[2Fe-2S]-Ferredoxin Binds Directly to Cysteine Desulfurase and Supplies an Electron for Iron–Sulfur Cluster Assembly but Is Displaced by the Scaffold Protein or Bacterial Frataxin

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Supporting Information

ABSTRACT: Escherichia coli [2Fe-2S]-ferredoxin (Fdx) is encoded by the isc operon along with other proteins involved in the ‘house-keeping’ mechanism of iron–sulfur cluster biogenesis. Although it has been proposed that Fdx supplies electrons to reduce sulfate sulfur (S\(^{0}\)) produced by the cysteine desulfurase (IscS) to sulfide (S\(^{-2}\)) as required for the assembly of Fe–S clusters on the scaffold protein (IscU), direct experimental evidence for the role of Fdx has been lacking. Here, we show that Fdx (in either oxidation state) interacts directly with IscS. The interaction face on Fdx was found to include residues close to its Fe–S cluster. In addition, C328 of IscS, the residue known to pick up sulfur from the active site of IscS and deliver it to the Cys residues of IscU, formed a disulfide bridge with Fdx in the presence of an oxidizing agent. Electrons from reduced Fdx were transferred to IscS only in the presence of L-cysteine, but not to the C328S variant. We found that Fdx, IscU, and CyaY (the bacterial frataxin) compete for overlapping binding sites on IscS. This mutual exclusion explains the mechanism by which CyaY inhibits Fe–S cluster biogenesis. These results (1) show that reduced Fdx supplies one electron to the IscS complex as S\(^{0}\) is produced by the enzymatic conversion of Cys to Ala and (2) explain the role of Fdx as a member of the isc operon.

Escherichia coli ferredoxin (Fdx) has been one of the most studied iron–sulfur (Fe–S) proteins since its first characterization in 1974. Fdx contains a [2Fe-2S] cluster with a redox potential of approximately −380 mV. The crystal structure of oxidized E. coli Fdx was found to be highly similar to those of bovine adrenodoxin and Pseudomonas putida putidaredoxin, as expected from their close sequence identity and conservation of the cysteine residues (C42, C48, C51, and C87) that coordinate the [2Fe-2S] cluster.

Despite its detailed biophysical characterization, hard evidence for the physiological role of E. coli Fdx has been elusive. Because the protein is encoded within the isc operon responsible for the production of proteins involved in the ISC Fe–S cluster biogenesis system (Figure S1 in Supporting Information), it has been proposed that Fdx functions in Fe–S cluster biosynthesis. Fdx has been shown to be critical for Fe–S cluster biosynthesis in Azotobacter vinelandii, yeast, and humans. Mitochondria contain a homologous ISC system, and defects in this system in humans have been linked to genetic diseases. The essential components of the ISC system in E. coli (Figure S1 in SI) include: IscS, the homodimeric pyridoxal phosphate–dependent cysteine desulfurase that generates sulfur by converting Cys to Ala and transfers it to other proteins; IscU, the scaffold protein on which Fe–S clusters are assembled and from which Fe–S clusters are transferred to various apo-proteins; and HscB, the DnaJ-type cochaperone, and HscA, the DnaK-like chaperone, both of which are involved in ATP-dependent cluster transfer. CyaY, the E. coli homologue of human frataxin, is not encoded by the isc operon, but has been found to inhibit Fe–S cluster assembly. The homodimeric cysteine desulfurase (IscS in E. coli and A. vinelandii, and Nfs1 in eukaryotes) produces S\(^{0}\), which needs to be reduced to sulfide (S\(^{-2}\)) in order for IscU to assemble the iron–sulfur cluster. Assuming that two electrons are supplied by the oxidation of two ferrous ions, two additional electrons from an external source are required to make one [2Fe-2S]\(^{2+}\) cluster and it has been speculated that Fdx is involved in this reduction.

Here, we demonstrate that Fdx interacts directly with IscS. We observed that most of the 1H–15N HSQC signals of [U-15N]-Fdx, in either its oxidized (Figure 1a) or reduced (Figure 1c) state, broadened beyond detection upon the addition of 1.5 equiv (subunit) of IscS (Figure 1b and 1d, respectively). See SI for detailed experimental procedures. We attribute the line broadening of signals from Fdx (12 kDa) to its association with the much larger homodimeric IscS (∼90 kDa). We confirmed the formation of the Fdx–IscS complex by inducing a disulfide bond between the two proteins (Figure 1e and 1f), in analogy to the disulfide-bonded complex of IscU and IscS obtained by exposing the IscU–IscS complex to an oxidizing agent. The covalent Fdx–IscS complex induced by an oxidizing agent failed to form when the Cys residue (C328) of IscS, which serves to transfer sulfur from the catalytic site of IscS to IscU, was substituted by Ser (Figure 1f). These results are consistent with the idea that Fdx interacts with IscS to provide one of the electrons needed to reduce S\(^{0}\) to S\(^{-2}\). The interaction between Fdx and IscS was also confirmed by a chemical cross-linking experiment (Figure S2 in SI).

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The addition of a substoichiometric quantity of IscS (0.5 equivalent subunit) to [U-15N]-Fdx led to broadening of subset of the 1H−15N HSQC cross peaks suggesting that these peaks correspond to residues in the interaction site (Figure S3 in SI). We used conventional methods to assign the NMR signals of [U-13C, U-15N]-Fdx in both its oxidized and reduced states (deposited in the Biological Magnetic Resonance data Bank, BMRB, under accession numbers 18991 and 18992, respectively, for oxidized and reduced Fdx). These assignments allowed us to identify the residues corresponding to the NMR signals that broadened preferentially upon the addition of IscS. For oxidized Fdx, these corresponded to the backbone signals from I27, L28, E39, C53, N68–G78, E80, E82–L85, and A89 (Figure 2a); for reduced Fdx, these corresponded to I8, E39, E67–M72, D74–E80, E82–R84, I100, Y103, T104, A108, and R109 (Figure 2b). In both oxidation states, these regions are adjacent to the [2Fe-2S] cluster.

To investigate the physiological relevance of the interaction between Fdx and IscS we took advantage of the large difference in the optical spectra of oxidized and reduced Fdx (Figure S4 in SI) to determine whether an electron from reduced-Fdx is transferred to IscS. We observed that the spectrum of Fdx alone (black trace in Figure 3a) and the spectrum of Fdx mixed with one equivalent (subunit) of IscS (red trace in Figure 3a) did not change over the period of 0.5 h. However, the addition of 5 equiv of l-cysteine to the mixture of reduced-Fdx and IscS led to rapid oxidation of Fdx (blue trace in Figure 3a). This result indicates that electrons from reduced-Fdx are transferred to the product of IscS and Cys. Intriguingly, we observed that reduced Fdx is more stable against air oxidation when complexed with IscS than when alone. Samples containing reduced-Fdx alone were found to oxidize over a period of 2 h, whereas reduced Fdx in a 1:1 mixture with IscS (subunit) failed to oxidize even after 6 h (data not shown). This result is consistent with the proposed binding interface, which shields the Fe–S cluster of Fdx from solvent.

We next examined whether reduced Fdx donates its electron for IscU-mediated Fe–S cluster reconstitution. In the presence of 5 equiv of ferrous ammonium sulfate and 5 equiv of l-cysteine, the UV/vis spectrum of reduced Fdx (black trace in Figure 3b) and the UV/vis spectrum of equimolar reduced Fdx and IscU (subunit) (scarlet trace in Figure 3b) remained unperturbed for over 1 h, indicating that Fdx remained reduced. By contrast, the addition of a catalytic amount (0.02 equivalent subunit) of IscS to the solution of equimolar reduced Fdx and IscU (subunit) (scarlet trace in Figure 3b) and the UV/vis spectrum of equimolar reduced Fdx and IscU (subunit) (scarlet trace in Figure 3b) yielded a UV/vis spectrum (Figure S5 in SI) similar to that of [2Fe-2S]IscU indicating cluster formation. These results indicate that donation of an electron...
from reduced Fdx is essential to cluster assembly on IscU. Notably, when the inactive IscS mutant, IscS(C328S), was substituted in place of wild-type IscS in the above experiments, electron transfer failed to occur even in the presence of L-cysteine (Figure S6 in SI).

CyaY has been reported to negatively regulate in vitro Fe−S cluster reconstitution and to form a ternary CyaY−IscU−IscS complex.Titration of [U-15N]-CyaY with unlabeled IscS argues against the formation of a reported ternary CyaY−IscU ternary complex.20 Titration of [U-15N]-IscS with unlabeled IscU by CyaY may account for this observation. Although our partially restored the desulfurase activity. The displacement of the desulfurase activity of IscS, yet the addition of CyaY was found to displace [U-15N]-Fdx from the [U-15N]-Fdx complex containing CyaY, IscU, and IscS by chemical cross-linking agents, and IscU and IscS were subsequently added in the absence of additional cross-linking reagents. It is known that chemical cross-linking can capture weak or transient protein−protein interactions.21

In eukaryotes, frataxin plays an opposite role.22 In forming a ternary complex with human cysteine desulfurase (Nfs1) and the human scaffold protein (ISCU), human frataxin, the eukaryotic orthologue of CyaY, accelerates the rate of Fe−S cluster biogenesis.23

Finally, we found that Fdx and IscU compete for overlapping binding sites on IscS. The addition of unlabeled Fdx to the [U-15N]-IscU−IscS complex resulted in the displacement of [U-15N]-IscU (Figure S8 in SI). This result suggests that Fdx binds more tightly to IscS than IscU. Together with the data for CyaY binding, it appears that the binding sites on IscS for CyaY, Fdx, and IscU are overlapping and that the order of affinity for IscS is CyaY > Fdx > IscU.

Our results suggest that one of the two electrons needed for reduction of S0 is transferred from Fdx bound to IscS prior to the transfer of the sulfur to IscU. Where this electron is bound remains to be determined, although it could be as a persulfide radical anion.24 It has been proposed that the second electron required to generate S2− is donated by the oxidation of Fe(II) to Fe(III).4 Whether this occurs before or after IscU displaces Fdx is currently under investigation. A second round of this reaction cycle would be required to deliver the second sulfur and iron required for [2Fe-2S] cluster assembly. Our earlier studies25,26 have suggested that the formation of a cluster or nascent cluster involving the Cys residues of IscU will perturb the IscU conformational equilibrium from the partially disordered state (D) to the structured state (S) state observed in the X-ray structure of [2Fe-2S]IscU.27

ASSOCIATED CONTENT

Supporting Information
Experimental procedures, the result of a chemical cross-linking experiment, additional NMR spectra, and additional UV/vis spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interests.

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