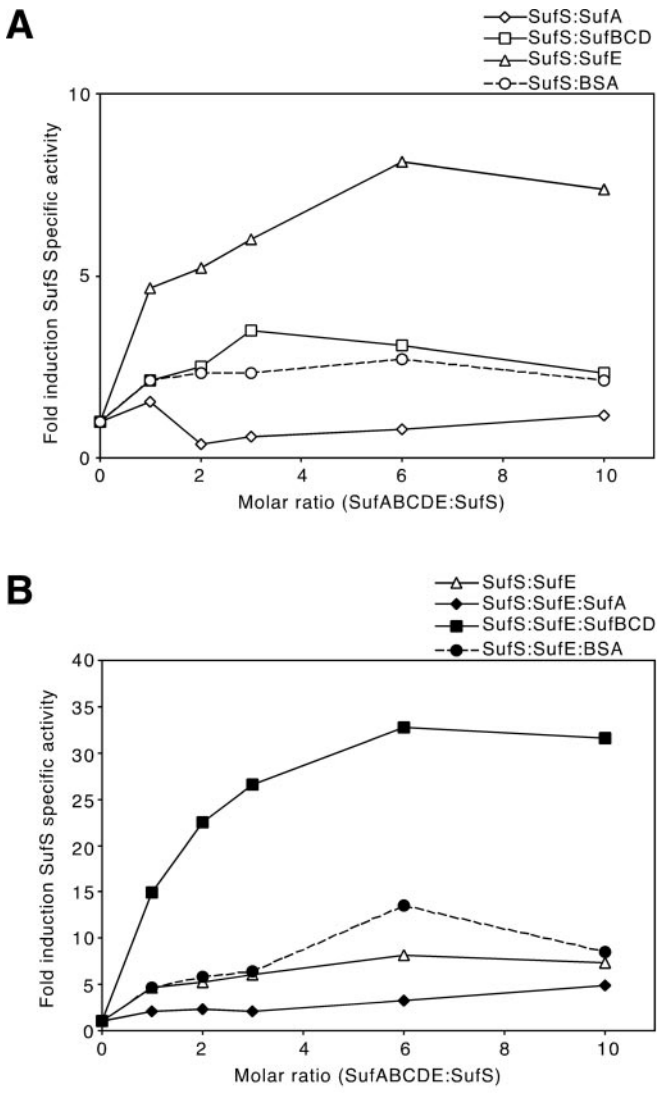

FIG. 2. **Enhancement of SufS cysteine desulfurase activity.** *A*, formation of sulfide from L-cysteine was measured after 20 min for samples containing a constant amount of $(His)_6$-SufS (500 nM) and increasing amounts of $(His)_6$-SufA, SufBCD, SufE, or BSA. *B*, formation of sulfide was measured for $(His)_6$-SufS (500 nM) combined with increasing amounts of SufE and $(His)_6$-SufA, SufBCD, or BSA. Specific activity is defined in units/mg where 1 unit catalyzes the formation of 1 μmol of product/min.

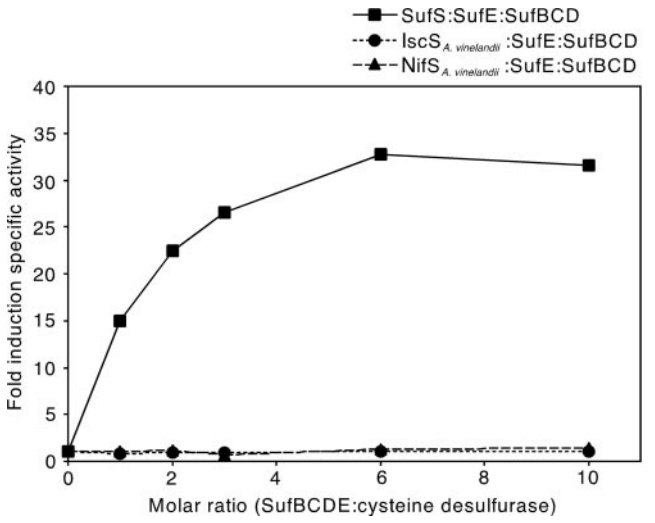

FIG. 3. **Enhancement by SufE and SufBCD is specific for SufS.** Formation of sulfide from L-cysteine by 500 nM *E. coli* $(His)_6$-SufS, $IscS_{A.\ vinelandii}$, or $NifS_{A.\ vinelandii}$ was measured after 20 min for desulfurase alone or with increasing molar ratios of *E. coli* SufE and SufBCD. Basal specific activities under these conditions were 0.0081 for IscS and 0.0112 for NifS.

no further effect on SufS activity, the addition of the SufBCD complex to SufE and $(His)_6$-SufS increased desulfurase activity from 8- to ~32-fold (Fig. 2*B*). The enhancement of desulfurase activity by SufE and SufBCD is specific for $(His)_6$-SufS as the addition of SufE and SufBCD to the NifS and IscS cysteine desulfurases from *A. vinelandii* had little effect on their activities (Fig. 3).

Cys^{51} Is Essential for SufE Function—It has been shown that IscS transfers sulfur to the Fe-S scaffold protein IscU via Cys^{63} on IscU and that this residue is required for IscU-dependent enhancement of IscS activity (12). An examination of the SufE amino acid sequence for conserved residues that might be involved in its function revealed the presence of a highly conserved cysteine at position 51 (Fig. 4*A*). This residue represents a candidate to accept sulfur mobilized from L-cysteine by $(His)_6$-SufS. We substituted this conserved cysteine with a serine, isolated the mutant protein, and assayed its effect on $(His)_6$-SufS desulfurase activity. As shown in Fig. 4*B*, the $SufE^{C51S}$ mutant lacks the ability to enhance $(His)_6$-SufS desulfurase activity. SufBCD enhancement of $(His)_6$-SufS activity also was abolished in the presence of $SufE^{C51S}$, further highlighting the importance of the Cys^{51} residue for SufE function (Fig. 4*B*).

SufE Functions as a Sulfur Acceptor Protein—Because SufE and SufBCD enhancement of $(His)_6$-SufS activity could involve specific transfer of sulfane sulfur to one of these proteins, we tested the ability of SufS to transfer sulfur to the Suf accessory proteins. We observed clear mobilization of ^{35}S from L-[^{35}S]cysteine to SufE via $(His)_6$-SufS (Fig. 5*A*). In contrast, ^{35}S labeling of $(His)_6$-SufA by $(His)_6$-SufS was essentially the same as that observed with the control protein BSA, indicating little to no specific transfer to this protein (Fig. 5*A*). Although there was more labeling of the SufBCD complex than $(His)_6$-SufA, the labeling was substantially less intense than the labeling of SufE, suggesting that there is no specific sulfur transfer between $(His)_6$-SufS and SufBCD. When SufBCD is added to $(His)_6$-SufS in the presence of SufE, we observed a diminished level of SufE monomer labeling and more high molecular weight labeling (Supplemental Fig. 1*A*). The high molecular weight species result from increased disulfide bonding among $(His)_6$-SufS, SufE, and SufB that occurs when the proteins are mixed in the presence of L-cysteine (Supplemental Fig. 1*B*).

The $SufE^{C51S}$ mutant is not labeled by SufS, indicating that this residue functions as the acceptor site for sulfur transfer to SufE (Fig. 5*A*). To determine whether sulfur transfer to SufE is mediated specifically by $(His)_6$-SufS or whether SufE can accept mobilized sulfur from other desulfurases, we repeated the transfer assay with IscS and NifS from *A. vinelandii*. The transfer of ^{35}S to SufE was reduced to background levels if the NifS or IscS cysteine desulfurases from *A. vinelandii* were used in place of SufS, indicating that sulfur transfer between SufS and SufE is highly specific (Fig. 5*B*).

A covalent SufE dimer also was observed in Fig. 5. This dimer could be the result of a sulfane sulfur linkage between two SufE monomers during the sulfur transfer reaction. When the transfer reaction was trapped using trichloroacetic acid precipitation and exposed cysteines were capped with iodoacetamide to avoid formation of nonspecific disulfides, we also observed a specific covalent complex between SufS and SufE on non-reducing gels (Supplemental Fig. 2). These interactions were disrupted if 250 mM β-mercaptoethanol was added, indicating that the SufE-SufE and SufS-SufE interactions are

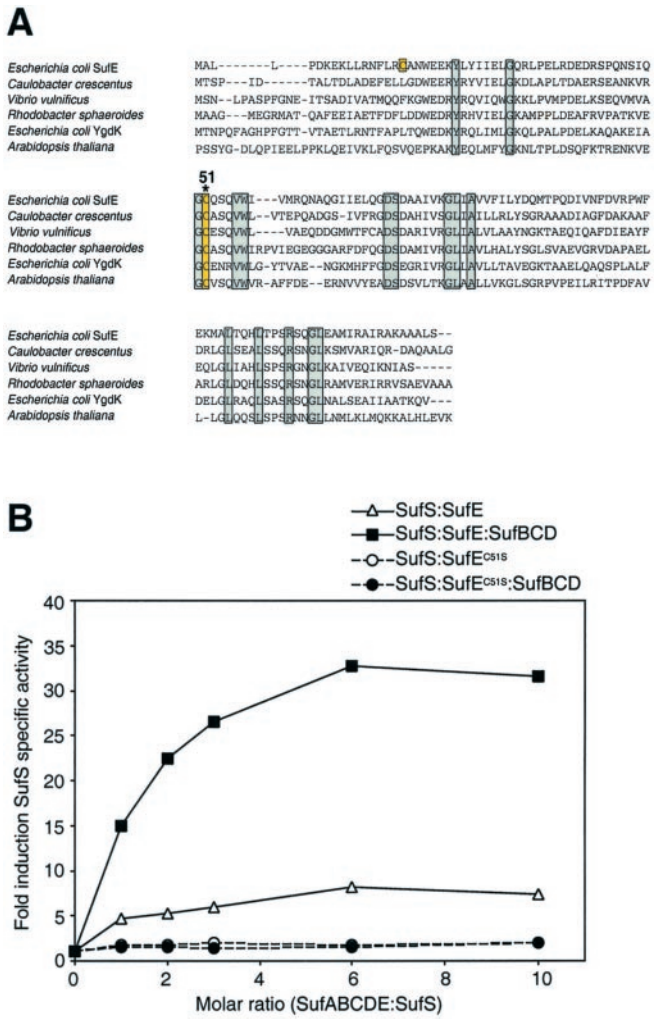

FIG. 4. **Identification of a critical cysteine in SufE.** *A*, sequence alignment of SufE with its closest homologues. Identical residues are shaded in *gray*. The highly conserved cysteine at position 51 in SufE is shaded in *yellow* (as is the non-conserved Cys[17]). *B*, SufS-dependent formation of sulfide from L-cysteine was measured after 20 min for samples containing a constant amount of $(His)_6$-SufS (500 nM) but increasing amounts of combinations of SufE or SufE^{C51S} and SufBCD.

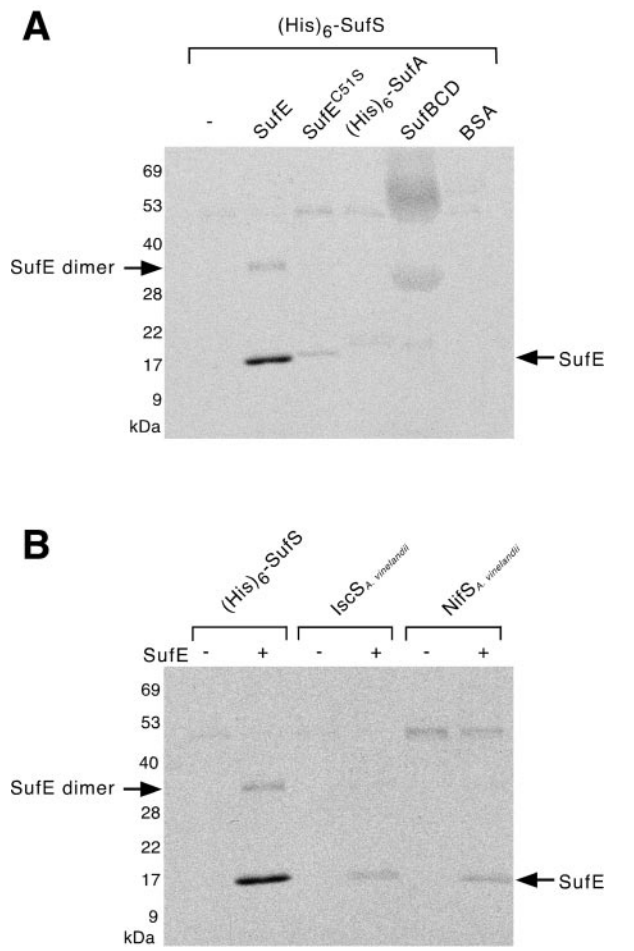

FIG. 5. **Sulfur transfer from SufS to SufE.** *A*, $(His)_6$-SufS (1 μM) was mixed with SufA, SufBCD, SufE, SufE^{C51S}, or BSA (20 μM) and incubated in the presence of L-[^{35}S]cysteine. *B*, *E. coli* $(His)_6$-SufS, IscS$_{A. vinelandii}$, or NifS$_{A. vinelandii}$ (1 μM) were mixed with *E. coli* SufE (20 μM) and incubated in the presence of L-[^{35}S]cysteine. Unincorporated cysteine was removed by spin column, and samples were separated on a non-reducing 10–20% Tris-glycine gel.

likely via a sulfane sulfur linkage between the two proteins. We noted that the sulfur transfer reaction itself was resistant to moderate levels of reductant since addition of up to 10 mM DTT during the reaction did not decrease ^{35}S labeling of SufE (Supplemental Fig. 3).

DISCUSSION

Overexpression and purification of each of the Suf proteins has allowed us to begin to characterize the functions of the SufB, SufC, SufD, and SufE accessory proteins. The addition of the SufBCD multiprotein complex and the SufE protein to the SufS cysteine desulfurase results in an enhancement of desulfurase activity of up to 32-fold over its basal levels. A recent study also demonstrated that SufE enhances SufS activity (33). In that study, a 50-fold induction of SufS activity by SufE was observed under different assay conditions containing higher levels of L-cysteine and reductant. Our work here reveals that the SufE and SufBCD enhancement of SufS is dependent upon Cys[51] of SufE and that transfer of sulfur from SufS to SufE occurs via this cysteine residue.

There are precedents for sulfur transfer intermediaries in other physiological processes that require mobilized sulfur. For example, during biosynthesis of 4-thiouridine, mobilized sulfur is transferred from IscS to the thiamin biosynthetic enzyme, ThiI, and from ThiI to tRNA^{Phe} (34). The need for accessory proteins to enhance SufS activity also is consistent with the SufS crystal structure (35, 36). The active site Cys[364] involved in desulfurase activity is located within a pocket created by dimerization of SufS monomers and appears solvent-excluded. The inability of reductants to reach this site to remove the persulfide and reset the cysteine for further reaction cycles may explain why SufS alone has such low *in vitro* activity. Protein-protein interactions between SufS and SufE, on the other hand, apparently mediate SufE access to this buried site and allow solvent-excluded transfer of sulfur specifically to SufE. The observation that the addition of 10 mM DTT did not decrease ^{35}S labeling of SufE is consistent with restricted solvent access to the sulfane sulfur during the transfer from SufS to SufE. In contrast, ^{35}S labeling of IscU by IscS is decreased by ~50% by the addition of 5 mM β-mercaptoethanol (11) and IscS labeling of ThiI is completely abolished by the addition of 5 mM DTT (34) during the transfer process.

The recently determined x-ray crystal structure of *E. coli* SufE (Protein Data Bank code 1MGZ)[2] coupled with our biochemical data supports the model that sulfur transfer between

[2] A. Kuzin, W. Edstrom, R. Xiao, T. Acton, B. Rost, G. T. Montelione, L. Tong, and J. F. Hunt, unpublished data.

SufS and SufE is shielded.[2] Based on the crystal structure, the SufE protein has a novel fold consisting of both α-helices and β-sheets and is a homodimer with the dimer interface comprising residues Glu^{21}, Tyr^{24}, Trp^{20}, Thr^{116}, Leu^{115}, and Arg^{119}, all of which are highly conserved among SufE homologues (Fig. 4*A*). In the structure, each SufE dimer contains two distinct Cys^{51} active sites located on opposing sides of the complex. Cys^{51} is positioned on a hydrophilic surface loop formed from residues Gln^{49}-Gln^{54}, but the Cys^{51} thiol is actually packed inward into the hydrophobic core of SufE and is largely solvent-excluded. This implies that SufE is in an inactive conformation as a homodimer. SufE interaction with SufS or possibly SufBCD may cause a conformational change in SufE, resulting in the exposure of the Cys^{51} thiol and reaction with the Cys^{364} persulfide on SufS.

Several models can explain the dramatic 32-fold increase in SufS activity observed upon the addition of the SufBCD complex to SufS/SufE. Given the numerous cysteine residues present on the SufBCD complex (4 highly conserved, 6 partially conserved), these proteins provide a large potential sink for mobilized sulfur. However, we only observed intermediate labeling of the SufBCD complex with L-[^{35}S]cysteine in comparison with the intense labeling seen with SufE. Another possibility is that the SufBCD complex facilitates sulfur transfer from SufS to SufE by binding to both components and co-localizing them and/or altering their conformations to promote sulfur transfer. Interestingly, we could no longer detect the SufE dimer when SufBCD was added to the sulfur transfer reaction, possibly indicating that SufE-SufBCD interactions limit SufE-SufE interactions. In contrast, the addition of BSA to the sulfur transfer reaction had no effect on the amount of SufE dimer observed (Supplemental Fig. 1*A*).

SufBCD probably has further roles in Fe-S cluster assembly in addition to the enhancement of SufS desulfurase activity. Because no ATP was added to our assays and a 60-fold excess of non-hydrolyzable ATP did not reduce stimulation by SufBCD, ATP hydrolysis by the SufBCD complex is not necessary for the enhancement of SufS activity. However, ATP may be required for some other step in cluster building such as iron acquisition. The theme of sheltering the Fe-S cluster assembly within a multiprotein complex may extend to iron acquisition as well as sulfur transfer. Because the *suf* operon is co-regulated by both iron starvation and oxidative stress, two conditions where cellular iron pools are disrupted, the SufBCD complex may function with SufS and SufE to acquire iron and protect it from cellular chelators and oxidants during the Fe-S cluster assembly. It has been shown that most *suf* mutants in *E. chrysanthemi* have increased levels of intracellular free iron (23), further supporting a connection between the Suf proteins and cellular iron pools.

Because of the marked enhancement of SufS desulfurase activity, it also is tempting to speculate that the SufBCD complex acts as a Fe-S assembly scaffold. However, it has been problematic to convincingly pinpoint any terminal sulfur acceptor within the SufBCD complex because all three proteins appear to be labeled with ^{35}S at low levels. Recent work has shown that a 2Fe-2S cluster can be reconstituted on SufA and that it might function as a scaffold for the Suf system (25). Alternatively, Suf may assemble clusters within the apoenzyme itself without an external scaffold. Further experiments are necessary to distinguish between these alternatives.

We propose the buried nature of the SufS active site, and the careful control of sulfur transfer between SufS and SufE protects the cell by minimizing the loss of sulfur to other cellular reductants. The magnitude of the SufS activity enhancement by SufE and SufBCD (>30-fold) is so great as to suggest that SufE and SufBCD together function as a reaction switch to effectively turn on or off SufS activity. In contrast, IscS has significantly higher basal activity than SufS and this activity is only enhanced 2-fold by ThiI (38) and 6-fold by IscU (12). This finding suggests that sulfur transfer from IscS is more facile and is less tightly controlled than from SufS. The recently solved crystal structure of IscS supports this hypothesis (39). In contrast to the buried active site in the SufS structure, the active site Cys residue of IscS is located on a flexible loop that is more exposed to the external environment.

In the case of the Suf system, the SufE/SufBCD switch might be a specific adaptation to help limit uncontrolled release of sulfide from SufS. Any sulfide released in an uncontrolled manner could react with the labile iron pool to form iron sulfides. Modulation of the SufS sulfur transfer process by SufE/SufBCD may be especially important under oxidative stress conditions given that iron sulfides are more efficient than ferrous iron alone at catalyzing the formation of highly damaging hydroxyl radicals from hydrogen peroxide (40). Our results reveal critical roles of the Suf accessory proteins and underscore the importance of characterizing their biochemical functions to fully understand how the *suf* operon protects the cell from environmental stresses.

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