Identification of the nucleophilic factors and the productive complex for the editing reaction by leucyl-tRNA synthetase

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Keywords: Error-editing reaction; Nucleophile; Water; Molecular dynamics simulation; QM/MM hybrid calculation.

Abbreviations: aaRS, aminoacyl-tRNA synthetase; LeuRS, leucyl-tRNA synthetase; ValRS, valyl-tRNA synthetase; PheRS, phenylalanyl-tRNA synthetase; ThrRS, threonyl-tRNA synthetase; MD, molecular dynamics; CP1, connective polypeptide 1; QM/MM, quantum mechanics / molecular mechanics; ES complex, enzyme•substrate complex.

Abstract

To ensure fidelity of translation, several aminoacyl-tRNA synthetases (aaRSs) possess editing capability to hydrolyse mis-aminoacylated tRNAs. In this report, based on our previously-modelled structure of leucyl-tRNA synthetase (LeuRS) complexed with valyl-tRNA^{Leu}, further structural modelling has been performed along with molecular dynamics simulations. This enabled the identification of the nucleophile, which is different from that suggested by the crystal structure of the LeuRS•Nva2AA complex. Our results revealed that the 3' hydroxyl group of A76 acts as a "gate" to regulate the accessibility of the nucleophile; thus, the opening of the gate leads to the productive complex for the reaction.

1. Introduction

Aminoacyl-tRNA synthetases (aaRSs) catalyse the attachment of their cognate amino acid to the 3'-end of the cognate tRNA (aminoacylation), through amino-acid activation via an aminoacyl-adenylate. The aaRSs are divided into two classes: the class I aaRSs are characterised by the nucleotide binding (Rossmann) fold, which contains the active site, whereas the active sites of the class II aaRSs comprise a characteristic seven-stranded β sheet with flanking α helices [1, 2]. The fidelity of translation is ensured by their strict discrimination of the cognate amino acids from the non-cognate ones. However, several enzymes, such as isoleucyl, valyl-, and leucyl-tRNA synthetases, denoted as IleRS, ValRS, and LeuRS, respectively, belong to a subclass, i.e., class Ia, and they have difficulties in discriminating their cognate amino acids. This is caused by the similar chemical structures of the three amino acids, and thus misactivated amino acids or misaminoacylated tRNAs are produced [3–11]. In fact, when isoleucine is replaced with valine in the IleRS system, the error rate is approximately 1 in 5 [12]. To avoid such incorrect products, these enzymes catalyse editing reactions by hydrolysing mis-products [3–11]. Two such reactions have been found: pre-transfer editing and post-transfer editing. Through pre-transfer editing, a misactivated amino acid is hydrolysed to the amino acid and AMP, whereas through post-transfer editing, the

misaminoacylated tRNA is hydrolysed to the amino acid and tRNA. In this way, the overall error rate in the above-mentioned isoleucine system is reduced to only 1 in 40,000 [13].

With respect to LeuRS, the crystal structures of the enzyme complexed with pre- and post-transfer editing inhibitors have been determined [4]. However, whether *Escherichia coli* LeuRS possesses the pre-transfer editing activity is apparently masked by a dominant post-transfer editing activity [14-16]. Quite recent experiments have indicated the presence of this activity in *Aquifex aeolicus* LeuRS [17], and this organism definitely possesses the post-transfer editing activity [4, 9, 10]. In this study, we thus have focussed on the post-transfer editing in LeuRS. The crystal structure of the *Thermus thermophilus* LeuRS complexed with $tRNA^{Leu}$ has been determined. In this structure, the 3' terminus of $tRNA^{Leu}$ is bound to the editing reaction active site located in the connective polypeptide 1 (CP1) domain, which interrupts the Rossmann-fold catalytic domain [18]. However, the structure does not include information essential for elucidating the mechanisms of the editing reaction by LeuRS: (i) a substrate is not included, i.e., no amino acid is attached to the terminus of the $tRNA^{Leu}$, and (ii) no crystallographic water molecules have been resolved. In fact, the detailed mechanisms of the catalysis have not yet been elucidated, even though biochemical analyses, such as

mutagenesis studies on LeuRS, have been conducted on the basis of the crystal structures [4, 9, 10]. Accordingly, in our previous study we constructed a fully-solvated structural model of the complex of the *Thermus thermophilus* LeuRS and misaminocylated (valyl) $tRNA^{Leu}$ [19].

In the present study, to identify the nucleophilic factors in the post-transfer editing reaction, we investigated the hydrated structures relevant to the editing reaction by performing further structural modelling with the use of our previously constructed model of the LeuRS•valyl-$tRNA^{Leu}$ complex. This led to the identification of the productive structure for the editing reaction by LeuRS.

2. Materials and methods

2. 1. Model construction of LeuRS complexed with misaminoacylated $tRNA^{Leu}$

We previously constructed a modelled structure of LeuRS complexed with valyl-$tRNA^{Leu}$, which was used as the initial structure for the MD simulations described below [19]. The model construction was performed using the crystal structure of *Thermus thermophilus* LeuRS complexed with $tRNA^{Leu}$, in which the 3′-terminus is bound to the editing active site (PDB accession code 2BYT [18]). The computational details of the structural modelling are described in Ref. 19.

2. 2. MD simulations

MD simulations were performed using the AMBER 9 program [20]. The parm99 force field was applied to the atoms of LeuRS and $tRNA^{Leu}$ [21], and the TIP3P model was used for the solvent water molecules. Electrostatic interactions were calculated by the particle-mesh Ewald (PME) method, using 1.0 as the dielectric constant [22]. A cut-off of 12 Å was used to calculate the direct space sum for PME; the electrostatic interactions beyond 12 Å were calculated in reciprocal space by the fast Fourier transform method. Thus, all electrostatic interactions between atoms were calculated. The SHAKE algorithm was used to restrain the bond lengths involving hydrogen atoms [23]. The time step for integration was set to 1 fs. The temperature and pressure were set using the Berendsen algorithm [24].

The initial coordinates used for the present MD simulations were from the modelled structure previously constructed for LeuRS complexed with valyl-$tRNA^{Leu}$ [19]. For the present system, we immersed the complex in a solvent box comprising 49,587 water molecules, and used the periodic boundary condition where the size of the unit box was $103.0 \times 138.3 \times 117.1$ $Å^3$. The total number of atoms in the system was 165,739. In the initial (relaxation) phase of the MD simulation, the water molecules were relaxed at 300 K for 10 ps, while the atoms of the protein and $tRNA^{Leu}$ were positionally

constrained by a harmonic function using a force constant of 500 kcal/mol·Å^2. The force constant was then reduced to 250, 125, 50, 25, 10 and 5 kcal/mol·Å^2 in six MD simulations. The time consumed by each simulation was 2 ps. A free dynamics simulation was subsequently performed at 300 K for 1 ns.

This equilibrated system was used for further structural modelling to investigate the hydrated structure relevant to the editing reaction, in which 1 ns MD simulations were performed in the presence of constraints to effectively explore the states. First, with respect to the atomic distance between the carbonyl carbon of the substrate and the oxygen atom of the identified nucleophilic water, a constrained MD simulation was performed to investigate the mechanisms of the approach of the water molecule. This simulation was started by using a harmonic potential as the distance constraint, where the force constant was set to 5 kcal/mol·Å^2. When the atomic distance was not reduced any further, despite the presence of the harmonic potential in the MD simulation (at about 470 ps), the force constant was increased up to 200 kcal/ mol·Å^2; this was exploited to mimic the first phase of a nucleophilic attack in the MD simulations, which enabled us to explore larger conformational spaces than with the first principles MD simulations. A similar protocol was applied in other constrained MD simulations for efficient explorations of conformational spaces. To obtain a PMF with respect to the

rotation of the dihedral angle, C4'–C3'–O3'–HO3', we employed the umbrella sampling technique, using fourteen windows in the range of 50 to 180 degrees. Umbrella sampling is a theoretical technique to efficiently search for not only energetically-favorable but also energetically-unfavorable conformations in a phase space of a system, combined with a bias potential (e.g. a harmonic potential) to overcome energy barriers. The force constants of the umbrella potential in those windows were set to 10.0 kcal/mol·rad^2.

The structures obtained using MD simulations were evaluated by exploiting quantum mechanics (QM) / molecular mechanics (MM) hybrid calculations in terms of the stability and reactivity. For this purpose, our interface program [25] was used to connect QM (gamess [26]) and MM (amber [20]) engines. The detailed computational procedures are described in Supplementary data (S2).

3. Results and discussion

3. 1. Identification of the nucleophile

In our previous study, to construct a structure of the LeuRS•valyl-tRNA^{Leu} complex, we performed an MD simulation for 500 ps in the last phase of the modelling scheme to equilibrate the system, and discovered that the catalytic site for the editing contains five

ordered water molecules. In fact, in the 500 ps MD simulation, these water molecules were very stable; the hydrogen bond networks formed between the interfacial ordered water molecules and the complex were completely preserved in the MD simulation [19]. In the present modelling study, to further confirm the structural stability of the complex, we first extended the MD simulation up to 1 ns, and again found that these ordered water molecules are stably bound to the catalytic site. The above-mentioned hydrogen bond networks are also fully maintained in the present MD simulation (Fig. 1a). Moreover, the RMSDs of the LeuRS•valyl-$tRNA^{Leu}$ complex with respect to the crystal structure of the LeuRS•$tRNA^{Leu}$ complex also show that the modelled structure is quite stable (Fig. 1b). Accordingly, in this study, we utilized the 1 ns snapshot of the MD simulation as the starting structure for subsequent analyses.

Among the five ordered water molecules identified in the interfacial regions around the catalytic site for the editing, one molecule, which is hydrogen-bonded to the 3′-hydroxyl group of A76 (3′-HO), was found to be closest to the carbonyl carbon of the valine attached in the scissile bond. The oxygen atom of this water is hydrogen-bonded to the hydrogen atom (which is referred to as 3'-HO here) of the 3'-hydroxyl group. The atomic distance is 3.4 Å (Fig. 2b), suggesting that this water molecule acts as a nucleophile in the post-transfer editing reaction (this configuration is referred to as State

1). In contrast, in the crystal structure of the complex with an inhibitor, 2′-(L-norvalyl) amino-2′-deoxyadenosine (Nva2AA) (PDB code: 1OBC), another water molecule, which is hydrogen-bonded to Asp344, was closest to the carbonyl carbon of the scissile bond in the editing reaction (Fig. 2a; the atomic distance is 3.95 Å) [4]. However, this water molecule is in an unfavourable position for a nucleophilic attack. In the crystal structure, the O_w, C, and O atoms defined in Fig. 2a are almost linear (the O_w–C–O angle is 160.6°), whereas the favourable angle for the reaction is 90°. In addition, a mutagenesis study on Asp342 of the *Escherichia coli* LeuRS (which corresponds to Asp344 in the *Thermus thermophilus* system) revealed that it does not affect the editing activity (3). On the other hand, in our modelled structure, the O_w–C vector is orthogonal to the plane defined by the O_w, C and O atoms (the O_w–C–O angle is 99.2°), as shown in Fig. 2b. Moreover, biochemical experiments indicated that the 3'-HO of A76 is important for the reaction (personal communication with M. Tukalo), in agreement with our computational results. The configurations of the ordered water molecule suggested by the X-ray crystallography and the MD simulation show discrepancies because of the difference in the chemical structures of the ligands, i.e., Nva2AA (used in the crystal structure) and the true substrate (used in the MD simulation); the 2' oxygen of the substrate is replaced with a nitrogen atom in Nva2AA (Figs. 1c and 1d). In the crystal

structure of the LeuRS•Nva2AA complex (1OBC), the closest water molecule can form a hydrogen bond with the hydrogen covalently bonded to the nitrogen at the 2' position (Fig. 2a). This allows the water molecule to be closer to the carbonyl carbon in the crystal structure.

Thus, we concluded that the water molecule hydrogen-bonded to the 3'-HO is the nucleophile. The trajectories of the O_w−C−O angle and the distances between the nucleophilic water and the three amino acid residues in its vicinity, i.e., Thr247, Thr248, and Asp344, show the high stability of the nucleophile (Figs. 3). This conclusion is further supported by the fact that such a water molecule is also present in other aaRSs that possess editing capability. In the crystal structures of a class Ia aaRS, the *Thermus thermophilus* IleRS [8], and a class IIa aaRS, the *Pyrococcus abyssi* ThrRS [27], the crystallographic water molecules hydrogen-bonded to the 3' and 2' hydroxyl groups, respectively, are located in positions favourable for nucleophilic attack, and are the closest to the C atom as compared to the other ordered water molecules present at the catalytic sites.

3. 2. Rotation of the 3'-HO leads to the productive complex

In this manner, we have identified the nucleophilic water, in good agreement with the available experimental results, as discussed earlier. However, the trajectory of the 1 ns MD simulation indicates that the averaged O_w–C distance, 3.4 Å, is still too long for a nucleophilic attack (Supplementary data S1; Fig. S1a), because the access of critical water is prevented by the 3'-HO, which forms a hydrogen bond with the nucleophilic water molecule (Fig. 2b). Based on these results, we performed further structural modelling to identify the productive structure in the editing reaction. For this purpose, we explored the mechanisms by which the water molecule could access the C atom of the carbonyl group, using MD simulations coupled with umbrella sampling techniques.

First, we performed a 1 ns MD simulation in which a distance constraint between the O_w and C atoms was imposed using a harmonic potential. In this MD simulation, the hydrogen bond between O_w and 3'-HO was not disrupted, and thus the water molecule could not approach the C atom (see Supplementary data S1; Fig S1b). This suggests that the rotation of the dihedral angle that includes the hydrogen atom, such as C4'–C3'–O3'–HO3', is necessary for the nucleophilic attack. It should be noted here that Thr248, which is well-conserved in the LeuRS, ValRS, and IleRS systems, is located near the 3'-OH. Based on these configurations, we performed another 1 ns MD simulation with constraints imposed on the dihedral angle, C4'–C3'–O3'–HO3', and on

the distance between the O_w and C atoms. As a result, the dihedral angle rotated, allowing the 3'-HO to form a hydrogen bond with the HO^{δ} of Thr248 (State 2). Moreover, water approaches the C atom, and the O_w–C distance is reduced to 2.4 Å (State 3). Instead of the disruption of the hydrogen bond between the 3'-HO and the O_w atom, a new hydrogen bond is formed between the 3' oxygen and a hydrogen atom of the nucleophilic water (Fig 2c; also see Supplementary data S1; Fig S1c).

Here, the difference in the degree of the accessibility of the nucleophile to the C atom is clearly demonstrated by comparing the distribution functions $g(r)$ of the O_w atom around the C atom, obtained in these two constrained MD simulations (Fig. 3). In the MD simulation where the 3'-HO forms the hydrogen bond with the nucleophile (State 1), the peak of the distribution function is observed at a distance around 3.0 Å, whereas in another MD simulation where the 3'-HO is rotated to form the hydrogen bond with the O^{δ} of Thr248 (State 2), the peak is shifted to shorter distance regions around 2.5 Å. In fact, the distribution functions of the O_w atom around the 3'-HO (Fig. 3) revealed that the access of the nucleophilic water is prevented by the presence of the hydrogen bond between the 3'-HO and the O_w atoms.

We then validated these conformational changes. To determine the rotation of the dihedral angle, we calculated the potential of mean force (PMF), using this dihedral

angle as the reaction coordinate (Fig. 4a). In the free energy profile, the minimum at about 70° of the dihedral angle corresponds to the state where the 3'-HO forms a hydrogen bond with the water molecule (State 1), whereas the other minimum, at about 170°, corresponds to the state where a hydrogen bond is formed between the 3'-HO and the HO^{δ} of Thr248 (State 2). The free energy of State 2 is greater than that of State 1 by 2.9 kcal/mol. The free energy barrier detected between the two minima was calculated as 3.7 kcal/mol, and is caused by the steric clash of the 3'-HO and H5' atoms of A76, and the amide hydrogen of the Thr247 backbone (Fig. 4b), indicating that the change in this dihedral angle can be driven by thermal fluctuations.

3.3. Evaluation by using QM/MM hybrid calculations

With respect to State 3 obtained by constrained MD simulations (Fig. 2c), we evaluated its stability by performing hybrid quantum mechanics (QM) / molecular mechanics (MM) calculations for the entire system of the solvated LeuRS•valyl-$tRNA^{Leu}$ complex. The calculation results suggested that its potential energy is comparable with that of State 2; the difference in the energy values is as small as 1.8 kcal/mol. The electronic structures of States 1 and 3 revealed that, in both states, the lowest unoccupied molecular orbital (LUMO) is localised on the carbonyl group of

the substrate, indicating that the C atom can accept electrons from the nucleophile (Fig. 6 and Fig. S2 in Supplementary data S2). With respect to the LUMO in State 3, the observed character of the antibonding orbital of the C–$O^{2'}$ bond suggests that the donation of electrons to the LUMO leads to cleavage of the bond (Fig. 6). Furthermore, in terms of the nucleophile, the energy difference between the LUMO and the molecular orbital (MO) where the contribution of *p*-orbitals of O_w is greatest significantly decreases through the changes from States 1 to 3 (see Fig. S2 in Supplementary data S2). This indicates that the nucleophilic attack is facilitated by the rotation of the dihedral angle and the formation of the hydrogen bond between 3' oxygen and the water, while the highest occupied molecular orbital (HOMO) is localised on the base moiety of the substrate (Fig. 6). Further approaches of the nucleophile would induce the elevation of the above-mentioned MO; i.e., the order of the MO would be raised up to the HOMO level, leading to the formation of a new bond between C and O_w (see Supplementary data S2 for more detailed discussions).

3.4. Thr248 acts as an opener of the H-gate

These evaluations confirm that this water molecule comes closer to the carbonyl group (State 3) by the formation of the hydrogen bond between the 3'-HO and HO^{δ}

(Thr248) atoms (State 2). However, the energy barrier between States 1 and 2 implies that the approach of the water molecule towards the C atom in the carbonyl group does not occur freely, but is limited by the 3'-HO, which is presumed to act as a "gate" (referred to as the "H-gate" here). In contrast, Thr248 (HO^{δ}) can open the H-gate, resulting in the formation of a hydrogen bond with the 3'-HO. These results show that the opened state of the H-gate (State 2) is the one followed by the approach of the nucleophile; thus, the opened state is supposed to be a productive enzyme•substrate (ES) complex, while the closed state, which does not allow the approach of the nucleophilic water, is non-productive.

It should be noted here that even though Thr248 is a highly conserved amino acid residue among the class Ia aaRSs, this threonine is replaced with valine in some organisms, such as the *Thermus thermophilus* and *Deinococcus radiodurans* ValRSs. The editing activity, k_{cat}/K_m, of the T248V mutant of the *Escherichia coli* LeuRS (the amino acid residue in *Escherichia coli* LeuRS corresponds to T248 in *Thermus thermophilus* LeuRS) was reduced by 6-fold [10]. This small reduction of the activity indicates that the nucleophile can actually approach the C atom of the substrate as well as the wild type (Fig. 2d). To elucidate the mechanisms of this change in the enzymatic activity, we further performed a 1 ns MD simulation for the complex in which the

wild-type LeuRS is replaced with the mutant T248V; the constraints for the O_w–C distance and the dihedral angle relevant to the 3'-HO were imposed in the MD simulation. As a result of this change, the 3'-HO of A76 hydrogen-bonds with a water molecule; this corresponds to State 2 of the wild-type LeuRS (the opened state of the H-gate), suggesting that this water molecule can act as the HO^{δ} of Thr248 (Fig. 2d). Thus, the water molecule can compensate for the functional role of threonine, thereby preserving the kinetic rate with only a slight reduction. In fact, the O_w can approach the C atom in this MD simulation of the mutant (see Supplementary data S1; Fig. S1d). However, the presence of the hydrophobic side-chain of the valine in the T248V mutant does not allow the water to reside at a sufficiently stable position to act as an opener of the H-gate. This may cause the slight reduction of the rate of the opened state of the H-gate (i.e., the probability of the productive ES complex), thus leading to a slight reduction in the accessibility of the nucleophile. In this manner, the apparently small reduction of the activity in T248V can be explained on the basis of the ratio of the productive/non-productive ES complexes.

3.5. Exploration of the reaction coordinates and the structural features for promoting the reaction

Our current investigations focussed on identifying the nucleophilic water molecule and the structural properties that lead to the productive complex essential for initiating the reaction (e.g., opening the H-gate). Here, we attempted to induce the water molecule to approach the C atom by using another scheme: in the MD simulation, a distance constraint between the 3′-HO and the Thr248 side chain (HO^{δ}) was imposed, using a force constant as small as 5.0 kcal/mol·Å^2. However, this constraint caused artifactual conformational changes of the Thr248 side chain: The hydrogen bond between 3′-HO and HO^{δ} was formed through the rotation of the dihedral angle; however, at the same time, the HO^{δ} of Thr248 also moved and approached the 3′-HO, leading to the artifactual structural transition of the Thr248 side chain, induced by forces acting on HO^{δ} derived from the distance constraint. Thus, from the technical aspect of the simulations, it should be noted here that to induce the conformational changes of the 3′-HO, such a distance constraint is not appropriate as a bias function for umbrella sampling. This information might be useful for future analyses, such as those for the estimation of the energy barrier of the enzymatic reaction using quantum chemistry calculations.

For the accurate estimation of energy barriers in the catalytic reaction, such careful structural analyses prior to the estimation are crucial, to provide an appropriate initial

structure and the proper reaction coordinates. Both of these have serious effects on the results of the estimation of activation barriers, even if one uses state-of-the-art methodologies. In the absence of the proper structural configurations and the reaction coordinates for initiating and promoting the catalysis, artifactual reaction pathways, some with similar activation barriers, are selected in the simulations. Actually, such confusion induced by distinct catalytic mechanisms or pathways with similar activation barriers was previously found in computational analyses of other enzymatic reactions, such as DNA polymerase β catalysis [28–33]. Even sophisticated methodologies would not always yield the correct results without critical information, such as a set of reaction coordinates (further discussions are provided in Supplementary data S3).

Therefore, the information on the structural features obtained by the present modelling is indispensable in subsequent analyses. In fact, the distance constraint between the 3′-HO and HO^{δ} (Thr248) atoms led to artifactual conformations of the Thr248 side chain, as discussed. In contrast, the alternative constraint with respect to the rotation of the dihedral angle relevant to the 3′-HO led to the identification of the nucleophilic attack by a water molecule. This suggests once again that the present modelling provides a solid basis for further investigations to elucidate the detailed mechanisms of the editing reaction.

3.6. Insights into the other aaRS systems

Our results indicated that the opening of the H-gate leads to the formation of the productive ES complex, which precedes the approach of the nucleophile. Accordingly, the lower stability of the ES complex limits the access of the nucleophile to the C atom, leading to the reduction of the catalytic activity. In fact, the proposed mechanism aptly explains the experimental data with respect to the mutant, T248V. The importance of the 2'- or 3'-HO of A76 has been identified in other aaRSs possessing the capability of editing beyond classes I and II (IleRS and ValRS in class Ia and two enzymes, ThrRS and PheRS, which belong to classes IIa and IIc, respectively) [11, 27, 34]. Furthermore, in the crystal structures of *Thermus thermophilus* IleRS [8] and *Pyrococcus abyssi* ThrRS [27], the crystallographic water molecules closest to the C atoms are ones which hydrogen-bond to the 3' and 2' hydroxyl groups, respectively. Thus, these experimental data suggest that the mechanism propounded by this study is shared among these aaRSs; i.e., the productive complex may be established through the opening of the H-gate, leading to a nucleophilic attack and the formation of the O_w−C bond by the mechanism discussed earlier. Thus, our results could serve as a platform for devising further experimental and theoretical investigations of the editing reactions of these aaRSs.

Acknowledgements

This work was partly supported by grants-in-aid from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), under contract Nos. 19019003, 19340108 and 20051003. Computations were performed using computer facilities under the 'Interdisciplinary Computational Science Program' at the Center for Computational Sciences, University of Tsukuba, the Computer Center for Agriculture, Forestry, and Fisheries Research, MAFF, Japan, and the facilities of the Supercomputer Center, Institute for Solid State Physics, University of Tokyo.

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Figure Legends

Figure 1. (a) Modelled structure of the LeuRS•valyl-tRNA^{Leu} complex. The entire structure of the complex (left panel) and the catalytic site for the editing (right panel) are depicted. The snapshot at 1 ns was extracted from the present MD trajectory and was used to render the figure. (b) RMSDs of the backbone and heavy atoms of the protein, and RNA moieties of the LeuRS•valyl-tRNA^{Leu} complex are represented in red and black, respectively. In the calculations of RMSDs, part of tRNA^{Leu} including the anti-codon loop, i.e., G30−A40, which is not recognised by LeuRS and is expected to have extremely high fluctuations, was excluded (in fact, for the excluded region, the B-factor values determined by X-ray crystallography are over 150 (11)). (c) (a) Chemical structure of a substrate for the editing by LeuRS. (b) Chemical structure of the inhibitor, Nva2AA, used in the crystal structure of a LeuRS•Nva2AA complex (PDB code: 1OBC).

Figure 2. (a) (left) Schematic representation of a configuration of the catalytic site for the editing reaction that is observed in the crystal structure of a LeuRS•Nva2AA complex in the absence of tRNA^{Leu} (PDB code: 1OBC). (right) The crystal structure

corresponding to the left panel. (b) (left) Schematic representation of a configuration where a nucleophilic water perpendicularly accesses the C atom of the carbonyl group of the substrate in the modelled structure of the complex of LeuRS and valyl-tRNA^{Leu} (State 1; see the text). (right) An MD snapshot corresponding to the left panel. (c) (left) Schematic representation of a catalytic configuration where the dihedral angle, defined as C4'–C3'–O3'–3'HO, is rotated by about 100 degrees in the modelled structure of the complex of LeuRS and valyl-tRNA^{Leu}, leading the nucleophilic water to approach the C atom (State 3; see the text). (right) An MD snapshot corresponding to the left panel. (d) (left) Schematic representation of a catalytic configuration in the MD simulation of the complex, using the T248V mutant of LeuRS. (right) An MD snapshot corresponding to the left panel.

Figure 3. (a) The trajectory of the O_w−C−O angle. The vertical axis shows the time (ps), and the equatorial axis shows the angle (degrees). (b) The trajectories of the distances between O_w and C_αs of Thr247, Thr248, and Asp344, respectively. The trajectory of O_w−C_α of Thr247 is coloured blue, that of O_w−C_α of Thr248 is coloured red, and that of O_w−C_α of Asp344 is coloured green. The vertical axis shows the time (ps), and the equatorial axis shows the distances (Å).

Figure 4. (a) The distribution function *g*(r) around the C atom obtained by the MD simulation where the H-gate is opened (closed) is coloured red (blue). (b) The *g*(r) around the hydrogen atom of the 3' hydroxyl group of A76 (3'-HO). The manner of the colour representation is identical to (a).

Figure 5. (a) Free energy profile of the rotation of the dihedral angle C4'–C3'–O3'–3'HO. Vertical and horizontal axes represent free energy (kcal/mol) and the dihedral angle (degrees), respectively. (b) Stereo view of the conformation of the transition state obtained in the calculated free energy profile.

Figure 6. (left) Schematic representation of State 3 where the H-gate is opened. (right) Stereo views of LUMO, which is localised on the carbonyl group of the valine moiety, and HOMO, which is localised on the base moiety.