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Review

The aminoacylation of transfer RNAs: a structural point of view†

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Summary — The aminoacylation of transfer RNAs is catalyzed by a family of enzymes named aminoacyl tRNA-synthetases. This family can be split into two classes characterized by a different type of active site, and therefore a different mechanism of aminoacylation. This review focuses on the class II enzymes, for which aspartyl tRNA-synthetase is taken as a representative example. The reaction requires ATP as the source of energy. The recognition of ATP is class specific, in contrast to that of the amino acid. The amino acid fits into a small pocket inside the active site of the enzyme. An extended set of hydrogen bonds maintains the shape of this pocket, which is sterically and electrostatically complementary to the amino acid. The crystal structures now at hand, complemented with site-directed mutagenesis experiments, enable us to propose a detailed mechanism of the aminoacylation reaction.

aminoacyl tRNA-synthetase / transfer RNA / ATP / aminoacylation

Résumé — Étude de l'aminoacylation des ARNs de transfert: son aspect structural. *L'aminoacylation des ARNs de transfert est catalysée par une famille d'enzymes nommée aminoacyl ARNt-synthétases. Cette famille peut être divisée en deux classes caractérisées par un type différent de site actif, et donc par un mécanisme différent de l'aminoacylation. Cette revue traite essentiellement des enzymes de classe II, pour lesquels l'aspartyl ARNt-synthétase est prise comme exemple représentatif. La réaction utilise de l'ATP comme source d'énergie. La reconnaissance de l'ATP, à la différence de celle de l'acide aminé, est spécifique de la classe. L'acide aminé se loge dans une poche à l'intérieur du site actif de l'enzyme. Un réseau étendu de liaisons hydrogène maintient la forme de cette poche, qui est stériquement et électrostatiquement complémentaire de l'acide aminé. Les structures cristallines présentement disponibles, complétées par des expériences de mutagénèse dirigée, nous permettent de proposer un mécanisme détaillé de la réaction d'aminoacylation.*

aminoacyl ARNt-synthétase / ARN de transfert / ATP / aminoacylation

Introduction

The fidelity of the translation of the genetic information relies upon the correct attachment of each amino acid (aa) to its appropriate transfer RNA (tRNA) prior to its incorporation into the elongating polypeptide chain on the ribosome. Aminoacyl-tRNA synthetases (aa*RS*) constitute the family of enzymes responsible for the accuracy of the aminoacylation reaction. This family encompasses 20 proteins, one for each amino acid, which have long been characterized by their large structural diversity, despite the fact that they all catalyze the same two-step reaction [1, 2]:

$$\text{aa} + \text{ATP} \underset{\text{Mg}^{++}}{\overset{\text{aa}RS}{\rightleftharpoons}} \text{aa-AMP} + \text{PPi}$$

$$\text{aa-AMP} + \text{tRNA} \overset{\text{aa}RS}{\rightleftharpoons} \text{aa-tRNA} + \text{AMP}$$

The first step uses adenine triphosphate (ATP) to form an intermediate adenylate by attachment of the amino acid to the adenine monophosphate (AMP) moiety, with a concomitant release of a pyrophosphate group (PPi). This part of the reaction requires magnesium ions, which in some systems can be replaced by manganese ions, albeit at a price of lesser efficiency. In most systems, it is possible to carry out the first step of the reaction in the absence of tRNA, but in some cases, for example in the glutaminyl system, it is necessary to have the tRNA bound before the ATP. The second step is the aminoacylation of the tRNA. The aminoacylated, or charged, tRNA is then released from the enzyme.

The first comprehensive classification of aaRSs was the result of primary sequence analysis, a few years ago [3]. The family could be partitioned into two classes of ten members each, on the basis of characteristic signature motifs formed by conserved peptides. Within each class of enzymes, subgroups of higher homology of

† Dedicated to Prof Raymond Weiss.

the primary sequence can also be detected (table I). This partition could immediately be correlated with a known property of these enzymes: their capacity to attach the amino acid to different primary targets on the ribose of the terminal adenosine of tRNA, namely the 2′-hydroxyl group for class I and the 3′-hydroxyl for class II synthetases (with the only exception of Phe*RS*).

Table I. The two classes of aminoacyl tRNA-synthetases.

Class I	*Class II*
Val, Ile, Leu, Cys, Met[22, 23]	His[20], Pro, Ser[7, 16, 24], Thr, Gly[25]
Glu [26], Gln[5, 13], Arg	Asp[6, 15, 18], Asn, Lys[19]
Tyr [27, 28], Trp [29]	Ala, Phe [21]

The references indicate crystal structure determinations. Synthetases of class I are characterized by two consensus sequences (HIGH and KMSKS) and an active site built around a Rossmann fold. The primary sequence of class II synthetases contains 3 conserved motifs, and the main structural feature of the active site is an antiparallel β-sheet. The primary attachment site of the amino acid on the tRNA is the 2′ oxygen of the terminal ribose in class I, and the 3′ oxygen in class II synthetases, with the exception of Phe*RS*. There is also a correlation with the type of oligomeric association (mostly monomers in class I and dimers in class II). Each class may be further divided into subgroups (the lines in the table). This subdivision is based on additional sequence homologies, usually related to analogies between additional domains in the three-dimensional structure.

The crystal structures of a number of aminoacyl tRNA-synthetases of both classes, alone or complexed with different substrates, and from different prokaryotic or eukaryotic organisms, are presently available (table I). They are entirely consistent with the partition based on the signature motifs. In class I synthetases the structure of the active site is a nucleotide binding fold, commonly called the Rossmann fold, essentially a five-stranded parallel β-sheet surrounded by α-helices, which is known as a classical binding domain for ATP and other mono- or dinucleotide cofactors. In class II, the central feature of the active site domain is a six-stranded antiparallel β-sheet in contact with two α-helices. This fold has also been recognized in the catalytic domain of the biotin synthetase/repressor protein (BirA) [4]. This enzyme also uses ATP as a cofactor, and catalyzes the formation of biotinyl adenylate. Luckily enough, the first two tRNA-synthetase cocrystals, *Escherichia coli* Gln*RS*-tRNA^{Gln} [5] and yeast Asp*RS*-tRNA^{Asp} [6] (fig 1), belong to different classes, and showed that the structural difference in the active site was associated with a different type of approach of the acceptor extremity of tRNA.

In addition to the class-specific fold of the active site domain, the crystallographic data emphasize a second important feature, the modular character of the synthetases. Each enzyme is made by the addition of a variety of different modules to a common core which contains the catalytic site. The essential function of the additional domains is the recognition of different parts of the tRNA substrate. These modules can be attached at the *C*- or *N*-terminal end of the polypeptide chain or inserted at different strategic points of the catalytic domain. The spatial location of these modules is correlated

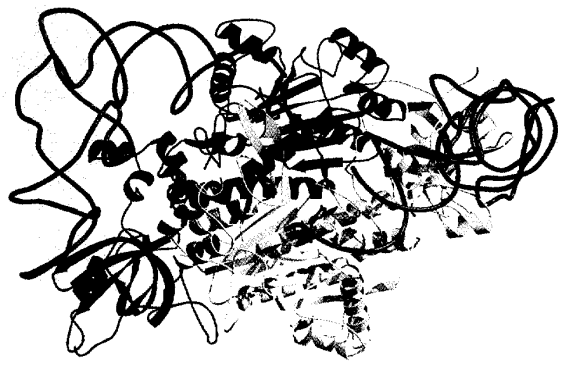

Fig 1. Molecular structure of aspartyl tRNA synthetase from yeast, cocrystallized with its cognate tRNA [6]. This enzyme is characteristic of the class II family of synthetases. The synthetase is a homodimer, shown here in yellow and blue. The α-helices and β-strands are materialized by coils and arrows. This dimeric enzyme is able to fix two tRNA molecules (the red lines, representing the phosphate backbone). The tRNA on the right-hand side is seen from the top, with the acceptor end, the attachment site of the amino acid, entering the active site. The characteristic L-shape is clearly seen on the other tRNA, with the acceptor arm at the top of the figure and the anticodon loop (which will be recognized by a complementary codon of the messenger RNA during the process of protein synthesis) at the bottom, in interaction with the *N*-terminal domain of the protein. This, and the other molecular structure representations, were made using programs *Molscript* [30] and *Raster3D* [31].

with the position of the identity determinants on the tRNA molecule, each of them being a target for the enzyme. One such determinant is often the anticodon, the triplet of bases which will recognize, through Watson–Crick interactions, a corresponding codon of the messenger RNA. This is the case with Gln*RS* and Asp*RS*, but not for example with Ser*RS* [7] (fig 2).

By contrast, the binding of the acceptor stem of the tRNA substrate to its cognate synthetase seems to be a class-specific feature. In Gln*RS*, the acceptor arm is approached by its minor groove site, thus requiring the single-stranded CCA-end to make a sharp turn in order to enter the active site. In the class II synthetases Asp*RS* and Ser*RS*, the approach is from the major groove side, thus permitting the terminal CCA-end to penetrate into the deep active side pocket without distorting the helical conformation (see fig 1).

In the rest of this paper, we will concentrate on the catalytic reaction and refer the reader to other reviews for the analysis of the specificity of tRNA recognition [8–10]. The aspartic acid system will be chosen to illustrate the mechanisms involved. As will be shown, this system may be considered as a prototype of all class II enzymes as far as the aminoacylation is concerned.

The aspartic acid system

The aspartic acid system is a good example of the modularity of aminoacyl-tRNA synthetases (fig 2). Despite

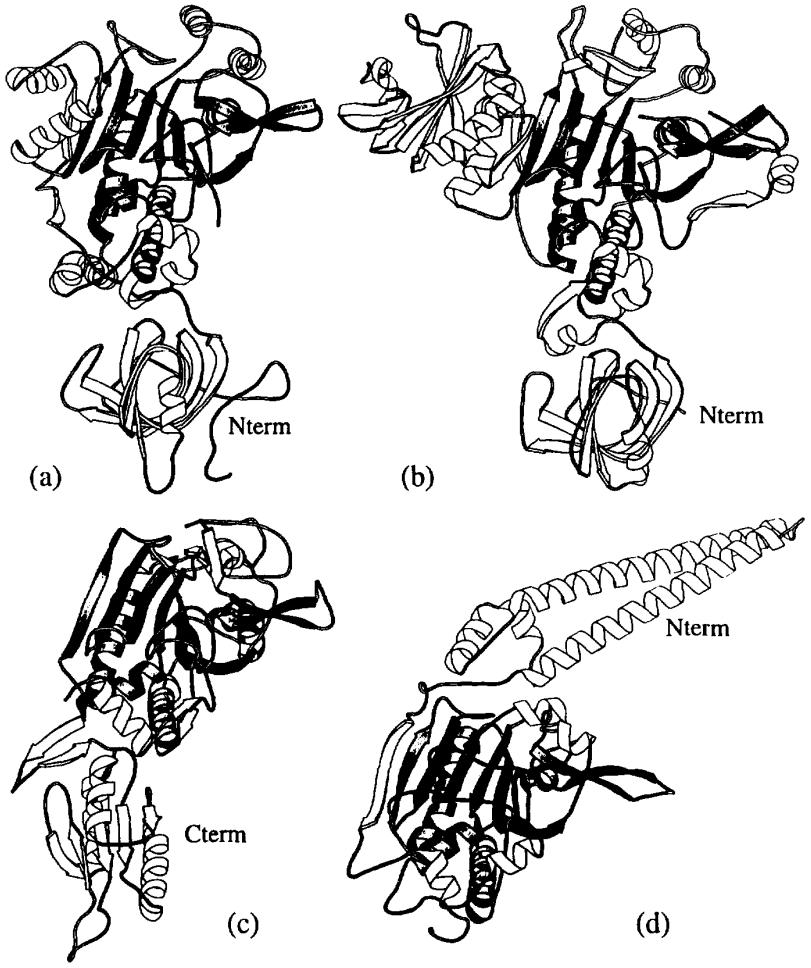

Fig 2. Synthetases are characterized by their modular structure. **a.** Eukaryotic (yeast) Asp*RS* [6]; **b.** prokaryotic (*T thermophilus*) Asp*RS* [11]; **c.** Gly*RS* from *T thermophilus* [25]; **d.** Ser*RS* from *E coli* [24]. For clarity, only one monomer of the homodimer is shown in all four cases. The parts highlighted form the active site. The other domains play a role in tRNA recognition. The β-barrel type domain formed by the *N*-terminal part of both Asp*RS* structures (**a** and **b**) encompasses the residues that recognize the anticodon of tRNA. The same type of folding, called oligonucleotide/oligosaccharide binding fold [32], was found recently in Lys*RS* [19], which belongs to the same subclass as Asp*RS* (see table I). Note the additional domain in the prokaryotic enzyme (**b**). The additional domain of Gly*RS* (**c**, bottom) is formed by the *C*-terminal part of the enzyme, but, very likely, is also used for the recognition ot the tRNA anticodon. A very similar domain was found in *E coli* His*RS* [20]. Ser*RS* (**d**) does not recognize the anticodon of tRNA, but the α-helical coiled coil at the upper side makes contacts with the long variable arm and the TΨ loop of $tRNA^{Ser}$ [7].

a similar molecular weight, Asp*RS* from *Thermus thermophilus* [11] and from yeast [6] share only two thirds of the polypeptide chain, the rest being specific prokaryotic or eukaryotic features. This marked additional subdivision of synthetases into eukaryotic and prokaryotic structures is not particular to the aspartyl system, although it is very pronounced in this case. The common part of eukaryotic and prokaryotic Asp*RS* encompasses the active site domain and the anticodon binding module at the *N*-terminal end, forming a total of 350 amino acids out of 560.

Asp*RS* is an $\alpha 2$ dimer like most class II enzymes. The dimer interface is very large with 4 800 $Å^2$ of buried surface for each monomer in the yeast enzyme and even more in the bacterial one. The first of the three class II signature motifs plays an important role in this interface. It provides a link between the two active site pockets. An essential proline residue conserved in most class II enzymes (Gly*RS* and Ala*RS* are the only exceptions) forms a key contact. Mutation of this residue has a direct effect on the active site of the adjacent subunit. A cooperativity of the two active sites could be demonstrated in this way [12].

The framework of the active site is constituted by a six-stranded antiparallel β-sheet flanked by an additional parallel strand and resting on two long α-helices. The second and third signature motifs, which contain the key residues for the catalytic reaction, constitute the first three strands and the conserved α-helices of the domain (fig 3). This antiparallel β-sheet forms the floor of a large flat pocket to which the three substrates of the reaction, ATP, the amino acid and the terminal adenosine of tRNA, bind.

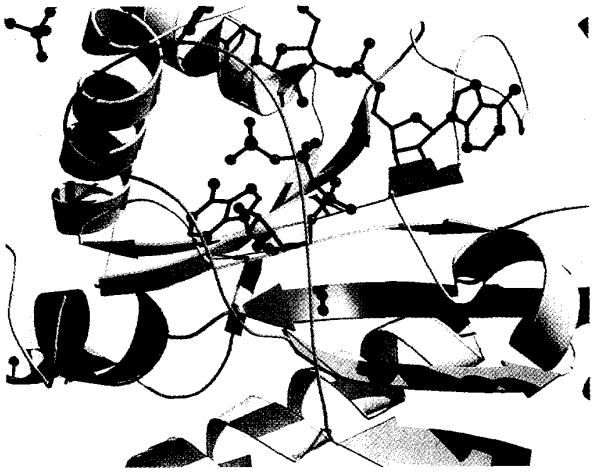

Fig 3. The active site in yeast aspartyl tRNA-synthetase, with ATP and the acceptor end of tRNA (at the top). Notice the bent conformation of ATP. The antiparallel β-sheet (arrows) forms the 'floor' of the active site pocket. The three first strands are part of conserved regions characterized by the second (in green) and third (in blue) signature motifs typical of class II synthetases.

ATP binding

The ATP molecule lies on the second and third strand, with which it makes a number of class II specific interactions. ATP is in a bent conformation, the γ-phosphate folding back close to the adenosine moiety. Analogous conformations, at least up to the β-phosphate, have been observed in the class I enzyme Gln*RS* [13] and in the class II enzyme Ser*RS* [14]. This conformation is stabilized by a Mg^{2+} ion interacting with the pyrophosphate group (the β- and γ-phosphates of ATP) and with two acidic residues, an Asp and a Glu, of the enzyme. Interestingly, in the crystal structure of the ternary complex Asp*RS* + tRNA + AMPPcP (an analog of ATP where the oxygen linking the β- and γ-phosphates is replaced by a methylene group), AMPPcP is in an elongated not bent conformation, probably because the interaction with Mg^{2+} is somewhat weaker in this case [15]. A similar elongated conformation has been reported in the case of Ser*RS*, for AMPPcP but also for ATP in the absence of divalent ions [7, 14]. These data indicate that ATP can easily switch from one conformation to the other. The bent conformation is necessary for activity because it leaves an empty pocket on one side of the α-phosphate, in which an aspartic acid molecule can fit. Everything is then in place for a nucleophilic attack on the α-phosphate by the peptidic carboxylate of the aspartic acid on one side, and release of the pyrophosphate on the other.

The adenine ring of ATP is stacked between the plane of a phenylalanine aromatic ring, which is part of motif 2, and the guanidine plane of an arginine belonging to motif 3 (see the schematic representation in fig 5a). This arginine also binds the γ-phosphate, thus contributing to the stabilization of the bent conformation of ATP. Another arginine, from motif 2, binds the α-phosphate of ATP. Discrimination between ATP and for example guanosine-5′-triphosphate (GTP) is ensured by two main-chain groups, NH and CO, again belonging to motif 2, which are hydrogen-bonded to N1 and to the amino group of the adenine base, respectively. Also noteworthy is the unusual number of glycine residues on the side of the β-sheet in contact with the ATP. These enable the ATP to come very close to the surface of the β-sheet, in an optimal position for the specific interactions mentioned. All the side chains and glycine residues involved are conserved in class II synthetases, suggesting that the mechanisms of recognition and positioning of the ATP are the same throughout this class of enzymes. This is confirmed by the structure of the Ser*RS* complex with ATP, for which essentially identical interactions with the various parts of ATP have been reported [16]. As expected, they differ from class I, even though a few similarities may be noted. Thus in Gln*RS*, the adenine ring of ATP is stacked between an arginine side chain and the polypetide backbone of the glycine of the conserved peptide HIGH, which plays thus the role of the conserved phenylanaline of class II. Furthermore, the same type of interactions between the adenine ring and main chain NH and CO groups discriminate against the binding of GTP [13].

tRNA acceptor arm

The tRNA acceptor end is on top of the ATP and makes contacts with the loops and helices which form the entrance of the active site pocket (fig 3). These contacts only involve one side of the acceptor arm, leaving an opening sufficient for the passage of the other substrates on the other. The fixation of ATP and of tRNA can thus

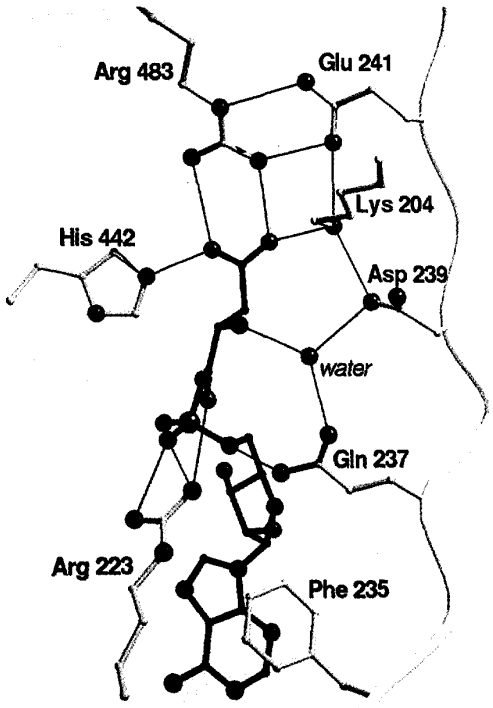

Fig 4. Aspartyl adenylate in its binding pocket in prokaryotic aspartyl tRNA-synthetase [18]. A part of the hydrogen-bond network surrounding the adenylate is shown.

occur in any order, and, after completion of the reaction, the pyrophosphate and the AMP can leave before the aminoacylated tRNA. However, once in place, the ATP leaves no access for the amino acid to its binding site, which means that the amino acid needs to take its place first. The contacts between the enzyme and the acceptor extremity of tRNA are not conserved within the Asp*RS* family, with the single exception of a hydrogen bond between the 2′-hydroxyl of the terminal adenosine A76 and a glutamine side chain. The acceptor stem itself makes a number of interactions on the major groove side with a long loop joining the two β-strands of motif 2 (in green in fig 3). This loop, variable in length and composition, is essential for the recognition of G73, with which two of its residues, Ser 329 and Thr 331, form specific hydrogen bonds. G73, the first base after the acceptor-end triplet CCA, is the so-called discriminator base, which in most tRNAs is essential for determining the tRNA's identity (ie, for defining unequivocally the amino acid that it is able to bind) [17].

Aminoacyl recognition

The best experimental data come from the crystal structure of the *T thermophilus* enzyme [18]. Availability of the crystal structure of the free enzyme, as well as of the enzyme bonding the aspartyl adenylate, allows an interesting insight into the recognition mechanism. The comparative analysis shows that the native structure is conserved within the accuracy of the experimental data for all side chains but one interacting with the amino-acid moiety. The exception is provided by a histidine residue attached to a loop located on top of the pocket. This loop moves and the rotation of the histidine residue brings its side chain in the vicinity of the aspartic acid side chain, close enough to form a hydrogen bond. All of the other residues involved in interactions are held together by a network of hydrogen bonds which create a three-dimensional template (fig 4). A rather rigid active site pocket is thus formed, which displays both stereochemical and electrostatic complementarity to aspartic acid. Every potential hydrogen bond donor or acceptor of the amino-acid substrate interacts with a partner group in this active site pocket. Many features seen in Asp*RS* are also present in the active site pocket of Ser*RS* [14], Lys*RS* [19] and His*RS* [20], where similar hydrogen bond networks were observed around the amino acid or the adenylate. It is interesting to note that Arg 485, which recognizes the side-chain carboxylate of the aspartic acid substrate in Asp*RS*, is replaced in Lys*RS* by a glutamic acid forming a salt bridge with the ε-amino group of lysine at a topologically equivalent position [19].

The mechanism of the aminoacylation reaction

The crystal structures of the enzyme alone or interacting with different substrates (tRNA, ATP, amino acid adenylate) provide several snapshots of the aspartylation reaction. These might be completed with site-directed mutagenesis experiments, which enable a functional correlation with the structural observations [15]. The combination of all these sources of information enables us to propose a complete scheme of the catalytic mechanism (fig 5).

The amino-acid substrate and ATP are maintained in place in their respective binding pockets by several specific electrostatic or hydrogen-bonding interactions. ATP is stabilized in a bent conformation, so that its pyrophosphate moiety and the amino acid occupy opposite locations relative to the α-phosphate. The peptidic carboxylic group of the amino acid then performs a nucleophilic attack (SN_2 type reaction) of the α-phosphate, resulting in a bipyramidal transition state with a pentacoordinated phosphorus. The bond with the β-phosphate, which is opposite to the newly formed bond with the amino acid, is then broken, resulting in an inversion of the α-phosphate, and release of the pyrophosphate group. The aminoacyl adenylate is thus formed. In the aspartic system, this reaction can occur in the absence of tRNA. One or several Mg^{2+} ions and arginine side chains play an essential role in the stabilization of the bent conformation of ATP and in lowering the energy barrier of the reaction, mainly by neutralizing the strong negative charge of the transition state. Mg^{2+} may also play a role in the stabilization of the leaving pyrophosphate group.

A second nucleophilic attack now occurs from the 3′-oxygen of the terminal adenosine of tRNA on the carboxylic carbon of the adenylate, resulting in the rupture of the bond between this atom and the α-phosphate. This same phosphate serves as acceptor for the proton released from the 3′-hydroxyl of tRNA. The crystal structures show that these reactions need practically no displacement of any of the substrates.

Quite interestingly, a very similar mechanism has been proposed for the class I synthetase Gln*RS* [13], even though the structure of the active site and the type of approach of the acceptor end of tRNA are completely different. The reason why the initial attachment site of the amino acid on the terminal ribose of tRNA is 2′ in Gln*RS* and 3′ in Asp*RS* becomes clear, since the tRNA ribose occupies opposite sides of the adenylate. In Gln*RS*, the 2′-hydroxyl is directed toward the α-phosphate while in Asp*RS* the 3′-hydroxyl takes this role.

Other class II synthetases

The similarities observed in the structure of the active site for all the synthetases of class II known today, the conservation of the residues involved in the interactions with the ATP and the need for Mg^{2+} ions suggest that the mechanism proposed for aspartic acid may be generalized to the other class II enzymes, even though a detailed comparison indicates some significant differences. Thus, in Ser*RS*, where magnesium had been replaced by manganese, three Mn^{2+} positions were observed around the ATP: a main site between the α- and the β-phosphates, and two minor sites between the β- and the γ-phosphates [16]. The divalent cations thus seem to display a dual function: catalytic for the one in the α-β-site (polarization of the α-phosphate in view of the nucleophilic attack), and structural (stabilization of the bent ATP) for the other two. Another

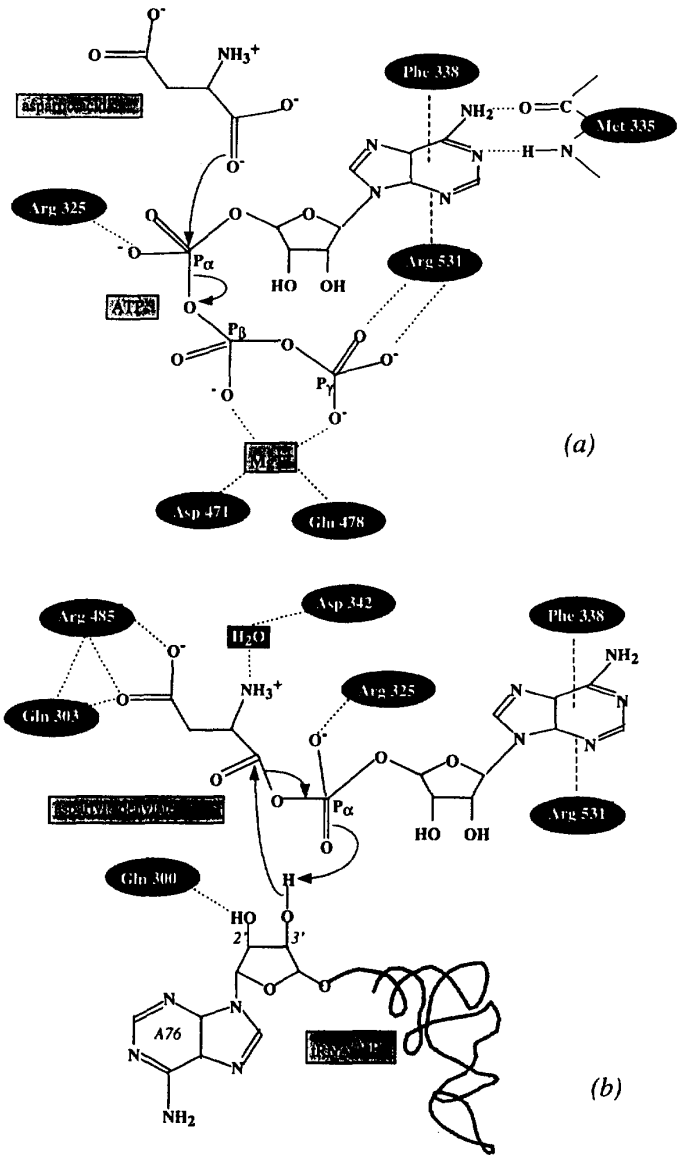

Fig 5. Mechanism involved in the two steps of the aminoacylation reaction in yeast aspartyl tRNA synthetase, as inferred from the crystal structures.

interesting case is that of *E coli* His*RS*. Here, the conserved glutamic acid that stabilizes the magnesium ion in Asp*RS* is absent, but the guanidinium group of an arginine comes close to the phosphate of the adenylate, and is likely to play a stabilizing role similar to that of Mg^{2+} [20].

Phe*RS* is the only class II system where the initial site of aminoacylation is the 2′-hydroxyl of the terminal ribose, as in class I. The recently published structure of Phe*RS* from *T thermophilus*, even without tRNA in the analyzed crystals, provides a clue to this anomaly [21]. The enzyme is an $(\alpha\beta)_2$ tetramer. The active site is on the α-subunits, but a domain of the β-subunits resembling closely the *N*-terminal domain of Asp*RS* contains probably, as in Asp*RS*, the recognition site of the anticodon. This would bring the tRNA in a different position relative to the synthetase, with contacts on the minor groove side of the acceptor stem, instead of the major groove side as in Asp*RS* or Ser*RS*. Thus, although the active site is very similar to what has been found in the other class II synthetases, the approach of tRNA is more like in class I, as observed for Gln*RS* [5].

Biological implications

A general picture of the active site of class II synthetases now emerges from the X-ray studies. It reveals two essential features of the binding mode of the amino acid substrate. First, there is a rigid template which suggests a lock and key process for the recognition of the amino acid. Stereochemical complementarity as well as electrostatic forces play an important role in this recognition process. From the limited quantity of informa-

tion available from other systems, it seems that this lock and key approach is more adapted to the class II enzymes than to class I. Since, with the exception of Phe*RS*, class II systems code predominantly for the amino acids with small and polar side chains, which have a limited possibility for hydrophobic contacts, this could be energetically advantageous. Second, the presence of a scaffold around the substrate holding residues together via a hydrogen bond network suggests a security control against evolutionary drift by single point mutations. Indeed, any single point mutation in the amino-acid active-site pocket or in its direct vicinity would dismantle or at least significantly perturb the hydrogen bond network and thus destabilize the pocket. Such a network thus provides an excellent protection against mutations, which could be highly damaging as they would endanger the amino-acid specificity.

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