

Role of Protein Electrostatics on the Post-transfer Editing Function of *Escherichia coli*



Prolyl-tRNA Synthetase

Bach Cao[†], Karl J. Meitzner[†], Mathew J. Tschudy[†], Karin Musier-Forsyth[‡], Sudeep Bhattacharyya[†], and Sanchita Hati[†]

[†]Department of Chemistry, University of Wisconsin–Eau Claire, WI-54702

[‡]Departments of Chemistry and Biochemistry, The Ohio State University, Columbus, OH, 43210

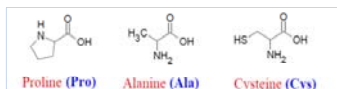
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Abstract

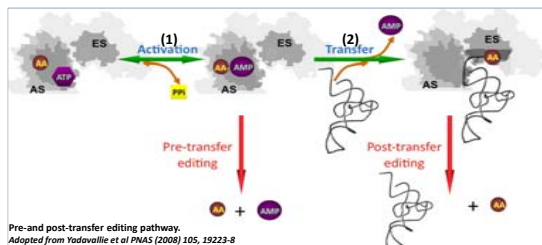
Prolyl-tRNA synthetases (ProRSs) are class II synthetases that catalyze covalent attachment of proline to the 3'-end of the tRNA^{Pro}. ProRSs from all three kingdoms of life have shown to misactivate noncognate alanine and cysteine, and form mischarged aminoacyl-tRNA^{Pro}. The insertion domain (~180 amino acids) of *Escherichia coli* ProRS is the post-transfer editing active site that hydrolyzes specifically mischarged alanyl-tRNA^{Pro}. The highly conserved lysine 279 (K279) in the insertion domain is critical for the post-transfer editing reaction and previous studies have shown that mutation of this lysine to alanine is detrimental to the post-transfer editing function of the enzyme. The exact mechanism through which K279 catalyzes the post-transfer editing reaction has remained poorly understood. In an attempt to gain insight into the mechanism of post-transfer editing reaction of *Escherichia coli* ProRS, the pK_a calculations of the K279 have been performed using combined quantum mechanical and molecular mechanical (QM/MM) simulations. Herein, we report the effect of charged residues on the pK_a of K279 and thereby, on the post-transfer editing function of *Escherichia coli* prolyl-tRNA synthetase. These computational results are also validated through site-directed mutagenesis.

Background

Prolyl-tRNA synthetases (ProRSs) are multi-domain proteins and members of class IIa synthetases. These enzymes catalyze the formation of prolyl-tRNA^{Pro} in a two step reaction: (1) and (2). However, bacterial ProRSs misactivate non-cognate alanine and cysteine and form alanyl-tRNA^{Pro} and cysteinyl-tRNA^{Pro}.



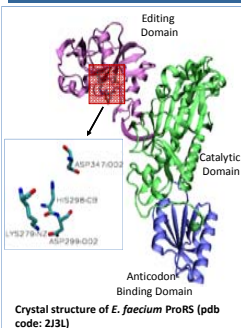
To maintain high fidelity in protein synthesis, several bacterial ARS's have developed pre- and post-transfer editing mechanisms to prevent misaminoacylation of tRNA.



- ✦ Editing domain is the site of post-transfer editing reaction in *Escherichia coli* (Ec) ProRS and K279 is critical for the post-transfer editing reaction.¹
- ✦ Proposed role: To position the 3'-CCA-end of tRNA^{Pro} in the editing active site through interaction with the phosphate group of A76.

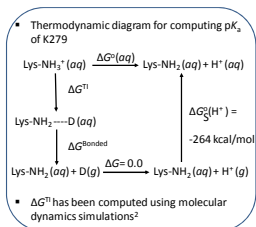
Objectives

- ✦ Explore the role of protein electrostatics on the pK_a of the K279.
- ✦ pK_a calculations of the WT enzyme using QM/MM method and verification of the theoretical results through mutational study.
- ✦ *E. Faecium* (Ef) and Ec ProRSs are prokaryotic-like ProRS's with an editing domain inserted between motifs 2 and 3 of the catalytic domain. These two bacterial ProRSs possess about 45% sequence identity.
- ✦ All calculations are done using 3D crystal structure of Ef ProRS and mutational studies are done using Ec ProRS.

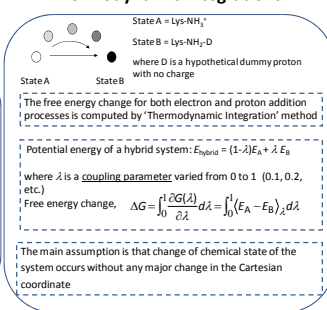


Computational Methodology

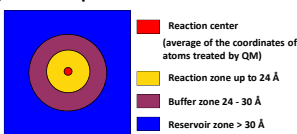
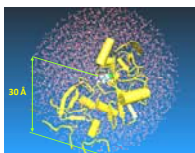
Quantum Mechanical/Molecular Mechanical (QM/MM) Simulations



Thermodynamic Integrations



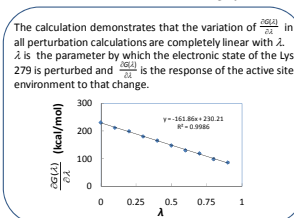
QM/MM Setup



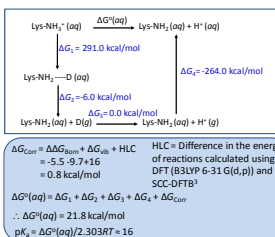
- 30 Å water sphere added around the editing active site center: Lys 279
- Deleting all atoms beyond 30 Å
- Stochastic boundary condition
- Explicitly treated water molecules are modeled by TIP3P
- The charge of the 30 Å solvated enzyme was made 0 by putting counter ions

Results

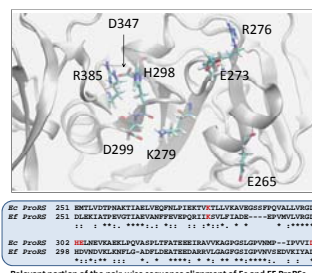
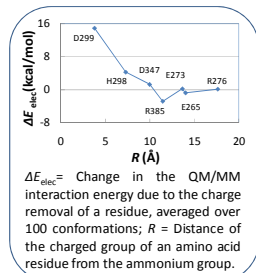
Linear Variation of $\frac{\partial G(\lambda)}{\partial \lambda}$



Free Energy Changes



Quantifying the Effects of the Electrostatic Environment

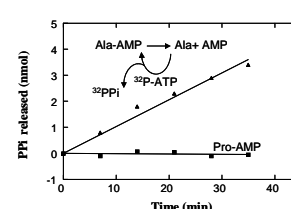


Experimental Methods

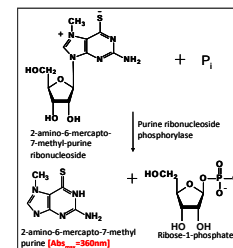
Pre-transfer Editing Reaction



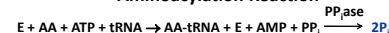
a) Radioactive Assay⁴



b) Spectroscopic Assay⁵



Aminoacylation Reaction

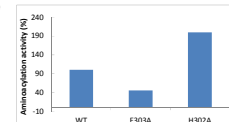


Experimental Results

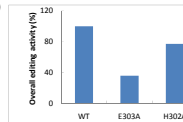
Relative amino acid activation efficiencies of WT and mutated proteins

Enzyme	Amino Acid	K_{cat} (s ⁻¹)	$K_{\text{cat}}/K_{\text{M}}$ (s ⁻¹ mM ⁻¹)	$k_{\text{cat}}/K_{\text{M}}$ (relative)
WT	Proline	076	7.96×10^4	10.3×10^3
	Alanine	5.37	8.67×10^4	1.61×10^4
E303A	Proline	702	7.97×10^4	1.13×10^3
	Alanine	5.48	1.59×10^4	0.29×10^4
H302A	Proline	1.023	1.28×10^3	1.25×10^3
	Alanine	1.16	3.93×10^4	3.38×10^4

B)



C)



Bar graph comparing A) the pre-transfer editing: stimulation of ATP hydrolysis in the presence of 5 mM alanine, B) the aminoacylation of tRNA^{Pro} in the presence of 10 μM tRNA and 1 mM proline and C) the overall editing in the presence 10 μM tRNA and 5 mM alanine by wild-type E. coli ProRS and two mutants (H302A and E303A), with the wild-type ProRS activity set at 100%. The enzyme concentrations used were 1 μM (BioRad concentration). Values reported are the average of two or three experiments with <20% difference between trials.

Conclusions

- ✦ The computed pK_a of K279 of Ef ProRS is ~16, which is five units higher than the free lysine (pK_a=10.8). The protonated state of the lysine is important for the interaction with the phosphate group of the tRNA^{Pro}.
- ✦ The protonated state of the lysine is stabilized by the surrounding charged residues like D299, H298, and D347, whereas destabilized by the charged residues like R385 and E265.
- ✦ The preliminary mutational data supports the theoretical findings that the salt-bridge interaction between D299 and K279 is critical for the post-transfer editing reaction by the *E. coli* ProRS.
- ✦ Previous mutational study by Wong *et al.*¹ has shown that D350 (D347 of Ef ProRS) has profound effect on post-transfer editing reaction by *E. coli* ProRS which is in agreement with the computed results.

Future Directions

- ✓ Compute the pK_a of K279 (Ef ProRS) by mutating the H298 and D299 to alanine.
- ✓ To determine the kinetic parameters for the amino acid activation and post-transfer editing reaction by Ec ProRS using active-site concentrations of enzymes.
- ✓ Examine the post-transfer editing reaction of the double mutant K279D and D299K in order to probe the exact role of K279.
- ✓ To explore the effect of R385A mutation on the post-transfer editing function.

References:

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