Journal of the Korean Magnetic Resonance Society **2020**, *24*, 86-90
DOI 10.6564/JKMRS.2020.24.3.086

Structural flexibility of *Escherichia coli* IscU, the iron-sulfur cluster scaffold protein

Bokyung Kim and Jin Hae Kim*

Department of New Biology, Daegu Gyeongbuk Institute of Science and Technology, Daegu 42988, Republic of Korea

Received Sep 15, 2020; Revised Sep 18, 2020; Accepted Sep 18, 2020

Abstract Iron-sulfur (Fe-S) clusters are one of the most ancient yet essential cofactors mediating various essential biological processes. In prokaryotes, Fe-S clusters are generated via several distinctive biogenesis mechanisms, among which the ISC (Iron-Sulfur Cluster) mechanism plays a house-keeping role to satisfy cellular needs for Fe-S clusters. The *Escherichia coli* ISC mechanism is maintained by several essential protein factors, whose structural characterization has been of great interest to reveal mechanistic details of the Fe-S cluster biogenesis mechanisms. In particular, nuclear magnetic resonance (NMR) spectroscopic approaches have contributed much to elucidate dynamic features not only in the structural states of the protein components but also in the interaction between them. The present minireview discusses recent advances in elucidating structural features of IscU, the key player in the *E. coli* ISC mechanism. IscU accommodates exceptional structural flexibility for its versatile activities, for which NMR spectroscopy was particularly successful. We expect that understanding to the structural diversity of IscU provides critical insight to appreciate functional versatility of the Fe-S cluster biogenesis mechanism.

Keywords iron-sulfur cluster, iron-sulfur cluster biogenesis, protein structure, protein dynamics, NMR spectroscopy

Introduction

Iron-sulfur clusters are essential and ubiquitous cofactors mediating various important biological activities.[1] Owing to superiority of iron ions in accepting and donating electrons, an Fe-S cluster is often employed for electron transport and redox mechanisms, while its usage is not limited to them but extended to cover various indispensable biological processes, such as iron and sulfur trafficking, enzyme catalysis, and gene regulation.[2]

In eukaryotes, proteins mediating Fe-S cluster biogenesis reside in mitochondria, where most Fe-S clusters are made and distributed to the entire cell.[3] Eukaryotic Fe-S cluster biogenesis mechanism is correlated with several essential physiological processes. For example, interference in mitochondrial Fe-S cluster biogenesis is a well-known cause for iron overload of mitochondria.[4] In addition, recent studies have shown that mitochondrial lipid metabolism is directly connected by the mitochondrial Fe-S cluster biogenesis mechanism.[5] Therefore, it is of great importance to elucidate the detailed mechanism how these Fe-S clusters are made and supplied to the cell.

Prokaryotic Fe-S cluster biogenesis mechanisms are mainly divided into three systems: NIF (Nitrogen Fixation), ISC (Iron-Sulfur Cluster), and SUF (Sulfur Utilization Factor), among which the ISC mechanism is known to act as a house-keeping mechanism.[2] The ISC mechanism is known to be conserved in

* Address correspondence to: **Jin Hae Kim,** Department of New Biology, Daegu Gyeongbuk Institute of Science & Technology, Daegu 42988, Republic of Korea, Tel: 82-53-785-1770; E-mail: jinhaekim@dgist.ac.kr

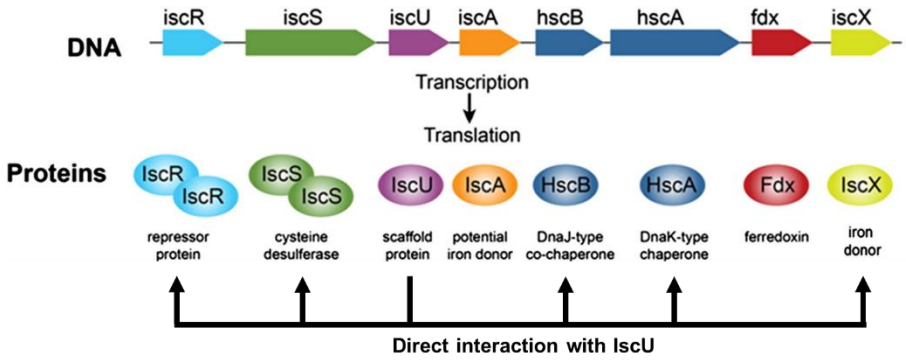

Figure 1. The components involved in *Escherichia coli* ISC Fe-S cluster biogenesis mechanism. Most proteins of the *E. coli* ISC mechanism are encoded in the *isc* operon, all the products of which play essential roles to mediate Fe-S cluster assembly and transfer. Notably, IscU, the key hub of this mechanism, interacts with IscR, IscS, HscB, HscA, and IscX. Adapted from [20] by permission from Elsevier.

mitochondria of eukaryotes.

Despite physiological significance, however, structural details of proteins involved in the Fe-S cluster biogenesis mechanism are still elusive, particularly due to dynamic features of themselves or upon forming a complex with the other proteins and ligands.[6,7] NMR spectroscopy made significant contribution to investigate structural flexibility and conformational dynamics of proteins and protein complexes and to elucidate the mechanistic details of Fe-S cluster assembly and transfer processes.[7–9] The present minireview discusses the recent NMR-based structural characterization for *E. coli* IscU, the key player of the ISC Fe-S cluster biogenesis processes. IscU exhibits extremely flexible structural features, which correlates with its capabilities to interact with various proteins and mediate essential physiological functions such as the Fe-S cluster assembly and transfer (Fig. 1).[7,10] We therefore expect that the current review of recent progresses in structural studies of IscU provides invaluable insights to appreciate the mechanistic details of the Fe-S cluster biogenesis mechanisms not only for prokaryotes but also for eukaryotes.

Structural flexibility of IscU

IscU is a U-type scaffold protein on which an Fe-S cluster is made, and from which the assembled cluster is transferred to recipient proteins.[11,12] IscU plays a key role in the processes of the Fe-S cluster biogenesis; it accepts iron and sulfur, assembles an Fe-S cluster, and transfer it by interacting with diverse proteins that require Fe-S clusters for their activity. Being consistent with these versatile and central functionalities, IscU has exceptional capabilities to adopt two distinctive states, the structured state (S-state) and the disordered state (D-state; Fig. 2).[7,13] A series of subsequent studies to characterize these structural features found that the equilibrium between two states are maintained in a delicate balance. For example, it was reported that a certain set of site-specific mutations (e.g. Asp39Ala, Asn90Ala, Ser107Ala, and Glu111Ala) stabilizes S-state, while the other set of mutations (e.g. Lys89Ala and Asn90Asp) stabilizes D-state.[7] On the other hand, metal ions, such as Zn^{2+}, Fe^{2+}, and Ga^{2+}, stabilize S-

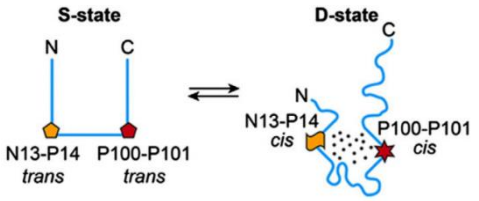

Figure 2. Structural plasticity of *E. coli* IscU. IscU maintains structural equilibrium to exhibit the S (structured) state and the D (disordered) state. The peptidyl-prolyl peptide bonds of N13-P14 and P100-P101 residues are in the *trans* conformation in the S-state, while they adopt the *cis* conformation in the D-state. Reprinted by permission from Elsevier.[20]

state of IscU.[7,14] Consistently, most structural models of IscU or its orthologs in different organisms were determined in Zn^{2+}-bound form.[15,16] Kim et al. determined solution structural model of *E. coli* apo-IscU in its S-state, which appeared to exhibit more dynamic features than the other structural models of Zn^{2+}-bound IscU.[17] It is notable that ISCU, the human ortholog of IscU, also maintains the structural equilibrium displaying both the S- and D-state in a physiological condition,[18] indicating that this structural plasticity is conserved at least from *E. coli* to human.

Subsequent studies have found that proline isomerization is involved in the structural transition of IscU; the peptidyl–prolyl peptide bonds for Asn13-Pro14 and Pro100-Pro101 are in *cis* conformations in the D-state, while they are in *trans* conformations in the S-state.[10,19] The *cis* conformation of peptidyl-prolyl peptide bond is energetically unfavorable, the presence of which in the D-state implies that the D-state may maintain at least some structural constraints to stabilize the unfavorable structural elements. More detailed investigation is necessary to appreciate structural features of the D-state.

Structural states of IscU in the Fe-S cluster biogenesis mechanism

Structural flexibility of IscU plays an essential role in the Fe-S cluster biogenesis mechanism. A series of studies showed that two distinctive states of IscU are alternately employed to mediate different functions for the Fe-S cluster assembly and transfer (Fig. 3).[20]

First of all, it appears that IscU maintains its apo-state-like flexibility upon forming a complex with IscS.[7] Several crystallographic studies have determined the structural models for IscU or ISCU (human ortholog) that are bound to IscS or NFS1 (human ortholog), in which the S-state of IscU/ISCU is stabilized.[21–23] In contrast, NMR-based studies have shown that IscU/ISCU stabilizes the D-state upon forming a complex with IscS/NFS1.[18] This inconsistency suggests that IscU may maintain its structural heterogeneity even upon forming a complex with IscS, which is consistent with the functional heterogeneity of IscU; IscU should remain flexible to accept iron and sulfur, while it also needs to be stable enough to sustain the assembled Fe-S cluster.

Once an Fe-S cluster is assembled on IscU, it is subsequently turned in to the Fe-S cluster transfer processes. In *E. coli*, the specialized Hsp70-type chaperone and Hsp40-type cochaperone pair, HscA and HscB respectively, is dedicated to mediate the transfer processes of an Fe-S cluster to a recipient protein. The cluster-bound IscU maintains its S-state as observed in *Aquifex aeolicus* IscU with X-ray crystallographic study.[24] The NMR spectroscopic studies confirmed that HscB preferentially binds to and further stabilizes the S-state of IscU.[13] This is consistent with the physiological role of HscB which guides Fe-S cluster-bound IscU to HscA. Notably, it was shown with X-ray crystallography and NMR spectroscopy that HscA preferentially binds to the D-state of IscU.[25,26] Therefore, the ternary complex between IscU, HscB, and HscA involves distinctive interaction network. The cluster-bound IscU interacts with HscB, while it does not interact with HscA; HscB acts as a bridge protein to maintain a ternary complex. In contrast, after an Fe-S cluster is dissociated from IscU, HscA now forms a tight complex with the D-state of IscU, and HscB is not able to form a stable complex with IscU, causing dissociation of HscB from the ternary complex.[20]

It should be stressed that despite its special functionalities, HscA retains its Hsp70-like features, such as ATPase activity, capabilities to accommodate interaction with a cochaperone (HscB), and conformational exchange between ADP-bound and ATP-bound states.[27] Indeed, the Fe-S cluster-specific roles of HscA can be substituted by rather general Hsp70 chaperones in eukaryotes (e.g. yeast Ssc1 and human mitochondrial HSP70).[28] In addition, an NMR spectroscopic study confirmed that human mitochondrial HSP70 prefers to interact with the D-state of ISCU.[18] These observations supports that IscU may be benefited from unfolding activity of Hsp70 chaperones to stabilize the D-state and facilitate Fe-S cluster transfer. Further studies are necessary to elucidate mechanistic details of these chaperone-

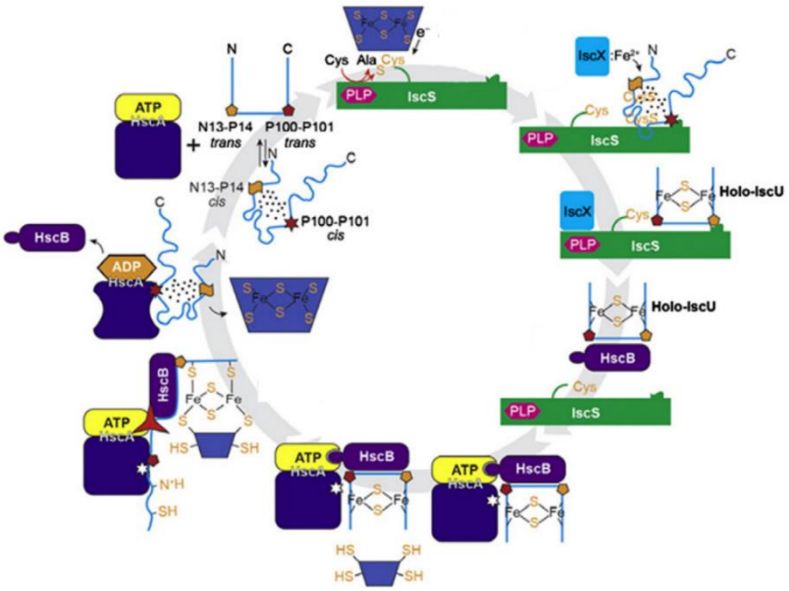

Figure 3. The proposed model of the *E. coli* ISC Fe-S cluster biogenesis mechanism. The S- and D-state of IscU plays differential roles of interacting different partner proteins and mediating distinctive physiological processes. For example, HscB and IscX preferentially interact with the S-state of IscU, whereas the D-state of IscU is exhibited upon interacting with IscS and HscA. Reprinted by permission from Elsevier.[20]

related activities in the Fe-S cluster biogenesis.

Conclusions and future directions

Despite evident importance in various indispensable cellular processes, structural dynamics of IscU and its functional relationship is still elusive. The metamorphic structural feature that is observed in IscU is relatively rare in nature,[29] implicating functional necessity of IscU to adopt exceptional flexibility in its structure. NMR spectroscopy is one of the most suitable tools to investigate a wide range of structural dynamics and biomolecular interactions in a physiologically relevant condition, and a series of NMR spectroscopic studies contributed much to reveal various structural features of IscU and to elucidate the related physiological events. Therefore, it is likely that the cutting-edge NMR spectroscopic techniques can work as one of the most suitable tools for elucidating further mechanistic details of IscU to mediate complex Fe-S cluster biogenesis by modulating its conformational diversity. In particular, recent structural studies for human ISCU and the related proteins showed that the Fe-S cluster biogenesis mechanism needs to be appreciated from the aspects of multi-protein complexes.[30,31] Due to their relatively large size, X-ray crystallography has been a major methodology to obtain their atomistic pictures,[32,33] yet we expect that NMR spectroscopic approaches also provide novel insights to elucidate structural heterogeneity of the Fe-S cluster biogenesis complexes and to allocate their physiological consequences.

Acknowledgements

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2020R1I1A2074335).

References

1. H. Beinert, R. H. Holm, and E. Münck, *Science* **277**, 653 (1997)
2. D. C. Johnson, D. R. Dean, A. D. Smith, and M. K. Johnson, *Annu. Rev. Biochem.* **74,** 247 (2005)
3. R. Lill, *Nature* **460**, 831 (2009)
4. T. A. Rouault and W. H. Tong, *Nat. Rev. Mol. Cell Biol.* **6**, 345 (2005)
5. D. R. Crooks, N. Maio, A. N. Lane, M. Jarnik, R. M. Higashi, R. G. Haller, Y. Yang, T. W. M. Fan, W. Marston Linehan, and T. A. Rouault, *J. Biol. Chem.* **293**, 8297 (2018)
6. F. Prischi, C. Pastore, M. Carroni, C. Iannuzzi, S. Adinolfi, P. Temussi, and A. Pastore, *Protein Expr. Purif.* **73**, 161 (2010)
7. J. Kim, M. Tonelli, and J. L. Markley, *Proc. Natl. Acad. Sci. U. S. A.* **109**, 454 (2012)
8. J. Kim, R. O. Frederick, N. M. Reinen, A. T. Troupis, and J. L. Markley, *J. Am. Chem. Soc.* **135**, 8117 (2013)
9. J. Kim, J. R. Bothe, R. O. Frederick, J. C. Holder, and J. L. Markley, *J. Am. Chem. Soc.* **136**, 7933 (2014)
10. Z. Dai, M. Tonelli, and J. L. Markley, *Biochemistry* **51**, 9595 (2012)
11. F. Bonomi, S. Iametti, D. Ta, and L. E. Vickery, *J. Biol. Chem.* **280**, 29513 (2005)
12. F. Bonomi, S. Iametti, A. Morleo, D. Ta, and L. E. Vickery, *Biochemistry* **47**, 12795 (2008)
13. J. Kim, A. K. Füzéry, M. Tonelli, D. Ta, W. M. Westler, L. E. Vickery, and J. L. Markley, *Biochemistry* **48**, 6062 (2009)
14. H. S. Alhajala, J. L. Markley, J. Kim, M. M. Al-Gizawiy, K. M. Schmainda, J. S. Kuo, and C. R. Chitambar, *Oncotarget* **11**, 1531 (2020)
15. T. A. Ramelot, J. R. Cort, S. Goldsmith-Fischman, G. J. Kornhaber, R. Xiao, R. Shastry, T. B. Acton, B. Honig, G. T. Montelione, and M. A. Kennedy, *J. Mol. Biol.* **344**, 567 (2004)
16. J. Liu, N. Oganesyan, D. H. Shin, J. Jancarik, H. Yokota, R. Kim, and S. H. Kim, *Proteins Struct. Funct. Genet.* **59**, 875 (2005)
17. J. Kim, M. Tonelli, T. Kim, and J. L. Markley, *Biochemistry* **51**, 5557 (2012)
18. K. Cai, R. O. Frederick, J. Kim, N. M. Reinen, M. Tonelli, and J. L. Markley, *J. Biol. Chem.* **288**, 28755 (2013)
19. Z. Dai, J. Kim, M. Tonelli, I. K. Ali, and J. L. Markley, *Biochemistry* **53**, 5290 (2014)
20. J. Kim, J. R. Bothe, T. R. Alderson, and J. L. Markley, *Biochim. Biophys. Acta* **1853**, 1416 (2015)
21. R. Shi, A. Proteau, M. Villarroya, I. Moukadiri, L. Zhang, J. F. Trempe, A. Matte, E. Armengod, and M. Cygler, *PLoS Biol.* **8**, e1000354 (2010)
22. M. T. Boniecki, S. A. Freibert, U. Mühlenhoff, R. Lill, and M. Cygler, *Nat. Commun.* **8**, 1287 (**2017)**
23. D. di Maio, B. Chandramouli, R. Yan, G. Brancato, and A. Pastore, A. *Biochim. Biophys. Acta* **1861**, 3154 (2017)
24. Y. Shimomura, K. Wada, K. Fukuyama, and Y. Takahashi, *J. Mol. Biol.* **383**, 133 (2008)
25. J. R. Cupp-Vickery, J. C. Peterson, D. T. Ta, and L. E. Vickery, *J. Mol. Biol.* **342**, 1265 (2004)
26. J. Kim, M. Tonelli, R. O. Frederick, D. C. F. Chow, and J. L. Markley, *J. Biol. Chem.* **287**, 31406 (2012)
27. R. Puglisi and A. Pastore, *FEBS Lett.* **592**, 4011 (2018)
28. R. Lill and U. Mühlenhoff, *Annu. Rev. Biochem.* **77**, 669 (2008)
29. M. Lella and R. Mahalakshmi *Biochemistry* **56**, 2971 (2017)
30. T. A. Rouault, *Biometals* **32**, 343 (2019)
31. N. Maio, A. Jain, and T. A. Rouault, *Curr. Opin. Chem. Biol.* **55**, 34 (2020)
32. S. A. Cory, J. G. Van Vranken, E. J. Brignole, S. Patra, D. R. Winge, C. L. Drennan, J. Rutter, and D. P. Barondeau, *Proc. Natl. Acad. Sci. U. S. A.* **114**, E5325 (2017)
33. N. G. Fox, X. Yu, X. Feng, H. J. Bailey, A. Martelli, J. F. Nabhan, C. Strain-Damerell, C. Bulawa, W. W. Yue, and S. Han, *Nat. Commun.* **10**, 2210 (2019)