Probing the Role of Highly Conserved Lysine (K279) Residue in the Editing Domain of Bacterial Prolyl-tRNA Synthetases

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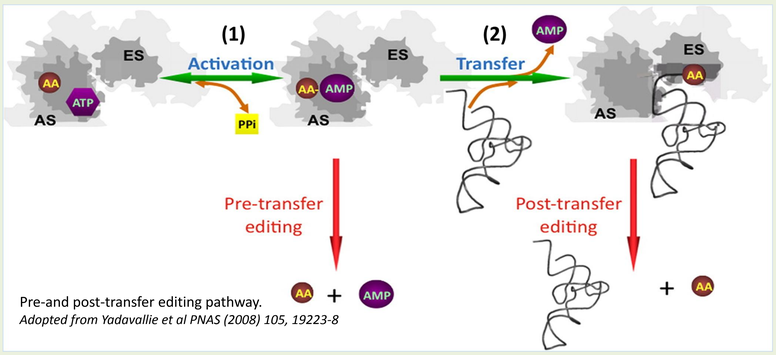
Abstract

The *Escherichia coli* (Ec) prolyl-tRNA synthetase enzyme possesses three distinct domains. Each of these three protein domains has a unique function towards attaching proline to $tRNA^{Pro}$, a charged $tRNA^{Pro}$. One of those domains, the editing domain, is critical for proofreading the charged $tRNA^{Pro}$, ensuring the correct amino acid has been attached to the tRNA molecule. In this editing domain, a highly conserved lysine has been found to be absolutely essential for proofreading, however, its exact role is not yet clear. Through site-directed mutagenesis and enzyme kinetic studies we have attempted to probe the role of this lysine residue (K279). Herein, we will present some of our preliminary results.

Background

- Prolyl-tRNA synthetases (ProRSs) are multi-domain proteins
- Members of class IIa synthetases
- Catalyze the formation of prolyl-$tRNA^{Pro}$ in a two step reaction: (1) and (2)
- Errors can occur in the reaction and a misactivated $tRNA^{Pro}$ is formed
- High fidelity is maintained by attaching a **pre** and **post-transfer** editing domain to ARS (aminoacyl-tRNA synthetases)

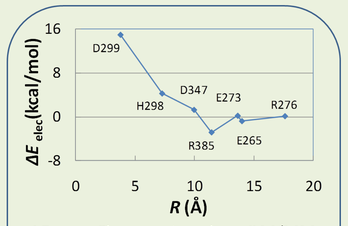


Pre-and post-transfer editing pathway.
Adopted from Yadavallie et al PNAS (2008) 105, 19223-8

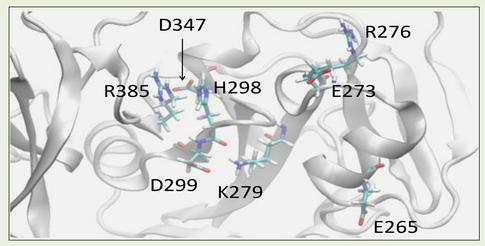
- Editing domain is the site of post-transfer editing reaction in Ec ProRS[1]
  - K279 is critical for the post-transfer editing reaction
- Proposed role: To position the 3'-CAA-end of $tRNA^{Pro}$ in the editing active site through interaction with the phosphate group of A76

Previous Work

- The computed pK_a (using Quantum Mechanical/Molecular Mechanical Method[3,4]) of K279 of Ef ProRS is ~16, which is five units higher than the free lysine (pK_a=10.8)
- H298, D299, and D347 of Ef ProRS correspond to H302, E303, and D350 of Ec ProRS
- Computational studies suggest that these three residues stabilized the protonated state of the K279 where as R385 destabilized it
- The protonated state of the lysine is important for either of the following two reasons-
  - the interaction with the phosphate group of the $tRNA^{Pro}$
  - stabilizing structure via a salt bridge with D299 or interacting with the tRNA backbone (phosphate)



ΔE_{elec}= Change in the QM/MM interaction energy due to the charge removal of a residue, averaged over 100 conformations; R = Distance of the charged group of an amino acid residue from the ammonium group.

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Ec ProRS   251 EMTLVDTPNAKTIAELVEQFNLPIEKTVRTLLVKAVEGSSFPQVALLVRGD
Ef ProRS   251 DLEKIATPEVGTIAEVANFFEVEPQRIIKSVLFIADE----EPVMVLVRGD
               ::  : **:. ****:.: *::  :: :*::*. * *      * :*****

Ec ProRS   302 HELNEVKAEKLPQVASPLTFATEEEIRAVVKAGPGSLGPVNMP--IPVVID
Ef ProRS   298 HDVNDVKLKNFLG-ADFLDEATEEDARRVLGAGFGSIGPVNVSEDVKIYAD
               *::*:** :::   *. *  ****: * *: ** **:****:.  : :  *
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Relevant portion of the pair-wise sequence alignment of Ec and EF ProRSs

Experimental Design

- Potential roles of K279 for the post-transfer editing reaction are to stabilize the over all tertiary structure of the post-transfer editing domain through a salt-bridge interaction with D299 and/or to position the 3'-CCA end of the misacylated–$tRNA^{Pro}$ into the editing active site was investigated by performing kinetic studies with wild-type and several mutant proteins. Site of mutations were determined based on computational results and three-dimensional structure analysis.

Spectroscopic Method

- Aminoacylation and editing activity of the wild-type and mutant variants were performed using the spectroscopic method developed by Lloyd et al.[5]

Aminoacylation Reaction:

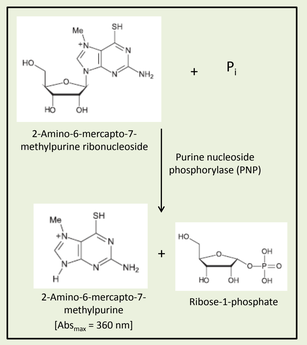
$E + Pro + ATP + tRNA^{Pro} \rightarrow Pro\text{-}tRNA^{Pro} + E + AMP + PP_i$

Pre-transfer Editing Reaction:

$E + ALA + ATP \leftrightarrows E.Ala\text{-}AMP + PP_i \rightarrow E + ALA + AMP + PP_i$

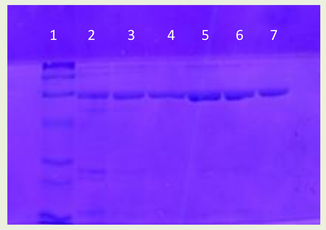
$PP_i + PP_i ase \rightarrow 2P_i$

Quantification of P_i using PNP

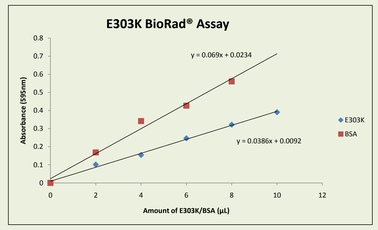


Experimental Results

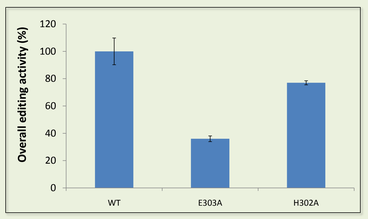
- We have purified some of the mutant proteins (shown in the gel picture)
- Protein concentration was determined through BioRad® assays



Purified mutant proteins: Lane 1 - Standard, Lane 2 - H302A, Lane 3 - E303K/K279E (DM), Lane 4 - E303D, Lane 5 - E303K, Lane 6 - E303A, Lane 7 - WT

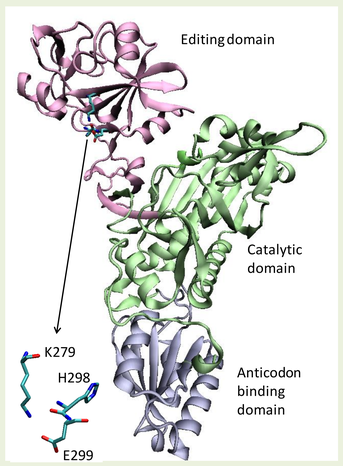


Determining the concentration of purified protein with a BioRad® assay by using bovine serum albumin for a standard curve and measuring the absorbance at 595 nm of the protein/ BioRad® dye solution. This graph shows the result of a BioRad® assay of a mutated ProRS, E303K.



The overall editing in the presence 10 µM tRNA and 5 mM alaline 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.

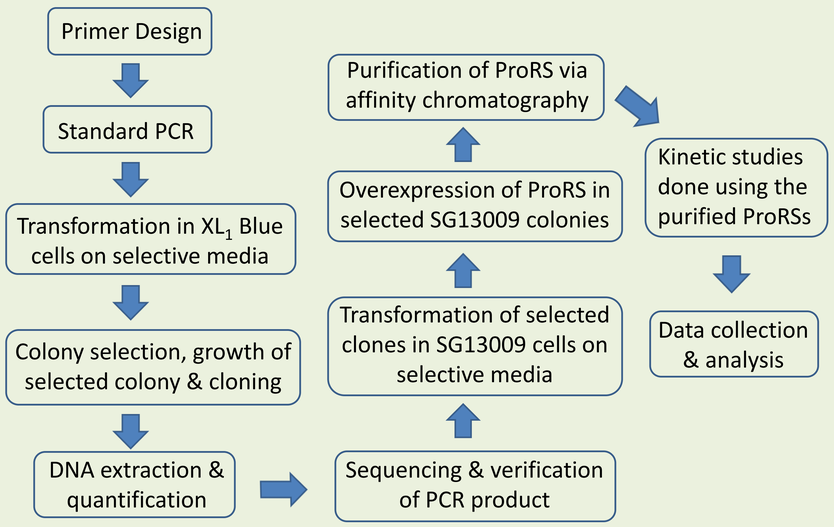
Objectives



Crystal structure of *E. faecium* ProRS (pdb code: 2J3L)[2]

- Explore the role of surrounding polar residues on the pK_a of K279
- Determine the post-transfer editing efficiency of ProRS variants as a result of site directed mutagenesis of the neighboring residues of K279
- *E. Faecium* (Ef) and Ec ProRSs are pro-karyotic-like ProRSs with an editing domain inserted between motifs 2 and 3 of the catalytic domain
- These two ProRSs possess about 45% sequence identity
- All calculations are done using 3D crystal structure of Ef ProRS
- Mutational studies are done using Ec ProRS

Experimental Methods



Conclusions

- Reduced editing activity efficiency of the H302A and E303A suggests that these two polar residues are indeed critical for stabilizing the protonated state of K279. However, detailed kinetic studies, especially with the double mutant (K279E/E303K), will help to better understand the exact role of K279 in the post-transfer reaction

Future Directions

- Perform kinetic studies for all the purified mutants after determining the active concentration of these proteins
- Specifically testing the post-transfer editing reaction - hydrolysis of the misactivated $tRNA^{Pro}$ (Ala-$tRNA^{Pro}$)

References:
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2. Crepin, T., Yaremchuk, A., Tukalo, M., and Cusack, S. (2006) Structure 14, 1511-1525.
3. Riccardi, D., Schaefer P., and Cui. Q.(2005) *Phys. Chem. B*, *109*, 17715-17733.
4. Rauschnot , J. C., Yang, C., and Yang, V., and Bhattacharyya, S. (2009) *J. Phys. Chem. B*, *113*, 8149-57.
5. Lloyd, A. J., Thomann, H. U., Ibba, M., and Soll, D. (1995) *Nucleic Acids Res., 23*, 2886-2892.