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# Binding and Enantiomeric Selectivity of Threonyl-tRNA Synthetase

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Homochirality is essential to life as we know it. With few exceptions, proteins are comprised exclusively of L-amino acids and the incorporation of even a single D-amino acid would prevent most proteins from folding into their native functional form.[1] Homochirality is primarily maintained during translation, via the action of aminoacyl-tRNA synthetases (aaRS's).[2] aaRS's combine two functions. First, they preferentially catalyze the binding of the cognate amino acid to the 2′ or 3′ hydroxyl of the terminal ribose of the corresponding tRNA.[3] Second, they contain an editing module that deacylates tRNAs charged with a noncognate amino acid.[4] This editing function prevents the misincorporation of noncognate amino acids and is essential for maintaining high fidelity in regard to both amino acid type and enantiomeric selectivity.

The editing domain of threonyl-tRNA synthetase (ThrRS) removes noncognate L-Ser and all D-amino acids attached to $tRNA^{Thr}$.[5] Fluorescence studies indicate that the N-terminal editing domain of ThrRS from *Pyrococcus abyssi* (Pab-NTD) preferentially binds L-Ser, L-Cys, and all D-amino acids.[6] Based on the crystal structures of Pab-NTD complexed with L-Ser (PDB 2HKZ) and the observation that the mutant Lys121Met binds only D-amino acids, it has been suggested that L-Ser binds such that the carboxylate of the ligand lies within 0.5 nm of the side chain of Phe117 and the ligand side chain hydroxyl group interacts with the side chain of Lys121 (Figure 1a).[7] While this binding mode might explain the preferential binding of L-Ser over L-Thr, it fails to explain how the editing domain recognizes all D-amino acids. Here we re-examine the binding and enantiomeric selectivity of amino acids bound to Pab-NTD using a combination of atomistic molecular dynamics (MD) simulations and free energy calculations. Specifically it is shown that the binding mode proposed by Hussain et al.[7] is unstable and that an alternative binding mode that explains both the binding of L-Ser and the recognition of all D-amino acids is preferred.

To investigate the stability of the binding mode of L-Ser suggested by Hussain et al.[7] a series of four MD simulations were started from the proposed model (PDB 2HKZ, 2.1 Å resolution, R 0.22, Rfree 0.286) using different initial velocities. In all cases the ligand (L-Ser) moved out of the binding pocket within <1 ns (Figure 1b) suggesting that the proposed binding mode is unstable. In contrast when the ligand (L-Ser) was placed in the pocket such that the negatively charged carboxylate group could interact with the positively charged side chain of Lys121 (Figure 1c) and the positively charged amino group was positioned in close proximity to the aromatic side chain of Phe117, the system was stable in multiple simulations of up to 10 ns (the average root mean squared positional deviation, rmsd, for all atoms of ligand during simulation was 0.196 nm). The observation that the alternative orientation is stable is not surprising. In addition to the salt-bridge and cation$-\pi$ interaction, a (water mediated) hydrogen bond forms between the side chain of L-Ser and the backbone carbonyl oxygen of Phe81. Note, an interaction between a negatively charged carboxylate and an aromatic side chain as proposed in the crystal structure is highly unusual.

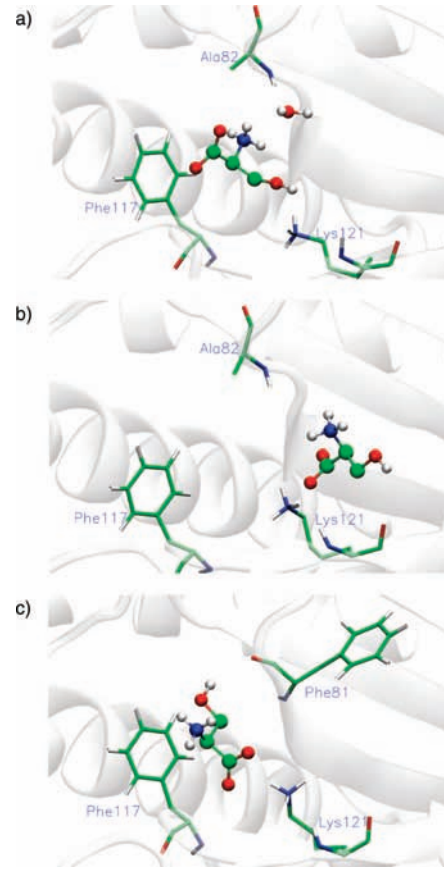


***Figure 1.*** (a) Binding mode of L-Ser to Pab-NTD proposed in the crystal structure 2HKZ. (b) Structure of the Pab-NTD- L-Ser complex with after 1 ns of MD simulation starting from the crystal structure 2HKZ. (c) Alternate model of the Pab-NTD L-Ser complex stable during multiple 10 ns simulations. The ligand (L-Ser) is shown in a ball and stick representation.

This new model suggests that the preference of L-Ser over L-Thr by Pab-NTD is due to the methyl group of Thr projecting into an unfavorable hydrophilic environment. To validate this model, the free energy (FE) of binding of a series of alternative amino acids to Pab-NTD relative to L-Ser was calculated (Table 1). In addition, Lys121 was mutated to Met121 in the presence of L-Ser. From Table 1 it can be seen that D-Ser, L-Cys, D-Cys, and D-Thr are all predicted to bind to Pab-NTD more strongly than L-Ser by between $-3.0$ and $-6.5$ kJ/mol which is in line with the experimental observation that Pab-NTD binds L-Ser, L-Cys, and all D-amino acids.

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***Table 1.*** Relative FE of Binding of the Specific Mutations in Pab-NTD Binding Pocket.

| mutation | fluorescence data[7] | relative FE (kJ/mol) |
|---|---|---|
| L-Ser to D-Ser | binding | $-3.5 \pm 0.6$ |
| L-Ser to L-Cys | binding | $-3.0 \pm 0.5$ |
| L-Ser to D-Cys | binding | $-6.5 \pm 1.1$ |
| L-Ser to D-Thr | binding | $-4.7 \pm 0.8$ |
| L-Ser to L-Thr | no binding | ligand |
| L-Ser to L-Ala | no binding | escapes |
| L-Ser to Gly | no binding | from pocket |
| Lys121Met in protein | no binding to L-Ser | |

In contrast when L-Ser was mutated to L-Thr, L-Ala, or Gly, the ligand escapes from the binding cavity, in line with the experimental observation that Pab-NTD does not bind to L-Thr, L-Ala, or Gly. The reason Gly does not bind may be related to the high entropy of free Gly. Finally, when Lys121 is mutated to Met121, L-Ser was lost from the binding pocket.

D-Ser, D-Cys, and D-Thr all spontaneously adopted a similar binding mode during the simulations. This is illustrated in Figure 2 for the case of D-Thr binding to Pab-NTD. The negatively charged carboxyl of D-Thr forms a salt bridge with the side chain of Lys121 as observed for the binding of L-Ser. The positively charged amino group again lies in close proximity to the aromatic side chain of Phe117 and forms a water-mediated hydrogen bond with the backbone carbonyl oxygen of Phe81. However, in contrast to the binding of L-Ser, the side chain of D-Thr (and by analogy all of other the D-amino acids) projects out of the pocket and is exposed to the solvent. This binding mode can explain how Pab-NTD binds all D-amino acids, including those with large side chains, with similar affinity and suggests a general mechanism for enantiomeric selectivity of the editing domain of aaRS's. The side chains of the L-amino acids project into the protein and are stabilized by specific interactions for a given set of amino acids. In contrast, the side chains of all D-amino acids project out of the active site and binding is nonselective (Figure 3). The main interactions involved in the recognition of the amino and carboxyl groups could vary between different aaRS's.

Taken together, calculations clearly suggest that the orientation of L-Ser proposed by Hussain et al.[7] in their structure of Pab-NTD (PDB 2HKZ) is incorrect. The positioning of small ligands within a binding cavity, however, can represent a major challenge in X-ray crystallography, especially in structures solved at moderate resolution. The ligands frequently show a higher degree of disorder than the surrounding protein, and unless the ligands are covalently bound to the protein, it is not possible to use the geometry of the protein chain to orientate the ligand. In addition, at the resolution at which most proteins are solved, it is not possible to directly correlate electron density to atom type. As a consequence the positioning of small ligands within the pocket of a protein is, at least in part, subjective and frequently based on assumptions in regard to specific local interactions. That critical groups in what is assumed to be an experimental structure can be positioned incorrectly is increasingly being recognized as a major problem in structure-based drug design.[8] A number of groups are, for example, turning to high level approaches in which the ligand and the surrounding interactions are treated quantum mechanically during the process of refinement.[9] While this is in principle the preferred approach, we have demonstrated the possibility of using low cost classical simulations with empirically based force fields in combination with free energy perturbation calculations as a means to verify the proposed binding modes of small ligands in proteins. In particular we have shown that using this approach we could propose a more appropriate model for the structure of the Pab-NTD-L-Ser complex leading to a better understanding of the mechanism of enantiomeric selectivity in aminoacyl-tRNA-synthetases.

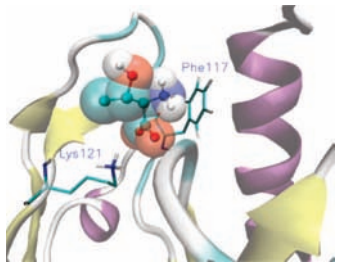


***Figure 2.*** Structure of Pab-NTD complexed with D-Thr. The ligand (D-Thr) is shown in a ball and stick as well as CPK representation.

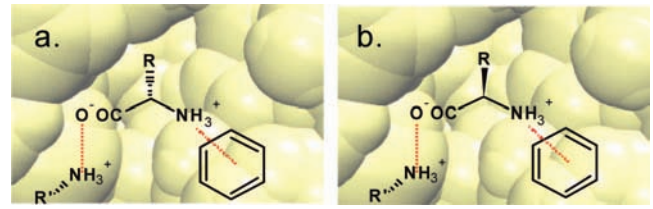


***Figure 3.*** Schematic representation of the mechanism of enantiomeric selectivity of the editing domain of threonyl-tRNA and potentially other aminoacyl-tRNA-synthetases. (a) The side chains of L-amino acids project into the protein forming specific interactions. (b) The side chains of D-amino acids project out of the active site of the editing domain such that all D-amino acids irrespective of size can bind. The broken red lines indicate the main interactions stabilizing the orientation of the amino acid in Pab-NTD (see text).

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**Supporting Information Available:** Details of computational methodology.This material is available free of charge via the Internet at http://pubs.acs.org.

## References

(1) Mason, S. *Chem. Soc. Rev.* **1988**, *17*, 347–359.
(2) (a) Mirande, M. *Prog. Nucleic Acid Res. Mol. Biol.* **1991**, *40*, 95–142. (b) Thompson, D.; Lazennec, C.; Plateau, P.; Simonson, T. *J. Biol. Chem.* **2007**, *282*, 30856–30868.
(3) Ibba, M; Soll, D. *Annu. Rev. Biochem.* **2000**, *69*, 617–650.
(4) Jakubowski, H.; Goldman, E. *Microbiol. Rev.* **1992**, *56*, 412–429.
(5) (a) Beebe, K.; Merriman, E.; Pouplana, L. R.; Schimmel, P. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 5958–5963. (b) Korencic, D.; Ahel, I.; Schelert, J.; Sacher, M.; Ruan, B.; Stathopoulos, C.; Blum, P.; Ibba, M.; Söll, D. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 10260–10265.
(6) Dwivedi, S.; Kruparani, S. P.; Sankaranarayanan, R. *Nat. Struct. Mol. Biol.* **2005**, *12*, 556–557.
(7) Hussain, T.; Kruparani, S. P.; Pal, B.; Dock-Bregeon, A.; Dwivedi, S.; Shekar, M. L.; Sureshbabu, K.; Sankaranarayanan, R. *EMBO J.* **2006**, *25*, 4152–4162.
(8) Davis, A. M.; St-Gallay, S. A.; Kleywegt, G. J. *Drug Discov. Today* **2008**, *13*, 831–841.
(9) Yu, N.; Li, X.; Cui, G.; Hayek, S. A.; Merz, K. M. *Protein Sci.* **2006**, *15*, 2773–2784.

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