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Mechanism and evolution of multidomain aminoacyl-tRNA synthetases revealed by their inhibition by analogues of a reaction intermediate, and by properties of truncated forms

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ABSTRACT

Many enzymes which catalyze the conversion of large substrates are made of several structural domains belonging to the same polypeptide chain. Transfer RNA (tRNA), one of the substrates of the multidomain aminoacyl-tRNA synthetases (aaRS), is an L-shaped molecule whose size in one dimension is similar to that of its cognate aaRS. Crystallographic structures of aaRS/tRNA complexes show that these enzymes use several of their structural domains to interact with their cognate tRNA. This mini review discusses first some aspects of the evolution and of the flexibility of the pentadomain bacterial glutamyl-tRNA synthetase (GluRS) revealed by kinetic and interaction studies of complementary truncated forms, and then illustrates how stable analogues of aminoacyl-AMP intermediates have been used to probe conformational changes in the active sites of *Escherichia coli* GluRS and of the nondiscriminating aspartyl-tRNA synthetase (ND-AspRS) of *Pseudomonas aeruginosa*.

Keywords: Multidomain Enzymes; tRNA; Aminoacyl-tRNA Synthetases; Truncated Enzymes; Steady-State Kinetics; Inhibitors; Mechanism; Evolution

1. THE MULTIDOMAIN ENZYMES: ASPECTS OF THEIR EVOLUTION AND OF THEIR FLEXIBILITY

Multidomain enzymes probably evolved from an ancestral domain containing the active site, to which were added during evolution of other domains which increased their catalytic efficiency (k_{cat}), and/or improved their specificity for their substrate(s). A beautiful and relatively simple illustration of this model, reviewed by Branden and Tooze [1] in their excellent "Introduction to protein structure", is the structure and mechanism of chymotrypsin, made of two antiparallel β-barrel domains; this serine protease which cleaves peptide bonds using the catalytic triad serine/histidine/aspartate (residues not adjacent in this polypeptide), harbors in one domain the serine (Ser) residue, which is the most important one for this catalysis, and harbors in the other domain the histidine (His) and aspartate (Asp) residues whose presence strongly increases the k_{cat} without altering significantly the K_M of this enzyme for its substrates. These results suggest that the His and Asp residues of the catalytic triad are not absolutely essential for the catalytic activity, and therefore the ancestral chymotrypsin may have been a single domain containing only the catalytic Ser residue.

The aminoacyl-tRNA synthetases (aaRS) are multidomain enzymes which play a central role in the correct translation of genetic information into proteins (reviewed by Ataide and Ibba [2], and by Giegé *et al*. [3]). Each member of this family of about 20 enzymes interacts with ATP and a specific amino acid to catalyze its activation in the form of an aminoacyladenylate, a high-energy intermediate where the α-COOH group of the amino acid forms an acid anhydride bond with the phosphate of AMP. This enzyme-bound intermediate then esterifies an OH group at the 3'-end of a tRNA corresponding to that amino acid according to the genetic code, yielding an aminoacyl-tRNA (reviewed by First [4]) which is used

either for the polymerisation of amino acid residues on the ribosome, or for other metabolic processes not related to translation biosynthetic (reviewed by Lapointe and Giegé [5]). As noted by Schimmel, Giegé, Moras and Yokoyama in their 1993 review [6], the aaRS structures are organized, to a rough approximation, into two major domains: one containing the active site which interacts with the ancestral part of tRNA including the acceptor end, and one generally less or not conserved which provides for interactions with the second domain of tRNA, including the anticodon.

Some structural domains in several multidomain enzymes change their relative orientations upon binding substrates or other ligands, often by rigid-body motions allowed by the flexible hinges linking adjacent domains. Using a bioinformatic method named "computational solvent mapping", Chuang *et al*. [7] compared ten different crystallographic structures of a multidomain enzyme, and were able to detect significant changes in binding sites and interdomain crevices at a higher resolution than that provided by superposing these X-ray structures, revealing conformational changes even at an overall root mean square deviation (RMSD) that is close to the expected error in the atomic coordinates. In the case of the pentadomain glutamyl-tRNA synthetase (GluRS) of *Thermus thermophilus*, crystallographic structures determined in the absence and in the presence of the cognate $tRNA^{Glu}$ substrate [8] revealed the reorientation, without changing their folds, by a 6˚ rotation of domain 4 relative to domain 3 (at the transition between the active site domains 1 to 3, and the anticodon arm-binding domains 4 and 5), and by a 8˚ rotation of the C-terminal domain 5 relative to domain 4. These $tRNA^{Glu}$-triggered domain reorientations result in a switch between ATP binding to a non-productive site in the absence of $tRNA^{Glu}$ (glutamate is not activated by GluRS alone) to a productive site in its presence.

2. ASPECTS OF GLURS MECHANISM AND EVOLUTION REVEALED BY KINETIC AND INTERACTION STUDIES OF COMPLEMENTARY TRUNCATED FORMS OF THIS PENTADOMAIN ENZYME

The above-mentioned GluRS domain reorientations prompted us to test the importance on GluRS activity of the covalent connectivity between some of these adjacent domains. For answering these questions, we used the GluRS of a mesophilic bacteria, *Escherichia coli*, because kinetic and interaction studies are performed more easily with it than with the corresponding enzyme of a thermophile. The high level of identity between the amino acid sequences of *E. coli* and *T. thermophilus* GluRSs allowed us to use an alignment of their sequences and the known crystalline structure of the latter [8] to identify the position of the hinges linking the structural domains of *E. coli* GluRS [9].

We constructed vectors allowing the overproduction of two sets of truncated GluRSs: domains 1 to 3, and domains 4 + 5; and domains 1 to 4, and free domain 5. Their kinetic characterization showed first that the two C-truncated GluRS (1 − 3) and GluRS (1 − 4) have very low k_{cat} in the tRNA glutamylation reaction (about 2000-fold lower than that of full-length GluRS), but that their K_M values for their substrates (ATP, glutamate and $tRNA^{Glu}$) are nearly identical to those of the full-length GluRS (1 − 5). A similar result for GluRS (1 − 3) was reported by Dasgupta *et al*. [10]. The major importance of k_{cat} in the recognition between aminoacyl-tRNA synthetases and tRNAs was initially reported by Ebel *et al*. in 1973 [11] (reviewed by Giegé and Springer [12]).

GluRS (1 − 3) glutamylation activity was not complemented by GluRS (4 + 5), indicating the importance of the covalent linkage between domains 3 and 4 for efficient activity. On the other hand, GluRS (1 − 4) activity was stimulated up to 100-fold by free domain 5 [9], located about 70 Å away from the active site. No interactions between the complementary GluRS (1 − 4) and free domain 5 were detected by white light interferometry with a nanoporous silicon biosensor [13], but K_D values of 0.11 and 1.2 μM, respectively, were measured for the interactions with $tRNA^{Glu}$ of these two truncated GluRSs (compared to 0.5 μM for full-length GluRS), using the quenching of the tryptophan fluorescence of these proteins. These results suggest, first, that an ancestral form of GluRS had only the first 4 domains, and that it evolved and became more efficient by the addition of an ancestral C-terminal domain 5, and secondly that at least a part of the information present in the anticodon identity elements of $tRNA^{Glu}$ is transferred to the active site of GluRS through the $tRNA^{Glu}$ backbone. Based on the structure of the *T. thermophilus* GluRS/$tRNA^{Glu}$ complex, domain 5 interacts with the first nucleotide of the anticodon, which in *E. coli* $tRNA^{Glu}$ is a modified U (5-methylamino, 2-thioU), which is an important identity element for the recognition of *E. coli* $tRNA^{Glu}$ by its GluRS ([14] and references therein).

For *E. coli* glutaminyl-tRNA synthetase, closely related to GluRS in its evolution and mechanism, but containing anticodon-binding domains with different topologies than those of GluRS (and therefore not related evolutionarily), a model of intraprotein communication between the anticodon-binding domains and the active site has been proposed by Weygand-Durasevic *et al*. [15].

3. STABLE ANALOGUES OF AMINOACYL-AMP INTERMEDIATES USED TO REVEAL CONFORMATIONAL CHANGES IN THE ACTIVE SITES OF *E. COLI* GLURS AND OF THE NONDISCRIMINATING ASPARTYL-TRNA SYNTHETASE (ND-AspRS) OF *PSEUDOMONAS AERUGINOSA*

As intermediates in the aminoacylation reaction, the aminoacyladenylates (aa-AMP) stand in the active sites of their cognate aaRSs. Stable analogues of some aa-AMP are good inhibitors of the cognate aaRS (reviewed by Chênevert *et al*. [16], and by Vondenhoff and Van Aerschot [17]. We used some of them as reporters of the influence of tRNA on the structure of the active site.

As mentioned above from crystallographic structures of *T. thermophiles* GluRS +/− $tRNA^{Glu}$, the presence of tRNA allows the correct positioning of ATP in the active site. Using isothermal microcalorimetry, we measured the free energy of binding of Glu-AMS (glutamylsulfamoyl adenosine, the strongest known inhibitor of GluRS, with a K_i of about 3 nM [18]) to *E. coli* GluRS, and found that the presence of $tRNA^{Glu}$ increases about 10-fold the binding energy of this inhibitor to this enzyme (Blais, S., Bonnaure, G., Kornblatt, J. and Lapointe, J., unpublished results), in qualitative agreement with the structural results.

We noticed a biphasic inhibition by L-aspartol adenylate of the ND-AspRS of *Pseudomonas aeruginosa*, when unfractionated tRNA containing both $tRNA^{Asp}$ and $tRNA^{Asn}$ from these bacteria was used as substrate in the aminoacylation reaction [19]. This nondiscriminating AspRS aspartylates both $tRNA^{Asp}$ and $tRNA^{Asn}$, and the incorrectly charged Asp-$tRNA^{Asn}$ is then transamidated into Asn-$tRNA^{Asn}$ by a tRNA-dependent amidotransferase [20], these two enzymes catalyzing the indirect pathway of Asn-tRNA biosynthesis (reviewed by Huot *et al*. [21]). We then separated these two tRNAs and found that the K_i of L-aspartoladenylate for this ND-AspRS was 41 µM for $tRNA^{Asp}$aspartylation, and 215 µM for $tRNA^{Asn}$ aspartylation, indicating that the two different tRNA substrates of this enzyme interact differently with its active site. This result is consistent with the observation of structural changes in the active site of yeast AspRS upon $tRNA^{Asp}$ binding [22].

More generally, we conclude from these results that aminoacyladenylate analogs, which are competitive inhibitors of their cognate aminoacyl-tRNA synthetase, can be used to probe rapidly the role of various structural elements in positioning the tRNA acceptor end in the active site.

REFERENCES

[1] Branden, C. and Tooze, J. (1999) Introduction to protein structure. 2nd Edition, Garland Publishing, Inc., New York.

[2] Ataide, S.F. and Ibba, M. (2006) Small molecules: Big players in the evolution of protein synthesis. *ACS Chemical Biology*, **1**, 285-297. http://dx.doi.org/10.1021/cb600200k

[3] Giege, R., Touze, E., Lorber, B., Theobald-Dietrich, A. and Sauter, C. (2008) Crystallogenesis trends of free and liganded aminoacyl-tRNA synthetases. *Crystal Growth & Design*, **8**, 4297-4306. http://dx.doi.org/10.1021/cg8007766

[4] First, E.A. (2005) Catalysis of the tRNA aminoacylation reaction. In: Ibba, M., Francklynand C. and Cusack, S., Eds., *The Aminoacyl-tRNASynthetases*, Landes Bioscience, Georgetown, pp. 328-352.

[5] Lapointe, J. and Giegé, R. (1991). Transfer RNAs and aminoacyl-tRNA synthetases in eukaryotes. In: H. Trachsel, Ed., *Translation in Eukaryotes*, CRC Press, pp 35-69.

[6] Schimmel, P., Giege, R., Moras, D. and Yokoyama, S. (1993) An operational RNA code for amino acids and possible relationship to genetic code. *Proceedings of the National Academy of Sciences of the United States of America*, **90**, 8763-8768. http://dx.doi.org/10.1073/pnas.90.19.8763

[7] Chuang, G.-Y., Mehra-Chaudhary, R., Ngan, C.-H., Zerbe, B.S., Kozakov, D., Vajda, S. and Beamer, L.J. (2010) Domain motion and interdomain hot spotsin a multidomain enzyme. *Protein Science*, **19**, 1662-1672. http://dx.doi.org/10.1002/pro.446

[8] Sekine, S.-I., Nureki, O., Dubois, D.Y., Bernier, S., Chênevert, R., Lapointe, J., Vassylyev, D.G. and Yokoyama, S. (2003) ATP binding by glutamyl-tRNA synthetase is switched to the productive mode by tRNA binding. *EMBO Journal*, **22**, 676-688. http://dx.doi.org/10.1093/emboj/cdg053

[9] Dubois, D.Y., Blais, S.P., Huot, J.L. and Lapointe, J. (2009) A C-truncated glutamyl-tRNAsynthetase specific for $tRNA^{Glu}$ is stimulated by its free complementary distal domain: Mechanistic and evolutionary implications. *Biochemistry*, **48**, 6012-6021. http://dx.doi.org/10.1021/bi801690f

[10] Dasgupta, S., Saha, R., Dey, C., Banerjee, R., Roy, S. and Basu, G. (2009) The role of the catalytic domain of *E. coli* GluRS in $tRNA^{Gln}$ discrimination. *FEBS Letters*, **583**, 2114-2120. http://dx.doi.org/10.1016/j.febslet.2009.05.041

[11] Ebel, J.P., Bonnet, J., Kern, D., Befort, N., Bollack, C., Fasiolo, F., Gangloff, J. and Dirheimer, G. (1973) Factors determining the specificity of the tRNA aminoacylation reaction; non absolute specificity of tRNA-aminoacyl-tRNA recognition and particular importance of the maximal velocity. *Biochimie*, **55**, 547-557. http://dx.doi.org/10.1016/S0300-9084(73)80415-8

[12] Giegé, R. and Springer, M. (2012) Aminoacyl-tRNA synthetases in the bacterial world. In: Curtiss, R., III, Kaper, J.B., Squires, C.L., Karp, P.D., Neidhardt, F.C. and Slauch,

J.M. Eds., *EcoSal*, ASM Press, Washington DC.

[13] Latterich, M. and Corbeil, J. (2008) Label-free detection of biomolecular interactions in real time with a nano-porous silicon-based detection method. *Proteome Science*, **6**, 31. http://dx.doi.org/10.1186/1477-5956-6-31

[14] Madore, E., Florentz, C., Giegé, R., Sekine, S.-I., Yokoyama, S. and Lapointe, J. (1999) Effect of modified nucleotides on *Escherichia coli* tRNAGlu structure and on its aminoacylation by glutamyl-tRNA synthetase; predominant and distinct roles of the mnm5 and s2 modifications of U34. *European Journal of Biochemistry*, **266**, 1128-1135. http://dx.doi.org/10.1046/j.1432-1327.1999.00965.x

[15] Weygand-Durasevic, I., Rogers, M.J. and Söll, D. (1994) Connecting anticodon recognition with the active site of *Escherichia coli* glutaminyl-tRNA synthetase. *Journal of Molecular Biology*, **240**, 111-118. http://dx.doi.org/10.1006/jmbi.1994.1425

[16] Chênevert, R., Bernier, S. and Lapointe, J. (2003) Inhibitors of aminoacyl-tRNA synthetases as antibiotics and tools for structural and mechanistic studies. In: J. Lapointe and L. Brakier-Gingras, Eds., *Translation Mechanisms*, Landes Bioscience/Eureka.com and Kluwer Academic/Plenum Publishers, pp. 416-428

[17] Vondenhoff, G.H.M. and Van Aerschot, A. (2011) Aminoacyl-tRNA synthetase inhibitors as potential antibiotics. *European Journal of Medicinal Chemistry*, **46**, 5227-5236. http://dx.doi.org/10.1016/j.ejmech.2011.08.049

[18] Bernier, S., Dubois, D.Y., Habegger-Polomat, C., Gagnon, L.-P., Lapointe, J. and Chênevert, R. (2005) Glutamyl-sulfamoyladenosine and Pyroglutamylsulfamoyladenosine are competitive inhibitors of *E. coli* glutamyl-tRNA synthetase. *Journal of Enzyme Inhibition and Medicinal Chemistry*, **20**, 61-67. http://dx.doi.org/10.1080/14756360400002007

[19] Bernard, D., Akochy, P.-M., Bernier, S., Fisette, O., Côté Brousseau, O., Chênevert, R., Roy, P.H. and Lapointe. J. (2007) Inhibition by L-aspartol adenylate of a nondiscriminating aspartyl-tRNA synthetase reveals differences between the interactions of its active site with $tRNA^{Asp}$ and $tRNA^{Asn}$. *Journal of Enzyme Inhibition and Medicinal Chemistry*, **22**, 77-82. http://dx.doi.org/10.1080/14756360600952316

[20] Akochy, P.-M., Bernard, D., Roy, P.H. and Lapointe, J. (2004) Direct glutaminyl-tRNA biosynthesis and indirect asparaginyl-tRNA biosynthesis in *Pseudomonas aeruginosa* PAO1. *Journal of Bacteriology*, **186**, 767-776. http://dx.doi.org/10.1128/JB.186.3.767-776.2004

[21] Huot, J.L., Lapointe, J., Chênevert, R., Bailly, M. and Kern, D. (2010) Glutaminyl-tRNA and asparaginyl-tRNA biosynthetic pathways and functions. In: Vederas, J.C., Ed., *Comprehensive Natural Products Chemistry II*, Vol. 5, Elsevier, Oxford, 383-431. http://dx.doi.org/10.1016/B978-008045382-8.00726-7

[22] Sauter, C., Lorber, B., Cavarelli, J., Moras, D. and Giegé, R. (2000) The free yeast aspartyl-tRNA synthetase differs from the $tRNA^{Asp}$-complexed enzyme by structural changes in the catalytic site, hinge region, and anticodon-binding domain. *Journal of Molecular Biology*, **299**, 1313-1324. http://dx.doi.org/10.1006/jmbi.2000.3791

