BIOCHEMISTRY
including biophysical chemistry & molecular biology

Rapid Report

pubs.acs.org/biochemistry

Molecular Investigation of Iron−Sulfur Cluster Assembly Scaffolds under Stress

Béatrice Blanc,[†,‡,§] Martin Clémancey,[†,‡,∥] Jean-Marc Latour,[†,‡,∥] Marc Fontecave,*[,†,‡,§,⊥] and Sandrine Ollagnier de Choudens*[,†,‡,§]

[†]Université Grenoble Alpes, iRTSV-LCBM, F-38000 Grenoble, France

[‡]CNRS, iRTSV-LCBM, F-38000 Grenoble, France

[§]CEA, iRTSV-LCBM-Biocat, F-38000 Grenoble, France

[∥]CEA, iRTSV-LCBM-pmb, F-38000 Grenoble, France

[⊥]Laboratoire de Chimie des Processus Biologiques, UMR 8229 CNRS, Université Pierre et Marie Curie-Paris 6, Collège de France, 11 Place Marcelin Berthelot, 75231 Paris Cedex 05, France

Supporting Information

ABSTRACT: Fe/S biosynthesis is controlled in *Escherichia coli* by two machineries, the housekeeping ISC machinery and the SUF system that is functional under stress conditions. Despite many *in vivo* studies showing that SUF is more adapted for Fe/S assembly under stress, no molecular data supporting this concept have been provided so far. This work focuses on molecular studies of key actors in Fe/S assembly, the SufB and IscU scaffolds under oxidative stress and iron limitation. We show that the IscU Fe_2S_2 cluster is less stable than the SufB Fe_2S_2 cluster in the presence of hydrogen peroxide, oxygen, and an iron chelator.

Iron−Sulfur (Fe/S) clusters, Fe_2S_2 and Fe_4S_4 clusters, are essential inorganic cofactors for a large set of proteins within all organisms, participating in a wide range of physiological processes, including respiration, photosynthesis, DNA repair, metabolism, and regulation of gene expression.[1] An increased number of cellular dysfunctions, including human pathologies, are found to be related to mutations in genes encoding Fe/S enzymes or systems synthesizing Fe/S clusters.[2] Iron limitation and oxidative stress constitute stresses under which Fe/S cluster homeostasis is disrupted, either because Fe/S building is made difficult by a decrease in iron availability or because Fe/S stability is endangered by direct oxidation by reactive oxygen species (ROS), the best illustration being the Fe_4S_4 cluster of dehydratases.[3] Fe/S cluster biosynthesis is achieved *in vivo* through dedicated machineries.[2,4] In *Escherichia coli*, it is accomplished by two machineries whose proteins are encoded by the *isc* (*iscSUA-hscBA-fdx-iscX*) and *suf* (*sufABCDES*) operons. Both machineries contain similar key molecular actors and share the same basic mechanisms for maturation of Fe/S proteins: a first step during which an Fe/S cluster is transiently built on a scaffold protein, IscU and SufB in the ISC and SUF systems, respectively, and a second step during which the assembled Fe/S cluster is transferred from the scaffold to target apoproteins.[5] Genetic and physiological observations established that the ISC system carries out Fe/S cluster assembly under normal conditions (iron, no ROS) while the SUF pathway is the stress-responsive system (ROS, low iron). Accordingly, the *suf* operon is under control of OxyR, Fur, and apo-IscR transcriptional regulators that respond to hydrogen peroxide stress and iron limitation, leading to a 10−20-fold induction of *suf* genes.[4] In addition, *E. coli suf* mutants are highly sensitive to oxidative stress and iron limitation in comparison to *isc* ones, and the activities of the Fe/S enzymes in *suf* mutant strains are more affected than those of the *isc* ones.[6−8] What are the intrinsic properties of the SUF system allowing it to protect Fe/S cluster homeostasis under stress conditions? Here, to address this issue biochemically, we characterize the reactivity of these clusters with regard to hydrogen peroxide, molecular oxygen, and iron chelation. Our data clearly demonstrate that the Fe/S cluster of SufB is more resistant to each of these stresses than that of IscU. These results provide a molecular explanation for the *in vivo* data.

Because IscU and SufB cannot be purified with their Fe/S cluster, the apoprotein preparations were reconstituted with iron and sulfide anaerobically *in vitro*. The IscU dimer that contains one Fe_2S_2 cluster was prepared as already described.[9] It contains 1.10 Fe and 1.07 S molecules per monomer and displays an Fe_2S_2 cluster-characteristic UV−visible spectrum (A_{456}/A_{280} = 0.22, and ε_{456} = 7.5 mM^{-1} cm^{-1}), in agreement with published data (Figure 1A).[9] Previous studies have established that SufB assembles an Fe_4S_4 cluster.[10,11] During this work, we discovered that in fact SufB stabilizes an Fe_2S_2 cluster, which is extensively described below. Such an Fe_2S_2 SufB form was obtained by anaerobic incubation of apo-SufB with a 3-fold molar excess of ferric iron and sulfide and purification onto an anion exchange column. It contains 2.0 Fe and 1.6 S atoms per SufB (A_{420}/A_{280} = 0.18, and ε_{420} = 8.5 mM^{-1} cm^{-1}). Figure 1B shows its UV−visible spectrum characteristic of an Fe_2S_2 cluster-containing protein with absorption bands at 420 and 320 nm. Some unspecific Fe/S clusters contribute to some absorption at 600 nm. The Mössbauer spectrum of SufB reconstituted with ^{57}Fe and sulfide under these conditions (Figure 1B inset) confirms the

Received: October 8, 2014
Revised: December 4, 2014
Published: December 8, 2014

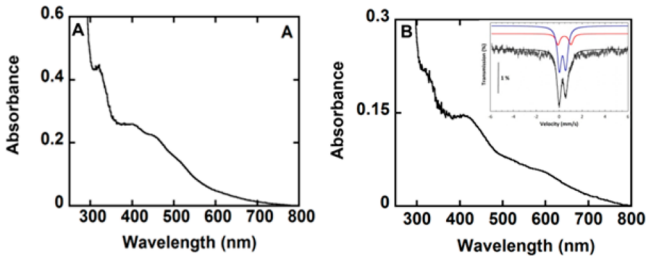

Figure 1. Characterization of reconstituted scaffold proteins. (A) Fe_2S_2 IscU (29 μM Fe_2S_2, 1.10 Fe atoms, and 1.07 S atoms per IscU), (B) Fe_2S_2 SufB (13 μM Fe_2S_2, 2.0 Fe atoms, and 1.6 sulfur atoms per SufB). The inset shows the Mössbauer spectrum of Fe_2S_2 SufB (250 μM SufB, 59% of Fe_2S_2 in blue and 16% of Fe_4S_4 in red).

cluster nuclearity with a major species whose parameters are characteristic of an Fe_2S_2 cluster (Table S1 of the Supporting Information). The Fe_2S_2 cluster of SufB is stable under anaerobic conditions for several days and can stand many freeze−thaw cycles (data not shown). The functionality of this Fe_2S_2 SufB form is demonstrated by its ability to achieve full maturation of apo-ferredoxin into Fe_2S_2 ferredoxin within 15 min (Figure S1 of the Supporting Information). The SufB Fe_2S_2 cluster can be converted to an Fe_4S_4 cluster through reduction as already observed for the IscU cluster.[12] Spectroscopic, biochemical, and functional properties of the Fe_4S_4 SufB form are presented in Figures S1A,D and S2 of the Supporting Information. In this study, we investigate the cluster stability of SufB and IscU in their Fe_2S_2 form toward stresses such as oxygen, hydrogen peroxide, and iron deprivation.

Reaction of IscU and SufB clusters with hydrogen peroxide was monitored by the absorbance variation at 420 nm as a function of time because cluster degradation is characterized by a bleaching of the solution (Figure S3 of the Supporting Information). To achieve complete cluster degradation within a given reaction time, more equivalents of H_2O_2 in the case of SufB (35, 70, 93, and 232 equiv) are required with regard to IscU (15, 21, and 61 equiv) (Figure 2). At each concentration of H_2O_2 in both cases, Fe/S degradation kinetics could be fit to a single exponential. Pseudo-first-order rate constants (k_{obs}) were plotted as a function of H_2O_2 concentration (inset of Figure 2). The second-order rate constant for IscU (4.6 M^{-1} s^{-1}) was 4-fold higher than that for SufB (1.1 M^{-1} s^{-1}) (Table 1). Altogether, these results show that the SufB Fe_2S_2 cluster is more resistant to hydrogen peroxide than the IscU Fe_2S_2 cluster.

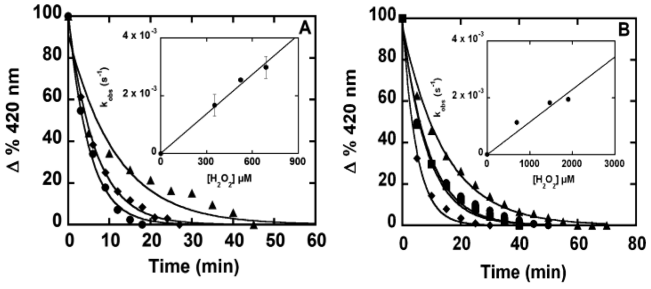

Figure 2. Kinetics of degradation of the IscU and SufB scaffold Fe_2S_2 clusters with H_2O_2. The kinetics of degradation of (A) the IscU Fe_2S_2 cluster (20 μM) in the presence of 15 (▲), 21 (◆), and 61 (●) equiv of H_2O_2 and (B) the SufB Fe_2S_2 cluster (22 μM) in the presence of 35 (▲), 70 (●), 103 (■), and 232 (◆) equiv of H_2O_2. Insets show pseudo-first-order rate constants plotted as a function of H_2O_2 concentration. Kinetic constants (k_{obs}) were determined by fitting the data to a linear fit.

Table 1. Second-Order Rate Constants [k (M^{-1} s^{-1})] for IscU and SufB

	IscU	SufB	IscU, HscA, and HscB	$SufBC_2D$
H_2O_2	4.6	1.1	2.5	0.5
O_2	4.8	0.7	4.1	*a*

[a]Not determined because of the stability of the $SufBC_2D$ cluster and O_2 concentration limitation in saturated buffer. HscA and HscB were added in stoichiometric amounts vs IscU. No ATP and KCl were added.

The kinetics of Fe/S degradation in the presence of air was also studied by UV−visible absorption spectroscopy (Figure S4 of the Supporting Information). A k_{obs} value of 8.8×10^{-4} s^{-1} and a half-life of 13 min could be extracted for IscU, whereas a k_{obs} value of 1.16×10^{-4} s^{-1} and a half-life of 99 min were obtained for SufB, showing that the SufB Fe_2S_2 cluster is much more stable to air than the IscU cluster (Figure S4 of the Supporting Information). To obtain second-order rate constants for Fe/S cluster degradation by oxygen, controlled amounts of O_2 (9, 12, and 15 equiv) were added to a known concentration of clusters assuming that the oxygen concentration in solution equilibrated with air is 284 μM (20 °C, 760 mmHg). In the case of IscU, pseudo-first-order rate constants (k_{obs}) were plotted as a function of O_2 concentration (Figure S5 of the Supporting Information), which resulted in a linear function. A second-order rate constant of 4.8 M^{-1} s^{-1} was calculated (Figure S5 of the Supporting Information and Table 1). In contrast to the IscU cluster, the SufB cluster was shown to be remarkably resistant to similar oxygen concentrations as no changes in the 300−700 nm region could be observed on the same time scale (2 h). For example, with 15 equiv of O_2, complete destruction of the cluster took 10 h (Figure S6 of the Supporting Information) with a half-life of 106 min. Under the same conditions (15 equiv of O_2), the IscU cluster had a half-life of 16.5 min. To be able to determine a pseudo-first-order rate constant for SufB Fe_2S_2 cluster disruption, we used 15 and 23 equiv of oxygen. A second-order rate constant of 0.7 ± 0.05 M^{-1} s^{-1} (Figure S6 of the Supporting Information and Table 1) was calculated, a value that is 7-fold lower than that for IscU, further illustrating that indeed the SufB Fe_2S_2 cluster is much more stable than the IscU Fe_2S_2 cluster in the presence of oxygen.

Fe/S cluster reactivity toward oxygen and hydrogen peroxide was also studied in the presence of their molecular chaperones, namely, HscA/HscB and SufC/SufD for IscU and SufB, respectively. Both chaperone systems form a complex with its corresponding scaffold and through their ATPase activity facilitate Fe/S cluster formation or transfer.[10,13,14] In the presence of hydrogen peroxide, both IscU/HscAB and $SufBC_2D$ clusters were degraded. The k_{obs} values calculated for IscU/HscAB and $SufBC_2D$ were plotted as a function of H_2O_2 concentration and demonstrated a linear function. A second-order rate of 2.5 M^{-1} s^{-1} was calculated for IscU/HscAB versus 0.5 M^{-1} s^{-1} for $SufBC_2D$ (Figure S7 of the Supporting Information and Table 1). These results show that chaperones of each Fe/S assembly system measurably increase the stability of the Fe/S cluster of their respective scaffold in the presence of H_2O_2, by 2.2-fold in the case of the SUF system

dx.doi.org/10.1021/bi5012496 | *Biochemistry* 2014, 53, 7867−7869

versus 1.8-fold with HscA/HscB proteins. Associated with the chaperones, the difference in stability between SufB and IscU clusters is slightly greater. In the presence of O_2, a second-order rate constant of 4.1 M^{-1} s^{-1} was calculated for IscU/HscAB (Table 1 and Figure S7 of the Supporting Information), showing no significant effect of HscAB on IscU Fe/S cluster stability under these conditions. For $SufBC_2D$, it was not possible to calculate a second-order rate constant as the cluster is perfectly stable at any used oxygen concentration.

Finally, we studied the sensitivity of Fe_2S_2 clusters with regard to ethylenediaminetetraacetic acid (EDTA), an iron chelator, with a K_{ass} for ferric iron of 10^{25} M^{-1},[15] a situation that would mimic iron deprivation. When IscU was incubated anaerobically with 64 equiv of EDTA, the solution became colorless quickly, with a concomitant decrease in light absorption in the 300−500 nm range (Figure S8 of the Supporting Information). An exponential decay was observed allowing us to calculate a k_{obs} value of $(7.0 \pm 2.2) \times 10^{-4}$ s^{-1} (Table 2). In contrast, the SufB Fe_2S_2 cluster proved to be much less sensitive to the same concentration of EDTA with a k_{obs} value of $\sim 6 \times 10^{-5}$ s^{-1} for 68 equiv of EDTA. We need to use close to 1000 equiv of EDTA to obtain a k_{obs} value on the same order of magnitude (10^{-4}) as that of IscU (Table 2), suggesting that the SufB cluster is more resistant to iron deprivation than the IscU cluster. The presence of chaperones had no influence on IscU cluster degradation by EDTA [k_{obs} of $(6.7 \pm 1.7) \times 10^{-4}$ with chaperones vs $(7.0 \pm 2.2) \times 10^{-4}$ s^{-1} without], in contrast to the case for the SufB cluster that was further protected by SufC and SufD (Table 2). Indeed, even with 1200 equiv of EDTA, the $SufBC_2D$ cluster did not show any degradation after incubation for 2 h.

Table 2. k_{obs} (s^{-1}) Values Determined with EDTA for IscU and SufB Clusters

		equiv	k_{obs} (s^{-1})
with EDTA	IscU	64	$(7.0 \pm 2.2) \times 10^{-4}$
	IscU, HscA, and HscB	65	$(6.7 \pm 1.7) \times 10^{-4}$
	SufB	68	6×10^{-5}
	SufB	975	2.3×10^{-4}
	$SufBC_2D$	51	*a*
	$SufBC_2D$	1200	*a*
without EDTA	$SufBC_2D$	0	*a*
	IscU, HscA, and HscB	0	$(3 \pm 1) \times 10^{-4}$

[a]Cannot be calculated because of the extreme stability of the Fe/S cluster. HscA and HscB were added in stoichiometric amounts vs IscU. No ATP and KCl were added.

In conclusion, we measured for the first time kinetic parameters for degradation of scaffold Fe/S clusters that reflect their relative reactivity with regard to oxygen, hydrogen peroxide, and iron chelators. Our data show unambiguously that the SufB Fe_2S_2 cluster is much more resistant to O_2, H_2O_2, and the iron chelator than the IscU Fe_2S_2 cluster (5−7-fold more resistant), which fits with the presumed function of SufB as a machinery for Fe/S cluster assembly under stress conditions and iron limitation. The molecular origin of that specificity is unclear. One possibility is that the SufB cluster is less accessible than the IscU cluster, and this behavior is further enhanced in the presence of SufC and SufD, which lead to an extremely stable SufB cluster. Structural data on each scaffold protein with their Fe/S cluster, still unavailable, could confirm this hypothesis. Additionally, we demonstrate here that SufB assembles a stable Fe_2S_2 cluster, raising the possibility that the Fe/S cluster of both SufB and IscU is an Fe_2S_2 cluster in their resting state, able to aid the maturation of Fe_2S_2 targets. When required, they convert to Fe_4S_4-containing protein, under reducing conditions, to aid the maturation of Fe_4S_4 proteins. These Fe_4S_4 clusters are extremely sensitive to oxidants, but whereas the IscU cluster fully degrades leading to apo-IscU, the SufB cluster better stabilizes an Fe_2S_2 cluster.

■ ASSOCIATED CONTENT

Supporting Information

Materials and methods and additional figures. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: sollagnier@cea.fr.

*E-mail: mfontecave@cea.fr.

Funding

S.O.d.C. thanks the Agence Nationale pour la Recherche for FeStreS Contract ANR-11-BSV3-022-02. J.-M.L. acknowledges the partial financial support of Labex ARCANE (ANR-11-LABX-0003-01).

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank V. Nivière (CBM/Biocatalyse) for help in kinetic studies and for scientific discussions.

■ REFERENCES

(1) Py, B., Moreau, P. L., and Barras, F. (2011) *Curr. Opin. Microbiol. 14*, 218−223.

(2) Lill, R., and Muhlenhoff, U. (2008) *Annu. Rev. Biochem. 77*, 669−700.

(3) Flint, D. H., Smyk-Randall, E., Tuminello, J. F., Draczynska-Lusiak, B., and Brown, O. R. (1993) *J. Biol. Chem. 268*, 25547−25552.

(4) Roche, B., Aussel, L., Ezraty, B., Mandin, P., Py, B., and Barras, F. (2013) *Biochim. Biophys. Acta 1827*, 455−469.

(5) Fontecave, M., and Ollagnier-de-Choudens, S. (2008) *Arch. Biochem. Biophys. 474*, 226−237.

(6) Jang, S., and Imlay, J. A. (2010) *Mol. Microbiol. 78*, 1448−1467.

(7) Nachin, L., Loiseau, L., Expert, D., and Barras, F. (2003) *EMBO J. 22*, 427−437.

(8) Outten, F. W., Djaman, O., and Storz, G. (2004) *Mol. Microbiol. 52*, 861−872.

(9) Agar, J. N., Krebs, C., Frazzon, J., Huynh, B. H., Dean, D. R., and Johnson, M. K. (2000) *Biochemistry 39*, 7856−7862.

(10) Wollers, S., Layer, G., Garcia-Serres, R., Signor, L., Clemancey, M., Latour, J. M., Fontecave, M., and Ollagnier de Choudens, S. (2010) *J. Biol. Chem. 285*, 23331−23341.

(11) Saini, A., Mapolelo, D. T., Chahal, H. K., Johnson, M. K., and Outten, F. W. (2010) *Biochemistry 49*, 9402−9412.

(12) Chandramouli, K., Unciuleac, M. C., Naik, S., Dean, D. R., Huynh, B. H., and Johnson, M. K. (2007) *Biochemistry 46*, 6804−6811.

(13) Hoff, K. G., Silberg, J. J., and Vickery, L. E. (2000) *Proc. Natl. Acad. Sci. U.S.A. 97*, 7790−7795.

(14) Silberg, J. J., Tapley, T. L., Hoff, K. G., and Vickery, L. E. (2004) *J. Biol. Chem. 279*, 53924−53931.

(15) Caris, C., Baret, P., Beguin, C., Serratrice, G., Pierre, J. L., and Laulhere, J. P. (1995) *Biochem. J. 312*, 879−885.