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Molecular dynamics simulation study of valyl-tRNA synthetase with its pre- and post-transfer editing substrates

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ABSTRACT

The main role of aminoacyl-tRNA synthetases (aaRSs) is to transfer the cognate amino acids to the 3′-end of their tRNA by strictly discriminating from non-cognate amino acids. Some aaRSs accomplish this via proofreading and editing mechanisms, among which valyl-tRNA synthetase (ValRS) hydrolyses the non-cognate amino acid, threonine. In ValRS, existence of pre-transfer editing process is still unclear, although crystal structure of editing site with pre-transfer substrate analog (Thr-AMS) was released. In the case of isoleucyl-tRNA synthetase (IleRS), editing mechanism is well studied and mutational analyses revealed the existence of post- and pre-transfer editing mechanisms. Our aim is to investigate the possibility of pre-transfer editing process by performing molecular dynamics (MD) simulation studies. Simulations were carried out for ValRS with pre-transfer substrates (Thr-AMP/Val-AMP) and post-transfer substrates (Thr-A76/Val-A76) to understand their binding pattern. Two important point mutation studies were performed to observe their effect on editing process. This study also intends to compare and contrast the pre-transfer editing with post-transfer editing of ValRS. Interestingly, the MD simulation results revealed that non-cognate substrates (Thr-AMP/Thr-A76) bind more strongly than the cognate substrates (Val-AMP/Val-A76) in both pre- and post-transfer editing respectively. The editing site mutations (Lys270Ala and Asp279Ala) severely affected the binding ability of pre-transfer substrate (Thr-AMP) by different ways. Even though pre- and post-transfer substrates bind to the same site, specific differences were observed which has led us to believe the existence of the pre-transfer editing process in ValRS.

1. Introduction

The fidelity of protein synthesis is guaranteed by the specific recognition of amino acids and tRNAs by aminoacyl-tRNA synthetases (aaRSs) [1–3]. The aaRSs catalyze the specific attachment of their cognate amino acid to the 3′ end of their cognate tRNA. The aminoacylation of tRNA proceeds in two steps: first, the synthesis of an aminoacyl-adenylate, as an active intermediate, from the amino acid and adenosine tri-phosphate (ATP), and second the transfer of the aminoacyl moiety from the adenylate to the 3′-terminal adenosine of tRNA [4]. However, the affinity difference is not large enough for the enzyme to discriminate strictly between similar and non-cognate amino acids from the cognate one [5]. Some aaRSs must discriminate their cognate amino acid from non-cognate ones. For example, isoleucyl-tRNA synthetase (IleRS) should discriminate the cognate isoleucine from the non-cognate valine, which differs by only one methylene group [6–8]. Valyl-tRNA synthetase (ValRS) should discriminate valine from threonine, which has a quite similar shape and size as valine with a hydroxyl group in its side chain instead of a methyl group [9]. Other aaRSs, such as the leucyl-, threonyl- and alanyl-tRNA synthetases also have editing activities [10–12].

To maintain high translational fidelity, these aaRSs catalyze proofreading (editing) reactions in which the mis-products are hydrolyzed. This editing reaction is carried out in the domain named as editing (or CP1) domain which is located more than 30 Å away from the aminoacylation site [13–22].

The editing reaction of ValRS comprises of two pathways depicted as follows [19]:

$$\mathrm{tRNA^{Val}.ValRS.Thr\text{-}AMP} \longrightarrow \mathrm{Thr\text{-}tRNA^{Val}.ValRS + AMP}$$
$$\downarrow$$
$$\mathrm{tRNA^{Val}.ValRS + L\text{-}threonine} \quad [1]\ \text{(Post-transfer editing)}$$

$$\mathrm{tRNA^{Val}.ValRS.Thr\text{-}AMP} \longrightarrow \mathrm{tRNA^{Val}.ValRS + L\text{-}threonine + AMP} \quad [2]$$
(Pre-transfer editing)

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In ValRS post-transfer editing, the threonine is cleared after being transferred to the 3′ end of $tRNA^{Val}$ (Thr-$tRNA^{Val}$ hydrolysis). However, in pre-transfer editing, the ATP-activated threonine is removed before being transferred to the 3′ end of $tRNA^{Val}$(Thr-AMP hydrolysis) [19,23], but still pre-transfer editing mechanism is not yet confirmed in ValRS. In IleRS, structure-based mutational experiments suggested the existence of two proximal, but distinct binding pockets in the editing domain for the pre- and post-transfer editing substrates [19,24]. IleRS reportedly performs editing reactions mainly (80–90%) via the pre-transfer pathway [7]. On the other hand, ValRS rejects a non-cognate amino acid, threonine, mainly by the post-transfer pathway [4,25], and whether ValRS also catalyzes the pre-transfer editing is still unknown. Recent mutational studies on ValRS pre-transfer editing has also not confirmed pre-transfer editing existence, but stated that the editing site may have been designed not only for the post-transfer editing but also for the pre-transfer editing [23]. In ValRS there may be a single binding site for pre-transfer and post-transfer editing substrates unlike IleRS. ValRS editing domain crystal structure with hypothetical pre-transfer substrate analogue (Thr-AMS) also speculates the possibility of pre-transfer editing in ValRS [23]. To investigate such a possibility we have performed molecular modeling studies on ValRS with pre- and post-transfer substrates. Initially molecular docking studies were performed to obtain the binding conformations of the substrates (Thr-AMP/Val-AMP) and the docked conformations were taken as the initial structures for the molecular dynamics (MD) simulation studies to understand the specificity of pre-and post-transfer substrates at ValRS editing site.

2. Methods

2.1. Molecular docking calculations

To obtain the starting structures of ValRS with its substrates Thr-AMP and Val-AMP for MD simulations we have performed molecular docking simulations. Automated docking simulations for the ligands were performed using the GOLD 3.1 software [26–28]. The GOLD uses a genetic algorithm (GA) in combination with scoring functions to predict binding poses. Molecular docking was achieved by using ValRS CP1/editing domain crystal structure with hypothetical pre-transfer substrate analog Thr-AMS (PDB ID: 1WK9) [23]. Active site was defined by 5 Å circle around the Thr-AMS which is bound to the editing site. The following genetic algorithm parameters were used: 100 population size, 1.1 for selection, 5 number of islands, 100,000 number of genetic operations. The Gold Score was opted to rank order the docked conformations.

2.2. Initial structure preparation for MD simulations

Molecular docking results of ValRS CP1 domain with Thr-AMP and Val-AMP were utilized to develop initial structures for MD simulation studies. These two initial conformations were used to perform CP1 domain (editing domain with substrates) simulations. We have developed six molecular systems for MD simulations with ValRS full structure; two with pre-transfer substrates, two mutants K270A, D279A with pre-transfer substrate Thr-AMP and two with post-transfer substrates. Though ValRS full structure in the presence of tRNA interacting with editing domain is available (PDB ID: 1GAX) [19], A76 with cognate/non-cognate amino acid or substrate analog like Thr-AMS is not available. For all the simulations with pre-transfer substrates (Thr-AMP/Val-AMP), the docked substrates with CP1 domain (1WK9) coordinates are taken into ValRS full structure by superimposition and the final base which occupies editing site (A76) was deleted as both substrate AMP and A76 will interact at the same site. For simulations with post-transfer substrates (Thr-A76/Val-A76), amino acids were connected at 2′-OH of A76. Both the zinc ions present in the ValRS structure were taken for the simulation of full structures. The InsightII [29] modules were used for initial structure preparation.

2.3. Molecular dynamics simulations

All the MD simulations were performed using GROMACS program (version 3.3.1) [30,31] with the *Amber99* force field. AM1-BCC charges [32,33] were calculated for substrates using *Antechamber* software [34]. The MD simulation protocol that we used is as follows. All hydrogens were added and the protonation state of ionizable groups was chosen appropriate to pH 7.0. The cysteine residues which interact with the zinc ions were chosen as negatively charged cysteines (CYM). Each system was inserted in a water box where the layer of the TIP3P water molecules was equal to 14 Å. The entire systems were neutralized by adding Na^+ counter ions by replacing solvent molecules. The systems were subjected to steepest descent energy minimization for 10,000 steps. Then the protein backbone was frozen and the solvent molecules with counter ions were allowed to move during a 100 ps position restrained MD run. The production run was for 5 ns for editing/CP1 domain with substrates and 2 ns for ValRS full structure (pre/post-transfer) simulations. The sizes of the studied systems are around 29,395 atoms for editing domain only and 280,200 for full structure simulations. The simulation period was chosen as a compromise between the quality of configuration space sampling and the calculation length. All simulations were run under periodic boundary conditions with NPT ensemble by using Berendsen's coupling algorithm for maintaining the temperature (300 K) and the pressure constant (1 bar). The SHAKE algorithm with a tolerance of 10^{-5} Å was applied to fix all bonds containing hydrogen atoms. The time step for the simulations was 2 fs. The electrostatic interactions were calculated by using the Particle-mesh Ewald (PME) algorithm, with an interpolation order of 4 and a grid spacing of 0.1 nm. The van der Waals forces were treated by using a cutoff of 10 Å and the coordinates were stored every 1 ps. All the analyses of the MD simulations were carried out by GROMACS and the computations were performed in a high performance Linux cluster computer.

3. Results and discussion

3.1. Molecular docking calculations

The details of ValRS pre-transfer substrate analog (Thr-AMS) orientation at the editing domain and the crucial amino acids which interact with adenosine along with the residues that are responsible for amino acid side chain recognition (threonine hydroxyl group) was clearly discussed in the recent publication [23]. As crystal structure information is not available for ValRS editing site with its real substrates, either for the post-transfer or the pre-transfer editing, molecular docking calculations were performed to investigate the initial conformations of substrates (Val-AMP and Thr-AMP) and their interactions with ValRS editing/CP1 domain.

The interaction pattern of Thr-AMS with the editing site in CP1 domain (Fig. 1a) has been analyzed from the crystal structure (PDB ID: 1WK9). The NH_2 group on the adenosine ring interacts through H-bonding with the backbone O atom of Phe264 and Glu261, main chain NH of Glu261 also forms H-bond with N1 of adenosine ring. These three H-bond interactions and hydrophobic interaction with Phe264 stabilize the adenosine ring at the editing domain. The sulphate group (mimic of phosphate group in substrate Val-AMP/Thr-AMP) forms H-bonds with side chain hydroxyl of Thr214, main chain NH of Val215 and guanidine group of Arg216. Threonine hydroxyl group is recognized by side chains of Lys270, Thr272 and Asp279 which are crucial hydrophilic residues for discriminating cognate valine from non-cognate threonine. Amino group of the threonine of Thr-AMS can interact with Asp276 and conserved

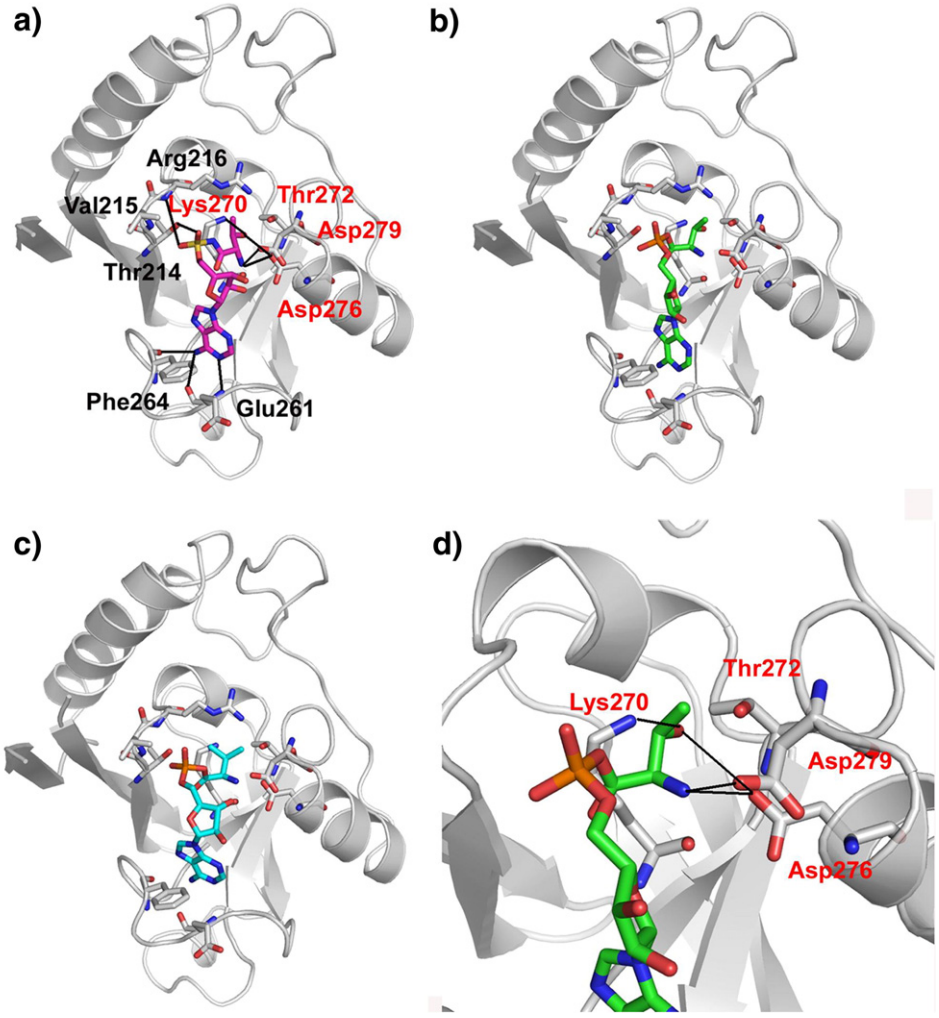

Fig. 1. Crystal structure of ValRS editing domain with Thr-AMS (a) and the docked conformation of Thr-AMP (b) and Val-AMP (c). The AdnBP residues were shown around the Thr-AMP (d). The ValRS residues interacting with the substrates are shown in stick model.

Asp279 side chains. For convenience, hereafter we mentioned "adenosine binding pocket" represented by residues Thr214, Val215, Arg216, Glu261, and Phe264 that interact with adenosine ring and phosphate/sulphate as "AdnBP"; and "amino acid binding pocket" representing Lys270, Thr272, Asp276, and Asp279 residues that interact with threonine hydroxyl group and amino group as "AABP".

After molecular docking simulations, the docked conformations of the substrates, Thr-AMP and Val-AMP were selected by comparing the resultant conformations with the crystal structure and by considering overall docking score, Gold Score. The final conformations are similar to the Thr-AMS conformation in the crystal structure. The docked conformation of Thr-AMP at ValRS CP1 domain demonstrates all the crucial interactions with the AdnBP and AABP similar to the Thr-AMS (Fig. 1b). The Thr-AMP forms H-bonds with the hydrophilic amino acids present in the AABP (Fig. 1d). However, the Val-AMP cannot form any interactions with the AABP although it binds in a similar manner with the AdnBP (Fig. 1c) due to lack of hydrophilic characteristics of the side chain. These docked conformations were used for MD simulation studies for pre- and post-transfer editing reactions.

3.2. Pre-transfer editing in CP1 domain structure

Two 5 ns MD simulations were performed to understand the binding pattern of the substrates (cognate/non-cognate amino acids) to ValRS editing site. The details of the MD simulation parameters and the size of the system are listed in Table 1. The MD simulations of CP1 domain (195–337) with its pre-transfer substrates, Val-AMP and

Table 1
Information of molecular dynamics simulations.

Simulation no.	Type of simulation	Type of substrate	Size of the system (total no. of atoms)	Simulation time (ps)
1	Pre-transfer (editing domain alone)	Thr-AMP	~29,400	5000
2	Pre-transfer (editing domain alone)	Val-AMP	~29,400	5000
3	Pre-transfer (ValRS full structure)	Thr-AMP	~280,200	2000
4	Pre-transfer (ValRS full structure)	Val-AMP	~280,200	2000
5	Pre-transfer (ValRS full structure with Lys270Ala mutation)	Thr-AMP	~280,200	2000
6	Pre-transfer (ValRS full structure with Asp279Ala mutation)	Thr-AMP	~280,200	2000
7	Post-transfer (ValRS full structure)	Thr-A76	~280,200	2000
8	Post-transfer (ValRS full structure)	Val-A76	~280,200	2000

Thr-AMP were performed as valine is the cognate amino acid and the threonine is the non-cognate one. The backbone root mean square deviation (RMSD) of the CP1 domain in the presence of Thr-AMP/Val-AMP with respect to the initial structure was analyzed (Fig. 2a). The average RMSDs for Thr-AMP and Val-AMP from 1001 ps to final snapshot are 0.127 nm and 0.133 nm, respectively. Both the figures are small enough to ensure that the two systems were very stable during the simulation time. Hereafter the term 'average' refers to the evaluation through 1001 ps to the final snapshot. The trajectory stability for total 5 ns simulation was monitored and confirmed to be stable by inspecting backbone RMSD plots for both the systems.

The binding ability of pre-transfer substrates with the editing site was analyzed by counting the number of intermolecular H-bonds (Fig. 2b) as well as by comparing two different energy functions (Fig. 2c and d). Number of H-bonds between ValRS editing site and the substrates were measured and the results showed that Thr-AMP formed high number of H-bonds which were maintained stably. On the other hand its cognate substrate (Val-AMP) formed less number of H-bonds initially and they decreased gradually (Fig. 2b). The average number of the intermolecular H-bonds for Thr-AMP and Val-AMP are 9.81 and 5.03, respectively. To elucidate in-depth binding ability of substrates with CP1 domain, the short range (SR) columbic and van der Waals (vdw) interaction energies were calculated and displayed (Fig. 2c and d). The SR columbic interaction energy between Thr-AMP and ValRS CP1 domain is comparatively less than Val-AMP as Thr-AMP forms stable hydrophilic interactions than Val-AMP. The columbic interaction between Thr-AMP and CP1 domain increased slightly at around 1 ns and thereafter stabilized throughout the 5 ns simulation with an average of −462.85 kJ/mol. In the case of Val-AMP, significant increment of columbic interaction energy with an average of −269.12 kJ/mol was observed from the starting of the simulation (Fig. 2c) but the vdw interaction was relatively stable (Fig. 2d). We assume that the ValRS editing site is mainly comprised of hydrophilic residues which are specifically designed for its non-cognate amino acid, threonine. Therefore, it will be more meaningful to study the columbic energy rather than vdw energy between ValRS CP1 domain and its substrates. Conformational changes were observed at GTG loop (Gly265, Thr266 and Gly267), in the presence of Val-AMP which affects the interaction between Phe264 CO and NH_2 of adenosine ring. On the contrary, the carbonyl group of Phe264 is forming stable interaction with adenosine ring of Thr-AMP. Though these analyses clearly show the binding ability difference between the two substrates and ValRS CP1 domain, concluding the results may not be accurate at this point as we have considered only CP1 domain. The conformational changes and binding ability differences may be attributed as artifacts because CP1 domain alone was considered. Therefore, these results have encouraged us to perform MD simulations of ValRS full structure with tRNA for further verification.

3.3. Pre-transfer editing using tRNA and ValRS full structure

Six MD simulations were performed with tRNA and ValRS full structures, among which two (2 ns each) were performed initially in the presence of pre-transfer editing substrates (Thr-AMP/Val-AMP). Due to the massive size of total ValRS with tRNA system (~280,000 atoms) the length of simulation was chosen for 2 ns when compared

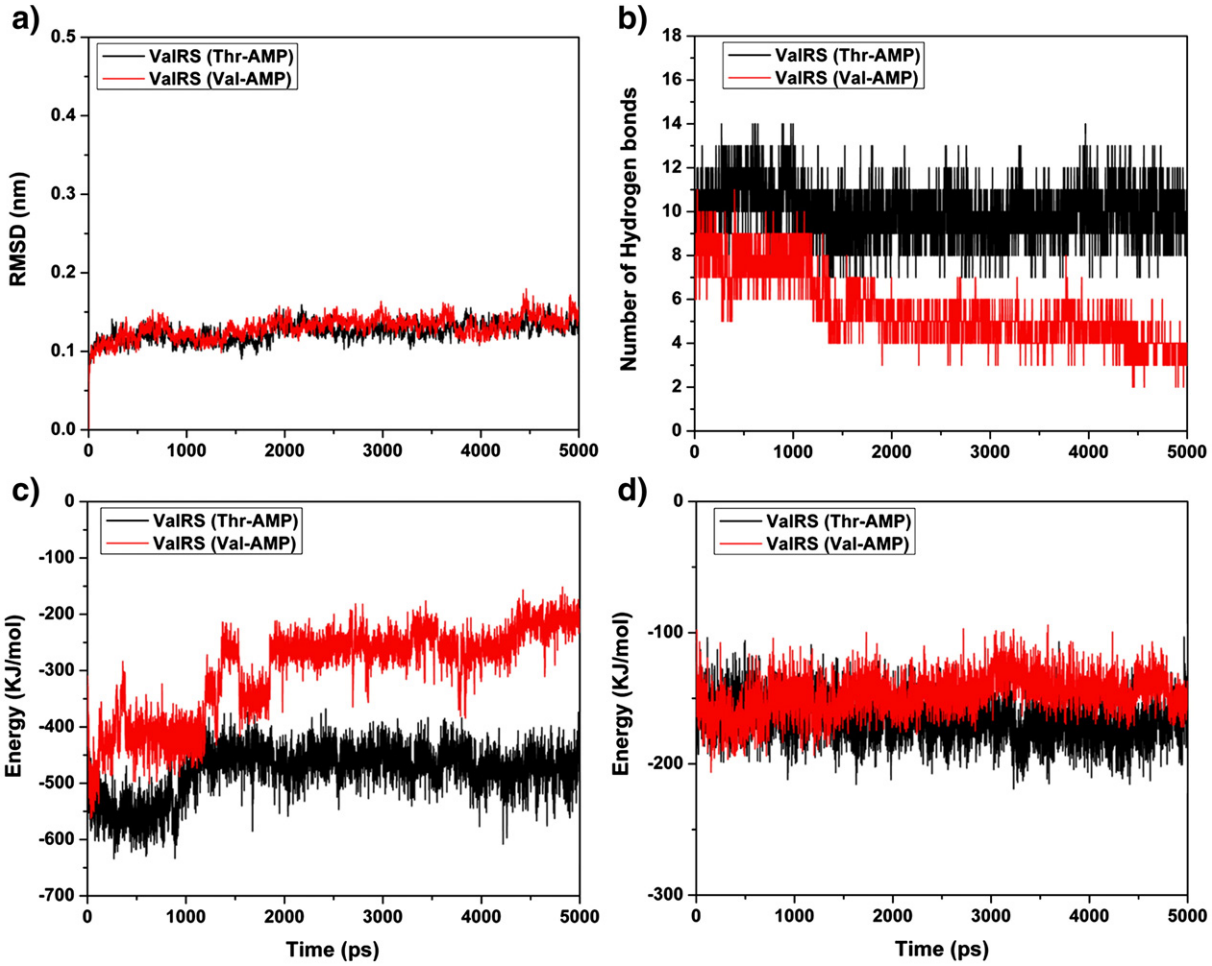

Fig. 2. The RMSD (a), the number of intermolecular H-bonds (b), short range columbic interaction energy (c), and short range Leonard–Jones potentials (d) of ValRS editing domain with Thr-AMP and Val-AMP for 5 ns MD simulation.

to the small domain simulation which was performed for 5 ns. The backbone RMSD of ValRS full structure with each substrate was evaluated and it was observed that the fluctuation is comparatively high in the presence of Thr-AMP when compared to the Val-AMP with an average of 0.277 and 0.187 nm respectively (Fig. 3a). Though the overall protein fluctuation is high, it is clearly evident that Thr-AMP interacts strongly with editing domain of ValRS than Val-AMP based on H-bond count and columbic interaction energy (Fig. 3b and c). The

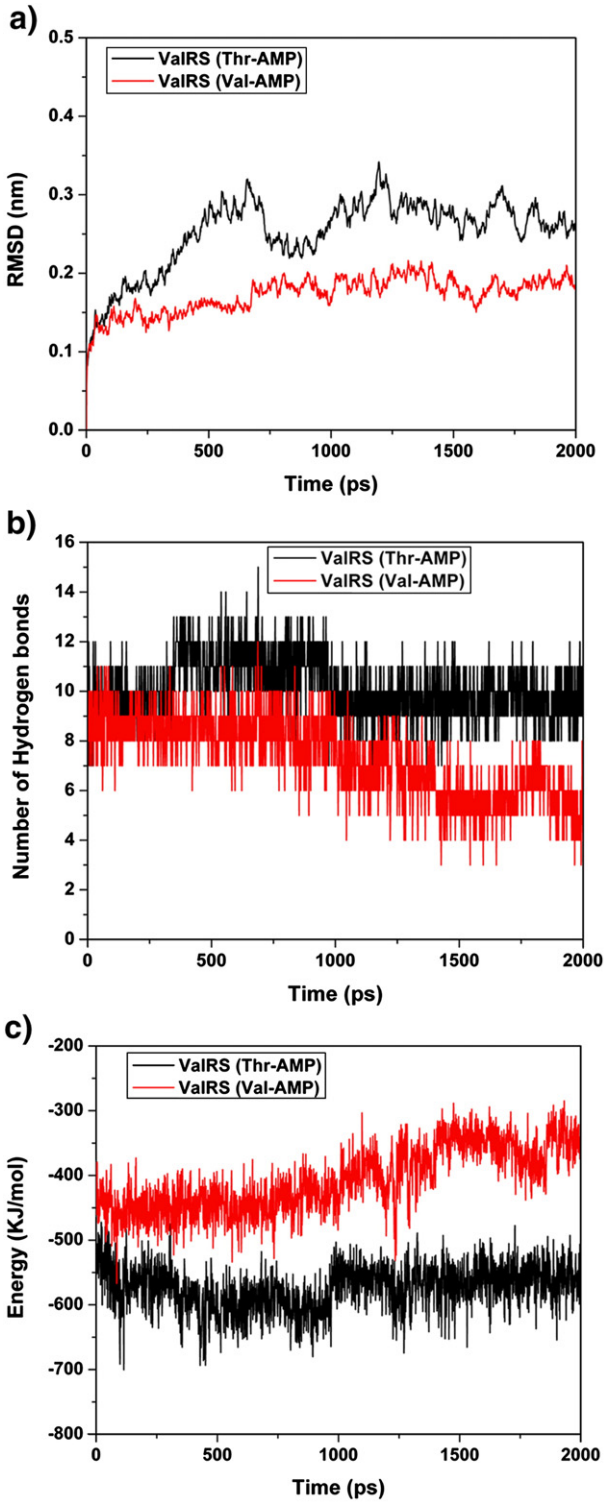

Fig. 3. The RMSD (a), the number of intermolecular H-bonds (b), and short range columbic interaction energy (c) of ValRS full structure with Thr-AMP and Val-AMP for 2 ns MD simulation.

Table 2
Occupancy rate of hydrogen bond interactions with editing domain and columbic interaction energy.

Type of interaction	Pre-transfer				Post-transfer	
	Thr-AMP	Val-AMP	Lys270Ala (Thr-AMP)	Asp279Ala (Thr-AMP)	Thr-A76	Val-A76
Phe264 main chain CO – NH of adenosine	0.998	**0.461**	**0**	**0.237**	0.944	**0.075**
Glu261main chain CO – NH of adenosine	0.919	0.826	0.992	0.407	0.902	0.724
Thr214 side chain OH – PO_4 group	1.0	**0.487**	1.0	1.0	NA[a]	NA[a]
Tyr337 side chain OH – PO_4 group	NA[a]	NA[a]	NA[a]	NA[a]	0.977	**0.338**
Lys270 side chain NH3+ – OH of Thr	0.829	**0**	**0**	**0**	0.826	**0**
Columbic interaction energy[b]	−562.5	−369.23	−439.87	−429.12	NA[a]	NA[a]

Occupancy calculated from 1001 ps to end of the simulation.
Highly deviated with reference to Thr-AMP/Thr-A76 highlighted with bold representation.
[a] Not applicable for particular simulation.
[b] Average columbic interaction energy between substrate and protein, values represented in KJ/mol.

average number of H-bonds is 9.60 and 6.17 and the average columbic interaction energy is −562.5 kJ/mol and −369.23 kJ/mol for Thr-AMP and Val-AMP with ValRS respectively (Table 2). The columbic interaction energy increased constantly between ValRS CP1 domain and its own substrate (cognate), Val-AMP which is not favorable for tight binding.

The binding ability of the substrates and the conformational changes during the MD simulation were analyzed. Not much change was observed in the binding conformation of Thr-AMP at editing domain as it was well accustomed and formed stable interactions with AdnBP and AABP (Fig. 4a and 4b). The interactions of Thr-AMP with AdnBP residues were analyzed by superimposing initial and final conformations (Fig. 4a). The two H-bonds formed by Thr214 side

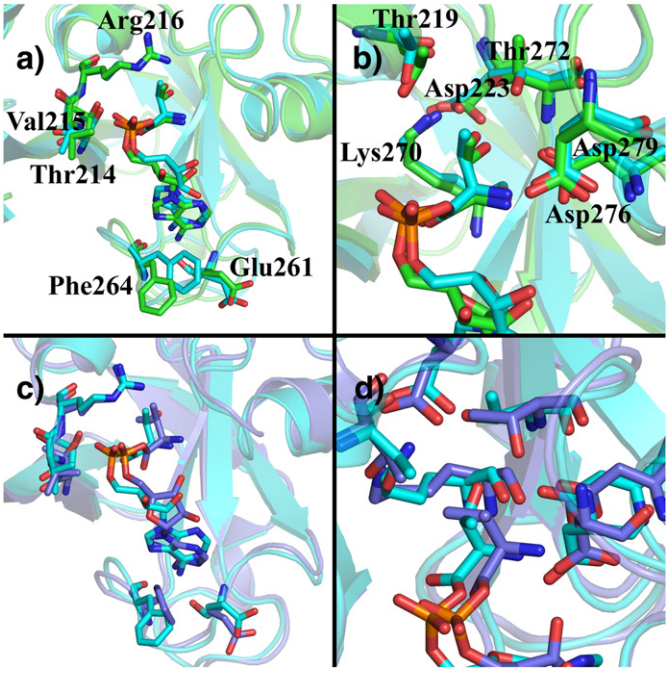

Fig. 4. The initial (green) and final (cyan) frames of ValRS full structure 2 ns MD simulation with Thr-AMP at AdnBP (a) and AABP (b). The superimposed final frames of Val-AMP (blue) on Thr-AMP (cyan) at AdnBP (c) and AABP (d). The protein is shown as ribbons; the crucial residues were shown in sticks with Thr-AMP as sticks and Val-AMP as ball and stick for clarity. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

chain OH group and Val215 main chain NH with PO_4 group of the Thr-AMP are stable throughout the simulation. The Phe264 present near GTG loop which is responsible for forming AdnBP is also well adjusted with Thr-AMP adenosine ring. The Phe264 CO group, Glu261 main chain carbonyl group also maintains the key H-bond interactions with NH_2 of adenosine ring. The Glu261 NH group also contributes an H-bond with N1 of adenosine ring. To determine the strength of the each H-bond, occupancy (defined as the number of snapshots having a particular H-bond from the total simulation snapshots) is calculated. The occupancy of five possible H-bond interactions between Thr-AMP and adenosine ring was 4.82 (Table 2). In a similar manner, Thr-AMP interacts strongly with AABP (Lys270, Thr272, Asp276 and Asp279). The residues Thr219 and Asp223 are also represented in figures as they form key interactions with other AABP residues like Lys270 and Thr272 to maintain the arrangement of the pocket. The side chains of Lys270, Asp279 form stable interactions with hydroxyl group of Thr-AMP. At the same time Asp279 and Asp276 side chain carboxyl groups form H-bond with α-NH_3^+ of Thr-AMP (Fig. 4b). According to the previous study, Thr272 side chain is speculated to form another H-bond with OH group of Thr-AMP [23]. In the present study Thr272 interacts less often with OH of Thr-AMP but maintains strong H-bond interaction with Asp223 residue. This result coincides with the experimental mutational studies where mutation of Thr272 has less effect on editing activity than Lys270 and Asp279.

Binding ability of Val-AMP with ValRS full structure was analyzed and it was observed that significant conformational changes were

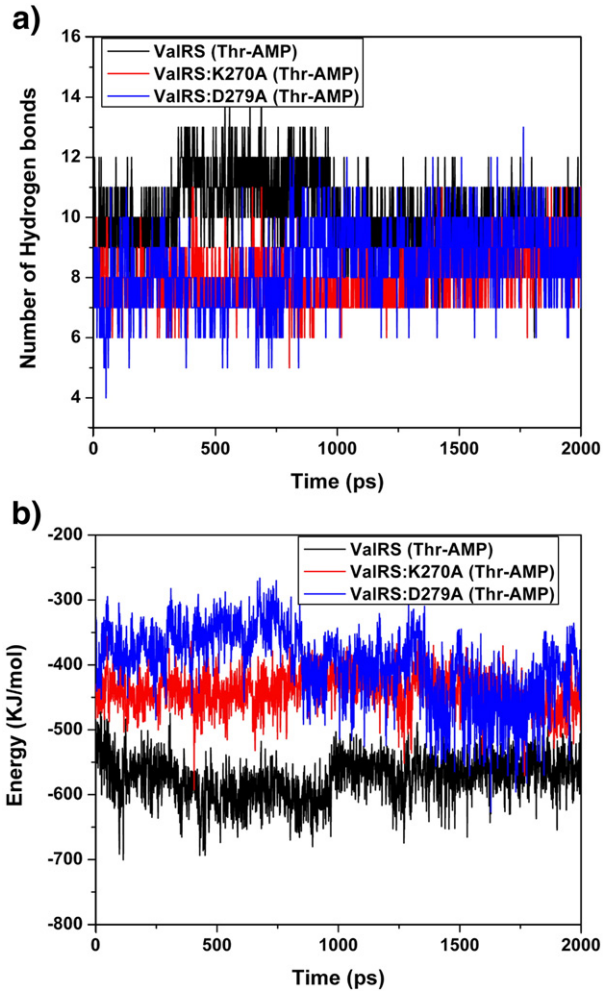

Fig. 5. The number of intermolecular H-bonds (a) short range columbic interaction energy (b) of Wild type ValRS full structure, Lys270Ala mutant and Asp279Ala mutant with Thr-AMP for 2 ns MD simulation.

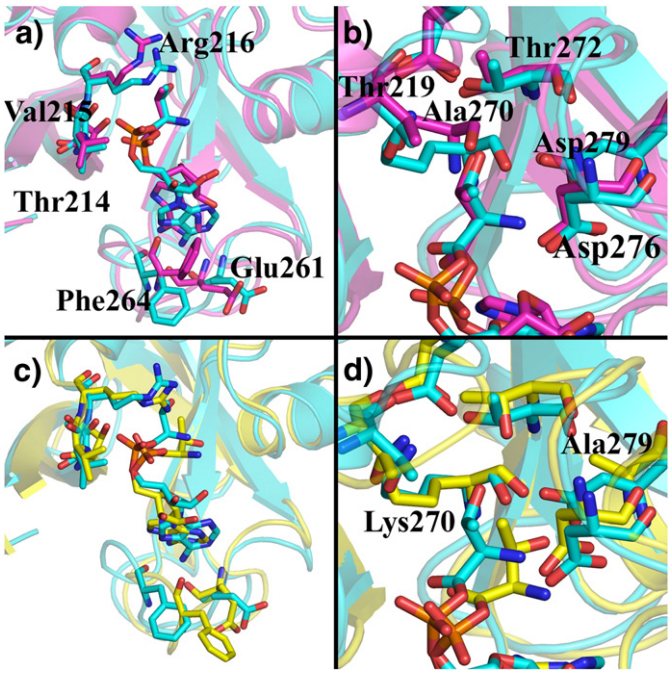

Fig. 6. The superimposed final frames of WT and Lys270Ala 2 ns MD simulation with Thr-AMP at AdnBP (a) and AABP (b). The superimposed final frames of WT and Asp279Ala 2 ns MD simulation with Thr-AMP at AdnBP (a) and AABP (b).The protein is shown as ribbons, the crucial residues were shown in sticks with Thr-AMP as sticks.

observed both in the AdnBP and AABP (Fig. 4c and d) unlike Thr-AMP. Although much fluctuation was not observed at GTG loop, crucial H-bond network was diminished between the NH_2 of adenosine ring and GTG loop amino acid residues like Phe264 and Glu261. The H-bond interactions at the AdnBP were effected as analyzed by measuring their occupancy (Table 2). The residues, Thr214 and Val215 which interact with PO_4 group of Thr-AMP through H-bonding are not able to maintain steady interactions with Val-AMP. Both the residues move away from the PO_4 group which causes loss of interaction (occupancy was 0.487 and 0.792 respectively, but in the case Thr-AMP it was 1.0 and 0.935). These results clearly showed that the binding ability of Val-AMP is fragile than the Thr-AMP at the AdnBP. Two residues Asp276, Asp279 formed H-bond interactions with α-NH_3+ group of Val-AMP at AABP. These results are also comparable with the CP1 domain MD simulation results (Fig. 2) and therefore gave confidence that 2 ns simulation of ValRS full structure with tRNA is sufficient to observe the important changes at the CP1 domain and the binding ability of substrates. Based on these results it can be established that non-cognate substrate (Thr-AMP) binds strongly at editing site and undergo hydrolysis, but Val-AMP interacts feebly with editing site and depart along with tRNA.

3.4. Mutational studies

Two most crucial residues present in the AABP (Lys270 and Asp279) were mutated to alanine experimentally and their results disclosed that editing site is not able to recognize the threonine side chain thereby finally losing its editing ability [23]. In order to observe these mutational effects on pre-transfer editing substrate (Thr-AMP), we have considered two point mutated systems (Lys270Ala, Asp279Ala) and performed 2 ns MD simulations each.

3.5. Lys270 mutation

The effect of Lys270Ala mutation on Thr-AMP binding ability was analyzed by intermolecular H-bond count and columbic interaction energy (Fig. 5a and b) resulting in an average of 8.16 (9.60

for wild type) and −439.87 kJ/mol (−562.5 kJ/mol for wild type) respectively. These two analyses together reveal that Lys270 mutation has a negative effect on the binding ability of Thr-AMP with the editing domain of ValRS. Fig. 6a illustrates the binding pattern of Thr-AMP with AdnBP of Lys270Ala ValRS mutant. The GTG loop which is crucial for adenosine ring binding is affected in such a way that the interaction between carbonyl group of Phe264 and NH_2 group of adenosine ring is completely vanished. The occupancy of Phe264 CO and adenosine NH_2 H-bonding was zero in stark contrast with wild type which has 0.998. Although the AABP residue, Lys270 was mutated not much effect was observed on amino acid side chain position of Thr-AMP. It formed all other interactions with Asp276 and Asp279 residues reasonably.

The main roles of Lys270 side chain is attributed to the recognition of Thr-AMP side chain along with maintaining key interaction network with residues Thr219 and Asp223 which may further help in the maintenance of AABP size. Effect of Lys270Ala was clearly observed on the secondary structural features. The mutation has directly affected the GTG loop, as it leads into the beta sheet containing Lys270. Due to this effect the Phe264 carbonyl group has turned away and lost the H-bond interaction with the adenosine NH_2 group. Mutation has been affected indirectly by losing the interaction with Asp223 and thereby losing secondary structural features from 228–257 residues (Fig. 7b). In the case of wild type and Asp279Ala mutant, this region is stable and maintains all the secondary structural features throughout the 2 ns simulations (Fig. 7a and c) unlike Lys270 mutant. The Lys270 in wild type forms key interactions with Thr219 but upon mutation to alanine the Arg216 side chain has moved away from AABP thus losing crucial hydrophobic interaction with methyl group of Thr-AMP. The Lys270Ala mutant has been affected directly and indirectly thereby leading to the loss of recognition of its cognate amino acid.

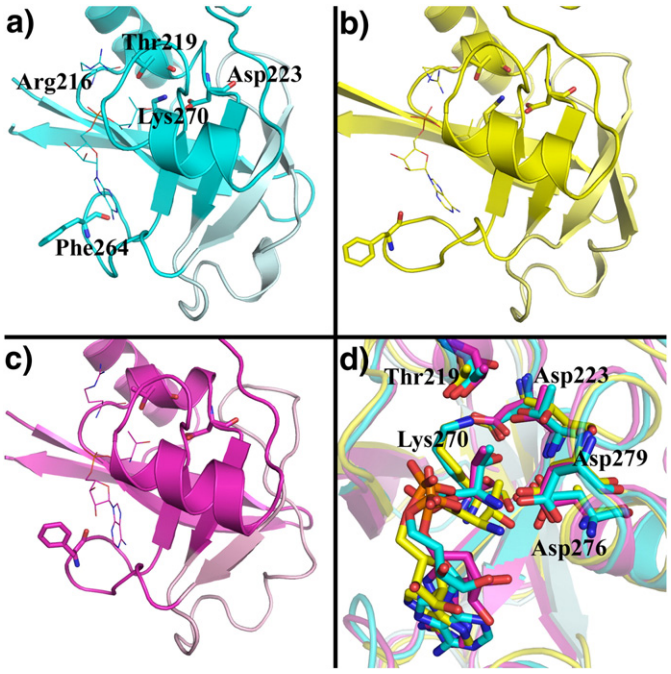

Fig. 7. The final frames of Wild type (a) Lys270Ala (b) Asp279Ala (c) full structure 2 ns MD simulation with certain residues shown in sticks and the substrate shown in lines. The region having observable secondary structure changes has been highlighted by pale colours in respective structures. The superimposed final frame structures of Wild type (cyan), Lys270Ala (magenta), and Asp279Ala (yellow) to highlight the dislocation of Thr-AMP from the AABP caused by Asp279 mutation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

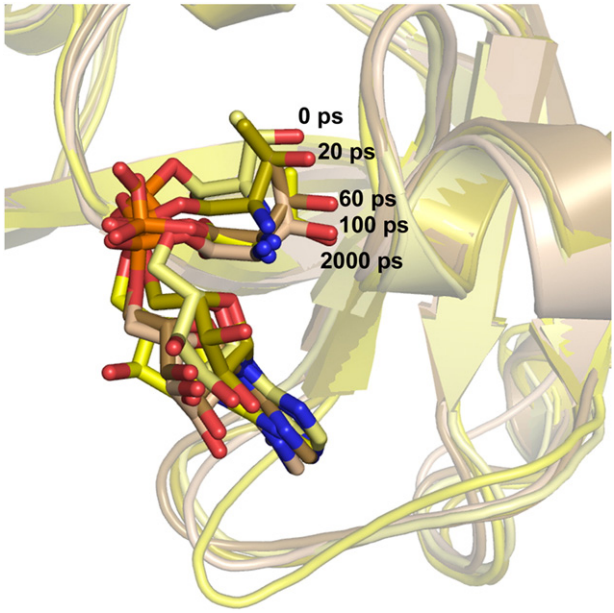

Fig. 8. The superimposed structures of initial (pale yellow), final (yellow) along with intermediate structures like 20 ps 60 ps and 100 ps snapshots to observe the orientational change of Thr-AMP in Asp279Ala mutation MD simulation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.6. Asp279 mutation

Asp279 present at the editing site is well conserved among all the three enzymes (ValRS, LeuRS and IleRS) in most of the organisms. In the case of *T. thermophilus* LeuRS (Asp347), IleRS (Asp328) H-bonds were formed with the α-NH_3+ which seem to play a vital role in the editing reaction. Mutation of the Asp328Ala in *T. thermophilus* IleRS is defective in the total editing activity [18,35]. In a similar manner Asp279Ala mutation in ValRS also demonstrated severe effect on total editing process (pre-transfer, if exists and post-transfer editing) [23]. In the case of ValRS, Asp279 forms H-bond with α-NH_3+ and also recognizes the side chain hydroxyl group of threonine by forming another H-bond.

The average number of H-bonds and the average columbic interaction energy were 8.63 (9.60 for wild type) and −429.12 kJ/mol (−562.5 kJ/mol for wild type) between Thr-AMP and Asp279 mutated ValRS. It is evident that the number of H-bonds was only 6 to 7 until 1 ns thereafter increasing and stabilizing around 8 to 9 between Thr-AMP and mutant ValRS (Fig. 5a). The columbic interaction energy also revealed a similar trend of results. The binding pattern of Thr-AMP with AdnBP of Asp279 mutant is illustrated in Fig. 6c. The mutational effect on GTG loop was similar to that of Lys270 mutation and also to wild type in the presence of Val-AMP substrate. The crucial H-bond between carbonyl group of Phe264 and NH_2 of Thr-AMP adenosine ring formed occasionally with an occupancy of 0.237 (0.998 for wild type). Another important H-bond between the carbonyl group of Glu261 and NH_2 of Thr-AMP adenosine ring was also affected severely with an occupancy of 0.407 (0.919 for wild type). The mutational effect on Thr-AMP binding and threonine side chain recognition was clearly observed in the case of Asp279Ala mutation (Fig. 6c and d). The threonine side chain of Thr-AMP was completely dislocated from the AABP which is not desirable for editing reaction (Fig. 6d). This dislocation is especially observed in Asp279 mutation case, while in the Lys270 case we have not observed much change in threonine side chain (OH group) position and is very similarly oriented as in wild type simulation (Fig. 7d). The superimposition of a few selected MD simulation frames (0, 20, 60, and 100 ps simulation time) until 100 ps revealed the dislocation process of threonine side chain from the AABP and after 100 ps not many changes were observed in threonine side chain position (Fig. 8). This can be

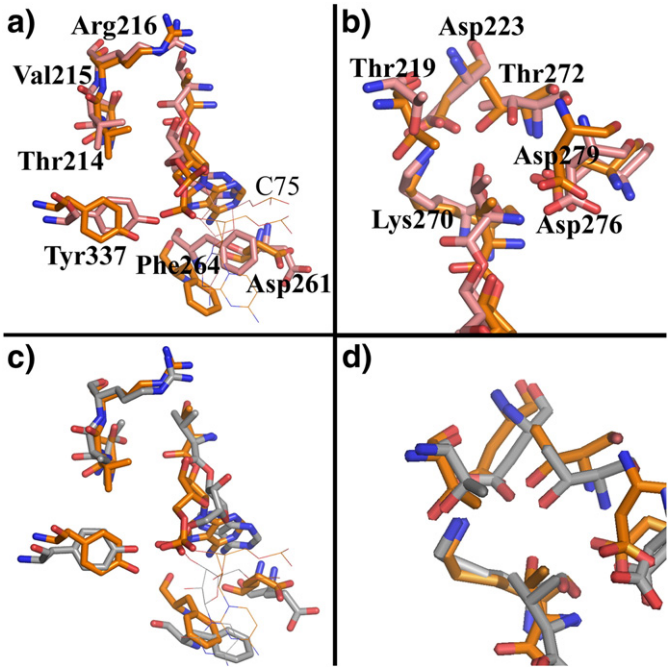

Fig. 9. Post-transfer MD simulation results. The superimposed initial (peach) and final (orange) snapshots of ValRS full structure with Thr-A76 at AdnBP (a) AABP (b). The superimposed final frames of Val-A76 (cement) and Thr-AMP (orange) snapshots of ValRS full structure at AdnBP (c) AABP (d). The protein is shown as ribbons, the crucial residues were shown in sticks with Thr-A76 as sticks and Val-A76 as ball and stick for clarity. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

further witnessed by superimposing simulation conformations (Fig. S1) at 0, 800, 1600 and 2000 ps.

Since the threonine side chain dislocated from the AABP, the crucial H-bond interaction between Lys270 side chain and OH group of Thr-AMP was completely missing (occupancy was zero). On the other hand the hydrophobic interaction between Arg216 side chain and threonine side chain was also affected. As amino acid side chain dislocated, main chain carbonyl group of Lys270 formed H-bond with hydroxyl group (amino acid side chain) of Thr-AMP. The Arg216 side chain moved inside the cavity due to a conformational change in the substrate binding and formed one to two H-bonds with PO_4 of Thr-AMP. Although Thr-AMP preserved the binding ability by forming H-bonds with these residues, the editing ability of ValRS have been lost because Thr-AMP dislocated from the AABP. The other H-bond interaction between α-NH_3+ group of Thr-AMP and carboxyl group of Asp276 was maintained well throughout the simulation. This reveals that although Asp276 can form H-bond with α-NH_3+ group, it is unable to resist the dislocation of amino acid side chain from the AABP. This demonstrates that Asp276 has not much importance in the editing process (pre-transfer) though it forms direct interaction with Thr-AMP. As we already know that Asp328Ala mutation in IleRS severely affects the editing process as it interacts with α-NH_3+ group. Most probably a similar type of side chain dislocation may occur from the AABP.

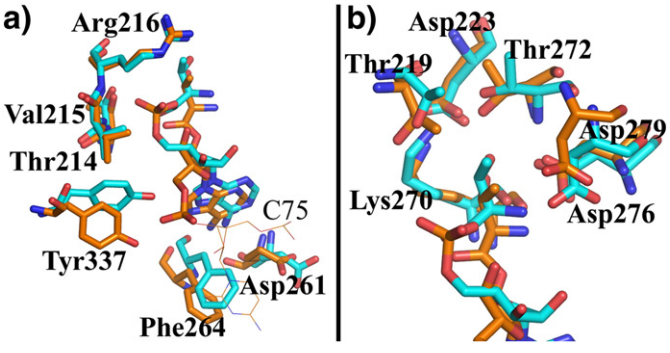

Fig. 10. The superimposed final frames of post-transfer (orange) and pre-transfer (cyan) snapshots of ValRS full structure at AdnBP (a) AABP (b). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.7. Post-transfer editing using tRNA and ValRS full structure

Two different 2 ns simulations were carried out to demonstrate the binding properties of post-transfer substrates (Thr-A76/Val-A76). The simulation stability was analyzed by backbone RMSD and total energy analyses. The average RMSD was 0.211 and 0.239 nm for Thr-A76 and Val-A76 respectively stabilizing soon after 500 ps (Fig. S2a). The total energy analyses also revealed that both the simulations were well stabilized without any deviations. The binding ability of post-transfer substrates was analyzed by the number of intermolecular H-bonds, which resulted in an average of 7.54 and 4.41 for Thr-A76 and Val-A76 with ValRS respectively (Fig. S2b). From the H-bond count analyses it is evident that Thr-A76 interacts with CP1 domain tightly compared with Val-A76 indicating that in both pre- and post-transfer process, the non-cognate substrates bind strongly.

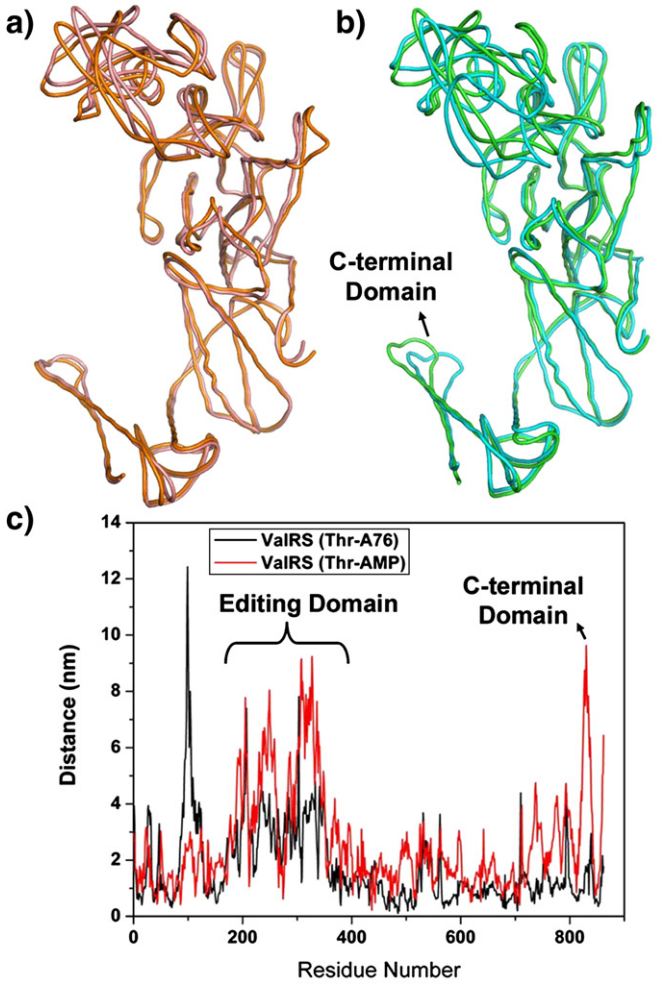

Fig. 11. The backbone structures of the extreme frames obtained from ED analyses results for 2 ns MD simulation of ValRS full structure with Thr-A76 (a) and Thr-AMP (b). The C-alpha distance measured between the two extreme frames of ED analyses (c) for post- and pre-transfer simulations.

The binding pattern of Thr-A76 with AdnBP of ValRS CP1 domain revealed that the GTG loop adjusted well in the presence of Thr-A76 and all the three crucial H-bond interactions were firmly maintained with adenosine ring of Thr-A76 (Fig. 9a) with an occupancy of ~0.9. The 5′-PO_4 group of Thr-A76 is forming firm H-bond interaction with Tyr337 hydroxyl group instead of Thr214 and Val215 (pre-transfer) resulting in occupancy of 0.977. There is no important role of Tyr337 in pre-transfer substrate binding but this residue specifically interacts with the 5′-PO_4 group of post-transfer editing substrates. The amino acid side chain hydroxyl of Thr-A76 interacts strongly with Lys270 and Asp279 similar to that of pre-transfer (Fig. 9b). The α-NH_3+ group of Thr-A76 interacts firmly with Asp279 side chain but the H-bond between Asp276 and α-NH_3+ group of Thr-A76 has been lost (occupancy is 0.222). This interaction loss was well correlated with a previous report and this interaction is specific for pre-transfer editing [23].

The binding pattern of Val-A76 with AdnBP and AABP of ValRS CP1 domain was analyzed by superimposing onto Thr-A76 for comparison (Fig. 9c and d). The GTG loop is affected in the presence of Val-A76 and the interaction between the carbonyl group of Phe264 and NH_2 of adenosine ring is affected thoroughly with an occupancy of 0.075 (0.944 for Thr-A76). In a similar manner the two H-bonds formed between Glu261 and adenosine ring are lost gradually. Another specific and important H-bond between Tyr337 hydroxyl group and 5′-PO_4 group of Val-A76 is also highly affected which is evident with an occupancy of 0.338 (0.977 for Val-A76).

3.8. Comparison of pre- and post-transfer editing

The results clearly demonstrate that both pre-transfer and post-transfer substrates (Fig. 10) can bind to the editing site with few differences (5′-PO_4 group can interact with Thr214, Val215 in the case of pre-transfer while with Tyr337 in post-transfer). The binding ability differences between cognate and non-cognate ones in both the editing process revealed that non-cognate substrates bind tightly with editing site to undergo hydrolysis. The essential dynamics analysis was carried out to understand the correlated domain motion differences between pre- and post-transfer systems. High fluctuation was observed in the case of pre-transfer especially at C-terminal domain and editing domain compared to post-transfer editing (Fig. 11a and b). For the comprehensible view Cα deviation graph was drawn for pre- and post-transfer simulations (Fig. 11c). The C-terminal domain which is interacting with D-loop of tRNA shows high fluctuation, suggesting that it may play an important role in translocation of tRNA in the presence of Thr-AMP (pre-transfer non-cognate substrate). This domain movement has caused high fluctuation in RMSD (Fig. 3a). Such C-terminal domain movement was not observed in the presence of Val-AMP (pre-transfer cognate substrate). Domain movement could be observed by superimposing pre- and post-transfer simulations final frames with Thr-AMP/Thr-A76 (Fig. S3). The mutational studies with pre-transfer substrate (Thr-AMP) also demonstrated reduced binding ability than wild type revealing that these mutations have such an effect that ValRS treats the non-cognate substrate as its own cognate substrate thereby loosing its ability to distinguish in the editing process. All these explanations disclose that there may be a single binding site for post- and pre-transfer editing and still have the possibility of the existence of pre-transfer editing in ValRS.

4. Conclusions

The possibility of pre-transfer editing process was investigated by comparing and contrasting the pre- and post-transfer substrates binding orientations at ValRS CP1 domain by performing molecular dynamics simulations. The GOLD docked conformations of Thr-AMP and Val-AMP at the ValRS CP1 domain were ideal as initial structures for MD simulation studies. In total six simulations with pre-transfer editing substrates (Thr-AMP/Val-AMP) and two simulations with post-transfer substrates (Thr-A76/Val-A76) were carried out. The simulations of pre-transfer substrates with ValRS CP1 domain (5 ns) and ValRS full structure (2 ns) has concomitantly revealed that the non-cognate substrate (Thr-AMP) binds strongly to the editing site than its cognate substrate. This was evident from the number of intermolecular H-bond and SR columbic interaction energy analyses. The strong binding is attributed to both the AdnBP and AABP of ValRS editing site.

The two simulations of the editing site mutants (Lys270Ala and Asp279Ala) were performed to probe their effect on pre-transfer editing. Both the mutants affected the pre-transfer editing process severely but the mechanism in which they affected was different. In the case of Lys270Ala mutant, the secondary structural features of the editing domain was affected as Lys270 interacts with Thr219 and Asp223 which together maintain the AABP. In the case of Asp279Ala mutant, the amino acid side chain of Thr-AMP was dislocated from the AABP of the editing domain. In both the cases the GTG loop fluctuated highly such that the interaction between Phe264 and adenosine ring were lost. The MD simulation studies of ValRS with post-transfer substrates also revealed that non-cognate substrate can bind strongly than cognate substrate. This indicates that in both pre- and post-transfer editing process strong binding is required for the hydrolysis of the non-cognate substrate. The binding site of both the pre- and the post-transfer substrates is the same except for the 5′-PO_4 group. A significant difference in the binding pattern of pre- and post-transfer substrates is that, 5′-PO_4 group interacts with Thr214 and Val215 while it interacts with Tyr337 respectively. It was mentioned elsewhere that Asp276 interacts with pre-transfer substrates alone. Our results also substantiate the same, but as both Asp276 and Asp279 interact with the amino group, the Asp276 mutant may not be strong enough to dislocate the amino acid side chain as observed in the case of Asp279 mutant. For distinguishing the pre- and post-transfer editing process the mutation of Thr214, Val215 or Tyr337 would be more helpful than earlier mutations like Arg216Ala, Phe264Ala, Lys270Ala, Thr272Ala, and Asp279Ala which play a crucial role in both editing mechanisms. Our results revealed that both pre-transfer and post-transfer substrates can bind to the same site with specific differences, thereby supporting the possibility of pre-transfer editing process.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bpc.2009.03.009.

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