The Binding and Enantiomeric Selectivity of Threonyl-tRNA Synthetase

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Supporting Information

Computational Details

Molecular Dynamics Simulations: All simulations were performed using the GROMACS 3.3.1[1] simulation package in conjunction with the GROMOS 53A6[2] force field. The initial structure of Pab-NTD (143 residues) was taken from crystal structure of the L-Ser complex given in the Protein Data Bank (PDB-code 2HKZ). The different complexes of interest (ligand: L-Ser, L-Thr and L-Ala) were generated based on the L-Ser complex using the package PyMol. Each complex was then placed in a rectangular periodic box and solvated with approximately 6270 simple point charge (SPC) water molecules.[3] The protonation state of titratable groups was choosen appropriate to pH 7.0 giving a total charge on the system of -4 e. No counter ions were added. The structure was energy minimized and the system equilibrated for 200 ps with the heavy atoms of the protein positionally restrained before commencing a series of unrestrained molecular dynamics (MD) simulations. All simulations were performed at constant temperature (298 K) and pressure (1 atm) using a Berendsen thermostat (coupling time of 0.1 ps) and barostat (coupling time of 1.0 ps and isothermal compressibility of 4.575×10^{-4} $(\text{kJ/mol/nm}^3)^{-1}$).[4,5] A triple-range cutoff was used. Interactions within a shorter-range cutoff of 0.8 nm were updated every step (0.002 ps). Interactions within the longer-range cutoff of 1.4 nm were updated very 0.010 ps together with the pairlist. To correct for the truncation of electrostatic interactions beyond the 1.4 nm long-range cutoff a reaction-field correction was applied using a dielectric permittivity of 78. The equations of motion were integrated using the leapfrog scheme. Initial velocities at a given temperature were taken from a Maxwell-Boltzmann distribution. All bonds were constrained using the SHAKE algorithm with a geometric tolerance of 0.0001.[6]

Free Energy Calculations: The change in Gibbs free energy associated with the mutation of the ligand from L-Ser (in the proposed binding mode) to D-Ser, L-Cys, L-Thr, L-Ala, and Gly as well as from D-Ser to D-Cys, D-Thr and Lys121Met mutation in protein was determined using the coupling parameter approach in conjunction with the thermodynamic integration formula.[7].

$$\Delta G = \int_{\lambda=0}^{\lambda=1} \left\langle \frac{\partial H}{\partial \lambda} \right\rangle_{\lambda} d\lambda \qquad 1$$

where $\lambda = 0$ corresponded to the initial state of the system and $\lambda = 1$ corresponded to the final state of the system. *H* is the Hamiltonian of the system and the brackets $\langle ... \rangle_{\lambda}$ correspond to an average over an equilibrium ensemble at λ. The relative free energy of binding $\Delta\Delta G$ was determined from the difference in the change in free energy of performing the same mutation free in solution and bound to the proteins. Equation one was integrated by performing separate simulations at a series of 25 (0.00, 0.01, 0.02 … 0.05, 0.10, 0.15, 0.20 … 0.90, 0.95, 1.00) λ points in both the bound and unbound states. The systems were first equilibrated for 200 ps, and 800 ps used to estimate $\langle \partial H / \partial \lambda \rangle_{\lambda}$. To prevent numerical instabilities as atoms were created or destroyed the soft-core potential as described by Beutler et al. was used[8,9] with $\alpha_{ij}^{LJ} = \alpha_{ij}^{C} =$ 0.5 nm^2. The area beneath the curve in 1 was estimated using a trapezoidal approximation. The statistical error at each λ-point was estimated using a block averaging technique.[10]

Note, an attempt was also made to estimate the free energy differences in the orientation of the ligand as originally proposed in the crystal structure but this was not possible as L-Ser either rapidly flips to adopt the proposed binding mode during the simulation or lost from the binding pocket.

References

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