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MOLECULAR DYNAMICS OF TRP40 FORM OF *B. TAURUS* TYROSYL-TRNA SYNTHETASE WITH REPLACEMENTS OF TRP87 AND TRP283 BY ALANINE

Aim. *Analysis of conformational flexibility of mutant Trp40 form of Bos taurus Tyr-tRNA-synthetase (BtTyrRS) where single Trp was localized near active site.* ***Methods.*** *Computational site directed mutagenesis of Trp87 and 283 residues using the AlphaFold3 server. In silico modeling of 3D structure and molecular dynamics (MD) simulation of mini Trp40 BtTyrRS mutant form.* ***Results.*** *Computational modeling of Trp40 mutant BtTyrRS form has not revealed significant changes at enzyme active site. A model of 3D structure of single-tryptophan form of BtTyrRS presents a compact structure of mutant protein and rigid microenvironment of Trp40 at the active site.* ***Conclusions.*** *The replacement of Trp residues at positions 87 and 283 in the amino acid sequence of mini BtTyrRS with alanine residues does not affect the structure of the enzyme active site. Stable mutant single-tryptophan mini BtTyrRS protein is suitable both for fluorescence studies of structural dynamic and catalytic properties of this enzyme.*

Keywords: *tyrosyl-tRNA synthetase, mutant form of mini TyrRS, computational modeling, molecular dynamics.*

Introduction

Aminoacyl-tRNA synthetases (ARSases) are key enzymes of the protein synthesis apparatus of pro- and eukaryotes. At the pre-ribosomal stage of translation, ARSases catalyze highly specific activation of amino acids and their attachment to homologous tRNAs, thus realizing the first stage of providing information about the protein structure [1—3].

Mammalian tyrosyl-tRNA synthetases (TyrRS) have a similar structure and are among the most studied ARSases of eukaryotes. The bos taurus tyrosyl-tRNA synthetase (528 aa, 59.2 kDa) consists of an N-terminal catalytic (corresponding to the shortened form of the enzyme mini TyrRS, 342 aa,

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39 kDa) and a C-terminal EMAP II-like (166 aa, 20 kDa) modules [3]. The N-terminal module, which contains the Rossmann fold, reveals the full catalytic function of activating and binding the amino acid to tRNA, while the C-terminal module is responsible for correction and stabilization of tRNA in the active site as cis-factor. In addition to the main function of tRNA aminoacylation, tyrosyl-tRNA synthetase also performs the important non-canonical functions in the cell. Both isolated mini TyrRS and C-terminal module after cleavage of the full-sized enzyme by elastase exhibit cytokine properties, which connect protein biosynthesis with cell signaling system [4, 5].

In mammalian cells, the functionally active form of *Bos taurus* tyrosyl-tRNA synthetase is an α2-type homodimer, the monomer of which is the full-length enzyme. When TyrRS was isolated from bull liver, it was shown that, along with the main form, a functionally active proteolytically modified N-terminal form of tyrosine-tRNA synthetase is also released with a molecular weight of 39 kDa, which has enzymatic activity in *in vitro* experiments, [3].

The study of the structural and dynamic properties of proteins is a necessary stage in the investigation of the mechanisms of their functioning. One of the most informative methods of studying conformational features and intramolecular interactions in the structure of proteins is fluorescence spectroscopy. The intrinsic fluorescence of proteins is due to aromatic amino acid residues, mainly tryptophan residues, which are probes in the spatial structure of the protein. They provide information about the properties of the microenvironment of the fluorophore and the dynamics of the protein in solution, and allow the assessment of conformational changes in the protein that have functional significance [6]. The experimental data are more meaningful in cases where it is possible to estimate the contribution to emission of individual tryptophan residues of the protein [7].

The amino acid sequence of the N-terminal catalytic module of *Bos taurus* tyrosyl-tRNA synthetase has three tryptophan residues, which are located in the active center of the enzyme (W40), in the dimerization region of the mini *Bt*TyrRS monomers (W87) and in the binding site of the $tRNA^{Tyr}$ anticodon triplet (W283). Previously, we cloned the cDNA of the catalytic module of tyrosyl-tRNA synthetase in the pET30a expressing plasmid and studied its expression [8]. Subsequently, using site-directed mutagenesis in the cloned mini *Bt*TyrRS cDNA, codons Trp87 and Trp283 were replaced by alanine codons and only one tryptophan codon was left in the catalytic center of the enzyme [9]. It was shown that amino acid substitutions did not affect the synthesis of mutant recombinant protein in *E. coli* [10].

The aim of this work is to perform computer modeling and long MD simulation of 3D structure of single-tryptophan mini BtTyrRS for further investigation of conformational changes at the active site of enzyme at the stage of tyrosyladenylate formation and during interaction with acceptor end of $tRNA^{Tyr}$.

Materials and methods

Structure modeling of TyrRS

The amino acid sequence of *Bos taurus* TyrRS was taken from NCBI Gene data-base (https://www.ncbi.nlm.nih.gov/protein/) under accession number DAA32266.1. Visualization and analysis of the protein structure was performed using the UCSF Chimera software [11]. The spatial structures of dimers of native *Bt*TyrRS and the double mutant *Bt*TyrRSW283 were modeled using the AlphaFold web server (https://alphafoldserver.com/), which is based on the AlphaFold 3 algorithm [12]. Refinement of the protein structure with high resolution and optimization of the structure was carried out using ModRefiner [13]. The final verification of the mini *Bt*TyrRS structure models was performed using the MolProbity server [14]. The surface area accessible to the solvent molecules of the amino acid residues of the mini *Bt*TyrRS protein was calculated on the GETAREA web server.

Molecular dynamics simulations

All-atom simulation of molecular dynamics (MD) of the dimers of native *Bt*TyrRS and the double mutant *Bt*TyrRSW283 was carried out in a time interval of 200 ns at temperature of 37 °C in a 150 mM NaCl salt solution.

The general calculation scheme was as follows. The coordinates of the structures of the proteins were converted from the pdb format to the internal GROMACS — gro format. In this case, the existing hydrogen atoms were removed from the pdb structure and then added back to the gro format file. This procedure was performed to create the correct topology. The CHARMM36 force field was used in all calculations. The structure was placed in a box that has the shape of a truncated octahedron with a minimum distance from the box walls to the protein atoms of 1 nm. Next, the energy of the system was minimized for 200 steps using the steep descent method. After that, the box was filled with the SPC216 (Single Point Charge) water model. The genion program was used to provide an ionic strength of 150 mM by adding Na^+ and Cl^- to the system. Energy minimization was performed using the steep descent method, after which the conjugate gradient (cg) method was used.

At the next stage, molecular dynamics was calculated with a harmonic binding of protein atoms to their initial coordinates for 50 ps (the resulting system was introduced as the initial one). Integration was performed with a step of 4 fs using the variable lead method, equivalent to the Verl*et algo*rithm. Atomic coordinates were recorded in a file with a periodicity of 1 ps. The bond length was maintained constant using the SHAKE algorithm. Electrostatic interaction was taken into account using the PME (Particle-Mesh Ewald sum) method. The cutoff parameter was set to 1 nm for all types of interactions. The system temperature (37 °C) and pressure (1 atm) were maintained constant. The trajectories were converted using the trjconv program. The root-mean-square deviations of Cα atoms from their initial positions were calculated using the g_rms and g_rmsf programs.

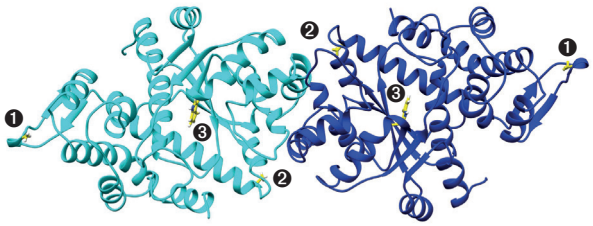
Computer calculations of molecular dynamics were performed in the Ukrainian National Grid Infrastructure (http://ung.in.ua/) using the services of the MolDynGrid virtual laboratory (http://moldyngrid.org/) [15].

Results and Discussion

Previously, we cloned and sequenced the nucleotide sequence of the full-length cDNA of the *Bos taurus* tyrosyl-tRNA synthetase gene [16]. On its basis, the plasmid construct pET-30a(+)-39KYRS was created with the cloned cDNA sequence of the N-terminal catalytic module of the synthetase. The recombinant mini *Bt*TyrRS protein expressed in *E.coli* strain BL21(DE3) retained the tRNA aminoacylation activity of synthetase.

Based on the X-ray crystal analysis of the N-terminal catalytic module of human tyrosyl-tRNA synthetase [17], the amino acid sequence of which is 96% similar to the amino acid sequence of the mini *Bt*TyrRS, and the computer model of the spatial structure of the full-sized *Bos taurus* tyrosyl-tRNA synthetase developed by us [18], a model of the structural organization of the homodimer of the N-terminal catalytic module of *Bt*TyrRS was built and the native tryptophan fluorescence of the native mini TyrRS was studied [18]. According to the data of X-ray structural analysis and the created three-dimensional model of the catalytic module of the synthetase, the tryptophan residues at the position 40 in the dimer of functional mini *Bt*TyrRS are located in the inner regions of the monomers.

To study the structural and dynamic properties of the enzyme in the process of catalysis by the methods of fluorescence spectroscopy in the cDNA of the catalytic module of the synthetase in the pET-30a(+)-39KYRS plasmid construct, the tryptophan triplets at positions 87 and 283 were replaced with alanine triplets by the site-directed QuikChange mutagenesis method [9]. The resulting plasmid construct pET-30a(+)-39KYRS40W has only one tryptophan codon in the catalytic center of the enzyme at the position 40 in the



❶ Ala283; ❷ Ala87; ❸ Trp40

Fig. 1. Trp40 mutant form of mini *Bt*TyrRS with the replacements of Trp87 and Trp283 by alanine. The different monomers of the homodimer are marked in cyan and blue

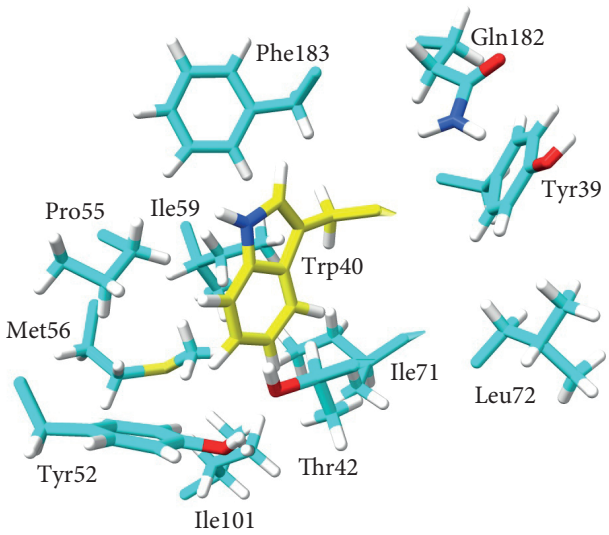


Fig. 2. Microenvironment of Trp40 in the structure of mini *Bt*TyrRS mutant form. Trp40 residue is marked in yellow, and the residues included in its microenvironment are highlighted in cyan

cloned cDNA sequence of the N-terminal synthetase module. The basis for choosing the amino acid alanine to replace tryptophan residues in the amino acid sequence of the mini *Bt*TyrRS was that its small hydrophobic radical does not interfere with the formation of the correct secondary structure of proteins.

It was established that the replacement of two tryptophan residues with alanine residues in the structure of the mutant protein does not affect its expression in the *E. coli* strain BL21 (DE3)pLysE in comparison with the synthesis of the native mini *Bt*TyrRS [10]. The fluorescence characteristics of the emission of tryptophan residues of the obtained recombinant native and mutant forms of the protein, in particular the width of the fluorescence spectrum $\Delta\lambda$ around 55 nm and the position of the fluorescence maximum around 330 nm, indicate that Trp40 in both forms of the enzyme is inside the protein globule in the non-polar microenvironment [10, 18, 19].

The parameters of the fluorescence spectra of tryptophan residues depend on the polarity of their microenvironment, as well as the ability of the tryptophan residue to relax during the fluorescence lifetime. The polarity of the microenvironment of tryptophan residue is determined by both its accessibility to solvent molecules and the protein's own polar groups, which are part of the microenvironment [6]. The analysis of the localization and properties of the microenvironment of 3 tryptophan residues responsible for the intrinsic fluorescence of the mini-TyrRS made it possible to characterize their accessibility in the enzyme dimer structure and the conformational mobility of their microenvironment [19, 20]. The characteristics of tryptophan fluorescence of the mutant mini-TyrRS with tryptophan residues in position 40 indicate the immobilization of the environment of tryptophan residues in the homodimer of the enzyme, its compact state and the preservation of the native conformation of the synthetase.

For a more in-depth assessment of the structure of the mutant form of mini *Bt*TyrRS in this work, we used the method of computer molecular dynamics simulation of the spatial structures of homodimers in parallel with the native and mutated catalytic modules of the synthetase in the time range of up to 200 ns, and also conducted a complete analysis of the microenvironment of tryptophan residue 40 in the catalytic region of both forms of mini TyrRS.

The spatial structure of double mutant *Bt*-TyrRSW87,283 was modeled using the AlphaFold web server [12], resulting model is shown in Fig. 1.

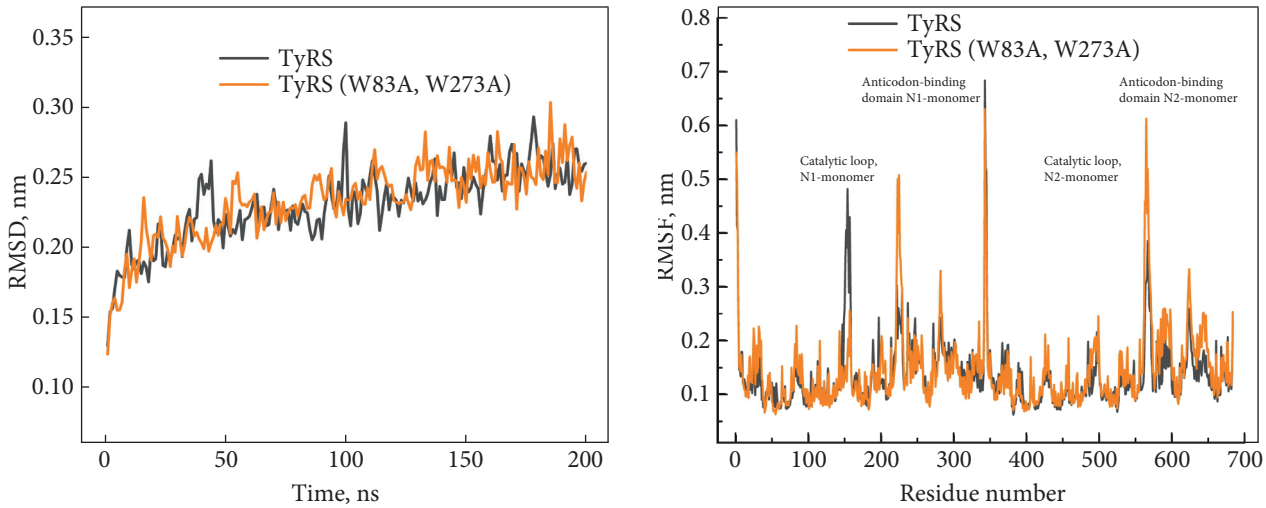


Fig. 3. Root Mean Square Deviation (RMSD) of Cα atoms of TyrRS which indicate the stability of protein structure. Relaxation period was observed after first 40—60 ns

Fig. 4. Root Mean Square Fluctuations (RMSF) per Cα atom of each amino acid residue in nanometers in the time interval 60—100 ns. The highest peak corresponds to the Met1 of monomer N1 and the beginning of monomer N2 (corresponds to number 343 on the X-axis)

When comparing the spatial structures of the native and mutant forms of synthetase, it is evident that Trp40 in both forms of mini *Bt*TyrRS is localized in the inner region of the proteins. The replacement of two tryptophan residues with alanine residues in the mini *Bt*TyrRS did not lead to visible

Table 1. **Amino acid microenvironment and solvent accessibility of Trp40 in native and mutant forms of mini *Bt*TyrRS**

Amino acid environment of Trp40 in *Bt*TyrRS (native form)	Distance between atoms, Å	Accessibility to solvent (Surface area, %)	Amino acid environment of Trp40 in *Bt*TyrRS (mutant form)	Distance between atoms, Å	Accessibility to solvent (Surface area, %)
Trp40		8.7	Trp40		7.3
Tyr39	2.3	0.6	Tyr39	2.3	0.6
Thr42	3.5	28.5	Thr42	3.5	30.8
Thr42	3.7	3.4	Thr42	3.6	3.2
Tyr52	2.6	13.8	Tyr52	2.8	13.7
Pro55	2.6	23.7	Pro55	2.6	25.5
Met56	2.9	0.0	Met56	2.9	0.0
Ile59	2.9	0.2	Ile59	2.9	0.1
Thr70	4.3	4.8	Thr70	4.2	4.8
Ile71	2.2	0.0	Ile71	2.9	0.0
Leu72	3.3	4.1	Leu72	3.3	4.1
Ile101	2.3	0.0	Ile101	2.3	0.0
Gln182	3.1	1.1	Gln182	3.1	1.1
Phe183	2.6	0.4	Phe183	2.6	0.4

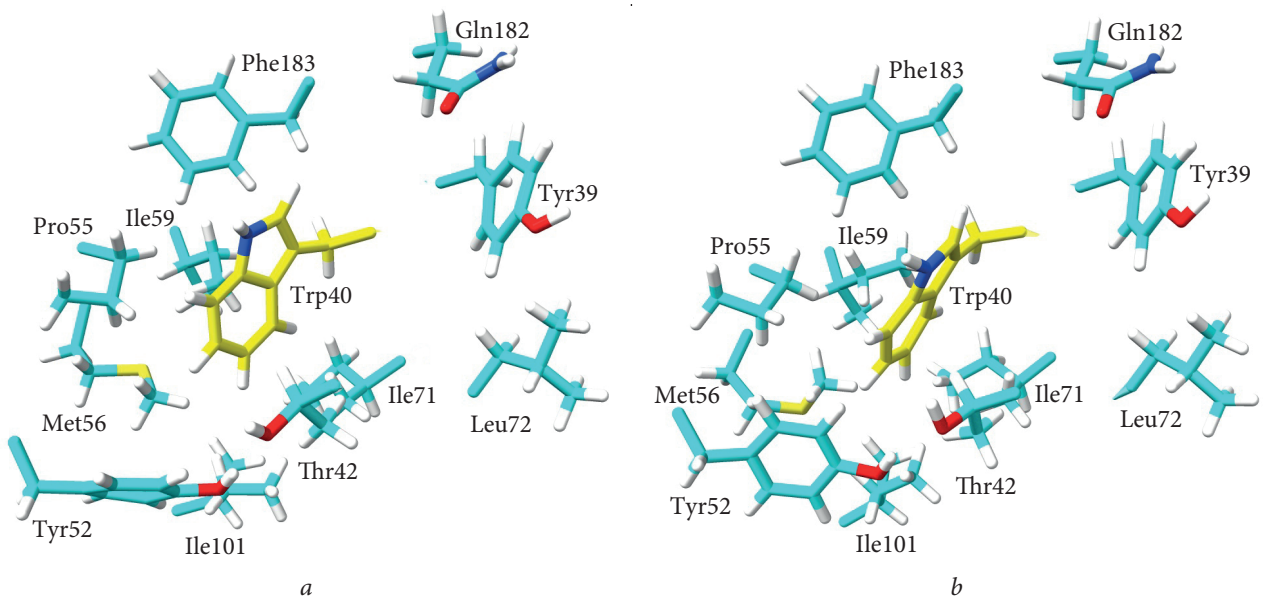


Fig. 5. Microenvironments of Trp40 after 200 ns MD simulation of native (*a*) and mutant (*b*) forms of *Bt*TyrRS

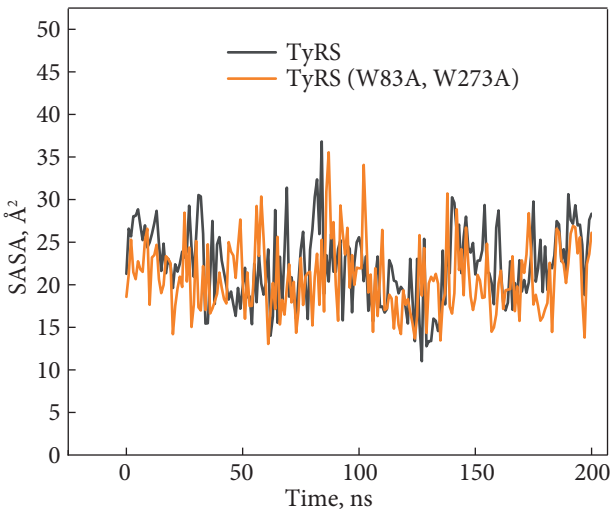


Fig. 6. Solvent accessibility of Trp40 in the native and mutant forms of mini *Bt*TyrRS during 200 ns MD simulation

changes in its three-dimensional structure, which is also confirmed by analysis of native and mutant forms of the enzyme using the ProtParam program. According to the data of ProtParam program, the instability was almost the same and equal to 36.2 and 37.23, respectively.

Similar data were obtained when comparing models of the spatial structure directly in the region of Trp40 localization in native and mutant forms of the mini *Bt*TyrRS (Fig. 2). There were no significant differences in the structure of single-tryptophan enzyme compared to the main form.

The obtained data showed that Trp40 is surrounded by 10 hydrophobic residues (Tyr39, Tyr52, Pro55, Met56, Ile59, Ile71, Leu72, Ile101, Gln182, Phe183) and one hydrophilic Thr42.

It is well known that both aminoacyladenylate formation and tRNA aminoacylation are associated with subtle conformational changes at the active site of TyrRS [2].

From the data presented in Table 1, it can be seen that microenvironment of Trp40 mostly not changed in the mutant form of mini *Bt*TyrRS in comparison with the native one. We observe only minor changes in the distance between the C_α atoms of Trp40 and some amino acid residues of microenvironment and accessibility to the solvent to Trp40 residue. These data indicated the similarity of 3D structures of native and mutant forms of TyrRS and testified the immobilization of Trp residue environment in its compact state.

Due to the calculations of MD simulations of mini-TyrRS and its double mutant form W87,273A several long 200 ns trajectories were obtained. Analysis of the root-mean-square deviations of Cα atoms (RMSD) indicates the stability of all trajectories during the simulation process with deviation of 0.2 and 0.35 nm (Fig. 3) for the trajectory of the wild-type enzyme and the mutant form W87,273A, respectively. The relaxation period corresponds to the first 50—60 ns, which was taken into account in the subsequent analysis of MD trajectories.

In order to analyze the mobility of individual protein regions, the root mean square fluctuations (RMSF) of individual atoms were calculated. The results indicated a pronounced asymmetry of monomer mobility (Fig. 4). The largest deviation values were found for amino acid residues of unstructured catalytic loop of synthetase, which contains the KMSKS-like catalytic motif in *Bt*TyrRS — KMSSS, and for anticodon binding site. The root-mean-square deviations of anticodon binding site were in average of 0.2 nm larger than for wild-type enzyme in monomer N2, which characterizes the asymmetry of movements in relation of N1 monomer.

We performed a comparative analysis of the 3D protein structures after 200 ns of MD simulation at the Trp40 localization region in the native and mutant forms of mini *Bt*TyrRS (Fig. 5). As a result, we found no significant differences in the structure of the single-tryptophan form compared to the native protein form.

Analysis of the molecular dynamics trajectories of TyrRS revealed no permanent changes in the solvent accessible surface area (SASA) of residue Trp40 (Fig. 6). The exposure area of Trp40 was calculated to be 8—10% for both native and mutant forms of *Bt*TyrRS. Throughout the 200 ns MD simulations, we observed small fluctuations of SASA parameter for Trp40 in the range of 11—37 Å^2, confirming the low mobility and rigid microenvironment of Trp40 residue.

Conclusions

Conformational flexibility of the mutant Trp40 form of *B. taurus* Tyr-tRNA-synthetase where a single Trp was localized near the enzyme active site has been explored. Computational site directed mutagenesis of Trp87 and 283 residues has been performed using the AlphaFold3 server. *In silico* modeling of 3D structure and MD simulation of the mini Trp40 *Bt*TyrRS mutant form does not revealed any significant changes in the enzyme active site. The 3D structure of single-tryptophan form of *Bt*TyrRS revealed a compact conformation of the mutant protein and rigid microenvironment of Trp40 at the active site. Hence, the replacement of Trp residues at positions 87 and 283 in the amino acid sequence of mini *Bt*TyrRS with alanine residues does not affect the structure of enzyme active site. The stable mutant single-tryptophan mini *Bt*TyrRS protein is suitable for the fluorescence studies on structural dynamic of active site and catalytic properties of this mutant enzyme.

Conflicts of Interests. The authors declare no conflict of interests.

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МОЛЕКУЛЯРНА ДИНАМІКА TRP40 ФОРМИ ТИРОЗИЛ-
ТРНК-СИНТЕТАЗИ *B. TAURUS* ЗАМІНАМИ TRP87 ТА TRP283 НА АЛАНІН

Мета. Аналіз конформаційної рухливості мутантної форми Trp40 *Bos taurus* Tyr-тРНК-синтетази (*Bt*TyrRS), де Trp40 локалізувався поблизу активного центру. ***Методи.*** Обчислювальний сайт-спрямований мутагенез залишків Trp87 та 283 за допомогою сервера AlphaFold3. Моделювання *in silico* 3D-структури та молекулярно-динамічне (МД) моделювання мутантної форми Trp40 *Bt*TyrRS. ***Результати.*** Обчислювальне моделювання мутантної форми *Bt*TyrRS з Trp40 не виявило суттєвих змін в активному центрі ферменту. Моделювання 3D структури та динаміки однотриптофанової форми *Bt*TyrRS вказує на компактну структуру мутантного білка та стабільне мікрооточення Trp40 в активному центрі. ***Висновки.*** Заміна залишків Trp у положеннях 87 та 283 в амінокислотній послідовності *Bt*TyrRS на залишки аланіну не впливає на структуру активного центру ферменту. Стабільний мутантний однотриптофановий білок *Bt*TyrRS підходить як для флуоресцентних досліджень структурної динаміки синтетази, так і для вивчення каталітичних властивостей цього ферменту.

***Ключові слова*:** тирозил-тРНК-синтетаза, мутантна форма міні TyrRS, комп›ютерне моделювання, молекулярна динаміка.