INFLUENCE OF SUBSTITUTIONS OF Isu1 RESIDUES ON BINDING TO Jac1 PROTEIN

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Abstract: The iron-sulfur (Fe/S) clusters are the most ancient co-factors of proteins involved in the most essential processes in bacterial systems and yeast, such as *Saccharomyces cerevisiae*. The main protein involved in the Fe/S cluster transfer is the Iron sulfur cluster assembly protein 1 (Isu1), which interacts with Jac1 during one of the stages of the Fe/S cluster biogenesis cycle forming a binary complex. In this work, the interaction interface of Isu1 was investigated by selective substitutions of amino-acid residues to understand their role in binding to the Jac1 protein. An initial alanine scan was done to limit the number of possible residues subjected to the replacement and to confirm the previously obtained results. Then, MD simulations using the coarse-grained UNRES force field were run for two selected mutants: $L_{63}V_{72}F_{94}$ and $L_{63}V_{64}G_{65}D_{71}$. The analysis of the dynamics and interaction patterns of the Isu1-Jac1 complexes confirmed that the investigated residues played an important role in their binding.

Keywords: Saccharomyces cerevisiae, Isu1-Jac1 protein complex, alanine scan, molecular dynamics, coarse-grained UNRES force field

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1. Introduction

Iron-sulfur (Fe/S) clusters widespread among organisms from prokaryote to eukaryote. They are a crucial part of many cellular processes like electron transfer, redox reactions, and catalysis of chemical reactions. Most of the proteins require the presence of chaperons to obtain and stabilize their structures. The release of the Fe/S cluster (ISC) from Isu1, and its transfer and incorporation into recipient apoproteins (Apo) is facilitated by late components of the Fe/S cluster assembly machinery including the ATP-dependent Hsp70 (heat shock protein) chaperone Ssq1 and the DnaJ-like co-chaperone Jac1. Any disturbance of the balance of

these processes can have very serious and dangerous consequences to an organism and turn into diseases such as cerebellar ataxia, myopathy, Friedreich's ataxia, and microcytic anemia. [1] In the *Saccharomyces cerevisiae* yeast, the Fe/S cluster biogenesis cycle is one of the mechanisms which are necessary for the organism but, notwithstanding the recent developments, still not well known. Although there are known bacterial equivalents of participating proteins: IscU for Isu1, HscB for Jac1, and DnaK for Ssq1, the process itself significantly varies between yeast and bacteria. Especially the stages involving transfer of the Fe/S cluster which require the presence of multiple proteins, such as Isu1 (scaffold protein), Jac1 (co-chaperon), Ssq1 (Hsp70 chaperon) and Grx5, make the system difficult to study. [2, 3] Isu1 influences the iron homeostasis within the mitochondrion where it is involved in the assembly of Fe/S proteins and can also be involved in the repair of Fe/S clusters. Jac1 is a two-domain J-protein and interacts with Isu1 and subsequently with Ssq1 in the Fe/S cluster biogenesis process in mitochondria.

In experimental studies of the Isu1-Jac1 complex, the influence of point and collective mutations on the fraction of bound Isu1-Jac1 complexes has been assessed, [4, 5] indicating which residues are important for binding. In later theoretical studies of structure and dynamics of the Isu1-Jac1 complex, the whole interaction interface has been presented. [3] Those studies have presented also the results of point and few cumulative mutations of Jac1 on binding to Isu1, as well as provided preliminary results on how amino-acids residues placed on Isu1 protein are involved in binding Jac1. Theoretical studies have shown which residues are necessary for Isu1-Jac1 interactions and which ones only stabilize the interaction site, confirming and extending the residues found experimentally. [3]

In this study Isu1 residues involved in forming and stabilization of the complex with Jac1 were investigated by point and collective mutations to alanine. Collective mutations are often found to cause a more distinct effect on the investigated systems [6, 7], allowing significant changes to be observed using even medium-resolution methods such as coarse-grained force fields. In previous theoretical studies [3] point (*e.g.*, Y_{163}) and several cumulative mutations on Jac1 only (*e.g.* L_{105}, L_{109} and Y_{163}) have been investigated and found to be an effective way to investigate the role of certain regions of Jac1 in binding to Isu1. In this work, an analysis of the alanine scanning of the most probable model of the Isu1 protein in complex with the Jac1 protein (structure E from ref [3]) was done using the Robetta Computational Interface Alanine Scanning server [8, 9] to confirm previous observations [3–5]. In order to determine which amino-acids residues found previously on Isu [3] were crucial and what their role in forming a complex with Jac1 was, two sets of amino-acids residues on Isu1 were chosen in the area of the interaction interface: $L_{63}V_{72}F_{94}$ and $L_{63}V_{64}G_{65}D_{71}$ [3–5] (Figure 1), to be substituted by alanine. The complexes thus obtained were subjected to molecular dynamics (MD) simulations in a coarse-grained UNRES force field [10–12]. Studies of conformational changes and interaction-patterns revealed functions of amino-acid residues placed on Isu1 and their importance to form a correct structure of the Isu1-Jac1 complex.

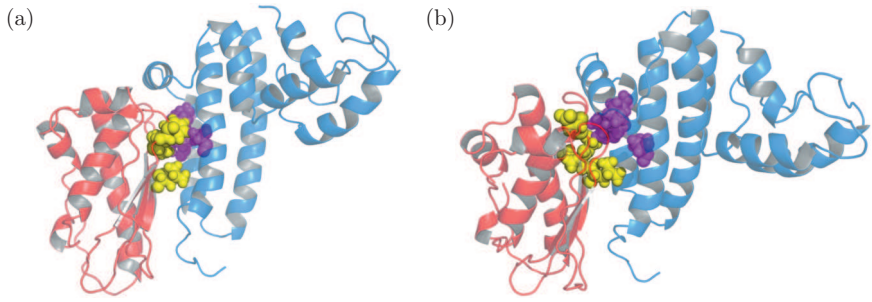

Figure 1. The starting structure of Isu1 (red) and Jac1 (blue) complexes: (a) with marked amino-acid residues $L_{63}V_{72}F_{94}$ (yellow spheres) on the Isu1 protein and $L_{105}L_{109}Y_{163}$ (magenta) on the Jac1 protein, (b) with marked amino-acid residues $L_{63}V_{64}G_{65}D_{71}$ (yellow spheres) on the Isu1 protein and $L_{105}L_{109}Y_{163}$ (magenta) on the Jac1 protein

2. Methods

The Robetta Computational Interface Alanine Scanning server [8, 9] is a simple tool which allows quick estimation of the relative free energies of binding for a given structure of the protein complex. As no dynamics of the system is included in this analysis, its use is restricted mostly to the role of the initial selector from a large interface area. Therefore, it was used only for comparison with the previous experimental [5] and theoretical [3] results.

In the next step MD simulations in a coarse-grained UNRES force field [10, 13–16] were performed because of the substantial size of the investigated systems. In the UNRES force field the polypeptide chain is represented by united peptide groups (p), each of which is placed between two consecutive Cα atoms, and united side chains (SC) (represented by ellipsoids of revolution) attached to the Cα atoms. It is only the SC and p centers that are the interaction sites – the α-carbon atoms serve to define the geometry of a chain only (Figure 2).

Canonical MD simulations were carried out with the Langevin scheme [17] and the VTS (variable time step) algorithm at temperature $T = 300$ K. In each of the simulations, sixteen independent trajectories were performed and each of the trajectories consisted of 40 million steps (approximately 0.2 ms of real time [17]).

3. Results and Discussion

The alanine scan performed by Robetta detected 8 major amino-acid residues on Isu1, which could be important for binding: V_{59}, L_{63}, V_{72}, R_{74}, Q_{76}, K_{78}, F_{94}, K_{136} (Table 1). Three of these residues have been previously found experimentally [4] and confirmed later in theoretical studies [3] as crucial for binding Jac1 – L_{63}, V_{72}, and F_{94}. The Robetta prediction of R_{74}, K_{78}, and K_{136} can be explained as too high contribution of the charged amino-acid residues to the interaction interface, which is a known problem [8] and residues V_{59} and Q_{76} have been previously found to play a supportive role in the stabilization of the conformation of the Isu1-Jac1 system. Other residues close to the interaction site (*e.g.* V_{64}, G_{65}, and D_{71}) have been detected as lightly interacting with Jac1 only.

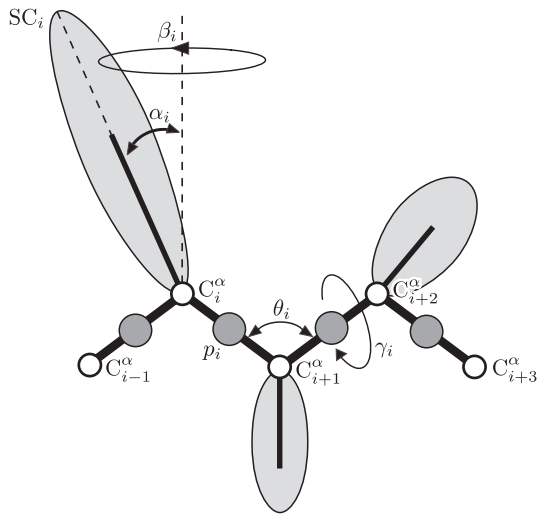

Figure 2. The UNRES model of polypeptide chains where two interaction sites: p_i (united peptide groups) and SC_i (united side-chains) are marked by grey circles and light-grey ellipsoids, respectively; Cα atoms (white circles) play a role in the geometry of the chain only and they are not the interaction site

Table 1. Estimations of the relative free energies of binding ($\Delta\Delta G$) for residues on Isu1 predicted by an alanine scan in Robetta for residues important for the interactions ($\Delta\Delta G > 0.5$ kcal/mol), based on the initial structure of the complex

Residue number	$\Delta\Delta G$ (kcal/mol)
59	1.02
63	1.46
72	0.56
74	2.08
76	1.42
94	3.05
136	1.39

To investigate the influence of alanine substitutions on the dynamics of the Isu1-Jac1 complex and changes to the interaction interface, two mutants of Isu1 were designed: (i) $L_{63}V_{72}F_{94}$ containing all the most important residues for Jac1 binding and (ii) $L_{63}V_{64}G_{65}D_{71}$, in which residues neighboring L_{63} and V_{72} were replaced to assess if there was a part of Isu1 which was responsible for the interaction, or rather whether these three residues helped stabilize the interaction site only.

During the simulation of the Isu1-Jac1 complex in which Isu1 was mutated in positions $L_{63}V_{72}F_{94}$, the structure of the complex lost its stability. It can be seen from Figure 3 that the interaction pattern between two proteins before and after mutations is different. During the simulation, Isu1 changed its placement

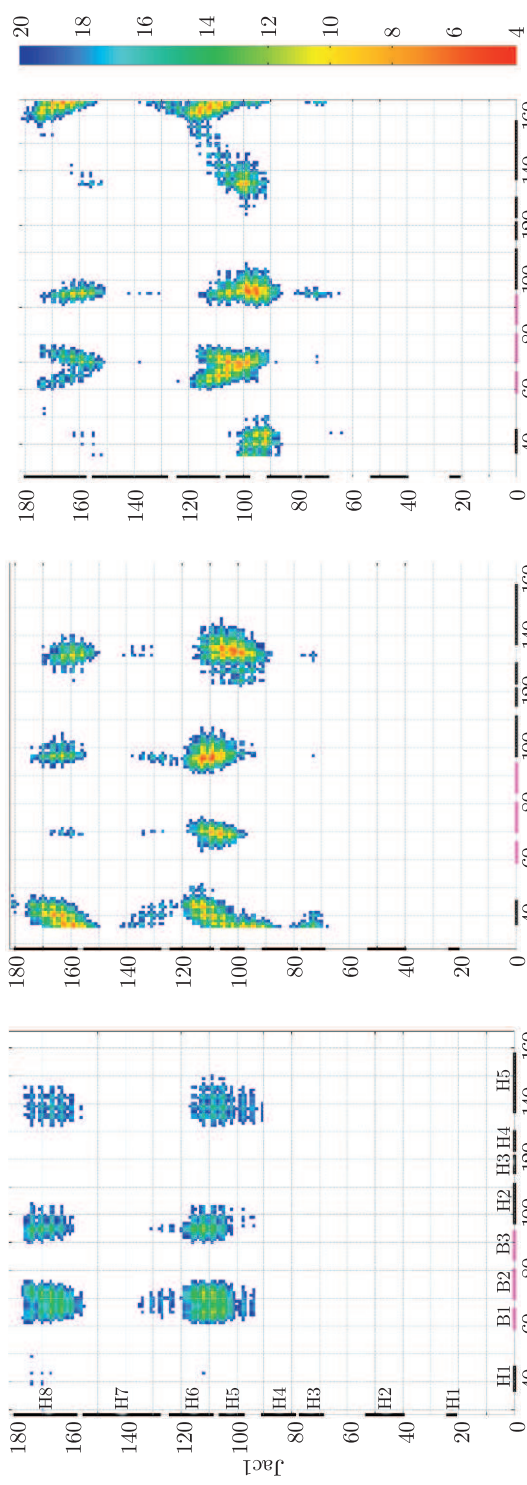

Figure 3. Contact maps (Cα···Cα atom distances smaller than 20 Å) of representative structures of the Isu1-Jac1 complex. The Cα···Cα atom distances are shown on the color scale in Å from red (smallest distances) to dark blue (20 Å); distances greater than 20 Å are mapped as blank fields; secondary structure elements are marked by lines at the bottom with labels of the respective helix (H, black) or β-strand (B, pink) on the x and y axes; residue numbers are marked on the x and y axes; (a) averaged contacts between the Isu1-Jac1 complex (panel nine in Figure 5 of reference [3]); contacts in the resulting structures after MD simulations of the Isu1-Jac1 complex with residues mutated to alanine: (b) $L_{63}V_{72}F_{94}$ and (c) $L_{63}V_{64}G_{65}D_{71}$

with regard to its starting position and rotated, in most of the cases, in the way it interacted by the 'upper side' of the protein (Figure 4). The analysis shows that V_{72} and F_{94} are the most important residues on Isu1, presumably, since they interact directly with L_{105}, L_{109} and Y_{163} which are crucial for the interactions from the Jac1 protein side (Figure 3) [3]. The important role of L_{63} indicates that it creates interactions with the region which stabilize the structure of the complex, but it is not necessary to form a stable complex, contrary to the other two residues.

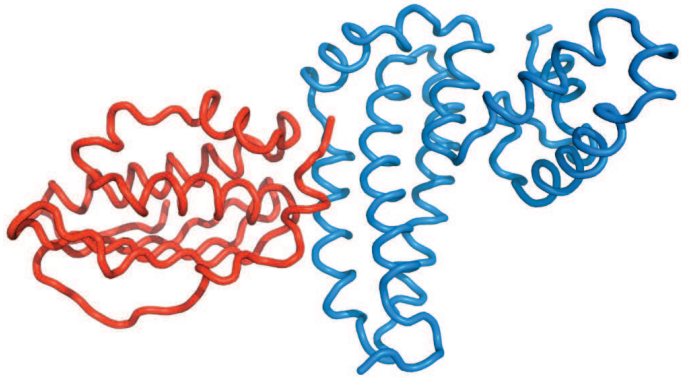

Figure 4. The most common structure of the Isu1-Jac1 complex with $L_{63}V_{72}F_{94}$ mutations on Isu1 obtained during the MD simulation

The second set of the mutations performed on the Isu1 protein ($L_{63}V_{64}G_{65}D_{71}$) showed that the structure of the Isu1-Jac1 complex also lost its stability and the interaction patterns between two proteins before and after mutations were different (Figure 3). The Isu1-Jac1 complex obtained a very similar conformation as in the case of the $L_{63}V_{72}F_{94}$ mutant (Figure 4). These simulations revealed that the role of D_{71} was similar to the L_{63} – it helped to stabilize the structure, but it was not necessary for binding.

The Isu1-Jac1 complex with both sets of mutations on Isu1 indicates the behavior to shift the interaction site on Isu1 from B1 (first β-sheet) and B2, which are the most important fragments in the wild structures, to H1 (first β-helix) and H5. While interactions of Jac1 with the H5 region on Isu1 can be observed also in the wild structure, interactions with H1 are present only after mutations of Isu1, especially with $L_{63}V_{72}F_{94}$ residues replaced by alanine.

4. Conclusions

Residues L_{63} and D_{71} on Isu1 are a part of the stabilizing region, but they are not necessary for the stability of the complex, whereas V_{64}, G_{65}, V_{72} and F_{94} are a part of the main interaction interface and their substitution to alanine significantly disturbs the dynamics and structure of the Isu1-Jac1 complex.

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