Prokaryotic and Eukaryotic Tetrameric Phenylalanyl-tRNA Synthetases Display Conservation of the Binding Mode of the tRNA^{Phe} CCA End†

Nina Moor,*,‡ Olga Lavrik,‡ Alain Favre,§ and Mark Safro||

Novosibirsk Institute of Bioorganic Chemistry, 630090 Novosibirsk, Russia, Institut Jacques Monod, CNRS-Université Paris 7-Université Paris 6, 75251 Paris Cedex 05, France, and Department of Structural Biology, Weizmann Institute of Science, 76100 Rehovot, Israel

Received May 6, 2003; Revised Manuscript Received July 21, 2003

ABSTRACT: The interaction of human phenylalanyl-tRNA synthetase, a eukaryotic prototype with an unknown three-dimensional structure, with the tRNA^{Phe} acceptor end was studied by s^{4}U-induced affinity cross-linking with human tRNA^{Phe} derivatives site-specifically substituted at the single-stranded 3′ end. Two different subunits of the enzyme bind two adjacent nucleotides of the tRNA^{Phe} 3′ end: nucleotide 76 is associated with the catalytic α subunit, while nucleotide 75 is in contact with the β subunit. The binding mode is similar to that revealed previously in structural and affinity cross-linking studies of the prokaryotic *Thermus thermophilus* phenylalanyl-tRNA synthetase. Our results suggest that the distinctive features of tRNA^{Phe} acceptor end binding are conserved for the eukaryotic and prokaryotic tetrameric phenylalanyl-tRNA synthetases despite their significant differences in the domain composition of the β subunits. The data from affinity cross-linking experiments with human phenylalanyl-tRNA synthetase complexed with small ligands (ATP and/or phenylalanine or a stable synthetic analogue of phenylalanyl adenylate) reveal that the location of the tRNA^{Phe} acceptor end varies with the presence and nature of other substrates. The lack of substrate activity of human tRNA^{Phe} substituted with s^{4}U at the 3′-terminal position suggests that base-specific interactions of the terminal adenosine are critically important for a productive interaction. The conformational rearrangement of the tRNA 3′ end induced by the other substrates and dictated by base-specific contacts of the terminal nucleotide is an additional means of ensuring the phenylalanylation specificity in both prokaryotic and eukaryotic systems.

Aminoacyl-tRNA synthetases (aaRSs)[1] are responsible for the correct translation of the genetic code, promoting accurate aminoacylation of tRNAs. Despite having similar functions, aaRSs exhibit a high degree of structural diversity (reviewed in refs *1* and *2*): most are homodimers, but monomers, heterodimers (*3*), and tetramers also exist. On the basis of sequence analysis and catalytic domain structure, aaRSs have been partitioned into two classes of 10 members each (*4*, *5*). The specificity of tRNA aminoacylation depends on a set of nucleotides arranged for most tRNAs in the anticodon loop and the acceptor stem; in specific cases, they are located in the core of the tRNA molecule (reviewed in refs *6* and *7*). Major determinants are conserved through evolution; however, they are utilized by species-dependent mechanisms (*6*, *8*−*10*). Eubacteria and eukaryotes differ in the location of the acceptor stem recognition elements of tRNA^{Gly} and tRNA^{Asp} and in the contribution of the tRNA backbone to tRNA^{Cys} aminoacylation (*11*−*13*). Changes in the mode of tRNA recognition are generally associated with structural differences in aaRSs between species, indicating codevelopment of a synthetase and cognate tRNAs in evolution.

Phenylalanyl-tRNA synthetase (PheRS) is one of the largest and most complex enzymes of the aaRS family; the tetrameric subunit organization [(αβ)$_2$] of cytoplasmic PheRS is markedly conserved through evolution. The three-dimensional (3D) structure was determined for *Thermus thermophilus* PheRS, both native and complexed with substrates (*14*−*17*). The enzyme belongs to class II (subclass IIc) according to its structural characteristics, but functionally it resembles a class I enzyme, aminoacylating the 2′-OH group of the tRNA terminal ribose (*18*−*20*). PheRS is a functional dimer (reviewed in ref *1*). The αβ heterodimer consists of 11 structural domains: three of them (a coiled-coil helical arm at the N-terminus, A1, and A2) belong to the α subunit and eight (B1−B8) to the large β subunit (*14*). Domains A1 and A2 create the class II conserved catalytic module. The β subunit contains a catalytic-like module (with a class II fold but no catalytic activity) as well as several other domains that are structurally similar to various functional domains found in other enzymes (reviewed in refs *14* and *15*). The CCA end and the acceptor stem of the tRNA^{Phe} molecule interact with the catalytic module and domains B1, B3, and B7 from the same heterodimer. The

† This work was supported by grants from the Russian Foundation for Basic Research to N.M. (99-04-49856 and 03-04-48384). N.M. is indebted to Université Paris 6 for an Invited Professor position.

* To whom correspondence should be addressed. E-mail: moor@niboch.nsc.ru. Fax: +7-383-2-33-36-77.

‡ Novosibirsk Institute of Bioorganic Chemistry.

§ CNRS-Université Paris 7-Université Paris 6.

|| Weizmann Institute of Science.

[1] Abbreviations: aaRS, aminoacyl-tRNA synthetase; PheRS, phenylalanyl-tRNA synthetase; ps^{4}Up, 4-thiouridine 3′,5′-diphosphate; APM, [(*N*-acryloylamino)phenyl]mercuric chloride; PheOH-AMP, L-phenylalaninyl adenylate; tRNA^{Phe}-s^{4}U76 and tRNA^{Phe}-s^{4}Up76, modified tRNA^{Phe} terminated with 4-thiouridine and 4-thiouridine 3′-phosphate, respectively, at the indicated position of the 3′ end; PAG, polyacrylamide gel; AspRS, aspartyl-tRNA synthetase.

10.1021/bi034732q CCC: $25.00
Published on Web 08/19/2003

anticodon loop and general shape of the $tRNA^{Phe}$ are recognized by the B8 domain and the coiled-coil arm of the α subunit of the second heterodimer. The interaction of one $tRNA^{Phe}$ molecule with all four subunits accounts for the necessity of the tetrameric structure for enzymatic activity. The $tRNA^{Phe}$ nucleotides required for recognition by PheRSs of evolutionarily diverged species have been analyzed by *in vitro* aminoacylation of mutant tRNAs (*21*−*27*). The anticodon nucleotides are the major determinants of $tRNA^{Phe}$ specificity in all systems, prevailing both in favoring aminoacylation catalysis and in the initial binding recognition as shown for the *T. thermophilus* system (*28*). The contribution of nucleotides 20 and 73 and of the A31·U39 and G30·C40 base pairs to phenylalanylation efficiency is phylum-dependent and varies greatly: the strongest effects of mutation are seen in humans (*23*).

Amino acid sequence analyses of PheRSs reveal significant differences in length between the bacterial (prokaryotic-like) and the archaeal/eukaryotic (eukaryotic-like) subunits: elongation of the polypeptide chains occurs mostly at the N- or C-terminal extremities of the subunits, rather than by insertion into the catalytic domain region (reviewed in refs *29* and *30*). The catalytic α subunit of human PheRS (the eukaryotic-like prototype) is ∼120 amino acids longer than the bacterial counterpart at its N terminus. However, the β subunit turns out to be approximately 200−250 residues (∼100 amino acids at both termini) shorter than the respective prokaryotic-like analogues. The amino acid residues located at the N-terminal region of the bacterial β subunit, partially conserved in eukaryotic-like PheRSs, form nonspecific contacts with $tRNA^{Phe}$. Coupled with base-specific interactions of the 3′-terminal adenosine with the catalytic subunit, they control the conformation of the single-stranded acceptor arm region (*15*). From structural (*17*) and biochemical (*31*) studies, it follows that the proper positioning of the $tRNA^{Phe}$ terminal nucleotide corresponding to the productive complex is promoted only in the presence of phenylalanyl adenylate. Here, we investigate the binding mode of the $tRNA^{Phe}$ acceptor end by human PheRS, a eukaryotic-like enzyme with an unknown structure, to compare it with that in the prokaryotic-like phenylalanine-specific system. The s^4U-induced affinity cross-linking assay with $tRNA^{Phe}$ derivatives substituted at the 3′ end has been used to localize the binding of the two terminal nucleotides (75 and 76) on the enzyme subunits in the absence or presence of other substrates (phenylalanine and/or ATP). The results show that despite structural differences between the two groups of tetrameric PheRSs, the mode of the initial binding of the $tRNA^{Phe}$ acceptor end and its correct positioning for the productive interaction are conserved through evolution from eubacteria to humans.

MATERIALS AND METHODS

Materials. 4-Thiouridine 3′,5′-diphosphate (ps^4Up) was synthesized by V. S. Bogachev (Novosibirsk Institute of Bioorganic Chemistry). [(*N*-Acryloylamino)phenyl]mercuric chloride (APM) was kindly provided by G. L. Igloi (Universität Freiburg, Freiburg, Germany). L-Phenylalaninyl adenylate (PheOH-AMP), an aminoacyl adenylate analogue with a methylene group substituted for the carbonyl group, was synthesized as described previously (*32*). Plasmid DNAs containing genes of human or *Escherichia coli* $tRNA^{Phe}$ under control of the phage T7 promoter were a generous gift of O. C. Uhlenbeck (University of Colorado, Boulder, CO). Prestained protein molecular mass markers in the range of 6.5−175 kDa were from Biolabs.

Homogeneous human PheRS (as evidenced by electrophoresis under denaturing conditions) with a specific activity of 190 units/mg was isolated from a recombinant *E. coli* strain as described previously (*33*). One unit of PheRS activity is defined as the amount of enzyme which catalyzes phenylalanylation of 1 nmol of $tRNA^{Phe}$ in 1 min at 25 °C. T7 RNA polymerase was isolated from *E. coli* BL21 cells harboring the pAR1219 plasmid. Calf intestine alkaline phosphatase and RNA ligase were from Pharmacia. Benzonase (benzon nuclease) was from Merck. Recombinant RNasin ribonuclease inhibitor was from Promega.

Preparation of $tRNA^{Phe}$ and s^4U-Containing $tRNA^{Phe}$ Derivatives. Human and *E. coli* $tRNA^{Phe}$s were synthesized using runoff transcription of synthetic genes with T7 RNA polymerase followed by electrophoretic isolation of full-length transcripts correctly ended with the CCA sequence as described previously (*28*). To prepare labeled tRNAs, transcriptions were run in the presence of [α-^{32}P]ATP.

$tRNA^{Phe}$ derivatives containing an s^4U residue at the 3′ end (incorporated as 3′,5′-diphosphate), $tRNA^{Phe}$-s^4Up77, $tRNA^{Phe}$-s^4Up76, and $tRNA^{Phe}$-s^4Up75, were synthesized by ligation of ps^4Up to the respective tRNA, full-length or truncated by one or two nucleotides at the 3′ end. The truncated tRNAs were prepared using a standard protocol for RNA stepwise degradation (*34*) followed by electrophoretic purification. Ligations were performed in reaction mixtures containing 50 mM HEPES-NaOH (pH 8.3), 10 mM $MgCl_2$, 5 mM DTT, 10% (v/v) dimethyl sulfoxide, 15% (v/v) glycerol, 100 μM ATP, 3 μM tRNA, 600 μM ps^4Up, 3 units/mL RNasin, and 460 units/mL RNA ligase. Incubations were carried out for 24 h at 4 °C, and reactions were stopped by phenol/chloroform extraction. Dephosphorylation of the derivatives to prepare their counterparts lacking the 3′-terminal phosphate ($tRNA^{Phe}$-s^4U77, $tRNA^{Phe}$-s^4U76, and $tRNA^{Phe}$-s^4U75) with calf intestine alkaline phosphatase was carried out according to a described procedure (*35*). The s^4U-containing tRNAs were further purified by affinity electrophoresis on an 8% denaturing polyacrylamide gel (PAG) containing 2 μg/mL APM, as described previously (*36*).

tRNA Aminoacylation. Aminoacylation of $tRNA^{Phe}$ transcripts (human or *E. coli*) was performed under conditions described previously (*33*). Eight tRNA concentrations ranging from 0.05 to 20 μM (depending on the tRNA) were used for the determination of kinetic parameters (K_m and k_{cat}). The K_i values for non-aminoacylable $tRNA^{Phe}$ derivatives were determined in experiments with three concentrations of the inhibitor varying in the range of 0.2−1.5 μM. The kinetic parameters were calculated using Microcal Origin 4.10; the type of inhibition was analyzed using double-reciprocal plots (*37*). The reported parameters represent the average of at least three determinations with experimental errors of less than 10% of the indicated values.

Cross-Linking of s^4U-Containing $tRNA^{Phe}$ Derivatives to PheRS and Analysis of Cross-Linked Products. Cross-linking of ^{32}P-labeled $tRNA^{Phe}$ derivatives to PheRS was carried out in a 10 μL reaction mixture containing 50 mM Tris-HCl (pH 8) and 30 mM $MgCl_2$. To optimize the reaction conditions,

the concentration of the protein or tRNA derivative was varied in the range of 0.1−3 μM at a fixed concentration of the second component (0.1 μM tRNA or 1 μM PheRS). The data presented were obtained at 1 μM PheRS and 0.4 μM photoreactive tRNA^{Phe}s. To study the effects of small substrates or an aminoacyl adenylate analogue on PheRS−tRNA cross-linking, reactions were performed in the presence of 5 mM ATP (K_m = 0.1 mM), 75−150 μM L-phenylalanine (K_m = 1.5 μM), or 10−50 μM PheOH-AMP (K_i = 0.2 μM). The specificity of photo-cross-linking was proven by competition experiments performed in the presence of 4−8 μM human tRNA^{Phe} (transcript). Samples were irradiated at 20 °C for 20 min with 365 nm light from an HBO 200W superpressure mercury lamp selected by means of a Bausch and Lomb monochromator. Cross-linked products were separated from free tRNA and free enzyme using denaturing electrophoresis on an SDS Laemmli gel consisting of a 5% stacking and a 10% separating gel. The gel was dried and subjected to autoradiography. The extent of cross-linking was quantified using a PhosphorImager (Molecular Dynamics). The values presented are the average of at least three separate determinations with standard deviations of 3−5% for major and 6−8% for minor products. They correspond practically to maximal levels of cross-linking as they increased by less than 5% (of the initial values) upon irradiation for a further 40 min. In all experiments described herein, the specific radioactivity of tRNA^{Phe}-s^{4}U77 and tRNA^{Phe}-s^{4}Up77 was 1.7-fold less than that of the other tRNA^{Phe} analogues. To identify PheRS subunits in the cross-linked products, the irradiated mixtures were incubated with benzonase (250 units/mL) at 37 °C for 30−60 min, and then products of hydrolysis were separated and analyzed as described above.

RESULTS

Preparation of Photoreactive tRNA^{Phe} Derivatives and Testing Their Interaction with Human PheRS. The structures of the photoreactive tRNA^{Phe} derivatives that were used are shown in Figure 1. Modified tRNA^{Phe}s containing 4-thiouridine 3′-phosphate at the 3′ end (tRNA^{Phe}-s^{4}Up77, tRNA^{Phe}-s^{4}Up76, and tRNA^{Phe}-s^{4}Up75) were synthesized by enzymatic attachment (with RNA ligase) of 4-thiouridine 3′,5′-diphosphate (ps^{4}Up) to a human tRNA^{Phe} transcript, full-length or truncated by one or two nucleotide(s) from the 3′ end by the method of stepwise degradation (*34*). Further phosphatase treatment of the analogues resulted in their nonphosphorylated counterparts (tRNA^{Phe}-s^{4}U77, tRNA^{Phe}-s^{4}U76, and tRNA^{Phe}-s^{4}U75). s^{4}U-containing tRNA^{Phe}s were separated from the initial tRNAs using the affinity electrophoresis technique (*38*) based on retardation of sulfur-containing nucleic acids by a specific interaction with an organomercurial derivative (APM) included in the gel.

Human tRNA^{Phe} synthesized by *in vitro* transcription is an efficient substrate of human PheRS, with the catalytic efficiency (k_{cat}/K_m) of aminoacylation being 2-fold lower than for the native tRNA^{Phe} (ref *33* and this work). None of the 3′ end-modified (nonphosphorylated) tRNAs, including the correct-length tRNA^{Phe}-s^{4}U76, revealed acceptor activity. But all modified tRNAs bind specifically to PheRS, since they are effective competitive inhibitors of aminoacylation. The inhibition constants (K_i) of some analogues presented in Table 1 are compared to each other and provisionally to the K_m parameter of the wild-type human tRNA^{Phe} transcript.

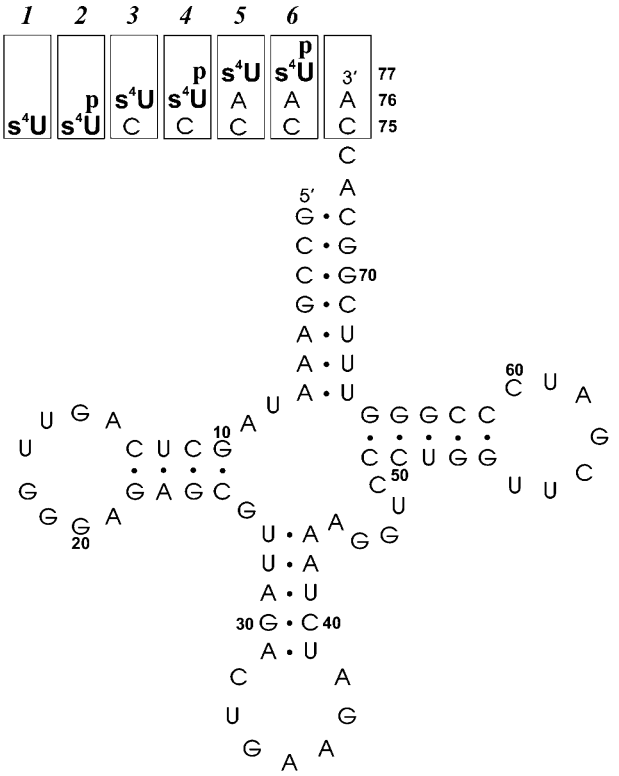


FIGURE 1: Structure of human tRNA^{Phe} (wild-type transcript). Modifications in the 3′-terminal sequence indicated in the frames correspond to the structures of reagents: tRNA^{Phe}-s^{4}U75 (1), tRNA^{Phe}-s^{4}Up75 (2), tRNA^{Phe}-s^{4}U76 (3), tRNA^{Phe}-s^{4}Up76 (4), tRNA^{Phe}-s^{4}U77 (5), and tRNA^{Phe}-s^{4}Up77 (6).

Table 1: Substrate Binding Properties of 3′-End-Modified Human tRNA^{Phe}s in the Aminoacylation Reaction Catalyzed by Human PheRS

tRNA^{Phe} (modification[a])	K_i (μM)	K_i/K_m[b]
tRNA^{Phe}-Cp75	0.69 ± 0.05	6.3 (5.3)[c]
tRNA^{Phe}-s^{4}U75	0.71 ± 0.04	6.4 (5.3)[c]
tRNA^{Phe}-s^{4}U76	0.64 ± 0.03	5.8 (4.8)[c]
tRNA^{Phe}-s^{4}U77	0.40 ± 0.02	3.6 (3.1)[c]
tRNA^{Phe}-s^{4}Up77	0.35 ± 0.03	3.2

[a] The 3′-terminal nucleotide (5′-monophosphate or 3′,5′-diphosphate) and its position in the modified tRNA are indicated. [b] Loss of binding affinity estimated from the K_i/K_m ratio; the K_m value for the wild-type human tRNA^{Phe} transcript is 0.11 μM. [c] For comparison, data obtained in the *T. thermophilus* Phe-specific system (*31*, *39*) are shown in parentheses.

The data suggest that substitution of the 3′-terminal adenosine with s^{4}U and its removal (tRNA^{Phe}-Cp75) reduced the binding affinity to a comparable extent. The decrease in affinity observed for tRNA^{Phe}-s^{4}U75 is in the same range and is evidently due to missing nucleotide 76. The attachment of s^{4}UMP as an additional nucleotide at the 3′ end (tRNA^{Phe}-s^{4}U77) reduced affinity less (1.6-fold) than its substitution for nucleotide 76. Phosphorylation of tRNA^{Phe} analogues at the 3′ end decreased their K_i values only slightly (within experimental error), as shown for tRNA^{Phe}-s^{4}Up77. The data evidence the functional importance of base-specific interactions of A76 for productive binding of tRNA^{Phe} to human PheRS.

Cross-Linking of s^{4}U-Containing tRNA^{Phe} Analogues to PheRS and Identification of the Cross-Linked Products. Photoreactive derivatives of human tRNA^{Phe} substituted with s^{4}U at the 3′ end were irradiated in the presence of PheRS

Table 2: Affinity Labeling of Human PheRS with $tRNA^{Phe}$ Derivatives

	relative yield[b] (%)					
product[a] (kDa)	s^4U75	s^4Up75	s^4U76	s^4Up76	s^4U77	s^4Up77
I (α) (63)	2.0	2.0	6.2	3.4	10	1.8
II (α) (69)	10	24	26	53	64	93
III (α) (73)			5.8	5.6		
IV (α) (75)	18	31	18	22	10	3.2
V (β) (84)	70	43	44	16	16	2.0
α/β[c]	0.43 (0.05)[d]	1.3	1.3 (1.9)[d]	5.2	5.2 (6.8)[d]	49
total cross-linking efficiency[e] (%)	23	22	23	25	37	62

[a] The products are listed according to their electrophoretic mobility. The subunit cross-linked to the tRNA is indicated in parentheses. [b] Yields of products in relation to total cross-linking of the α and β subunits. [c] Cross-linking ratio for α and β subunits. [d] Data shown in parentheses were obtained for the *T. thermophilus* Phe-specific system (*31*, *39*). [e] Defined as the amount of cross-linked tRNA normalized to the total amount of tRNA in the irradiated sample.

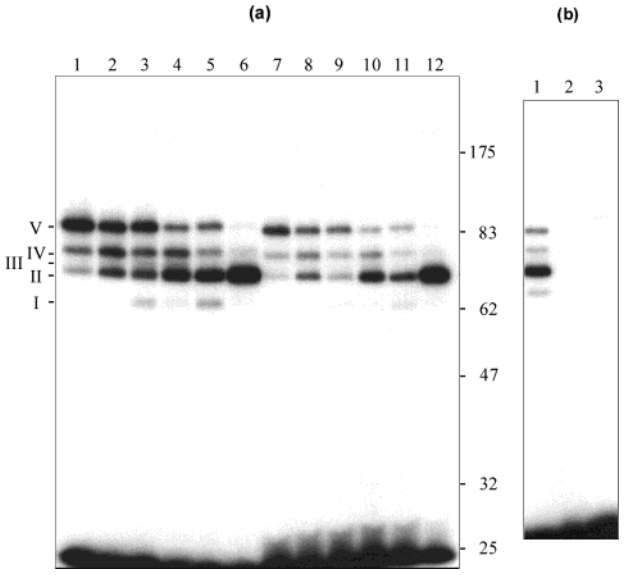


FIGURE 2: Photoaffinity labeling of human PheRS with $tRNA^{Phe}$ derivatives. (a) The derivatives of human $tRNA^{Phe}$ ($tRNA^{Phe}$-s^4-U75 in lanes 1 and 7, $tRNA^{Phe}$-s^4Up75 in lanes 2 and 8, $tRNA^{Phe}$-s^4U76 in lanes 3 and 9, $tRNA^{Phe}$-s^4Up76 in lanes 4 and 10, $tRNA^{Phe}$-s^4U77 in lanes 5 and 11, and $tRNA^{Phe}$-s^4Up77 in lanes 6 and 12 at 0.4 μM) were irradiated with 365 nm light in the presence of PheRS (1.0 μM) under identical conditions in the absence (lanes 1−6) or presence (lanes 7−12) of the wild-type human $tRNA^{Phe}$ transcript (8.0 μM). Equal aliquots of the samples were separated on a 10% SDS gel. Products of photo-cross-linking (described in detail in the text) are specified on the left of the autoradiogram. Positions of molecular mass (kilodaltons) markers are indicated on the right. Free tRNA migrated to the bottom of the gel. (b) Comparative labeling of PheRS with s^4U77-containing derivatives of human (0.4 μM, lane 1) and *E. coli* $tRNA^{Phe}$ (0.4 and 0.8 μM in lanes 2 and 3, respectively).

with long-wavelength UV light. Cross-linked products were separated by SDS−PAGE (Figure 2a). Five products of cross-linking to PheRS, marked I−V on the autoradiogram, migrated with apparent molecular masses in the range of 63−84 kDa (evaluated relative to marker proteins). Their relative yields (determined by normalization to the total enzyme cross-linking) and efficiencies of formation varied for the six analogues that were used (Table 2). The specificity of the photo-cross-linking reaction is evidenced from its inhibition in the presence of the wild-type human $tRNA^{Phe}$ transcript (see Figure 2a, lanes 7−12): under the indicated conditions, the efficiency of cross-linking decreased by 60−63% for $tRNA^{Phe}$-s^4U75, $tRNA^{Phe}$-s^4Up75, and $tRNA^{Phe}$-s^4-U76 and by 48, 54, and 33% for $tRNA^{Phe}$-s^4Up76, $tRNA^{Phe}$-s^4U77, and $tRNA^{Phe}$-s^4Up77, respectively. The addition of the nonreactive $tRNA^{Phe}$ equally reduced the yields of all the products, indicating that cross-linking occurs within the specific complex of PheRS with the $tRNA^{Phe}$ analogue. The apparent weak inhibition observed with competing tRNA (added in a 20-fold excess) is mainly due to its modest affinity for PheRS: a dissociation constant K_d of ~2 μM was determined by a gel-retardation assay (N. Moor et al., unpublished data). Cross-linking experiments with $tRNA^{Phe}$-s^4U77 obtained in the background of *E. coli* $tRNA^{Phe}$ (Figure 2b) showed a 40-fold decrease in the efficiency of photocoupling between human PheRS and the heterologous $tRNA^{Phe}$ as compared to that observed for the respective human $tRNA^{Phe}$ derivative under identical conditions. This is a further indication of the high specificity of the cross-linking reaction between human PheRS and the photoreactive $tRNA^{Phe}$ derivatives that were used. Indeed, the wild-type *E. coli* $tRNA^{Phe}$ transcript is a poor substrate of human PheRS: its catalytic efficiency of aminoacylation is 380-fold less than that of the wild-type human $tRNA^{Phe}$ transcript.

To determine the nature of cross-linked products, we digested them with benzonase, an endonuclease that degrades RNA to small oligonucleotides. SDS−PAGE analysis of the cross-linked tRNA after nuclease hydrolysis (Figure 3) revealed two radiolabeled bands, comigrating with the enzyme subunits (α and β, visualized by Coomassie blue staining). Such experiments (data not shown) were made with the six derivatives in the absence or presence of small substrates, whose addition had distinctive effects on relative yields of the products as discussed below. The distribution of radioactivity between the two subunits in hydrolyzed samples was analyzed and compared to the relative yields of cross-linked products in the respective probes not treated with nuclease. The relative yield of the band corresponding to the large β subunit [~66 kDa derived from the sequence (*29*)] was always consistent with that of product V. We thus conclude that the slowly migrating product is a β subunit−tRNA cross-link, while the other four products (marked I−IV) result from cross-linking to the small α subunit [~57 kDa (*29*)]. The formation of four products (with different electrophoretic mobility) for the same (α) subunit can be explained only by attachment of the reagent to distinct amino acid residues of the protein. The polyanionic RNA can influence SDS binding and hence the hydrodynamic shape and the net charge of the SDS-coated complex in a different way depending on the nature and position of the cross-linked

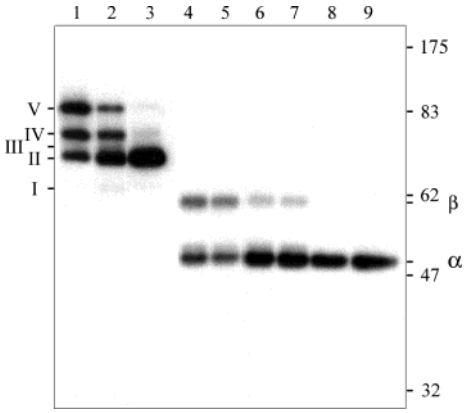


FIGURE 3: Identification of the cross-linked PheRS subunits in the photoproducts. Cross-linked products of tRNA^{Phe}-s^{4}Up75 (lanes 1, 4, and 5), tRNA^{Phe}-s^{4}Up76 (lanes 2, 6, and 7), and tRNA^{Phe}-s^{4}Up77 (lanes 3, 8, and 9) with PheRS were separated directly after irradiation (lanes 1−3) or after benzonase hydrolysis of the irradiated samples (30 min hydrolysis for lanes 4, 6, and 8 and 60 min hydrolysis for lanes 5, 7, and 9). Products of photo-cross-linking are specified on the left of the autoradiogram; positions of molecular mass (kilodaltons) markers and PheRS subunits are indicated on the right. The relative yield (determined by normalization to the total PheRS cross-linking) of product V in the probe not treated with nuclease is equal to that of the band comigrating with the β subunit in the respective hydrolyzed sample (43% for lanes 1, 4, and 5, 16% for lanes 2, 6, and 7, and 2% for lanes 3, 8, and 9).

amino acid residue. Chemical modification (or mutation) of a single amino acid residue has been shown to affect the electrophoretic migration (in SDS gels) of a number of proteins (*40*, *41*, and references therein). The behavior of the α subunit products (I to IV) is primarily due to the position of the branching site as their apparent molecular masses vary by 6−12 kDa, whereas a single amino acid modification (with small reagents) triggers at most a ±2 kDa shift in the apparent molecular mass. Recently, we revealed differently migrating products of both the α subunit and β subunit when analogous s^{4}U-substituted tRNA^{Phe} derivatives were cross-linked to *T. thermophilus* PheRS (*31*).

Product V is a major product of PheRS cross-linking with tRNA^{Phe}-s^{4}U75 (see Table 2); the labeling of the β subunit in this case was 2.3 times higher than that of the α subunit. Repositioning of the s^{4}U residue by one or two nucleotide units to the 3′ end (tRNA^{Phe}-s^{4}U76 or tRNA^{Phe}-s^{4}U77) increased the relative level of α subunit cross-linking by 26 or 54%, respectively. Phosphorylation of the s^{4}U residue (at the 3′-OH ribose group) resulted in the same effect on the cross-linking ratio for α and β subunits as did its repositioning to the adjacent position (as evidenced from comparing PheRS cross-linking with tRNA^{Phe}-s^{4}Up75 and tRNA^{Phe}-s^{4}U76 or with tRNA^{Phe}-s^{4}Up76 and tRNA^{Phe}-s^{4}U77). However, the two structural changes had different effects on the relative yields of different α subunit cross-links. Formation of product III was observed for only two derivatives, tRNA^{Phe}-s^{4}U76 and tRNA^{Phe}-s^{4}Up76. The 3′-end phosphorylation of tRNA^{Phe}-s^{4}U77 caused a further increase in the level of α subunit cross-linking, which became prevalent (98%). tRNA^{Phe}-s^{4}Up77 revealed a significantly higher efficiency of photocoupling with PheRS than its nonphosphorylated counterpart (1.7-fold) and the other four derivatives (from 2.5- to 2.8-fold) cross-linked with identical or closely similar efficiencies. This is obviously related to the higher binding affinity of tRNA^{Phe}-s^{4}Up77 for the enzyme, which follows from comparison of the extents of cross-linking inhibition by the nonreactive tRNA^{Phe} (see Figure 2a and its description above).

The results of cross-linking experiments show that two different subunits of human PheRS bind the CCA end of human tRNA^{Phe}. The 3′-terminal nucleotide is located at the catalytic α subunit, and the adjacent nucleotide is at the large β subunit. Formation of a covalent s^{4}U-induced cross-link implies a close contact between the reacting groups (*42*), indicating that the tRNA and enzyme regions involved are close to each other. Therefore, the absence of strict selectivity of the cross-linking reaction between the site-specifically substituted tRNAs and the two enzyme subunits as well as multiple α subunit cross-links suggests conformational flexibility of the 3′ end of the bound tRNA^{Phe}.

Cross-Linking of tRNA^{Phe} Derivatives to PheRS Complexed with Small Substrates or Their Analogues. Low-molecular mass functional ligands of PheRS (Phe, ATP, and Phe+ATP) added at subsaturating concentrations affect both the total cross-linking efficiency and, even more relevant here, the cross-link distribution (Figure 4). The total cross-linking efficiency reflects both local rearrangement and a possible change in binding affinity, whereas the cross-link distribution depends solely upon local rearrangement. The ratio of cross-links directed to the α subunit versus the β subunit decreased, whatever ligand was added (compare the data presented in Table 2 and panels b and d of Figure 4). As none of the cross-links observed in the absence of ligands was abolished and no new cross-link appeared, it can be concluded that the tRNA 3′ end retains some conformational flexibility, which is modulated by ligand binding. Changes in the yields of the α subunit cross-links can result from substrate-induced structural rearrangements or blockage of functional residues located in the active site area. In turn, these effects will modulate the conformations accessible to the tRNA 3′ end, and in particular, stereochemical interference for the 3′-extended tRNAs is likely to occur. The influence of small substrates on the cross-linking of the correct-length tRNA^{Phe}-s^{4}U(p)76 or 3′-truncated tRNA^{Phe}-s^{4}U(p)75 to the β subunit is expected to be due solely to rearrangement of the 3′-terminal nucleotide since binding of the substrates should not alter the structure of this subunit as shown for the *T. thermophilus* system (*16*, *17*). Phenylalanine displays no appreciable effect on the cross-linking of 3′ phosphate free tRNA^{Phe} analogues to the β subunit (V), while the α subunit cross-links (I−IV) appear to be differentially quenched (see Figure 4b). Remarkably, the relative yields of the cross-links obtained with all three of the phosphorylated derivatives when Phe substrate was added are close to those of their nonphosphorylated counterparts in the absence of ligands (Figure 4c). This finding suggests that the phosphate group appended to the 3′ end of the tRNAs competes with the substrate for enzyme binding. This nonfunctional interaction (realized in the absence of phenylalanine) significantly influences the orientation of the photoreactive nucleotide at all the positions that have been tested (see the previous section). Its stabilizing effect on 3′ end binding, which is most obvious for tRNA^{Phe}-s^{4}Up77 (as revealed by its high cross-linking selectivity and the weak protective effect of the nonreactive tRNA^{Phe}), may account for the more pronounced influence of phenylalanine on the β subunit cross-linking with the phosphorylated analogues (see Figure 4b).

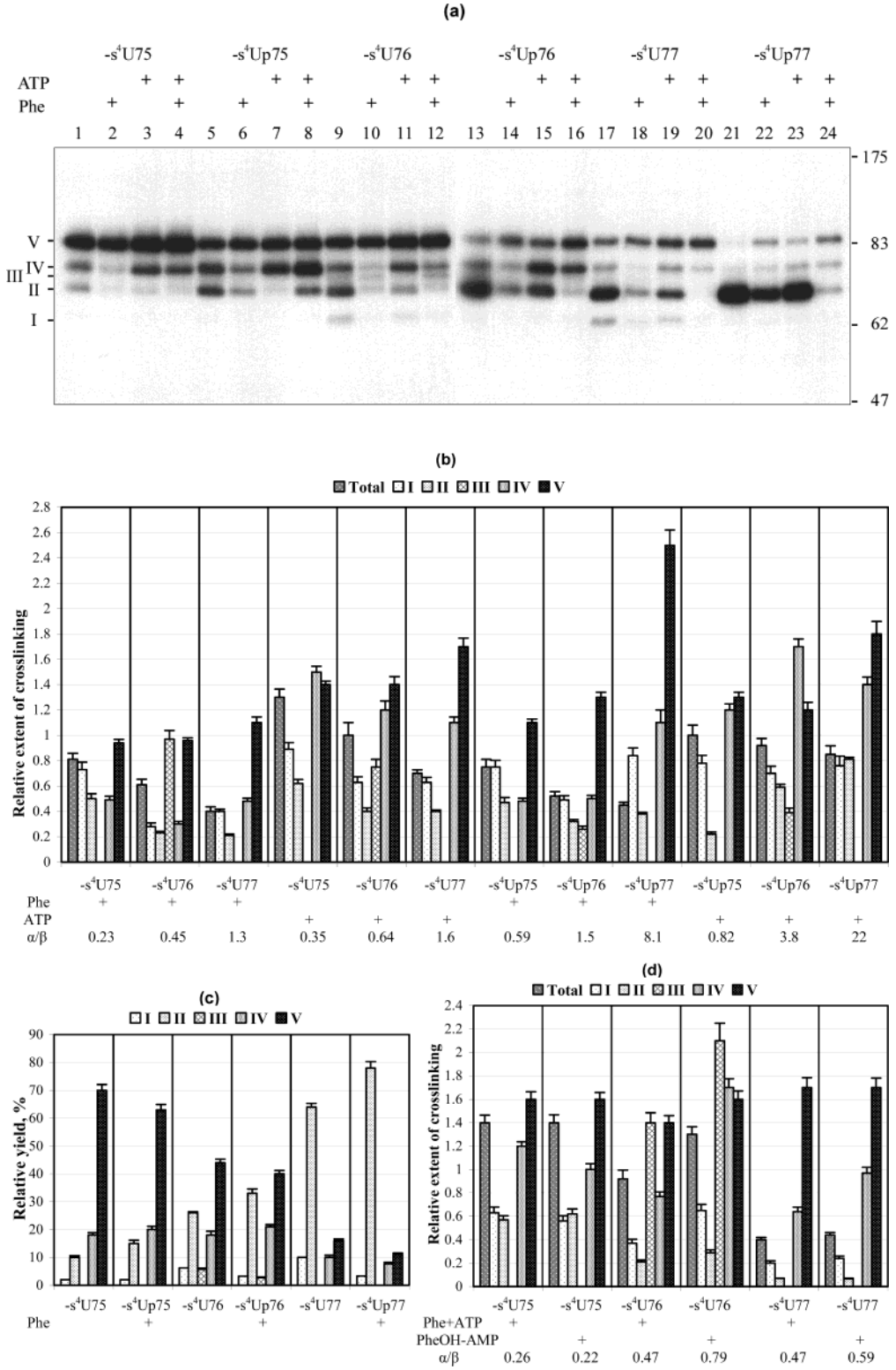


FIGURE 4: Influence of low-molecular mass ligands on human PheRS cross-linking with human $tRNA^{Phe}$ derivatives. (a) Autoradiogram of a gel after separation of cross-linked products obtained with $tRNA^{Phe}$-s^4U75 (lanes 1−4), $tRNA^{Phe}$-s^4Up75 (lanes 5−8), $tRNA^{Phe}$-s^4U76 (lanes 9−12), $tRNA^{Phe}$-s^4Up76 (lanes 13−16), $tRNA^{Phe}$-s^4U77 (lanes 17−20), or $tRNA^{Phe}$-s^4Up77 (lanes 21−24) in the absence (lanes 1, 5, 9, 13, 17, and 21) or presence of substrates L-phenylalanine (lanes 2, 6, 10, 14, 18, 22), ATP (3, 7, 11, 15, 19, and 23), and L-phenylalanine and ATP (lanes 4, 8, 12, 16, 20, and 24). (b) Analysis of the effects of substrate (phenylalanine or ATP) on the efficiency of total cross-linking or α (I−IV) and β (V) subunit cross-link formation. The relative extent of the total enzyme cross-linking or individual product formation was determined from the ratio of the total cross-linking efficiency or the product yield in the presence vs the absence of small substrates. (c) The distribution of cross-links obtained with the 3′-phosphorylated derivatives in the presence of the Phe substrate is parallel to that for the respective nonphosphorylated counterparts in the absence of small ligands. Relative yields of products were determined in relation to total cross-linking of the α and β subunits. (d) Comparison of the effects of phenylalanyl adenylate and its synthetic analogue (PheOH-AMP) on the cross-linking. Values represent the mean (±standard error) from at least three experiments. The small ligands were added at subsaturating concentrations (75 μM Phe, 5 mM ATP, and 50 μM PheOH-AMP) based on their K_m (K_i) values (specified in Materials and Methods). The cross-linking ratio for α and β subunits is indicated below the histograms.

Table 3: Interatomic Contacts between PheRS and Functional Ligands[a]

protein residue[b]	tRNA^{Phe} [c]	Phe substrate[c]	ATP (AMP)[c]
Metβ-1	C74 O2P (es)		
Argβ-2	C74 O1P (es), A73 O2P (es)		
Valβ-160 (Leu/Ile)	C75 O2P (hb)		
Argβ-362	C74 O2′ (hb)		
Glnα-207	C69 O2P (hb)		
Trpα-149	A76 N7 (hb), A76 base (aa)	O (hb)	
Serα-180	A76 N6 (hb)	N (hb)	
Gluα-220 (Asp)	A76 N6 (hb)	Phe ring (vdw), N (hb)	
Pheα-258	A76 base (aa)	Phe ring (aa)	
Pheα-260 (Tyr)	A76 base (aa)	Phe ring (aa)	
Metα-148			O1P (hb), O2P (hb)
Arg*α-204		O (hb)	O1P (hb)
Gluα-206 (Asp)			N6 (hb)
Hisα-212			adenine (vdw)
Gluα-213			N1 (hb), N6 (hb)
Phe*α-216			adenine (vdw)
Glnα-218		O (hb), N (hb)	O4′ (hb), O5′ (hb)
Gluα-279			O3′ (hb)
Leuα-280 (Val/Ile)			O2′ (hb), O3′ (hb)
Glyα-281			O3′ (hb)
Glyα-318			O2′ (hb), N3 (hb)
Arg*α-321			adenine (vdw), O2′ (hb), N3 (hb)
Ileα-332			adenine (vdw)

[a] Determined in binary complexes of *T. thermophilus* PheRS with cognate tRNA, Phe substrate, or Phe-AMP (*15*−*17*). [b] Amino acid residues conserved (invariant or replaced with similar residues indicated in parentheses) in the PheRSs are bold type; those with asterisks are class II conserved. [c] The type of interaction (es, electrostatic interaction; hb, hydrogen bond; vdw, van der Waals contact; and aa, aromatic−aromatic interaction) is indicated in parentheses.

In the presence of ATP, the yields of three α subunit cross-links (I−III) decreased to different extents, while those of products IV and V increased noticeably (see Figure 4b). The effects on β subunit cross-linking are identical for tRNA^{Phe}-s^{4}U76 and tRNA^{Phe}-s^{4}U75 (or slightly stronger for tRNA^{Phe}-s^{4}Up75 than for tRNA^{Phe}-s^{4}Up76), showing that both nucleotide 76 and nucleotide 75 are involved in the rearrangement promoted by ATP. Moreover, the influence of ATP on the β subunit cross-linking with the nonphosphorylated derivatives is much more pronounced than that of phenylalanine and retained (for tRNA^{Phe}-s^{4}U76 and tRNA^{Phe}-s^{4}U77) or slightly increased (for tRNA^{Phe}-s^{4}U75) when the two substrates were added simultaneously (compare the data presented in panels b and d of Figure 4). The effects of aminoacyl adenylate (synthesized by the enzyme from Phe and ATP) on PheRS cross-linking with three derivatives (nonphosphorylated) were compared to those of its synthetic analogue (PheOH-AMP) with a methylene group substituted for the carbonyl group (see Figure 4d). The most striking differences are seen for tRNA^{Phe}-s^{4}U76. The yield of the unique α subunit cross-link (product III, observed only for s^{4}U76-substituted derivatives) increased remarkably (1.4-fold) in the presence of aminoacyl adenylate and much more (2.1-fold) in the presence of PheOH-AMP, while it remained practically unchanged or reduced upon addition of phenylalanine or ATP, respectively. The formation of product IV (a major α subunit cross-link) was quenched in the presence of the true intermediate, but strengthened remarkably (1.7-fold) in the presence of the analogue. The efficiency of β subunit cross-linking increased to a higher level upon addition of PheOH-AMP than in the presence of aminoacyl adenylate, indicating a different orientation of nucleotide 76 in the two complexes of PheRS and thus suggesting that the positioning of this nucleotide is controlled by amino acid residues interacting with the carbonyl group of the aminoacyl adenylate (or the Phe substrate).

These data suggest that phenylalanine binding triggers a local rearrangement within the active site, thus influencing mainly the positioning of the tRNA^{Phe} 3′-terminal nucleotide, while ATP binding modulates the interactions of the whole single-stranded 3′ end with PheRS.

DISCUSSION

The results of our study show that two different subunits of human PheRS bind two adjacent nucleotides of the tRNA^{Phe} 3′ end: the terminal nucleotide is located on the catalytic α subunit, while nucleotide 75 is on the β subunit. A given mode of acceptor end binding was previously revealed for *T. thermophilus* PheRS (*15*) and evidenced by affinity cross-linking experiments (*31*, *39*). Multiple cross-links to the α and β subunits formed by 3′-end-substituted (with s^{4}U or 6-thioguanosine) tRNA^{Phe}s strongly suggest conformational flexibility of the acceptor end in the PheRS−tRNA^{Phe} complex under functional conditions. The same type of human tRNA^{Phe} derivatives used in this work revealed a lower selectivity of cross-linking to human PheRS (compare the cross-linking ratio for α and β subunits presented in Table 2 for the two systems), suggesting that CCA-end binding in this system is less rigid than in the *T. thermophilus* system. The conformation of the single-stranded 3′ ACCA end of tRNA^{Phe} in the complex with *T. thermophilus* PheRS (*15*) is maintained by base-specific interactions of the terminal adenosine with five amino acid residues of the α subunit and a network of backbone-mediated contacts with four residues of the β subunit, Metβ-1, Argβ-2, and Valβ-160 from domain B1 and Argβ-362 from domain B3 (Table 3). The multiple-sequence alignment of five prokaryotic-like (eubacterial) and four eukaryotic-like (three from the cytoplasm of eukaryotes and one from archaea) PheRSs (Figure 5) shows that the sequence of the eukaryotic-like β subunit is significantly shorter at the N-terminus. Valβ-160 located in the proximity of the most conserved (P/A)NRxDxL (where

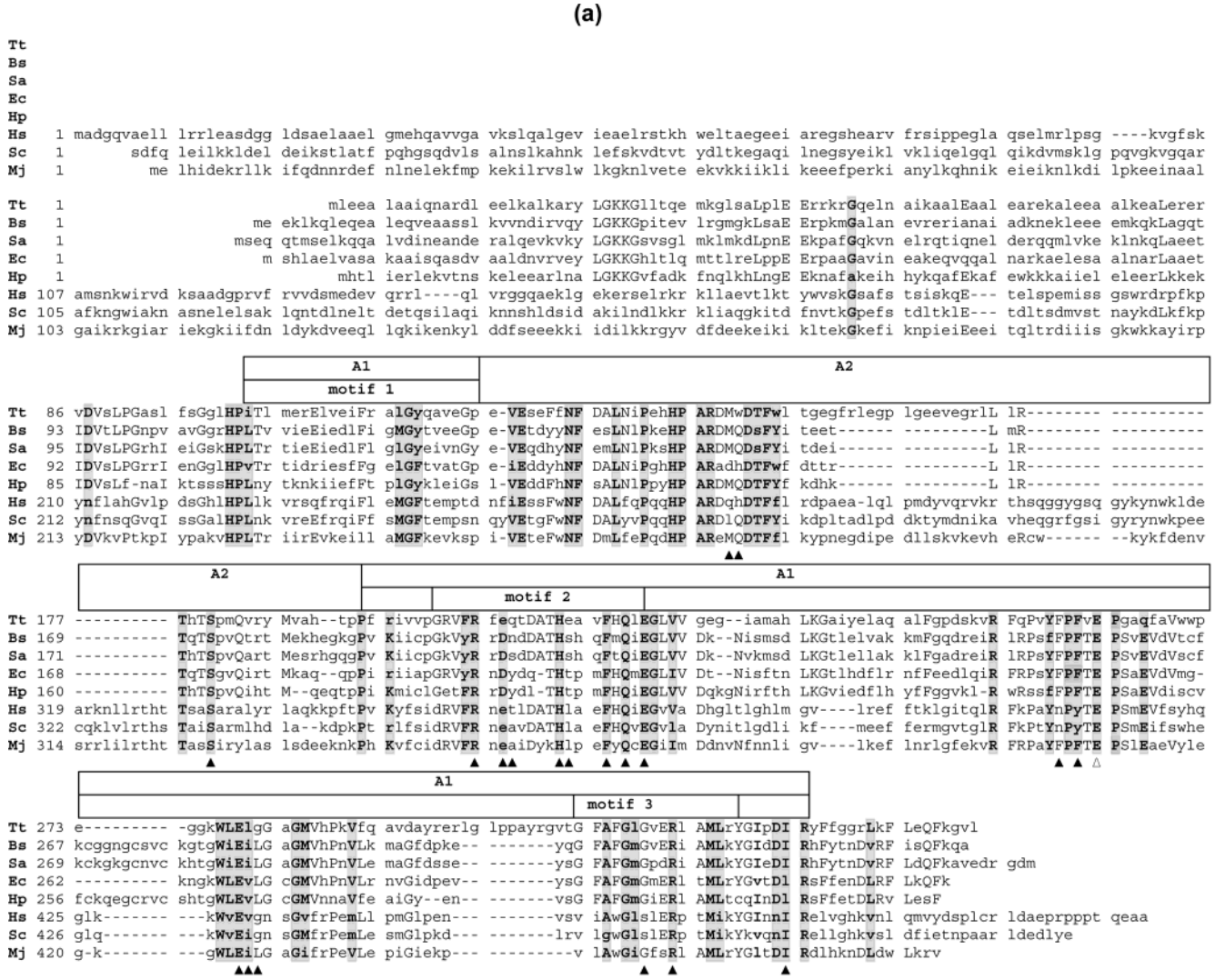
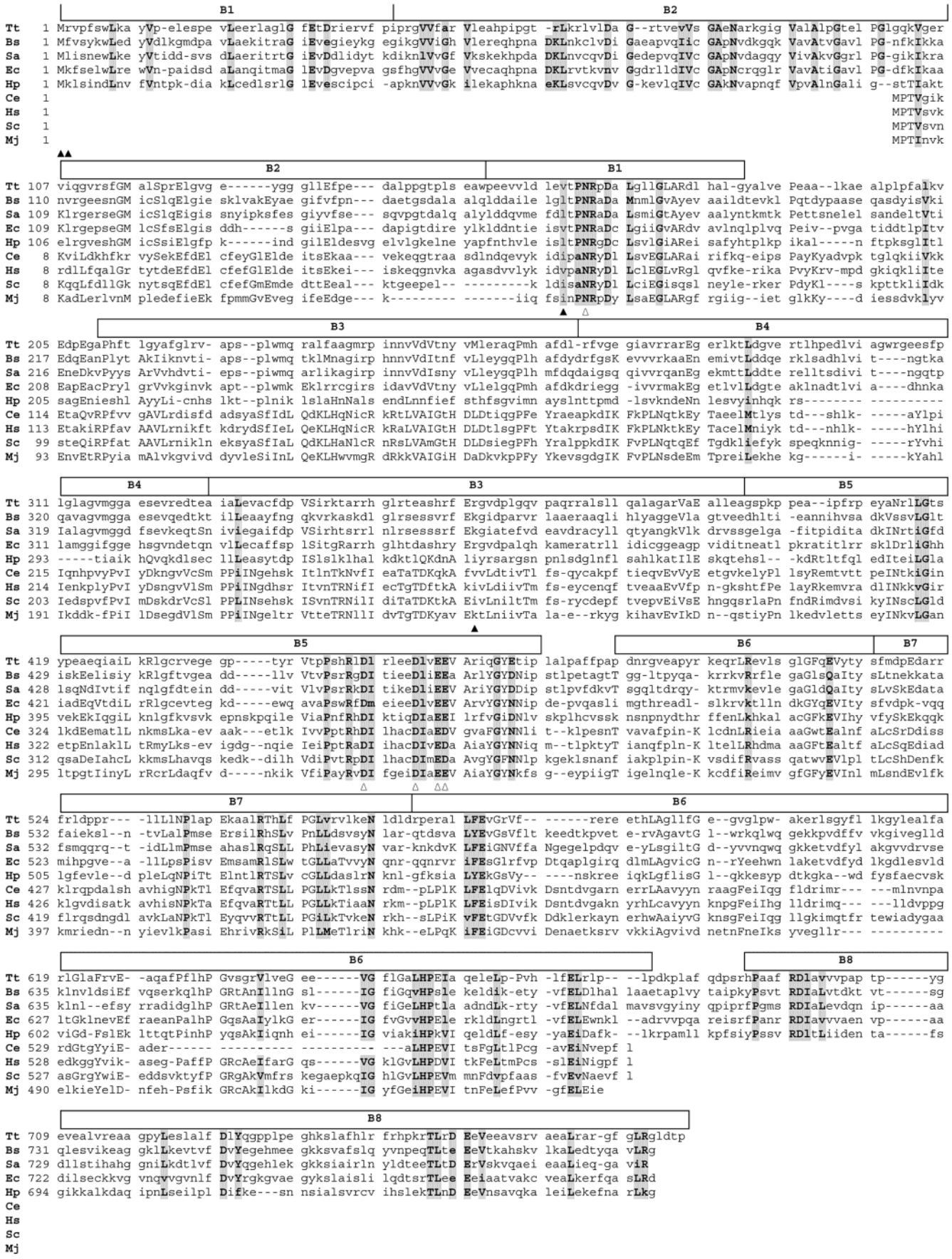


FIGURE 5: Sequence alignment of the α (a) and *β* subunits (b) of tetrameric PheRSs from different organisms [*T. thermophilus* (Tt), *Bacillus subtilis* (Bs), *Staphylococcus aureus* (Sa), *E. coli* (Ec), *Helicobacter pylori* (Hp), *Caenorhabditis elegans* (Ce), *Homo sapiens* (Hs), *Saccharomyces cerevisiae* (Sc), and *Methanococcus jannaschii* (Mj)]. The location of domains and motifs is depicted above the sequences with labeled boxes. Shaded boxes denote residues that are conserved (identical or similar) in all the sequences or in the terminal extensions of the bacterial *β* subunits. Black triangles below the sequences mark amino acid residues interacting with substrates (as identified for the *T. thermophilus* enzyme and summarized in Table 3). The positions of six residues constituting the metal binding site at the α−*β* subunit interface are indicated by white triangles. Database searching was performed using the National Center for Biotechnology Information (NCBI), Aminoacyl-tRNA synthetase database Y2K (*43*). The multiple-sequence alignment was carried out using MultAlin (*44*).

x is any residue) motif of the B1 domain is replaced with nearly isosteric amino acids in different sequences. Arg*β*-362 is a highly conserved basic amino acid only in the bacterial enzymes, while Arg*β*-2, contributing mainly to stabilization of the single-stranded acceptor end, is missing in the eukaryotic-like sequences and not conserved in the bacterial subunits. Our recent study revealed human PheRS to be inactive in aminoacylation when three amino acids are appended to the N-terminus of the *β* subunit (*33*), suggesting that this region is essential for enzyme functioning. Strict conservation of its four residues [MPT(V/I)] in the eukaryotic-like sequences reinforces this assumption. Two motifs of the PheRS *β* subunit, (P/A)NRxDx(L/M) from domain B1 and RxDx$_5$D(L/I)xE(E/D) from domain B5, are essential for the catalytic activity due to their indirect involvement in stabilization of the phenylalanine-binding loop (residues 257−263 of the α subunit in *T. thermophilus* PheRS) by coordinating a metal ion at the α−*β* subunit interface (*16*, *17*). The phenylalanine-binding loop and the two motifs of the *β* subunit are strictly conserved, strongly implying the presence of the metal-binding site in all tetrameric PheRSs and implying that the N-terminal region of the *β* subunit is near the active site. This suggests that the N-terminal MPT-(V/I) motif of human PheRS is implicated in the interaction with the tRNA CCA end. Thus, while significantly different between the two groups of PheRS but fairly well conserved within each group, the N-terminal region of the PheRS *β* subunit conserves its function in tRNA binding through evolution. The revealed similarity between human and *T. thermophilus* PheRSs in positioning the tRNA 3′ end at the α−*β* subunit interface and the phylogenetic analyses suggest that this mode of acceptor end binding is universal for tetrameric PheRSs. Species-specific differences in PheRS interactions with this tRNA region are reflected in phylum-dependent recognition of A73, whose contribution to the phenylalanylation efficiency is noticeably higher in humans (*23*) and yeast (*22*, *27*) than in *T. thermophilus* (*26*) and *E. coli* (*25*).

(b)


B1
B2
Tt 1 MrvpfswLka yVp-elespe vLeerlaglG fEtDriervf piprgVVfar Vleahpipgt -rLkrlvlDa G--rtvevVs GAeNarkgig ValAlpGtel PGlgqkVger
Bs 1 MfvsykwLed yVdlkgmdpa vLaekitraG iEvegieykg egikgVViGh Vlereqhpna DKLnkclvDi Gaeapvqiic GApNvdkgqk VavAtvGavl PG-nfkIkka
Sa 1 MlisnewLke yVtidd-svs dLaeritrtG iEvDdlidyt kdiknlVvGf Vkskekhpda DKLnvcqvDi Gedepvqivc GApNvdagqy VivAkvGgrl PG-gikIkra
Ec 1 MkfselwLre wVn-paidsd aLanqitmaG lEvDgvepva gsfhgVVvGe Vvecaqhpna DKLrvtkvnv GgdrlldIVc GApNcrqglr VavAtiGavl PG-dfkIkaa
Hp 1 MklsindLnv fVntpk-dia kLcedlsrlG lEvescipci -apknVVvGk ilekaphkna eKLsvcqvDv G-kevlqIVc GAkNvapnqf VpvAlnGali g--stTIakt
Ce 1 MPTVgik
Hs 1 MPTVsvk
Sc 1 MPTVsvn
Mj 1 MPTInvk
B2
B1
Tt 107 viqgvrsfGM alSprElgvg e------ygg gllEfpe--- dalppgtpls eawpeevvld levtPNRpDa LgllGLARdl hal-gyalve Peaa--lkae alplpfalkv
Bs 110 nvrgeesnGM icSlqElgie sklvakEyae gifvfpn--- daetgsdala alqlddaile lgltPNRaDa MnmlGvAyev aaildtevkl Pqtdypaase qasdyisVki
Sa 109 KlrgerseGM icSlqEigis snyipksfes giyvfse--- sqvpgtdalq alylddqvme fdltPNRaDa LsmiGtAyev aalyntkmtk Pettsnelel sandeltVti
Ec 109 KlrgepseGM lcSfsElgis ddh------s giiElpa--- dapigtdire ylkllddntie isvtPNRaDc LgiiGvARdv avlnqlplvq Peiv--pvga tiddtlpItv
Hp 106 elrgveshGM icSsiElgfp k------ind gilEldesvg elvlgkelne yapfnthvle isltPNRgDc LsvlGiARei safyhtplkp ikal-----n ftpksglItl
Ce 8 KviLdkhfkr vySekEfdEl cfeyGlElde itsEkaa--- vekeqgtraa sdlndqevyk idipaNRyDl LsvEGLARai rifkq-eips PayKyadvpk tglqkiiVkk
Hs 8 rdlLfqalGr tytdeEfdEl cfefGlElde itsEkei--- iskeqgnvka agasdvvlyk idvpaNRyDl LclEGLvRgl qvfke-rika PvyKrv-mpd gkiqkliIte
Sc 8 KqqLfdllGk nytsqEfdEl cfefGmEmde dttEeal--- ktgeepel-- ---------k ldisaNRyDl LciEGisqsl neyle-rker PdyKl----s kpttkliIdk
Mj 8 KadLerlvnM pledefieEk fpmmGvEveg ifeEdge--- k--------- -------iiq fsinPNRpDy LsaEGLARgf rgiig--iet glkKy----d iessdvklyv
B3
B4
Tt 205 EdpEgaPhft lgyafglrv- aps--plwmq ralfaagmrp innvVdVtny vMleraqPmh afdl-rfvge giavrrarEg erlktLdgve rtlhpedlvi agwrgeesfp
Bs 217 EdqEanPlyt AkIiknvti- aps--plwmq tklMnagirp hnnvVdItnf vLleyggPlh afdydrfgsK evvvrkaaEn emivtLddqe rklsadhlvi t----ngtka
Sa 216 EneDkvPyys ArVvhdvti- eps--plwmq arlikagirp innvVdIsny vLleyggPlh mfdqdaigsq qivvrqanEg ekmttLddte relltsdivi t----ngqtp
Ec 208 EapEacPryl grVvkginvk apt--plwmk Eklrrcgirs idavVdVtny vLlelgqPmh afdkdriegg -ivvrmakEg etlvlLdgte aklnadtlvi a----dhnka
Hp 205 sagEnieshl AyyLi-cnhs lkt--plnik lslaHnNals endLnnfief sthfsgvimn ayslnttpmd -lsvkndeNn lesvyinhqk rs-------- ----------
Ce 114 EtaQvRPfvv gAVLrdisfd adsyaSfIdL QdKLHqNicR kRtLVAIGtH DLDtiqgPFe YraeapkdIK FkPLNqtkEy TaeelMtlys td---shlk- -----aYlpi
Hs 113 EtakiRPfav AAVLrnikft kdrydSfIeL QeKLHqNicR kRaLVAIGtH DLDtlsgPFt YtakrpsdIK FkPLNktkEy TacelMniyk td---nhlk- -----hYlhi
Sc 99 steQiRPfat AAVLrnikln eksyaSfIaL QdKLHaNlcR nRsLVAmGtH DLDsiegPFh YralppkdIK FvPLNqtqEf Tgdkliefyk speqknnig- -----rYvhi
Mj 93 EnvEtRPyia mAlvkgvivd dyvleSiInL QeKLHwvmgR dRkkVAIGiH DaDkvkpPFy YkevsgdgIK FvPLNsdeEm TpreiLekhe kg------i- -----kYahl
B4
B3
B5
Tt 311 lglagvmgga esevredtea iaLevacfdp VSirktarrh glrteashrf Ergvdplgqv paqrralsll qalagarVaE alleagspkp pea--ipfrp eyaNrlLGts
Bs 320 qavagvmgga esevqedtkt ilLeaayfng qkvrkaskdl glrsessvrf Ekgidparvr laaeraaqli hlyaggeVla gtveedhlti -eannihvsa dkVssvLGlt
Sa 319 Ialagvmggd fsevkeqtSn iviegaifdp VSirhtsrrl nlrsesssrf Ekgiatefvd eavdracyll qtyangkVlk drvssgelga -fitpidita dkINrtiGfd
Ec 311 lamggifgge hsgvndetqn vlLecaffsp lSitgRarrh glhtdashry Ergvdpalqh kameratrll idicggeagp viditneatl pkratitlrr sklDrliGhh
Hp 293 -----tiaik hQvqkdlsec llLeasytdp ISlslklhal kdktlQKdnA liyrsargsn pnlsdglnfl sahlkatIlE skqtehsl-- -kdRtltfql edIteiLGla
Ce 215 IqnhpvyPvI yDkngvVcSm PPiINgehsk ItlnTkNvfI eaTaTDKqkA fvvLdtivTl fs-qycakpf tieqvEvVyE etgvkelyPl lsyRemtvtt peINtkiGin
Hs 214 IenkplyPvI yDsngvVlSm PPiINgdhsr ItvnTRNifI ecTgTDftkA kivLdiivTm fs-eycenqf tveaaEvVfp n-gkshtfPe layRkemvra dlINkkvGir
Sc 203 IedspvfPvI mDskdrVcSl PPLINsehsk ISvnTRNilI diTaTDKtkA EivLnilTm fs-rycdepf tvepvEiVsE hngqsrlaPn fndRimdvsi kyINscLGld
Mj 191 Ikddk-fPiI lDsegdVlSm PPiINgeltr VtteTRNllI dvTgTDKyav EktLniivTa la-e--rkyg kihavEvIkD n--qstiyPn lkedvletts eyINkvLGan
B5
B6
B7
Tt 419 ypeaeqiaiL kRlgcrvege gp-----tyr VtpPshRlDl rleeDlvEEV AriqGYEtip lalpaffpap dnrgveapyr keqrLRevls glGFqEVyty sfmdpEdarr
Bs 429 iskEelisiy kRlgftvgea dd-----llv VtvPsrRgDI tieeDliEEa ArlYGYDNip stlpetagtT gg-ltpyqa- krrkvRrfle gaGlsQaIty sLtnekkata
Sa 428 lsqNdIvtif nqlgfdtein dd-----vit VlvPsrRkDI tikeDliEEV AriYGYDDip stlpvfdkvT sgqltdrqy- ktrmvkevle gaGldQaIty sLvSkEdata
Ec 421 iadEqVtdiL rRlgcevteg kd-----ewq avaPswRfDm eieeDlvEEV ArvYGYNNip de-pvqasli mgthreadl- slkrvktlln dkGYqEVIty sfvdpk-vqq
Hp 395 vekEkIqgiL knlgfkvsvk epnskpqile ViaPnfRhDI ktiqDIaEEI lrfvGiDNlv skplhcvssk nsnpnydthr ffenLkhkal acGFkEVIhy vfySkEkqqk
Ce 324 lkdEematlL nkmsLka-ev aak---etlk IvvPptRhDI lhacDIaEDV gvaFGYNNli t--klpesnT vavafpin-K lcdnLRieia aaGwtEalnf aLcSrDdiss
Hs 322 etpEnlaklL tRmyLks-ev igdg--nqie IeiPptRaDI ihacDIvEDa AiaYGYNNiq m--tlpktyT ianqfpln-K ltelLRhdma aaGFtEaltf aLcSqEdiad
Sc 312 qsaDeIahcL kkmsLhavqs kedk--dilh VdiPvtRpDI lhacDImEDa AvgYGFNNlp kgeklsnanf iakplpin-K vsdifRvass qatwvEVlpl tLcShDenfk
Mj 295 ltpgtIinyL rRcrLdaqfv d-----nkik VfiPayRvDI fgeiDIaEEV AiaYGYNkfs g--eypiigT igelnqle-K kcdfiReimv gfGFyEVInl mLsndEvlfk
B7
B6
B8
Tt 524 frldppr--- -llLlNPlap EkaalRThLf PGLvrvlkeN ldldrperal LFEvGrVf-- ------rere ethLAgllfG e--gvglpw- akerlsgyfl lkgylealfa
Bs 532 faieksl--n -tvLalPmse ErsilRhSLv PnLLdsvsyN lar-qtdsva LYEvGsVflt keedtkpvet e-rvAgavtG l--wrkqlwq gekkpvdffv vkgiveglld
Sa 532 fsmqqrq--t -idLlmPmse ahaslRqSLL PhLievasyN var-knkdvK LFEiGNVffa Ngegelpdqv e-yLsgiltG d--yvvnqwq gkketvdfyl akgvvdrvse
Ec 523 mihpgve--a -llLpsPisv EmsamRlSLw tGLLaTvvyN qnr-qqnrvr iFEsGlrfvp Dtqaplgirq dlmLAgvicG n--rYeehwn laketvdfyd lkgdleesvld
Hp 505 lgfevle--d pleLqNPiTt ElntlRTSLv cGLLdaslrN knl-gfksia LYEkGsVy-- ----nskree iqkLgflisG l--qkkesyp dtkgka--wd fysfaecvsk
Ce 427 klrqpdalsh avhigNPkTl EfqvaRTSLL PGLLkTlssN rdm--pLPlK LFElqDVivk Dsntdvgarn errLAavyyn raagFeiIqg fldrimr--- ---mlnvnpa
Hs 426 klgvdisatk avhisNPkTa EfqvaRTtLL PGLLkTiaaN rkm--pLPlK LFEisDIvik Dsntdvgakn yrhLcavyyn knpgFeiIhg lldrimq--- ---lldvppg
Sc 419 flrqsdngdl avkLaNPkTl EyqvvRTtLL PGiLkTvkeN rkh--sLPiK vFEtGDVvfk Ddklerkayn erhwAaiyvG knsgFeiIqg llgkimqtfr tewiadygaa
Mj 397 kmriedn--n yievlkPasi EhrivRkSiL PlLMeTlriN khk--eLPqK iFEiGDcvvi Denaetksrv vkkiAgvivd netnFneIks yvegllr--- ----------
B6
B8
Tt 619 rlGlaFrvE- -aqafPflhP GvsgrVlveG ee------VG flGaLHPEIa qeleLp-Pvh -lfELrlp-- --lpdkplaf qdpsrhPaaf RDlavvvpap tp------yg
Bs 635 klnvldsiEf vqserkqlhP GRtAnIllnG sl------IG fiGqvHPsle keldik-ety -vfELDlhal laaetaplvy taipkyPsvt RDIaLvtdkt vt------sg
Sa 635 klnl--efsy rradidglhP GRtAeIllen kv------VG fiGeLHPtla adndLk-rty -vfELNfdal mavsvgyiny qpiprfPgms RDIaLevdqn ip------aa
Ec 627 ltGklnevEf raeanPalhP GqsAaIylkG er------IG fvGvvHPEle rkldLngrtl -vfELEwnkl --adrvvpqa reisrfPanr RDIavvvaen vp------aa
Hp 602 viGd-FslEk lttqtPinhP yqsAkIiqnh ei------IG viakiHPkVI qeldLf-esy -yaEiDafk- --lkrpamll kpfsiyPssv RDltLiiden ta------fs
Ce 529 rdGtgYyiE- ader------ ---------- ---------- ---aLHPEVI tsFgLtlPcg -avEiNvepf l
Hs 528 edkggYvik- aseg-PaffP GRcAeIfarG qs------VG klGvLHPDVI tkFeLtmPcs -slEiNigpf l
Sc 527 asGrgYwiE- eddsvktyfP GRgAkVmfrs kegaepkqIG hlGvLHPEVm mnFdvpfaas -fvEvNaevf l
Mj 490 elkieYelD- nfeh-Psfik GRcAkIlkdG ki------IG yfGeiHPEVI tnFeLefPvv -gfELEie
B8
Tt 709 evealvreaa gpyLeslalf DlYqgpplpe ghkslafhlr frhpkrTLrD EeVeeavsrv aeaLrar-gf gLRgldtp
Bs 731 qlesvikeag gklLkevtvf DvYegehmee gkksvafslq yvnpeqTLte EeVtkahskv lkaLedtyqa vLRg
Sa 729 dllstihahg gniLkdtlvf DvYqgehlek gkksiairln yldteeTLtD ErVskvqaei eaaLieq-ga viR
Ec 722 dilseckkvg vnqvvgvnlf DvYrgkgvae gykslaisli lqdtsrTLee Eeiaatvakc veaLkerfqa sLRd
Hp 694 gikkalkdaq ipnLseilpl Difke---sn nsialsvrcv ihslekTLnD EeVnsavqka leiLekefna rLkg
Ce
Hs
Sc
Mj

The striking resemblance of human PheRS to its bacterial counterpart in the interaction with the $tRNA^{Phe}$ acceptor end is further evidenced from the relative contribution of base-specific interactions of A76 to productive binding. Noticeably, the human and *T. thermophilus* enzymes show a significant difference in their initial binding of the homologous $tRNA^{Phe}$s, which is 400-fold stronger in the prokaryotic system (*28*). However, they are quite similar in their K_m values: 0.10 and 0.17 μM for native and *in vitro* transcript human $tRNA^{Phe}$, respectively (ref *33* and this work), as compared to 0.12 and 0.18 μM for native and unmodified *T. thermophilus* $tRNA^{Phe}$, respectively (ref *26* and our unpublished data). Losses of $tRNA^{Phe}$ productive binding affinity for human PheRS (estimated from the K_i/K_m ratio; see Table 1) caused by modifications of the CCA end are comparable to those observed for the *T. thermophilus* system. The kinetic experiments with $tRNA^{Phe}$s substituted with different nucleotides at position 76 have shown that lack of the exocyclic amino group or the imidazole ring of the terminal nucleotide substantially decreases the catalytic efficiency of aminoacylation, and the absence of both elements causes a loss of charging activity in both the *T. thermophilus* and *E. coli* systems (*31*, *45*). The lack of substrate activity of human $tRNA^{Phe}$ substituted with s^4U for A76 strongly suggests that the same structural elements of the terminal nucleotide are responsible for its productive interaction in the eukaryotic system. The base-specific contacts of A76 may dictate the conformational rearrangement of the tRNA in the aminoacylation reaction, the necessity of which follows from structural and biochemical studies.

The position of the terminal adenosine in the PheRS−$tRNA^{Phe}$ complex (*15*) partially interferes with the location of the Phe substrate in the active site (*16*, *17*): all five residues involved in the binding of base A76 contact the phenylalanine, in both the free and activated state (see Table 3). As suggested from steady-state kinetic experiments with prokaryotic (*E. coli*) and eukaryotic (yeast) PheRS (*46*, *47*), all substrates may bind to the enzyme randomly. This implies that $tRNA^{Phe}$ binding to both the free enzyme and its complexes with phenylalanine and/or ATP is relevant. The data presented here and obtained previously for *T. thermophilus* PheRS (*31*) give a clear indication of acceptor end rearrangement in the presence of other substrates. Evidently, the proper positioning of the $tRNA^{Phe}$ acceptor end corresponding to the productive complex is promoted only in the presence of phenylalanyl adenylate, and this additional means of ensuring the phenylalanylation specificity is conserved through evolution. On the basis of the currently available structural data, we can analyze amino acid residues involved in nonproductive binding of the 3′-terminal nucleotide; nevertheless, most of them preserve their contacts in the proposed model of simultaneous positioning of phenylalanyl adenylate and the $tRNA^{Phe}$ 3′ end in the active site (*17*). Both Serα-180 and Gluα-220, anchoring the amino group of A76, are strictly conserved in oligomeric PheRSs (see Table 3 and Figure 5a). Pheα-260 is a conserved aromatic residue; Pheα-258 is invariant only in eubacteria and archaea, and Trpα-149 is not conserved (replaced with Gln or His in other PheRSs). ATP revealed more pronounced effects (in comparison with the amino acid substrate) on the binding of the $tRNA^{Phe}$ 3′ end in the complex with human PheRS as well as with *T. thermophilus* PheRS (*31*), influencing the orientation of at least two of the terminal nucleotides. A concerted movement of the acceptor arm with the motif 2 (a signature peptide of class II aaRSs) loop can be proposed as a mechanism that accounts for the ATP-induced changes in the acceptor end binding. Indeed, the motif 2 loop (residues 206−213) shifts as a whole toward the active site to make contacts with the AMP portion of adenylate (*16*, *17*). While Gluα-206 is involved in the interactions with the nucleotide portion of aminoacyl adenylate, the next residue of the loop, Glnα-207, contacts the tRNA acceptor stem (see Table 3). Most of the residues anchoring the AMP are class II invariant or strictly conserved in the reported PheRSs sequences. Glnα-207 is not conserved in different PheRSs, but its location within the signature motif and the observed similarity between human and *T. thermophilus* PheRSs in the ATP-induced rearrangement of the $tRNA^{Phe}$ 3′ end strongly suggest that the acceptor arm binding by the motif 2 loop of PheRS is conserved through evolution.

The importance of prior binding of small substrates for ordered and productive binding of the tRNA 3′ end has been shown for other aaRSs (*48*−*50*). The final step in AspRS−$tRNA^{Asp}$ adaptation occurring within the catalytic module of the yeast and *E. coli* enzymes involves the motif 2 and flipping loops, which in their closed conformation anchor the tRNA acceptor end in the catalytic site in an optimal position for aminoacylation (*12*, *51*). Two variants of the proposed general mechanism of aspartylation involve several conserved and one species-specific functional interactions. Our experiments provide evidence that the precise positioning of the $tRNA^{Phe}$ 3′ end in the catalytic site of human PheRS and its prokaryotic counterpart of known structure, *T. thermophilus* PheRS, is ensured by a general molecular mechanism. The phylogenetic analyses suggest that the binding mode of the $tRNA^{Phe}$ acceptor end at the $\alpha-\beta$ subunit interface and the mechanism of its adjustment for the productive interaction by involvement of both conserved and species-specific amino acid residues and motifs are most likely to be universal for tetrameric PheRSs.

ACKNOWLEDGMENT

We are very grateful to O. C. Uhlenbeck for the plasmid containing the wild-type human $tRNA^{Phe}$ gene, to V. S. Bogachev for synthesis of ps^4UpP, and to G. Igloi for APM. We thank our colleagues I. A. Vasil'eva and D. Tvorowski for technical assistance and R. D'Ari for careful reading of the manuscript.

REFERENCES

1. Meinnel, T., Mechulam, Y., and Blanquet, S. (1995) Aminoacyl-tRNA synthetases: occurrence, structure and function, in *tRNA: Structure, Biosynthesis and Function* (Söll, D., and RajBhandary, U., Eds) pp 251−292, American Society for Microbiology, Washington, DC.
2. Ibba, M., and Söll, D. (2000) Aminoacyl-tRNA synthesis, *Annu. Rev. Biochem. 69*, 617−650.
3. Gouda, M., Yokogawa, T., and Nishikawa, K. (2002) The β subunit of *Aquifex aeolicus* leucyl-tRNA synthetase is responsible for cognate tRNA recognition, *Biochem. Biophys. Res. Commun. 297*, 950−955.
4. Eriani, G., Delarue, M., Poch, O., Gangloff, J., and Moras, D. (1990) Partition of tRNA synthetases into two classes based on mutually exclusive sets of sequence motifs, *Nature 347*, 203−206.

5. Cusack, S., Berthet-Colominas, C., Härtlein, M., Nassar, N., and Leberman, R. (1990) A second class of synthetase structure revealed by X-ray analysis of *E. coli* seryl-tRNA synthetase at 2.5 Å resolution, *Nature 347*, 249−255.
6. Giege, R., Sissler, M., and Florentz, C. (1998) Universal rules and idiosyncratic features in tRNA identity, *Nucleic Acids Res. 26*, 5017−5035.
7. Beuning, P. J., and Musier-Forsyth, K. (1999) Transfer RNA recognition by aminoacyl-tRNA synthetases, *Biopolymers 52*, 1−28.
8. Delagoutte, B., Moras, D., and Cavarelli, J. (2000) tRNA aminoacylation by arginyl-tRNA synthetase: induced conformations during substrate binding, *EMBO J. 19*, 5599−5610.
9. Shimada, A., Nureki, O., Goto, M., Takahashi, S., and Yokoyama, S. (2001) Structural and mutational studies of the recognition of the arginine tRNA-specific major identity element, A20, by arginyl-tRNA synthetase, *Proc. Natl. Acad. Sci. U.S.A. 98*, 13537−13542.
10. Burke, B., Yang, F., Chen, F., Stehlin, C., Chan, B., and Musier-Forsyth, K. (2000) Evolutionary coadaptation of the motif 2-acceptor stem interaction in the class II prolyl-tRNA synthetase system, *Biochemistry 39*, 15540−15547.
11. Nameki, N., Tamura, K., Asahara, H., and Hasegawa, T. (1997) Recognition of tRNA^{Gly} by three widely diverged glycyl-tRNA synthetases, *J. Mol. Biol. 268*, 640−647.
12. Eiler, S., Dock-Bregeon, A.-C., Moulinier, L., Thierry, J.-C., and Moras, D. (1999) Synthesis of aspartyl-tRNA^{Asp} in *Escherichia coli*: a snapshot of the second step, *EMBO J. 18*, 6532−6541.
13. Ming, X., Smith, K., Suga, H., and Hou, Y.-M. (2002) Recognition of tRNA backbone for aminoacylation with cysteine: evolution from *Escherichia coli* to human, *J. Mol. Biol. 318*, 1207−1220.
14. Mosyak, L., Reshetnikova, L., Goldgur, Y., Delarue, M., and Safro, M. (1995) Structure of phenylalanyl-tRNA synthetase from *Thermus thermophilus*, *Nat. Struct. Biol. 2*, 537−547.
15. Goldgur, Y., Mosyak, L., Reshetnikova, L., Ankilova, V., Khodyreva, S., Lavrik, O., and Safro, M. (1997) The crystal structure of phenylalanyl-tRNA synthetase from *Thermus thermophilus* complexed with cognate tRNA^{Phe}, *Structure 5*, 59−68.
16. Reshetnikova, L., Moor, N., Lavrik, O., and Vassylyev, D. (1999) Crystal structures of phenylalanyl-tRNA synthetase complexed with phenylalanine and a phenylalanyl-adenylate analogue, *J. Mol. Biol. 287*, 555−568.
17. Fishman, R., Ankilova, V., Moor, N., and Safro, M. (2001) Structure at 2.6 Å resolution of phenylalanyl-tRNA synthetase complexed with phenylalanyl-adenylate in the presence of manganese, *Acta Crystallogr. D57*, 1534−1544.
18. Fraser, T. H., and Rich, A. (1975) Amino acids are not all initially attached to the same position on transfer RNA molecules, *Proc. Natl. Acad. Sci. U.S.A. 72*, 3044−3048.
19. Sprinzl, M., and Cramer, F. (1975) Site of aminoacylation of tRNAs from *E. coli* with respect to the 2′- or 3′-hydroxyl group of the terminal adenosine, *Proc. Natl. Acad. Sci. U.S.A. 72*, 3049−3053.
20. Stepanov, V. G., Moor, N. A., Ankilova, V. N., Vasil'eva, I. A., and Lavrik, O. I. (1995) Mechanism of bis-2′,3′-O-phenylalanyl-tRNA formation in the reaction of tRNA^{Phe} aminoacylation catalyzed by phenylalanyl-tRNA synthetase from *Thermus thermophilus*, *Mol. Biol. (Moscow) 29*, 392−396.
21. Sampson, J. R., DiRenzo, A. B., Behlen, L. S., and Uhlenbeck, O. C. (1990) Role of the tertiary nucleotides in the interaction of yeast phenylalanine tRNA with its cognate synthetase, *Biochemistry 29*, 2523−2532.
22. Sampson, J. R., Behlen, L. S., DiRenzo, A. B., and Uhlenbeck, O. C. (1992) Recognition of yeast tRNA^{Phe} by its cognate yeast phenylalanyl-tRNA synthetase: an analysis of specificity, *Biochemistry 31*, 4161−4167.
23. Nazarenko, I. A., Tinkle Peterson, E., Zakharova, O. D., Lavrik, O. I., and Uhlenbeck, O. C. (1992) Recognition nucleotides for human phenylalanyl-tRNA synthetase, *Nucleic Acids Res. 20*, 475−478.
24. Perret, V., Florentz, C., Puglisi, J. D., and Giege, R. (1992) Effect of conformational features on the aminoacylation of tRNAs and consequences on the permutation of tRNA specificities, *J. Mol. Biol. 226*, 323−333.
25. Tinkle Peterson, E., and Uhlenbeck, O. C. (1992) Determination of recognition nucleotides for *Escherichia coli* phenylalanyl-tRNA synthetase, *Biochemistry 31*, 10380−10389.
26. Moor, N. A., Ankilova, V. N., and Lavrik, O. I. (1995) Recognition of tRNA^{Phe} by phenylalanyl-tRNA synthetase of *Thermus thermophilus*, *Eur. J. Biochem. 234*, 897−902.
27. Aphasizhev, R., Senger, B., Rengers, J. U., Sprinzl, M., Walter, P., Nussbaum, G., and Fasiolo, F. (1996) Conservation in evolution for a small monomeric phenylalanyl-tRNA synthetase of the tRNA^{Phe} recognition nucleotides and initial aminoacylation site, *Biochemistry 35*, 117−123.
28. Vasil'eva, I. A., Ankilova, V. N., Lavrik, O. I., and Moor, N. A. (2002) tRNA discrimination by *T. thermophilus* phenylalanyl-tRNA synthetase at the binding step, *J. Mol. Recognit. 15*, 188−196.
29. Rodova, M., Ankilova, V., and Safro, M. (1999) Human phenylalanyl-tRNA synthetase: cloning, characterization of the deduced amino acid sequences in terms of the structural domains and coordinately regulated expression of the α and β subunits in chronic myeloid leukemia cells, *Biochem. Biophys. Res. Commun. 255*, 765−773.
30. Woese, C. R., Olsen, G. O., Ibba, M., and Söll, D. (2000) Aminoacyl-tRNA synthetases, the genetic code, and the evolutionary process, *Microbiol. Mol. Biol. Rev. 64*, 202−236.
31. Vasil'eva, I. A., Ankilova, V. N., Lavrik, O. I., and Moor, N. A. (2000) Interaction of *T. thermophilus* phenylalanyl-tRNA synthetase with the 3′-terminal nucleotide of tRNA^{Phe}, *Biochemistry (Moscow) 65*, 1157−1166.
32. Lavrik, O. I., Moor, N. A., and Nevinsky, G. A. (1978) Synthesis of analogs of L-phenylalanyl-adenylate and study of their interactions with *E. coli* phenylalanyl-tRNA synthetase, *Bioorg. Khim. 4*, 1480−1487.
33. Moor, N., Linshiz, G., and Safro, M. (2002) Cloning and expression of human phenylalanyl-tRNA synthetase in *Escherichia coli*: comparative study of purified recombinant enzymes, *Protein Expression Purif. 24*, 260−267.
34. Paulsen, H., and Wintermeyer, W. (1984) Incorporation of 1,N^6-ethanoadenosine into the 3′ terminus of tRNA using T4 RNA ligase, *Eur. J. Biochem. 138*, 117−123.
35. Silberklang, M., Gillum, A. M., and RajBhandary, U. L. (1979) Use of *in vitro* ^{32}P labeling in the sequence analysis of nonradioactive tRNAs, *Methods Enzymol. 59*, 58−109.
36. Moor, N. A., Ankilova, V. N., Lavrik, O. I., and Favre, A. (2001) Determination of tRNA^{Phe} nucleotides contacting the subunits of *Thermus thermophilus* phenylalanyl-tRNA synthetase by photoaffinity crosslinking, *Biochim. Biophys. Acta 1518*, 226−236.
37. Cornish-Bowden, A., and Wharton, C. W. (1976) in *Enzyme Kinetics* (Rickwood, D., and Male, D., Eds.) IRL Press, Oxford, England.
38. Igloi, G. L. (1988) Interaction of tRNAs and of phosphorothioate-substituted nucleic acids with an organomercurial. Probing the chemical environment of thiolated residues by affinity electrophoresis, *Biochemistry 27*, 3842−3849.
39. Moor, N. A., Ankilova, V. N., Favre, A., and Lavrik, O. I. (1998) Localization of the binding site for the 3′-terminal sequence of tRNA^{Phe} in subunits of phenylalanyl-tRNA synthetase from *Thermus thermophilus*, *Biochemistry (Moscow) 63*, 1051−1056.
40. Billings, P. C., Orf, J. W., Palmer, D. K., Talmage, D. A., Pan, C. G., and Blumenfeld, M. (1979) Anomalous electrophoretic mobility of *Drosophila* phosphorylated H1 histone: is it related to the compaction of satellite DNA into heterochromatin? *Nucleic Acids Res. 6*, 2151−2164.
41. Noel, D., Nikaido, K., and Ames, G. F.-L. (1979) A single amino acid substitution in a histidine-transport protein drastically alters its mobility in sodium dodecyl sulfate-polyacrylamide gel electrophoresis, *Biochemistry 18*, 4159−4165.
42. Favre, A. (1990) 4-Thiouridine as an intrinsic photoaffinity probe of nucleic acid structure and interactions, in *Bioorganic Photochemistry: Photochemistry and the Nucleic Acids* (Morrison, H., Ed.) pp 379−425, Wiley, New York.
43. Szymanski, M., and Barciszewski, J. (2000) Aminoacyl-tRNA synthetase base Y2K, *Nucleic Acids Res. 28*, 326−328.
44. Combet, C., Blanchet, C., and Geoujon, C. (2000) NPS@: network protein sequence analysis, *Trends Biochem. Sci. 5*, 47−150.
45. Moor, N. A., Repkova, M. N., Yamkovoy, V. I., and Lavrik, O. I. (1994) Alterations at the 3′-CCA end of *Escherichia coli* and *Thermus thermophilus* tRNA do not abolish their acceptor activity, *FEBS Lett. 351*, 241−242.
46. Santi, D. V., Danenberg, P. V., and Satterly, P. (1971) Phenylalanyl transfer ribonucleic acid synthetase from *Escherichia coli*. Reaction parameters and order of substrate addition, *Biochemistry 10*, 4804−4812.

47. Thiebe, R. (1978) Analysis of the steady-state mechanism of the aminoacylation of $tRNA^{Phe}$ by phenylalanyl-tRNA synthetase from yeast, *Nucleic Acids Res. 5*, 2055−2071.
48. Biou, V., Yaremchuk, A., Tukalo, M., and Cusack, S. (1994) The 2.9 Å crystal structure of *Thermus thermophilus* seryl-tRNA synthetase complexed with $tRNA^{Ser}$, *Science 263*, 1404−1410.
49. Cusack, S., Yaremchuk, A., and Tukalo, M. (1996) The crystal structures of *T. thermophilus* lysyl-tRNA synthetase complexed with *E. coli* $tRNA^{Lys}$ and a *T. thermophilus* $tRNA^{Lys}$ transcript: anticodon recognition and conformational changes upon binding of a lysyl-adenylate analogue, *EMBO J. 15*, 6321−6334.
50. Yaremchuk, A., Cusack, S., and Tukalo, M. (2000) Crystal structure of a eukaryote/archaeon-like prolyl-tRNA synthetase and its complex with $tRNA^{Pro}$(CGG), *EMBO J. 19*, 4745−4758.
51. Sauter, C., Lorber, B., Cavarelli, J., Moras, D., and Giege, 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, *J. Mol. Biol. 299*, 1313−1324.

BI034732Q