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Mitochondrial Cysteine Desulfurase and ISD11 Coexpressed in *Escherichia coli* Yield Complex Containing Acyl Carrier Protein

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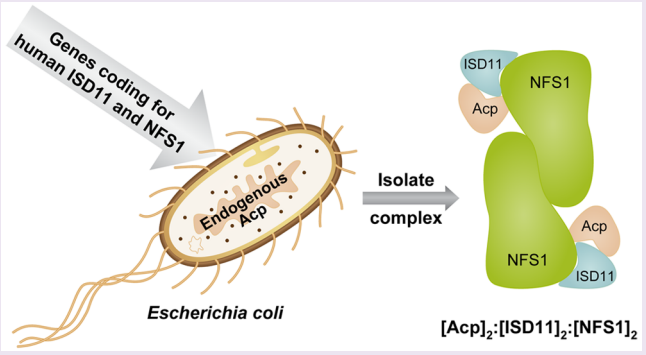
Supporting Information

ABSTRACT: Mitochondrial cysteine desulfurase is an essential component of the machinery for iron−sulfur cluster biosynthesis. It has been known that human cysteine desulfurase that is catalytically active *in vitro* can be prepared by overexpressing in *Escherichia coli* cells two protein components of this system, the cysteine desulfurase protein NFS1 and the auxiliary protein ISD11. We report here that this active preparation contains, in addition, the holo-form of *E. coli* acyl carrier protein (Acp). We have determined the stoichiometry of the complex to be $[Acp]_2$:$[ISD11]_2$:$[NFS1]_2$. Acyl carrier protein recently has been found to be an essential component of the iron−sulfur protein biosynthesis machinery in mitochondria; thus, because of the activity of $[Acp]_2$:$[ISD11]_2$:$[NFS1]_2$ in supporting iron−sulfur cluster assembly *in vitro*, it appears that *E. coli* Acp can substitute for its human homologue.



It has been known for several years that catalytically active mitochondrial cysteine desulfurase can be prepared from *Escherichia coli* cells by coexpressing two essential proteins, the mitochondrial cysteine desulfurase (NFS1) and a small accessory protein (ISD11, also known as LYRM4).[1−12] Each of these proteins has proved difficult to prepare recombinantly on its own, and the presence of ISD11 appears to stabilize the structure of NFS1. We recently discovered that His-tagged human ISD11 when overexpressed in *E. coli* cells pulls down the holo-form of *E. coli* acyl carrier protein (Acp). The complex with Acp appears to stabilize ISD11, which on its own is intrinsically disordered and has a tendency to aggregate (Tonelli, M., Frederick, R.O., Cai, K., Markley, J.L., manuscript in preparation). In addition, Van Vranken and co-workers found that holo-acyl carrier protein interacts with ISD11 and NFS1 and serves as an essential component of the machinery for *in vivo* iron−sulfur (Fe−S) cluster biogenesis.[13] Combined, these findings prompted us to investigate whether ISD11:NFS1 complexes prepared recombinantly from *E. coli* cells also might contain *E. coli* Acp.

Four samples were prepared for analysis as described in Methods. Sample 1 was the size exclusion chromatography (SEC) purified product from coexpression of NFS1 and ISD11 in *E. coli* cells. Sample 2 was an aliquot of sample 1 to which excess human scaffold protein (ISCU) was added, and the complex purified by SEC. Sample 3 was an aliquot of sample 1 to which excess ISCU and human frataxin (FXN) were added, and the complex purified by SEC. Sample 4 was the product of expression of *E. coli* cysteine desulfurase (IscS) in *E. coli* cells after purification by ion-exchange chromatography and SEC.

Sample 1 was digested with trypsin and analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS), which identified peptides from *E. coli* Acp (Figure 1A, Table S1, 44% sequence coverage). To confirm the results, another aliquot of sample 1 was digested with endoproteinase Glu-C (V-8 protease) and analyzed by LC-MS/MS, which also identified peptides from *E. coli* Acp (Figure 1B, Table S2, 88% sequence coverage). SDS gel electrophoresis (SDS-PAGE) of purified cysteine desulfurase complex exhibited a faint band corresponding to Acp (8.6 kDa) in addition to those from NFS1 and ISD11 (Figure 1C). The same faint band was also shown in the SDS-PAGE of purified cysteine desulfurase:ISCU complex (Figure 1D). Acp stains poorly on gels, and this may explain why the protein was not discovered earlier as a component of ISD11:NFS1 complexes. MS/MS fragmentation analysis of the peptide ^{23}VTNNASFVEDLGADSLDTVE^{42} from endoproteinase Glu-C digestion (Table S2, red) indicated that it contained 4′-phosphopantetheine conjugated to the invariant residue S37 (Figure S2, Table S3); thus the complex contains holo-acyl carrier protein (Acp).

To determine the relative stoichiometry of each complex, samples 1−4 were submitted for amino acid analysis, and the results (Table S5) were fitted to different assumed protein compositions (Figure S3). The best fits were $[Acp]_1$:$[ISD11]_1$:$[NFS1]_1$ for sample 1, $[Acp]_1$:$[ISD11]_1$:$[NFS1]_1$:$[ISCU]_1$ for sample 2, and $[Acp]_1$:$[ISD11]_1$:$[NFS1]_1$:$[ISCU]_1$:$[FXN^{81-210}]_1$

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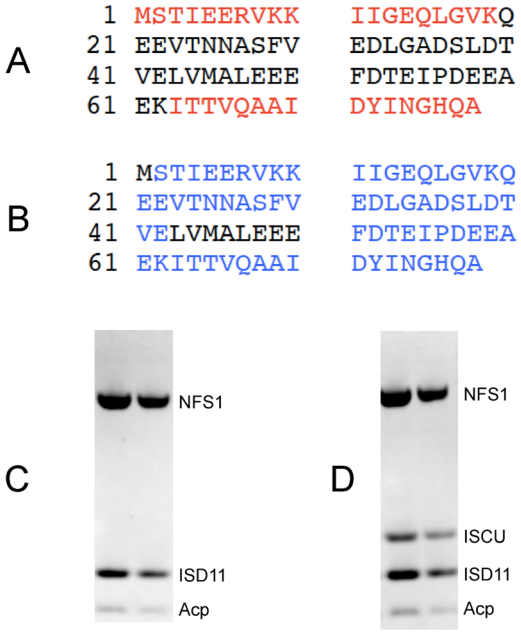

Figure 1. Results from LC-MS/MS and SDS-PAGE showing that human cysteine desulfurase (NFS1) and ISD11 coexpressed in *E. coli* cells form a complex that contains *E. coli* acyl carrier protein (Acp). (A) Mass spectrometry analysis of sample 1 following trypsin digestion revealed the presence of peptides shown in red from the sequence of *E. coli* Acp. (B) Mass spectrometry analysis following digestion of sample 1 with endoproteinase Glu-C identified peptides shown in blue from the *E. coli* Acp amino acid sequence. (C) SDS-PAGE analysis of sample 1 revealed a faint band from Acp in addition to those from ISD11 and NFS1. (D) SDS-PAGE analysis of sample 2 revealed a faint band from Acp in addition to those from ISD11, NFS1, and ISCU.

for sample 3 (Table 1). The relative stoichiometry of the NFS1:ISD11 complex has been reported as either 1:1[8] or 1:2;[19] the results here clearly are in agreement with 1:1 relative stoichiometry. By contrast, the amino acid analysis of sample 4 better fitted that predicted for $[IscS]_1$ than $[Acp]_1:[IscS]_1$ (Figure S3 and Table 1), even though Acp has been reported to bind to IscS.[20,21] LC-MS/MS analysis of trypsin-digested sample 4 failed to indicate the presence of Acp (data not shown).

The molecular weights of IscS and three human mitochondrial cysteine desulfurase complexes (Table 2) were estimated from analysis of small-angle X-ray scattering (SAXS) data (Figure S4). Combined with the relative stoichiometries from amino acid analyses, these results reveal that the absolute stoichiometries are homodimeric in each protein component (Table 2).

Table 2. Results from Small-Angle X-ray Scattering (SAXS) Analysis of Cysteine Desulfurase Complexes

assumed stoichiometry of the complex	R_g (Å) from SAXS	D_{max} (Å) from SAXS	MW (kDa) from SAXS	MW from assumed stoichiometry
sample 1: $[Acp]_2:[ISD11]_2:[NFS1]_2$	41.9 ± 0.2	147 ± 5	125 ± 5	132.2
sample 2: $[Acp]_2:[ISD11]_2:[NFS1]_2:[ISCU]_2$	44.4 ± 0.3	150 ± 5	160 ± 5	159.4
sample 3: $[Acp]_2:[ISD11]_2:[NFS1]_2:[ISCU]_2:[FXN]_2$	38.1 ± 0.1	133 ± 3	175 ± 5	186.5
sample 4: $[IscS]_2$	33.4 ± 0.1	118 ± 3	92 ± 2	90.2

One may question whether other mitochondrial cysteine desulfurase complexes produced from *E. coli* cells reported in the literature contained Acp.[1−12] None of these studies tested for the presence of Acp, and the preparations presumably would have been inactive without Acp.[13] In one of these studies, because the authors provided an amino acid analysis of their complex,[12] it has been possible to test the hypothesis that the complex contained Acp. The authors coexpressed ISD11, NFS1, ISCU, and FXN^{42-210} (an immature form of frataxin) in *E. coli* cells and used amino acid analysis in support of the relative stoichiometry $[ISD11]_1:[NFS1]_1:[ISCU]_1:[FXN^{42-210}]_1$ for the complex.[12] Our linear least-squares fit of the experimental amino acid composition reported in the article to the theoretical composition $[ISD11]_1:[NFS1]_1:[ISCU]_1:[FXN^{42-210}]_1$ yielded $R_2 = 0.962$, whereas its fit to the theoretical composition containing Acp, $[Acp]_1:[ISD11]_1:[NFS1]_1:[ISCU]_1:[FXN^{42-210}]_1$, yielded the improved value of $R_2 = 0.980$, consistent with the presence of Acp in the complex (Figure S5).

Two independent reports have provided evidence for interaction between IscS and Acp.[20,21] The first report proposed a role for Acp in the cysteine desulfurase reaction.[21] We have detected Acp to be present by MS in partially purified samples of IscS; however, sample 4, which was purified by SEC under reducing conditions, contained no detectable Acp. Furthermore, in our hands, purified samples exhibited desulfurase activity and supported *in vitro* Fe−S cluster assembly.[22] No Acp was observed in the X-ray structures of

Table 1. Results from Linear Correlation Analysis between the Experimental Amino Composition of Each Sample and the Amino Acid Composition Predicted from an Assumed Relative Stoichiometry

samples and assumed relative stoichiometry	correlation (R^2)
sample 1: [ISD11]1:[NFS1]1	0.895
sample 1: [ISD11]2:[NFS1]1	0.857
sample 1: [Acp]1:[ISD11]1:[NFS1]1	0.983
sample 2: [ISD11]1:[NFS1]1:[ISCU]1	0.959
sample 2: [ISD11]2:[NFS1]1:[ISCU]1	0.927
sample 2: [Acp]1:[ISD11]1:[NFS1]1:[ISCU]1	0.982
sample 3: [ISD11]1:[NFS1]1:[ISCU]1:$[FXN^{81-210}]$1	0.959
sample 3: [ISD11]2:[NFS1]1:[ISCU]1:$[FXN^{81-210}]$1	0.938
sample 3: [Acp]1:[ISD11]1:[NFS1]1:[ISCU]1:$[FXN^{81-210}]$1	0.992
sample 4: $[IscS]_1$	0.982
sample 4: $[Acp]_1:[IscS]_1$	0.958

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IscS[23] or the IscS:ISCU complex.[24] Thus, it is somewhat ironic that Acp, which had been proposed to play a functional role in *E. coli* Fe−S cluster assembly,[21] has been found to play a critical role in the biosynthesis of Fe−S proteins in mitochondria[13] but not in bacteria.

Recent single-particle electron microscopy studies of other LYRM proteins (LYRM3 and LYRM6, components of complex I) show them bound to Acp, with the 4′-phosphopantetheine group occupying a hydrophobic cavity formed by the LYRM protein.[25−27] Our working model is that overproduced ISD11 becomes structured upon binding endogenous Acp and that the resulting complex binds to overexpressed NFS1 forming an active complex. Although the $[Acp]_2$:$[ISD11]_2$:$[NFS1]_2$ complex has been shown to support Fe−S cluster assembly *in vitro*,[14] it remains to be determined whether the complex would be more or less active with a mitochondrial Acp in place of the bacterial homologue.

METHODS

Protein samples and complexes were prepared as described in detail elsewhere.[14] Briefly, ISD11-His_6 and His_6-SUMO-NFS1 were coexpressed in *E. coli* cells, and the complex was isolated by immobilized metal affinity chromatography (IMAC) and then cleaved with SUMO protease; the released His_6-SUMO was removed by size exclusion chromatography (SEC) yielding a complex containing ISD11 and NFS1 (sample 1). His_6-SUMO-ISCU and His_6-SUMO- FXN^{81-210} (mature form of frataxin) were expressed in *E. coli* cells; ISCU and FXN^{81-210} were each isolated by IMAC followed by subtractive IMAC to remove His_6-SUMO, and then the proteins were purified by SEC. Sample 2 was prepared from an aliquot of sample 1 to which ISCU was added; the complex was subsequently purified by SEC. Sample 3 was prepared from an aliquot of sample 1 to which equimolar ISCU and FXN^{81-210} were added; the complex was subsequently purified by SEC. Compositions of each complex were analyzed by SDS-PAGE (Figure 1, Figure S1) and mass spectrometry (Tables S2−S4). Sample 4 consisted of *E. coli* cysteine desulfurase (IscS), which was prepared as described earlier.[15]

Solutions used for SAXS contained 0.05 mM protein in 20 mM HEPES buffer at pH 7.6 with 150 mM NaCl and 2 mM TCEP. SAXS data were collected immediately after SEC to ensure monodispersion. SAXS experiments were carried out on a Bruker Nanostar benchtop SAXS system (Bruker AXS) at the National Magnetic Resonance Facility at Madison (NMRFAM) equipped with a rotating anode (Cu) Turbo X-ray Source and a Vantec-2000 (2048 × 2048 pixel) detector. The sample-to-detector distance was set at ∼1 m, allowing for the detection range $0.012 > q > 0.300$ Å^{-1}. Then, 40 μL of protein and buffer samples were loaded separately into a capillary cell with 1 mm diameter, and scattering data were collected for 3 h with frames recorded every hour. Each frame was compared to check for radiation damage, and none was detected over the course of the experiments. The SAXS data sets were then averaged and converted to 1D scattering profiles for further analysis. The ATSAS software suite[16] was used to process the SAXS data. The radius of gyration (R_g) for each protein or protein complex was determined by using the Guinier approximation in the q range (q_{max}·R_g) < 1.3. Pairwise distance distribution functions (P_r) were obtained using the software GNOM[17] to yield D_{max}. Molecular mass was determined by the V_c approach.[18]

Mass spectrometry was carried out at the University of Wisconsin—Madison Mass Spectrometry/Proteomics Facility. Amino acid analyses were performed by AAA Service Laboratory Inc., Damascus, Oregon, United States.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acschembio.6b01005.

LC-MS results from analysis of cysteine desulfurase complexes, experimental amino acid analyses of samples, SDS-PAGE of Sample 3, MS/MS fragmentation analysis, correlations between experimental amino acid compositions and the amino acid compositions of assumed stoichiometries, experimental SAXS data for cysteine desulfurases and their complexes, comparison of the correlation between the reported amino acid composition of a human mitochondrial cysteine desulfurase complex[12] and theoretical compositions without and with equimolar Acp (PDF)

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Notes

The authors declare no competing financial interest.

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