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Interaction of the tRNA^{Phe} Acceptor End with the Synthetase Involves a Sequence Common to Yeast and *Escherichia coli* Phenylalanyl-tRNA Synthetases†

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ABSTRACT: Modified lysines resulting from the cross-linking of the 3′ end of tRNA^{Phe} to yeast phenylalanyl-tRNA synthetase (an enzyme with an $\alpha_2\beta_2$ structure) have been characterized by sequencing the labeled chymotryptic peptides that were isolated by means of gel filtration and reversed-phase chromatography. The analysis showed that Lys131 and Lys436 in the α subunit are the target sites of periodate-oxidized tRNA^{Phe}. Mutant protein with a Lys → Asn substitution established that each lysine contributes to the binding of the tRNA but is not essential for catalysis. The major labeled lysine (K131) belongs to the sequence IALQDKL (residues 126-132), which shares three identities with the peptide sequence ADKL found around the tRNAox-labeled Lys61 in the large subunit of *Escherichia coli* phenylalanyl-tRNA synthetase [Hountondji, C., Schmitter, J. M., Beauvallet, C., & Blanquet, S. (1987) *Biochemistry 26*, 5433-5439].

Periodate-oxidized tRNA (tRNAox)[1] has been used as a specific affinity label in a number of aminoacyl-tRNA synthetases to probe the lysine residues that interact with the CCA end of tRNA (Baltzinger et al., 1979; Fayat et al., 1979; Hountondji et al., 1979, 1985, 1986a, 1987; Hill & Schimmel, 1989). Covalent cross-linking of tRNA proceeds via the formation of a Schiff base between the 2′- or 3′-aldehyde group

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[1] Abbreviations: PheRS, phenylalanyl-tRNA synthetase; SDS, sodium dodecyl sulfate; tRNAox, periodate-oxidized tRNA; HPLC, high-performance liquid chromatography.

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of tRNAox and the ϵ-NH_2 group of one or several lysine residues that are supposed to be located in or near the active site pocket. This covalent tRNA binding results in a total inactivation of both aminoacylation and ATP–PP_i exchange activities for *Escherichia coli* methionyl- and tyrosyl-tRNA synthetases (Hountondji et al., 1979, 1986a) but not for phenylalanyl-tRNA synthetases (PheRS) from yeast (Baltzinger et al., 1979) or *E. coli* (Hountondji et al., 1987). In the latter two cases, only the aminoacylation activity was lost. Alanyl-tRNA synthetase represents an intermediate case where ATP–PP_i exchange is partially inactivated whereas tRNA aminoacylation is completely lost (Hill & Schimmel, 1989).

The tRNAox-labeled amino acid residues of aminoacyl-tRNA synthetases were identified, and in most cases, their functional role was assessed by site-directed mutagenesis. The three lysine residues identified in *E. coli* tyrosyl-tRNA synthetase (lysines-229, -234, and -237) are homologous to residues Lys225, Lys230, and Lys233 of the same enzyme from *Bacillus stearothermophilus* (Hountondji et al., 1986a). These residues are located at the enzyme surface, and model-building studies suggest that the 2′- and 3′-OH of the terminal adenosine of the CCA make contact with the Lys230 and Lys233 side chains but not with that of Lys225 (Labouze & Bedouelle, 1989). However, mutagenesis experiments establish a role that is important in adenylate synthesis (Bedouelle & Winter, 1986), which explains the effect on the ATP–PP_i exchange activity. The labeled lysine in alanyl-tRNA synthetase (Lys73) is in the domain responsible for adenylate synthesis, and kinetic analysis of the mutant Lys → Gln indicates that Lys73 is important for a $tRNA^{Ala}$-dependent step (i.e., the alignment of the CCA end in the active site) but has little influence on the kinetic parameters for ATP and alanine in the ATP–PP_i exchange reaction. Therefore, partial inactivation of the alanine-dependent ATP–PP_i exchange is due to a steric hindrance by the large covalently bound $tRNA^{Ala}$ (Hill & Schimmel, 1989).

The mutagenesis experiments were also useful to clarify the role of the signature sequence KMSKS that has been deduced from sequence comparisons around the tRNAox-labeled lysines of *E. coli* methionyl- and tyrosyl-tRNA synthetases (Hountondji et al., 1986b). The major tRNAox-labeled Lys335 of methionyl-tRNA synthetase which belongs to the KMSKS sequence has been substituted for glutamine by means of site-directed mutagenesis. This resulted in the total loss of both the ATP–PP_i exchange and $tRNA^{Met}$ aminoacylation activities of the enzyme (Brunie et al., 1987). Detailed analysis of the properties of the mutant enzyme indicates that Lys335 of the *E. coli* methionyl-tRNA synthetase participates in the binding of the γ-phosphate of ATP (Mechulam et al., 1990). The involvement of the MSK sequence in the binding of the CCA end of $tRNA^{Gln}$ to glutaminyl-tRNA synthetase has been ruled out by resolution at the atomic level of the crystal structure of the enzyme complexed with both ATP and $tRNA^{Gln}$ (Rould et al., 1989). However, the MSK sequence could participate in the binding of ATP to the glutaminyl-tRNA synthetase.

Labeling of *E. coli* PheRS by the cognate oxidized tRNA led to the identification of lysines-2, -61, and -106 as the sites of covalent attachment of $tRNA^{Phe}$ox to the large α subunit of PheRS (Hountondji et al., 1987). None of the labeled peptides showed sequence similarity with the KMSKS sequence although one, Lys61, pointed at a somewhat divergent RVTK sequence (at positions 63–66). Similar studies carried out on the yeast PheRS had identified Lys325 of the small subunit as the target of $tRNA^{Phe}$ox (Renaud et al., 1982). Both enzymes have $\alpha_2\beta_2$ structures and require the two subunits to function (Fasiolo et al., 1975; Ducruix et al., 1983).

We have tested the role of Lys325 in the yeast PheRS by site-directed mutagenesis. For example, substituting Asn for Lys suppresses the potentiality of the ϵ-NH_2 group to form an ionic bond with the phosphate backbone of the tRNA while keeping the hydrophilic nature. To achieve this goal, we recently constructed an operon with the two structural genes of α and β subunits from yeast (FRS1 and FRS2; Sanni et al., 1990). This construction enabled us to perform mutagenesis and expression from the same plasmid in a minimum of time. The change of Lys325 into Asn did not alter the kinetics of aminoacylation, a result which is not consistent with the loss of enzyme activity following covalent attachment of $tRNA^{Phe}$ox. This result casts doubt on the validity of this lysine previously identified as target site for $tRNA^{Phe}$ox (Renaud et al., 1982). In order to solve this apparent contradiction, we have undertaken a reinvestigation of the lysine residue(s) actually labeled by oxidized $tRNA^{Phe}$.

Materials and Methods

Enzymes. Yeast PheRS (cytoplasmic enzyme) was a gift from Dr. P. Remy. The enzyme was purified from commercial bakers' yeast and had a specific activity of 1600 units/mg (k_{cat} 7 s^{-1}) at the time of the assays reported here. Chymotrypsin was from Worthington and ribonuclease T1 from P-L Biochemicals, Inc. Restriction enzymes were purchased from Boehringer Mannheim.

Materials. TSK2000 SW (30 cm × 7.5 mm i.d.) was from Bio-Rad. A Superspher RP-18 column (250 mm × 4 mm, particles size 4 μm, Merck) was used for the HPLC separation of labeled peptides. The composition of buffers and gradients used for these two chromatography steps is as described in Beauvallet et al. (1988).

$tRNA^{Phe}$. Yeast $tRNA^{Phe}$ was obtained by countercurrent distribution. Its purity estimated by its accepting capacity was about 60%. It was further purified by HPLC on a Phenomenex column (250 mm × 22.5 mm, 5-μm particles) with a reverse gradient of ammonium sulfate. Buffer A was 200 mM potassium phosphate, pH 7–2-propanol (0.75% v/v), and buffer B was the same as buffer A, supplemented with 2.4 M ammonium sulfate. The total gradient volume was 2 L, and the flow rate was 10 mL/min. The purified $tRNA^{Phe}$ had an acceptance capacity of 1600 pmol of phenylalanine/A_{260} unit.

Radiolabeling and Periodate Oxidation of $tRNA^{Phe}$. $tRNA^{Phe}$ (1.3 nmol) was labeled at the 3′ end by use of tRNA nucleotidyl transferase (TNT) after removal of the CCA end by limited hydrolysis with venom phosphodiesterase (1.25 μg). The reaction mixture (30 μL) containing 1.3 nmol of $tRNA^{Phe}$(-CCA), 6 μmol of CTP, 50 μCi of [α-^{32}P]ATP (400 Ci/mmol), 5 μg of TNT, 50 mM Tris-HCl (pH 8.6), 10 mM $MgCl_2$, and 8 mM dithioerythreitol was incubated at 37 °C for 30 min. Then, the mixture was subjected to polyacrylamide gel electrophoresis under denaturating conditions. Radiolabeled tRNA comigrated with untreated cold $tRNA^{Phe}$. After recovery from the gel, the integrity of the acceptor end was further verified by sequencing from the 3′-terminal end. The labeled tRNA was mixed with 29 nmol of unlabeled $tRNA^{Phe}$ to yield a specific activity of 580 cpm/nmol. The dialdehyde derivative of $tRNA^{Phe}$ was obtained according to the procedure previously described (Renaud et al., 1982).

Covalent Complex Formation between $tRNA^{Phe}$ox and PheRS. Yeast PheRS (6.7 μM) was covalently labeled with $tRNA^{Phe}$ox (21 μM) in a reaction mixture containing 20 mM imidazole hydrochloride, pH 8, 25% glycerol, 10 mM $MgCl_2$,

and 2 mM sodium cyanoborohydride according to Hountondji et al. (1986a, 1987).

Purification of tRNA-Labeled Chymotryptic Peptides of Yeast PheRS. Labeled peptides were isolated by means of a combination of techniques involving chymotryptic digestion of the enzyme, gel filtration on TSK2000, ribonuclease T1 digestion of tRNA, rechromatography on TSK2000, and reverse-phase chromatography on an RP18 column. Details for each step are described in Beauvallet et al. (1988).

PTH-Amino Acid Analysis. Peptides were subjected to automated N-terminal degradation with phenylisothiocyanate (PITC) in a gas-phase sequencer (Applied Biosystems Model 470A). Samples were applied to a polybrene-treated and precycled glass-fiber filter. Amino acid phenylthiohydantoins (PTH) were identified by chromatography on a C18 column Brownlee (2.1 mm × 200 mm) coupled with the PTH analyzer.

Oligonucleotide-Directed Mutagenesis. The Amersham mutagenesis system based on the method of Eckstein (Sayers et al., 1988) was used to create single base pair mutations in the artificial FRS1-FRS2 operon described in Sanni et al. (1990). After transformation of TG1 strain, positive clones were identified by sequencing the DNA of four AmpR colonies according to the Sanger dideoxy method (1977).

Determination of Enzymatic Activities. Yeast wild type and mutant PheRS expressed from the FRS2-FRS1 operon was tested in crude extracts from exponentially growing cells carrying the plasmid-recombinant pUC-ASab2. Cells were resuspended in the aminoacylation buffer and subjected to ultrasonic disintegration.

Aminoacylation reactions were carried out at 37 °C under the following conditions: 144 mM Tris-HCl, pH 7.8, 5 mM dithiothreitol, 2 mM ATP, 10 mM $MgCl_2$, 0.1 mM [^{14}C]-phenylalanine (25 μCi/μmol), and 6 mg/mL unfractionated yeast tRNA (the fraction of $tRNA^{Phe}$ is 2.5%) or purified fractions as indicated. At various time intervals 40-μL aliquots from a 200-μL reaction mixture were spotted onto Whatman paper disks and quenched by 5% trichloroacetic acid. Radioactivity was counted in a toluene-based scintillant. The ATP–pyrophosphate exchange assays were done at 37 °C with 144 mM Tris-HCl, pH 7.8, 2 mM ATP, 2 mM [^{32}P]PP_i (0.35 μCi/μmol), 2 mM phenylalanine, 10 mM potassium fluoride (an inhibitor of endogenous pyrophosphatase), 0.1 mM phenylmethanesulfonyl fluoride, and various amounts of crude enzyme. Labeled ATP was adsorbed onto charcoal and washed with water, and the radioactivity was monitored by scintillation counting as above.

Western Blotting. Protein samples (20 μg) were run on 10% polyacrylamide gels in the presence of 0.1% sodium dodecyl sulfate. Conditions for the transfer of proteins to nitrocellulose membranes were as described in Schleicher & Schuell Manual No. 2. The protein band corresponding to PheRS was detected by use of DEAE-Sephadex-purified antibodies (100 μg per nitrocellulose membrane of about 150 cm^2) and ^{125}I-labeled protein A (0.7 mCi at 50 mCi/mg). Antibodies were obtained from intradermal injections into rabbits of native PheRS emulsified with complete Freund's adjuvent.

Peptide Synthesis. The tryptic peptide YNWK-PEECQKLVLRT containing the labeled Lys325 was synthesized by the method of Merrifield (1963). Antibodies were obtained as above with 100 μg of peptide.

Immunological Tests. Enzyme-linked immunosorbant assay (ELISA) was carried out as follows: walls were coated for 2 h at 37 °C in 0.05 M carbonate buffer, pH 9.6, with the synthetic peptide (10–200 ng) or with PheRS (10–200 μg). After being rinsed, the plates were incubated with 1% bovine serum albumin for 1 h at 37 °C. Antisera diluted in phosphate buffer (pH 7.4) containing 0.05% Tween 20 were added for 2 h at 37 °C. After further rinsing, goat anti-rabbit globulins conjugated with alkaline phosphatase were added as described by Muller et al. (1982). The absorbance at 405 nm was read on a Titertek Multiskan MC photometer.

Results

Substitution of Cys323 and Lys325 in the β Subunit of the Yeast PheRS by Site-Directed Mutagenesis. Lys325 previously identified as the target for $tRNA^{Phe}$ox attachment (Renaud et al., 1982) belongs to a dodecapeptide containing the unique cysteine residue of the β subunit. This sequence is part of a polypeptide that connects two blocks of homologies between yeast and *E. coli* PheRS sequences (Sanni et al., 1988). We tested the probability that Lys325 is close to the 3′ end of tRNA when bound to the synthetase, by replacing it with Asn.

We also replaced the unique Cys323 by Ala because sulfhydryl groups have been postulated to play a role in catalysis according enzyme inactivation by dithiobis(nitrobenzoate) (Raffin et al., 1978). The protein level of the two PheRS mutants detected by immunoblotting is unchanged compared to that of the wild-type enzyme (data not shown), allowing a comparison of their specific activity. The specific activity of tRNA aminoacylation was found identical for the three enzyme species (mutants and wild type). These results rule out (i) a catalytic role for Cys323 and (ii) the participation of Lys325 in the correct orientation of the CCA end during the catalytic step. At best, it could be on the edge of the normal CCA binding site in which case its labeling would be rather a consequence of the mobility of the CCA end. To test this possibility, we raised antibodies against the peptide sequence YNWKPEECQKLVLRT, corresponding to the sequence identified by $tRNA^{Phe}$ox cross-linking. Binding of the antibodies to PheRS was assessed by ELISA tests. No inhibition of aminoacylation could be observed when the antibodies were incubated in the presence of PheRS. Because the Cys → Ala substitution had no effect on the activity, steric hindrance by the bound chemical reagent [dithiobis(nitrobenzoate)] is most likely responsible for the enzyme inactivation.

Isolation and Sequencing of the Labeled Peptides. Schiff base formation between yeast PheRS and $tRNA^{Phe}$ox was conducted at large scale in the presence of sodium cyanoborohydride. This causes irreversible inactivation of the synthetase in an apparent first-order process (results not shown) due to covalent complex formation (Baltzinger et al., 1979; Hountondji et al., 1979). A 90% inactivation was obtained within 60 min of reaction, whereas control experiments performed in the absence of oxidized tRNA showed that inactivation occurred at a much slower rate (40% at 90 min). Similar observations were made in the case of alanyl-tRNA synthetase upon incubation under reducing conditions with oxidized $tRNA^{Ala}$ (Hill & Schimmel, 1989). This unspecific inactivation is not a major problem since our goal was to characterize an ϵ-NH_2 of lysine modified at 100% and to check its role by site-directed mutagenesis.

The labeled peptide(s) were recovered from the covalent radioactive complex after chymotryptic digestion by a combination of HPLC chromatographic steps (Beauvallet et al., 1988). From the first TSK column a unique radioactive peak was eluted in the void volume that contained the tRNA-bound peptide(s). After T1 ribonuclease digestion followed by a second chromatographic step on the same TSK column, a

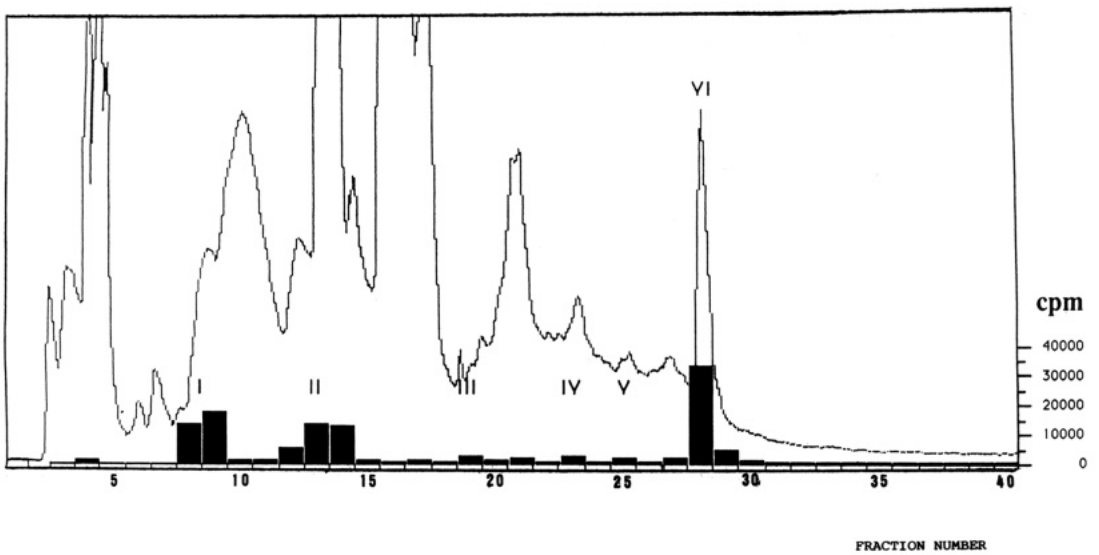

FIGURE 1: RPLC separation on an RP18 Superspher column of the labeled tRNA^{Phe}ox–enzyme complex subjected to chymotrypsin and ribonuclease T1 digestions. Shown is the absorbance at 215 nm (solid line) as well as the ^{32}P radioactivity profile (black rectangles) corresponding to cpm for 10-μL aliquots of each collected fraction (400 μL). The radioactive peaks along the column profile are indicated in Roman letters. Solvent A was 0.1% trifluoroacetic acid, and solvent B was 80% acetonitrile in 0.1% trifluoroacetic acid. The flow rate was 0.4 mL/min. Radioactive peaks I–VI were applied to the sequencer. Nonradioactive light-absorbing peaks (not numbered) were analyzed but not sequenced.

Table I: Sequences of the tRNA^{Phe} ox-Labeled Peptides[a]

Cycle number	1	2	3	4	5	6	7	8		
Peptide 1 (peak IV)	A	N	P	X	T	L	E	Y		
Peptide 2 (peaks V and VI)	I	A	L	Q	D	X	L			
Known sequence for peptide 1	L ↓	A	N	P	K	T	L	E	Y ↓	Q
Residue number	432	433	434	435	436	437	438	439	440	441
Known sequence for peptide 2	F ↓	I	A	L	Q	D	K	L ↓	H	
Residue number	125	126	127	128	129	130	131	132	133	

[a] Peptide sequences of tRNA^{Phe} ox-labeled peptides were determined on an Applied Biosystems 470 sequencing apparatus. Amino acid residues listed for each cycle correspond to chromatograph peaks showing the strongest increase over the previous cycle. Cycles that produced either no known amino acid signal or no signal increase over the previous cycle are designed as X. The known sequences corresponding to peptides 1 and 2 are indicated. Arrows indicate the chymotryptic cleavage sites.

retarded radioactive fraction was obtained that contained free and peptide(s)-bound oligonucleotides. This fraction was eluted as a peak and a shoulder (data not shown). Both were run separately under the same conditions on a reverse-phase C18 column and gave an identical elution profile. Figure 1 shows the elution profile obtained with the peak fraction.

Peptide Sequences. All the radioactive peaks eluted from the reverse-phase C18 column were analyzed for their amino acid composition. Only three of these peaks turned out to contain peptidic material, namely, peaks IV, V, and VI (Figure 1). From the results of scintillation counting it was shown that the major peptide (peak VI) and the two minor peptides (peaks IV and V) corresponded to respectively 87%, 6.5%, and 6.5% of the total radioactivity incorporated into the protein. These peaks were then subjected to gas-phase sequencing, and in each case a unique sequence was established: both peaks V and VI contained the same peptide, IALQDKL, whereas the minor peak IV corresponded to the peptide ANPKTLEY (Table I). These two peptides could be unambiguously assigned to residues 126–132 and 433–440, respectively, of the α subunit (Table I). These two peptides arise from normal chymotryptic cuts after Phe125, Leu132, Leu432, and Tyr440. The fact that peak V contained the same peptide as the major peak VI is probably the result of partial hydrolysis by T1 ribonuclease. In other words, the same major peptide is attached to two oligonucleotides of different length; hence, the two peaks V and VI eluted at different positions.

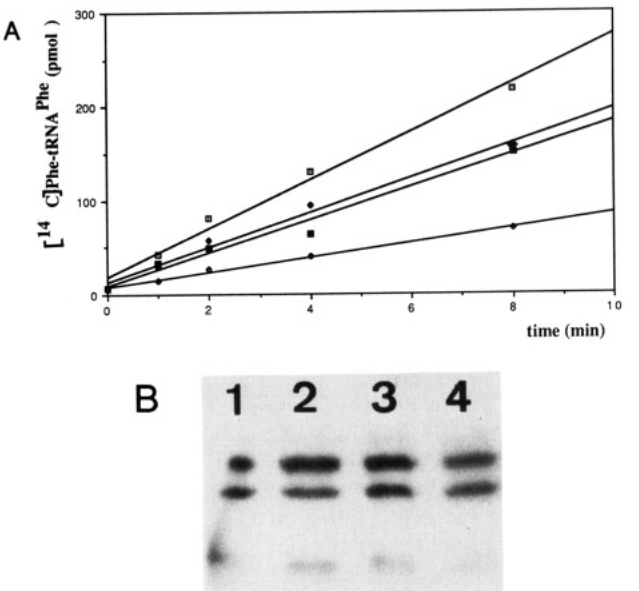

FIGURE 2: Comparison of aminoacylation activities of wild-type and Lys → Asn mutants at the tRNAox cross-linking sites. Crude protein extracts (1 μg) were assayed for phenylalanine acceptance activity as described under Materials and Methods. (A) Kinetics of aminoacylation: (⊡) wild type; (■) mutant K131N; (◆) mutant K436N; (◇) double mutant K131N, K436N. (B) Immunoblot analysis of wild-type and mutant PheRS. Visualization of the α subunit (upper band) and the β subunit (lower band) was done with antibodies raised against native PheRS: (1) wild-type PheRS; (2) mutant K131N; (3) mutant K436N; (4) double mutant K131N, K436N.

Substitution of Lys131 and Lys436 by Asn in the α Subunit of Yeast PheRS. The labeled Lys131 and Lys436 were replaced by Asn either independently or simultaneously, and the effect of these replacements was examined by measuring initial rates of aminoacylation and ATP–PP$_i$ exchange reactions. From the slope of the curves shown in Figure 2, a 30% inhibition of tRNA aminoacylation for each single mutation and a 75% inhibition for the double mutation could be measured. No variation in the rate of the ATP–PP$_i$ exchange was ob-

Table II: Kinetic Parameters of Aminoacylation of $tRNA^{Phe}$ by Wild-Type and Mutant PheRS[a]

enzyme	K_M (μM) (tRNA)	relative k_{cat}	ΔG_S (kcal/mol)
wild type	0.2	1	
K131N	0.6	0.7	0.67
K436N	0.6	0.7	0.67
K131N, K436N	1.2	0.25	1.1

[a] K_M values for tRNA were determined with pure yeast $tRNA^{Phe}$ (Böerhinger). The concentration of specific PheRS in the crude poly(ethylene glycol) fraction (5–17.5%) was quantitated by densitometry of Western blots. No correction was made for small variations in yield for the mutants. The specific activity relative to that of the wild-type PheRS is indicated. $\Delta G_S = -RT \ln [K_M(wt)/K_M(mt)]$ (Fersht, 1985).

served for either mutant analyzed (the values differ by about 10% which corresponds to the statistical error of the measure). The influence of these mutations on the K_M values for tRNA was estimated with partially purified enzymes. For the single mutants K131N[2] and K436N, a value of 0.6 μM was measured as compared to 1.2 μM for the double mutant (Table II). With the $tRNA^{Phe}$ concentration used in the aminoacylation tests of Figure 2 ($[tRNA^{Phe}] = 5K_M$) nearly saturating substrate concentration was used (83%). The slope of the curve is therefore a good approximation of the V_{max} value. A 4-fold decrease in V_{max} was estimated from the kinetics of aminoacylation of the double mutant.

Discussion

Previous photoaffinity labeling experiments conducted on the tetrameric $\alpha_2\beta_2$ yeast PheRS (α, M_r 63 000; β, M_r 55 000) had shown that the core of $tRNA^{Phe}$ binds to the β subunit (Baltzinger et al., 1979). The CCA binding site was localized on the β subunit as well, by use of periodate-oxidized $tRNA^{Phe}$, and Lys325 was identified as the target of oxidized $tRNA^{Phe}$ (Renaud et al., 1982). The binding sites for the small ligands were characterized by UV cross-linking experiments, which indicated that ATP binds to the β subunit and that the site for phenylalanine lies between the two subunits (Baltzinger et al., 1979). The results of this study suggest a major functional role for the small β subunit whereas the large α subunit bears only some determinants for phenylalanine binding. Alternatively, the α subunit is only required to generate activity at the subunit interface by inducing a conformational change in the β catalytic subunit. Both interpretations explain why a structural integrity ($\alpha_2\beta_2$) is necessary for the enzyme to function.

We have tested the functional relevance of Lys325 by replacing it by Asn. This change, as well as immunoinactivation assays with antibodies raised against the labeled peptide containing Lys325, had no noticeable effect on the aminoacylation reaction. These results indicate that the region encompassing peptide 316–325 of the β subunit is not the CCA binding site. Rather, it is part of a domain at the enzyme surface that allows access to large molecules like antibodies. The hydrophilicity of the peptide region 316–325 further underlines its surface localization. Besides, replacement of the Cys323 by Ala had no adverse effect on the enzyme activity, a result which further corroborates the nonessential role of the peptide region 316–325. In view of these negative results, we have undertaken a reinvestigation of the peptides actually labeled by $tRNA^{Phe}$ox. Taking advantage of the strategy developed by Hountondji et al. (1987) and Beauvallet et al. (1988), we could isolate two labeled peptides, each containing one modified lysine. The major labeled lysine (Lys131) belongs to the sequence IALQDKL, which shares three identities with the peptide sequence ADKL found around the labeled Lys-61 in the large subunit of *E. coli* PheRS.

In order to check whether any of the two labeled lysines, Lys131 and Lys436, have a functional role, point mutations were introduced at each position. Each Lys → Asn mutation at either position results in a 3–4-fold increase of the K_M value for $tRNA^{Phe}$ with no significant change in V_{max}. When both mutations were simultaneously introduced, the most significant effect was a 6-fold decrease in the apparent affinity of PheRS for $tRNA^{Phe}$, whereas V_{max} was only affected by a factor of 4. These results suggest that each of these two lysines partly contributes to the functional alignment of the CCA arm and that these contributions are additive (within the limits of experimental error, the K_M for the double mutant is the product of the individual alterations). However, these two lysines are not essential for catalysis since the catalytic efficiency is only reduced by a factor of 4. The 4-fold decrease in V_{max} of the aminoacylation reaction for the double mutant appears to be more a consequence of an improper positioning of the CCA end in the active site. From the variation in K_M for an individual lysine substitution, it was calculated that each lysine side chain contributes 0.67 kcal/mol to the binding energy of the substrate. This suggests that the positive charge of the ϵ-NH_3^+ group makes a weak electrostatic interaction, if any, with the negatively charged phosphate of the tRNA. Alternatively, altered binding results from the modification of the local protein structure following lysine substitution.

The results from this study showing that the 3′-oxidized ribose of $tRNA^{Phe}$ binds to the α subunit of yeast PheRS agree with those of Roques et al. (1989), who could cross-link to this subunit an alkylating derivative of $tRNA^{Phe}$ that has modified adenine and cytosine residues in the amino acid acceptor arm. This indicates that the tRNA binding occurs across the two subunits and is not confined to the β subunit as was deduced before. This mode of binding better describes the interaction with the $tRNA^{Phe}$ that involves recognition nucleotides located at extreme distal parts of the tRNA molecule: the anticodon region, G20 of the D loop, and A73 of the acceptor arm (Sampson et al., 1989).

We have aligned the Lys61 and Lys131 regions from the *E. coli* and yeast PheRS to check for homology. There is only a stretch of three identities and one conservative replacement out of 11 compared residues. The significance of this homology is poor when considered outside the context of their functional role. It is interesting to note that the similarities around the tRNAox-labeled lysines in the *E. coli* methionyl- and tyrosyl-tRNA synthetases that lead to the discovery of the signature sequence KMSKS are not much more pronounced: two conservative replacements and three identities including the reactive lysine are interspaced by two nonhomologous amino acids. Moreover, these lysines are topologically equivalent: they occupy identical positions in the central part of the two synthetase molecules close to the ATP binding site and are exposed toward the outside of the molecule (Hountondji et al., 1986b). Therefore, even poor sequence similarities in the functional regions of the PheRS from *E. coli* and yeast that carry the same reaction may be noteworthy.

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[2] One-letter code is used to designate mutant protein; i.e., K131N designates an asparagine replacement at position 131.

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Registry No. PheRS, 9055-66-7; Lys, 56-87-1.

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Kinetics and Mechanism of the Pressure-Induced Lamellar Order/Disorder Transition in Phosphatidylethanolamine: A Time-Resolved X-ray Diffraction Study†

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ABSTRACT: By using synchrotron radiation, a movie was made of the X-ray scattering pattern from a biological liquid crystal undergoing a phase transition induced by a pressure jump. The system studied includes the fully hydrated phospholipid dihexadecylphosphatidylethanolamine in the lamellar gel ($L_{\beta'}$) phase at a temperature of 68 °C and a pressure of 9.7 MPa (1400 psig). Following the rapid release of pressure to atmospheric the $L_{\beta'}$ phase transforms slowly into the lamellar liquid crystal (L_α) phase. The pressure perturbation is applied with the intention of producing a sudden phase disequilibrium followed by monitoring the system as it relaxes to its new equilibrium condition. Remarkably, the proportion of sample in the L_α phase grows linearly with time, taking 37 s to totally consume the $L_{\beta'}$ phase. The time dependencies of radius, peak intensity, and width of the powder diffraction ring of the low-angle (001) lamellar reflections were obtained from the movie by image processing. The concept of an "effective pressure" is introduced to account for the temperature variations that accompany the phase transition and to establish that the observed large transit time is indeed intrinsic to the sample and not due to heat exchange with the environment. The reverse transformation, L_α to $L_{\beta'}$, induced by a sudden jump from atmospheric pressure to 9.7 MPa, is complete in less than 13 s. These measurements represent a new approach for studying the kinetics of lipid phase transitions and for gaining insights into the mechanism of the lamellar order/disorder transition.

A fundamental characteristic of a lipid phase transition is its intrinsic transit time, the shortest possible time in which the sample can completely transform from one phase to another. To tackle the problem of measuring the transit time, various temperature-jump methods have been used in which a high-energy pulse is applied to a sample with the intent of instantaneously and uniformly raising its temperature by a measurable amount, ΔT, aiming to render the initial phase unstable and observing its relaxation to the new equilibrium condition. Achieving a rapid, uniform, and nondestructive increase in temperature in a controlled and reproducible

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