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Molecular Docking and Rare Codons Evaluation in the *Luciola Lateralis* luciferase, an in Silico Study

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ABSTRACT

Luciferase enzymes are involved in the bioluminescence reaction (light emission by living organisms). The bioluminescence process is a widespread phenomenon in the Nature. These enzymes are identified in some domains of life, but the luciferases from lampyrid genus are considered of for biological applications. The molecular cloning of a new type of firefly luciferase from *Luciola lateralis* was reported, previously. Here, we study its substrate binding site and rare codon with molecular docking and bioinformatics studies. By molecular modelling, some rare codons were identified that may have a critical role in structure and function of this luciferase. AutoDock Vina was used in the molecular docking that recognizes some residues that yield closely related with luciferin and AMP binding site. These types of studies help in the discovery of the light production reaction. Evaluation of these hidden information's can improve the knowledge of luciferases folding and protein expression challenges and help in design of new drugs.

Keywords: *Luciola lateralis*, Luciferase, Rare codon, Docking, Substrate binding site

INTRODUCTION

Firefly luciferase (EC 1.13.12.7) enzyme is responsible for the bioluminescence reaction. It catalyzes the oxidation of firefly luciferin with molecular oxygen in the presence of ATP and Mg^{2+} [1,2]. At the end of reaction, the yellow-green light emit [3]. The formation of luciferyl adenylate is the initial reaction that catalyzed by luciferase with the release of PPi. In the following, with reaction of luciferyl adenylate and molecular oxygen, the CO, AMP, oxyluciferin and light are released. Heretofore, luciferase genes and enzymes have been identified and isolated from diverse firefly species and their characteristics have been studied [4-7]. In the previous study, two sequence of cDNA encoding of Iranian luciferases as *Lampyris turkestanicus* and *Lampyroidea maculata* (lampyrid genus) were cloned [4,8]. So far, the luminescence reaction mechanism has not been completely resolved [9,10] but some of the binding and catalytic residues are identified to be important for enzyme catalysis [11,12]. The enzymatic bioluminescence assay is rapid, sensitive and nonradioactive [13] and is widely used in various areas of biotechnology as ATP detection [14], genetic reporter [13], phosphatase activity detection [15], and as a tool for monitoring *in vivo* protein folding [16].

The results of previous studies show that rare codons have an important role in protein folding and activity [17,18]. However, these studies indicate that ribosomal pausing in the rare codons have involved in the proper protein folding [19]. Evaluation of these rare codons, can provide a new insights in problem solving of protein challenges [20]. Previously, Tatsumi *et al.* reported the

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molecular cloning and expression of new Japanese luciferase from *Luciola lateralis* firefly (AAB00229) [21]. Here, the rare codons of this gene and their situation in the structure of this firefly luciferase were studied using the ATGme (http://atgme.org/) [22], Rare codon calculator (RaCC) (http://nihserver.mbi.ucla.edu/RACC/), LaTcOm (http://structure.biol.ucy.ac.cy/latcom. html) [23], and Sherlocc program (http://bcb.med.usherbrooke.ca/ sherlocc.php) [24] servers. The 3D structure of this firefly luciferase enzyme was modeled in the Swiss Model [25] and I-TASSER server [26]. In the following, the situation of these rare codons were studied in the this model using SPDBV [27] and PyMOL software [28]. For better insight, the *in silico* docking simulation of AMP and luciferin binding site was also conducted using AutoDock Vina [29]. Evaluation of these hidden information (rare codons) can help in illustrating the role of these codons in structure and function of firefly luciferase.

MATERIALS AND METHODS

Detection of Rare Codon Clusters in Gene and Structure of Luciferase

For evaluation of rare codons, the Pfam accession number of *lampyrid* luciferase enzymes was found in the uniprot database, (http://www.uniprot.org/). This Pfam was analyzed in Sherlocc program. The nucleotide sequence of the *L. lateralis* luciferase was obtained from NCBI (https://www.ncbi.nlm.nih.gov/nuccore/ DQ137139.1) and analyzed in the rare codon detection software as ATGme, LaTcOm and RaCC. By codon usage table of *E. coli B* [gbbct]: 11 CDS's (3771 codons, http://www.kazusa.or.jp/codon/) in the ATGme, the rare and highly rare codons are detected. The codons of problematic residues as Arg, leu, Ile, and Pro were detected in the RaCC. By use of three RCC detection algorithms as MSS, sliding window and %MINMAX In LaTcOm web tool [23], the RCC of *L. lateralis* luciferase were analyzed.

Study of Rare Codon in Structure of Luciferase

The three dimensional model of *L. lateralis* luciferase enzyme was conducted in the Swiss Model [25] and I-TASSER web server [26] based on the multiple-threading alignments of LOMETS [30]. The suitable models ("Confidence Score" and Z-score) were chosen. In the following, the situation of these rare codons were evaluated using the PyMOL [28] and SPDBV [31]. The crystal structure of some luciferase enzymes as Japanese *Luciola cruciata* Luciferase (PDB: 2d1r) [32] and *Photinus pyralis* Luciferase (PBD: 1ba3) (33) were used as the template in these molecular modeling database. The physicochemical parameters of this model was studied by the Expasy ProtParam (http://us.expasy.org/tools/protparam.html) server. Hydrogen bonds were computed using the WHAT IF [29] and PIC web server [30].

Molecular Docking Using AutoDock Vina

For evaluation of *L. lateralis* luciferase binding site, the docking process was studied between the luciferase enzyme and luciferin and AMP. In AutoDock Vina (version 1.1.2) [29], the 3D model of *L. lateralis* luciferase enzyme was converted to PDBQT format by MGL tools (version 1.5.4) [34] and treated as a receptor. The SDF format of luciferin and AMP was obtained from PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and were converted to PDB format by Open Babel (version 2.3.1) [35] and to PDBQT format by means of MGL tools (version 1.5.4). The search region and dimensions of grid box was selected as similar situation of substrate binding site of luciferase enzyme that crystal structure were determined [33]. By means of MGL tools, the best result of the luciferase and luciferin docking was selected and converted to the PDBQT format. In the following, the secondary docking was studied between this PDBQT format and AMP as a receptor. The docking experiments were performed at exhaustiveness value of 25.

RESULTS

Detection of Rare Codon Cluster

The Pfam accession numbers of *L. lateralis* luciferase enzyme was identified as PF00501 in the uniprot database, (http://www.uniprot.org/). This Pfam accession numbers was studied in the Sherlocc program [24] for rare codon cluster detection that did not identify any RCC in this protein sequence of luciferase enzyme (Table 1).

In the following, the *L. lateralis* luciferase nucleotide

Table 1. The Characteristics of PF00501 ID that was Analyzed in the Sherlocc Program

PFAM ID	Pfam Name	Number of rare codon clusters	Rare codon frequency threshold	Size of largest cluster	Number of sequences	Number of unique organisms
Your query gave 0 match.						

ATG GAA AAC ATG GAG AAC GAT GAA AAT ATT GTA TAT GGT CCT GAA CCA TTT TAC CCT ATT GAA GAG GGA TCT GCT GGA GCA CAA TTG
CGC AAG TAT ATG GAT CGA TAT GCA AAA CTT GGA GCA ATT GCT TTT ACT AAC GCA CTT ACC GGT GTC GAT TAT ACG TAC GCC GAA TAC
TTA GAA AAA TCA TGC TGT CTA GGA GAG GCT TTA AAG AAT TAT GGT TTG GTT GTT GAT GGA AGA ATT GCG TTA TGC AGT GAA AAT TGT
GAA GAA TTC TTT ATT CCT GTA TTA GCC GGT TTA TTT ATA GGT GTC GGT GTG GCT CCA ACT AAT GAG ATT TAC ACT CTA CGT GAA TTG
GTT CAC AGT TTA GGC ATC TCT AAG CCA ACA ATT GTA TTT AGT TCT AAA AAA GGA TTA GAT AAA GTT ATA ACT GTA CAA AAA ACG GTA
ACT GCT ATT AAA ACC ATT GTT ATA TTG GAC AGC AAA GTG GAT TAT AGA GGT TAT CAA TCC ATG GAC AAC TTT ATT AAA AAA AAC ACT
CCA CCA GGT TTC AAA GGA TCA AGT TTT AAA ACT GTA GAA GTT AAC CGC AAA GAA CAA GTT GCG CTT ATA ATG AAC TCT TCG GGT TCT
ACC GGT TTG CCA AAA GGT GTG CAA CTT ACT CAT GAA AAT GCA GTC ACT AGA TTT TCT CAC GCT AGA GAT CCA ATT TAT GGA AAC CAA
GTT TCA CCA GGC ACG GCT ATT TTA ACT GTA GTA CCA TTC CAT CAT GGT TTT GGT ATG TTT ACT ACT TTA GGC TAT CTA ACT TGT GGT
TTT CGT ATT GTC ATG TTA ACA AAA TTT GAC GAA GAA ACT TTT TTA AAA ACA CTG CAA GAT TAC AAA TGT TCA AGC GTT ATT CTT GTA
CCG ACT TTG TTT GCA ATT CTT AAT AGA AGT GAA TTA CTC GAT AAA TAT GAT TTA TCA AAT TTA GTT GAA ATT GCA TCT GGC GGA GCA
CCT TTA TCT AAA GAA ATT GGT GAA GCT GTT GCT AGA CGT TTT AAT TTA CCG GGT GTT CGT CAA GGC TAT GGT TTA ACA GAA ACA ACC
TCT GCA ATT ATT ATC ACA CCG GAA GGC GAT GAT AAA CCA GGT GCT TCT GGC AAA GTT GTG CCA TTA TTT AAA GCA AAA GTT ATC GAT
CTT GAT ACT AAA AAA ACT TTG GGC CCG AAC AGA CGT GGA GAA GTT TGT GTA AAG GGT CCT ATG CTT ATG AAA GGT TAT GTA GAT AAT
CCA GAA GCA ACA AGA GAA ATC ATA GAT GAA GAA GGT TGG TTG CAC ACA GGA GAT ATT GGG TAT TAC GAT GAA GAA AAA CAT TTC
TTT ATC GTG GAT CGT TTG AAG TCT TTA ATC AAA TAC AAA GGA TAT CAA GTA CCA CCT GCT GAA TTA GAA TCT GTT CTT TTG CAA CAT
CCA AAT ATT TTT GAT GCC GGC GTT GCT GGC GTT CCA GAT CCT ATA GCT GGT GAG CTT CCG GGA GCT GTT GTT GTA CTT GAA AAA GGA
AAA TCT ATG ACT GAA AAA GAA GTA ATG GAT TAC GTT GCA AGT CAA GTT TCA AAT GCA AAA CGT TTG CGT GGT GGT GTC CGT TTT GTG
GAC GAA GTG CCT AAA GGT CTT ACT GGT AAA ATT GAC GGT AAA GCA ATT AGA GAA ATA CTG AAG AAA CCA GTT GCT AAG ATG

Fig. 1. The situation of rare and very rare codons are displayed in orange and red, respectively.

sequence was studied for its rare codons in the ATGme server (http://atgme.org/). By use of codon usage table of *E.coli* B [gbbct]: 11 CDS's, this nucleotide sequence was studied. However, this gene has GC%: 37.04 and AT%:62.96 in the original sequence. Figure 1 shows the rare and highly rare codons that are highlighted in orange and red, respectively.

For better understanding of these results, the codons of problematic residue as Arg, Leu, Ile and Pro in the *L. lateralis* luciferase codon usage was evaluated in RaCC server. Our study demonstrate that this gene has six single rare codons of Arg, three rare codons of Leu, three rare codons of Ile and six rare codon of Pro (Fig. 2).

In the following, this nucleotide sequence was analyzed in LaTcOm web tool [23]. This web tool used three algorithms of %MINMAX, sliding window, and MSS. For our analysis, the Codon_usage_Dong_*et al.* 1996 [36] was used as the reference scale (Fig. 3).

In this analysis, different rare codon cluster have been identified based on internal criteria. In the following,

Fig. 2. The position of the rare codons of Arg, Leu, Ile, and Pro in the *L. lateralis* luciferase gene red, green, blue and orange color, respectively).

based on the important of large clusters of rare codon [37,38], these common rare codons were accurately evaluated. According to the structural important, some of these rare codons were selected and studied in the structure of luciferase enzyme.

Enzyme-substrate Docking

For conduction of enzyme-substrate docking, the 3D structure of luciferase was modelled in the I-TSSAR server. The best model of five models that generated has the 0.99 + -0.04 value of TM-Score, 2.00 value of overall C-score, and Exp. RMSD of 3.3 + -2.3. The physicochemical properties of luciferase enzyme that was analysed by ProtParam tool (Table 2).

The study and comparison of our luciferase model with the crystallography structures of Japanese *Luciola cruciata* luciferase (2d1r) [32] and *P. pyralis* luciferase (1ba3) [33] show that their active sites were analogous. For evaluation of luciferin and AMP binding site, the computer-simulated docking studies were performed using AutoDock Vina [29]. The molecular model of *L. lateralis* luciferase was treated as a receptor, while the luciferin, and the AMP were treated as a small molecule ligands. As mentioned, the search space was designed according to the active site of Japanese *Luciola cruciata* luciferase. We evaluated the results of molecular docking with the different box sizes. The *L. lateralis* luciferase enzyme-luciferin complex has a network of diverse non-covalent interactions (Fig. 4).

Finally, the *L. lateralis* luciferase enzyme-luciferin complex was converted to the PDBQT format and considered as a receptor in the second docking process with AMP as a ligand. The *L. lateralis* luciferase enzyme-luciferin-AMP complex that obtained from docking results is shown in Fig. 5. As mentioned, the network of different non-bonded interactions are observed at these complexes. As shown, a few hydrogen bonds can be formed between the enzyme-luciferin AMP (Fig. 5).

In following, by use of ChExVis method the molecular channel of this enzyme was extracted based on the alpha complex representation [35]. The ChExVis method computes geometrically feasible channels. Then, stores the volume occupied by the channel and reports important channels [35]. The results of this analysis is shown at Fig. 6.

Study of Rare Codon in Structure of Luciferase Enzyme

In the following, the locations of this these rare codons

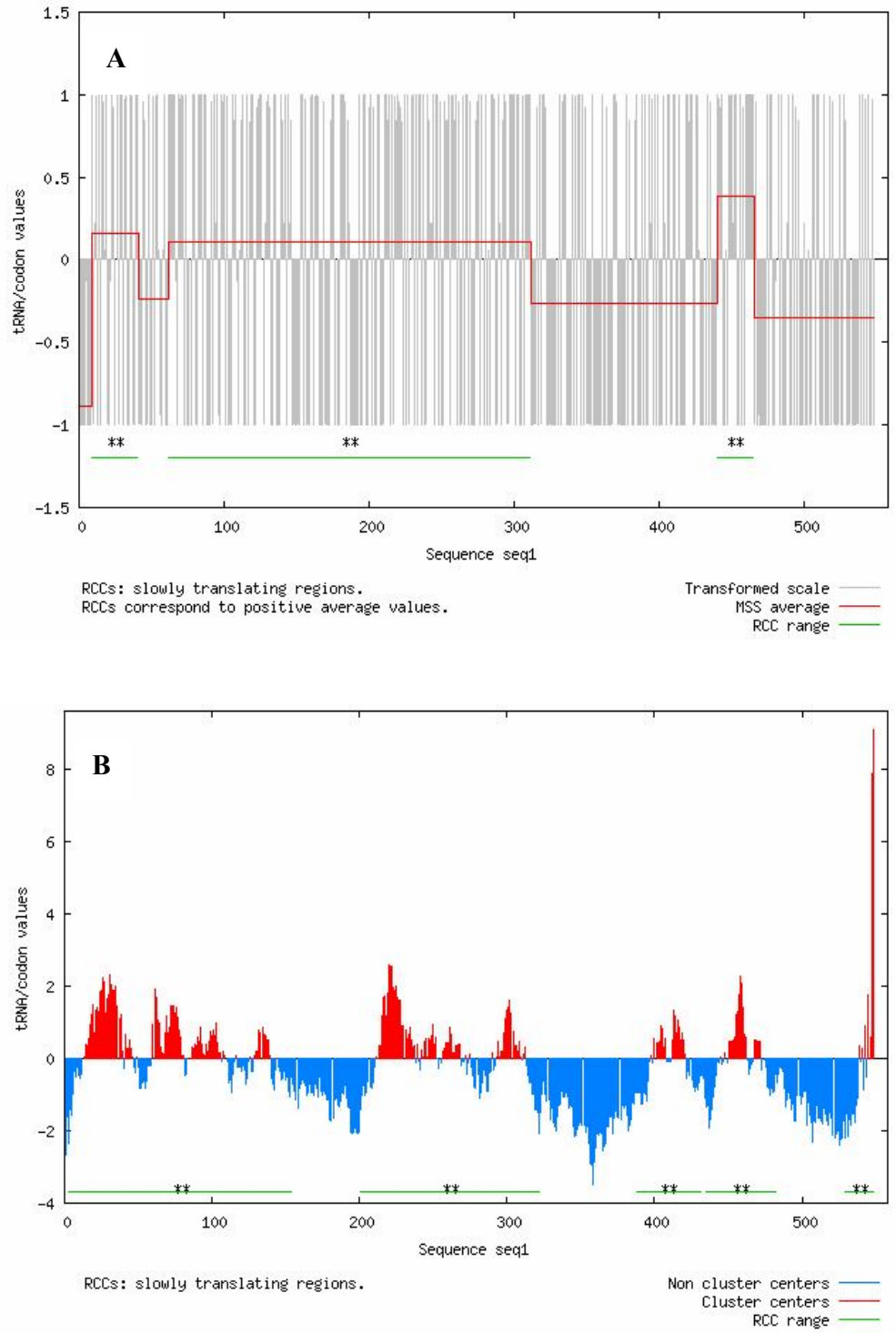

Fig. 3. The position of rare codon clusters in the *L. lateralis* luciferase gene. The results show that analysis of this gene in the MSS (A), minmax (B) and sliding_window algorithm (C) have recognized various numbers of rare codon clusters, which shows the diversity capability of these algorithms in assessment of RCC.

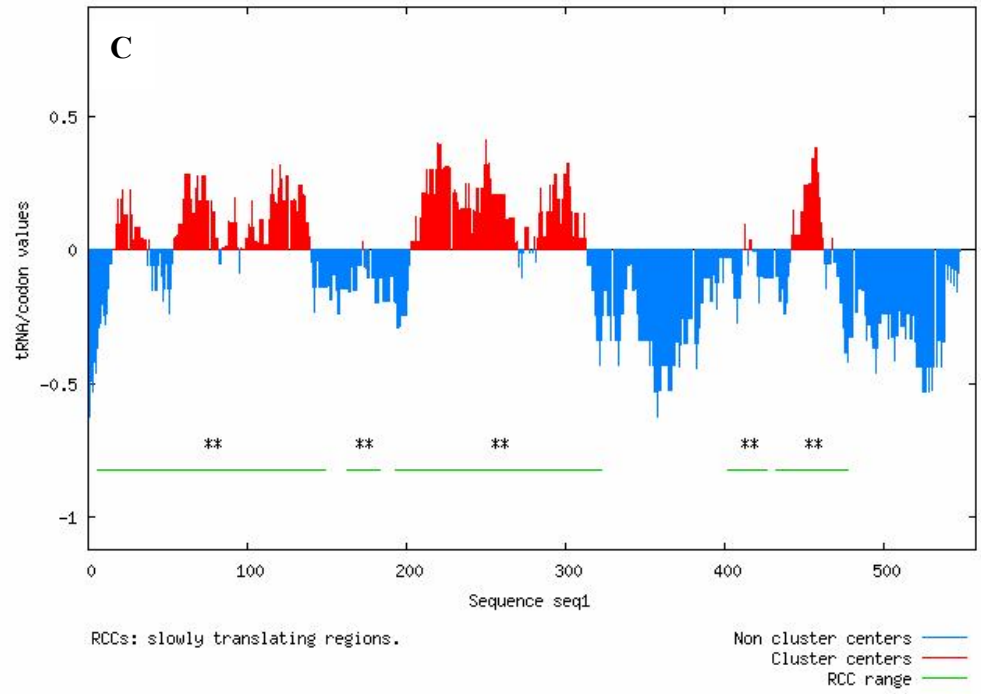

Fig. 3. Continued.

Table 2. *In-silico* Physicochemical Properties of Luciferase Enzyme Obtained from Prot Param Tool. *First Value is Based on the Assumption that both Cysteine Residues form the Cystine and the Second Assumes that both Cysteine Residues are Reduced

No.	Parameters	Luciferase
1	Theoretical pI	7.96
2	Molecular weight	60048.41
3	Sequence length	548
4	Extinction coefficients (M-1 cm-1at 260 nm)*	38655-38280
5	Asp + Glu	65
6	Arg + Lys	67
7	Instability index	26.91
8	Grand average of hydropathicity	-0.107
9	Aliphatic index	92.63

were evaluated in structural models of luciferase enzyme. This study show that these rare codons were located at distinct regions of luciferase structure. This structure along with some rare codons, luciferin and AMP are shown in Fig. 7.

This Figure shows positions of these rare codons in the

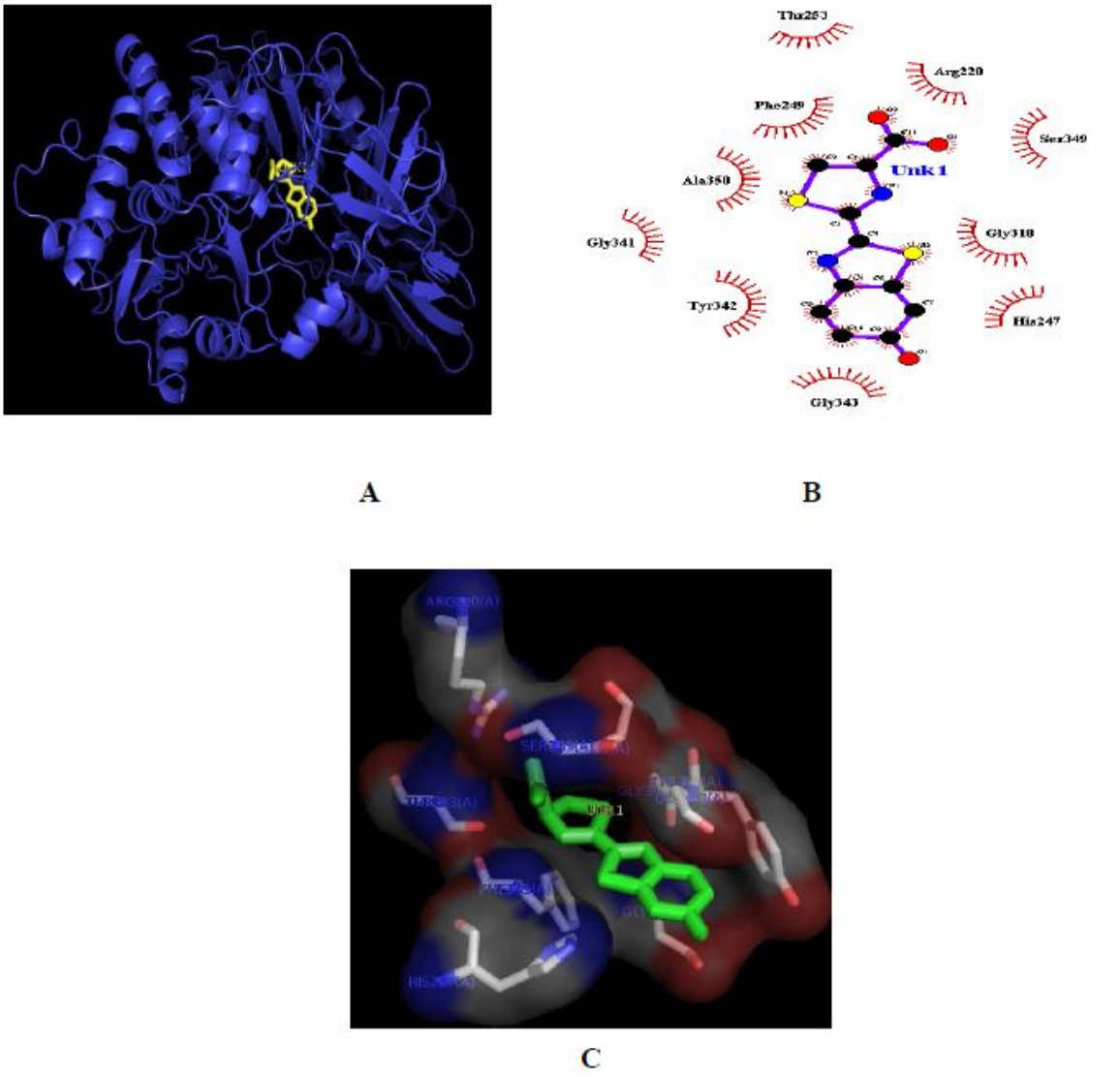

Fig. 4. A) Stereo presentation of docking situation of luciferin into the *L. lateralis* luciferase conducted in the PyMOL (Blue color: luciferase enzyme structure and yellow stick: luciferin). B) LIGPLOT result that polar interactions are shown as cyan colored lines. The plot was generated using LIGPLOT program [39]. C) PyMOL diagram showing the interaction of luciferin with the *L. lateralis* luciferase enzyme (Green stick: heme).

L. lateralis enzyme (Fig. 6). However, these rare codon of Arg construct some hydrogen bond with other residue and our initial review on the location of these Arg predicts the important roles of these residues in the proper folding of luciferase.

DISCUSSIONS

In the luciferase reaction, light emission can be detected and allowed the observation of biological processes [40]. Luciferase can be synthesized and inserted

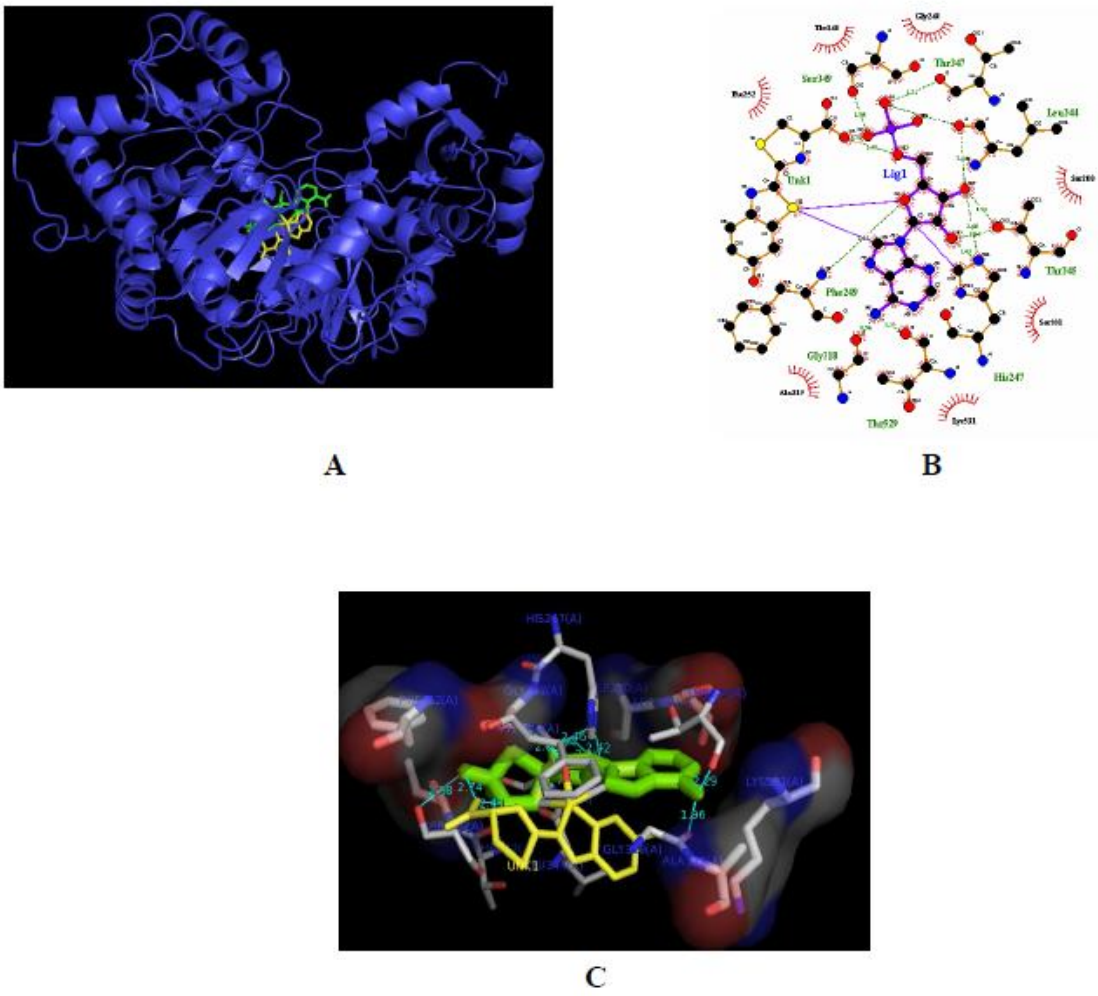

Fig. 5. A) Stereo presentation of docking situation and AMP into the *L. lateralis* luciferase enzyme-luciferin complex (Blue color: *L. lateralis* luciferase enzyme and yellow stick: luciferin and green stick: AMP acid). Polar interactions are shown as cyan colored lines. B) The luciferin-AMP interaction plot was generated using LIGPLOT program [39]. C) The interaction of AMP with the *L. lateralis* luciferase enzyme-luciferin complex in the PyMOL diagram (red stick: AMP).

into organisms or transfected into cells in the lab through genetic engineering for a number of purposes. In biological research, luciferase is commonly used as a reporter to assess the transcriptional activity in cell [41], detection the of cellular ATP level in cell viability assays [42], whole animal imaging for studying cell populations in live animals [43] and following the tumorigenesis and response of tumors to treatment in animal models [44]. However, environmental factors and therapeutic interferences may cause some disorder in the bioluminescence intensity in relation to changes in proliferative activity. Furthermore, the intensity of the signal may depend on various factors, such as intracellular pH and the amount of proper folded luciferase [39].

Previously, the molecular cloning and expression of *L. lateralis* luciferase was conducted [21]. Although, some molecular tools have been developed for expression of recombinant proteins in *E. coli*, evaluation of new

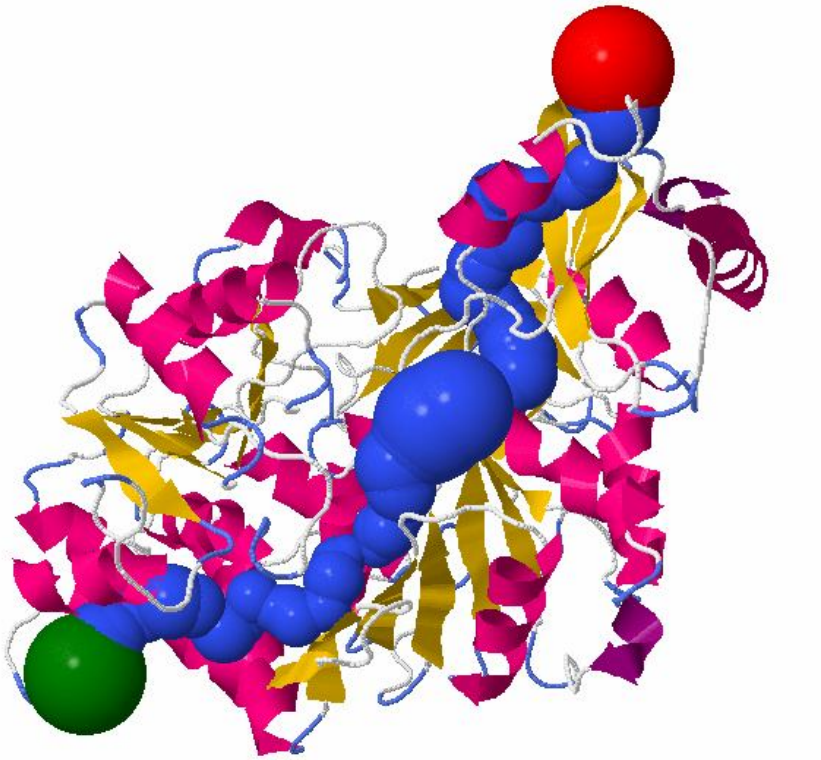

Fig. 6. Channel extraction in Top ranked pore shown using the skin surface (blue) and the luciferase structure.

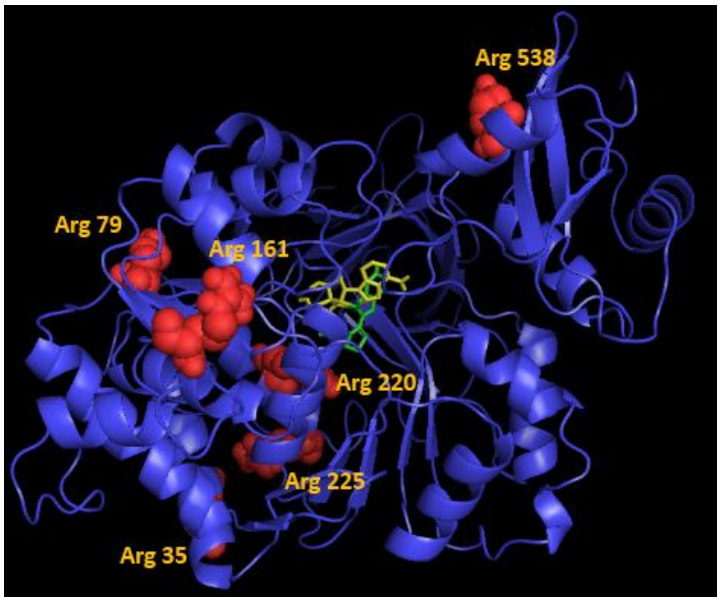

Fig. 7. The ribbon diagram of luciferase enzyme along with luciferin (green), AMP (red) and rare codon residues of Arg (yellow).

problematic challenges in the protein expression as "rare" codons are critical. Furthermore, evaluation of substrate binding site has a particular function role in protein engineering as site directed mutagenesis and protein expression. Since, there is no equivalent study on this enzyme, in this study, a survey of rare codons and substrate binding site in the gene and protein structures of this luciferase was conducted. Formerly, we have a good experience of *in silico* studies on the vaccine and HCV and HBV proteins [45,46].

Although, this new luciferase enzyme can be considered for biological application, but there are some unresolved topic as the substrate binding site. For better evaluation of the catalytic mechanism, some approaches were studied as docking and SDM. The luciferase enzyme utilizes an ATP and luciferin to process the light emission by certain amino acids that were identified to play a role in this process. These amino acids along with rare codons must be studied through bioinformatics study since the structural position of these residues are very important in mutations design. In this regard, the situation of these critical residues in the luciferase gene and protein were studied.

As shown, Sherlocc program identified no rare codon clusters in the luciferase protein family (Table 1). However, ATGme web server identified 131 rare codons and 24 highly rare codons. These results were summarized in the RaCC server as identified six rare codons of Arg, three rare codons of Ile, six rare codons of Pro, and three rare codons of Leu (Fig. 2). In the following, with three algorithms of minmax, MSS and sliding_window in the LaTcOm web tool ([3], different number of RCCs in the luciferase gene was detected (Fig. 1). Evaluation of these rare codons is very difficult, hence we considered on the repetitive rare codons.

In the following, some rare codons of arginine were selected and studied in the molecular model structure of luciferase enzyme. Further analyses indicated that these Arg codons established some non-covalent bonds with other residues (data not shown). These hydrogen bonds hold together the different domains of luciferase enzyme and their formation may be time consuming. This process reduce the rate of protein folding in these positions. However, other analyzes and experimental evidences are needed to evaluate that if such pauses is essential for *L. lateralis* luciferase folding.

For molecular docking studies, the molecular model of *L. lateralis* luciferase as a macromolecule and AMP and luciferin as ligands were submitted to AutoDock Vina [29]. The crystal structure of the Japanese *Luciola cruciata* luciferase (PDB: 2d1r) [32] show that active site of this enzyme was similar to our model of *L. lateralis* luciferase and hence, the grid box was designed based on the substrate binding site of *Luciola cruciata* luciferase and the docking results were studied using the PyMOL and Ligplot [39].

As shown in Figs. 4 and 5, luciferin and AMP constitute some hydrogen bonds with each other and luciferase structure. The docking process were conducted with various search space sizes (Data not shown). However, all of the predictable substrate binding site residues did not find in docking results. This is may be due to eliminating of Mg^{2+} ion in the docking proceeding and adaptation of two conformations by rotation of C terminal domain. Mg^{2+} ion has a critical role in catalytic activity but was deleted from our docking process as the simultaneous docking of Mg^{2+} and substrate is very problematic. We will try to redock these study by introduction the Mg^{2+} ion in the further studies.

These results show that these rare codons may have an important role in proper establishment of the substrate binding site. One of the best methods for study of luciferase activity, is conduction of new mutations based on these results. In this study some of important residues that may have a critical roles in substrate binding site or proper folding were determined. This study can also enable the design of new biosensor in the biological science.

CONflICT OF INTEREST

The authors declare that there is no conflict of interest.

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