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Role of Low-Molecular-Weight Substrates in Functional Binding of the $tRNA^{Phe}$ Acceptor End by Phenylalanyl-tRNA Synthetase

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Abstract—The functional roles of phenylalanine and ATP in productive binding of the $tRNA^{Phe}$ acceptor end have been studied by photoaffinity labeling (cross-linking) of *T. thermophilus* phenylalanyl-tRNA synthetase (PheRS) with $tRNA^{Phe}$ analogs containing the s^4U residue in different positions of the 3′-terminal single-stranded sequence. Human and *E. coli* $tRNA^{Phe}$s used as basic structures differ by efficiency of the binding and aminoacylation with the enzyme under study. Destabilization of the complex with human $tRNA^{Phe}$ caused by replacement of three recognition elements decreases selectivity of labeling of the α- and β-subunits responsible for the binding of adjacent nucleotides of the CCA-end. Phenylalanine affects the positioning of the base and ribose moieties of the 76th nucleotide, and the recorded effects do not depend on structural differences between bacterial and eukaryotic $tRNA^{Phe}$s. Both in the absence and presence of phenylalanine, ATP more effectively inhibits the PheRS labeling with the s^4U76-substituted analog of human $tRNA^{Phe}$ ($tRNA^{Phe}$-s^4U76) than with *E. coli* $tRNA^{Phe}$-s^4U76: in the first case the labeling of the α-subunits is inhibited more effectively; the labeling of the β-subunits is inhibited in the first case and increased in the second case. The findings analyzed with respect to available structural data on the enzyme complexes with individual substrates suggest that the binding of phenylalanine induces a local rearrangement in the active site and directly controls positioning of the $tRNA^{Phe}$ 3′-terminal nucleotide. The effect of ATP on the acceptor end positioning is caused by global structural changes in the complex, which modulate the conformation of the acceptor arm. The rearrangement of the acceptor end induced by small substrates results in reorientation of the 3′-OH-group of the terminal ribose from the catalytic subunit onto the noncatalytic one, and this may explain the unusual stereospecificity of aminoacylation in this system.

Key words: phenylalanyl-tRNA synthetase, *Thermus thermophilus*, affinity modification, tRNA, acceptor end, 4-thiouridine

Accuracy in translation of the genetic code depends on specificity of the codon–anticodon interaction of tRNAs with messenger RNAs and synthesis of aminoacyl-tRNAs. The two-stage esterification of tRNAs with the cognate amino acids is catalyzed by aminoacyl-tRNA synthetases (aaRSs). During the first stage, the amino acid (aa) is activated by ATP with production of the enzymatically bound aminoacyladenylate (aa-AMP) and release of pyrophosphate (PP_i); during the second stage the activated amino acid is transferred onto the 3′-terminal ribose of the cognate tRNA:

1) aaRS + aa + ATP → aaRS·aa-AMP + PP_i;

2) aaRS·aa-AMP + tRNA → aa-tRNA + AMP + aaRS.

aaRSs extremely vary in their size and primary and secondary structures [1, 2]. The discovery of signature motifs characterizing variations in topology of active sites resulted in their subdivision into two classes [3, 4]. The class I enzymes (mainly monomers) contain two conservative sequences His-Ile-Gly-His and Lys-Met-Ser-Lys-Ser which form the nucleotide-binding fold of the active site. The class II aaRSs (usually dimers or tetramers) are characterized by three motifs: motif 1 is involved in for-

Abbreviations: APM) *p*-(N-acryloylamino)phenylmercuric chloride; aaRS) aminoacyl-tRNA synthetase; XCA) X-ray crystallographic analysis; s^6G) 6-thioguanosine; s^4U) 4-thiouridine; ps^4Up) 4-thiouridine-3′,5′-diphosphate; $tRNA^{Phe}$-s^4U76 (Ec) or (Hs)) analog of *E. coli* or human $tRNA^{Phe}$ containing the s^4U residue in the position indicated; Phe-AMP) phenylalanyl-adenylate; PheRS) phenylalanyl-tRNA synthetase (similar abbreviation is used for other aminoacyl-tRNA synthetases, with their specificity indicated according to conventional names of amino acids).

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mation of intersubunit contacts, motifs 2 and 3 contain two conservative Arg residues and form a part of the active site. The structure of two complexes with specific tRNAs is known for two enzymes of the first class and for six enzymes of the second class [2, 5, 6]. The class I enzymes interact with the acceptor stem from the minor groove side, with the variable loop exposed into solution. The class II aaRSs bind the acceptor stem from the major groove side, and the variable loop contacts with the protein. The 3′-CCA-end has bent or straight conformation in complexes of the class I and II enzymes, respectively. The subdivision into two classes correlates with the difference in stereochemistry of aminoacylation: aaRSs of the I and II class attach the amino acid to the 2′- and 3′-OH-group, respectively, of the 3′-terminal adenosine of tRNA. Structural principles of aaRS interaction with low-molecular-weight substrates have been studied for the majority of aaRSs [2]; catalytic mechanisms of activation are common inside each class. However, each system has an individual mode of tRNA binding. By new approaches using mutant tRNAs [7, 8], identity determinants (recognition elements) have been determined for all systems from *E. coli* and many other organisms. The known structures of a number of complexes allows us to understand the mechanism responsible for provision of the specificity on the atomic level, but for the majority of systems the problem of productive binding of the tRNA acceptor end remains unclear. X-Ray crystallographic analysis (XCA) data on complexes with the proper positioning of the CCA-end are available only for glutaminyl- and arginyl-tRNA synthetases (GlnRS and ArgRS) of class I and for aspartyl- and seryl-tRNA synthetases of class II [6, 9-13]. The presence of small substrates in the active site is needed for functional binding of the tRNA 3′-end not only for tRNA-dependent aaRSs (GlnRS and ArgRS which synthesize aminoacyladenylate in the presence of tRNA). In complexes of lysyl- and prolyl-tRNA synthetases with specific tRNAs co-crystallized with stable analogs of aminoacyladenylate the CCA-end remains disordered [14, 15].

Phenylalanyl-tRNA synthetase (PheRS) from *Thermus thermophilus* is one of the most complicated aaRS enzymes; it is a functional dimer with a rare subunit structure $(\alpha\beta)_2$ [16]. The motifs of its active site are characteristic for class II, but similarly to class I enzymes, it transfers the amino acid to the 2′-OH-group of the terminal adenosine [17-19]. Each αβ heterodimer consists of 11 structural domains: three domains of the small α-subunit (A1, A2, and N-terminal fragment) and eight domains of the large β-subunit (B1-B8). The active site is located on the α-subunit. The structure of PheRS complexes with individual substrates (phenylalanine, $tRNA^{Phe}$) and phenylalanyl-adenylate (Phe-AMP) has been determined [20-22]. Each molecule of $tRNA^{Phe}$ interacts with all subunits of the enzyme. For functional (corresponding to structure of the productive complex) binding of the $tRNA^{Phe}$ acceptor end low-molecular-weight substrates are required. The preparation of aaRS complexes with all substrates was a great success because of limitations by conditions of crystallization and the occurrence of second stage of aminoacylation in crystals [6]; the use of non-hydrolysable adenylate analogs is not always favorable for ordered binding of the tRNA acceptor end [13-15]. Affinity modification seems to be an alternative approach to study the structure of aaRS complexes under functional conditions. In the present work, we studied the functional role of phenylalanine and ATP in the productive binding of the 3′-end of $tRNA^{Phe}$ using photoaffinity labeling of PheRS with 3′-s^4U-substituted analogs of $tRNA^{Phe}$ (bacterial and eukaryotic).

MATERIALS AND METHODS

Reagents used were as follows: ATP, UTP, GTP, CTP, EDTA, L-phenylalanine, and a set of marker proteins with molecular weight from 36 to 193 kD were from Sigma (USA); acrylamide, N,N′-methylenebisacrylamide, β-mercaptoethanol, Tris, and HEPES were from Fluka (Switzerland); SDS was from USB (USA); glycine and dithiothreitol were from Serva (Germany). L-Phenyl-[2,3-^{3}H]alanine (1.96 TBq/mmol) was produced in the Institute of Applied Chemistry (St. Petersburg, Russia), [α-^{32}P]ATP (30 TBq/mmol) was from ICN (USA). A preparation of *p*-(N-acryloyl-amino)phenylmercuric chloride (APM) was presented by Prof. G. Igloi (Freiburg University, Germany). Plasmids with incorporated genes of *E. coli* and human $tRNA^{Phe}$s were constructed in the laboratory of Prof. O. Uhlenbeck (Colorado University, USA). The following enzyme preparations were used: alkaline phosphatase from calf intestine and T4 RNA ligase from Pharmacia Biotech (USA), benzonase from Merck (Germany), inhibitor of ribonucleases from Promega (USA). Phenylalanyl-tRNA synthetase (EC 6.1.1.20) was isolated from *T. thermophilus* HB8 as described earlier [23]. T7 RNA polymerase was isolated from *E. coli* BL21 cells carrying the pAR1219 plasmid.

4-Thiouridine-3′,5′(2′,5′)-diphosphate (ps^4Up) was synthesized as described in [24] with modifications. 4-Thiouridine (ammonium salt, 120 μmol) from Serva (Germany) was dissolved in pyridine (6 ml) with water (6 μl) added, and the solution was evaporated in vacuum. The residue was dried by successive distillations of azeotropic mixtures with pyridine (3 × 0.5 ml) and acetonitrile (3 × 0.4 ml) and then in vacuum above P_2O_5. The dry residue was dissolved in 0.4 ml of trimethylphosphate containing pyridine (48 μl, 600 μmol), cooled to 0-2°C, and $POCl_3$ (27 μl, 3 μmol) was added. The reaction mixture was kept for 10 min at the same temperature, and the reaction was stopped by addition of 4 ml of 0.5 M triethylammonium bicarbonate (TEAB) (pH 8.0). The reaction

products were fractionated on a column with Polysil SA-500 (30-40 μm, 100 ml) with a linear gradient of 0.05-0.30 M ammonium sulfate in 30% ethanol (total volume 2 liters). The target product was re-chromatographed on a column with DEAE-Sephadex A-25 (50 ml) in TEAB gradient of 0.0-0.5 M (pH 7.5) (500 ml) and isolated as the tetrasodium salt by precipitation with 1 M NaI in acetone. The resulting mixture of 4-thiouridine-3′,5′- and 2,′5′-diphosphates (34 mg, 52%) was used without separation because only nucleoside-3′,5′-diphosphates are substrates of T4 RNA ligase [25]. HPLC: 100%, the retention time t_r = 8.73 min; pT (control) t_r = 4.48 min. HPLC was performed on a Milikhrom-4 chromatograph (Nauchpribor, Russia) on a column (2 × 62 mm) with Polysil SA-500 (15 μm) in a stepwise (7 × 0.3 ml) gradient of 0.0-0.3 M potassium phosphate buffer (pH 7.0) in 30% CH_3CN, at the elution rate of 200 μl/min. ^{31}P-NMR-spectrum (δ, ppm): 2.60 (m, 2P); ^{1}H-NMR-spectrum (δ, ppm): 7.55 (d, 1H, H5), 6.71 (d, 1H, H6), 6.12 (s, 1H, H1′), 5.18 (m, 3H, H2′, H3′, H4′), 4.16 (m, 2H, H5′, H5″). ^{1}H- and ^{31}P-NMR-spectra were recorded using an AM-400 spectrometer (Bruker, Germany) at 25°C in D_2O at frequencies of 400.13 and 161.977 MHz, respectively, with tetramethylsilane and 85% phosphoric acid as external standards. UV-spectrum (H_2O): λ_{max} = 330 nm (ε = 19,600 $M^{-1}\cdot cm^{-1}$), λ_{min} = 280 nm (ε = 7400 $M^{-1}\cdot cm^{-1}$) at pH 7.0; λ_{max} = 315 nm (ε = 20,900 $M^{-1}\cdot cm^{-1}$), λ_{min} = 260 nm (ε = 11,800 $M^{-1}\cdot cm^{-1}$) at pH 12.0.

Transcripts corresponding to the *E. coli* and human tRNA^{Phe} sequences were synthesized *in vitro* using T7 RNA polymerase as described in [26]. Labeled transcripts were prepared by incorporation of [^{32}P]AMP into the tRNA structure by addition of [α-^{32}P]ATP (0.2-1 MBq) into the reaction mixture (20 μl) for transcription.

Photoactivated tRNA^{Phe} analogs containing s^{4}U residue on the 3′-end (tRNA^{Phe}-s^{4}U77, tRNA^{Phe}-s^{4}U76, tRNA^{Phe}-s^{4}U75) were prepared by ligation of ps^{4}Up to full-length or shortened tRNA^{Phe} transcripts as described in [27] with some modifications. tRNA was shortened from the 3′-end by one (two) nucleotide(s) by the standard procedure [28]. The reaction mixture for the ligation contained 660 μM ps^{4}Up, 10 μM tRNA^{Phe}-transcript, ribonuclease inhibitor (2300 U/ml), 5 mM dithiothreitol, 0.1 mM ATP, 50 mM HEPES-NaOH buffer (pH 8.3), 10 mM $MgCl_2$, 10% DMSO, 15% glycerol, and T4 RNA ligase (230 U/ml). The reaction was performed for 16 h at 4°C and stopped by extraction with phenol. ps^{4}Up-Completed tRNAs were dephosphorylated in reaction mixture containing 4-5 μM ps^{4}Up-tRNA, 50 mM Tris-HCl buffer (pH 8.5), 0.1 mM EDTA, 2 mM dithiothreitol, 2000 U/ml ribonuclease inhibitor, and 50 U/ml alkaline phosphatase. The mixture was incubated for 30 min at 37°C and for 30 min at 55°C on addition of a fresh portion of the enzyme at the same concentration. The enzyme was removed by extraction with phenol. s^{4}U-Containing tRNA was isolated by affinity electrophoresis [29] in denaturing 8% polyacrylamide gel copolymerized with APM (2 μg/ml). tRNA was eluted from the gel by shaking with 0.15 M NaCl in the presence of 2 mM dithiothreitol for 4 h at 4°C and precipitated with ethanol, with a subsequent washing of the precipitate with 75 and 96% ethanol. The yield of s^{4}U-containing products was 60-70%.

tRNA^{Phe} was aminoacylated at 37°C as described in [19]. To determine values of K_m and V_{max}, five different concentrations of tRNA^{Phe} (0.1-1 μM) were used. The K_i values for non-aminoacylable derivatives of tRNA^{Phe}-s^{4}U76 and tRNA^{Phe}-s^{4}U77 were determined using three different concentrations of the inhibitor. The kinetic parameters were calculated using the Microcal Origin 4.1 program; standard errors of the values were no more than 20% for the parameter K_m and 10% for V_{max}; mean deviations of K_i values were no more than 10%.

Modification of phenylalanyl-tRNA synthetase by photoreactive derivatives of tRNA^{Phe} and analysis of the modification products. Reaction mixtures (10 μl) contained 50 mM Tris-HCl buffer (pH 8.5), 15 mM $MgCl_2$, 0.05-2 μM enzyme, and 0.05-0.4 μM ^{32}P-labeled tRNA^{Phe}-s^{4}U. Samples were UV-irradiated by an HBO 200 W high-pressure mercury lamp supplemented with a Bausch and Lamb monochromator (wavelength 365 nm) for 30-60 min at 25°C. The modification products were separated in 10% polyacrylamide gel in the presence of SDS according to Laemmli [30] without predenaturation of the protein at 100°C. The gel was dried, exposed with a PhosphorImager screen (Molecular Dynamic, USA), and visualized by scanning using a Storm 860 system (Molecular Dynamic). The radioactivity distribution in the gel was analyzed with the ImageQuant program version 5.0. Mean values of two-three independent determinations of relative yields of products of the subunit labeling are presented, which virtually correspond to a plateau on the kinetic curves of the photo-cross-linking. Molecular weights of the products were determined from the calibration curve for marker proteins.

Nuclease hydrolysis of products of phenylalanyl-tRNA synthetase modification. After irradiation, the reaction mixtures were treated with benzonase as described in [31, 32] and the hydrolysis products were analyzed by electrophoresis in SDS-polyacrylamide gel.

RESULTS

As basic structures, *E. coli* and human tRNA^{Phe}s were used (Fig. 1). tRNA^{Phe} from *E. coli* (both *in vitro* synthesized and modified) is an effective substrate of PheRS from *T. thermophilus*: *E. coli* tRNA^{Phe}-transcript and *T. thermophilus* tRNA^{Phe} have very similar values of kinetic parameters of aminoacylation and the same values of dissociation constants (K_d) of complexes with the enzyme [26, 33, 34]. The fivefold decrease in the catalytic effi-

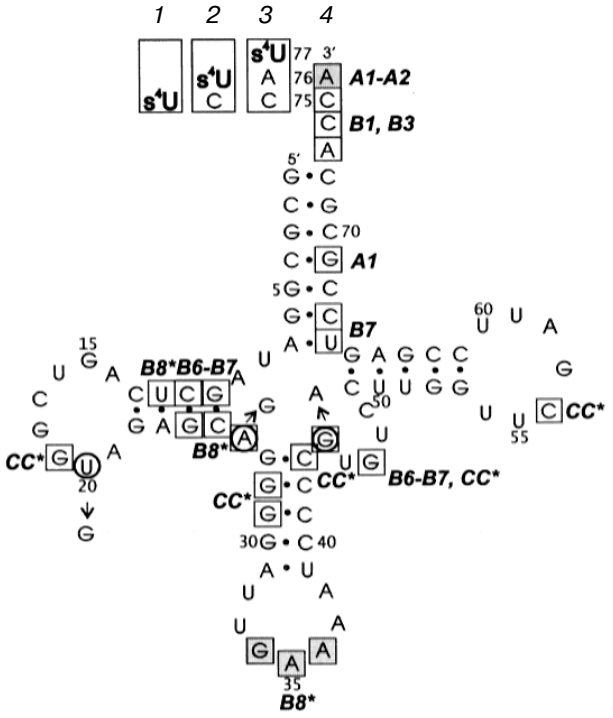

Fig. 1. Structures of $tRNA^{Phe}$ and its reactive analogs. The 3′-terminal sequence of the reagents is presented in separate frames: $tRNA^{Phe}$-s^4U75 (*1*), $tRNA^{Phe}$-s^4U76 (*2*), $tRNA^{Phe}$-s^4U77 (*3*). The main sequence (*4*) corresponds to the *E. coli* $tRNA^{Phe}$-transcript; the arrows show nucleotide replacements in human $tRNA^{Phe}$ in functionally significant positions (indicated with circles). Nucleotides shown to contact PheRS in the crystal structure [21] are placed in rectangular frames; base specific contacts are hatched. Protein domains of the α- (A1, A2, and the N-terminal coiled coil (CC)) and β-subunits (B1-B8) which bind $tRNA^{Phe}$ are indicated; the fragments marked with asterisks belong to the second αβ-heterodimer of the functional $(\alpha\beta)_2$-dimer.

ciency of aminoacylation (k_{cat}/K_m) of human $tRNA^{Phe}$-transcript with this enzyme compared to bacterial $tRNA^{Phe}$ is caused by replacement of some recognition elements [26, 34]. Photoreactive derivatives of $tRNA^{Phe}$ were produced by ligation of ps^4Up to the 3′-end of the transcripts (shortened by one-two nucleotides or full-length) with T4 RNA ligase and subsequent dephosphorylarion. s^4U-Containing transcripts were separated from the original tRNA by affinity electrophoresis in denaturing polyacrylamide gel copolymerized with organomercurial compound (APM) [29].

It was earlier shown [31, 32] that the affinity modification of PheRS with *E. coli* $tRNA^{Phe}$ analogs, $tRNA^{Phe}$-s^4U75 (Ec) and $tRNA^{Phe}$-s^4U76 (Ec), results in many products of labeling of the α- and β-subunits with different electrophoretic mobility (table). $tRNA^{Phe}$-s^4U75 is mainly cross-linked to the large β-subunit, whereas $tRNA^{Phe}$-s^4U76 more effectively generates products of α-subunit labeling. These findings are consistent with XCA data on the PheRS complex with $tRNA^{Phe}$ [21]. Two adjacent nucleotides of the $tRNA^{Phe}$ acceptor end are bound with different subunits: the A76 base forms specific contacts with the catalytic α-subunit, whereas the nucleotide in the position 75 and other nucleotides of the acceptor arm are involved in a network of nonspecific interactions (with involvement of ribose-phosphate groups) with the β-subunit (Fig. 1). Kinetic studies on aminoacylation of $tRNA^{Phe}$ analogs with the modified CCA-end have shown that specific contacts of the exocyclic amino group and imidazole ring of A76 play the leading role in the productive interaction of the acceptor end with PheRS: lack of any of these structural elements (on replacement by s^6G or C) significantly decreases the catalytic efficiency of aminoacylation, and the lack of both elements results in the loss of the $tRNA^{Phe}$ substrate activity [32, 35]. Although $tRNA^{Phe}$-s^4U76 is not a substrate, its selectivity in the α-subunit labeling is not significantly lower than that of other reagents the structure of which ensures partial or complete retention of specific contacts. The ratio of labeling levels of the α- and β-subunits with $tRNA^{Phe}$-s^4U76 (1.9) is comparable to the corresponding values for $tRNA^{Phe}$-s^6G76 (2.1) and $tRNA^{Phe}$-$A_{ox}76$ (2.2) prepared by periodate oxidation of the 3′-terminal ribose [32]. The broad specificity of $tRNA^{Phe}$-s^4U76-reagent (unlike $tRNA^{Phe}$-$A_{ox}76$) allows it to be used as an effective test for conformational dynamics of the PheRS interaction with the 3′-end of $tRNA^{Phe}$, and the absence of phenylalanine transfer onto it (unlike $tRNA^{Phe}$-s^6G76) allows the PheRS complex with $tRNA^{Phe}$ and aminoacyladenylate to be "frozen".

The findings of the present work have shown that $tRNA^{Phe}$-s^4U77 (Ec) (with the s^4U residue in the additional position on the 3′-end of the full-length chain) generates the same set of modification products as $tRNA^{Phe}$-s^4U75 (Ec) and $tRNA^{Phe}$-s^4U76 (Ec) (Fig. 2a, lane *2* compared to *1*; table). The ligation of the reactive nucleotide to the native 3′-CCA end of $tRNA^{Phe}$ changes the main address of the modification. The more effective cross-linking of $tRNA^{Phe}$-s^4U77 than of $tRNA^{Phe}$-s^4U76 to the α-subunit is more likely due to the stronger binding of the 3′-terminal As^4U-fragment capable of producing specific contacts with involvement of A76. Actually, the affinity of $tRNA^{Phe}$-s^4U77 for the enzyme (determined from the value of K_i = 0.58 μM) is 1.6-fold higher than the affinity of $tRNA^{Phe}$-s^4U76 (K_i = 0.92 μM). In production of RNA–protein cross-links induced by s^4U only closely located partners can be involved [36]. Thus, the lack of strict selectivity in labeling of the subunits along with formation of several products of modification of the two subunits observed for the three reagents with differently located s^4U suggest that the $tRNA^{Phe}$ 3′-CCA-end should have conformational mobility in the complex with PheRS.

The modification of PheRS with 3′-s^4U-substituted analogs of human $tRNA^{Phe}$ also results in a significant

Affinity modification of *T. thermophilus* PheRS with s^{4}U-substituted derivatives of *E. coli* and human tRNA^{Phe}s

tRNA^{Phe} [a]	Relative yield of products[b], %						α/β[c]
	α		β				
	56-58	67*, 70	100	108*, 110	120*, 132	140-155*, 155-165	
-s^{4}U75(Ec)	1.5	3.2	8.0	2.0	2.3	83	0.05
-s^{4}U76(Ec)	12	53	4.0	2.4	3.6	25	1.9
-s^{4}U77(Ec)	83	4.2	1.6	1.4	0.8	9.0	6.8
-s^{4}U75(Hs)	3.2	14*	5.3	4.5*		73*	0.21
-s^{4}U76(Hs)	13	56*	3.7	5.6*	2.7*	19*	2.2
-s^{4}U77(Hs)	75	4.2*	8.6	5.5*		6.7*	3.8

[a] Prepared by *in vitro* transcription of the wild-type gene of *E. coli* (Ec) and human (Hs) tRNA^{Phe}s. Position of the reactive nucleotide is indicated.
[b] The yield of labeled products of the subunits with respect to the total level of the enzyme labeling. Apparent molecular weights (kD) of the products are indicated: products with similar electrophoretic mobilities are grouped in the same column; the range of molecular weights is given for the set of products that are not resolved completely (resulting in an enlarged or double band). Yields of the products and the total labeling efficiency are determined by normalizing the amount of the cross-linked analog (in an individual product or to the protein on the whole) to its total amount in the sample. The enzyme concentration is 2 μM, concentrations of *E. coli* and human tRNA^{Phe} analogs are 0.2 and 0.4 μM, respectively. Mean results of three determinations are presented; mean deviations of the results were 6-8% for minor products (with the relative yield not more than 10%) and 2-4% for other products.
[c] Ratio of labeling levels of the α- and β-subunits.

number of products, and only two of them have the same electrophoretic mobilities as the products of cross-linking of *E. coli* tRNA^{Phe} analogs (Fig. 2a, lanes *3-5* compared to lanes *1* and *2*). Hydrolysis of the modification products (without their electrophoretic pre-isolation) with nuclease results in two bands with mobilities corresponding to those of the α- and β-subunits (Fig. 2b). Comparison of the radioactivity distribution in the modification products treated and untreated with benzonase has shown that the products with apparent molecular weight in the range from 56 to 67 kD are results of the tRNA cross-linking to the α-subunit, whereas other products are results of its attachment to the β-subunit. The general pattern of increase in the relative efficiency of the α-subunit labeling is retained on repositioning of the reactive nucleotide from the 75th position by one-two units to the 3′-end (table). However, relative levels of labeling of the α- and β-subunits differ for tRNA^{Phe} analogs of different origin with the identically located s^{4}U residue. Relative efficiencies of tRNA^{Phe}-s^{4}U75 cross-linking to the β-subunit and of tRNA^{Phe}-s^{4}U77 to the α-subunit significantly decrease on the replacement of bacterial tRNA^{Phe} by the eukaryotic one. This decrease in the labeling selectivity suggests higher conformational mobility of the acceptor end of human tRNA^{Phe} in the complex with the enzyme compared to *E. coli* tRNA^{Phe}. The difference in electrophoretic mobility of some modification products can be caused by both different nature of the modified protein residues and dissimilar contribution of polyanionic chains of two tRNA^{Phe}s that are much different in structure. Experimental findings described below support the second hypothesis.

The interaction of PheRS with both ATP and Phe-substrate affect the cross-linking of tRNA^{Phe}-s^{4}U76 (Ec): yields of all products decrease or increase differently (Figs. 3a, 4a, and 4b). The findings suggest that amino acid residues of the α-subunit involved in production of photo-cross-links are located in the active site, and levels of their labeling depend on the functional state, which changes in the presence of small substrates. Changes in the labeling efficiency of the noncatalytic β-subunit in the presence of Phe or ATP can be caused only by substrate-induced changes in positioning of the reactive nucleotide. The effect of ATP on yields of all products of the tRNA^{Phe}-s^{4}U76 (Ec) cross-linking to the β-subunit is more clearly pronounced compared to the Phe-substrate (Fig. 4, a and b). Not surprising, the effect of the latter has not been detected in experiments on tRNA^{Phe}-A$_{ox}$76 [32], which has narrower specificity compared to that of s^{4}U-containing reagents.

Effects of Phe-substrate on tRNA^{Phe}-s^{4}U76 (Ec) and tRNA^{Phe}-s^{4}U77 (Ec) are significantly different: the yields of the tRNA^{Phe}-s^{4}U76 cross-links to the β-subunit decrease or increase by not more than 17%, whereas for the second analog these yields increase 1.8-2.6-fold (Fig. 4a). Changes in the s^{4}U residue positioning in the presence of Phe markedly decrease the efficiency of tRNA^{Phe}-s^{4}U77 attachment to the catalytic subunit and slightly increase the efficiency of tRNA^{Phe}-s^{4}U76 cross-linking;

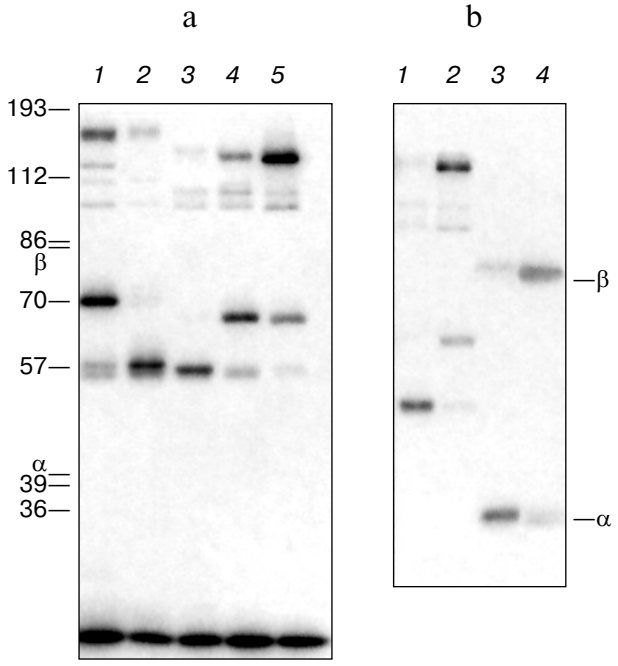

Fig. 2. a) Electrophoretic separation in SDS-polyacrylamide gel of products of the covalent cross-linking of ^{32}P-labeled derivatives of *E. coli* tRNA^{Phe} (lanes *1* and *2*) and human tRNA^{Phe} (lanes *3-5*) to PheRS: tRNA^{Phe}-s^{4}U76 (lanes *1* and *4*); tRNA^{Phe}-s^{4}U77 (lanes *2* and *3*); tRNA^{Phe}-s^{4}U75 (lane *5*). The enzyme concentration was 2 μM; those of *E. coli* and human tRNA^{Phe} reagents were 0.2 and 0.4 μM, respectively. Positions of the α- and β-subunits of the enzyme and molecular weights of marker proteins are shown to the left. b) Identification of the subunits in the products of photoaffinity labeling of PheRS with human tRNA^{Phe} analogs by hydrolysis with nuclease. The control (lanes *1* and *2*) and benzonase-treated (lanes *3* and *4*) protein samples were irradiated in the presence of tRNA^{Phe}-s^{4}U77 (lanes *1* and *3*) and tRNA^{Phe}-s^{4}U75 (lanes *2* and *4*). Positions of the α- and β-subunits are shown to the right.

thus, the significant difference between the two analogs in the labeling selectivity of different subunits disappears. However, on comparison of the enzyme modification with s^{4}U76-substituted analogs of *E. coli* and human tRNA^{Phe}s in the presence of Phe-substrate no significant difference is found: efficiencies of generation of the majority of products and labeling levels of the α- and β-subunits change similarly. The greater dependence of the effects on the s^{4}U position in the 3′-terminal sequence than on the structural difference between the bacterial and eukaryotic tRNA^{Phe}s (located more distantly) suggests the influence of Phe on the binding directly of the 3′-terminal (the 76th) nucleotide of tRNA^{Phe}. The positioning of s^{4}U base in the enzyme complex with tRNA^{Phe}-s^{4}U76 and Phe-substrate is more favorable for coupling with the catalytic subunit than its positioning in the binary complex of the enzyme with tRNA^{Phe} analog. On the contrary, changes in the positioning of ribose residue of A76, whose 3′-OH-group is ligated with the photoreactive nucleotide in tRNA^{Phe}-s^{4}U77, significantly decreases the relative level of the α-subunit labeling. Based on the comparable effects recorded for tRNA^{Phe}-s^{4}U76 (Ec) and tRNA^{Phe}-s^{4}U76 (Hs) it is suggested that the products with similar values of apparent molecular weight (as they are grouped in the table and Fig. 4) should be generated with involvement of the same protein residues, and their difference should be due to dissimilar contribution of two tRNA^{Phe}s to the electrophoretic mobility.

Changes in yields of the majority of the β-subunit cross-links caused by the presence of ATP are comparable for tRNA^{Phe}-s^{4}U76 (Ec) and tRNA^{Phe}-s^{4}U77 (Ec), but are significantly different for tRNA^{Phe}-s^{4}U76 (Ec) and tRNA^{Phe}-s^{4}U76 (Hs) (Fig. 4b). In the presence of ATP, the general efficiency of cross-linking to the protein decreases more significantly (3.2-fold) for tRNA^{Phe}-s^{4}U76 (Hs) than for tRNA^{Phe}-s^{4}U76 (Ec) (1.3-fold), but both analogs are more effectively coupled with the β-subunit (with similar relative levels of the subunits labeling). However, in the case of tRNA^{Phe}-s^{4}U77 (Ec) the α-subunit is labeled preferentially. These differences in the effects cannot be explained by the influence of ATP on the binding only of the 3′-terminal nucleotide; obviously, the more profound changes in the structure of the PheRS complex with tRNA^{Phe} induced by the nucleotide substrate determine the positioning of the acceptor end. ATP similarly increases the cross-linking efficiency of both *E. coli* tRNA^{Phe} analogs to the β-subunit, but the labeling level of the α-subunit decreases more significantly in the case of tRNA^{Phe}-s^{4}U76 (2.2-fold) than in the case of tRNA^{Phe}-

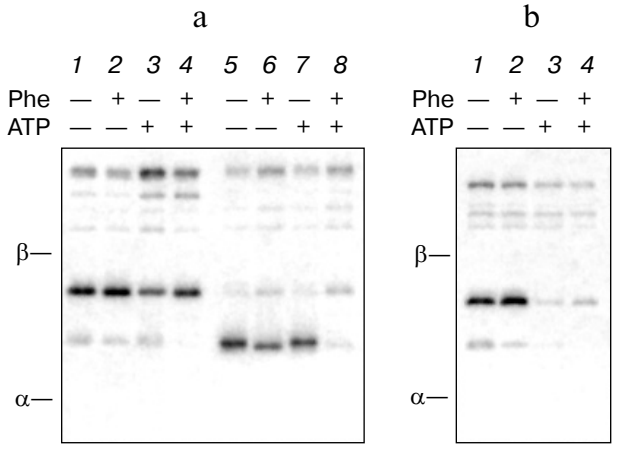

Fig. 3. Comparative analysis of cross-linked products of PheRS with *E. coli* (a) and human tRNA^{Phe} (b) analogs in the absence and presence of low-molecular-weight substrates: tRNA^{Phe}-s^{4}U76 (lanes *1-4* (a) and *1-4* (b)); tRNA^{Phe}-s^{4}U77 (lanes *5-8* (a)). The enzyme concentration was 2 μM, those of *E. coli* and human tRNA^{Phe}-reagents were 0.2 and 0.4 μM, respectively, of ATP 5 mM, Phe 50 μM. Positions of the subunits are shown to the left.

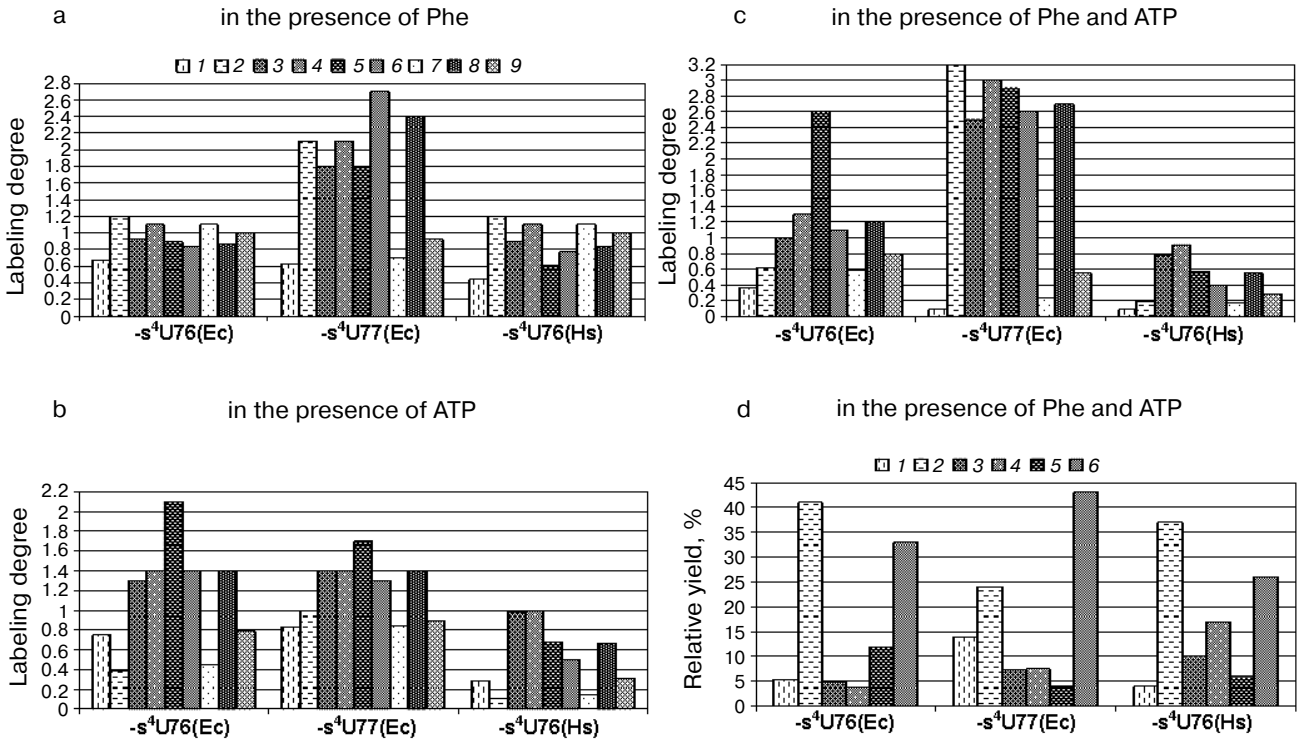

Fig. 4. Effect of low-molecular-weight substrates on labeling of PheRS with s^{4}U-substituted derivatives of *E. coli* and human tRNA^{Phe}s. The labeling degree was determined as the ratio of yields of individual products ((*1*) 56-58 kD, (*2*) 67*, 70 kD, (*3*) 100 kD, (*4*) 108*, 110 kD, (*5*) 120*, 132 kD, (*6*) 140-155*, 155-165 kD; asterisks indicate products of labeling with human tRNA^{Phe} derivatives which differ in mobility from products of labeling with *E. coli* derivatives), or of all products of labeling of the subunits ((*7*) α, (*8*) β), or of total efficiency of labeling of the protein (*9*) (in the presence of substrates (Phe (a), ATP (b), Phe and ATP (c)) to the corresponding values in the absence of the substrates. d) Relative yields of labeling products (% of the total labeling of the protein) in the presence of Phe and ATP. Mean values of two experiments are presented: mean deviations of the values were no more than 10%.

s^{4}U77 (1.2-fold). This can be caused by the stronger binding of tRNA^{Phe}-s^{4}U77 to the α-subunit (due to specific contacts of A76) and also due to ATP influence on the positioning of the base and ribose in the 76th nucleotide.

The effects recorded for two concurrently available substrates are not results of addition of their separate effects (Fig. 4, a-c). Thus, a product of the tRNA^{Phe}-s^{4}U76 (Ec) cross-linking to the β-subunit (132 kD) is most effectively produced in the presence of both substrates (or of Phe-AMP synthesized from them), whereas its yield decreases in the presence of Phe and increases in the presence of ATP. The total efficiency of the enzyme modification with s^{4}U76-substituted analogs of both tRNA^{Phe}s remains nearly the same as in the presence of ATP, but in the case of tRNA^{Phe}-s^{4}U77 (Ec) it is significantly (by 35%) decreased because of the sharply decreased generation of the major products of the α-subunit labeling (56-58 kD). In the presence of Phe and ATP, tRNA^{Phe}-s^{4}U77 (Ec) is more effectively attached to the β-subunit, whereas in the presence of only one of the substrates the relative level of the α-subunit labeling remains higher. The inhibitory effect of each substrate separately on generation of products of the α-subunit modification is comparable for tRNA^{Phe}-s^{4}U76 (Ec) and tRNA^{Phe}-s^{4}U77 (Ec), or is even significantly lower for the second analog that excludes a significant competition of the reactive nucleotide in tRNA^{Phe}-s^{4}U77 with low-molecular-weight substrates. Therefore, the position of s^{4}U unfavorable for the tRNA^{Phe}-s^{4}U77 cross-linking to the catalytic subunit in the presence of ATP and Phe is caused, first, by changes in the positioning of its carrier, the carbohydrate residue. The same products of the tRNA^{Phe}-s^{4}U76 (Ec and Hs) cross-linking are predominant in the absence or presence of both substrates: their relative yields varied 1.3-1.5-fold (table and Fig. 4d). In the case of tRNA^{Phe}-s^{4}U77, the minor products (155-165 kD) of the β-subunit labeling turn to the major ones, with their relative yield 4.8-fold increased, whereas the relative yield of the major products (in the absence of substrates) of the α-subunit labeling (56-58 kD) is 5.9-fold decreased. Thus, the interaction of PheRS with two small substrates more markedly affects the ribose residue positioning than that of the 76th nucleotide base. In total, the findings suggest that the binding of Phe and ATP and synthesis of amino-

acyladenylate additionally correct the positioning of the $tRNA^{Phe}$ 3′-terminal nucleotide in the complex with the enzyme.

DISCUSSION

PheRS of *T. thermophilus* binds human $tRNA^{Phe}$ less effectively than *E. coli* $tRNA^{Phe}$; K_d values of the corresponding complexes differ 16-fold [26]. The most fundamental differences in sequences of the two $tRNA^{Phe}$s are nucleotide replacements in the 20th position (G instead of U) and in the tertiary pair A26-G44 (by G26-A44) (Fig. 1). Significant changes in the binding to the enzyme are suggested to occur just in these regions of $tRNA^{Phe}$ based on XCA data [21] and protection of thiophosphate groups introduced into tRNA against iodine-induced hydrolysis [37]. Conformational mobility and deformability of these regions are confirmed by results of the photoaffinity modification; nucleotides in the 20th and 45th positions produce s^4U-induced cross-links [38], although have no direct contacts with the protein. The present work findings suggest that changes in the complex structure caused by nucleotide replacements in the central regions of $tRNA^{Phe}$ affect the interaction of PheRS with the acceptor end even at the stage of the initial binding (in the absence of low-molecular-weight substrates): the conformational mobility of the latter increases due to destabilization of the complex.

Conformation of the 3′-ACCA-end of $tRNA^{Phe}$ in the complex with PheRS (in the absence of other substrates) is stabilized due to specific interactions of A76 with four amino acid residues of the active site and to nonspecific interactions of the other nucleotides with residues of B1 and B3 domains of the large subunit (Fig. 5). Specific contacts with A76 are realized with involvement of Serα180 and Gluα220 (with the exocyclic amino group), Trpα149 (with the N7 atom of the imidazole ring), and Pheα258 (with the aromatic ring). All these residues interact with Phe-substrate or its part in Phe-AMP, and three of them (except Serα180) change their positioning [22]. The comparison of these models suggests a necessity of the conformational rearrangement to provide the functional binding of the $tRNA^{Phe}$ acceptor end. The findings of the present work have shown that the binding of the $tRNA^{Phe}$ 3′-terminal nucleotide is really changed in the presence of Phe-substrate, which affects the positioning of ribose and base residues. Only the so-called Phe-binding loop (residues α257-263) of all structural motifs of the α-subunit responsible for the enzyme interaction with Phe is in contact with the β-subunit B1-domain that is involved in the binding of $tRNA^{Phe}$. This contact occurs through a metal ion coordinated by six residues, including Gluα262 from the Phe-binding loop and Asnβ163 from the B1-domain. In its turn, Valβ160 from the B1-domain is involved in the binding of the C75-nucleotide of $tRNA^{Phe}$. Both structural motifs of the α- and β-subunits are well ordered in the native protein and do not change their overall conformation in the PheRS complexes with Phe, Phe-AMP, or $tRNA^{Phe}$ [20-22]. Thus, changes in the $tRNA^{Phe}$ acceptor end positioning induced by Phe-substrate are likely to be caused only by local rearrangements in the active site.

According to XCA data on the enzyme complex with Phe-AMP or its synthetic analog [20, 22], the interaction with ATP occurs with involvement of class II signature motifs. The motif 2 loop (residues α205-214), which along with Pheα216 (strictly conserved for class II) is responsible for most of the contacts with the nucleotide moiety of adenylate, is involved in the binding of the $tRNA^{Phe}$ acceptor stem: Glnα207 interacts with phosphate of the C69-nucleotide. Another region of this loop contact with the $tRNA^{Phe}$ acceptor stem is mediated by intersubunit interactions: three residues of the α-subunit (α213-215) from the motif 2 responsible for the interaction with ATP form hydrophobic contacts with the B7-domain of the β-subunit, which in turn interacts with the 67th and 66th nucleotides of $tRNA^{Phe}$ [21]. Conformation of the motif 2 loop is ordered in the absence of functional ligands and unchanged upon PheRS binding with $tRNA^{Phe}$, but it is shifted as a whole fragment into the active site region in the complex with Phe-AMP or its synthetic analog [20-22]. Thus, the effect of ATP on the $tRNA^{Phe}$ acceptor end positioning seems to be, first, due to changes in the acceptor stem position as a result of the coordinated movement with the loop. Dissimilar effects of ATP on the PheRS interaction with the acceptor end of *E. coli* and human $tRNA^{Phe}$s are more likely caused by differences in the complex structures on the level of the acceptor stem. The weaker binding of human $tRNA^{Phe}$ with *T. thermophilus* PheRS compared to bacterial $tRNA^{Phe}$ (*E. coli* and *T. thermophilus*) is caused by nucleotide replacements in positions 20, 26, and 44, which are responsible for conformational adaptation of the substrate on complexing [26]. The complex can be destabilized because of disorders in contacts with the acceptor arm, which in the system studied lacks specificity determinants of the complexing and seems to be bound last. Actually, the findings of the present work suggest that the 3′-CCA-end of human $tRNA^{Phe}$ in the complex with PheRS has increased conformational mobility compared to that of *E. coli* $tRNA^{Phe}$. In the presence of ATP or Phe-AMP, the acceptor end of the eukaryotic $tRNA^{Phe}$ becomes still more flexible, and this significantly decreases (3.2-3.4-fold compared to the 1.3-fold decrease in the case of bacterial $tRNA^{Phe}$) the efficiency of its cross-linking to the protein. However, in the presence of adenylate the difference in positioning of the 3′-end of two $tRNA^{Phe}$s with respect to residues of the catalytic subunit is less significant, as it is shown by similar relative yields of the corresponding products of labeling with their analogs (Fig. 4d). These findings correlate with kinetic characteristics of aminoacylation: the 5-fold decrease in

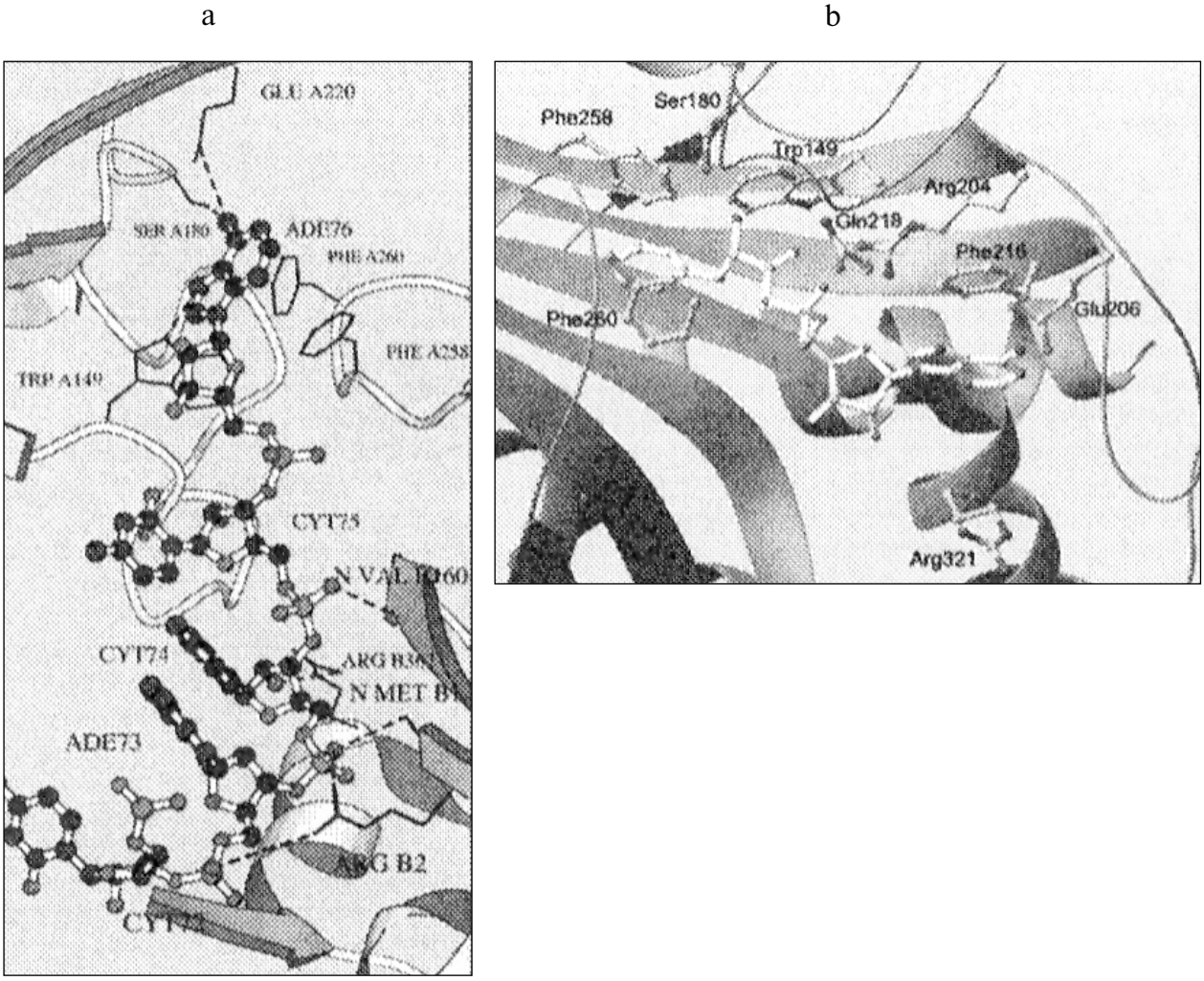

Fig. 5. Fragments of the structure of PheRS complexes with tRNA^{Phe} (a) and Phe-AMP (b). The 3′-terminal adenosine of tRNA^{Phe} interacts with residues of the α-subunit (designated with the letter A) and the other nucleotides of the ACCA-end interact with residues of the β-subunit (designated with the letter B). Two Phe residues from the Phe-binding loop (257-263), Trp149, Ser180, and Glu220 are in contact with A76 (a) and Phe-substrate (b). The loop of motif 2 (206-213) and conservative residues of motifs 2 (Phe216) and 3 (Arg321) interact with the nucleotide substrate. The data are reproduced from earlier publications [21, 22] with permission of the publisher (The International Union of Crystallography).

the catalytic efficiency of aminoacylation of human tRNA^{Phe} compared to *E. coli* tRNA^{Phe} is mainly caused by decrease in the affinity: the K_m values differ 4.6-fold and the apparent constants of the catalytic rate differ only 1.1-fold [26]. Thus, the conformational mobility of the tRNA^{Phe} acceptor arm is functionally significant for the proper positioning of the acceptor end in the productive complex with all substrates and for the catalytic process as it is.

The structure of class II aaRS complexes with specific tRNAs produced in the absence or presence of low-molecular-weight ligands (or their analogs) has been studied in detail for aspartyl-tRNA synthetase (AspRS) from various organisms [3, 11, 12] and for seryl-tRNA synthetase from *T. thermophilus* [13]. In both systems the motif 2 loop is involved in binding of both the acceptor stem and ACCA-sequence which contain identity determinants, but the productive binding of the acceptor ends of tRNA^{Ser} and tRNA^{Asp} is realized by different molecular mechanisms. The acceptor arm of tRNA^{Ser} forms contacts with the protein only in complex with a non-hydrolysable analog of seryladenylate, although two 3′-terminal nucleotides are not ordered. Different conformations of the loop in the absence or presence of tRNA^{Ser} are stabilized by different interactions with involvement of the same amino acid residues. The tRNA^{Asp} acceptor arm binds without small substrates, but in their absence, A76 takes the position of ATP; ATP induces conforma-

tional changes in the CCA-end and acceptor stem. In the productive interaction of AspRS with $tRNA^{Asp}$ along with the motif 2 loop a so-called flipping loop is involved, whose conformation depends on the presence and nature of substrates. In the enzyme complex with homologous $tRNA^{Asp}$ and aspartyladenylate an open conformation of this loop stabilizes the proper positioning of A76 for the Asp-substrate transfer. In the inactive complex of *E. coli* AspRS with yeast $tRNA^{Asp}$ (a bad substrate), this loop adopts the "closed" conformation, and A76 points outwards from the active site [39]. The regulatory role of $tRNA^{Asp}$ in the conformational change of the flipping loop is played by the acceptor stem.

A unique feature of PheRS is complete ordering of the active site in the absence of substrates; the only change with involvement of the whole fragment is the ATP-induced shift of the motif 2 loop without changing its overall conformation. Functional conformations of structural motifs of the active sites of other class II aaRSs are produced upon interaction with the substrates [10, 11, 13, 14, 40]. Modulation of the tRNA acceptor arm conformation in the productive complex in these systems is accompanied by significant changes in the protein itself. The available XCA data on the PheRS interaction with substrates together with results of the present study suggest that the productive binding of the 3′-end of $tRNA^{Phe}$ in the complex with all substrates should be provided by correction of the acceptor arm conformation. In the active site structure, the positioning varies only in individual residues that are responsible for the binding of A76 and small substrates. The absence of multiple contacts between the $tRNA^{Phe}$ acceptor arm and PheRS is functionally significant for the conformational mobility of the arm. The structure of the class II aaRS complexes with tRNA (a classic example is the AspRS complex with $tRNA^{Asp}$) is specified by a straight conformation of the single-stranded acceptor end, whereas in the class I enzyme complexes (the GlnRS-$tRNA^{Gln}$ complex is a prototype) the CCA-end is bent. The conformation of the $tRNA^{Phe}$ acceptor arm in the complex with PheRS is different from classic ones; it is more like the structure of the 3′-end of $tRNA^{Asp}$, but it has a small bend in the A73 region. The coordinated movement of the acceptor stem with the motif 2 loop has to result in additional deformation of the acceptor arm in this region because the CCA-end is fixed by the network of interactions with well-ordered fragments in the subunits interface. The A73 base plays a minor role as the recognition element in this system; mutation of this base affects the catalytic rate but not the initial binding of $tRNA^{Phe}$ [26, 34]. Thus, this role is limited to stabilization of the acceptor end conformation in the productive complex. The same function is served by pyrimidine bases of the CCA-end, which fail to produce specific contacts with the protein but are necessary for the substrate activity of $tRNA^{Phe}$ [35]. Specific contacts of A76 with involvement, first, of amino group and imidazole ring (crucial for the substrate activity) are controlled by Phe-substrate and determine the proper positioning for the transfer. The findings of the present work suggest that the conformational rearrangement of the acceptor end induced by small substrates significantly affects the terminal ribose of $tRNA^{Phe}$, which results in reorientation of its 3′-OH-group from the catalytic subunit onto the noncatalytic one. Thus, the unusual stereospecificity of aminoacylation of $tRNA^{Phe}$ is caused by specific features of the structural composition of domains whose interactions with all substrates determine the conformation of the acceptor arm and positioning of the terminal nucleotide in the productive complex.

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REFERENCES

1. Meinnel, T., Mechulam, Y., and Blanquet, S. (1995) in *tRNA: Structure, Biosynthesis and Function*, Chap. 14 (Söll, D., and RajBhandary, U., eds.) American Society for Microbiology, Washington, pp. 251-292.
2. Ibba, M., and Söll, D. (2000) *Annu. Rev. Biochem.*, **69**, 617-650.
3. Eriani, G., Delarue, M., Poch, O., Gangloff, J., and Moras, D. (1990) *Nature*, **347**, 203-206.
4. Cusack, S., Berthet-Colominas, C., Hartlein, M., Nassar, N., and Leberman, R. (1990) *Nature*, **347**, 249-255.
5. Arnez, J. G., and Moras, D. (1997) *Trends Biochem. Sci.*, **22**, 211-216.
6. Delagoutte, B., Moras, D., and Cavarelli, J. (2000) *EMBO J.*, **19**, 5599-5610.
7. Giegé, R., Sissler, M., and Florentz, C. (1998) *Nucleic Acids Res.*, **26**, 5017-5035.
8. Beuning, P. J., and Musier-Forsyth, K. (1999) *Biopolymers*, **52**, 1-28.
9. Rath, V. L., Silvian, L. F., Beijer, B., Sproat, B. S., and Steitz, T. A. (1998) *Structure*, **6**, 439-449.
10. Cavarelli, J., Eriani, G., Rees, B., Ruff, M., Boeglin, M., Mitschler, A., Martin, F., Gangloff, J., Thierry, J. C., and Moras, D. (1994) *EMBO J.*, **13**, 327-337.
11. Eiler, S., Dock-Bregeon, A.-C., Moulinier, L., Thierry, J.-C., and Moras, D. (1999) *EMBO J.*, **18**, 6532-6541.
12. Sauter, C., Lorber, B., Cavarelli, J., Moras, D., and Giegé, R. (2000) *J. Mol. Biol.*, **299**, 1313-1324.
13. Cusack, S., Yaremchuk, A., and Tukalo, M. (1996) *EMBO J.*, **15**, 2834-2842.
14. Cusack, S., Yaremchuk, A., and Tukalo, M. (1996) *EMBO J.*, **15**, 6321-6334.
15. Yaremchuk, A., Cusack, S., and Tukalo, M. (2000) *EMBO J.*, **19**, 4745-4758.
16. Mosyak, L., and Safro, M. (1993) *Biochimie*, **75**, 1091-1098.

17. Fraser, T. H., and Rich, A. (1975) *Proc. Natl. Acad. Sci. USA*, **72**, 3044-3048.
18. Sprinzl, M., and Cramer, F. (1975) *Proc. Natl. Acad. Sci. USA*, **72**, 3049-3053.
19. Stepanov, V. G., Moor, N. A., Ankilova, V. N., Vasil'eva, I. A., Sukhanova, M. V., and Lavrik, O. I. (1998) *Biochim. Biophys. Acta*, **1386**, 1-15.
20. Reshetnikova, L., Moor, N., Lavrik, O., and Vassylyev, D. G. (1999) *J. Mol. Biol.*, **287**, 555-568.
21. Goldgur, Y., Mosyak, L., Reshetnikova, L., Ankilova, V., Khodyreva, S., Lavrik, O., and Safro, M. (1997) *Structure*, **5**, 59-68.
22. Fishman, R., Ankilova, V., Moor, N., and Safro, M. (2001) *Acta Crystallogr.*, **D57**, 1534-1544.
23. Ankilova, V. N., Reshetnikova, L. S., Chernaya, M. M., and Lavrik, O. I. (1988) *FEBS Lett.*, **227**, 9-13.
24. Yoshikawa, M., Kato, T., and Takenishi, T. (1969) *Bull. Soc. Chem. Jap.*, **42**, 3505-3508.
25. Uhlenbeck, O. C., and Gumport, R. I. (1981) in *The Enzymes* (Boyer, P. D., ed.) 3rd Edn., Vol. 15, Academic Press, New York, pp. 31-58.
26. Vasil'eva, I. A., Ankilova, V. N., Lavrik, O. I., and Moor, N. A. (2002) *J. Mol. Recogn.*, **15**, 188-196.
27. England, T. E., and Uhlenbeck, O. C. (1978) *Nature*, **275**, 560-561.
28. Paulsen, H., and Wintermeyer, W. (1984) *Eur. J. Biochem.*, **138**, 117-123.
29. Igloi, G. L. (1988) *Biochemistry*, **27**, 3842-3849.
30. Laemmli, U. K. (1970) *Nature*, **227**, 680-685.
31. Moor, N. A., Ankilova, V. N., Favre, A., and Lavrik, O. I. (1998) *Biochemistry (Moscow)*, **63**, 1051-1056.
32. Vasil'eva, I. A., Ankilova, V. N., Lavrik, O. I., and Moor, N. A. (2000) *Biochemistry (Moscow)*, **65**, 1157-1166.
33. Moor, N., Nazarenko, I., Ankilova, V., Khodyreva, S., and Lavrik, O. (1992) *Biochimie*, **74**, 353-356.
34. Moor, N. A., Ankilova, V. N., and Lavrik, O. I. (1995) *Eur. J. Biochem.*, **234**, 897-902.
35. Moor, N. A., Repkova, M. N., Yamkovoy, V. I., and Lavrik, O. I. (1994) *FEBS Lett.*, **351**, 241-242.
36. Favre, A., Saintomé, C., Fourrey, J.-L., Clivio, P., and Laugâa, P. (1998) *J. Photochem. Photobiol. B: Biol.*, **42**, 109-124.
37. Kreutzer, R., Kern, D., Giegé, R., and Rudinger, J. (1995) *Nucleic Acids Res.*, **23**, 4598-4602.
38. Moor, N. A., Ankilova, V. N., Lavrik, O. I., and Favre, A. (2001) *Biochim. Biophys. Acta*, **1518**, 226-236.
39. Moulinier, L., Eiler, S., Eriani, G., Gangloff, J., Thierry, J. C., Gabriel, K., McClain, W. H., and Moras, D. (2001) *EMBO J.*, **20**, 5290-5301.
40. Yaremchuk, A., Tukalo, M., Grøtli, M., and Cusack, S. (2001) *J. Mol. Biol.*, **309**, 989-1002.