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Deletion of the Proposed Iron Chaperones IscA/SufA Results in Accumulation of a Red Intermediate Cysteine Desulfurase IscS in *Escherichia coli*[*]

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Background: Iron-sulfur cluster biogenesis requires the coordinated delivery of iron and sulfur.
Results: Deletion of IscA/SufA or depletion of intracellular iron produces a red-colored cysteine desulfurase IscS in *Escherichia coli* cells.
Conclusion: Deficiency of accessible iron results in the accumulation of red IscS in cells.
Significance: IscA/SufA may work in concert with IscS in delivering iron and sulfur for iron-sulfur cluster biogenesis.

In *Escherichia coli*, sulfur in iron-sulfur clusters is primarily derived from L-cysteine via the cysteine desulfurase IscS. However, the iron donor for iron-sulfur cluster assembly remains elusive. Previous studies have shown that, among the iron-sulfur cluster assembly proteins in *E. coli*, IscA has a unique and strong iron-binding activity and that the iron-bound IscA can efficiently provide iron for iron-sulfur cluster assembly in proteins *in vitro*, indicating that IscA may act as an iron chaperone for iron-sulfur cluster biogenesis. Here we report that deletion of IscA and its paralog SufA in *E. coli* cells results in the accumulation of a red-colored cysteine desulfurase IscS under aerobic growth conditions. Depletion of intracellular iron using a membrane-permeable iron chelator, 2,2′-dipyridyl, also leads to the accumulation of red IscS in wild-type *E. coli* cells, suggesting that the deletion of IscA/SufA may be emulated by depletion of intracellular iron. Purified red IscS has an absorption peak at 528 nm in addition to the peak at 395 nm of pyridoxal 5′-phosphate. When red IscS is oxidized by hydrogen peroxide, the peak at 528 nm is shifted to 510 nm, which is similar to that of alanine-quinonoid intermediate in cysteine desulfurases. Indeed, red IscS can also be produced *in vitro* by incubating wild-type IscS with excess L-alanine and sulfide. The results led us to propose that deletion of IscA/SufA may disrupt the iron delivery for iron-sulfur cluster biogenesis, therefore impeding sulfur delivery by IscS, and result in the accumulation of red IscS in *E. coli* cells.

In the past decade, a group of highly conserved proteins has been identified as essential for iron-sulfur cluster biogenesis in bacteria (1, 2) and eukaryotes (3). Among these identified proteins, cysteine desulfurases (NifS (4), IscS (5, 6), and SufS (7)) catalyze the desulfurization of L-cysteine and provide sulfur for iron-sulfur cluster assembly in the scaffold proteins IscU (8–10) or the SufBCD complex (11, 12). The scaffold proteins then transfer the assembled clusters to target proteins. Two heat shock cognate proteins, HscB and HscA, interact with the scaffold protein IscU and regulate the iron-sulfur cluster transfer from IscU to target proteins (10, 13, 14). Specific glutaredoxins are also involved in storing and transporting iron-sulfur clusters from scaffold proteins to target proteins (15–17). Nevertheless, the iron donor for iron-sulfur cluster biogenesis remains elusive. Frataxin, a mitochondrial protein associated with the human neurodegenerative disease Friedreich ataxia (18), has been proposed previously as a putative iron chaperone for iron-sulfur cluster biogenesis (19, 20). Frataxin is highly conserved from bacteria to humans and interacts with iron-sulfur protein aconitase (21), mitochondrial electron transfer components (22), and the iron-sulfur cluster assembly proteins IscS (23–27) and IscU (28). However, frataxin has a weak iron-binding activity under physiological conditions (29–31), and deletion of frataxin has little or no effect on iron-sulfur proteins in *Saccharomyces cerevisiae* (32), *Salmonella enterica* (33), and *Escherichia coli* (34). Only in iron-rich medium does the deletion of frataxin have a mild effect on iron-sulfur proteins in *E. coli* cells (26). It has therefore been postulated that frataxin may act as a gatekeeper to regulate iron-sulfur cluster biogenesis by activating cysteine desulfurase (27) or directing the sulfur flux from cysteine desulfurase (23, 25).

Among the iron-sulfur cluster assembly proteins encoded by the gene cluster *iscSUA-HscBA-fdx-iscX* in *E. coli* (1), IscX has also been proposed as a possible iron donor for iron-sulfur cluster biogenesis (35). However, like frataxin, IscX has a weak iron-binding activity (36), and deletion of IscX has very little effect on iron-sulfur proteins in *E. coli* cells (26), indicating that IscX may have functions other than directly providing iron for iron-

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sulfur cluster assembly under physiological conditions. In contrast, IscA, a proposed alternative scaffold protein (37–39), has a unique and strong iron-binding activity (40–44) with an iron association constant of $\sim 3.0 \times 10^{19}$ M^{-1} (45, 46). The iron center in IscA can be readily mobilized by L-cysteine (43, 44, 47) and transferred for iron-sulfur cluster assembly in proteins *in vitro* (43, 48). These results led us to postulate that IscA may recruit intracellular iron and deliver iron for iron-sulfur cluster biogenesis. It has also been reported that depletion of IscA in *Azotobacter vinelandii* results in a null-growth phenotype in modified Burks minimal medium under elevated oxygen conditions (49). In *E. coli* cells, deletion of IscA and its paralog SufA has the same null-growth phenotype in M9 minimal medium under aerobic conditions (50, 51). In *S. cerevisiae*, depletion of IscA homologs leads to accumulation of iron in mitochondria and dependence on lysine and glutamate for cell growth (52, 53). In cultured human cells, depletion of IscA1 significantly decreases iron-sulfur cluster assembly activity in mitochondria and in the cytosol (54). Recent studies also revealed that IscA and its homologs have an essential role in [4Fe-4S] cluster assembly in *E. coli* (55), *S. cerevisiae* (42), and human cells (56) under aerobic conditions.

If IscA and its paralog SufA act as iron chaperones (48), deletion of IscA and SufA would disrupt iron delivery for iron-sulfur cluster biogenesis, therefore impeding sulfur delivery by the cysteine desulfurase IscS, a major sulfur donor in *E. coli* cells (6). In this study, we find that deletion of IscA and SufA indeed results in the accumulation of a red-colored IscS in *E. coli* cells under aerobic growth conditions. Red IscS also accumulates in wild-type *E. coli* cells in which intracellular iron is depleted by using a membrane-permeable iron chelator 2,2′-dipyridyl, suggesting that deletion of IscA/SufA can be emulated by depleting intracellular iron in *E. coli* cells. Purified red IscS has a distinct absorption peak at 528 nm in addition to the absorption peak at 395 nm of pyridoxal 5′-phosphate (5, 6). When purified red IscS is oxidized by H_2O_2, the absorption peak at 528 nm is shifted to 510 nm, which is reminiscent of an alanine-quinonoid intermediate in cysteine desulfurases from other organisms (4, 57). The results suggest that deletion of IscA/SufA or depletion of intercellular iron in *E. coli* cells leads to accumulation of red IscS, which likely contains a highly conjugated quinonoid intermediate, and that the proposed iron chaperones IscA/SufA and cysteine desulfurase IscS may work in concert in delivering iron and sulfur for iron-sulfur cluster biogenesis.

Experimental Procedures

Protein Purification—Recombinant *E. coli* IscS was expressed in the *E. coli iscA/sufA* mutant or its parental wild-type strain MC4100 cells. *E. coli iscA/sufA* mutant was constructed as described previously (50). The *E. coli* gene encoding IscS was cloned to an expression plasmid, pBAD (Life Technologies). The *E. coli* cells hosting the expression plasmid were grown in LB (Luria-Bertani) medium to $A_{600\ nm}$ of 0.6 under aerobic conditions. Arabinose (at a final concentration of 0.02%) was then added to the cell cultures to induce the expression of recombinant IscS in the cells. IscS was purified as described in Ref. 48. The purity of purified IscS was over 95%, judging from the SDS-polyacrylamide gel electrophoresis staining with Coomassie Brilliant Blue. The protein concentration of IscS was measured from the absorption peak at 280 nm using an extinction coefficient of 39.8 $mM^{-1}cm^{-1}$. The UV-visible absorption spectra were recorded using the Beckman DU640 UV-visible spectrometer equipped with a temperature controller.

Site-directed Mutagenesis of E. coli IscS—Two pairs of primers (IscS-K206A-1, 5′-CTTTCTCCGGTCACGCAATCTATGGCCCG-3′; IscS-K206A-2, 5′-CGGGCCATAGATTGCGTGACCGGAGAAAG-3′; IscS-C328S-1, 5′-CAGTTTCTTCAGGTTCCGCCTCTACGTCAGCAAGCC-3′; IscS-C328S-2, 5′-GAGGCGGAACCTGAAGAAACTGCGAGGTCTTTCAGC-3′) were synthesized for construction of IscS mutants IscS-K206A and IscS-C328S, respectively. The site-directed mutagenesis of IscS was carried out using the QuikChange kit (Agilent Technologies), and the mutations were confirmed by direct sequencing (Eurofins Co.).

Treatment of 2,2′-Dipyridyl—Different amounts of 2,2′-dipyridyl were added to LB medium before the overnight *E. coli* culture was inoculated. Cells were grown at 37 °C under aerobic conditions. The cell growth was measured after cells were washed once with fresh LB medium to remove 2,2′-dipyridyl.

Cysteine Desulfurase Activity Assay—Cysteine desulfurase activity of purified *E. coli* IscS was measured using the sulfide detection method by Siegel (58). Briefly, purified cysteine desulfurase (1 μM) was incubated with L-cysteine (1 mM) in buffer containing Tris (20 mM (pH 8.0)), NaCl (200 mM), and dithiothreitol (2 mM) at 37 °C for 10 min. Reactions were terminated by addition of *N,N*-dimethyl-*p*-phenylene-diamine sulfate (20 mM) (in 7.2 N HCl) and $FeCl_3$ (30 mM) (in 1.2 N HCl). Color was allowed to develop for 20 min at room temperature before quantifying methylene blue at 670 nm. Freshly prepared Na_2S was used as the standard for sulfide quantification in the enzyme reaction.

Results

Deletion of IscA/SufA Results in the Accumulation of a Red-colored IscS in E. coli Cells—To explore the physiological interplay between IscA, a putative iron donor (44), and IscS, a major sulfur donor for iron-sulfur cluster biogenesis (6), we expressed recombinant IscS in the *E. coli* wild type and *iscA/sufA* mutant cells grown in LB medium under aerobic conditions. Fig. 1 shows that, although IscS purified from wild-type *E. coli* cells is yellow (indicative of pyridoxal 5′-phosphate) (5), IscS purified from the *iscA/sufA* mutant cells is bright red. In parallel experiments, IscS was also expressed in *E. coli* mutant cells in which the scaffold protein IscU (8–10) was deleted. Unlike in *iscA/sufA* mutant cells, IscS expressed in *iscU* mutant cells has the same yellow color as that produced in wild-type *E. coli* cells (data not shown), suggesting that deletion of IscU does not significantly affect IscS in *E. coli* cells under aerobic conditions.

UV-visible absorption measurements showed that IscS purified from *E. coli iscA/sufA* mutant cells has a distinct absorption peak at 528 nm in addition to the absorption peak at 395 nm of pyridoxal 5′-phosphate (5, 6) (Fig. 1). Metal content analyses showed that IscS proteins purified from wild-type or *iscA/sufA* mutant cells did not contain any detectable amounts of transition metals (data not shown), suggesting that the red color of IscS is not due to the metal binding in the protein.

Formation of Red IscS in E. coli iscA/sufA Mutant Cells Requires an Active Catalytic Site in IscS—To examine whether the formation of red IscS in the *E. coli iscA/sufA* mutant cells is an enzymatic process, we constructed two IscS mutants, IscS-C328S and IscS-K206A, by site-directed mutagenesis. Cys-328 in IscS directly attacks substrate L-cysteine (4), whereas Lys-206 is the ligand for pyridoxal 5′-phosphate (5). Both mutant proteins were expressed in wild-type *E. coli* cells grown in LB medium under aerobic conditions. As expected, purified IscS-C328S and IscS-K206A are not active to catalyze the desulfurization of L-cysteine (data not shown). UV-visible absorption measurements showed that purified IscS-C328S has an absorption peak at 395 nm of pyridoxal 5′-phosphate (Fig. 2*A*), which is identical to that of wild-type IscS (Fig. 1). To our surprise, purified IscS-K206A has two new absorption peaks, at 338 nm and 428 nm (Fig. 2*A*). Nevertheless, binding of pyridoxal 5′-phosphate to the enzyme without the ligand lysine is not unprecedented because substitution of the active-site Lys-313 with alanine in *E. coli* aminolevulinate synthase produces external aldimine intermediates (59). Because the absorption peaks at 338 nm and 428 nm have been assigned to the Cys-ketimine and Cys-aldimine intermediates in other cysteine desulfurases, respectively (4, 57), it is likely that Cys-ketimine and Cys-aldimine intermediates are trapped in the IscS-K206A mutant when expressed in wild-type *E. coli* cells.

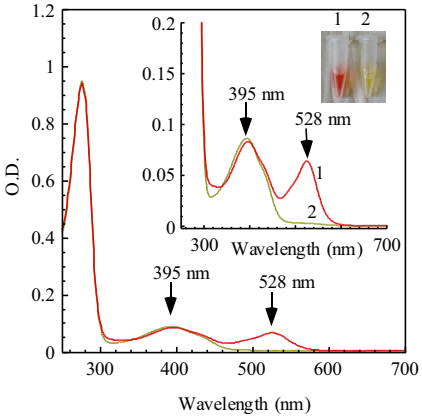

FIGURE 1. **UV-visible spectra of IscS purified from the *iscA/sufA* mutant and parental wild-type *E. coli* cells.** Recombinant *E. coli* IscS was expressed in *E. coli iscA/sufA* mutant cells (*spectrum 1*) or parental wild-type cells (*spectrum 2*). The protein concentration was calibrated to about 22 μM in buffer containing NaCl (500 mM) and Tris (20 mM (pH 8.0)). *Inset*, a photograph of IscS proteins purified from the *E. coli iscA/sufA* mutant cells (*1*) and parental wild-type cells (*2*).

IscS-C328S and IscS-K206A were then expressed in *E. coli iscA/sufA* mutant cells grown in LB medium under aerobic conditions. As shown in Fig. 2*B*, purified IscS-C328S and IscS-K206A have essentially the same absorption peaks as those purified from wild-type *E. coli* cells (Fig. 2*A*), suggesting that the formation of red IscS in the *iscA/sufA* mutant cells requires an active catalytic center in IscS.

Red IscS Also Accumulates in Wild-type E. coli Cells with Depletion of Intracellular Iron—If deletion of IscA/SufA disrupts iron delivery for iron-sulfur cluster biogenesis and results in the accumulation of red IscS in *E. coli* cells under aerobic growth conditions (Fig. 1), then we reasoned that depletion of intracellular iron would also produce a red-colored IscS in wild-type *E. coli* cells under the same experimental conditions.

To test this idea, wild-type *E. coli* cells expressing recombinant IscS were grown in LB medium supplemented with or without 2,2′-dipyridyl, a membrane-permeable iron chelator (31), under aerobic growth conditions. Fig. 3 shows that IscS purified from wild-type *E. coli* cells grown in LB medium supplemented with 0.4 mM 2,2′-dipyridyl is indeed red, with an absorption peak at 528 nm (spectrum 2). The addition of 0.4 mM ferrous ammonium sulfate to the LB medium containing 0.4 mM 2,2′-dipyridyl completely suppresses the formation of red IscS in wild-type *E. coli* cells (spectrum 3). Therefore, depletion of intracellular iron can also result in the accumulation of red IscS in wild-type *E. coli* cells.

E. coli cells expressing recombinant IscS were then grown in LB medium supplemented with different concentrations of 2,2′-dipyridyl under aerobic growth conditions. As shown in Fig. 4*A*, when the concentration of 2,2′-dipyridyl in LB medium increases from 0 to 1.0 mM, cell growth is severely inhibited to a level close to that of the *E. coli iscA/sufA* mutant grown in LB

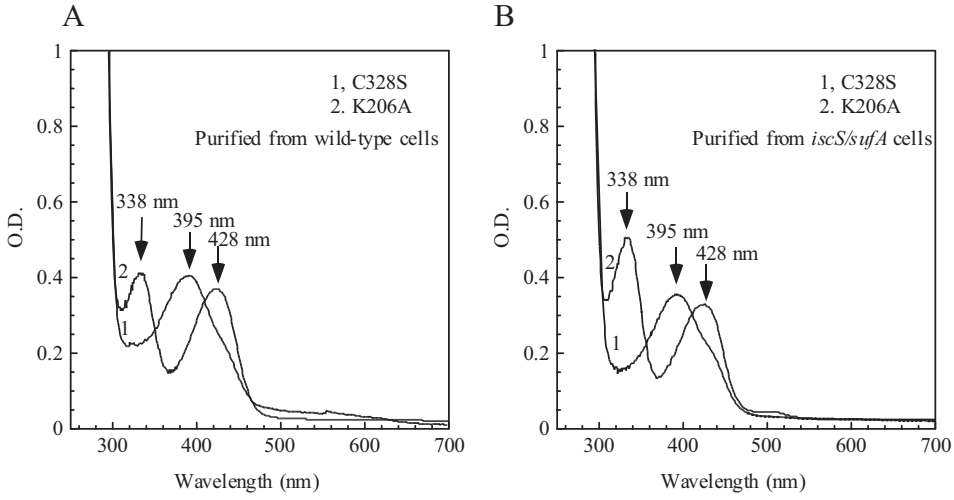

FIGURE 2. **An active catalytic site is required for the formation of red IscS in *E. coli iscA/sufA* mutant cells.** IscS-C328S and IscS-K206A were expressed in wild-type (*A*) or *iscA/sufA* mutant *E. coli* cells (*B*). Purified proteins (50 μM) were subjected to UV-visible absorption measurements. Spectrum 1, IscS-C328S; spectrum 2, IscS-K206A.

medium without 2,2′-dipyridyl. Recombinant IscS was also purified from the wild-type *E. coli* cells grown in LB medium supplemented with different concentrations of 2,2′-dipyridyl. As the concentration of 2,2′-dipyridyl in LB medium increases from 0 to 1.0 mM, the absorption peak at 528 nm of purified IscS increases gradually (Fig. 4*B*). Therefore, deletion of IscA/SufA can be emulated by depletion of intracellular iron in inhibiting cell growth and in producing red IscS in *E. coli* cells under aerobic growth conditions.

Redox Property of Red IscS—To the best of our knowledge, the red-colored IscS has not been previously purified or characterized. To explore the redox property of red IscS, we incubated the purified protein with sodium borohydride, a strong reducing reagent. As shown in Fig. 5, *A* and *B*, the absorption peak at 395 nm of pyridoxal 5′-phosphate in red IscS and wild-type IscS is shifted to 330 nm upon reduction with sodium borohydride. However, the absorption peak at 528 nm of red IscS is not affected by sodium borohydride (Fig. 5*A*). This result suggests that purified red IscS may contain two distinct IscS: one with an absorption peak at 395 nm of pyridoxal 5′-phosphate and the other with an absorption peak at 528 nm of an unknown intermediate. Unfortunately, attempts to separate the IscS with an absorption peak at 395 nm from that with an absorption peak at 528 nm using various chromatographic approaches were not successful (data not shown), likely because of the subtle difference between these intermediates of IscS.

Purified red IscS was also oxidized with H_2O_2. As shown in Fig. 5, *C* and *D*, although the absorption peak at 395 nm of red IscS and wild-type IscS is not changed upon addition of H_2O_2, the absorption peak at 528 nm of red IscS is quickly shifted to 510 nm (Fig. 5*C*). The results further suggest that the absorption peaks at 395 nm and 528 nm of purified red IscS represent two distinct IscS. It should be pointed out that the absorption peak at 510 nm of red IscS is not stable at 37 °C and decreases quickly (Fig. 5*C*). However, when dithiothreitol is added to the incubation solution right after the addition of H_2O_2, the absorption peak at 510 nm is partially reversed back to 528 nm (data not shown), suggesting that oxidation of red IscS by H_2O_2 is at least partially reversible.

The cysteine desulfurase activity was also measured for purified red IscS and wild-type IscS when the proteins were oxidized or reduced. The enzyme activity of purified red IscS and wild-type IscS varies in different preparations and closely correlates with the amplitude of the absorption peak at 395 nm of the prepared proteins. Interestingly, although oxidation with H_2O_2 has very little or no effect on the enzyme activity of red IscS and wild-type IscS, reduction with sodium borohydride

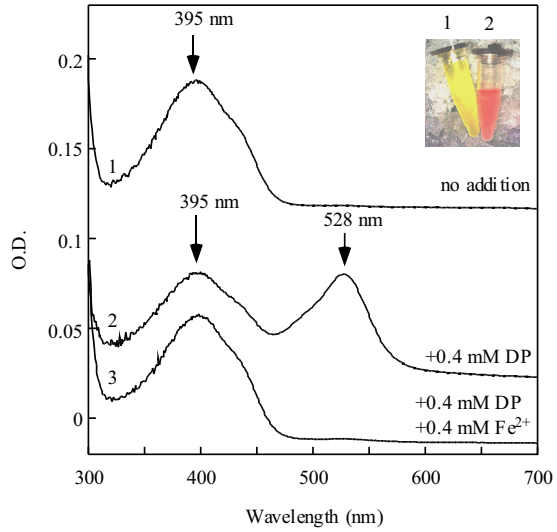

FIGURE 3. **Red IscS also accumulates in wild-type *E. coli* cells with depletion of intracellular iron.** Recombinant IscS was expressed in wild-type *E. coli* cells grown in LB medium supplemented with 0 (*spectrum 1*), 0.4 mM 2,2′-dipyridyl (*spectrum 2*), or 0.4 mM 2,2′-dipyridyl and 0.4 mM $Fe(NH_4)_2(SO_4)_2$ (*spectrum 3*). Purified IscS proteins (20 μM) were subjected to UV-visible absorption measurements. *Inset*, a photograph of IscS proteins purified from wild-type *E. coli* cells grown in LB medium supplemented with 0 (*1*) or 0.4 mM 2,2′-dipyridyl (*2*).

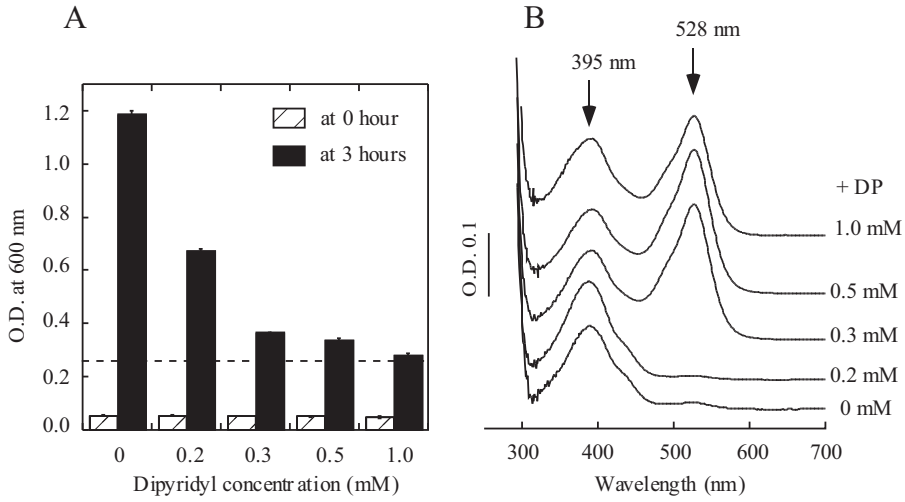

FIGURE 4. **Effects of depletion of intracellular iron on cell growth of wild-type *E. coli* cells and on recombinant IscS expressed in wild-type *E. coli* cells.** *A*, inhibition of cell growth of wild-type *E. coli* by 2,2′-dipyridyl. The same amount of overnight *E. coli* cultures was inoculated in fresh LB medium supplemented with the indicated concentrations of 2,2′-dipyridyl (0–1.0 mM). Cell growth was measured at 0 (*white columns*) and 3 h (*black columns*) under aerobic growth conditions. The *dotted line* represents the cell growth of the *E. coli iscA/sufA* mutant in LB medium without 2,2′-dipyridyl for 3 h under aerobic growth conditions. *B*, recombinant IscS was expressed in wild-type *E. coli* cells grown in LB medium supplemented with the indicated concentrations of 2,2′-dipyridyl (0–1.0 mM). Purified IscS proteins (30 μM) were subjected to UV-visible absorption measurements. The results are representatives from three independent experiments.

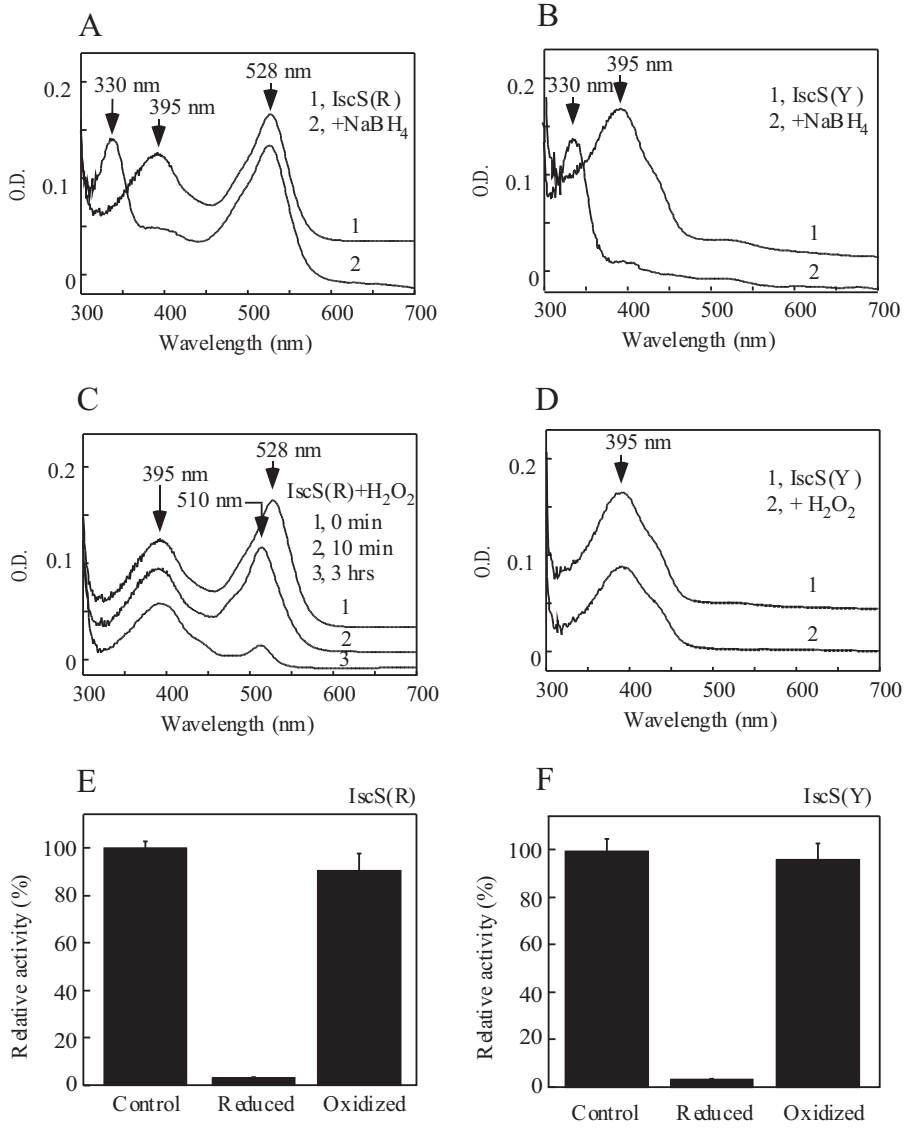

FIGURE 5. **Redox property of red IscS.** *A*, reduction of red IscS by sodium borohydride. Purified red IscS (*IscS(R)*, 30 μM, *spectrum 1*) was incubated with $NaBH_4$ (100 μM, *spectrum 2*) at 4 °C for 30 min. *B*, reduction of wild-type IscS by sodium borohydride. Purified wild-type IscS (*IscS(Y)*, 30 μM, *spectrum 1*) was incubated with $NaBH_4$ (100 μM, *spectrum 2*) at 4 °C for 30 min. *C*, oxidation of red IscS by H_2O_2. Purified red IscS (30 μM, *spectrum 1*) was incubated with H_2O_2 (10 mM, *spectrum 2*) at 4 °C for 10 min (*spectrum 2*) or 3 h (*spectrum 3*). *D*, oxidation of wild-type IscS by H_2O_2. Purified wild-type IscS (30 μM, *spectrum 1*) was incubated with 10 mM H_2O_2 (*spectrum 2*) at 4 °C for 3 h. *E*, relative cysteine desulfurase activity of red IscS after being treated with sodium borohydride (*reduced*) or H_2O_2 (*oxidized*). *F*, relative cysteine desulfurase activity of wild-type IscS after being treated with sodium borohydride or H_2O_2. The enzyme activity of purified red IscS varied in different preparations and closely correlated with the amplitudes of the absorption peak at 395 nm of pyridoxal 5′-phosphate. The total enzyme activity of red IscS shown was ~75% of that of wild-type IscS. The results are the representatives from three independent different experiments.

completely inactivates red IscS and wild-type IscS (Fig. 5, *E* and *F*). Because the reduced red IscS is inactive (Fig. 5*E*) but still has the absorption peak at 528 nm (Fig. 5*A*), and the oxidized red IscS remains largely active (Fig. 5*E*) but without 528 nm (Fig. 5*C*), we propose that the IscS fraction with the absorption peak at 528 nm is inactive to catalyze desulfurization of L-cysteine.

Formation of Red IscS in Vitro—The transient absorption peak at 510 nm of purified red IscS after oxidization with H_2O_2 (Fig. 5*C*) is very close to the absorption peak at 506 nm of the proposed alanine-quinonoid intermediate in cysteine desulfurases from other organisms (4, 57). Conceivably, the absorption peak at 528 nm of purified red IscS could represent an alanine-quinonoid-like intermediate. To test this idea, we incubated wild-type IscS with L-alanine and sulfide, the two products produced from L-cysteine by IscS (4).

Fig. 6*A* shows that, when wild-type IscS is incubated with excess L-alanine and sulfide under aerobic conditions, red IscS is gradually formed. In parallel experiments, incubation of wild-type IscS with D-alanine and sulfide or glycine and sulfide fails to produce red IscS (Fig. 6*B*), demonstrating that L-alanine is essential for the formation of red IscS *in vitro*. Red IscS formed *in vitro* has an absorption peak at 510 nm (Fig. 6*A*), which is the same as that of red IscS purified from *E. coli* cells after oxidation with H_2O_2 (Fig. 5*C*). Similar to that shown in Fig. 5*C*, the absorption peak at 510 nm of red IscS produced *in vitro* is not stable at 37 °C and disappears quickly (data not shown). Taken

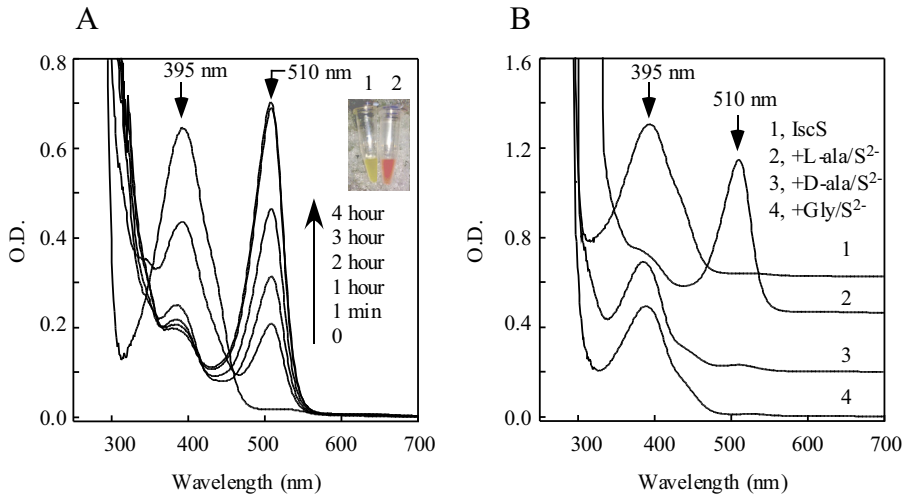

FIGURE 6. **Formation of red IscS *in vitro*.** *A*, purified wild-type IscS (100 μM) was incubated with L-alanine (100 mM) and Na_2S (100 mM) in the presence of Tris (120 mM (pH 8.0)) at room temperature. The UV-visible absorption spectra were taken at 0, 1 min, 1 h, 2 h, 3 h, and 4 h. *Inset*, a photograph of IscS proteins before (*1*) and after (*2*) incubation with L-alanine and Na_2S at room temperature for 3 h. *B*, L-alanine is essential for formation of red IscS *in vitro*. Purified wild-type IscS (100 μM) was incubated with buffer (*spectrum 1*), L-alanine (100 mM, *spectrum 2*), D-alanine (100 mM, *spectrum 3*), or glycine (100 mM, *spectrum 4*) in the presence of Na_2S (100 mM) and Tris (120 mM (pH 8.0)) at room temperature. Spectra were taken after incubation at room temperature for 3 h.

together, the results suggest that the absorption peaks at 528 nm and 510 nm of red IscS purified from *E. coli* cells with deletion of IscA/SufA represent the quinonoid-like intermediates at different redox states.

Discussion

In this study, we report that deletion of the proposed iron chaperones IscA/SufA or depletion of intracellular iron results in the accumulation of a red-colored cysteine desulfurase IscS in *E. coli* cells under aerobic growth conditions. Red IscS can also be produced *in vitro* by incubating wild-type IscS with excess L-alanine and sulfide under aerobic conditions. We propose that deletion of IscA/SufA may block iron delivery for iron-sulfur cluster biogenesis, therefore impeding the sulfur delivery of cysteine desulfurase IscS, and result in the accumulation of red IscS intermediate in *E. coli* cells. The results also represent the first evidence for the physiological interplay between the proposed iron chaperones IscA/SufA and major cysteine desulfurase IscS in iron-sulfur cluster biogenesis in *E. coli* cells under aerobic growth conditions.

IscA is highly conserved from bacteria to humans (41). Although IscA and its homologs have been characterized previously as alternative scaffold proteins or carriers (15, 37–39), IscA has a unique and strong iron-binding activity *in vitro* (40–44) and *in vivo* (31) under aerobic conditions. The three invariant cysteine residues (45), and possibly an oxygen ligand (43), are likely involved in iron binding in IscA. Furthermore, the iron center in IscA can be readily mobilized by L-cysteine and transferred for iron-sulfur cluster assembly *in vitro* under aerobic conditions (43, 44, 47). Recently, we also reported that *E. coli* IscA has its unique activity to bind copper *in vivo* and *in vitro* and that excess copper can compete with iron for the metal binding sites in IscA and block iron-sulfur cluster biogenesis (60). These results led us to propose that IscA and its homologs may act as iron chaperones for iron-sulfur cluster biogenesis. Interestingly, although IscA and its homologs are essential for iron-sulfur cluster biogenesis in bacteria and eukaryotic cells under aerobic conditions (42, 49–56), deletion of IscA and its homolog SufA has very little or no effect on iron-sulfur proteins in *E. coli* cells under anaerobic growth conditions (31). One of the simplest explanation is that, under anaerobic conditions, the intracellular iron concentration may be sufficient to facilitate iron-sulfur cluster assembly in proteins without IscA and its homologs (31). Consistent with this idea, IscA and its homologs are absent in most anaerobic organisms (61). Here we found that, although deletion of IscA/SufA results in the accumulation of red IscS in *E. coli* cells under aerobic conditions, deletion of IscA/SufA has no apparent effects on IscS in *E. coli* cells under anaerobic growth conditions (data not shown). Therefore, IscA and its homologs may have a crucial role in recruiting intracellular iron and delivering iron for iron-sulfur cluster biogenesis under aerobic conditions but not under anaerobic conditions.

In *E. coli* cells, IscS is not only a major sulfur donor for iron-sulfur cluster biogenesis (6) but also provides sulfur for biogenesis of thiamine, tRNA thiolation (62), DNA phosphorothioation (63), molybdopterin (64), and other biological thiolation processes (65). Although the chemical nature of the red chromophore in IscS accumulated in the *E. coli* cells with deletion of IscA/SufA or depletion of intracellular iron could not be ascertained, a similar absorption peak at 521 nm of the serine-glyoxylate aminotransferase intermediate from *Hyphomicrobium methylovorum* has been reported previously (66). The absorption peak at 521 nm has been attributed to a highly conjugated quinonoid intermediate produced from hydroxyaminoacrylate and pyridoxal 5′-phosphate (66). In this context, we propose that the absorption peak at 528 nm of red IscS may also represent a highly conjugated quinonoid intermediate. With deficiency of accessible iron for iron-sulfur cluster biogenesis in *E. coli* cells, IscS may accumulate persulfide on the catalytic residue Cys-328 (67). Accumulated persulfide in Cys-328 may promote the transfer of a hydride from substrate L-cysteine to produce hydrogen sulfide and a thiocarbonyl group in L-cys-

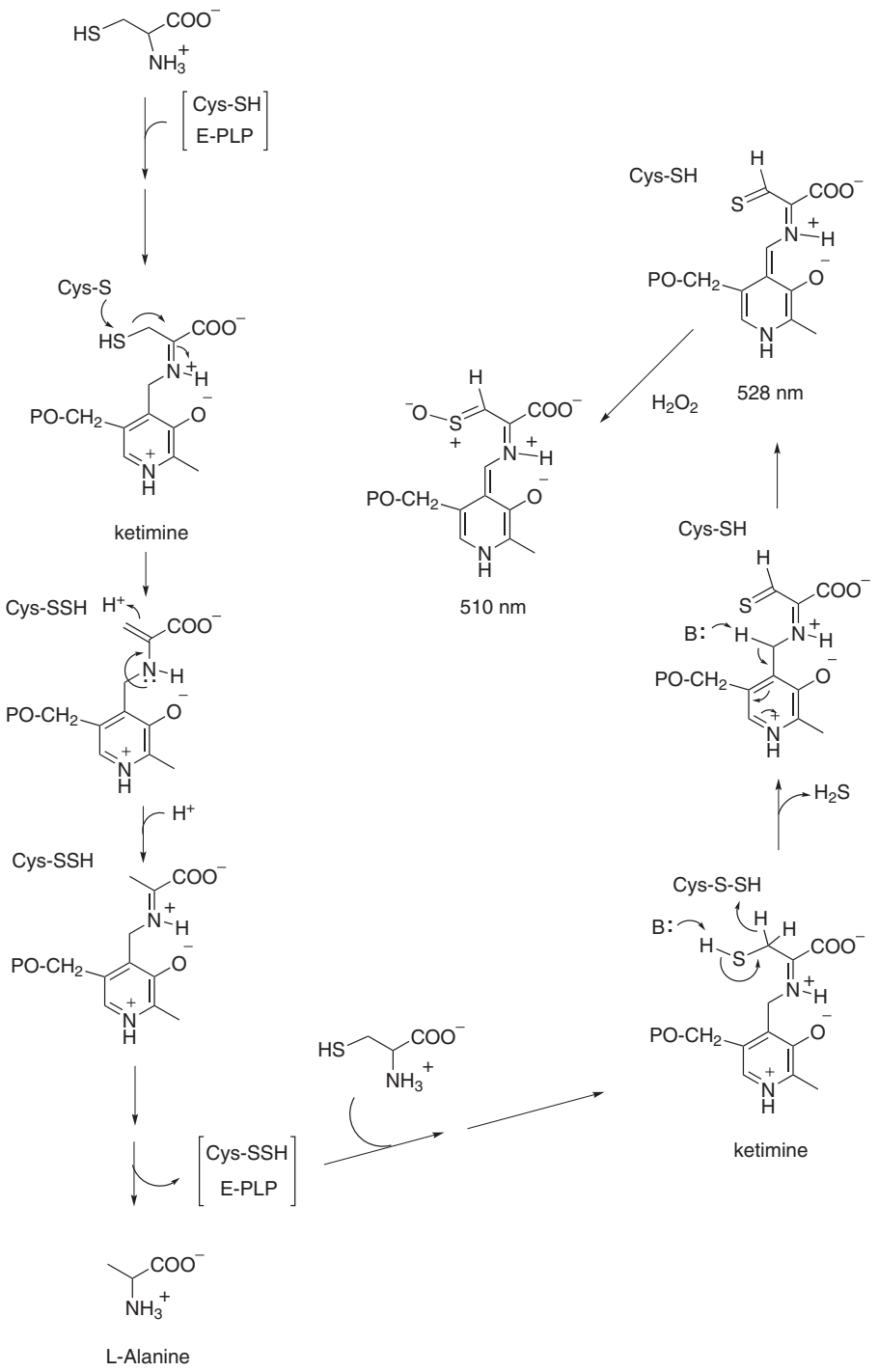

FIGURE 7. **Proposed intermediates of IscS formed in *E. coli* cells with deficiency of accessible iron for iron-sulfur cluster biogenesis.** In *E. coli* cells with deletion of IscA/SufA or depletion of intracellular iron, IscS accumulates persulfide in Cys-328, which may transfer a hydride from the thiol group of L-cysteine to produce H_2S and a thiocarbonyl group in L-cysteine. The thiocarbonyl group may form a highly conjugated quinonoid intermediate in red IscS that has an absorption peak at 528 nm. Oxidation of red IscS with H_2O_2 may modify the thiocarbonyl group and generate an unstable intermediate with an absorption peak at 510 nm.

teine that forms a highly conjugated quinonoid represented by an absorption peak at 528 nm (Fig. 7). When red IscS is oxidized by H_2O_2, the thiocarbonyl group could be modified to form a new intermediate with an absorption peak at 510 nm. Such an intermediate IscS may also be produced *in vitro* by incubating wild-type IscS with excess L-alanine and sulfide under aerobic conditions. Nevertheless, the exact nature of red chromophores in IscS formed in *E. coli* cells remains to be further investigated.

In *E. coli*, the cysteine desulfurase IscS, the scaffold protein IscU, and the proposed iron chaperone IscA are encoded by the same operon (1), which is regulated by the global transcription factor IscR (68) and the small regulatory RNA RyhB (69). It is likely that IscS, IscU, and IscA work in concert for iron-sulfur

cluster biogenesis (44, 48). The physical interaction between IscS and IscU has been well characterized (70). Dimeric IscU interacts with dimeric IscS to form a functional protein complex (9). However, the protein-protein interactions between IscA and IscS or IscA and IscU have not been reported. The finding that deletion of IscA and its paralog SufA results in the accumulation of red IscS in *E. coli* cells under aerobic conditions (Fig. 1) provides the first evidence of the physiological interplay between the sulfur donor IscS and the proposed iron chaperones IscA/SufA during iron-sulfur cluster biogenesis under aerobic growth conditions.

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