Escherichia coli SufE Sulfur Transfer Protein Modulates the SufS Cysteine Desulfurase through Allosteric Conformational Dynamics

**Background:** SufS cysteine desulfurase mobilizes sulfur for stress-responsive iron-sulfur cluster biogenesis in bacteria. Interaction with the sulfur transfer protein SufE triggers conformational changes in the SufS active site. SufE participates in allosteric regulation of SufS activity in addition to being a sulfur acceptor. New insight into sulfur mobilization and transfer during iron-sulfur cluster metallocofactor assembly is provided.

**Results:** Interaction with the sulfur transfer protein SufE triggers conformational changes in the SufS active site.

**Conclusion:** SufE interactions and protein dynamics in solution. HDX-MS exchange mass spectrometry (HDX-MS) to characterize SufS–SufE sulfur transfer interactions and protein dynamics in solution. HDX-MS analysis shows that SufE binds near the SufS active site to accept persulfide from Cys-364. Furthermore, SufE binding initiates allosteric changes in other parts of the SufS structure that likely affect SufS catalysis and alter SufS monomer-monomer interactions. SufE enhances the initial l-cysteine substrate binding to SufS and formation of the external aldimine with pyridoxal phosphate required for early steps in SufS catalysis. Together, these results provide a new picture of the SufS–SufE sulfur transferase pathway and suggest a more active role for SufE in promoting the SufS cysteine desulfurase reaction for Fe–S cluster assembly.

Fe-S clusters are critical metallocofactors required for cell function. Fe–S cluster biogenesis is carried out by assembly machinery consisting of multiple proteins. Fe–S cluster biogenesis proteins work together to mobilize sulfide and iron, form the nascent cluster, traffic the cluster to target metalloproteins, and regulate the assembly machinery in response to cellular Fe–S cluster demand. A complex series of protein–protein interactions is required for the assembly machinery to function properly. Despite considerable progress in obtaining static three-dimensional structures of the assembly proteins, little is known about transient protein–protein interactions during cluster assembly or the role of protein dynamics in the cluster assembly process. The Escherichia coli cysteine desulfurase SufS (EC 2.8.1.7) and its accessory protein SufE work together to mobilize persulfide from l-cysteine, which is then donated to the SufB Fe–S cluster scaffold. Here we use amide hydrogen/deuterium exchange mass spectrometry (HDX-MS) to characterize SufS–SufE interactions and protein dynamics in solution. HDX-MS analysis shows that SufE binds near the SufS active site to accept persulfide from Cys-364. Furthermore, SufE binding initiates allosteric changes in other parts of the SufS structure that likely affect SufS catalysis and alter SufS monomer-monomer interactions. SufE enhances the initial l-cysteine substrate binding to SufS and formation of the external aldimine with pyridoxal phosphate required for early steps in SufS catalysis. Together, these results provide a new picture of the SufS–SufE sulfur transferase pathway and suggest a more active role for SufE in promoting the SufS cysteine desulfurase reaction for Fe–S cluster assembly.

Fe-S clusters are small, inorganic cofactors in metalloproteins that are electron carriers in redox reactions, regulatory sensors, and catalysts (1). Because both iron and sulfide ions are toxic, Fe–S clusters do not assemble spontaneously in vivo. Instead, a series of proteins is required to synthesize Fe–S clusters in a carefully controlled process that is regulated by iron bioavailability and Fe–S cluster demand. Although these proteins may vary among organisms, the functional steps for cluster biogenesis are well conserved. These steps include mobilization of sulfide, formation of the nascent Fe–S cluster, and incorporation of the cluster into target proteins (2). In bacteria, the three common Fe–S cluster biogenesis systems are Nif (nitrogen fixation), Isc (iron-sulfur cluster assembly), and Suf (sulfur formation) (3).

In many Gammaproteobacteria such as Escherichia coli, Fe–S cluster biogenesis is carried out by the Isc system under normal cellular conditions (4). However, if the cell experiences oxidative stress or iron starvation, the Suf system is the major biogenesis pathway (5). The sufABCDSE operon encodes six proteins SufA, SufB, SufC, SufD, SufS, and SufE. Dimeric SufS is an 88.8-kDa pyridoxal 5'-phosphate (PLP)3 containing cysteine desulfurase that mobilizes sulfur from l-cysteine substrate, resulting in an enzyme-bound persulfide intermediate at Cys-364 in the active site (Fig. 1) (5, 6). Persulfides readily react with oxidants, so the active site of SufS is more buried as compared with housekeeping cysteine desulfurases such as IscS (7). The monomeric 15.8-kDa SufE co-substrate protein interacts with the SufS dimer to stimulate cysteine desulfurase activity and accepts sulfane sulfur through a persulfide transfer reaction (8, 9). This sulfur transfer reaction, which proceeds via a ping-pong mechanism, is important for limiting sulfide release under oxidative stress conditions (10, 11). SufE transfers the persulfide to SufB of the SufBC2D complex, which is a scaffold complex that assembles [4Fe–4S] clusters (12–14). Once nas-
cent Fe-S clusters are formed, SufA may transfer the clusters to apo-Fe-S proteins (13). After SufS mobilizes sulfur from l-cysteine, a covalent persulfide intermediate with Cys-364 is formed in the active site (Fig. 1). In apo-SufS, Cys-364 resides in a small loop and the S lies relatively far (7.5 Å) from the C4’ atom of PLP; therefore, loop movement should be required for desulfurase activity (15, 16). The slowest step in the desulfurase activity corresponds to the nucleophilic attack of the Cys-364 thiolate ion on the substrate cysteine-PLP ketimine adduct (Fig. 1) (17). In the presence of SufE, SufS cysteine desulfurase activity is increased by an order of magnitude (8, 9). The invariant Cys-51 of SufE acts as a co-substrate for SufS and accepts the sulfur from Cys-364 of SufS, thereby enhancing the catalytic rate (9, 11, 18). It is also possible that interaction with SufE may elicit changes in structural dynamics near the active site that facilitate the desulfuration reaction (5). Because the thiol group of Cys-51 of SufE is buried in a solvent-inaccessible hydrophobic region, a conformational change is also likely to accompany the interaction with SufS (19). Thus, coupled conformational changes may accompany the SufS-SufE interaction.

To fully understand the mechanistic details of the cysteine desulfurase activity of SufS and subsequent transfer of sulfur from SufS to SufE, the interaction interface and catalytically relevant changes in protein conformational dynamics were characterized by amide hydrogen/deuterium exchange mass spectrometry (HDX-MS). A variety of factors influence amide hydrogen exchange rates, but their dependence on hydrogenbonding, solvent accessibility, and environment make HDX a useful reporter of conformational changes that coincide with SufS-SufE complex formation (20). In general, the extent of deuterium incorporation within the first few seconds of exchange indicates regions that are highly dynamic and solvent-accessible (e.g. loops). Amides that are buried in the protein interior or involved in hydrogen bonding (e.g. α-helices and β-sheets) exchange at slower rates (minutes to days) because exchange is dependent on unfolding/folding equilibria or breathing motions (20, 21). The protection of amides within a protein-protein interface leads to a decrease in deuterium incorporation in the backbone and can be localized through pepsin digestion of the proteins and analysis of the peptides by mass spectrometry (22). Peptides outside the region of interaction may also have altered solvent deuterium incorporation due to coupled or allosteric conformational changes, so complete evaluation of the HDX solvent accessibility and kinetics is required to obtain a full picture of the SufS-SufE interaction in different intermediate states. HDX deuterium trapping also was employed as an alternative method to confirm regions of interaction.

These studies revealed that SufE binds near the active site entrance of SufS and also influences backbone dynamics in the active site, particularly near PLP and Cys-364. Under conditions where sulfur transfer is stalled at Cys-364 of the persulfide intermediate of SufS, the SufE interaction leads to dynamic changes in the dimer interface that could influence the reactivity of the other SufS active site. The results suggest that SufE plays an active role in stimulating the SufS cysteine desulfurase reaction through modulation of conformational dynamics, which enhances l-cysteine substrate binding to SufS and the formation of the external aldimine with PLP. The mechanistic implications for Fe-S cluster assembly by the Suf system are discussed.

**EXPERIMENTAL PROCEDURES**

*Protein Expression and Purification—E. coli SufSapo and SufEapo were independently expressed and purified as described previously (10). All SufS preparations contained the cofactor PLP. The term “apo” refers to SufS or SufE proteins.*
that do not contain a persulfide sulfur covalently attached to the active site Cys residue. Purified proteins were concentrated, frozen as drops in liquid nitrogen, and stored at −80 °C until further use.

**Formation of the Persulfide SufS Intermediate (SufSper)**—The 1.5 mM SufSapo stock in 25 mM Tris-HCl, 150 mM NaCl, 10 mM 2-mercaptoethanol, pH 7.4, was buffer-exchanged into Buffer A (25 mM Tris-HCl, 150 mM NaCl, pH 7.4) in an anaerobic Vacuum Atmospheres glove box. Twenty-five microliters of 900 μM SufSapo was incubated with 5 μl of 200 mM cysteine for 30 min and then desalted using spin columns (Thermo Scientific). Desalted SufSper was aliquoted, sealed under nitrogen atmosphere, and immediately taken for HDX experiments.

**Carbamidomethylation of SufEapo (SufEalk)**—A 1.5 mM SufEapo stock in 25 mM Tris-HCl, 150 mM NaCl, 10 mM 2-mercaptoethanol, pH 7.4, was buffer-exchanged into Buffer A in an anaerobic glove box. Twenty-five microliters of 900 μM SufSapo was incubated with 5 μl of 200 mM cysteine for 30 min and then desalted using spin columns. The number of free thiols before and after alkylation was determined by a 5,5-di-thio-bis(2-nitrobenzoic) acid assay (23). SufEalk retained 10% of free thiol, and tandem MS/MS sequencing did not identify nonalkylated peptides containing Cys-51, as described below.

**Identification of Pepsin-generated Peptides—SufSapo, SufSper, SufEapo, and SufEalk** were separately digested with pepsin, and the subsequent peptides were sequenced using MS/MS collision-induced dissociation (24). Twenty-five microliters of 10 μM protein was incubated with 25 μl of quench buffer (0.1 M potassium phosphate, pH 2.3) followed by 2 μl of 5 mg/ml porcine pepsin in 10 mM potassium phosphate, pH 7, for 5 min on ice. The generated peptides were loaded onto a Phenomenex 50 × 2-mm microbore C18 HPLC column pre-equilibrated with solvent A (HPLC grade 98% H2O, 2% acetonitrile, 0.4% formic acid). The digested peptides were eluted over 26 min at 0.1 ml/min on an Agilent 1100 HPLC using a linear gradient of 0–50% HPLC grade solvent B (98% H2O, 2% acetonitrile, 0.4% formic acid). Peptides were sequenced using a Bruker HCT Ultra PTM Discovery mass spectrometer in positive ion mode by data-dependent MS/MS. Peptide identification was performed with PEAKS Client 6 (Bioinformatics Solutions Inc.) using the appropriate modifications. The SufSapo and SufEapo pepsin peptide digest maps generated from peptide identification are shown in Fig. 2, A and B.

**HDX-MS**—Separate HDX-MS experiments were performed with 125 μM stocks of SufSapo, SufEapo, and the 1:1 SufSapo-SufEapo complex in Buffer A with 5 mM DTT, pH 7.4. The SufSapo-SufEapo complex was generated by combining equal volumes of 250 μM SufSapo and 250 μM SufEapo. The HDX reaction was initiated by the addition of 23 μl of 99.9% atm D2O to 2 μl of 125 μM protein (24). Samples were incubated at 25 °C for 15 s to 1 h, after which the reaction was quenched with 25 μl of quench buffer and transferred to ice. The sample was imme-

![Figure 2](image-url)
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adiately digested on ice using 2 µl of 5 mg/ml pepsin for 5 min. The digested peptides were separated over 15 min at 0.1 ml/min using a 0–50% gradient of solvent B. All samples for HDX were prepared individually and were run on the same day.

The appropriate HDX control samples corresponding to the natural isotope distribution pattern for various peptides (m_{100%}) and the amount of deuterium back-exchange from fully deuterated peptides (m_{100%}) were also performed. For the m_{100%} control, 2 µl of 125 µM protein was incubated with 23 µl of water at 25 °C followed by quenching and pepsin digestion as described above. For the m_{100%} control, 2 µl of 125 µM protein was incubated with 12-fold excess of D_{2}O at 37 °C for 16 h and then quenched and digested as above. The spectra from each HDX-MS sample were analyzed using HDExaminer (Sierra Analytics). Each experiment was repeated in triplicate and averaged. The percentage of deuterium incorporated for each peptide was plotted as a function of log time using Kaleida-Graph (Synergy Software), and the resulting plot was fit to the sum of first-order rate expressions using Equation 1,

\[ D = N - \sum_{i=1}^{n} A_i \times e^{-kt} \]

where \( N \) is the total number of exchangeable hydrogens and \( A_i \) is the number of amide protons that exchange at the rate \( k_i \) for the exchange time \( t \) (20).

Amide H/D Exchange Deuterium Trapping—HDX trapping experiments were performed at protein concentrations well above the measured dissociation constant for the SufSapo–SufEapo complex (14) to ensure that the complex does not dissociate during exchange. The incubation times for on-exchange and back-exchange of deuterium were experimentally optimized for this system. Stock solutions of SufSapo and SufEapo (1.5 mM) were prepared in Buffer A with 5 mM DTT. For the 1:1 SufSapo–SufEapo complex, 2 µl of 1.5 mM SufSapo and 2 µl of 1.5 mM SufEapo were separately incubated with 23 µl of D_{2}O for 8 min at 25 °C, mixed, and then incubated for 2 min at 25 °C (25, 26). The 25–µl reaction was back-exchanged with 250 µl of H_{2}O at 25 °C for 2 min and immediately quenched with 3 µl of 7.5% formic acid at 4 °C. Deuterium retention for both SufSapo and SufEapo as individual proteins after 10 min in D_{2}O was also measured. Two microliters of 1.5 mM SufSapo or SufEapo was incubated with 23 µl of D_{2}O for 8 min at 25 °C. An additional 25 µl of D_{2}O was added (to mimic the addition of the other protein during complex formation) and incubated at 25 °C for 2 min. Next, 250 µl of H_{2}O was added and incubated at 25 °C for 2 min.

To determine the extent of deuterium incorporation into each protein without back-exchange with water, 2 µl of 1.5 mM protein was incubated with 23 µl of D_{2}O for 8 min followed by the addition of 25 µl of D_{2}O for 2 min. The reaction was quenched using 250 µl of 0.15% formic acid at 4 °C. The quenched solution was digested for 5 min on ice with 5 µl of 5 mg/ml porcine pepsin in 0.01 M potassium phosphate, pH 7.4, at 0 °C. The m_{30%} and m_{100%} controls were prepared as before for HDX-MS time course experiments. Samples were analyzed by mass spectrometry as described. Each experiment was repeated in triplicate and averaged. The percentage of retained deuterium in the complex after back-exchange with water is based on the total amount of deuterium incorporated after 10 min in D_{2}O for SufSapo and SufEapo individually. HDX trapping was also performed with the SufSapo–SufEalk and SufE_{apo,pe}–SufE_{alk} complexes using the same procedure.

Cysteine Binding Assays—All assays were performed at room temperature in Buffer A. L-Cysteine binding was evaluated by monitoring the immediate ΔΔ_{420} or ΔΔ_{340} elicited by the addition of increasing concentrations of L-cysteine to 25 µM SufSapo or 25 µM SufSapo with an equal amount of SufE_{alk} (27). Protein was first added to the cuvettes, and then L-cysteine was added and mixed for ~5 s prior to a wavelength scan from 200 to 650 nm. As L-cysteine concentrations increased, the 420 nm PLP peak intensity (internal aldimine) decreased and the new 340 nm peak intensity (external aldimine) increased. Data were analyzed with Prism software (GraphPad). SufSapo-Cys data were best fit with the one-site-specific binding with the Hill slope model. SufSapo–SufE_{alk}–Cys data were best fit with a one-site-specific binding model.

Isothermal Titration Calorimetry—Isothermal Titration Calorimetry (ITC) measurements were performed on a VP-ITC calorimeter (MicroCal) at 27 °C. For the SufSapo and SufEapo ITC experiment, SufSapo present in the cell (1.44 ml at 108 M) was titrated with 45 6-µl injections of 1.1 mM SufE (a 10-fold molar excess over SufSapo). The duration of each injection was 7.2 s (1.2 s/µl) with an interval of 200 s between injections. For the SufSapo and SufE_{alk} ITC experiment, SufSapo present in the cell (1.44 ml at 108 M) was titrated with 40 5-µl injections of 1.1 mM iodoacetamide-treated SufE (SufE_{alk}). The duration of each injection was 6 s with an interval of 360 s between injections. Titrations were performed in Buffer A. Each experiment was corrected for the endothermic heat of injection resulting from the titration of SufE/SufE_{alk} into buffer. SufSapo–SufEapo ITC data were analyzed with the two sequential binding sites model in MicroCal Origin using a SufSapo dimer concentration of 54 µM, SufSapo–SufE_{alk} ITC data were analyzed with the one-site model in MicroCal Origin using a SufSapo monomer concentration of 108 µM.

RESULTS

SufSapo–SufEapo Interaction

Solvent Accessibility and Backbone Dynamics—Previously, it has been shown that E. coli SufSapo interacts with SufEapo even in the absence of L-cysteine substrate when SufS is not active (9, 10, 18). We employed HDX-MS to characterize the interaction between SufSapo and SufEapo to determine whether these interactions are relevant to the sulfur transfer mechanism. Here we use the term apo only to refer to SufS or SufE proteins without persulfide sulfur covalently attached to the active site Cys residue (Cys-364 for SufS or Cys-51 for SufE). In all experiments, SufS contains the PLP cofactor.

The amount of deuterium exchange into backbone amides as a function of time for SufSapo and SufEapo was compared with that for the SufSapo–SufE_{apo,pe} complex. It is useful to compare the D_{2}O accessibility of amides that exchange with a very fast rate (i.e. before the 15-s time point) to identify regions that are altered by the interaction of the two proteins (20, 22). Decreased deuterium levels denote regions that become
shielded from D$_2$O by the interaction (22). There are only minor differences in solvent accessibility for SufSapo upon the formation of the SufSapo-SufEapo complex (Fig. 3A). This indicates that SufEapo does not significantly protect a large surface area of SufSapo. For SufEapo, only residues 66–83 have a >10% decrease in deuterium incorporation in the SufSapo-SufEapo complex (Fig. 3B). This peptide forms one side of a structural groove into which the SufE Cys-51 thiolate is oriented (19).

Changes in deuterium uptake over longer time periods (i.e. beyond 15 s) occur through shifts in protein unfolding/folding equilibria, caused by constrained protein backbone dynamics upon interaction of SufSapo and SufEapo (20, 28). HDX-MS kinetic traces show that two regions in SufSapo lose conformational flexibility when in complex with SufEapo. Kinetic traces for peptides 356–366 and 225–236 reveal a 2- and 4-fold decrease in the rate of deuterium incorporation, respectively.
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FIGURE 5. HDX-MS kinetic traces comparing deuterium incorporation as a function of time for SufE<sub>apo</sub> and the SufS<sub>apo</sub>-SufE<sub>apo</sub> complex. HDX reactions were initiated by the addition of 23 µl of D<sub>2</sub>O to 2 µl of 125 µM SufE<sub>apo</sub> or SufS<sub>apo</sub>-SufE<sub>apo</sub>. Samples were incubated at 25 °C for 15 s to 1 h, after which the reactions were quenched and digested with pepsin for 5 min at 4 °C. A and B, SufE peptides 38–56 (A) and 66–83 (B) show decreased rates of deuterium incorporation in the SufS<sub>apo</sub>-SufE<sub>apo</sub> complex. C, the peptides 38–56 (teal) and 66–83 (pink) along with Cys-51 in stick format are depicted on the structure of SufE<sub>apo</sub> (PDB: 1MZG) (19). The data were fit to a sum of first-order rate expressions, which can be found in supplemental Table S1. The uptake plots for all peptides are found in supplemental Fig. S2.

for the SufS<sub>apo</sub>-SufE<sub>apo</sub> complex (Fig. 4, A and B). Peptide 356–366 is a loop that extends from the surface of SufS to the active site channel and includes the sulfur-accepting residue Cys-364 (15, 16). In contrast, residues 225–236 are located at the bottom site channel and includes the sulfur-accepting residue Cys-364. 366 is a loop that extends from the surface of SufS to the active site cavity (Fig. 7). These results suggest that SufE<sub>apo</sub> binding near the surface of the active site channel (residues 356–366) leads to an allosteric change in conformational dynamics near the catalytic PLP cofactor (residues 225–236).

Changes in SufE<sub>apo</sub> backbone dynamics near Cys-51, the sulfur acceptor, are also observed upon formation of the SufS<sub>apo</sub>-SufE<sub>apo</sub> complex. Peptide 38–56, which is a surface loop containing Cys-51 (19), has an ~3-fold reduced rate of deuterium incorporation in the SufS<sub>apo</sub>-SufE<sub>apo</sub> complex (Fig. 5, A and C). Another peptide in close proximity to Cys-51 (residues 66–83) also has altered deuterium uptake in the complex (Fig. 5B), but this is more reflective of decreased solvent accessibility because it is protected within 15 s of D<sub>2</sub>O incubation (see also Fig. 3B). Thus, residues within 66–83 are most likely involved in the SufS<sub>apo</sub> interaction, which may cause conformational changes that are propagated to the Cys-51 loop (residues 38–56).

Deuterium Trapping—One of the limitations with traditional HDX-MS is that some regions in the individual proteins may not incorporate a significant amount of deuterium after 15 s of D<sub>2</sub>O incubation. If a change in solvent accessibility does occur after complex formation, it might be too small to accurately measure because of normal deuterium loss during HPLC analysis. This was observed for SufS<sub>apo</sub> for which there was little change in solvent accessibility upon SufE<sub>apo</sub> binding (Fig. 3A). Therefore, a simple technique was sought to overcome this limitation.

Modified HDX deuterium trapping was used to identify deuterated amides in the SufS<sub>apo</sub>-SufE<sub>apo</sub> complex that are not easily off-exchanged with water (i.e., “trapped”) (26, 29, 30). By preincubating SufS<sub>apo</sub> and SufE<sub>apo</sub> individually with D<sub>2</sub>O for 10 min, highly/moderately solvent-accessible amides will exchange. This leads to a greater percentage of deuterium incorporation, which is inherently easier to measure by MS. SufS<sub>apo</sub> and SufE<sub>apo</sub> are then mixed to form the SufS<sub>apo</sub>-SufE<sub>apo</sub> complex and further diluted into H<sub>2</sub>O to off-exchange solvent-accessible amide deuterons. The percentage of deuterium retained in peptides from the SufS<sub>apo</sub>-SufE<sub>apo</sub> complex after off-exchange with water was compared with the amount of deuterium retained within the individual proteins after off-exchange. The percentage of retention was based on the total amount of deuterium incorporated before off-exchange with water (i.e., after a 10-min incubation in D<sub>2</sub>O). Amides that retain more deuterium in the complex are either involved in the interaction between SufS<sub>apo</sub> and SufE<sub>apo</sub> or highly protected by associated conformational changes that influence stable hydrogen bonding (26). Note that some amide deuterons at the protein-protein interface may still back-exchange for hydrogen if they are accessible to water. This method only surveys amides whose exchange rates have been significantly reduced by complex formation (26, 29).

Deuterium trapping analysis showed that SufS<sub>apo</sub> peptides 356–366 and 225–236 retain more deuterium in the SufS<sub>apo</sub>-SufE<sub>apo</sub> complex as compared with free SufS<sub>apo</sub> (Fig. 6, A and B). These are the same peptides identified by the traditional HDX-MS method (Fig. 4). The increased retention confirms that SufE<sub>apo</sub> binding protects deuterated SufS<sub>apo</sub> amides in those regions from back-exchange with water. Peptide 356–366 is at the opening of the cavity leading to the active site (Fig. 6, C and D), so the deuterium protection could be from direct interaction with SufE<sub>apo</sub> which must gain access to Cys-364 for sulfur transfer (9). Because residues 225–236 are at the bottom of the active site cavity, it is unlikely that they directly interact with SufS<sub>apo</sub> (16). Increased protection of this peptide suggests that SufE<sub>apo</sub> binding leads to significant allosteric changes in hydrogen bonding around the active site PLP or that SufE interacts with PLP, which is covalently bound to Lys-226.

Two peptides from SufE<sub>apo</sub> (38–56 and 66–83) retain >10% amide deuteration in the SufS<sub>apo</sub>-SufE<sub>apo</sub> complex as compared with free SufE<sub>apo</sub> (Fig. 7, A and B). Peptide 38–56 contains the sulfur acceptor Cys-51 (Fig. 7C) (19). Because these two regions form a surface around Cys-51, the increased deuterium retention in the complex suggests direct interaction with SufS<sub>apo</sub> or a significant change in the conformation of SufE<sub>apo</sub> upon binding to SufS<sub>apo</sub>. This is consistent with kinetic HDX-MS results (Fig. 5).

Deuterium Trapping with the SufS Persulfide Intermediate

The previous experiments determined that SufE<sub>apo</sub> affected SufS<sub>apo</sub> conformation and mapped the interacting regions of both proteins simultaneously in the absence of a Cys-364 persulfide. It is known, however, that SufE<sub>apo</sub> binding to the per-
sulfide intermediate form of SufS (SufSper) stimulates SufS desulfurase activity by providing an acceptor for the persulfide species via direct sulfur transfer (9, 11, 18). Thus, SufS could have a different conformational response to SufE that is dependent on the SufS catalytic intermediate state. To obtain a better understanding of whether the SufS-SufE interaction interface is modulated by the Cys-364 persulfide, we performed deuterium trapping assays with SufSper in complex with SufE.

In these experiments, sulfur transfer to SufEapo needs to be blocked to prevent turnover of the SufSper species. Therefore, SufEapo Cys-51 was alkylated with iodoacetamide (SufEalk) to specifically prohibit sulfur transfer from SufSper Cys-364 to SufEapo Cys-51. HDX-MS time course experiments reveal that carbamidomethylation of SufS yields increased solvent accessibility and dynamics around Cys-51.4 A loss of hydrogen bonding with increased backbone solvent accessibility suggests that alkylation of SufE triggers a conformational switch in the loop that could mimic the “sulfur-accepting” state, possibly by forcing the modified Cys-51 side chain out of its groove and into an exposed conformation (31). This interpretation is supported by previous studies showing that SufEalk is a potent inhibitor of SufS enhancement by SufEapo (10).

Before mapping SufEalk binding to SufSper, we first determined whether SufE alkylation affected the interaction with SufSapo. ITC was used to compare the binding affinity of both SufEapo and SufEalk for SufSapo. The ITC isotherm shows that SufEapo binding to SufSapo has a biphasic behavior with an initial exothermic phase at lower SufEapo concentrations and an endothermic phase at higher SufEapo concentrations (Fig. 8A, supplemental Table S2). The SufEapo binding data are best fit by a sequential two-site binding model with a higher affinity site (Kd1 = 3.59 𝜇M) and a lower affinity site (Kd2 = 312 𝜇M). Each SufSapo dimer has two active sites (one per monomer), but the ITC data suggest that the two sites are not equivalent for SufEapo binding. Instead, the observed SufEapo binding behavior is consistent with a flip-flop mechanism of allosteric regulation where binding of SufEapo to one active site on the SufS dimer diminishes further SufEapo binding to the second active site. A similar mechanism has been proposed for SufS-SufU interactions in the Bacillus subtilis Suf system (32). This type of allosteric regulation may also explain the previously reported

4 H. Singh, D. Kim, and L. S. Busenlehner, unpublished observations.
substrate inhibition behavior exhibited by SufE on SufS at low L-cysteine concentrations (10).

In stark contrast, SufE_{alk} binding to SufS_{apo} was primarily exothermic, and the binding data were well fit using a one-site binding model (Fig. 8B, supplemental Table S2). The SufE_{alk} K_d for binding to SufS_{apo} was $0.26 \text{ M}$, indicating that it binds SufS_{apo} 10-fold more tightly than SufE_{apo}. The number of SufE_{alk} binding sites on SufS calculated from ITC is only 0.73, suggesting that SufE_{alk} binding may induce negative allosteric regulation of the second SufS monomer, albeit at a significantly lower level than that observed for SufE_{apo}. This result is consistent with SufE_{alk} being locked into a conformation that mimics the sulfur-accepting state of SufE_{apo}, which would likely bind more tightly to SufS.

HDX trapping assays indicate that, like the SufS_{apo}-SufE_{apo} complex, peptides 356–366 and 225–236 have increased deuterium retention in the SufS_{apo}-SufE_{alk} complex (Fig. 6, A and B). An additional area of protection within residues 262–274 is also observed (Fig. 6E). In the SufS_{apo} dimer, residues 262–274 from one SufS monomer chain form a surface above and covering the active site channel of the second SufS monomer, which we refer to as the active site "lid" (Fig. 6, C and D) (16, 33). Increased retention of deuterium in the active site lid indicates that additional interactions are detectable at the SufS_{apo}-SufE interface when Cys-51 of SufE is alkylated. This may contribute to the higher affinity observed for SufS_{apo} as well as the ability to partially override the negative cooperativity observed for SufE_{apo} binding to SufS_{apo} (Fig. 8A).

Once SufE_{alk} contributions to HDX deuterium trapping were determined for SufS_{apo}, SufE_{apo} complex, peptides 356–366 and 225–236 have increased deuterium retention in the SufS_{apo}-SufE_{alk} complex (Fig. 6, A and B). An additional area of protection within residues 262–274 is also observed (Fig. 6E). In the SufS_{apo} dimer, residues 262–274 from one SufS monomer chain form a surface above and covering the active site channel of the second SufS monomer, which we refer to as the active site "lid" (Fig. 6, C and D) (16, 33). Increased retention of deuterium in the active site lid indicates that additional interactions are detectable at the SufS_{apo}-SufE interface when Cys-51 of SufE is alkylated. This may contribute to the higher affinity observed for SufS_{apo} as well as the ability to partially override the negative cooperativity observed for SufE_{apo} binding to SufS_{apo} (Fig. 8A).

Once SufE_{alk} contributions to HDX deuterium trapping were determined for SufS_{apo}, assays with the SufS_{apo}-SufE_{alk} stalled sulfur transfer complex were performed. Like SufS_{apo}-SufE_{apo} and SufS_{apo}-SufE_{alk}, protection of deuterated amides is observed for SufS peptides 356–366, 225–236, and 262–274 in the SufS_{apo}-SufE_{alk} complex (Fig. 6, A, B, and E). Thus, these regions of SufS are protected by SufE regardless of the presence of the SufS Cys-364 persulfide (and regardless of SufC Cys-51 modification). Surprisingly, peptide 243–255 shows a loss of deuterated amide protection in the SufS_{apo}-SufE_{alk} complex (Fig. 6F). These residues are part of a long loop at the SufS_{apo} dimer interface with several interactions between the two SufS monomers (Fig. 6, C and D) (16, 33). HDX trapping suggests that SufE_{alk} binding to SufS_{apo} increases the solvent accessibility at the dimer interface, leading to more back-exchange with water. Therefore, residues within the dimer interface respond to both persulfuration of SufS Cys-364 and the orientation of SufE Cys-51, which may coordinate active site cooperativity in the SufS dimer.

**SufE_{alk} Alters L-Cysteine Binding to SufS**

Because SufE binding to SufS_{apo} leads to conformational changes within the SufS peptide containing the PLP ligand Lys-226, it was important to test whether SufE binding alters the reactivity of PLP for L-cysteine substrate (Fig. 1). If so, this could provide additional functional insight into how SufE activates SufS. This assay required SufE_{alk} to prevent sulfur transfer from SufS to SufE (i.e. SufS turnover) and to allow us to exclusively examine the first step of the reaction, L-cysteine binding to SufS PLP in the presence of SufE. When l-cysteine binds to PLP, it displaces the internal aldimine with Lys-226 and forms an external aldimine at the same position (Fig. 1) (33). The initial binding of L-cysteine substrate to resting SufS_{apo} was compared with the SufS_{apo}-SufE_{alk} complex by following the formation of the external aldimine with L-cysteine, which absorbs at 340 nm, and the disappearance of the internal aldimine, which absorbs...
at 420 nm (Fig. 9, A and C). Fitting of the $\Delta A_{420}$ measurements to a one-site binding model shows that the $K_d$ of SufS for L-cysteine decreases 3-fold from 61 ± 1.5 µM for SufS alone to 18 ± 1.6 µM for SufS with one equivalent of SufEalk (Fig. 9, B and D). We should note that these values are not true dissociation constants because this assay does not distinguish between L-cysteine binding and the rate of external aldimine formation once L-cysteine is bound. Either step or both steps might be promoted by SufEalk in this equilibrium assay. Regardless, the results indicate that the binding of SufE may actively remodel the SufSapo active site, leading to changes in deuterium incorporation by the peptides present around the active site cavity, to promote catalysis at the PLP site.

**DISCUSSION**

Conformational dynamics may be essential for the catalytic activity of many enzymes (20). It is important to define structural and conformational changes in the vicinity of the active/binding site, but also in the surrounding regions that may have allosteric responses. This holistic view provides insight into how structural dynamics are related to catalytic and allosteric mechanisms. Many enzymes interact with other proteins as part of their function, so localization of the interaction interface is of primary importance. However, if binding leads to allosteric effects, the conformational changes resulting from the interaction are also relevant. This point is illustrated by the results presented here on the interaction between the SufS cysteine desulfsulfurase and its co-substrate SufE as part of the Suf Fe-S cluster biosynthesis system in *E. coli*. The enzyme-bound PLP cofactor in the active site of SufS forms an external aldimine with substrate L-cysteine to catalyze abstraction of sulfur by SufS Cys-364 followed by sulfur transfer to Cys-51 of SufEapo (14). However, the structure of the *E. coli* SufSapo–SufEapo complex and potential conformational changes that result from their interaction are not defined. SufE binding increases the SufS desulfurase activity, at least in part, by acting as a co-substrate for the ping-pong reaction pathway that depends on SufE Cys-51 (9, 11). In this study, we used HDX-MS, HDX deuterium trapping, and biochemical assays to better define the role protein dynamics plays in possible SufE allosteric activation of SufS catalytic activity.

Characterization of the SufSapo–SufEapo Interaction—HDX-MS and deuterium trapping experiments indicated that SufSapo residues within 38–56 and/or 66–83 interact with SufEapo and undergo a conformational change upon complex formation (Fig. 5). SufEapo peptide 38–56 contains the sulfur acceptor Cys-51 as part of the loop connecting the $\beta_1$ and $\beta_2$ strands (Fig. 7C) (19). In the static structure of SufE, the thiolate side chain of Cys-51 is in a solvent-inaccessible, hydrophobic pocket partially formed from residues within 66–83 (Fig. 7D). A conformational change in both regions could expose the Cys-51 thiolate for sulfur transfer upon docking with SufS (19). Our SufSapo HDX data show that the Cys-51 loop is moderately solvent-accessible and that binding to SufSapo decreases its backbone dynamics. A recent crystallographic structure of a complex between two *E. coli* proteins related to SufS and SufE, CsdA and CsdE, is consistent with the conformational change and
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dynamic stabilization we observed for the Cys-51 loop (PDB: 4LW4) (31). In the CsdAapo-CsdEapo co-structure, the CsdE Cys-61 loop region underwent an ~11 Å shift upon interaction with CsdA. Based on the CsdAapo-CsdEapo co-structure (31), HDX trapping assays, and sequence alignments, there are many SufSapo surface residues that could form stabilizing side chain and backbone hydrogen bonds that constrain dynamics within residues 38–56 and 66–83 including Gln-52, Gln-54, and Asp-74.

Despite the change in CsdEapo conformation, there were no noticeable structural changes to the CsdAapo cysteine desulfurase backbone in the CsdAapo-CsdEapo complex (31). This is in contrast to our HDX-MS studies with SufSapo that showed altered deuterium uptake for two active site peptides, residues 225–236 and 356–366, in the presence of SufEapo (Fig. 4, A and B). HDX trapping experiments further confirmed that deuterated amides within these regions are highly solvent-protected in the presence of SufEapo and SufEalk (Fig. 6, A and B). The location of peptide 356–366 near the surface suggests that some residues may be directly involved in the interaction with SufE (15). The CsdAapo structure in complex with CsdEapo is partially consistent with our HDX experiments and showed that CsdA residues Gln-356 (SufS His-362) and Gln-360 (SufS Met-366) directly interact with CsdE (31). It is possible that some of the interactions within this region provide specificity for SufS/SufE to limit cross-reactivity with CsdA/CsdE (34).

Based on the CsdAapo-CsdEapo co-structure, it was proposed that residues within 343–354 (helix16), 355–378 (the Cys-364 Cys-51 loop region) underwent an 11 Å shift upon interaction with SufE (31). The strong protection of backbone hydrogen bonds that constrain dynamics within residues 38–56 and 66–83 including Gln-52, Gln-54, and Asp-74.

Because residues 225–236 are buried in the active site cavity, the stabilizing changes in conformation observed around PLP upon SufEapo binding may be transmitted via the 356–366 active site loop, which we propose is involved in the SufEapo interaction based on HDX trapping experiments and the CsdAapo-CsdEapo co-structure (31). The strong protection of deuterated amides (Fig. 6B) and the decrease in backbone dynamics (Fig. 4B) within 225–236 suggested that SufEapo alters the PLP environment, which is also supported by the enhanced formation of the PLP-l-cysteine external aldimine in the presence of SufEalk (Fig. 9). The PLP binding site is highly conserved for both type I and type II cysteine desulfurases (7). It is possible that SufEapo binding shifts the SufS equilibrium toward a conformation optimal for substrate l-cysteine binding or external aldimine formation. The results suggest that SufEapo subtly remodels SufSapo architecture in the vicinity of the internal aldimine (between Lys-226 and PLP) to promote catalysis and may not activate SufS solely through a passive persulfide acceptor role.

Because SufE stimulates SufS cysteine desulfurase activity during the ping-pong reaction, we also considered that the binding interactions could be modulated by the presence of the SufS Cys-364 persulfide (where SufE is primed for sulfur transfer to SufEapo) or by the conformation/orientation of SufE Cys-51 thiolate. In the SufS persulfide intermediate (SufSper) structure, the Cys-364 persulfide moiety is facing the entrance to the active site cavity, presumably to orient it toward Cys-51 of SufE (16). It has been postulated that the Cys-51 thiolate must reach into the active site channel to carry out a nucleophilic attack on the Cys-364 persulfide for sulfur transfer (3). It is also known that in the absence of a further sulfur acceptor (e.g. SufB or a thiol reductant), Cys-51 of SufE can accept multiple sulfur groups, thereby forming a polymeric sulfur species (8, 11). This indicates that both apo and persulfurated/polysulfurated SufE can bind to SufS, presumably with protrusion of the Cys-51 side chain into the active site cavity, as was observed in the co-structure of CsdA-CsdE in which the CsdE Cys-61 thiolate is exposed and oriented toward Cys-358 of CsdA (31). It is unclear whether SufS stabilizes this particular sulfur-accepting conformation of SufE or vice versa.

We simulated the sulfur-accepting SufE conformation by alkylating Cys-51 (SufEalk). HDX-MS indicated that alkylation led to increased solvent exposure in the SufE Cys-51 loop peptide as compared with native SufEapo consistent with loss of stabilizing internal hydrogen bonds and potential exposure of Cys-51 from its secluded pocket.4 We demonstrated through deuterium trapping assays that alkylation of SufE led to additional amide deuterium retention within SufS residues 262–274 for both SufSapo and SufSper (Fig. 6E). The additional interactions were not dependent on the state of SufS, but entirely mediated by the presumed change in SufE conformation when Cys-51 is modified. Residues 262–272 are part of a surface β-hairpin loop that forms a lid over the SufS active site (Fig. 6, C and D). This β-hairpin structure is not found in type I PLP-dependent cysteine desulfurases and is proposed to have a specialized functional role in SufS enzymes (33). Unlike what we observed for SufSapo-SufEalk, the lid region from the related type II cysteine desulfurase enzyme CsdA does not interact
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with CsdE or exhibit a significant conformational change in the CsdA-CsdE complex (31). Consistent with the additional interactions observed by HDX trapping, SufE_alk bound SufS_apo with a higher affinity than SufE_apo, indicating that SufE_alk bound SufS_apo with a higher affinity than SufE_apo, and SufE_alk stimulates the formation of the SufS-S_cysteine external aldimine (Fig. 9). Thus, the shift in SufE equilibrium toward the sulfur-accepting conformation of Cys-51 may enhance the interaction with SufS and impact the SufS active site architecture, possibly altering PLP cofactor reactivity via changes in residues 225–236 (Fig. 4).

Further analysis of the SufS_per-SufE_alk complex revealed that SufE binding to the Cys-364 persulfide intermediate of SufS led to increased solvent accessibility at the SufS dimer interface. Deuterium trapping assays indicate that ~75–80% of the dimer interface is located on the backbone of peptide 243–255, which is contained within peptide 243–255 (16). However, no significant changes in deuterium retention were noted in deuterium trapping assays with SufSapo and SufSper enzymes (Fig. 6F). It is only in the SufSper-SufE_alk complex (i.e. the stalled sulfur transfer complex) that some of these stable hydrogen bonds between the SufS monomers are broken, leading to more back-exchange with water. The changes at the SufS dimer interface suggest that formation of the stalled sulfur transfer complex with SufE_alk may partially uncouple the SufS per monomers. This uncoupling, which could decrease the flip-flop regulation of SufS, is consistent with the different binding data obtained for SufE_alk as compared with SufE_apo (Fig. 8 and supplemental Table S2).

Residues 243–255 comprise a loop connecting the entrance of the active site cavity to the dimer interface (Fig. 6, C and D). In the SufS per persulfide intermediate crystal structure, the Cys-364 persulfide is oriented toward the entrance of the dimer interface and stabilized through formation of a hydrogen bond with the amide of Ser-254, which is contained within peptide 243–255 (16). However, no significant changes in deuteron retention levels were noted in deuteron trapping assays with individual SufS_apo and SufS_per enzymes (Fig. 6F). The levels of deuteron retention in this region for SufS_apo in complexes with SufE_apo and SufE_alk were also similar to the SufS_apo protein alone, indicating that SufE or SufE_alk has little effect on hydrogen bonding at the dimer interface of SufS_apo. However, if SufS Cys-364 is in the persulfide state, binding of SufE_alk leads to a destabilization of hydrogen bonding within residues 243–255 at the dimer interface (resulting in the observed alterations in amide exchange). Functionally, this might suggest that in the absence of SufE, the SufS Cys-364 persulfide is stabilized by residues within 243–255 (especially the amide hydrogen bond to Ser-254). If the SufE sulfur acceptor is bound to SufS, the persulfide stabilization is diminished to facilitate nucleophilic attack by SufE Cys-51 on the SufS Cys-364 persulfide for direct sulfur transfer. The SufE-mediated changes in the 243–255 loop could also provide an allosteric mechanism for one SufS monomer to alter the reactivity of the other SufS monomer via a flip-flop mechanism. Unfortunately, we were unable to directly assay SufE_apo binding to SufS_per because this would lead to SufS turnover on the timescales of HDX and greatly complicate data analysis and interpretation.

Conclusions—The results presented here provide a clearer picture of the dynamic interactions between SufS and SufE during sulfur liberation and transfer for Fe-S cluster biogenesis by the Suf system in E. coli. The observed changes around the SufS PLP cofactor binding site suggest that SufE actively promotes external aldimine formation between l-cysteine and PLP. This SufE-dependent effect may also provide a mechanistic explanation for the observation that the SufS-SufE complex has a higher level of activity at lower l-cysteine levels than other Type I cysteine desulfurases such as IscS (10). An active role for SufE in stimulating the first step of the ping-pong reaction is also consistent with the fact that SufE_alk binds well to SufSapo, which would be unlikely if SufE were purely a passive co-substrate for the second step of the reaction. Finally, the results suggest that the sulfur-accepting conformation of SufE (mimicked by SufE_alk) is able to trigger additional changes in SufSper that help facilitate sulfur transfer and/or provide allosteric regulation of the other SufS monomer. Further detailed mechanistic and structural studies are underway to fully test these intriguing hypotheses.

Acknowledgments—We thank Dr. Qiaoli Liang (The University of Alabama) for maintenance and help with the mass spectrometer and Dr. Leslie Lovelace and Dr. Lukasz Lebioda (The University of South Carolina) for assistance with use of their ITC instrument. The University of Alabama Mass Spectrometry Facility was supported by Chemistry Research Instrumentation and Facilities Program (CRIF) Grant CHE 0639003.

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