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Molecular docking and molecular dynamics simulation studies on *Thermus thermophilus* leucyl-tRNA synthetase complexed with different amino acids and pre-transfer editing substrates

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Aim. To investigate the structural bases for the amino acid selectivity of the *Thermus thermophilus* leucyl-tRNA synthetase (LeuRSTT) aminoacylation site and to disclose the binding pattern of pre-transfer editing substrates. **Methods.** Eight amino acids proposed as semi-cognate substrates for aminoacylation and eight aminoacyl-adenylates (formed from AMP and eight amino acids) were prepared in zwitterions form. The protein structure with a co-crystallized substrate in the aminoacylation site [PDBID: 1OBH] was taken from RCSB. Docking settings and evaluation of substrate efficiency were followed by twofold docking function analysis for each conformation with Gold CCDC. The molecular dynamics simulation was performed using Gromacs. The procedures of relaxation and binding study were separated in two different subsequent simulations for 50ns and 5ns. **Results.** The evaluation of substrate efficiency for 8 amino acids by twofold docking function analysis, based on score values,has shown that the ligands of LeuRSTT can be positioned in the following order: Leu>Nva>Hcy>Nle>Met>Cys>Ile >Val. MD simulation has revealed lower electrostatic interactions of isoleucine with the active site of the enzyme compared with those for norvaline and leucine. In the case of aminoacyl-adenylates no significant differences were found based on score values for both GoldScore and Asp functions. Molecular dynamics of leucyl-, isoleucyl- and norvalyl-adenylates showed that the most stable and conformationally favorable is leucine, then follow norvaline and isoleucine. It has been also found that the TYR43 of the active site covers carboxyl group of leucine and norvaline like a shield and deflected towards isoleucine, allowing water molecules to come closer. **Conclusions.** In this study we revealed some structural basis for screening unfavorable substrates by shape, size and flexibility of a radical. The results obtained for different amino acids by molecular docking and MD studies correlate with the experimental data on the first stage of aminoacylation. MD study of the aminoacyl-adenylates revealed that difficulty of some amino acids activation can be caused by two reasons: excessive flexibility due to the size or structure and quick hydrolysis of intermediate substrate with nucleophilic attack by water molecules.

Keywords: leucyl-tRNAsynthetase, editing, amino acids, aminoacyl-adenylate, molecular docking, MD simulations

Introduction

An aminoacyl-tRNA synthetase (aaRS) is an enzyme, which binds three substrates (amino acid, ATP, tRNA) and catalyzes a transfer and attachment of an appropriate amino acid to its tRNA. The substrate specificity of these enzymes is crucial for the accurate translation of the genetic code [1]. In general, aaRS provides 2–3 stages with formation of an intermediate compound – aminoacyl-adenylate, transferring an amino acid from the intermediate to tRNA and generation of aminoacyl-tRNA and, in some cases, elimination of misactivated or mischarged substrates ("editing"). There are two distinct

classes of aaRS(class I and class II) which were distinguished by the structural differences of the catalytic domain [2, 3]. At the same time, within each class, there exist subclasses a, b and c according to their sequence and structural features. Ten of all aaRS families are unable to distinguish accurately enough the cognate from the non-cognate amino acids in the synthetic reactions and therefore possess additional editing activities for hydrolysis of the misactivated amino acids (pre-transfer editing) and misacylated tRNAs(post-transfer editing) [4]. There are two different 'sieves' which allow aaRSs to achieve a high fidelity in the selection of a necessary amino acid [5]. The first or a "coarse" sieve is implemented at the activation step, and is based on a size component of recognition and activates the cognate amino acids as well as isosteric or closely related amino acids that fit into the binding pocket. The second is "fine" sieve, which prevents pass of the cognate aminoacyl-tRNA but hydrolyzes the mischarged tRNA. For hydrolysis of misacylated tRNA many aaRSs have an alternative active center which is responsible only for editing [4].

The architecture of aaRS consists of individual domains or modules. These modules of various length and structure could be found either on the ends of the protein molecule or within the core part of the catalytic domain, forming the aminoacylation site of the enzyme. Thus, the general pattern structure of aaRS includes the core part, which contains the active site, and additional modules with the editing domain. The aminoacylation active site of the class I aaRSs has a Rossmann fold [1,4]. Rossmann fold is represented with six parallel β-strands, alternateing with α-helical sections, forming a flat surface similar to a fan. Another important feature of class I enzymes is the two conservative motifs, HIGH and KMSKS, inside a Rossmann fold. These motifs are necessary for binding ATP and an amino acid and for promoting the catalysis [1, 4].Leucyl-tRNA synthetase is one of the most interesting representatives of this class, because of its multi-domain structure. The class II aaRS is characterized by a completely different structure of the active center: seven anti-parallel β-strands [2, 3]. Other typical peptide motifs are also located in the active site, but instead of HIS a conservative ARG from the second motif is involved in the binding of ATP. Thus, binding the similar substrates by aaRS from different classes occurs in a different way, with participation of specially organized domains.

Study on the crystal structure of *Thermus thermophilus* LeuRS in a complex with sulfamoyl analogue of leucyl-adenylate (LeuAMS) has revealed the structure of synthetic site of the enzyme and the details of substrate binding [6]. The large hydrophobic pocket for the leucine has enough room to accommodate the amino acids of different size. The biochemical studies have shown that besides leucine, LeuRS activates non-cognate isoleucine, methionine, norvaline, norleucine and homocysteine [6–9]. To avoid errors LeuRS has adopted both pre- and post-transfer editing activities which were investigated in many laboratories [6–11]. Despite the intensive research, the structural basis for the amino acids discrimination and mechanisms of editing by LeuRS remain unresolved. Especially this problem is important in the case of isoleucine, which is generally used in the biochemical research of LeuRS's editing [12–14].

Here, we employed *in silico* approaches (a molecular docking and molecular dynamics (MD) simulation) to define the basis for amino acid discrimination by the LeuRSTT aminoacylation site and to understand the binding pattern and stability of the pre-transfer substrates.

Materials and Methods

Marvin Sketch was used for drawing, displaying and characterizing chemical structures, substructures and reactions, Marvin 5.5, 2011, ChemAxon (http://www.chemaxon.com). Eight amino acids proposed as semi-cognate substrates for aminoacylation and eight aminoacyl-adenylates (formed from AMP and amino acids) were prepared in zwitterions form. We used AMP instead of ATP to reconstruct the conditions for the aminoacyl-adenylate formation. For this purpose AMP pose was taken from leucyl-ade-

nylate coordinates. To avoid repulsion from COO- of amino acids one oxygen atom was removed from phosphate group. Antechamber package from AmberTools was applied to produce topologies for all ligands in amber99 force field and am1-bcc charge model [15]. VMD was used as a basic package for study and visualization of structural features of the synthetases [16]. The protein structure with co-crystallized substrate in the aminoacylation site [PDBID: 1OBH] was taken from RCSB.

Genetic algorithm implemented in Gold CCDC was applied to dock ligands into the protein structure [17]. All parameters have been selected in accordance with the recommendations guide. Based on the literature and visual analysis of the aminoacylation sites a group of residues were set as flexible, taking into account the degree of freedom for each atom. Docking settings and evaluation of substrate efficiency were followed by twofold docking function analysis for each conformation of a ligand. The first docking function was GoldScore, which takes into account hydrogen bonds, van der Waals interaction and favorable localization of a ligand. The second one was ASP (the Astex Statistical Potential), based on the statistic of conformations. Rescoring method gives good results with complex compounds and highly specialized binding site. The superior value of each function is a numerical representation of both: the realistic pose and internal energy which suggests the ability to form favorable interactions between a ligand and the protein.

The molecular dynamics simulation was performed using Gromacs on the IMBG cluster [18, 19]. The procedures of relaxation and binding study were separated in two subsequent simulations with different duration. The first one lasted for 50ns and resulted in more reliable geometry of the protein structure in the water environment comparing to its unnatural state during the protein purification and desalting. And the second one which lasted only for 5ns was acceptable to catch the activation state, which naturally occurs almost immediately. The LINCS algorithm was used to constrain all bond lengths during equilibration step and 5ns free MD [20]. To reduce artifacts in calculation of stacking interaction amber99sb force field was used with TIP3P type of water molecule [21, 22]. The energy minimization step was performed in steepest descent and conjugate gradient methods; it was followed by 100 ps of equilibration step, imposing positional restraints on the non-H atoms. The simulation was conducted at a constant temperature (333 K) natural for this type of thermophilic proteins, coupling each component separately to a temperature bath using the Berendsen coupling method [23]. A cutoff of 1.0 nm was selected for Coulomb interaction and 0.9 nm for Lennard Jones interaction. The time step was 2 fs, with coordinates stored after every 2 ps. MD simulation was performed for 5ns.

Results and Discussion

Study of amino acids and AMP located in LeuRSTT active site

For the identification of correct poses of amino acids in the LeuRSTT synthetic site we have used molecular docking. After mapping the surface (steric conditions) and determination of possible hydrogen bonds we have identified a set of amino acids and their rotamers for docking. We computationally studied eight LeuRSTT- AMP-amino acid complexes with Leu and its analogs Nva, Hcys, Nle, Met, Cys, Ile, Val. Fig. 1 shows visual differences primarily in distance between the carboxyl oxygen of amino acids and the phosphate group of AMP as well as the spatial orientation of the carboxyl groups, which could be considered as the ability of bond formation. Thus, even those poses that have high rates of value functions, but did not meet the predicted orientation, were rejected. H-bonds between the amino group of a substrate and the carboxylic groups of ASP80 and PHE41 were also taken into account. A special feature for AMP was the spatial orientation of the purine ring that plays a major role in stabilizing a whole substrate. Evaluation of substrate efficiency was followed by twofold docking function analysis (see Materials and Methods for the docking details) for each conformation of a ligand (Table 1).The results

obtained well correlate with the experimental data of the aminoacylation first stage. In the case of *E.coli* LeuRS on the basis of an activation rate the amino acids can be positioned in the following order: Leu>Nva>Hcy>Nle>Met>Ile [7, 9] and in the case of LeuRS from thermophilic bacteria *Aqui fexaelicus,* in the following order: Leu>Nva> Met > Ile [8].

Further study was carried out with the long term molecular dynamics of each complex, consisting of the active site, amino acid and AMP. MD simulation was performed for 5ns. The degree of "affinity" for a substrate was predicted based on its stable position during the MD of the complex, the distance between its atoms and amino acid environment, i.e. the ability to form h-bonds and high values of Coulomb energy (van der Waals interaction is insignificant for small molecules). As it turned out, the most meaningful data could be obtained only for leucine, isoleucine and norvaline. Graphs with fluctuations of energy and atoms' mobility do not express noticeable differences enough to explain the biochemical experiments. The reason for the inability of adequate interpretation and data comparison for valine and cysteine is a small size causing an increased mobility. Despite excessive flexibility of methionine, homocysteine and norleucine the docking results showed that these amino acids could become substrates of LeuRS. It can be confirmed with the fact that the distance between leucine, norvaline, homocysteine amino groups and ASP80 was stable and sufficient for the formation of h-bond and keeping the substrate in the pocket of the site. The average positions for these amino acids in the site that corresponds to the most frequent frames during dynamics are represented in Fig.1. Thus, we can conclude that the main factor influencing the rate of activation of amino acids is COO- group oriented on the phosphate of AMP. Of course, such a comparison makes sense only for similar amino acids.

Concerning best triad of amino acids the analysis showed significant differences between novaline and isoleucine. Leucine molecule was used as a test ligand, as it is a cognate amino acid. Graph of RMSD (Fig. 2 *A*) shows the shift of isoleucine's atoms and confirms a different level of ligand stability in the binding site. Graph of electrostatic interactions (Fig.2 *B*) between ligands and the synthetase, presented below, shows the similarity of trends for noravline and leucine, which are higher than the rates of isoleucine. It correlates with decrease in binding energy for isoleucine. The reasons for these differences should be in a rigid scaffold of isoleucine and its large surface area. Although isoleucine also oriented to ASP80 the distance to the AMP phosphate group is large enough.

Study on aminoacyl-adenylates in LeuRSTT active site

To understand the binding pattern of pre-transfer substrates at the LeuRSTT aminoacylation site we have performed molecular docking studies for several aminoacyl-adenylates. The results of docking for 8 aminoacyl-adenylates presented in (Table 2) and (Fig. 3). As can be seen, the aminoacyl-adenylates have absolutely distinct binding properties comparing to the amino acids. In the case of aminoacyl-adenylates no significant differences were found based on score values for both GoldScore and Asp functions (Table 2). The reason is a loss of degree of freedom and the existence of hydrophobic adenine. A spatial orientation of a plane of purine ring plays a significant role in stabilizing any aminoacyl-adenylate. Presumably, the tension that occurs in the phosphate group passed on and affects the ribose conformation. Note, that a group of aligned compounds (leucyl-, norvalyl-, homocysteil, isoleucyl-adenylates) occupied a special place, based on their conformations.

Based on these results we initiated the molecular dynamics to determine the changes in internal energy of the aminoacyl-adenylate which can be assessed by watching its conformations. Its simulation should reflect preconditions of amino acid transfer on tRNA. Molecular dynamics of leucine', isoleucine' and norvaline' derivatives showed that the most stable and conformationally favorable is leucine, then norvaline and isoleucine (Fig. 4). This order can be explained by the internal energies of aminoacyl-ade-

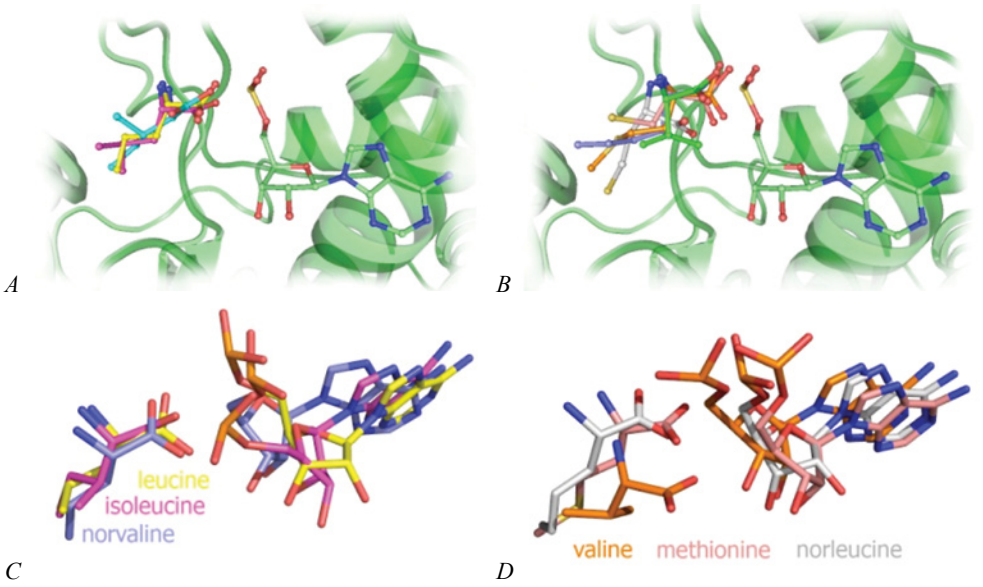


Fig.1. Docking results for Leu, Nva and Ile (*A*) and Hcys, Nleu, Met, Cys, Val (*B*) zwitterions in active site of LeuRSTT. There is a comparative alignment of average coordinate for last 3 ns of MD for Leu, Nva and Ile (*C*) and Val, Met, Nleu (*D*) in the active site and a disorder in the orientations of functional groups in AMP and amino acids.

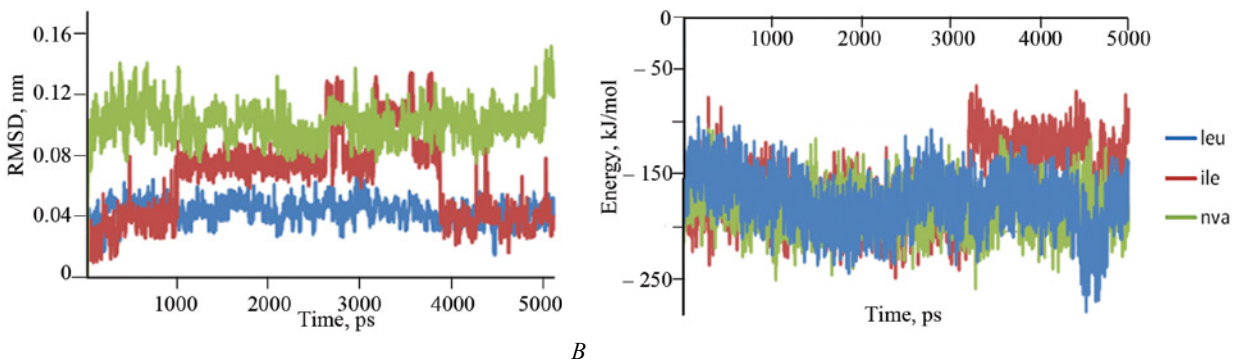


Fig. 2. Graph of RMSD calculated for amino acid substrates and values of electrostatic energy interactions between ligands and synthetase, calculated from 5ns MD.

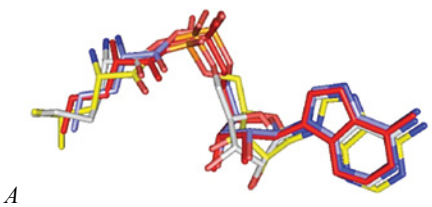
nylates that affect the conformation or shape of amino acid. In the case of isoleucine its rigid structure makes it very difficult to settle and fix itself in the site for a time, which would be sufficient to perform a transfer on CCA-end of tRNA. This fit well with the experimental data on isothermal titration calorimetry shown that K_d value for the binding of isoleucyl-adenylate to *E.coli* LeuRS was more than 8000-fold lover of that for leucyl-adenylate [9].

Table 1. **Result of the docking of amino acids in a site of LeuRS from T.thermophilus in the presence of AMP.**

Amino acid	GoldScore	ASP	Amino acid	GoldScore	ASP
leu	56.7	22.3	met	46.3	10.1
nva	52.2	15.3	cys	40.6	7.9
hcys	50.5	15.3	ile	44.1	6.4
nle	48.2	10.5	val	37.8	5.3

Table 2. **Result of the docking of aminoacyl-adenylates in a site of LeuRS from T.thermophilus.**

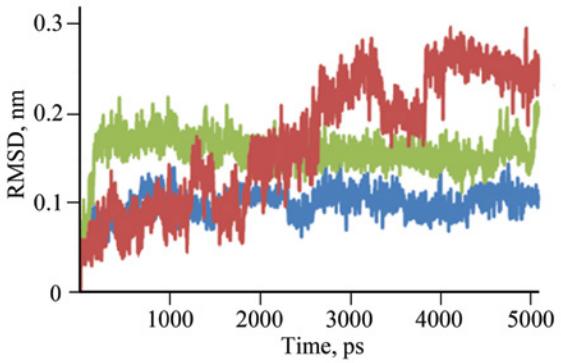
Amionacyl-adenylate	GoldScore	ASP	Amionacyl-adenylate	GoldScore	ASP
leu	94.3	44.6	met	90.6	40.8
hcys	95.9	43.3	ile	74.4	49
nva	84.4	40.8	val	70.9	41.6
nle	87	41.1	cys	72	35.1



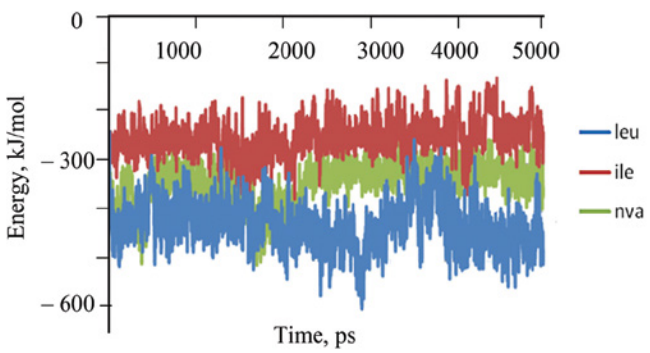
A

B

Fig. 3. *A*– Crystal structures of analog of norvalyladenylate (red) leucyl- (yellow) homocysteyil- (gray) norvalyl- (blue). *B* – Crystal structure of analog of norvalyladenylate (red) cysteyil- (green) metionil- (pink) valil- (orange) isoleucyl- (green).

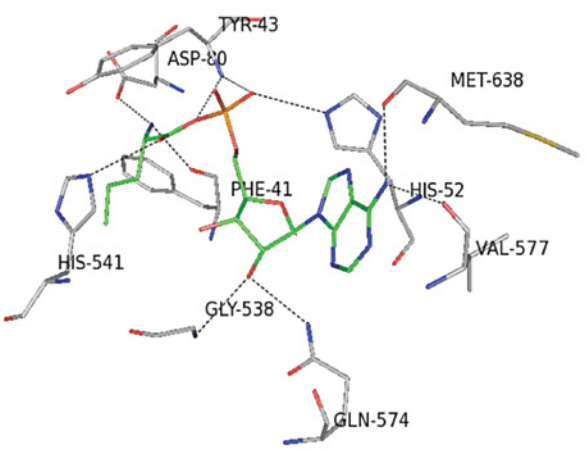


A



B

Fig. 4. Graphs of RMSD *A* – which was designed for amionoacyl-adenylates, and fluctuations in electrostatic energy of interaction between ligands and synthetase during 5 ns of MD.



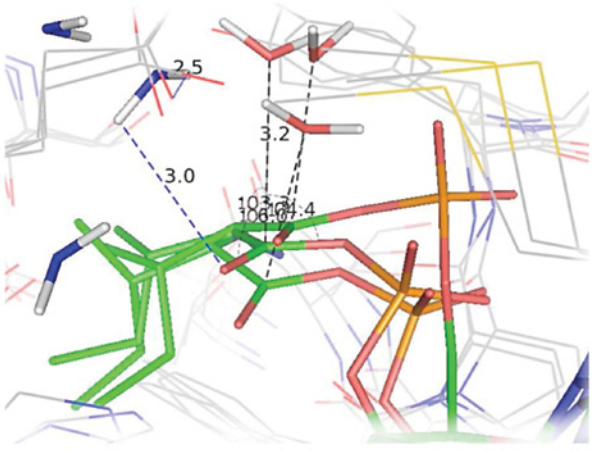


Fig. 5. The figure to the left shows the contacts that form amino acid part of norvalyl-adenylate with its environment. From right hand three frames of MD of isoleucyl-adenylate are imposed, clearly showing that the carboxyl carbon is exposure to water molecules (red) that can form contacts with it.

It should be noted that a tension that occurs at the end of an amino acid radical can be transmitted to a phosphate group and a ribose ring, as it was shown after docking (Fig. 3) and in the graph of RMSD (Fig. 4 *A*). In this case, a rather electronegative phosphate group is not able to make a significant contribution to the stabilization of the substrate by means of electrostatic interaction. Interestingly, after the formation of aminoacyl-adenylate such amino acids as cysteine and valine have become less mobile, because of the rigid ether bond with a phosphate group and the hydrogen bond formed between the amino group of the amino

acids and ASP80 (Fig.5*A*). Although for such amino acids as methionine, norleucine and homocysteine the reduction of degree of freedom and partial charge redistribution influenced in a way that, despite the decline in mobility, a value of electrostatic interaction energy decreased. Presumably, the amino acids with long radical faced with steric obstacles of the site.

Quite interesting is the fact that the TYR43 of the active site covers the carboxyl group of leucine and norvaline like a shield and deflected towards near isoleucine. This deflect allows the water molecules to come closer and act as a nucleophile in the hydrolysis of isoleucyl-adenylate (Fig. 5). Note, TYR43 together withPRO42 forms a conserved motif PY, found in all leucyl, valyl- and isoleucyl-tRNA synthetases. Our finding fit well to the recent date which have shown that substitution of highly conserved tyrosine with threonine within the synthetic site of IleRS modulates both editing and aminoacylation [24].

Conclusions

To study the structural bases for the amino acid selectivity of the LeuRSTT aminoacylation site and to understand the binding pattern of pre-transfer substrates we used the molecular docking and molecular dynamics simulation approaches. We revealed some structural basis for screening of unfavorable substrates by shape, size and flexibility of a radical. Using at the first step the two fold docking function analysis of amino acids and AMP we have obtained data on conformation of the ligands which well correlate with the experimental results. The MD simulation data revealed that the differences in a rigid scaffold of isoleucine and its large surface area result in more low electrostatic interactions with the active site of the enzyme compared with those for norvaline and leucine. The experimental and our data indicate that norvaline as well as leucine are much more acceptable substrates in the activation reaction as compared to isoleucine. Further study on the aminoacyl-adenylates showed that the difficulty in some amino acids activation can be caused by two factors: the excessive flexibility due to the amino acid size or structure and quick hydrolysis of the intermediate substrate with nucleophilic attack by the water molecules. The first one refers to the small cysteine or valine residues and the second one can be explained by the movement of TYR43 in response to the internal stress of some aminoacyl-adenylates.

REFERENCES

1. *Ibba M, Soll D.* Aminoacyl-tRNA synthesis. *Annu Rev Biochem.* 2000;**69**:617–50.
2. *Cusack S, Berthet-Colominas C, Härtlein M, Nassar N, Leberman R.* A second class of synthetase structure revealed by X-ray analysis of Escherichia coli seryl-tRNA synthetase at 2.*5 A*. Nature. 1990;**347**(6290):249–55.
3. *Eriani G, Delarue M, Poch O, Gangloff J, Moras D.* Partition of tRNA synthetases into two classes based on mutually exclusive sets of sequence motifs. *Nature.* 1990;**347**(6289):203–6.
4. *Yadavalli SS, Ibba M.* Quality control in aminoacyl-tRNA synthesis its role in translational fidelity. *Adv Protein Chem Struct Biol.* 2012;**86**:1–43.
5. *Fersht AR.* Editing mechanisms in protein synthesis. *Rejection of valine by the isoleucyl-tRNA synthetase.* Biochemistry. 1977;**16**(5):1025–30.
6. *Cusack S, Yaremchuk A, Tukalo M.* The 2 A crystal structure of leucyl-tRNA synthetase and its complex with a leucyl-adenylate analogue. *EMBO J.* 2000;**19**(10):2351–61.
7. *Martinis SA, Fox GE.* Non-standard amino acid recognition by Escherichia coli leucyl-tRNA synthetase. *Nucleic Acids Symp Ser.* 1997;**36**:125–8.
8. *Zhu B, Yao P, Tan M, Eriani G, Wang ED.* tRNA-independent pretransfer editing by class I leucyl-tRNA synthetase. *J Biol Chem.* 2009;**284**(6):3418–24.
9. *Cvetesic N, Palencia A, Halasz I, Cusack S, Gruic-Sovulj I.* The physiological target for LeuRS translational quality control is norvaline. *EMBO J.* 2014;**33**(15):1639–53.
10. *Lincecum TL Jr, Tukalo M, Yaremchuk A, Mursinna RS, Williams AM, Sproat BS, Van Den Eynde W, Link A, Van Calenbergh S, Grøtli M, Martinis SA, Cusack S.* Structural and mechanistic basis of pre- and posttransfer editing by leucyl-tRNA synthetase. *Mol Cell.* 2003;**11**(4):951–63.
11. *Dulic M, Cvetesic N, Perona JJ, Gruic-Sovulj I.* Partitioning of tRNA-dependent editing between pre- and post-transfer pathways in class I aminoacyl-tRNA synthetases. *J Biol Chem.* 2010;**285**(31):23799–809.
12. *Boniecki MT, Vu MT, Betha AK, Martinis SA.* CP1-dependent partitioning of pretransfer and posttransfer editing in leucyl-tRNA synthetase. *Proc Natl Acad Sci USA.* 2008;**105**(49):19223–8.
13. *Zhai Y, Martinis SA.* Two conserved threonines collaborate in the Escherichia coli leucyl-tRNA synthetase amino acid editing mechanism. *Biochemistry.* 2005;**44**(47):15437–43.

14. *Tan M, Zhu B, Zhou XL, He R, Chen X, Eriani G, Wang ED.* tRNA-dependent pre-transfer editing by prokaryotic leucyl-tRNA synthetase. *J Biol Chem.* 2010;**285**(5):3235–44.
15. *Case DA, Cheatham TE 3rd, Darden T, Gohlke H, Luo R, Merz KM Jr, Onufriev A, Simmerling C, Wang B, Woods RJ.* The Amber biomolecular simulation programs. *J Comput Chem.* 2005;**26**(16):1668–88.
16. *Humphrey W, Dalke A, Schulten K.* VMD: visual molecular dynamics. *J Mol Graph.* 1996;**14**(1):33–8, 27–8.
17. *Jones G, Willett P, Glen RC, Leach AR, Taylor R.* Development and validation of a genetic algorithm for flexible docking. *J Mol Biol.* 1997;**267**(3):727–48.
18. *Pronk S, Páll S, Schulz R, Larsson P, Bjelkmar P, Apostolov R, Shirts MR, Smith JC, Kasson PM, van der Spoel D, Hess B, Lindahl E.* GROMACS 4.*5: a high-throughput and highly parallel open source molecular simulation toolkit.* Bioinformatics. 2013;**29**(7):845–54.
19. *Salnikov AO, Sliusar IA, Sudakov OO, Savytskyi OV, Kornelyuk AI.* MolDynGrid virtual laboratory as a part of Ukrainian Academic Grid infrastructure. *2009 IEEE International Workshop on Intelligent Data Acquisition and Advanced Computing Systems: Technology and Applications.* 2009; **237–40.**
20. *Páll S, Abraham MJ, Kutzner C, Hess B, Lindahl E.* Tackling exascale software challenges in molecular dynamics simulations with GROMACS. *Solving Software Challenges for Exascale.* 2015; **8759**:3–27.
21. *Hornak V, Abel R, Okur A, Strockbine B, Roitberg A, Simmerling C.* Comparison of multiple Amber force fields and development of improved protein backbone parameters. *Proteins.* 2006;**65**(3):712–25.
22. *Jorgensen WL, Chandrasekhar J, Madura JD, Impey RW, Klein ML.* Comparison of simple potential functions for simulating liquid water. *J Chem Phys.* 1983;**79**(2):926–35.
23. *Berendsen HJC,van der Spoel D,van Drunen R.* GROMACS: A message-passing parallel molecular dynamics implementation. *Comput Phys Commun.* 1995; **91**(1–2):43–56.
24. *Dulic M, Perona JJ, Gruic-Sovulj I.* Determinants for tRNA-dependent pretransfer editing in the synthetic site of isoleucyl-tRNA synthetase. *Biochemistry.* 2014;**53**(39):6189–98.

Вивчення комплексів лейцил-тРНК синтетази *Thermus thermophilus* з різними амінокислотами та субстратами пре-трансферного редагування методами молекулярного докінгу і молекулярної динаміки

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Мета. Вивчення структурних особливостей, що забезпечують селективність аміноацилюючого сайту лейцил-тРНК синтетази із *Thermus thermophilus* (LeuRSTT) по відношенню до амінокислот, і пояснення механізму зв'язування субстрату претрансферного редагувааня. **Методи.** Вісім амінокислот, запропонованих в якості субстратів аміноацилювання і вісім аміноациладенилатів (сформованих з АМФ і восьми амінокислот) були підготовлені у вигляді цвіттер-іонів. Кристалічна структура білка із субстратом в аміноацилюючому сайті [PDBID: 1OBH] була отримана із RCSB бази даних. Параметри докінгу та оцінка ефективності лиганда припускали дворазовий аналізу конформацій за допомогою пакету Gold CCDC. Моделювання молекулярної динаміки проводилось з використанням Gromacs. Процедури релаксації і детального дослідження були розділені на два послідовних моделювання тривалістю 50ns і 5ns. **Результати.** Оцінка ефективності зв'язування 8 амінокислот була визначена завдяки аналізу на основі двох оціночних функцій і показала, що ліганди LeuRSTT за цими властивостями можна розташувати в наступному порядку: Leu> Nva> Hcy> Nle> Met> Cys> Ile > Val. МД показала нижчі електростатичні взаємодії ізолейцину з активним сайтом ферменту у порівнянні з норваліном і лейцином. У випадку аміноациладенилатів істотних відмінностей не було знайдено. Молекулярна динаміка лейцил-, ізолейцил- і норваліладенилату показала, що найбільш стабільним і конформаційно вигідним субстратом є лейцин, а не норвалін і ізолейцин. Крім того, було виявлено, що TYR43 в активному сайті екранує карбоксильні групи лейцину і норваліну і відхиляється убік в присутності ізолейцина, дозволяючи молекулі води наблизитися. **Висновки.** В цьому дослідженні ми виявили деякі причини відбору субстратів в залежності від форми, розміру і гнучкості радикала. **Результати** для різних амінокислот, отримані за допомогою молекулярного докінгу і МД, добре корелюють з експериментальними даними по першому етапу аміноацилювання. МД аміноациладенилатів показала, що складність в активації деяких амінокислот може бути викликана двома причинами: надмірною гнучкістю через розмір радикалу або структуру і швидким гідролізом проміжного субстрату під час нуклеофільної атаки молекулами води.

Ключові слова: лейцил–tRNA синтетаза, редагування, амінокислоти, аміноацил-аденилат, молекулярний докінг, МД

Изучение комплексов лейцил-тРНК синтетазы *Thermus thermophiles* с различными аминокислотами и субстратами пре-трансферного редактирования методами молекулярного докинга и молекулярной динамики

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Цель. Изучение структурных особенностей, обеспечивающих селективность аминоацилирующего сайта лейцил-тРНК синтетазы *Thermus thermophilus* (LeuRSTT) в отношении связывания аминокислоты, и механизма связывания субстрата пре-трансферного редактирования. **Методы.** Восемь аминокислоты предложенных в качестве родственных субстратов аминоацилирования и восемь аминоациладенилатов (сформированых из АМФ и восьми аминокислот) были подготовлены в виде цвиттер-ионов. Кристаллическая структура белка в комплексе с субстратом в аминоацилирующем сайте [PDBID:

1OBH] была взята из базы данных RCSB. Параметры докинга и оценка эффективности лиганда предполагали двукратный анализ каждой конформации при помощи Gold CCDC. Моделирование молекулярной динамики проводилось с использованием Gromacs. Этап релаксации и, собственно, изучение связывания были разделены на два последовательных моделирования по 50ns и 5ns. **Результаты.** Оценка эффективности связывания 8 аминокислот проводилась на основе значений двух оценочных функций и показала, что лиганды можно расположить по активности в следующем порядке: Leu> Nva> Hcy> Nle> Met> Cys> Ile > Val. МД симуляции показали более низкие электростатические взаимодействия изолейцина с активным сайтом фермента по сравнению с таковыми для норвалина и лейцина. В случае аминоациладенилатов существенных различий не было обнаружено. МД лейцил-, изолейцил- и норвалиладенилата показала, что наиболее стабильной и благоприятной является конформация лейцина, а не норвалина и изолейцина. Кроме того, было обнаружено, что TYR43 активного сайта экранирует карбоксильную группу лейцина и норвалина, но отклоняется в сторону в присутствии изолейцин, позволяя молекуле воды приблизиться. **Выводы.** В этом исследовании мы описали некоторые причины отсева различных по форме, размеру и гибкости радикала субстратов. **Результаты** для различных аминокислот, полученные в результате молекулярного докинга и МД симуляций, хорошо коррелируют с экспериментальными данными первого этапа аминоацилирования. Отдельное изучение аминоациладенилатов показало, что сложность активации некоторых аминокислот может быть вызвана двумя причинами: чрезмерной гибкостью, в связи с размером или формой радикала, и быстрым гидролизом промежуточного субстрата в момент нуклеофильной атаки молекулами воды.

Ключевые слова: лейцил–tRNA синтетаза, редактирование, аминокислоты, аминоацил-аденилат, молекулярный докинг, МД

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