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Interplay of IscA and IscU in Biogenesis of Iron-Sulfur Clusters*

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Increasing evidence suggests that sulfur in ubiquitous iron-sulfur clusters is derived from L-cysteine via cysteine desulfurases. In Escherichia coli, the major cysteine desulfurase activity for biogenesis of iron-sulfur clusters has been attributed to IscS. The gene that encodes IscS is a member of an operon iscSLUA, which also encodes two highly conserved proteins: IscU and IscA. Previous studies suggested that both IscU and IscA may act as the iron-sulfur cluster assembly scaffold proteins. However, recent evidence indicated that IscA is an iron-binding protein that can provide iron for the iron-sulfur cluster assembly in IscU (Ding, H., Harrison, K., and Lu, J. (2005) J. Biol. Chem. 280, 30432–30437). To further elucidate the function of IscA in biogenesis of iron-sulfur clusters, we evaluate the iron-sulfur cluster binding activity of IscA and IscU under physiologically relevant conditions. When equal amounts of IscA and IscU are incubated with an equivalent amount of ferrous iron in the presence of IscS, L-cysteine and dithiothreitol, iron-sulfur clusters are assembled in IscU, but not in IscA, suggesting that IscU is a preferred iron-sulfur cluster assembly scaffold protein. In contrast, when equal amounts of IscA and IscU are incubated with an equivalent amount of ferrous iron in the presence of IscS and dithiothreitol but without L-cysteine, nearly all iron is bound to IscA. The iron binding in IscA appears to prevent the formation of the biologically inaccessible ferric hydroxide under aerobic conditions. Subsequent addition of L-cysteine efficiently mobilizes the iron center in IscA and transfers the iron for the iron-sulfur cluster assembly in IscU. The results suggest an intriguing interplay between IscA and IscU in which IscA acts as an iron chaperon that recruits “free” iron and delivers the iron for biogenesis of iron-sulfur clusters in IscU under aerobic conditions.

Iron-sulfur clusters are one of the most ancient and ubiquitous redox centers in almost all living organisms (1–3). Throughout evolution, iron-sulfur clusters have become integral parts of diverse biological processes including energy conversion and the regulation of gene expression. It is now clear that biogenesis of iron-sulfur clusters is not a spontaneous process. The pioneering work by Dean’s group (4) revealed that sulfur in iron-sulfur clusters is derived from L-cysteine via cysteine desulfurases, a group of pyridoxal 5-phosphate-dependent enzymes that are conserved from bacteria to humans (5–7). In Escherichia coli, there are at least three cysteine desulfurases: IscS (5), SulS (8), and CSD (cysteinate desulfinase) (9, 10). Deletion of gene iscS greatly diminishes the specific activities of iron-sulfur proteins in E. coli (11, 12), suggesting that IscS is the major cysteine desulfurase for biogenesis of iron-sulfur clusters. Gene iscS is a member of an operon iscSLUA, which also encodes two highly conserved proteins: IscU and IscA (13, 14). Biochemical studies indicated that IscS catalyzes desulfurization of L-cysteine and transfers sulfane sulfur for the iron-sulfur cluster assembly in IscU via specific protein-protein interactions (15–18). Accordingly, IscU was characterized as an iron-sulfur cluster assembly scaffold protein (15–21).

The function of IscA, however, still remains elusive. Previous studies suggested that IscA is an alternative iron-sulfur cluster assembly scaffold protein (22–29), because IscA, like IscU, can bind iron-sulfur clusters in the presence of ferrous iron and sulfide in vitro. On the other hand, recent studies indicated that IscA is a novel iron binding protein with an iron association constant of 2.0–3.0 \( \times 10^{19} \) M\(^{-1} \) in the presence of the thioredoxin reductase system (30) or dithiothreitol (31) and that the iron-loaded IscA can provide iron for the iron-sulfur cluster assembly in IscU (32, 33). To reconcile the two models proposed for the function of IscA, here we re-evaluated the iron-sulfur cluster binding activity of IscA and IscU under physiologically relevant conditions and found that in the presence of ferrous iron, L-cysteine, and cysteine desulfurase IscS, IscU is a preferred iron-sulfur cluster assembly scaffold protein. On the other hand, when L-cysteine is not present in the incubation solution, IscA acts as an iron chaperon that binds “free” iron. The iron binding in IscA appears to prevent the formation of the biologically inaccessible ferric hydroxide under aerobic conditions. Subsequent addition of L-cysteine mobilizes the iron center in IscA and transfers the iron for the iron-sulfur cluster assembly in IscU even under aerobic conditions. The potential interplay between IscA and IscU for biogenesis of iron-sulfur clusters will be discussed.

**EXPERIMENTAL PROCEDURES**

**Protein Preparation**—Recombinant E. coli IscA (31), IscU (31), and IscS (34) were purified using a Ni-agarose column (Qiagen) followed by a HiTrap Desalting column (Amersham...
Biosciences) as described previously. The His-tag in the purified proteins was removed with thrombin and re-purified using a Mono Q column (Amersham Biosciences) (31). E. coli thioredoxin 1 (TrxA) and thioredoxin reductase (TrxB) were produced from the expression vectors pDL59 (35) and pTrR301 (36), respectively, and purified as described in (30). The expression vectors pDL59 (35) and pTrR301 (36) were kindly provided by Dr. Scott B. Mulrooney (University of Michigan). Both TrxA and TrxB were purified as native form without any tags. The purity of purified proteins was greater than 95% as judged by electrophoresis analysis on a 15% polyacrylamide gel containing SDS followed by staining with Coomassie Blue. The monomeric concentrations of apo-IscA were determined according to the extinction coefficient at 260 nm of 2.4 mM protein samples.

Iron-Sulfur Cluster Assembly in IscA and IscU—For the iron-sulfur cluster assembly reactions, apo-IscA and/or apo-IscU were incubated with Fe(NH4)2(SO4)2 and IscS in the presence of the thioredoxin reductase system (containing thioredoxin 1 (TrxA) (5 µM), thioredoxin reductase (TrxB) (0.5 µM) and NADPH (500 µM)) or dithiothreitol (2 mM) in buffer containing NaCl (200 mM) and Tris (20 mM) (pH 8.0) anaerobically at 37 °C. While both the thioredoxin reductase system and dithiothreitol were effective for the iron-sulfur cluster assembly in IscU (30), the absorption peak at 340 nm of NADPH in the presence of dithiothreitol (2 mM) anaerobically at 37 °C. The UV-visible absorption spectra were taken at 0 min (thin lines) and 30 min (thick lines) after l-cysteine (1 mM) was added. A, no apo-IscU; B, with 6.3 mM apo-IscU; C, with 12.5 mM apo-IscU; D, with 25 µM apo-IscU.

Iron Binding in IscA and IscU—For the iron binding experiments, apo-IscA and apo-IscU were incubated with Fe(NH4)2(SO4)2 and IscS in the presence of dithiothreitol (2 mM) in buffer containing 200 mM NaCl and 20 mM Tris (pH 8.0) anaerobically at 37 °C for 30 min. IscA and IscU were then re-purified from the incubation solutions using a Mono Q column. The UV-Vis absorption spectra of the protein samples and their total iron contents were measured as described above. The iron association constants of IscA in the presence or absence of IscU were analyzed using a physiological iron binding competitor citrate (40) as described previously (31).

EPR Measurements—The EPR spectra were recorded at X-band on a Bruker ESR-300 spectrometer using an Oxford Instruments ESR-9 flow cryostat (Chemistry Department/Louisiana State University). The EPR conditions were: microwave frequency, 9.45 GHz; microwave power, 20 milliwatt; modulation frequency, 100 kHz; modulation amplitude, 2.0 mT; sample temperature, 4.5 K; receive gain, 1.0 × 10³.

RESULTS

IscU Is a Preferred Iron-Sulfur Cluster Assembly Scaffold Protein—Previously, both IscU (15–21) and IscA (22–29) were characterized as alternative iron-sulfur cluster assembly scaffold proteins. However, their physiological relevance is still not fully understood. It would be of interest to compare the relative iron-sulfur cluster binding activity of IscU and IscA under physiologically relevant conditions.

For the iron-sulfur cluster assembly in IscA, we incubated apo-IscA (25 µM) with cysteine desulfurase IscS (0.5 µM), l-cysteine (1 mM) and ferrous iron (25 µM) in the presence of dithiothreitol (2 mM) anaerobically at 37 °C for 30 min. After incu-
Distinct Functions of IscA and IscU

bation, an absorption peak at 415 nm that was assigned to the iron-sulfur clusters in IscA (22, 23) appeared (Fig. 1A). The absorption spectrum was essentially identical to that reported previously for the [2Fe-2S] cluster-bound IscA (22, 23). When apo-IscA (25 μM) was incubated with apo-IscU (6.3 μM) in the presence of IscS (0.5 μM), l-cysteine (1 mM), and dithiothreitol (2 mM) anaerobically at 37 °C for 30 min. After incubation, IscA and IscU were re-purified using a Mono Q column as described under “Experimental Procedures.” Panel a, the elution profile of IscA and IscU from a Mono Q column. Panel b, the SDS-polyacrylamide gel electrophoresis analysis of the eluted fractions. The positions of IscA and IscU were indicated on the left of the panel. Panels c and d, the UV-visible absorption spectra of the eluted fraction 8 (IscA) and fraction 10 (IscU), respectively. The inset in panel d is a full scale absorption spectrum of fraction 10 (IscU). The high absorption at 280 nm of IscU is due to its large extinction coefficient (11.2 mM⁻¹ cm⁻¹): B, same as in A, except Fe(NH₄)₂(SO₄)₃ (25 μM) was included in the incubation solution.

The EPR measurements of the eluted IscA and IscU samples showed no EPR signals even after the samples were treated with a 5-fold excess of dithionite (data not shown). This observation is consistent with the previous report that the iron-sulfur clusters in IscU are EPR silent and are quickly decomposed after reduction with dithionite (15).

The total iron content analyses of the IscA and IscU fractions shown in Fig. 2B revealed that the ratio of iron to the IscA dimer in fraction 8 was 0.05 ± 0.03 (n = 3), whereas the ratio of iron to the IscU dimer in fraction 10 was 0.85 ± 0.08 (n = 3). The amount of sulfide in fraction 8 was not detectable. In contrast, the ratio of sulfide to the IscU dimer in fraction 10 was 0.78 ±

FIGURE 2. IscU is a preferred iron-sulfur cluster assembly scaffold protein. A, apo-IscA (25 μM) was incubated with apo-IscU (25 μM) in the presence of IscS (0.5 μM), l-cysteine (1 mM), and dithiothreitol (2 mM) anaerobically at 37 °C for 30 min. After incubation, IscA and IscU were re-purified using a Mono Q column as described under “Experimental Procedures.” Panel a, the elution profile of IscA and IscU from a Mono Q column. Panel b, the SDS-polyacrylamide gel electrophoresis analysis of the eluted fractions. The positions of IscA and IscU were indicated on the left of the panel. Panels c and d, the UV-visible absorption spectra of the eluted fraction 8 (IscA) and fraction 10 (IscU), respectively. The inset in panel d is a full scale absorption spectrum of fraction 10 (IscU). The high absorption at 280 nm of IscU is due to its large extinction coefficient (11.2 mM⁻¹ cm⁻¹): B, same as in A, except Fe(NH₄)₂(SO₄)₃ (25 μM) was included in the incubation solution.
Distinct Functions of IscA and IscU

Iron-sulfur cluster assembly in IscU, IscA, and bovine serum albumin. a, apo-IscU (25 μM) was incubated with IscS (0.5 μM) and Fe(NH₄)₂(SO₄)₂ (25 μM) in the presence of dithiothreitol (2 mM) anaerobically at 37 °C. The UV-Vis absorption spectra were taken 30 min after L-cysteine (1 mM) was added. b, same as in a, except apo-IscU (25 μM) was used instead of apo-IscU. c, same as in a except BSA (5 μM) was used instead of apo-IscU. d, same as in a except no apo-IscU was added.

0.10 (n = 3). These results further suggest that under physiologically relevant conditions, iron-sulfur clusters were assembled in IscU but not in IscA.

No absorption peaks from 400 nm to 600 nm were observed without apo-IscA or apo-IscU in the incubation solution (Fig. 3d), indicating that the observed iron-sulfur clusters were assembled in the proteins. However, a number of proteins containing free thiol groups have been shown to form nonspecific iron-sulfur clusters in the presence of ferrous iron and sulfide in vitro (41). To test this idea, we used BSA as an example for the iron-sulfur cluster assembly. BSA contains 35 cysteine residues, which are about five times as many cysteine residues in the IscA or IscU dimer. When BSA (5 μM) was incubated with IscS (0.5 μM) and ferrous iron (25 μM) in the presence of dithiothreitol (2 mM) anaerobically at 37 °C for 30 min, an absorption peak at 415 nm representing the iron-sulfur cluster assembly appeared (Fig. 3c). The observed spectrum of BSA was almost indistinguishable to that of IscA (Fig. 3b) but was very different from that of IscU (Fig. 3a), suggesting that the iron-sulfur cluster assembly in IscA could in fact be nonspecific.

IscA Acts as an Iron Chaperon When L-Cysteine Is Not Present—The iron-sulfur cluster assembly requires a coordinated delivery of both iron and sulfide. It has been well established that sulfide in iron-sulfur clusters is derived from L-cysteine via cysteine desulfurases (4–7). Conceivably, the intracellular L-cysteine content could be a major determining factor for the overall biogenesis of iron-sulfur clusters.

To emulate the conditions at which the intracellular L-cysteine is limited, we incubated apo-IscA (25 μM), apo-IscU (25 μM), and IscS (0.5 μM) with ferrous iron (25 μM) in the presence of dithiothreitol (2 mM) anaerobically at 37 °C for 30 min. After incubation, both IscA and IscU were re-purified using a Mono Q column. Fig. 4c shows that after incubation, apo-IscA was converted to an iron-loaded IscA with a major absorption peak at 315 nm and a shoulder around 435 nm as described previously (30–33). The ratio of the absorption amplitude at 315–260 nm was about 1.02, indicating that the iron binding in IscA was nearly saturated (31). The total iron content analyses of the IscA fraction showed that the ratio of iron to the IscA dimer was 0.94 ± 0.07 (n = 3), consistent with the previous report that each IscA dimer binds one iron in the iron-loaded IscA (31). In contrast, the ratio of iron to the IscU dimer in fraction 10 was only 0.07 ± 0.04 (n = 3). The UV-visible and EPR measurements of the re-purified IscU (Fig. 4, e and f) confirmed that there was no significant amount of iron binding in IscU. A slight increase of the UV-visible absorption at around 330 nm (Fig. 4e) could reflect the small amount of iron associated with IscU (0.07 ± 0.04 iron per IscU dimer), because the absorption at around 330 nm could be completely removed by an iron chelator EDTA (data not shown). No acid-labile sulfide was detected in both IscA and IscU samples, demonstrating that no iron-sulfur clusters were assembled in IscA or IscU under the experimental conditions.

The EPR measurements of the eluted IscA fraction revealed a broad EPR signal at around the g = 4–6 region (Fig. 4d), which represents a unique S = 3/2 ground spin state of the ferric iron center in the protein (31). The observed EPR signal of IscA was identical to that found in as-purified IscA from E. coli cells (31). The broad EPR signal indicates substantial heterogeneity, which prevents the reliable assessment of spin Hamiltonian parameters. The lack of an EPR signal at g = 4.3 in the spectrum suggests that there was very little non-specifically bound “junk” iron in the IscA sample. The S = 3/2 ground spin state EPR signal of IscA differs significantly from that of the S = 5/2 ground spin iron center in desulfoferredoxin or rubredoxin (42) but is somewhat similar to that of the S = 3/2 ground spin state observed in the reduced [4Fe-4S] cluster of the nitrogenase Fe-protein from Azotobacter vinelandii (43). It should be pointed out that the S = 3/2 ground spin state of the [4Fe-4S] cluster in the nitrogenase Fe-protein can be observed only after the sample is reduced with dithionite (43). In contrast, reduction of the ferric iron center in IscA with dithionite completely eliminates the S = 3/2 ground spin state of the protein (31). Taken together, the results suggest that the iron center in...
IscA has its unique redox property, which remains to be further investigated.

Previous studies revealed that *E. coli* IscA has an iron association constant of about 2.0–3.0 M⁻¹ in the presence of the thioredoxin reductase system or dithiothreitol (30, 31). It would be important to determine whether the iron binding activity of IscA can be modulated by IscU and IscS. To test this idea, we re-analyzed the iron association constant of IscA in the presence and absence of IscU and IscS. Using sodium citrate as a physiological iron binding competitor (30, 31), we found that the iron association constant of IscA was essentially unchanged by an equal amount of IscU or IscS under the experimental conditions (data not shown).

**Distinct Functions of IscA and IscU**

IscA acts as an iron chaperon when L-cysteine is not present. Apo-IscA (25 μM) was incubated with apo-IscU (25 μM) in the presence of IscS (0.5 μM), Fe(NH₄)₂(SO₄)₂ (25 μM), and dithiothreitol (2 mM) anaerobically at 37 °C for 30 min. IscA and IscU were re-purified using a Mono Q column as described under "Experimental Procedures." a, the elution profile of IscA and IscU from a Mono Q column. b, the SDS-polyacrylamide gel electrophoresis analysis of the eluted fractions. The positions of IscA and IscU were indicated on the left of the panel. c, and e, UV-visible absorption spectra of the eluted fraction 8 (IscA) and 10 (IscU), respectively. The inset in e is a full scale absorption spectrum of fraction 10 (IscU). d and f, EPR spectra of the eluted fraction 8 (IscA) and 10 (IscU), respectively.

FIGURE 4. IscA acts as an iron chaperon when L-cysteine is not present.
Distinct Functions of IscA and IscU

IscA and IscU are the two key members of the iron-sulfur cluster assembly machinery in E. coli (12, 13). Both proteins are conserved in organisms ranging from bacteria to humans. However, the specific functions of IscA and IscU in biogenesis of iron-sulfur clusters are not fully understood. In previous studies, both IscU (15–21) and IscA (22–29) were characterized as alternative iron-sulfur cluster assembly scaffold proteins. Here we report that under physiologically relevant conditions, IscU is a preferred iron-sulfur cluster assembly scaffold protein, whereas IscA acts as an iron chaperon and delivers the iron for the iron-sulfur cluster assembly in IscU under aerobic conditions.

The observed iron binding activity of IscA is consistent with the x-ray crystal structure models, which reveal that IscA exists as a tetramer with a central channel formed by the association of four IscA monomers (45, 46). Within the channel, the three invariant cysteine residues (Cys-35, Cys-99, and Cys-101) from each IscA monomer are projected to form a “cysteine pocket” which could readily accommodate the mononuclear iron binding in the protein (31, 45, 46). The site-directed mutagenesis studies further showed that substitution of Cys-99 or Cys-101 with serine (C99S and C101S) completely abolishes the iron binding activity of E. coli IscA (31), suggesting that Cys-99 and Cys-101 are likely involved in the iron binding of the protein.

Previous characterizations of IscA and its homologues as an iron-sulfur cluster assembly scaffold protein were primarily based on the observation that IscA can bind iron-sulfur clusters in the presence of ferrous iron and sulfide in vitro (22–29). However, many proteins containing free thiol groups such as BSA are also able to form nonspecific iron-sulfur clusters (Fig. 3) (41). The nearly identical UV-visible absorption spectra of IscA and BSA after reconstitution with iron-sulfur clusters (Fig. 3) strongly suggest that the iron-sulfur cluster assembly in IscA could in fact be nonspecific. In addition, the iron-sulfur cluster formation in IscA does not require the invariant cysteine residues 99 and 101, because both IscA mutants C99S and C101S have the same iron-sulfur cluster binding activity as the wild-type IscA (data not shown). Similar results were also reported for the IscA homologue from Schizosaccharomyces pombe in which substitution of the three invariant cysteine residues with alanine had little or no effect on the iron-sulfur cluster assembly activity in these mutant proteins in vitro (47).

Furthermore, here we reported that little or no iron-sulfur clusters were assembled in IscA when an equal amount of IscU was present in the incubation solution (Fig. 1), indicating that IscA is not a preferred iron-sulfur cluster binding protein. While we could not exclude the possibility that IscA may act as a transient iron-sulfur cluster assembly scaffold and transfer the assembled cluster to IscU or other target proteins, we propose that the primary function of IscA is to bind intracellular free iron, not iron-sulfur clusters.

In contrast, IscU has a strong iron-sulfur cluster binding activity and a weak iron binding activity under physiologically...
relevant conditions (Figs. 2 and 4). The NMR studies (48, 49) reveal that IscU and IscA are two structurally very different proteins. The compact core of the globular structure of IscU has a sandwich architecture with a three-stranded antiparallel sheet and four helices. The unique structure of IscU could be the bases for the observed iron-sulfur cluster binding activity. Alternatively, IscS may preferably transfer sulfur to IscU for the iron-sulfur cluster assembly because of the specific protein-protein interactions between IscU and IscS (15–17). It is worth mentioning that IscU also interacts with two heat shock cognate proteins HscB and HscA (50, 51). Both HscB and HscA are the members of the proteins encoded by the gene cluster iscULATION which is responsible for general biogenesis of iron-sulfur clusters in E. coli and other bacteria (12, 13). HscB and HscA appear to regulate the processes of the iron-sulfur cluster assembly in IscU and/or the transfer of the assembled clusters from IscU to target proteins (50, 51). Thus, IscU is likely a dominating scaffold protein for biogenesis of iron-sulfur clusters.

In the literature, almost all iron-sulfur cluster assembly reactions in proteins were carried out under anaerobic conditions. Perhaps, the major obstacle for the iron-sulfur cluster assembly under aerobic conditions is rapid oxidation of ferrous iron to the biologically inaccessible ferric hydroxide, which would effectively avert the iron-sulfur cluster assembly (Fig. 5A). On the other hand, iron-sulfur clusters must be assembled in aerobically growing bacteria (14) or in oxygen-rich mitochondria in eukaryotic cells (44). It has been speculated that specific proteins must be involved in stabilizing the iron and facilitating the iron-sulfur cluster assembly under aerobic conditions. One candidate for the iron chaperon function was proposed to be frataxin (52), a small mitochondrial protein that has been linked to Friedreich’s ataxia, an autosomal recessive neurodegenerative human disease. However, deletion of the frataxin homologue in E. coli (53) and in Saccharomyces cerevisiae (54) had no apparent effect on biogenesis of iron-sulfur clusters in these organisms. Furthermore, unlike the frataxin homologues from E. coli and S. cerevisiae, human frataxin has little tendency to bind iron (55), suggesting that iron binding of frataxin could be a non-conserved part of its more complex cellular function. In this context, we find that IscA can bind free iron and effectively prevent the formation of the biologically inaccessible ferric hydroxide under aerobic conditions (Fig. 5). The iron binding activity of IscA appears to be conserved from bacteria to humans, as the recombinant human IscA homologue has a similar iron association constant as E. coli IscA. Finally, the otherwise stable iron center in IscA can be readily mobilized by L-cysteine for the iron-sulfur cluster assembly in proteins (33) (Fig. 6). These results led us to suggest that IscA may act as a major iron chaperon for the iron-sulfur cluster assembly under aerobic conditions.

Since IscS, IscU, and IscA are encoded by the same operon iscSUA in E. coli and other bacteria (12, 13), it is conceivable that IscS, IscU, and IscA may work in concert for biogenesis of iron-sulfur clusters. We propose that IscS (a sulfur donor), IscA (an iron chaperon), and IscU (a scaffold) represent the core of the iron-sulfur cluster assembly machinery. Fig. 7 shows a proposed “USA” model for biogenesis of iron-sulfur clusters. Under the conditions where intracellular L-cysteine content is limited, IscA recruits intracellular free iron and forms an iron-loaded IscA. When L-cysteine becomes available, L-cysteine mobilizes the iron center in IscA (33) and forms a transient...
The L-cysteine-Fe complex is well known, but it is still not clear how its synthesis is regulated (56). The genetic control of the L-cysteine biosynthesis is still not clearly understood (57). The role of the cellular iron supplies is sufficient. While the L-cysteine biosynthesis has been well investigated (56), the genetic control of the L-cysteine biosynthesis remains to be investigated. How the intracellular L-cysteine content is regulated and then regulates biogenesis of iron-sulfur clusters remains to be investigated.

### REFERENCES