Biogenesis

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$(IscS\text{-}IscU)_2$ Complex Structures Provide Insights into Fe_2S_2 Biogenesis and Transfer**

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Iron–sulfur clusters (FeS clusters), ancient prosthetic groups found in proteins belonging to the three kingdoms of life, display a variety of functions, including electron transfer, formation of thiol-containing cofactors, catalysis, metal trafficking, regulation of gene expression, DNA repair/replication, and tRNA thiolation.[1] The numerous functional roles of FeS clusters reflect nature's exploitation of the chemical, structural, and electronic plasticity of these simple structures.[2] Three distinct systems, designated NIF (nitrogen fixation), SUF (sulfur mobilization), and ISC (iron–sulfur cluster), have been shown to catalyze biological formation of FeS clusters and their subsequent transfer to target proteins. NIF was first identified in the assembly of nitrogenase metal centers but related systems are also found in non-nitrogen-fixating microorganisms.[3] SUF, which is operational under O_2 stress and Fe deprivation, is the only FeS cluster assembly system in chloroplasts and cyanobacteria.[4] The ISC system is found in a large number of prokaryotes and in mitochondria,[5] and defects in FeS cluster assembly in the latter can cause human pathologies.[6]

The three systems start cluster biogenesis with the persulfuration by a pyridoxal phosphate (PLP)-dependent cysteine desulfurase of its active cysteine residue.[7] The activated S species is subsequently delivered to assembly scaffold protein(s) where FeS clusters are formed.[8] The primary scaffold can transfer the cluster either directly to client proteins or to an "A-type" carrier that also has the capacity to transfer clusters[9] and, possibly, other intermediate carriers such as NfuA.[10] Concerning the ISC system, structures for the IscS desulfurase,[11] the IscU scaffold,[12] and an apo $(IscS\text{-}IscU)_2$ complex from *Escherichia coli* (*Ec*) are available.[13] Previous work with the diazotroph bacterium *Azotobacter vinelandii* (*Av*) has shown that the substitution of a conserved Asp from a Cys-Gly-Asp motif by Ala in IscU results in the in vivo trapping of a nondissociating $(IscS\text{-}IscU^{D39A})_2$ complex that contains an oxygen-resistant FeS species.[14] The same substitution either hinders[15] or completely abolishes[16] FeS cluster transfer from IscU to client proteins, thus suggesting that IscS-IscU dissociation and IscU-mediated cluster transfer follow related processes.

Herein, we propose processes of FeS cluster biogenesis and IscS-IscU complex dissociation based on the 2.55 Å resolution crystal structure of the anaerobically purified, dithiothreitol (DTT)-treated $(Fe_2S_2\text{-}(IscS\text{-}IscU^{D35A}))_2$ complex from *Archaeoglobus fulgidus VC-16* (*Af*) (Figure 1 A–C; and see Materials and Methods in the Supporting Information, Tables S1 and S2). We also show that the reported oxygen resistance of the FeS cluster in the corresponding *Av* complex[14] is only partial because the 2.75 Å resolution structure obtained from an $Af(IscS\text{-}IscU^{D35A})_2$ crystal grown under air contains an oxidized Fe_2S–S cluster (see Figure S1 in the Supporting Information). The disorder in the crystal of an $IscU^{D35A}$ region involved in cluster coordination strongly suggests that this structure represents a trapped intermediate of cluster O_2-induced degradation.

In all known IscS structures, including the one in the *Ec* apo $(IscS\text{-}IscU)_2$ complex, the active site Cys is located on a disordered region here called the Cys loop.[11,13] Conversely, in our FeS cluster-containing structures, the IscS active site Cys loop is well ordered and points at the cluster-binding site of $IscU^{D35A}$ (Figure 1 A). A comparison of our $Af(Fe_2S_2\text{-}(IscS\text{-}IscU^{D35A}))_2$ complex with the *Synechocystis* IscS-like SufS structure, where the conserved Cys points at the active site PLP cofactor,[17] indicates that the IscS Cys loop, Cys321, moves almost 14 Å during cluster assembly (Figure 1 B). As shown by the apo *Ec* complex,[13] the Cys loop is not essential for the interaction between IscS and IscU (see Figure S2 in the Supporting Information). Consequently, the Cys loop should be able to oscillate between the two conformations discussed above within the complex. In addition, there is a wide tunnel connecting the $IscU^{D35A}$ assembly site and the IscS active site in our complex structures, thus allowing for both cysteine diffusion to the IscS active site and unhindered Cys-loop conformational changes (Figure 1 C). These observations suggest that the persulfided Cys321 can donate successive S^0 atoms to IscU without IscS-IscU complex dissociation.

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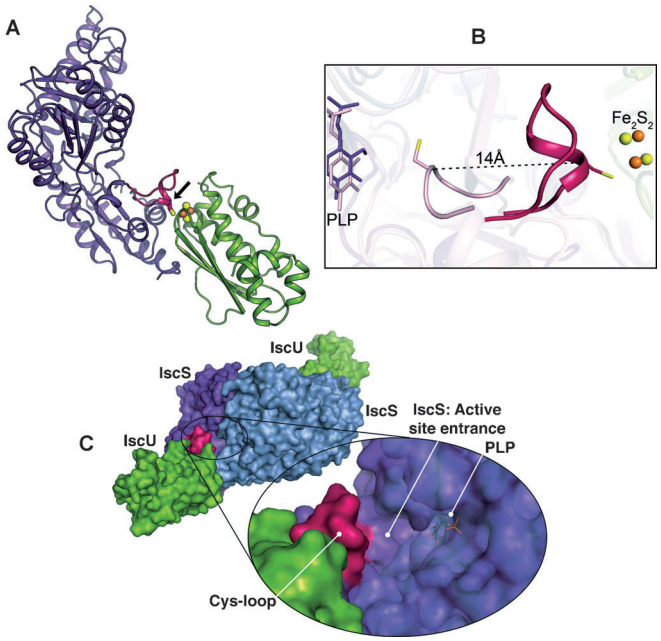

Figure 1. *Af*$(Fe_2S_2$-(IscS-IscU^{D35A}))$_2$ complex structure. A) Ribbon representation of the heterodimer Fe_2S_2-(IscS-IscU^{D35A}). IscS and IscU^{D35A} are represented in purple and green, respectively. The black arrow points at the Cys loop depicted in pink. B) Different conformations for the Cys loop in the IscU^{D35A}-bound *Af*IscS (pink, this work) and in *Synechocystis* SufS (light pink).[17] C) Surface representation of the complex. The two IscS molecules are depicted in purple and gray-blue and the two IscU^{D35A} molecules in green; the IscS Cys loop is shown in pink. Close-up view: the semitransparent IscS surface highlights the PLP position, located deep inside a tunnel. The Cys loop does not occlude this tunnel, which allows for cysteine diffusion to the IscS active site and Cys-loop movements during the cluster assembly process without complex dissociation. This view is rotated by 180° relative to (A).

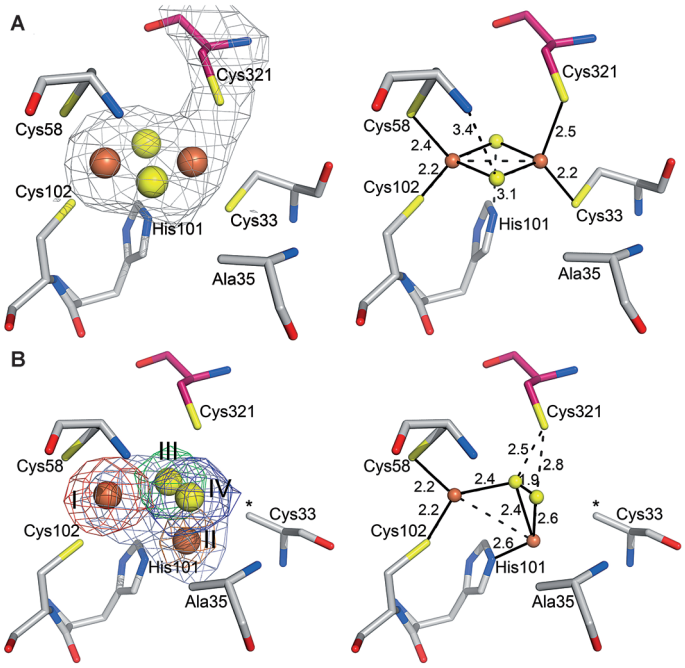

Figure 2. A) The Fe_2S_2 complex with its classical cluster depicted as spheres and IscS Cys321, and the corresponding omit electron density map (left) and bond distances (right). B) The partially oxidized complex with the Fe_2S–S atoms (I–IV) depicted as spheres, their corresponding omit map peaks, and the global omit map (light blue, left). The μ_2-η^3 sulfido ligation has been observed in a model compound[19] (see Figure S5 in the Supporting Information). The maps are contoured at the 3σ level. The -S(H) (indicated by a star) from Cys33 is not visible in the map due to disorder and, consequently, has not been included in the model. Carbon atoms are colored gray and pink in IscU and IscS, respectively.

The crystallographic analysis of the Fe_2S_2 complex also shows that the cluster is coordinated by the three conserved IscU residues Cys33, Cys58, and Cys102, and, remarkably, by Cys321 from the Cys loop of IscS (Figure 2A). The rhomb is further stabilized by interactions between the semiconserved His101 and one of the bridging sulfide ions and between the main-chain NH of Cys58 and the other sulfide ion. The ionic character of the interaction between His101 and one of the sulfide ions of the cluster should be preserved in the related SufU where this residue is replaced by lysine.[5a] Our Fe_2S_2 complex structure supports previous propositions that the semiconserved His residue is not a natural cluster iron ligand.[18]

The proximity of the sulfur-donating IscS Cys321 thiol to both the persulfido ligand and the iron pair in our partially oxidized complex (Figure 2B and Figure S1 in the Supporting Information) shows that direct reductive S transfer to the nascent iron cluster by the active IscS Cys is stereochemically feasible. However, this observation alone does not determine the order of Fe and S arrival to the IscU cluster assembly site, a problem still unsolved. It is known that the three conserved IscU Cys residues can be polysulfurated in the absence of iron,[8a,20] thus underscoring the possibility of intra- or intermolecular S transfer during the assembly process involving the IscU Cys residues and the active site IscS Cys. However, persulfuration of *Thermotoga maritima* (*Tm*) apo IscU has been reported to lead to a dead-end product that does not bind iron.[21] Conversely, mononuclear iron binding in the absence of IscS has been observed for apo forms of *Av*NifU and NifU-1[22] and *Tm*IscU,[21] but this binding only takes place at much lower-than-physiological temperatures. In yeast mitochondria, it is thought that the initial iron binding by Isu1, the eukaryotic orthologue of IscU, takes place at a site different from the cluster assembly pocket. Indeed, Cook et al. have reported that after being transferred from Yfh1, ferrous iron bound to Isu1^{D37A}, the eukaryotic orthologue of the *Af*IscU^{D35A} variant, has two unique nearest-neighbor ligand coordination environments, both O/N-based, without evidence of sulfur ligation.[6] Addition of sulfide mobilizes the iron to the assembly site in Isu1 and an Fe_2S_2 cluster is formed (probably involving the opportunistic histidine side chain (Figure 2B and Figure S1 in the Supporting Information). If a similar situation is assumed in the prokaryotic case, iron migration to the IscU assembly site could be elicited by the arrival of the persulfide form of IscS Cys321 to that region. Persulfide ligation to iron should be possible, as suggested by our partially oxidized complex structure (Figure 2B). Consequently, an alternative to models invoking the initial delivery of either S or Fe during cluster

assembly[20,21] is the simultaneous delivery of persulfurated IscS Cys321 and iron to the IscU assembly site, the metal possibly coming from a storage site in IscU.[6]

Because Cys321 successively donates two S^0 atoms to IscU, a total of four electrons are required to assemble the Fe_2S_2 cluster. The first two electrons are likely to come from the oxidation of two ferrous ions to ferric at the cluster-assembly site,[23] thereby generating the first sulfido iron ligand. The second S^0 reduction event, which could be direct or mediated by reduction of the two ferric ions to ferrous, requires an external two-electron donor. Because the persulfurated Cys321 must deliver the second sulfane at, or very near to, the nascent FeS cluster, the first step could involve nonredox transfer of S^0 to the S^{2-} ion already present at the cluster site, to form a transient $^-S–S^-$ species. The unprecedented formation of an iron persulfido ligand in our partially oxidized crystalline complex (Figure 2B) is compatible with this idea. Subsequent reduction and rearrangement of the cluster atoms would generate the observed Fe_2S_2 center.

Taking advantage of the crystal structures reported here, we carried out loop sampling and hybrid quantum mechanical/molecular mechanical (QM/MM) calculations[24] to explore the functional role of the Cys-Gly-Asp motif contained in all U-type scaffolds (see the Supporting Information). First, we placed the Asp residue at the substituted Ala35 position in the X-ray Fe_2S_2 complex structure. The geometry-optimized model shows only minor structural readjustments because the substituted protein complex can easily accommodate a buried -COO(H) fragment, away from the Fe_2S_2 cluster (Figure 3A and Figure S3 in the Supporting Information). This observation further suggests that Asp35 is

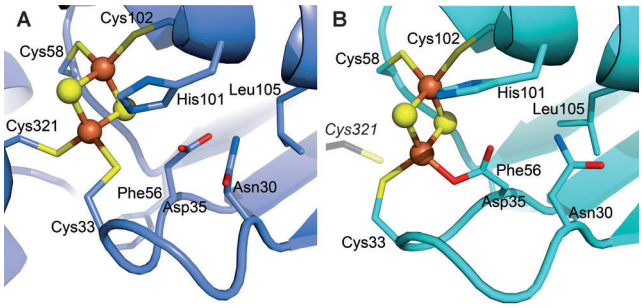

Figure 3. Models from QM/MM calculations. Geometry-optimized structure of A) the *Af*$(Fe_2S_2$-(IscS-IscU))$_2$ complex and B) a model of *Af*Fe_2S_2-IscU where sampling of loop 30–35 was performed to evaluate whether Cys33 and Asp35 could coordinate the cluster. Cys33 replaces IscS Cys321 (see the Supporting Information for details).

not directly involved in Fe_2S_2 cluster assembly. We conclude that our Fe_2S_2 complex is likely to represent a stabilized version of an intermediate in the assembly process just before complex dissociation, which occurs after the two S^0 atoms have been transferred to the cluster assembly site and reduced to S^{2-}.

An alternative role for the conserved Asp35 would be to become a cluster ligand upon dissociation of IscU from IscS, as previously suggested.[5a] To explore this possibility, we performed the sampling of the Cys33 loop using the geometry-optimized, Asp35-substituted Fe_2S_2-IscU model described above after IscS removal. Only those energetically favored solutions with distances for Cys33Sγ–Fe and one of the Asp35Oδ–Fe distributed about 2.3 Å were selected (see the Supporting Information). The assembly site conformer with the lowest energy loop was further geometry-optimized using hybrid QM/MM potentials. The model converged to a regular Fe_2S_2 cluster, coordinated by Cys33, Cys58, Cys102, and Asp35 (Figure 3B). The QM/MM model structure suggests that upon IscS-IscU complex dissociation, the replacement of the IscS Cys321 ligand involves a small translation of the Fe_2S_2 cluster with Cys33 and Asp35 acting as a "clamp" on the more exposed iron ion. A similar, but possibly physiologically irrelevant, clamp function is also indicated by the Zn^{2+} coordination of equivalent Cys and Asp residues in *Streptococcus pyogenes* IscU (see Figure S4 in the Supporting Information).[12a] The clamp prevents the excessive exposure of the cluster that would result if there were no iron coordination changes upon complex dissociation (Figure 4). Bonomi et al. have recently found the coordination of the Fe_2S_2 cluster by IscU dimers to be quite variable.[18] We thus suggest that cluster coordination variability in IscU is generally based on the highly flexible Cys33 loop.

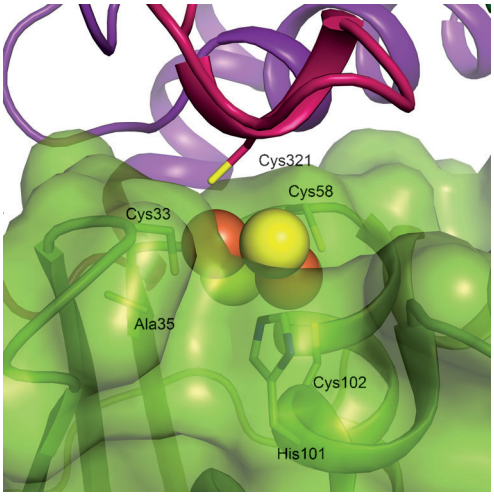

Figure 4. Close-up view of the IscS/IscU^{D35A} interface. Without a reorganization of the Fe_2S_2 cluster coordination sphere, dissociation of the IscS-IscU complex would labilize the cluster, especially the iron coordinated by the flexible Cys33, which is exposed to the solvent medium. Same color code as in Figure 1.

In summary, once the apo form of the (IscS-IscU)$_2$ complex is formed, all subsequent steps of the Fe_2S_2 cluster assembly are likely to take place without complex dissociation. We postulate that during Fe_2S_2 cluster biogenesis the conserved Asp35 remains a spectator because its replacement by alanine does not interfere with this process. Conversely, based on our structural and theoretical data, Asp35 appears to play a fundamental role in complex dissociation by displacing Cys33 and indirectly causing Cys321 dissociation (Figure 3B). This proposition nicely explains why the D35A mutation impairs the complex dissociation step and therefore stabilizes the IscS-IscU complex.

After complex dissociation, Fe_2S_2 cluster integrity will depend on the stability of the resulting IscU species existing either as a monomer, or associating itself to other proteins. A plausible option for the latter is the formation of IscU dimers, which is well documented in the literature.[18,25] In this respect, it would be very informative to solve native and Ala35 mutant structures of IscU with bound Fe_2S_2 clusters.

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