Role of Coupled-Domain Motions on the Catalytic Activity of *Escherichia coli* Prolyl-tRNA Synthetase

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Abstract

Prolyl-tRNA synthetases (ProRSs), which 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-$tRNA^{Pro}$. It has been found that the insertion domain (≈ 180 amino acids) of *Escherichia coli* (Ec) ProRS is the post-transfer editing active site that hydrolyzes specifically mischarged alanyl-$tRNA^{Pro}$. Earlier studies demonstrated that deletion of this editing domain has a profound impact on the amino acid activation efficiency of Ec ProRS, suggesting inter-domain communication may play a critical role in this enzyme's function. To explore the role of specific structural element(s) on domain-domain communication, we have employed a combination of computational and biochemical strategies. Herein, we report the effect of mutations on highly conserved residues (G217 and E218) located on the loop connecting the catalytic and editing domains of Ec ProRS. These two residues are about 15 Å apart from a catalytically significant proline-binding loop (residues 198-206). Normal mode analysis (NMA) of Ec ProRS revealed that the E218 containing loop is engaged in strong correlated motion with the proline-binding loop. The combined NMA and mutational studies suggest that coupled domain motions facilitate domain-domain communication in this enzyme and, therefore, are critical for efficient catalysis.

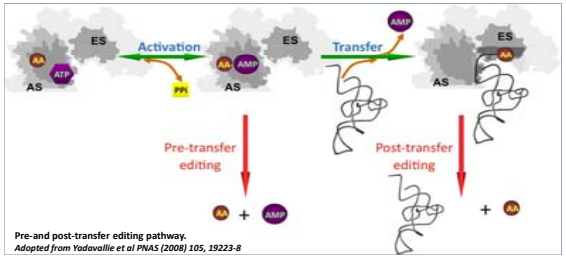
Background

Aminoacyl-tRNA synthetases (ARS's) are multi-domain proteins which are responsible for catalyzing the aminoacylation of tRNA in a two step reaction.

$$AA + ATP + ARS \rightarrow AA\text{-}AMP.ARS + PP_i \quad (i)$$

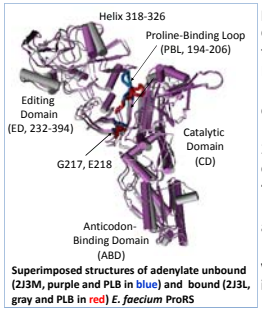
$$AA\text{-}AMP.ARS + tRNA \rightarrow AA\text{-}tRNA + AMP + ARS \quad (ii)$$

This two-step aminoacylation process is a critical step in the translation of the genetic code and involves a series of events including the selection of the correct amino acid and its activation in the presence of ATP, recognition of the cognate tRNA, transfer of the activated amino acid onto the cognate tRNA, release of the aminoacylated tRNA from the enzyme active site. To maintain high fidelity in protein synthesis, several bacterial ARS's have developed **pre-**and **post-transfer** editing mechanisms to prevent misaminoacylation of tRNA.



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

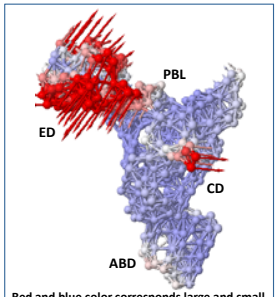
Objectives



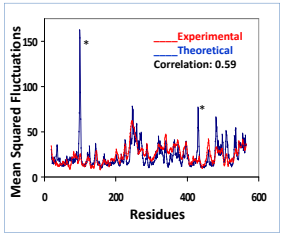
Superimposed structures of adenylate unbound (2J3M, purple and PLB in blue) and bound (2J3L, gray and PLB in red) *E. faecium* ProRS

❖*E. Faecium* (Ef) and *E. coli* (Ec)ProRSs are prokaryotic-like ProRS's with an editing domain inserted between motifs 2 and 3 of the catalytic domain. These two bacterial ProRS possess about 45% sequence identity.
❖Editing domain is the site of post-transfer editing reaction in Ec ProRS.[1]
❖Deletion of the editing domain resulted in a 200-fold increase of K_m for proline. The overall activation efficiency was decreased by ~1200-fold relative to the wild-type enzyme.[2]
❖Closed conformation of PBL is important for adenylate binding.
➢**Explore the role of coupled motions of various structural elements of *E. coli* ProRS in its enzymatic function.**
➢**Examine the effect of GLOBAL motion on the LOCAL motion of ProRS**.

Collective Motions in Ef ProRS



Red and blue color corresponds large and small fluctuations, respectively, of C_α atoms.

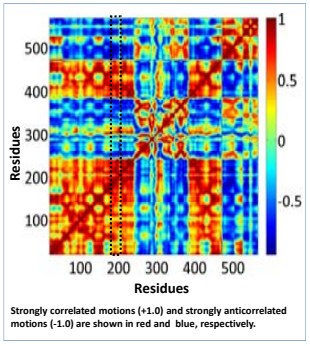


Properties	Mode 1	Mode 2	Mode 3
Overlap	0.65	0.67	0.67
Correlation	0.04	0.12	0.24
Collectivity	0.59	0.54	0.40

❖The online server http://ignmtest.ccbb.pitt.edu/cgi-bin/anm/anm1.cgi was used to calculate normal modes and analyze the functional motions of Ef ProRS.
❖**Overlap** measures the extent to which a mode describes the experimentally observed displacements.
❖**Correlation** describes relative magnitude of the atomic displacements determined experimentally and in a given mode.
❖**Collectivity** describes the extent by which various structural elements undergo a conformational change together.

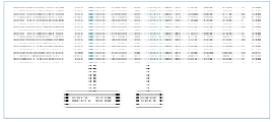
➢In the present study, all three lowest-frequency modes (1 -3) have very similar overlap values, so a combination of these three modes has been used to identify the correlated /anticorrelated motions among various protein segments.

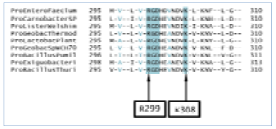
Cross-correlations Map



Strongly correlated motions (+1.0) and strongly anticorrelated motions (-1.0) are shown in red and blue, respectively.

➢It has been reported that nature has conserved all residues that are critical for domain dynamics[5,6]





➢Alanine-scanning mutagenesis was performed for these five residues.

Various Structural Elements of Ef ProRS	Motion with respect to PBL (194-206)
Residues 64-81, 128-164, and 435-465 of CD	Highly correlated
Residues 232- 292 and residues 310-380 of ED	Mainly anticorrelated
Residues 293-309 of ED	Highly correlated
G217 and 218 containing loop	Highly correlated
Residues 506-565 of ABD	Anticorrelated

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➢ UWEC-ORSP
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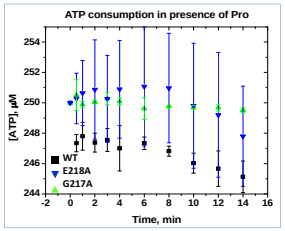
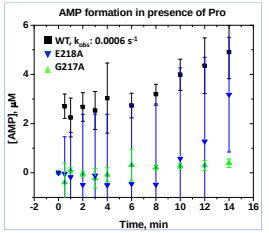
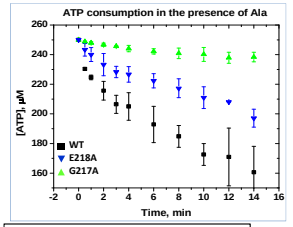
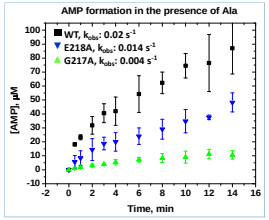
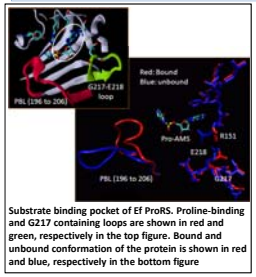
Experimental Results











Substrate binding pocket of Ef ProRS. Proline-binding and G217 containing loops are shown in red and green, respectively in the top figure. Bound and unbound conformation of the protein is shown in red and blue, respectively in the bottom figure

➢G217 is critical for amino acid activation.
➢ E218A activates alanine comparable to WT enzyme but proline activation is affected by the mutation of E218.
➢ E218A hydrolyzes Ala-AMP but weaker than WT enzyme.
➢Although it has been reported that the interaction between E218 and R151 is important for stabilizing the bound adenylate,[7] our work suggests that this G217-E218 containing loop is important for maintaining protein dynamics of other critical structural elements like proline-binding loop.

Conclusions

- The present normal mode analysis demonstrated that a strong anticorrelated motion exists between the editing domain and the proline-binding loop of ProRS. This very existence of anticorrelated motion is critical for the conformational change of PBL required for adenylate binding. Deletion of the editing domain, therefore, had such a drastic effect on amino acid activation Ec ProRS.[2]
- The conformational change of the proline-binding loop, essential for adenylate binding, is also affected by the motion of the G217-E218 containing loop that joins the editing domain with the catalytic domain. Any disturbances in this loop (~15 Å apart from the proline-binding loop) has impact on proline activation.
- The preliminary pre-transfer editing results (AMP formation) also demonstrate that coupled motions of G217-E218 containing loop and catalytic domain is important for editing function of Ec ProRS.

References

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