Pyrophosphatase mediates the effect of certain tRNA mutations on aminoacylation of yeast tRNA\textsuperscript{Phe}

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ABSTRACT

The influence of pyrophosphate hydrolysis by inorganic pyrophosphatase on homologous aminoacylation of different yeast tRNA\textsuperscript{Phe} mutants was studied. The addition of pyrophosphatase significantly improved the aminoacylation efficiency of tRNA\textsuperscript{Phe} structural mutants as well as the mutant with substitution at position 20, while having no effect on the charge of wild-type tRNA\textsuperscript{Phe}. Aminoacylation of tRNA\textsuperscript{Phe} anticodon and discriminator base (N\textsubscript{73}) mutants was not affected by pyrophosphatase. Activation of wild-type tRNA\textsuperscript{Phe} transcript aminoacylation by inorganic pyrophosphatase was observed only at low Mg\textsuperscript{2+} concentrations due to distortion of the tRNA\textsuperscript{Phe} structure under these conditions. Our results demonstrate that pyrophosphatase dissociation becomes a rate-limiting step in the reaction in yeast phenylalanyl-tRNA synthetase catalyzed aminoacylation of tRNA\textsuperscript{Phe} variants with altered tertiary structure. A possible mechanism of pyrophosphate-mediated inhibition of tRNA mutants aminoacylation is discussed.

INTRODUCTION

Specificity and efficiency of tRNA aminoacylation by cognate aminoacyl-tRNA synthetase (aaRS) relies on the correct presentation of a certain number of nucleotides in the tRNA molecule to the enzyme. Any substitution at these key positions (called identity elements) generally results in a dramatic decrease of the aminoacylation efficiency of such mutant tRNAs (1,2).

The formation of aminoacyl-tRNA by aminoacyl-tRNA synthetases is generally described as a two-step reaction (1,3). Firstly, the amino acid (AA) is activated by forming enzyme-bound aminoacyladenylate (AA-AMP) with the release of pyrophosphate (PP\textsubscript{i}).

$$E + AA + ATP \rightarrow E\cdot AA-AMP + PP\textsubscript{i}$$

In the second step, the activated amino acid is transferred to the terminal ribose of tRNA, followed by dissociation of AMP and aminoacylated tRNA.

$$E\cdot AA-AMP + tRNA \rightarrow AA\cdot tRNA + AMP + E$$

The process of tRNA aminoacylation consists of multiple elementary steps, such as binding of substrates and dissociation of products, conformational changes of both tRNA and aminoacyl-tRNA synthetase, and chemical reactions occurring in the enzyme active site (1–3). The observed overall reaction rate of tRNA aminoacylation is determined by the slowest elementary step of the reaction. The point mutations in tRNA may, as earlier predicted (4), differentially affect the rate of specific elementary steps in the aminoacylation reaction (for example by influencing the binding efficiency or by impeding the conformational changes in the enzyme–substrate complex). Although the effects of numerous tRNA mutations on kinetic parameters of aminoacylation have been described, surprisingly little is known about the correlation between a given mutation and the reaction step affected.

It is logical to expect that mutations in tRNA would affect transfer of the amino acid to tRNA. Indeed, substitutions of discriminator base (N\textsubscript{73}) of tRNA\textsuperscript{Glu} directly affected $k$\textsubscript{cat} of the amino acid transfer reaction (5), probably through interference with the proper orientation of the tRNA CCA terminus in the active site (6,7). However, substitutions of tRNA\textsuperscript{Glu} identity elements affect aminoacylation by an entirely different mechanism, through interference with aminoacyladenylate formation by decreasing the enzyme affinity for Gln (8,9).

These examples demonstrate the limits of our current knowledge of the biochemical functions of tRNA identity elements. In this paper we present evidence that PP\textsubscript{i} dissociation is selectively affected by certain tRNA mutations and becomes the limiting step in tRNA aminoacylation catalyzed by yeast phenylalanyl-tRNA synthetase (PheRS).

Recognition of yeast tRNA\textsuperscript{Phe} (GAA) is determined by five major identity elements (nucleotides G\textsubscript{39}, G\textsubscript{34}, A\textsubscript{35}, A\textsubscript{36} and A\textsubscript{73}) located in three separate regions of the tRNA molecule (the D-loop, the anticodon and the acceptor stem) (10). Previously we have shown that dissociation of the pyrophosphate is the rate-limiting step in the case of heterologous aminoacylation of Escherichia coli tRNA\textsuperscript{Phe} by yeast PheRS (11). This slow PP\textsubscript{i} dissociation correlates with the single difference in the identity element sets between yeast and E. coli tRNA\textsuperscript{Phe} (substitution G→U at position 20). In this paper, we have screened the set of tRNA\textsuperscript{Phe} variants to identify mutations resulting in pyrophosphate-dependent inhibition of aminoacylation.

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MATERIALS AND METHODS

Materials
1-[3H]Phenylalanine (21 Ci/mmol) was from Amersham, α- and γ-[32P]ATP were from NEN, PEI-cellulose plates were from Selecto Scientific, yeast PheRS was purified as described previously (12), purified T7 RNA polymerase was a kind gift of Dr R. Aphasizhev (Institute of Molecular Biology, Moscow, Russia). Restriction enzyme BsrNI was from New England Biolabs. Plasmids containing yeast tRNA^{Phe} variants (pYF₀, pYF₁, pYF₂, pYF₃, pYF₄, pYF₆₆ and pYF₉₁) were kindly provided by Prof. O. Uhlenbeck (University of Colorado, Boulder, CO) and described previously (10,13). Inorganic pyrophosphatase (PPase) was from Sigma.

In vitro transcription
All tRNA transcripts used in this study were obtained by in vitro transcription of the corresponding synthetic genes placed under T7 promoter (14). Transcription was performed in the reaction mixture containing 40 mM Tris–HCl pH 8.1 (at 37°C), 22 mM MgCl₂, 5 mM dithiothreitol, 0.01% Triton X-100, 1 mM spermidine, 1 mM each nucleotide triphosphate, 5 mM GMP, 0.1 mg/ml linearized plasmid and 2.5 U/ml T7 RNA polymerase. Incubation was performed for 3 h at 37°C and the reaction terminated by phenol/chloroform extraction. Full-length tRNA transcripts were purified by single-nucleotide resolution electrophoresis on 12% polyacrylamide denaturing gels and recovered by electroelution. Concentration of tRNA transcripts was measured by optical density at 260 nm. Functional integrity of tRNA transcripts was verified by their aminoacylation at high PheRS concentration (200 nM). Aminoacylation plateau values of all the transcripts used in this study generally exceed 90% (81% for the YF₂ mutant).

Aminoacylation assay
Aminoacylation reactions were performed in the reaction buffer containing 100 mM Tris–HCl pH 7.5, 15 mM MgCl₂ (unless indicated), 3 mM ATP, 30 mM KCl, 5 μM [3H]phenylalanine, between 50 nM and 100 nM PheRS, depending on the nature of the transcript and the observed reaction rate. When indicated, PPase was added to the reaction mixtures to a final concentration of 20 U/ml. Renaturation of all tRNA transcripts was performed by heating in the presence of 1 mM MgCl₂ for 90 s at 65°C followed by slow cooling to room temperature. Incubation was done at 37°C and aminoacylated tRNA samples were precipitated by 5% trichloroacetic acid, collected on GF/C filters (Whatman) and radioactivity was counted. The kinetic constants were calculated from Lineweaver–Burk plots and non-linear regression of the data. All values represent an average of the results obtained in at least two independent experiments. The relative efficiency of aminoacylation for different variants as compared to the wild-type tRNA^{Phe} transcript was estimated using apparent kinetic parameters (Michaelis–Menten approximation) and in particular by comparison of the specificity constant k_{cat}/K_{m}.

Rate of ATP-PP₆ exchange was measured in reaction buffer containing either 5 or 15 mM MgCl₂, 1 mM PP₆, 2 mM [γ-32P]ATP (~2000 c.p.m/pmol) and 1 nM PheRS. Reaction progress was measured by the rate of ATP consumption by spotting 1 μl aliquots of the reaction mixture on PEI-cellulose plates. Plates were developed in 0.8 M LiCl and quantified using a phosphor-imager.

Rate of AMP production was measured in the standard reaction buffer containing either 5 or 15 mM MgCl₂, 2 μM tRNA and 30 μM [α-32P]ATP at 100 mCi/mmol. PheRS concentration was 2 nM for YF₀ aminoacylation at 15 mM MgCl₂, 10 nM at 5 mM MgCl₂ and 100 nM for aminoacylation of YF₆₆. The reaction was monitored by TLC as described above.

PP₆ inhibition was measured by addition of PP₆ into standard reaction mixtures containing either 15 or 5 mM MgCl₂, 1 μM YF₀ tRNA and 0.5 or 2 nM PheRS, respectively. PP₆ concentration was varied between 1 and 100 μM. K_{i} was deduced either from apparent K_{m} of YF₀ aminoacylation with and without 10 μM PP₆, or from Dixon plots, using tRNA concentrations between 0.5 and 2 μM and PP₆ concentrations between 1 and 50 μM.

RESULTS

Selective enhancement of tRNA^{Phe} mutants aminoacylation in the presence of PP₆ase
In order to identify mutations leading to pyrophosphate-dependent reduction of the aminoacylation rate, we screened a set of yeast tRNA^{Phe} transcripts with mutations in all three major identity regions (10); two mutants with the disrupted G₁₉-C₅₆ tertiary base pair, which occur between the D- and T-loops, and the compensatory mutant containing the inverted G₁₅-C₅₆ base pair (Fig. 1). The aminoacylation of these tRNA transcripts was tested both in the absence and in the presence of yeast inorganic pyrophosphatase in the reaction mixture. In agreement with our previous results (14), the addition of the PP₆ase had almost no effect on the aminoacylation of wild-type tRNA^{Phe} transcript (YF₀) under the test conditions (15 mM MgCl₂), while the charging of the YF₁ mutant (G₁₅-C₅₆ substitution) was considerably activated (Fig. 2 and Table 1). However, aminoacylation of the mutants with the A₁₀₁-U substitution in the anticodon (YF₆₆) and the A₅₀-U substitution at the discriminator base position (YF₉₁) were almost insensitive to the presence of PP₆ase in the reaction.

Figure 1. Cloverleaf structures of yeast tRNA^{Phe} mutants used in this study. tRNA^{Phe} identity elements are shaded. Mutations are shown by arrows. Corresponding mutants names are shown in parentheses.
mixture (Table 1)—only 2-fold activation was observed. Both tRNA
Phe mutants with disrupted 3D structure (YF 1 and YF 4 )
appeared to be rather poor substrates for PheRS in our experimental
conditions, but their aminoacylation was significantly
improved in the presence of PPase (Fig. 2 and Table 1).
Aminoacylation of the YF2 mutant with the compensatory
mutations (G 19 C and C 56 G), which by itself is a much better
substrate than both structural variants, was also improved by
PP i cleavage but to a lesser extent (Table 1). It is worth
mentioning that the influence of the mutations on the kinetic
parameters of aminoacylation measured in this study is
qualitatively the same but quantitatively stronger, than pre-
viously reported (10,12). Most probably, this may be attributed
to the differences in the conditions used for renaturation and
amino-acylation of tRNA transcripts.

Kinetic constants for aminoacylation of mutant tRNA transcripts
are summarized in Table 1. Pyrophosphate hydrolysis generally
results in the increase of \( k_{cat} \) and decrease of \( K_m \) values,
although the extent of their variations differs considerably
from one mutant to another. Taking into account the rather
complex nature of the observed (apparent) \( K_m \) and \( k_{cat} \) parameters,
we mostly use the specificity factor (\( k_{cat}/K_m \)) value as a
measure of the overall catalytic efficiency, rather than consider
the variations on these kinetic constants independently.

**PPase-dependent activation correlates with distortion of
tRNA tertiary structure**

If inhibition of the aminoacylation reaction by PP i is related to
the compromised tRNA tertiary structure, it should be observed
even for wild-type tRNA Phe transcript aminoacylation in conditions

![Figure 2](image-url)
where its tertiary structure is disrupted. Since distortion of the correct 3D structure of tRNA Phe transcript occurs at low Mg\(^{2+}\) concentration (14,15), we have studied the effect of PPase on the aminoacylation of wild-type transcript at different Mg\(^{2+}\) concentrations (Fig. 3). At Mg\(^{2+}\) concentrations below 5 mM, addition of PPase considerably activated the aminoacylation of wild-type transcript, while it had no effect at higher Mg\(^{2+}\) concentrations. PPase-dependent activation of wild-type tRNA Phe aminoacylation at low Mg\(^{2+}\) concentration is mostly due to the \(\sim\)10-fold decrease of \(K_m\) value in the presence of PPase (Table 2). The influence of PP i cleavage on the aminoacylation of YF 4 mutant (G 20 U), tested at low Mg\(^{2+}\) concentration, was much more profound even than at 15 mM (Fig. 3).

Enhancement of tRNA aminoacylation by PPase is not related to the amino acid activation step of the reaction

Since it is possible that the rate of amino acid activation can be altered at 5 mM MgCl\(_2\), the rate of phenylalanyl-adenylate formation was measured at both 15 and 5 mM Mg\(^{2+}\). Activation rates, as measured by ATP–PP i exchange appeared to be the same (data not shown). Thus the lower rate of tRNA aminoacylation at 5 mM Mg\(^{2+}\) may be attributed to an alteration of tRNA structure.

PPase-dependent activation of aminoacylation at low Mg\(^{2+}\) presumes a stronger sensitivity of reaction to PP i inhibition in these conditions. Inhibition experiments showed that PP i, is a potent inhibitor of aminoacylation even at standard conditions with \(I_S^{50}\) of 8 \(\mu\)M (Table 2). However, a comparison of PP i inhibition of aminoacylation at low and high Mg\(^{2+}\) concentrations did not reveal substantial differences. In both cases PP i inhibition is competitive to tRNA with only a 2-fold decrease in \(K_i\) and \(I_S^{50}\) at 5 mM Mg\(^{2+}\).

One may expect that the observed PPase activation effect is due to the higher rate of PP i production (i.e. higher ATP hydrolyzed/Phe-tRNA formed ratio) upon aminoacylation of mutated tRNAs or wild-type transcript at low Mg\(^{2+}\) concentration. This possibility was tested by parallel measuring of tRNA aminoacylation and AMP production rates in the course of aminoacylation of the YF 4 mutant and wild-type transcript at low MgCl\(_2\) concentration compared to YF 0 aminoacylation in standard conditions. No significant enhancement of AMP production was found under our experimental conditions (data not shown).

**DISCUSSION**

By screening the set of tRNA\(^{Phe}\) mutants we found that they fall into two groups according to sensitivity of their aminoacylation to PP i inhibition. Aminoacylation of tRNA mutants with single substitution of the discriminator base (N 73 ) and in the anticodon is not activated in the presence of PPase. On the contrary, aminoacylation of the mutant with substitution at position 20 as well as of the structural mutants is significantly improved upon the cleavage of PP i. Since point mutations in the anticodon and at position 73 have no significant impact on the overall structure of the tRNA molecule (16), our results indicate that PP i dissociation becomes the limiting step of the reaction only when tRNA tertiary structure is disturbed. For both structural variants tested here, the introduced mutations disrupt the tertiary base pair stabilizing the interaction between D- and T-loops, thus changing significantly the overall structure of the molecule (16). Aminoacylation of these mutants is significantly improved in the presence of PPase, mainly due to the increase in the \(k_{cat}\). The double mutant with the reconstructed N 73-N 56 base pair, and thus mostly restored tertiary structure (16), still shows the increased \(K_m\) value. It may reflect the presence

<table>
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<th>PPase (nM)</th>
<th>(K_m) (nM)</th>
<th>(k_{cat}/K_m) relative</th>
<th>(k_{cat}/K_m) ratio (+/-)</th>
<th>(K_i) (PP i) ((\mu)M)</th>
<th>(I_S^{50}) (PP i) ((\mu)M)</th>
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<tr>
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<td>1</td>
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<td>15 + 370</td>
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<td>1.2</td>
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Table 2. Influence of inorganic pyrophosphatase and PP i on the kinetic parameters of wild-type tRNA\(^{Phe}\) (YF 0 ) aminoacylation at different Mg\(^{2+}\) concentrations

\(a\)Apparent \(K_m\), \(K_i\) and \(k_{cat}\) values obtained in replicate experiments varied by, at most, 20%.

\(b\)\(I_S^{50}\) (PP i) was measured at 1 \(\mu\)M of YF 0 transcript.

Figure 3. Mg\(^{2+}\) dependence of PPase activation effect on YF 0 (circles) and YF 4 (squares) aminoacylation. Initial rate of aminoacylation was measured at different MgCl\(_2\) concentrations. PheRS concentration was 0.5 nM for YF 0 and 5 nM for YF 4 aminoacylation. Concentration of transcripts was 1 \(\mu\)M. Activation is calculated as a ratio of initial rate of aminoacylation in the presence of PPase to the rate in its absence at a given MgCl\(_2\) concentration.
Figure 4. Hypothetical kinetic scheme of PPase-dependent activation of tRNA aminoacylation (detailed in text).

of some residual structural distortions, probably due to the impaired stacking interaction involving G67, G18 and G19 (17), which are apparently sensed by PheRS. Hydrolysis of PPi in this case results in a several-fold decrease of $K_m$.

The G20U substitution, unlike the other point mutations of identity elements, displayed the same kinetic phenotype as the structural mutations. Mutations introduced at this position do not change dramatically the overall tRNA structure, as was shown by Pb2+ cleavage test (16). Crystallographic data also indicates that G20 is not involved in any interactions with other nucleotides in tRNA$^{Phe}$ (18). Structural studies of yeast tRNA$^{Phe}$ indicate that the base of G20 is implicated in the formation of the Mg$^{2+}$ binding site (19). Thus the substitutions introduced at this position may considerably affect magnesium binding through altering a local conformation of tRNA$^{Phe}$ at the ‘angle’ region. This suggestion is in good agreement with the observed higher sensitivity to PPi inhibition of the G20 U tRNA ‘angle’ region. This suggestion is in good agreement with the observed higher sensitivity to PPi inhibition of the G20U transcript aminoacylation at low Mg$^{2+}$ concentrations. The other possibility is that G20 becomes involved either in tertiary interactions within the tRNA molecule or interacts with the protein upon the transitional conformational change of the tRNA–synthetase complex. The former possibility is well illustrated by the structural rearrangement of the conserved base G20b in tRNA$^{Ser}$ upon interaction with synthetase (20). Insertion of G20b from the D-loop into the tRNA core determines the correct orientation of the long variable loop of tRNA in the complex and directs the acceptor stem of the tRNA into the active site.

Correlation between tRNA structure and the phenomena of PPi inhibition is further confirmed by the PPase effect on Mg$^{2+}$ dependence of aminoacylation. PPi dissociation becomes limiting at low Mg$^{2+}$ concentration, where tRNA conformation is altered (14,15). Aminoacylation of wild-type transcript is not affected by PPase when Mg$^{2+}$ concentration is sufficient to maintain native tRNA structure.

Thus the observed PPi-dependent inhibition of tRNA$^{Phe}$ mutants aminoacylation correlates with the distortions in the tertiary structure of the tRNA substrate. At the same, time point mutations in other major identity determinants such as anticodon and discriminator base clearly inhibit tRNA aminoacylation by another mechanism.

What could be a mechanism of PPi inhibition or, put another way, how could the distorted tertiary structure of the tRNA molecule be responsible for the observed change in the reaction mechanism? One possible explanation is that PPi dissociation is required for the enzyme to adopt the catalytically active conformation and the correctly folded tRNA molecule serves as a trigger of this process. Philosophically, from the aminoacyl-tRNA synthetase point of view, tRNA is just a rather complex device for the correct positioning of the terminal adenosine in the enzyme active site. For the vast majority of tRNAs, with identity elements located far away from the acceptor stem, this positioning may be achieved through the conformational changes of both the enzyme and tRNA (1–3). Evidence that both tRNA and aminoacyl-tRNA synthetase undergo significant mutual conformational changes upon the interaction comes both from biochemical experiments (1–3,21) and crystallographic data (20,22). Of special interest is the comparison of mutually exclusive adenylyl- (A-) and tRNA-bound (T-) conformations of seryl-tRNA synthetase (23). Upon tRNA binding, a number of motif 2 active site residues previously found interacting with ATP or adenylyl switch to tRNA binding. The switch from A- to T-conformation is most likely triggered by successful completion of the serine activation step. Pyrophosphate release may free Arg271 to interact with C74 and thus to stabilize correct tRNA$^3$ end positioning in the active site to permit the aminoacylation step. Vice versa, tRNA binding may facilitate PPi release from the active site.

Our results can be described by a simplified scheme (Fig. 4). The proposed hypothetical mechanism is based on two assumptions: first, that PPi dissociation from enzyme–adenylate complex (complex I) is slower than the rate of its generation from ATP, which makes this complex a predominant adenylyl-containing form of enzyme; and secondly that PPi-containing complex will react with tRNA (complex II) is catalytically inactive or marginally active. The second assumption is supported by the fact that complete inhibition by PPi can be achieved. According to the scheme, the major reaction pathway is through complex I to complex IV via complex II. tRNA binding to complex I triggers PPi dissociation with formation of the active complex IV. Apparently, certain mutant tRNA molecules cannot displace PPi, and the reaction is blocked by formation of the inactive complex II. Since aminoacylation of neither anticodon nor N73 position substitutions is rescued by the PPi cleavage, it seems logical to suggest that interactions outside the identity elements are required to induce PPi dissociation. Besides identity elements, tRNA forms multiple backbone contacts with the enzyme (1,2) and it seems likely that this type of enzyme–tRNA interaction is responsible for the triggering of PPi dissociation. Distortion of the backbone interactions in structurally altered tRNA molecules apparently may slow down this process. Another question is why PPi dissociation is required for the productive interaction between tRNA and synthetase. As mentioned above, it is possible that the enzyme adopts a catalytically active conformation only after PPi dissociation. PPase may activate mutant aminoacylation by facilitating PPi dissociation from complex I and bypassing
inactive complex II by rerouting the reaction mechanism through complex III. This scheme presumes that the reaction mechanism may be different in the presence or absence of PPase. Such PPase-induced changes of the aminocoylation mechanism have been observed for several aminocytyl-tRNA synthetases (3,24,25). Further experiments are required to confirm this hypothetical mechanism.

Details of the PPase-dependent activation mechanism are still unclear. The most obvious explanation would be that activation occurs due to the removal of product inhibition. But activation takes place at low levels of tRNA aminocoylation where the concentration of PP, formed (<1 μM) is well below its SI (Table 1) and thus cannot have any strong inhibitive effect unless its production, upon aminocoylation, of ‘wrong’ tRNAs is tremendously enhanced. However, such enhancement is not observed under our experimental conditions. Thus PPase activation occurs at very low PP, concentration and the simple explanation of uplifting of the product inhibition displayed by external PP, in inhibition experiments may not necessarily be the right one. Similar observations were made earlier when PPase-induced changes in the kinetic mechanism of tRNA aminocoylation by several aminocytyl-tRNA synthetases were found (24,25). Thus a more complicated mechanism may exist. It does not seem unreasonable to hypothesize that prior to dissociation PP, forms a very tight complex with the enzyme and its dissociation may become a limiting step in the reaction. Formation of such a complex by yeast PheRS has been demonstrated (12). Hydrolysis of PP, may either facilitate dissociation of this complex or PP, in this complex may be directly accessible to PPase and its cleavage may result in the observed activation.

Is the PP, inhibition phenomena unique for aminocoylation catalyzed by yeast PheRS? We do have reason to believe that it may be of general importance. First of all high sensitivity of tRNA aminocoylation (especially in heterologous systems) to PP, inhibition was noticed a long time ago (12,26–30). Moreover, covalent binding of PP, to various aminocytyl-tRNA synthetases (aaRSes) has been demonstrated, suggesting the existence of a covalent intermediate between certain aaRSes and PP, (3,1,32). Finally, results very similar to those described above for PheRS were also obtained for tRNA aminocoylation by yeast aspartyl-tRNA synthetase (A.M.Khorovoa, A.D.Wolfson and R.Giege, manuscript in preparation).

Our data suggest that PP, dissociation is an important, active step of the aminocoylation reaction. It may be associated with substantial conformational transitions within the tRNA–synthetase complex. Only binding of correctly folded tRNA molecules to aminocytyl-tRNA synthetase efficiently triggers PP, dissociation, which becomes a rate-limiting step in aminocoylation of tRNAs with altered tertiary structure. Further studies, probably using a modification interference approach, may allow the determination of the exact locations of nucleotides responsible for the induction of PP, dissociation and thus directly involved in the conformational rearrangement of the complex.

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REFERENCES