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Comparison stability Rusticyanin 23270 wild-type and mutant His143Leu using molecular dynamics simulation

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ABSTRACT

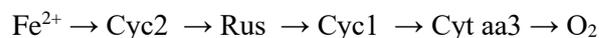
The *Acidithiobacillus ferrooxidans* bacterium plays an important role in the bioleaching process of uranium. The rusticyanin protein is the second most crucial component in the electron transport chain in the membrane of the *Acidithiobacillus ferrooxidans* bacterium. This protein belongs to the large family of copper blue proteins. The protein sequence rusticyanin 23270 was derived from UniProtKB database. A suitable template for modeling was prepared from the Swiss model server, and the best protein model was made with Modeller software. The His143Leu mutation was developed using the Pymol software in the protein. The effect of the mutation on the stability of the protein structure was investigated by analysing the results of molecular dynamics simulation on the wild-type and mutant protein. The values RMSD and RMSF are the same for both wild-type and mutant. The amount of Rg in mutant protein is reduced. His143Leu mutation in the rusticyanin 23270 protein does not affect the secondary structure protein and slightly increases the folding and stability of the tertiary structure.

Keywords: Rusticyanin, *Acithiobacillus ferrooxidans* 23270, Mutation His143Leu, Molecular dynamics simulation

I. INTRODUCTION

Acidithiobacillus ferrooxidans is a gram-negative, gamma proteobacterium, that grows optimally at 30°C and pH 2; however, it can grow at pH 1 or lower [1]. This bacterium can oxidize insoluble ferrous iron and convert it to soluble ferric iron, which is why it has been studied so much. The primary way to provide metabolic energy for this compulsive prokaryote is to oxidize sulfur-reducing compounds or ores containing Fe²⁺ under acidic conditions using intracellular O₂ as an oxidant [2]. By oxidizing Fe²⁺, electrons are transmitted in two directions. Most of them reach the O₂ through the potential slope in the downhill

path, while a small number of them move uphill in the opposite direction of the potential slope [1,3]. The model of the downhill electron transfer path is as follows [4]:



These proteins are encoded by *rus* operon. The *rus* operon includes a set of eight genes encoding a cytochrome c (Cyc2), located in the outer membrane, a blue copper protein, rusticyanin (RcY), a periplasmic cytochrome c4 (Cyc1), an OM protein with indefinite function (ORF1), and a cytochrome oxidase aa3 in the membrane [5].

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Acidithiobacillus ferrooxidans has a comprehensive and essential application in the process of bioleaching of uranium [6]. Bioleaching is the use of microorganisms to extract metals from mines [7]. Rusticyanin is a type 1 blue copper monomeric protein with 155 amino acids and is a significant component in the electron respiration chain of the acidic bacterium *Acidithiobacillus ferrooxidans* [8]. This protein has the highest redox potential among copper blue proteins (+ 680mV) and is stable and active at pH=2 [9]. This periplasmic component forms part of the iron/oxygen oxidizing supercomplex oxidizer, which traverses the outer and inner membranes of the *Acidithiobacillus ferrooxidans* [10, 11]. Mutation affects the efficiency of the uranium bioleaching process. In this study, the aim was to create His143Leu point mutation in the rusticyanin protein from the *Acidithiobacillus ferrooxidans* 23270, and compare stability wild-type and mutant protein using molecular dynamics simulation.

II. COMPUTATIONAL METHOD

A. Homology Modeling

In the RCSB PDB, there is a three-dimensional model of the rusticyanin 23270 with the identification code 1Rcy which contains 151 amino acids. The complete structure of the protein has not been determined. Therefore, homology modeling was used to construct the tertiary complete structure of the protein using Modeller software.

Determining the appropriate template: The amino acid sequence of the rusticyanin protein 23270 was derived from UniProtKB database. This sequence has 155 amino acids. First, the amino acid sequence was given to Swiss model server to find

the suitable template, and 50 templates were produced. 1Cur was chosen as the best template with 99% identity.

Modeling: To make the appropriate protein model from the selected template, Modeller 9.12 software used. According to the order given to the Modeller software, 100 models were made. Models made by Modeller software were sorted from small to large based on the Discrete Optimized Protein Energy (DOPE) evaluation equation. The first 25 models with the lowest Dope were separated to select the best model.

Validation and selection of the best model: To choose the optimal protein model from the 25 separated models, we used two servers Prosa Web and Rampage that the best model, PDB58 was chosen. The selected model was used to make the mutation and then molecular dynamics simulation. Create a mutation His143Leu: This mutation was created using Pymol software in the selected model sample.

Preliminary evaluation of mutant protein stability: I-mutant 2.0 server was used to evaluate the effects of this mutation on the stability of the mutant protein.

Molecular Docking: In order to place copper in the right place in the protein, molecular docking was done using the molecular docking program on Wild-type and mutant proteins.

Molecular Dynamics Simulation: The simulation of the molecular dynamics of the wild-type and mutant model on PDB files (initial covariances) was performed using Gromax version 1. 4.5 with a force field of Gromus 96. The protein was placed in a tetrahedron box. The simulation was performed at 100 ns under NPT and NVT conditions, and atomic coordinates were stored

every 2 ft. The simulation results were then analyzed using existing software.

III. RESULTS

Table 1

The Results of the Evaluation of the PDB58 Sample Model rusticyanin

rusticyanin	Prosa-Web Z-Score	Rampage favoured and allowed regions
23270 Wild-type	-5.83	98%

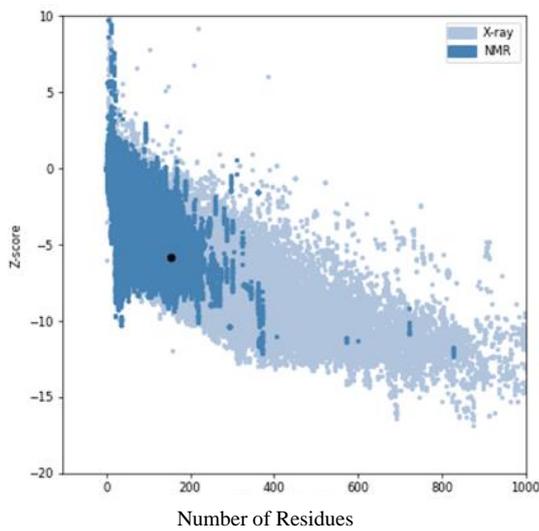


Fig. 1. Prosa Web diagram model PDB58 of the rusticyanin 23270 Wild-type

Table 2

Initial Assessment of the Stability of rusticyanin His143Leu

	DDG	Stability
I-Mutant 2.0	0.6	Increase

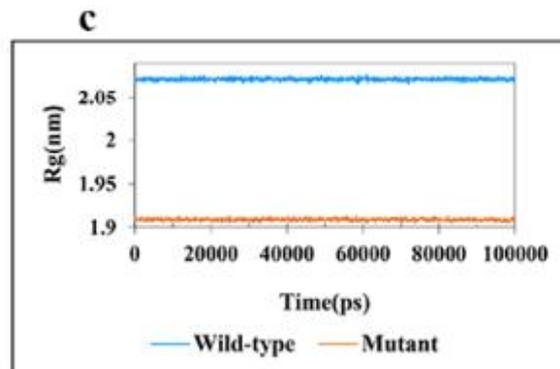
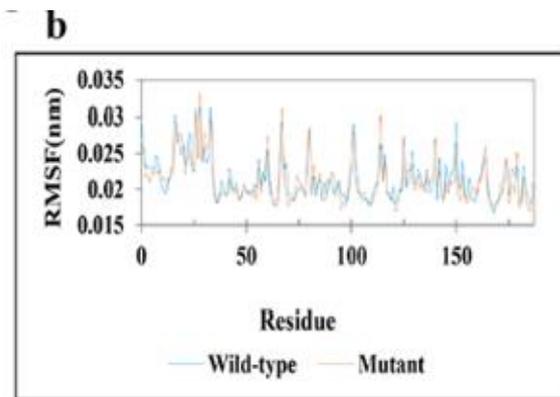
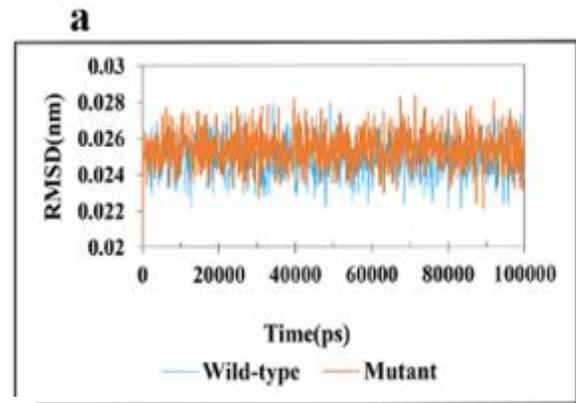


Fig. 2. Analysis (a)RMSD, (b)RMSF, (c)Rg of rusticyanin 23270 wild-type and mutant.

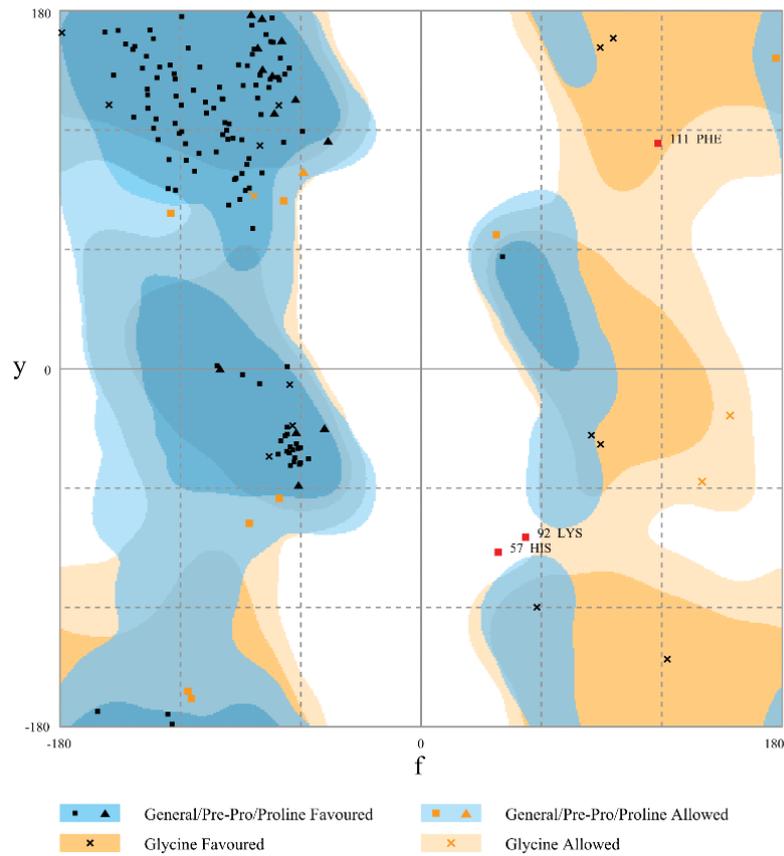


Fig. 3. Rampage Plot of PDB58 rusticyanin 23270 Wild-type

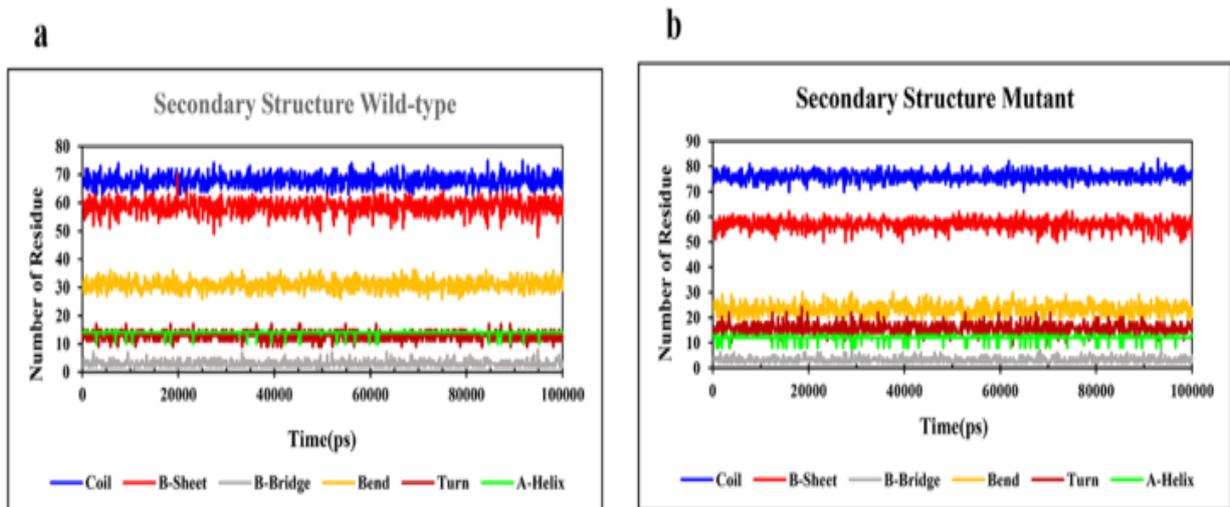


Fig. 4. Amount of secondary structure elements in the rusticyanin 23270 (a)Wild-type and (b)mutant His143Leu.

Table 3.

The percentage of the secondary structure elements in rusticyanin 23270 the wild-type and mutant His143Leu.

Secondary Structure%	B-Sheet	A-Helix	Turn	B-Bridge	Coil	Bend	3-Hlix
Wild-type	0.31	0.07	0.07	0.02	0.36	0.17	0.00
Mutant (His143Leu)	0.31	0.06	0.08	0.02	0.41	0.12	0.00

Table 4.

The mean values of RMSD, RMSF, and Rg rusticyanin wild-type and mutant His143Leu.

rusticyanin	RMSD (nm)	RMSF (nm)	Rg (nm)
Wild-type	0.025	0.021	2.072
Mutant (His143Leu)	0.025	0.021	1.909

Crystallographic and NMR studies of rusticyanin protein have shown that the protein is composed of a beta-sandwich core with a copper location very similar to other copper blue proteins. Histidine 85, cysteine 138, and histidine 143, in the equatorial position, and methionine 148 in the axial position of the ligands attached to copper, are in the active position of the protein [12,13]. In RCSB PDB there is a rusticyanin 23270 with identification (ID) code 1Rcy with 151 amino acids [14]. Complete protein 155 amino acids rusticyanin 23270 has not been modeled and determined by laboratory methods. Although the first four amino acids of this protein may not play a significant role in protein function, according to the purpose of this study, preferably the PDB file of the complete protein model was made using the homology modeling method. Modeling the three-dimensional structure of the rusticyanin 23270 was done using the 1Cur rusticyanin as a template. The selected template, 1Cur, is a form of rusticyanin reduction that contains 155 amino acids and was established in 1996 by the NMR method [12]. The identity template, 1Cur, with 23270 protein, is 99%. Two evaluation servers were used to evaluate the 25 selected models and to select the best model. Using

the Prosa Web tool, errors in the designated three-dimensional structure can be detected. The designated model is faulty if the z-score is outside the specific range for native proteins of the same size. According to the result, the selected model has a Z-score of -5.83 and is in the appropriate range (Table 1 and Figure 1). The Ramachandran plot protein was investigated using the Rampage tool. The Ramachandran plot is an x-y diagram of the dihedral ϕ and Ψ angles between the flat peptide bonds of N-C α and C α -C in a backbone protein. In the Ramachandran plot, if more than 90% of the residues are in appropriate area, the quality of the selected model is acceptable. According to the result selected model's evaluation, 98% of the residues are in the appropriate area (Table 1 and Figure 2). Therefore, based on the results of the two evaluation tools, the PDB58 model was selected as the best model.

In the active site rusticyanin four residues on the structure and function of the redox center protein are more effective than other residues. For this reason, the mutation of ligands that are firmly attached to copper in the equatorial position is expected to have a significant effect on protein properties [15]. Rusticyanin forms a potent

complex with cytochrome c4 (Cyc1), and Histidine 143 is exposed, probably a critical residue in complex formation [16]. The potential redox of rusticyanin in the Cyc1 complex is about 100 mV less than that of free rusticyanin. This suggests that the Histidine 143 acts as a redox switch. This residue is in the electron transfer pathway and exposed to solvent and is the subject of SDM in some blue family copper proteins [15]. Researches show various studies have been done on different mutations in rusticyanin, which is mainly focused on methionine in the axial position, including the mutations of Met148Leu, Met148Glu, and Met148Lys. The effects of these mutations on the structure and physical-chemical properties of proteins have been investigated by laboratory methods [9]. His143Leu mutation is the only mutation in the equatorial position that in rusticyanin has been studied. In a study, the structure of this mutation was determined with a resolution of 1.10 Å. In this study, for the first time, the crystal structure of native rusticyanin with a resolution of 1.27 was determined [15], and it was shown that in His143Leu, the redox potential of 400 mV is higher than that of the wild-type protein. The redox potential has been altered by noticeable structural changes in the mutant protein.

A. Investigating the effect of mutation on protein structure using molecular dynamics simulation

A preliminary study of the His143Leu mutation by server I-Mutant 2.0 shows replacing leucine with histidine in the equatorial position increases the stability of the protein to a small extent (Table 2). Root Mean Square Deviation (RMSD) is the average distance between protein atoms compared to its original state and is calculated in simulation for the protein alpha carbon atom. This parameter

is used to compare the stability of the wild-type and the mutant. In mutant protein, the RMSD value is 0.025nm. That has not changed compared to the wild-type (Table 4, fig3a). Therefore, according to this parameter, the stability of the protein due to this mutation has not changed. Root Mean Square Fluctuation (RMSF) is used to check flexibility and structure movement. The higher the RMSF value, the greater the particle motion. The average RMSF of 155 amino acids is the same in wild-type and mutant protein (Table 4 and Fig 3b). This indicates that the motions and flexibility of the residues have not changed significantly in the two types of proteins. In the analysis of simulation results, Rg is a good criterion for folding or unfolding protein. The smaller Rg indicates that the protein is denser and therefore more stable, and the folding increases, and vice versa. The amount of Rg in His143Leu mutant protein is significantly reduced compared to the wild-type (Table 4 and Fig3c). This indicates an increase in protein folding, resulting in a more stable mutant protein than the wild-type. To examine the changes in the elements of the secondary structure in the mutant protein relative to the wild-type, for each sample, the graph of the number of residues in each secondary structure type was plotted against the simulation time (Fig 4, Table 3). The results show that this mutation causes a minimal decrease in Alpha-helix and a slight increase in the coil. The amount of bend element has also decreased slightly. So, in general, these minor changes are not significant, and this mutation does not significantly affect the elements of the secondary structure.

B. Total resulting

Replacing the leucine residue with the histidine 143 in the rusticyanin protein 23270 causes a slight stabilization of the protein, and it does not affect its structure. Since the structure of the protein does

not change as a result of this mutation, it is expected that the function, physical, and chemical properties of the protein, including the potential of redox, have not changed under the influence of this mutation.

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