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The *E. coli* SufS–SufE sulfur transfer system is more resistant to oxidative stress than IscS–IscU

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ABSTRACT

During oxidative stress in *Escherichia coli*, the SufABCDSE stress response pathway mediates iron–sulfur (Fe–S) cluster biogenesis rather than the Isc pathway. To determine why the Suf pathway is favored under stress conditions, the stress response SufS–SufE sulfur transfer pathway and the basal housekeeping IscS–IscU pathway were directly compared. We found that SufS–SufE cysteine desulfurase activity is significantly higher than IscS–IscU at physiological cysteine concentrations and after exposure to H_2O_2. Mass spectrometry analysis demonstrated that IscS–IscU is more susceptible than SufS–SufE to oxidative modification by H_2O_2. These important results provide biochemical insight into the stress resistance of the Suf pathway.
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1. Introduction

Iron–sulfur (Fe–S) clusters in metalloproteins carry out myriad cellular functions [1,2]. Fe–S cluster biogenesis requires proteins that donate sulfur and iron, pre-assemble clusters, and traffic Fe–S clusters to target metalloproteins [3–5]. Fe–S cluster biogenesis is sensitive to oxygen due to the proclivity of iron, sulfide, and protein sulfhydryl groups to be modified by oxygen or reactive oxygen species [6]. In *Escherichia coli*, the Isc system carries out Fe–S cluster assembly under normal conditions while the Suf pathway is required for Fe–S cluster biogenesis under oxidative stress conditions [7–10].

Both Isc and Suf use superficially similar mechanisms to mobilize sulfur for Fe–S cluster assembly. The homodimeric IscS and SufS cysteine desulfurase enzymes catalyze the pyridoxal-phosphate (PLP)-dependent removal of sulfur from L-cysteine substrate resulting in a protein-bound persulfide (R–S–SH) intermediate. This persulfide S^0 species (also referred to as sulfane sulfur) is reduced and incorporated into the Fe–S cluster as sulfide (S^{2-}) during assembly on a scaffold protein (IscU or the $SufBC_2D$ complex) [11–21]. Due to the reactivity of both the persulfide intermediate and active site sulfhydryl groups on the enzymes [22,23], oxidative stress has the potential to block the sulfur donation step of Fe–S cluster biogenesis. Genetic evidence has shown that the Isc system is not efficient at Fe–S cluster assembly under oxidative stress, raising the question of whether sulfur trafficking by the Suf pathway may be more resistant to disruption than the Isc system [24].

IscU and SufE are structural (but not sequence) homologues that each interact with their cognate cysteine desulfurase enzymes to accept S^0 via a thiol exchange mechanism [17,19,20,25]. While IscU is a bona fide scaffold protein where the full Fe–S cluster can be assembled, SufE uses a single active site cysteine residue (C51) for accepting S^0 and does not bind a nascent Fe–S cluster [20]. SufE then further traffics the S^0 to SufB within the $SufBC_2D$ scaffold complex where the nascent cluster is assembled [21]. SufE enhances the cysteine desulfurase activity of SufS, although the exact mechanism of enhancement is unclear. $SufBC_2D$ further increases SufE-dependent enhancement of SufS via an unknown mechanism [20]. In contrast, IscU was recently shown to not enhance the desulfurase activity of IscS [26].

To determine if sulfur trafficking by the Suf pathway is more resistant to oxidative stress than the Isc pathway, we directly compared the oxidative stress resistance of the SufS–SufE sulfur transfer pathway to that of the *E. coli* IscS–IscU system. We discovered that SufS–SufE are more active than IscS–IscU at physiological concentrations of L-cysteine and that SufS–SufE activity is more resistant to H_2O_2 exposure than IscS–IscU. Furthermore, IscS and IscU

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are more sensitive to oxidative modification by H_2O_2 than SufS and SufE. The functional ramifications of these results for defining the relative roles of Isc and Suf are discussed.

2. Materials and methods

See Supplementary data.

3. Results

3.1. Kinetic analysis of SufS activity in the presence of SufE

Native SufS, SufE, $SufBC_2D$, IscS, and IscU proteins were purified to homogeneity and PLP cofactor occupancy was greater than 90% for IscS and SufS (Supplementary Fig. 1). Using 2 mM L-cysteine with 2 mM DTT, SufS liberated 2.6 nmol of S^{2-} min^{-1} mg^{-1}, which is 20 times lower than IscS (51.7 nmol of S^{2-} min^{-1} mg^{-1}) (Supplementary Fig. 2). Based on this low turnover number, under these reaction conditions SufS is hardly able to qualify as a bona fide enzyme. Previously, activities of 19 nmol of S^{2-} min^{-1} mg^{-1} for SufS and 380 nmol of S^{2-} min^{-1} mg^{-1} for IscS were measured using 12 mM cysteine and 50 mM DTT [14]. Under the same conditions used in the previous study, we observed activities of 7.9 nmol of S^{2-} min^{-1} mg^{-1} for SufS and 312.8 nmol of S^{2-} min^{-1} mg^{-1} for IscS (data not shown). Addition of 4 molar equivalents of SufE (adding 2 μM SufE to 0.5 μM SufS) increases SufS activity to 41.9 nmol of S^{2-} min^{-1} mg^{-1} so that it is comparable to IscS (Supplementary Fig. 2). Further addition of 4 molar equivalents of the $SufBC_2D$ complex (2 μM $SufBC_2D$ complex) to SufS and SufE further enhanced SufS activity to 172.6 nmol of S^{2-} min^{-1} mg^{-1}, making SufS a more efficient sulfur mobilization enzyme than IscS under these conditions (Supplementary Fig. 2). In agreement with recently published reports, we found that IscU, the sulfur receptor for IscS, did not enhance IscS activity under these conditions (Supplementary Fig. 2) [26].

SufS removes sulfur from L-cysteine and forms persulfide (S^0) on the active site residue C364. The persulfide intermediate of *E. coli* SufS directly transfers the sulfur atom to residue C51 of SufE and SufS activity is enhanced specifically by SufE [19,20]. To further probe the SufS–SufE reaction, we performed kinetic analyses of *E. coli* SufS while varying both components, L-cysteine and SufE, using the methylene blue assay to quantify sulfide production [20]. This in vitro reaction requires a non-physiological reductant (such as DTT) to reduce persulfide (S^0) to sulfide (S^{2-}) on SufS and SufE thereby allowing the sulfide to react with DMPD. The concentration of cysteine was varied from 0 to 500 μM in the presence of 4 μM SufE (Fig. 1A) while the concentration of SufE was varied from 0 to 15 μM SufE at a fixed 2 mM concentration of L-cysteine (Fig. 1B). Under these conditions, SufS showed Michaelis–Menten enzyme kinetics for L-cysteine and SufE as its two substrates. The kinetic parameters are listed in Table 1. Previous studies of the *Erwinia chrysanthemi* SufS–SufE reported that the SufS–SufE K_m for L-cysteine was 500 μM and the V_{max} = 900 mU/mg, which are both higher than the values measured for *E. coli* SufS–SufE (Table 1) suggesting that the *E. coli* system has a higher affinity for the L-cysteine substrate but is a somewhat slower system [18]. We also found that SufE where C51 has been covalently blocked with iodoacetamide ($SufE_{alk}$) was able to inhibit SufS activity in the presence of unalkylated SufE with a K_i of 0.19 μM (Supplementary Fig. 3). This inhibition occurred regardless of the presence of the $SufBC_2D$ complex.

3.2. SufS displays non-Michaelis–Menten kinetics at low but physiological cysteine concentrations

SufS activity deviated from Michaelis–Menten enzyme kinetics when it was measured as a function of different concentrations of SufE but over a wider range of fixed L-cysteine levels (10 μM–20 mM) (Fig. 2A). At L-cysteine concentrations below 300 μM, increasing the concentration of SufE actually decreased sulfide formation by SufS (Fig. 2A). As long as the L-cysteine concentration remained at 500 μM or higher the inhibition by SufE was not observed and SufS showed Michaelis–Menten kinetics (compare Figs. 1B and 2A). Intracellular L-cysteine concentrations in *E. coli* are variable depending on growth conditions but can often be in the range of 100–200 μM [27], which is below the mM levels often used for in vitro cysteine desulfurase enzyme assays, so the deviation of SufS–SufE from Michaelis–Menten behavior under these conditions may be physiologically relevant.

To test whether inhibition by SufE affects $SufBC_2D$ enhancement of SufS at lower cysteine concentrations, we assayed $SufBC_2D$ enhancement at 50 μM cysteine where SufE showed inhibition of SufS (Fig. 2B). For comparison $SufBC_2D$ enhancement at 2 mM L-cysteine (where SufE inhibition does not occur) is also shown in Fig. 2B. The enhancement normally provided by the $SufBC_2D$ complex diminished as the fixed concentration of SufE increased, in stark contrast to the $SufBC_2D$-dependent enhancement seen at higher L-cysteine levels (Fig. 2B). These results indicate that

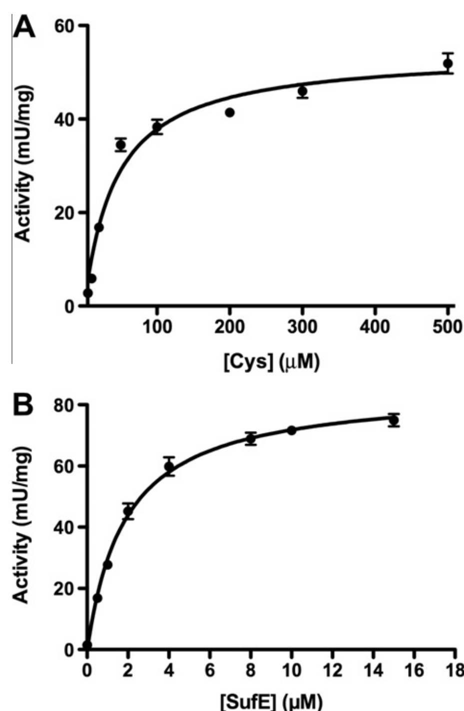

Fig. 1. Kinetic analysis of SufS activity in response to varied substrate concentrations. The reactions contained (A) 0.5 μM SufS, 4 μM SufE, 2 mM DTT and 10–500 μM L-cysteine or (B) 0.5 μM SufS, 0–15 μM SufE, 2 mM DTT, and 2 mM L-cysteine. The lines are the best fits to the Michaelis–Menten equation obtained using GraphPad Prism. A unit of activity is defined as one micromole of sulfide formation by the desulfurase enzyme per minute. Activity is shown as mU per mg of enzyme.

Table 1
Kinetic parameters of the SufS cysteine desulfurase.

	Cysteine dependent[a]	SufE dependent[b]
K_m (μM)	43.5 ± 5.8	1.9 ± 0.1
V_{max} (mU/mg)	54.3 ± 1.9	85.4 ± 1.8
R^2	0.95	0.99

[a] Reaction conditions were: 0.5 μM SufS, 4 μM SufE, 2 mM DTT and 5–500 μM L-cysteine.
[b] Reaction conditions were: 0.5 μM SufS, 0–15 μM SufE, 2 mM DTT, and 2 mM L-cysteine.

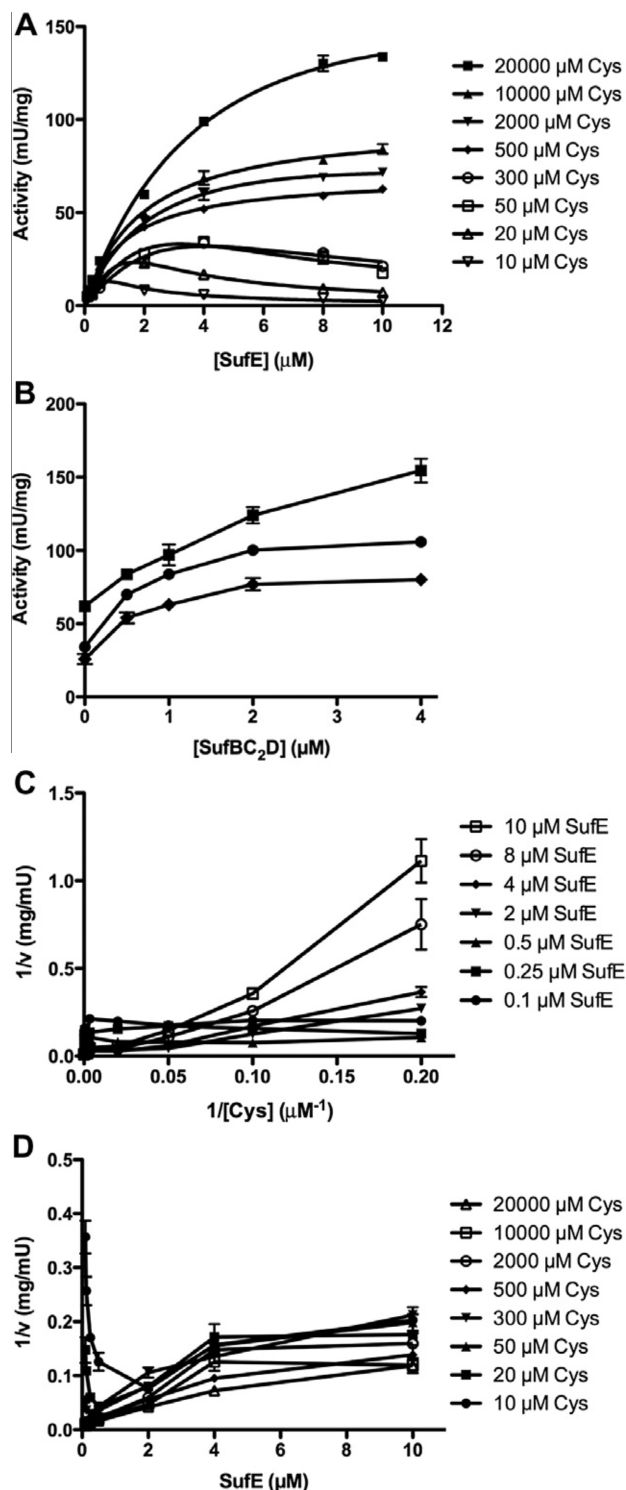

Fig. 2. Inhibition of SufS by SufE at lower concentrations of L-cysteine. (A) The reactions contain 0.5 μM SufS, 0–10 μM SufE, 2 mM DTT, and 10–20,000 μM L-cysteine (see embedded legend). (B) The reactions contain 0.5 μM SufS, 50 μM cysteine, 2 mM DTT, 4 μM (●) or 8 μM (♦) SufE with increasing concentrations of $SufBC_2D$ (0–4 μM). A control reaction with 2 mM cysteine, 2 mM DTT, 0.5 μM SufS, and 8 μM SufE with increasing concentrations of $SufBC_2D$ (0–4 μM) is also shown (■). Double reciprocal plots of kinetic data. Activity of 0.5 μM SufS, 2 mM DTT, and (C) varied 10–20,000 μM L-cysteine at several fixed concentrations of SufE or (D) varied 0.1–10 μM SufE at several fixed concentrations of L-cysteine. See embedded legend for symbol explanations.

$SufBC_2D$ cannot reverse the SufE inhibition of SufS that is seen at low cysteine concentrations.

The double reciprocal transformations of the kinetic data clearly show the SufS deviation from Michaelis–Menten behavior at lower cysteine concentrations (Fig. 2C and D). At low fixed SufE concentrations, parallel lines are observed when initial velocity as a function of L-cysteine is plotted (Fig. 2C). As the fixed concentration of SufE becomes inhibiting (2 μM SufE and above), the slopes of the reciprocal plots increase and the lines begin to cross at high L-cysteine concentrations (approaching the $1/v$ axis) as the SufE concentration approaches the substrate inhibition K_i (Fig. 2C). Similarly, when L-cysteine is fixed at concentrations below 500 μM and initial velocity is plotted as a function of SufE, we observed that as SufE concentration increases (approaching the $1/v$ axis), the initial velocity sharply decreases (turns sharply upward) (Fig. 2D). The activity plot and double reciprocal plots are qualitatively similar to those of *O*-acetylserine sulfhydrylase, a PLP-dependent enzyme that reacts via a ping-pong mechanism with substrate inhibition [28]. We attempted to fit our data with the appropriate rate equation for this type of substrate inhibition [28]. Unfortunately the quality of the fit was insufficient to instill confidence in the values for the substrate inhibition constant and other kinetic constants (data not shown). This leaves open the question of whether SufE inhibition is due to substrate inhibition. Previously it was shown that *E. coli* SufS itself (even in the absence of SufE) deviates from Michaelis–Menten kinetics, which may explain the difficulty in fitting the rate equation described for other enzymes [14].

3.3. The SufS–SufE system is more active at physiological cysteine concentrations than IscS and IscS–IscU

Next we directly compared the efficiency of the SufS–SufE sulfurtransferase system to that of the *E. coli* IscS and IscS–IscU proteins under the same conditions. The desulfurase activities of SufS–SufE, IscS alone, and IscS–IscU were measured at different concentrations of L-cysteine. A 1:3 molar ratio of SufS to SufE or IscS to IscU was used throughout. At a 1:3 molar ratio of SufS (0.5 μM) to SufE (1.5 μM), SufE does not show measurable inhibition of SufS activity over the range of L-cysteine concentrations used (30 μM–10 mM). For ease of comparison, the activity of SufS–SufE at each L-cysteine concentration was divided by the activity of IscS alone or IscS–IscU measured under the same conditions and these activity ratios were plotted as a function of L-cysteine (Fig. 3). For the activity ratios generated by these calculations, values greater than one indicate that SufS–SufE have a higher activity than IscS or IscS–IscU at those specific L-cysteine concentrations (Fig. 3). This comparison reveals that the SufS–SufE system has higher cysteine desulfurase activity than IscS or the IscS–IscU system at physiological L-cysteine concentrations (up to

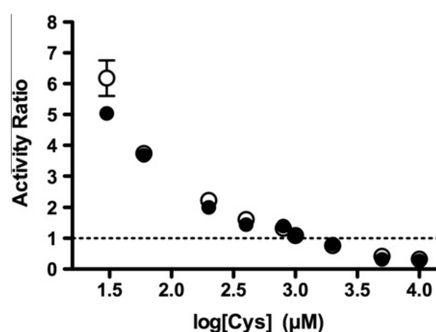

Fig. 3. Direct activity comparison of the SufS–SufE and IscS–IscU sulfur transfer systems. SufS–SufE activity was divided by IscS activity (closed circles ●) or the IscS–IscU activity (open circles ○) and the ratios were plotted as a function of the L-cysteine concentration in the reaction. The reactions contain 0.5 μM SufS or IscS, 1.5 μM SufE or IscU, and 0.03–10 mM L-cysteine and DTT.

200 μM). At 30 μM L-cysteine SufS–SufE activity was 6-fold higher than IscS or the IscS–IscU system and remained at least 2-fold higher until the L-cysteine concentration exceeded 200 μM. Only at high L-cysteine concentrations above 1 mM did IscS or the IscS–IscU system begin to exceed SufS–SufE activity. These results also showed no activity difference between IscS alone compared to the IscS–IscU mixture over the range of L-cysteine tested (Fig. 3).

3.4. IscS–IscU activity is more sensitive to H_2O_2 exposure than SufS–SufE

The Suf pathway is activated to build Fe–S clusters during oxidative stress in *E. coli* and deletion of the *suf* operon causes disruption of Fe–S cluster biosynthesis by oxidative stress [7–9,29,30]. In contrast, the Isc system is unable to carry out Fe–S cluster assembly in vivo upon exposure to reactive oxygen species like H_2O_2 [24]. Active site cysteine residues and persulfide intermediates in sulfur trafficking may react with oxidants like H_2O_2 depending on their exact pK_a values [22,23]. To test if the SufS–SufE or IscS–IscU sulfur trafficking pathways are maintained under oxidative stress, we compared their relative in vitro H_2O_2 sensitivity. It is difficult to test for H_2O_2 sensitivity in the present of DTT due to the propensity for DTT itself to react with and consume H_2O_2 and the ability of DTT to reverse some H_2O_2-mediated thiol oxidation products, such as sulfenic acid [31,32]. Therefore the desulfurase reactions were carried out in the presence of H_2O_2 but in the absence of DTT under anaerobic conditions (Fig. 4). Since the SufE and IscU sulfur acceptors may not be as efficiently recycled in the absence of DTT (see above), they were used in a 10:1 excess over SufS and IscS. The concentration of L-cysteine was increased to 2 mM to ensure adequate activity could be measured in the presence of H_2O_2. Interestingly, in the absence of DTT, excess IscU was now able to enhance IscS desulfurase activity by 1.5-fold (Fig. 4A). This result suggests that if DTT is present it will normally outcompete IscU to release persulfide from IscS and explains why IscU enhancement is not usually observed in the unmodified assay where DTT is present.

Using this modified assay, we found that as the H_2O_2 concentration increased from 0 to 400 μM, sulfide production by IscS and IscS–IscU decreased by 50% or more (Fig. 4). In contrast, sulfide production by SufS–SufE only decreased by about 10–15%. The percent decrease in IscS–IscU activity was greater than the percent decrease in the activity of IscS alone, suggesting that IscU enhancement of IscS is largely abolished in response to H_2O_2, possibly due to oxidative damage to IscU (Fig. 4B). Furthermore, total sulfide production by SufS–SufE was always from 3- to 9-fold higher than IscS or IscS–IscU throughout the entire range of H_2O_2 concentrations used (Fig. 4A). Together these results demonstrate that SufS–SufE sulfide production is more resistant to oxidative stress exposure than sulfide production by IscS or IscS–IscU.

3.5. Oxidation of IscS–IscU and SufS–SufE residues after H_2O_2 exposure

The decrease in IscS and IscS–IscU activity in response to H_2O_2 suggests that important active site residues or reaction intermediates are damaged by oxidative stress. To map the sites of oxidation in the Isc and Suf sulfur transfer proteins, anaerobic cysteine desulfurase reactions were carried out in the presence of H_2O_2 as described above (in the absence of DTT) except that the reactions were quenched and trapped by the addition of tricholoroacetic acid (TCA) rather than by heating. TCA-trapped samples were alkylated, trypsinized, and analyzed by LC–MS without any further reduction steps as described in Supplementary data. Oxidation of the active site Cys residues C328 from IscS, C51 from SufE, and Cys 364 from SufS as well as conserved C63 and C106 in IscU were confirmed by MS/MS analysis of those peptides. The different oxidative modifications detected for the active site Cys residues or their reaction intermediates are summarized in Supplementary Table 2. Using this protocol, stable sulfenic acid modifications were not observed but the more stable sulfinic and sulfonic acid oxidation products were detected. The *m*/*z* peak areas for each modified peptide were separately quantified (see Supplementary data). For ease of comparison, the signal intensity for the oxidized forms of each specific Cys-containing peptide were pooled and divided by the total signal intensity for all forms of that Cys-containing peptide (Fig. 5). These values can be used for relative comparisons between samples.

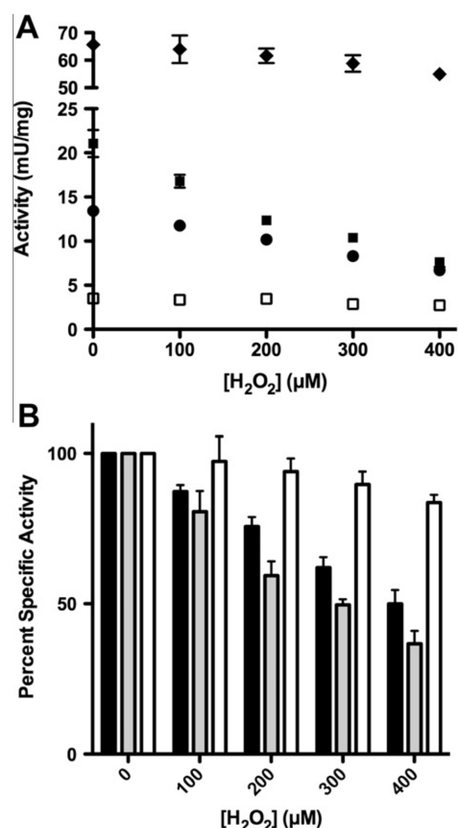

Fig. 4. The sensitivity of SufS–SufE and IscS–IscU to H_2O_2 during the cysteine desulfurase reaction. 1 μM SufS or IscS and (where indicated) 10 μM SufE or IscU were mixed for 5 min. 2 mM L-cysteine was added to initiate the reaction followed immediately by 0–400 μM H_2O_2. After 30 min the reaction was quenched by heating at 95 °C for 5 min, followed by the addition of 2 mM DTT to reduce and release sulfide for measurement as described in Section 2. All steps were carried out anaerobically. (A) Desulfurase activity of SufS (□), IscS (●), IscS–IscU (■) and SufS–SufE (♦). (B) Percent activity of IscS (black bar), IscS–IscU (light grey bar), and SufS–SufE (white bar) compared to their activity without H_2O_2.

For IscS treated with 400 μM H_2O_2, peptides with oxidative modification to the active site C328 accounted for 54% of the total signal intensity (Fig. 5A), in rough agreement with the decrease in IscS activity observed under the same conditions (Fig. 4). IscS C328 was more protected when IscU was added since the oxidized forms of C328 only represented 16% of the total signal intensity in that sample. In contrast, the total oxidation of IscU C63 by 400 μM H_2O_2 was 40% for IscU alone and 52% for IscU in the presence of IscS. IscU C106 was fairly similar with 58% oxidation for IscU alone and 46% for IscU in the presence of IscS (Fig. 5A). These results show that IscS is sensitive to oxidation by H_2O_2 during the desulfurase reaction cycle. While IscU seems to help prevent direct oxidation of IscS C328, probably by binding to and protecting IscS, we did detect disulfide bond formation between IscS–IscU (see below), which was not directly quantified by MS. IscU itself is oxidized by H_2O_2 even if IscS is present. Oxidized IscU can no longer enhance

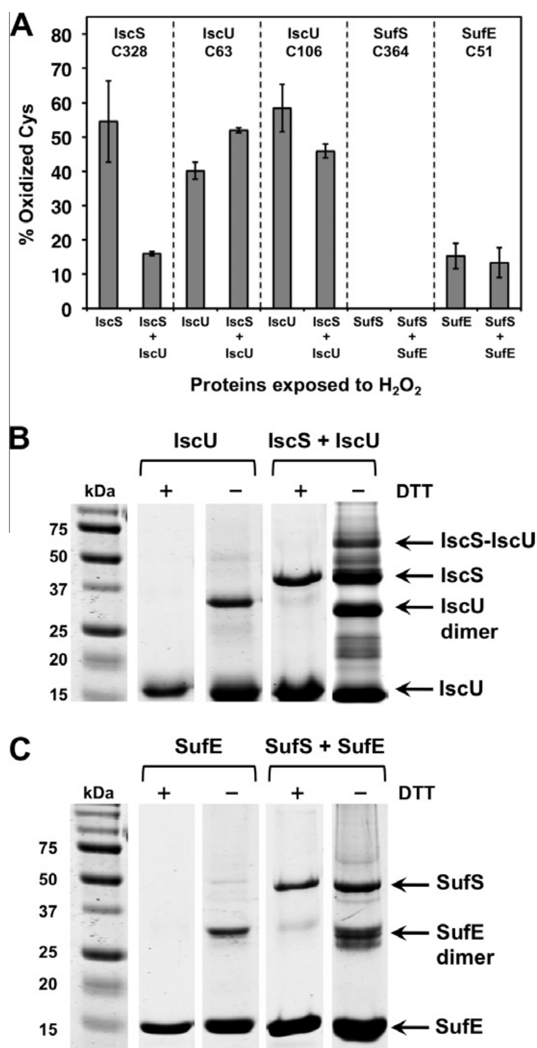

Fig. 5. (A) Percent oxidation of active site Cys residues in the IscS, IscU, SufS, and SufE proteins after H_2O_2 exposure during the cysteine desulfurase reaction. (B) and (C) Reducing and non-reducing 12% SDS–PAGE gel separation of H_2O_2 treated proteins. The proteins were treated the same way as the samples for mass spectrometry analysis (see text). Proteins were precipitated with 10% TCA and dissolved in 1X SDS loading buffer with or without DTT. Samples were heated at 95 °C for 10 min before loading on the gel. (B) IscU and IscS–IscU gel separation. (C) SufE and SufS–SufE gel separation.

IscS activity and may also actively decrease IscS activity by acting as an inhibitor that competes with undamaged IscU for access to IscS. The percent oxidation of IscU C63 and C106 correlates with the percent decrease in IscS–IscU activity upon exposure to 400 μM H_2O_2 (Fig. 4).

In contrast to IscS, oxidized forms of the peptides containing the SufS active site C364 were not detected after H_2O_2 exposure under these conditions, indicating that residue has intrinsic resistance to oxidative damage (Fig. 5A). Peptides with oxidative modification to the SufE active site C51 were observed but only accounted for 15% of total signal in the absence of SufS and 13% in the presence of SufS. The generally lower levels of Cys oxidation in the SufS and SufE proteins correlate with their higher activity in the presence of H_2O_2 (Fig. 4).

Disulfide bond formation is another potential consequence of H_2O_2 oxidation of Cys thiolates. The MS analysis was conducted without a reduction step (to allow detection of oxidized sulfane sulfur species) and may not adequately detect disulfide-bonded fragments, which tend to poorly ionize. Therefore, we also analyzed each oxidized sample qualitatively for the formation of mixed disulfides. After 400 μM H_2O_2 treatment, TCA-trapped samples were resuspended and separated by SDS–PAGE under both reducing (+DTT) and non-reducing (−DTT) conditions (Fig. 5B and C). Regardless of H_2O_2 treatment, no high molecular weight species were detected for SufS and IscS alone and each protein migrated at its monomer molecular weight irrespective of DTT addition (data not shown). However, both SufE and IscU form disulfide bonded homodimers that are clearly delineated in the non-reducing gel (Fig. 5B and C). Quantification of the intensity of the gel bands indicates that the relative level of SufE homodimer is fairly constant at about 18% of the total protein regardless of the addition of H_2O_2 (data not shown and Fig. 5C). In contrast, the relative amount of IscU homodimer increases from 12% to 28% of total IscU protein upon exposure to H_2O_2. In the samples containing both IscS and IscU, the IscU homodimer increased to 39% of total IscU protein and we also observed the appearance of a new higher molecular weight species that runs at the expected size for a covalent IscS–IscU heterodimer. The IscS–IscU heterodimer band was excised from the gel and analyzed by mass spectrometry. The mass spectrometry results showed the presence of an H_2O_2-induced disulfide or polysulfide bond between IscS C328 and IscU C63, which is consistent with previous studies [33]. A disulfide bonded SufS–SufE heterodimer was not observed under our experimental conditions although small amounts of such a species have been seen for ^{35}S-labeled SufS–SufE analyzed on a non-reducing gel [20]. Based on these results it appears that upon exposure to H_2O_2, both IscU and the IscS–IscU complex have a greater propensity to form covalently linked dimers compared to SufE and the SufS–SufE complex, providing an additional mechanism by which IscS activity may be inhibited by H_2O_2 exposure. While we did not absolutely quantify the total levels of IscU-IscU and IscS–IscU disulfide linked dimers, their formation would likely contribute to the decrease in IscS–IscU activity observed upon H_2O_2 treatment.

4. Discussion

4.1. Substrate inhibition of SufS by SufE may be a physiological adaptation

Using label transfer assays and surface plasmon resonance measurements we previously showed that SufS–SufE interact in the absence of L-cysteine with a K_D of 0.36 μM [21]. Furthermore, previous yeast two-hybrid experiments indicate that the SufS C364S mutant, which cannot form a persulfide intermediate, interacts as well with SufE as the wild type SufS [34]. These published studies confirm that SufE interacts strongly with SufS regardless of SufS persulfide state, which is consistent with the potential substrate inhibition we observe at lower L-cysteine levels. Substrate inhibition by SufE could be a mechanism to limit SufS activity when cellular L-cysteine pools drop below a critical threshold. Measurable inhibition by SufE begins to occur if L-cysteine levels drop below 500 μM and if the ratio of SufE:SufS simultaneously increases beyond 4:1(which in the experiment is 2 μM SufE to 0.5 μM SufS). Depending on the exact in vivo ratio of SufE:SufS, which has not currently been measured, substrate inhibition may occur in vivo. Further experiments are necessary to fully explore this enzymatic behavior and its physiological relevance.

4.2. SufS–SufE provide a more robust sulfur transfer system than the Isc pathway

We found that SufS–SufE has higher cysteine desulfurase activity than IscS or IscS–IscU at physiological L-cysteine concentrations

(200 μM and below), especially if the SufE:SufS ratio is maintained at 3:1 or lower. The higher activity of SufS–SufE at lower cysteine concentrations may be physiologically important for its oxidative stress resistance. Cysteine biosynthetic genes are upregulated under oxidative stress possibly to replenish free cysteine used for glutathione biosynthesis or replacement of oxidized protein thiols [35,36]. There is also evidence that L-cysteine is actively exported to the periplasm during oxidative stress to protect that sub-cellular compartment [37]. Since SufS–SufE has a higher desulfurase activity than IscS–IscU at lower cysteine concentrations the Suf system may be better able to maintain Fe–S cluster biosynthesis under conditions where L-cysteine availability decreases.

We observed a pronounced activity difference between the Isc and Suf sulfur trafficking proteins when they were exposed to H_2O_2 during the cysteine desulfurase reaction cycle. Under these conditions, IscS and IscS–IscU activity was inhibited while SufS–SufE activity was largely resistant to the H_2O_2 stress. Similar results were obtained when the resting proteins were exposed to H_2O_2 stress prior to initiating the desulfurase reaction (Supplementary Fig. 4). MS analysis of the proteins shows that during enzyme turnover the active site Cys residues of IscS and IscU are sensitive to oxidation, forming dead-end sulfinic and sulfonic acid species as well as mixed disulfide heterocomplexes. In contrast, active site C364 of SufS remained unmodified throughout the stress. In addition, MS analysis revealed that the highly reactive S^0 persulfide intermediates on IscS, IscU, and SufE, could also react with H_2O_2 to form cysteine-*S*-sulfonate derivatives (Supplementary Table 2). This is not surprising given that persulfides tend to have lower pK_a values than thiols, making them an "activated" form of sulfur that could readily react with oxidants. Indeed in some organisms a cysteinyl persulfide is the substrate for enzymatic sulfur-oxidation rather than elemental sulfur (S_8) and is oxidized to a cysteine-*S*-sulfonate derivative as part of the reaction cycle [38–40]. The relative stress resistance of the SufS–SufE system indicates that the active site Cys thiolates and persulfide intermediates for this sulfur transfer pathway are partially protected from reactive oxygen species compared to IscS–IscU.

In summary, the results above show that the SufS–SufE and SufS–SufE -SufBC$_2$D (see Supplementary Fig. 4C) sulfur transfer partners maintain higher desulfurase activity upon exposure to oxidative stress than the analogous IscS and IscS–IscU systems. The robust activity of SufS–SufE at physiological cysteine concentrations, coupled with the resistance of SufS–SufE activity to oxidative stress, indicate that the *E. coli* Suf pathway is well-suited to carry out Fe–S cluster biogenesis when it is induced under stress conditions.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2012.10.001.

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