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Understanding the role of dynamics in the iron sulfur cluster molecular machine

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ABSTRACT

Background: The bacterial proteins IscS, IscU and CyaY, the bacterial orthologue of frataxin, play an essential role in the biological machine that assembles the prosthetic Fe—S cluster groups on proteins. They form functionally binary and ternary complexes both in vivo and in vitro. Yet, the mechanism by which they work remains unclear.
Methods: We carried out extensive molecular dynamics simulations to understand the nature of their interactions and the role of dynamics starting from the crystal structure of a IscS-IscU complex and the experimentally-based model of a ternary IscS-IscU-CyaY complex and used nuclear magnetic resonance to experimentally test the interface.
Results: We show that, while being firmly anchored to IscS, IscU has a pivotal motion around the interface. Our results also describe how the catalytic loop of IscS can flip conformation to allow Fe—S cluster assembly. This motion is hampered in the ternary complex explaining its inhibitory properties in cluster formation.
Conclusions: We conclude that the observed 'fluid' IscS-IscU interface provides the binary complex with a functional adaptability exploited in partner recognition and unravels the molecular determinants of the reported inhibitory action of CyaY in the IscS-IscU-CyaY complex explained in terms of the hampering effect on specific IscU-IscS movements.
General significance: Our study provides the first mechanistic basis to explain how the IscS-IscU complex selects its binding partners and supports the inhibitory role of CyaY in the ternary complex.

1. Introduction

The bacterial proteins IscS and IscU are essential cellular components which take part in iron-sulfur cluster biogenesis [1,2]. Iron-sulfur clusters are evolutionary ancient prosthetic groups found in several different pathways where they provide their unique redox potential. IscS is the desulfurase which converts cysteine to alanine and forms highly reactive persulfide species. It is not only involved in iron-sulfur cluster formation as it is the enzyme that provides sulfur also in the molybdenum cofactor (Moco) biosynthesis and tRNA thiolation [3–5]. IscU is the scaffold protein which hosts transiently the iron-sulfur cluster and eventually delivers it to the final acceptors. IscS and IscU form a hetero-tetrameric complex together and are highly conserved from bacteria to primates, with the eukaryotic orthologues being known as Nfs1 and Isu1, respectively.

Several structures of these two proteins are available, both in isolation and in a complex [6–8]. From them, we have learned that, when in the test tube and not in a complex, IscU can be in at least two conformational states, one compactly folded (S state), the other partially unfolded (D state), where the N-terminal helix is detached from the rest of the structure and highly flexible in solution [9]. Free IscU is an intrinsically unstable protein able to undergo both cold and heat denaturation at detectable temperatures [10,11]. However, such flexibility is not observed in the crystal structures of IscS-IscU complexes or in solution when bound to IscS or to zinc [6–8,11,12]. In these complexes, IscU appears fully folded, putting in doubt the physiological importance of the D state. It is also possible that, also in the absence of a binding partner, crowding stabilizes the protein in the cell to its compact form.

Abbreviations: NMR, nuclear magnetic resonance; MD, molecular dynamics; PCA, principal component analysis; ED, essential dynamics; RMSD, root-mean square deviation; RMSF, root-mean-square fluctuation.

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In the IscS-IscU complex, the obligate IscS dimer interacts with two copies of IscU. Each copy sits close but not in direct contact with the catalytic site of IscS and binds the roughly prolate ellipsoidal IscS dimer at the opposite extremes of the two poles, far away from each other. The active site of IscS contains a covalently bound pyridoxal phosphate (PLP) group, a catalytic cysteine (C328 in the *E. coli* structure numbering) hosted in a flexible loop, and a lysine (K206). During catalytic activity aimed at the production of iron-sulfur clusters, the loop is thought to move from the active site to IscU to deliver the persulfide which willl form the cluster together with iron. Additionally, IscS forms complexes with several other partners, such as the proteins TusA [8], ferredoxin [13,14], CyaY [15], and the ancillary protein YfhJ (also known as IscX) [8,16], which all belong to the machine that is responsible for iron-sulfur cluster biogenesis. Of these, only the structure of the TusA-IscS complex has been determined at high resolution [8]. Experimentally-based models exist for the complexes with frataxin, ferredoxin and YfhJ [14,15,17]. Interestingly, all these proteins seem to compete for the same surface of IscS, which is close to the active site but distinct from the region which hosts IscU [8,14,15].

Among these interactions, a particularly interesting one is that with CyaY, since this protein is the bacterial ortholog of frataxin which, in humans, is linked to the neurodegenerative Friedreich's ataxia (MIM 229300). This disease is caused by reduced levels of frataxin [18]. Previous studies have shown that frataxin intervenes in regulating the speed of iron-sulfur cluster formation, although, paradoxically, in prokaryotes appears to act as an inhibitor while in eukaryotes is an activator [19].

Several mechanistic questions remain open about the IscS-IscU and IscS-IscU-CyaY complexes. We still do not know, for instance, what part the dynamical behaviour of IscU and its intrinsic flexibility plays in complex formation with IscS. How the catalytic loop of IscS bearing the persulfide group moves from the catalytic site to the acceptor (i.e., IscU) and what is the role of CyaY in such movements are other important aspects that need elucidation. In one of the crystal structures of the complex, the catalytic loop is so flexible not to be observable [8]. This is not the case for the structure of the IscS-IscU complex from *A. fulgidus* where the loop is close to IscU providing a potential alternative ligand for the cluster [6,7]. Only a low resolution model of the ternary IscS-IscU-CyaY complex is available, hampering direct evaluation of how its presence close to the catalytic loop might affect it. Finally, it is also unclear what is the pathway followed by iron to reach IscU [15].

To address these open questions and to provide more insights into the dynamics of the IscS-IscU complex, both in the presence and in the absence of CyaY, we have carried out an extensive study based on molecular dynamics (MD) simulations and experimental validation. We show that the binary IscS-IscU complex is stably folded and features a likely functionally relevant pivotal motion of IscU around the interface with IscS. We also show how the mobility of the catalytic loop of IscS is strongly reduced by the presence of CyaY and suggest the mechanism by which this protein stabilizes the IscS-IscU interaction and acts as an inhibitor. We validated experimentally, using nuclear magnetic resonance (NMR), the nature of the IscS-IscU interface and found that, despite the tight anchoring of IscU, the side chains of some groups are trapped in different conformations according to what we can predict from our simulations. Overall, our findings bear important consequences for understanding the role of frataxin in iron-sulfur cluster biogenesis.

2. Materials and methods

2.1. IscS-IscU complex model

The *E. coli* IscS-IscU complex (PDB code: 3LVL) [8] was used for molecular dynamics study of the binary complex. A missing segment of the IscS loop bearing the catalytic cysteine (residues 328–334) was reconstructed using the Modeller [20] module (v 9.12) as implemented into the UCSF-Chimera software package [21]. Five different conformations of the loop were generated. We selected as the starting conformation the one where the loop was most equidistant from both IscS and IscU so as not to artificially orient the dynamics of the loop towards one side or another of the binding interface. The same coordinates were applied to both missing loop segments to avoid possible inconsistencies. The complex was then solvated in a ~23 Å layer cubic water box using the TIP3P water model parameters. Na^+ and Cl^- ions were added to ensure system electroneutrality and to set the final concentration to 0.15 M. The final system size was 188 Å × 95 Å × 105 Å for a total number of atoms of ~170,000. The system was minimized in two stages: first, a 20,000-step run was carried out with restraints on all the protein atoms (5 kcal/mol/Å^2). A further 10,000-step minimization was carried out by applying the restraints on the cofactor and C_α protein atoms only. A short (200 ps) NPT simulation at 200 K and 1 atm was performed with restraints on all the protein atoms (5 kcal/mol/Å^2), to adjust the volume of the simulation box, while preserving the minimized protein structure obtained in the previous steps. The system was slowly heated up to 300 K over a 3 ns period, again applying the restraints on the cofactor and C_α atoms only and gradually releasing them to 1 kcal/mol/Å^2 along the thermalization process. Subsequently, the system was equilibrated for 2 ns, gradually reducing the restraints to zero.

2.2. IscS-IscU-CyaY complex model

The *E. coli* IscS-IscU-CyaY complex structure [15] was used as the starting point for the molecular dynamics of the ternary complex. The procedure to prepare the system was slightly different to minimize the steric clashes between the CyaY monomers and the IscS-IscU subunits. First, the side chains of the residues at the interface between the CyaY monomers and the corresponding IscS-IscU subunits were manually adjusted by means of the UCSF-Chimera software package [21]. The simulation box was prepared as for the IscS-IscU complex. The final system size was 194 Å × 102 Å × 111 Å for a total number of atoms of ~205,000. The system was then minimized and equilibrated in the following fashion. A first round of 15,000-step energy minimization was performed with restraints on the C_α protein atoms and on the cofactor atoms (5 kcal/mol/Å^2), followed by a subsequent 20,000-step energy minimization run with no restraints to minimize as much as possible steric clashes in the first stage. A short (200 ps) NPT simulation at 200 K and 1 atm was performed restraining all atoms (5 kcal/mol/Å^2) to adjust the volume of the simulation box, while preserving the minimized protein structure obtained in the previous steps. Afterwards, the system was slowly heated up to 300 K over a 3 ns period, again applying the restraints on the cofactor and C_α atoms only and gradually releasing them to 1 kcal/mol/Å^2 along the thermalization process and finally equilibrated for 6 ns, gradually reducing the restraints to zero.

2.3. Molecular dynamics simulation and analysis

Production runs were performed under NPT conditions at 1 atm and 300 K and extended up to 400 ns for both complexes. A 10 Å cutoff (switched at 8.0 Å) was used for atom pair interactions. The long-range electrostatic interactions were computed by means of the particle mesh Ewald (PME) method using a 1.0 Å grid spacing in periodic boundary conditions. The RATTLE algorithm [22] was applied to constrain bonds involving hydrogen atoms, and thus a 2 fs time step could be used. All the systems were simulated with NAMD [23] (v. 2.9), using the ff99SBildn Amber force field parameters [24,25] for protein and ions. The parameters for PLP were generated in two steps. Initially, charges were computed using the restrained electrostatic potential (RESP) fitting procedure [26]. The ESP was calculated by means of the Gaussian09 package [27] using a B3LYP/6-31G* level of theory. The RESP charges were obtained by a two-stage fitting procedure using the program RED [28,29]. Missing bond, angle, torsion and improper torsion angle parameters were generated using Antechamber