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Structures of SUF Machinery Proteins and their Implications for Iron-Sulfur Cluster Biosynthesis

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Abstract

SUF machinery in *Echerichia coli,* responsible for the biosynthesis of iron-sulfur clusters, is composed of six protein components (SufABCDSE), among which SufB, SufC, and SufD associate in a complex. We have determined the structures of SufA, SufC, and SufD by X-ray crystallography. SufA is a dimer, in which C-terminal segments containing essential cysteine residues (Cys-Gly-Cys) are positioned to allow coordination of an Fe-S cluster and/or an Fe atom. SufC has the overall structure similar to that of ABC-ATPase but takes an inactive form. SufD has a β-helix flanked with α-helical domains. We also studied the functional roles of the residues in SufD by mutagenesis and determined the crystal structure of SufCD complex. Molecular mechanism of Fe-S cluster biosynthesis is discussed on the basis of the structural and functional evidence.

Introduction

Iron-sulfur (Fe-S) proteins are found in a variety of organisms and are required for a number of processes essential to cells, such as respiratory and photosynthetic electron transfer and the regulation of gene expression (1). Fe-S proteins ligate [4Fe-4S], [3Fe-4S], and/or [2Fe-2S] clusters via primarily the thiolate side-chain of cysteine residues. Recent genetic studies revealed that Fe-S clusters are biosynthesized by the independent systems, termed ISC system and SUF system, in addition to NIF system (2, 3). Except for SufS, which has been well characterized to act as cysteine desulfurase to provide sulfur for Fe-S cluster synthesis, little is known concerning what exact roles of the other components are and how the components interact to each other and/or form complexes. Here will be discussed the molecular mechanism of SUF machinery proteins on the basis of the crystal structures and mutational results.

Results and Discussion

Each component protein was overproduced in *E. coli* and purified by the standard chromatography. SufCD complex was prepared by co-expressing SufC and SufD in *E. coli.* Crystallization was performed

with the hanging drop vapor-diffusion method. X-ray diffraction data were collected at 100K using synchrotron radiation at SPring-8 and processed using the HKL2000 package. The structures were refined using CNS and modeling program Xfit. Resolution limits are 2.7 Å for SufA, 2.5 Å for SufC, and 2.0 Å for SufD. R/R_{free} values were 0.232/0.279 for SufA, 0.224/0.293 for SufC, and 0.187/0.235 for SufD. These structures are shown in Figure 1.

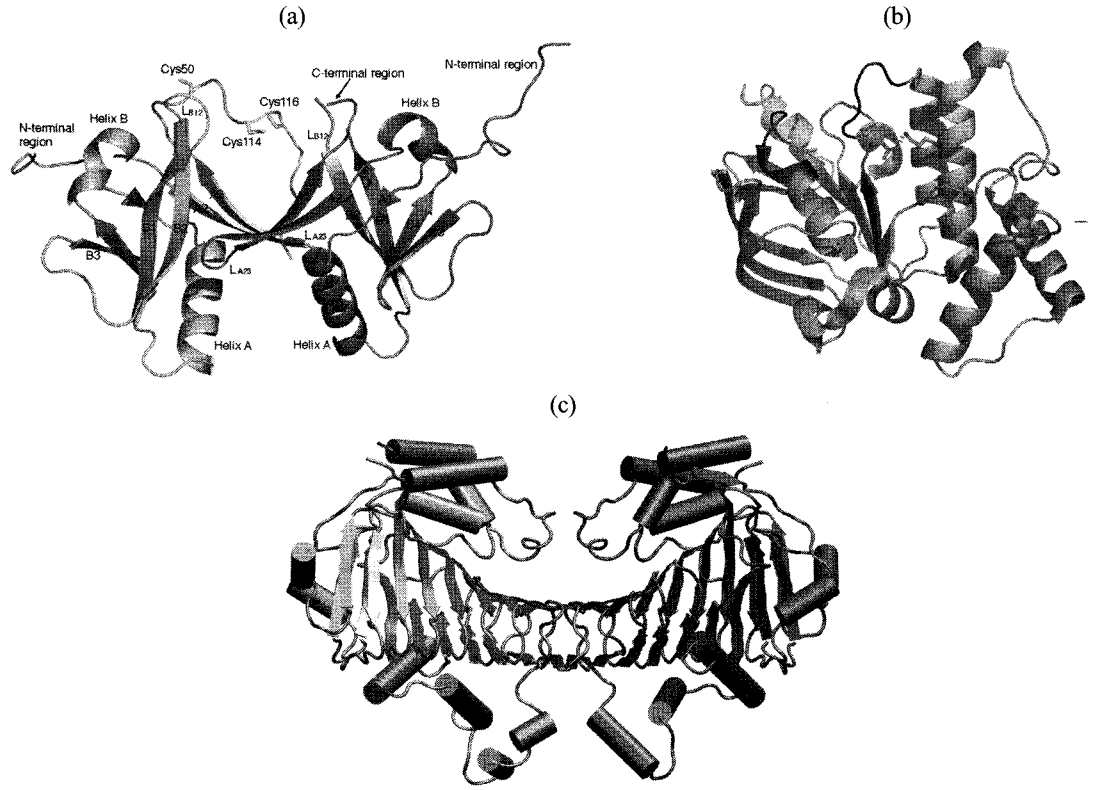

Figure 1. Crystal structures of SUF machinery proteins. (a) SufA (dimer), (b) SufC, and (c) SufD (dimer).

SufA plays crucial roles in Fe-S cluster biosynthesis, as its paralogous protein IscA. SufA forms a dimer, in contrast to the tetrameric organization of IscA (4). The structure of SufA subunit is similar to that of IscA subunit, but notably the C-terminal segment of SufA containing essential cysteine residues (Cys-Gly-Cys) was ordered in one subunit. The computer modeling of this segment of the other subunit suggests that the four cysteine residues are positioned in close proximity at the dimer interface. The arrangement of these cysteine residues suggests that SufA dimer may allow coordination of an Fe-S cluster and/or an Fe atom.

SufC is an ATPase component of the SUF machinery. SufC exists as monomer in solution and in the crystal. Although the overall structure of SufC is similar to that of ABC-ATPase, notable differences were observed (5); Glu171, an invariant residue involved in ATP hydrolysis, is rotated away from the nucleotide-binding pocket to form a salt bridge with Lys152. Consequently the loop that follows Glu171 is flipped out to the molecular surface, which may sterically inhibit the formation of an active dimer.

This salt bridge may regulate ATPase activity to prevent wasteful ATP hydrolysis.

SufD has a unique β-helix, which is flanked with the N-terminal and C-terminal α-helical domains. The β-helices are jointed together to form SufD dimmer; its interface is held primarily by hydrogen bonds between anti-parallel β-strands. Genetic study has shown that mutations/deletions of the residues in the C-terminal α-helical domain affected severely the function whereas those in the N-terminal α-helical domain affected little. We also found that mutations of some residues located inside of the β-helix affected the function. These results suggest that SufD interact with the other SUF machinery proteins through the C-terminal α-helical domain and that SufD may change its quaternary structure.

Recently we determined the crystal structure of SufCD complex. On the basis of the structures so far determined as well as genetic and biochemical results, the molecular mechanisms of SUF machinery proteins will be discussed.

Acknowledgements

We thank Drs. M. Kawamoto, H. Sakai, N. Shimizu, and K. Hasegawa (JASRI) for their aid with data collection at SPring-8. This work was supported by a grant from the National Project on Protein Structural and Functional Analysis from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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