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Phenylalanyl-tRNA Synthetase and Isoleucyl-tRNA^{Phe}: A Possible Verification Mechanism for Aminoacyl-tRNA

(misaminoacylation/coding/recognition)

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ABSTRACT **The synthesis of isoleucyl-tRNA^{Phe} (*Escherichia coli*) proceeds at an appreciable rate under normal *in vitro* conditions in the presence of isoleucyl-tRNA synthetase (EC 6.1.1.5) from *E. coli*. The misacylated product is shown here to be hydrolyzed by highly purified phenylalanyl-tRNA synthetase from *E. coli*, with release of isoleucine and active tRNA^{Phe}. Thus, phenylalanyl-tRNA synthetase possesses a previously unrecognized activity, which deacylates a mistakenly acylated tRNA^{Phe}; the enzyme is inactive toward correctly matched aminoacyl tRNAs. Such a mechanism could serve to verify aminoacyl-tRNAs, deacylating those that are misacylated. Thus, a common generalization needs to be modified: an amino acid is not necessarily committed to a given (incorrect) anticodon when it is incorporated into aminoacyl-tRNA. It may be possible to correct it thereafter.**

I have shown previously that homogeneous isoleucyl-tRNA synthetase (EC 6.1.1.5) from *Escherichia coli* can catalyze the synthesis of Ile-tRNA^{Phe} (*E. coli*). Such isoleucine residues would be incorporated into polypeptides in response to phenylalanine codons (1).

Does this occur *in vivo*, and if not, how is it prevented? With Ile-tRNA^{Phe} as a probe, two previously unnoted mechanisms for insuring the correctness of the catalog of aminoacyl-tRNAs have been demonstrated. This paper describes the first of these, a hydrolytic deacylation of Ile-tRNA^{Phe} by purified phenylalanyl-tRNA synthetase.

MATERIALS AND METHODS

Transfer RNAs were, with one exception, purified isoacceptors. Ala-tRNA was synthesized from unfractionated tRNA (2): 0.53 nmol Ala/10 A_{260} tRNA. Purified Ile-tRNA^{Ile} (*E. coli*) has been described (3). The [^{3}H]Phe-tRNA^{Phe} was synthesized from ($\geq$ 78% pure) tRNA^{Phe} (*E. coli*) (4); the purified Phe-tRNA synthetase described below was used: the product carried 6.5 nmol of [^{3}H]Phe/10 A_{260} tRNA. Absorbance is measured after dilution in 0.01 M NaOH. Other aminoacyl-tRNAs were synthesized by use of mixed synthetases from *E. coli* MRE 600 (5); the purified tRNAs are from the tRNA purification project at Oak Ridge (4). The acylated tRNAs, isolated from the aminoacylation reaction by phenol extraction followed by alcohol precipitation in the presence of 0.5 M NaCl, had the following activities per 10 A_{260} units: [^{14}C]Met-tRNA$_f^{Met}$, 11.2 nmol; [^{14}C]Arg-tRNA^{Arg}, 8.9 nmol; [^{14}C]Val-tRNA^{Val}, 5.6 nmol. These aminoacyl tRNAs were readily deacylated in the presence of their respective synthetase, AMP, and PP_i.

Ile-tRNA^{Phe} was prepared under normal acylation conditions; purified Ile-tRNA synthetase (1) and 20% methanol were added: the two preparations of [^{14}C]Ile-tRNA^{Phe} used here had, per 10 A_{260} units, 2.7 nmol and 3.6 nmol Ile-tRNA. Phe-tRNA synthetase from *E. coli* B was purified by a modification of the method of Fangman and Neidhardt (6), which is itself a modification of the procedure of Conway, Lansford, and Shive (8). An additional step was added to the previous procedure (6): chromatography of the diethylaminoethyl (DEAE)-cellulose fraction on an hydroxylapatite (Biogel HTP, Biorad Laboratories) column (0.8 × 13 cm). This column was eluted with a linear gradient of 0.01–0.20 M potassium phosphate buffer, pH 6.8 (250 ml total volume), with 7 mM 2-mercaptoethanol and 10% glycerol. The flow rate was 15 ml/hr at 2°.

Treatment with nitrous acid has been described (3). The product used here is [^{14}C]hydroxymethylvaleryl-tRNA^{Phe} (HOMeVal-tRNA^{Phe}, from Ile-tRNA^{Phe}).

The reaction mixtures (100 μl) used to study the hydrolysis of Ile-tRNA^{Phe} duplicate our standard aminoacylation reaction mixture, except for the absence of 2 mM Mg·ATP. They contained 0.10 M sodium cacodylate (pH 7.0)–3 mM $MgCl_2$–1 mM dithiothreitol–50 μg/ml carboxymethyl-bovine serum albumin (7)–80 mM NH_4OAc–0.1 mM sodium salt of ethylene diaminetetraacetate.

Two assays were used to follow the disappearance of Ile-tRNA^{Phe}. Acid-precipitability was measured, on addition of 3 ml of 2 N HCl with 200 μg of yeast RNA carrier, followed by filtration on Whatman GF/C filters. If precise kinetics were needed, amino acid released was measured directly in the supernatant after addition of 0.10 ml carrier RNA (1 mg/ml) and 0.20 ml cetyltrimethylammoniumbromide (1 mg/ml). An aliquot of the supernatant (12,000 × g for 10 min) was assayed after complete dissolution in a water-compatible scintillant: 5 g of 2,5-diphenyloxazole, 100 ml of Bio-Solv solubilizer (Beckman formula BBS-3), and toluene to 1000 ml. Cetyltrimethylammoniumbromide, unlike the salt in neutralized acid supernatant fluids, is soluble in this scintillation fluid. Reconstruction experiments established that (*a*) this procedure precipitates aminoacyl-tRNA quantitatively, (*b*) cetyltrimethylammoniumbromide supernatants do not quench ^{14}C, (*c*) addition of carrier and cetyltrimethylammonium bromide stops the reaction of Ile-tRNA^{Phe} with Phe-tRNA synthetase, and (*d*) [^{14}C]isoleucine is recovered quantitatively in the supernatants.

The assay for contamination with RNase in Phe-tRNA

Abbreviation: HOMeVal, hydroxymethylvalerate or hydroxymethylvaleryl residue.

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TABLE 1. *Purification of Phe-tRNA synthetase from E. coli B*

Step number	Preparation	Total volume (ml)	Total activity (aminoacylation units)	Specific activity (units/mg)*	Reference
1	Lysate, 45 g *E. coli*	45	96,000	21.4	6
2	Supernatant, 200,000 × *g*, 2 hr	25	42,500	85.0	—
3	Redissolved pH 4.5 precipitate	5	18,500	206	6
4	Redissolved ammonium sulfate precipitate, 45–55% saturation	10	12,000	480	6
5	Pooled DEAE-column fractions	16.4	12,000	10,700	6
6	Hydroxylapatite column fractions	10.2	5,300	31,000	(see *Methods*)

* Protein estimation by a modified Lowry (9) procedure with bovine-serum albumin as standard.
† An aminoacylation unit transfers 1 nmol of amino acid to tRNA in 15 min at 37°.

synthetase was release of soluble radioactivity from [^{3}H]-poly(U) or [^{3}H]poly(A) (Miles Laboratories, 0.1 A_{260}/ml). Incubation for up to 130 min with 12 units of Phe-tRNA synthetase at 37° was performed under standard acylation conditions.

Other synthetases were assayed under the usual acylation conditions with the addition of the appropriate ^{14}C-labeled amino acids (Schwarz–Mann) and 5 A_{260} units of mixed tRNA from *E. coli* B.

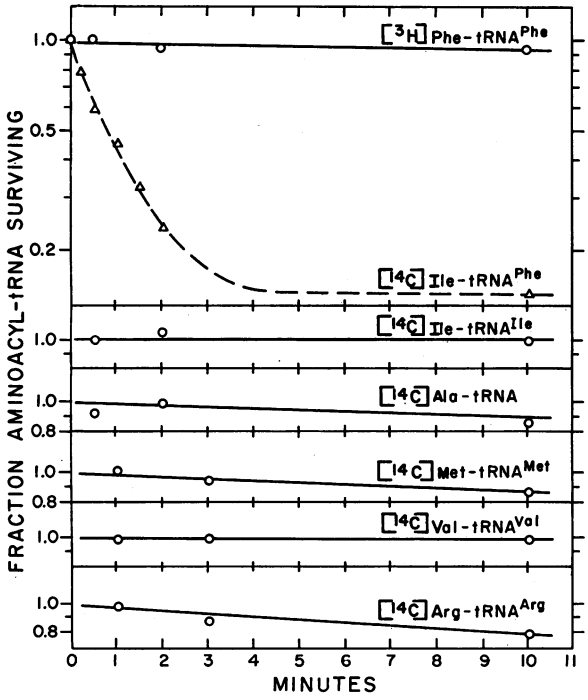

FIG. 1. The effects of purified Phe-tRNA synthetase on some aminoacyl-tRNAs. The reaction in the *top panel* contained, in 200 μl, 3.3 pmol [^{3}H]Phe-tRNA^{Phe}, 4.8 pmol [^{14}C]Ile-tRNA^{Phe}, and 0.6 aminoacylation units of Phe-tRNA synthetase. 40-μl Samples were withdrawn and precipitated with acid after intervals at 37°. The experiments in the other panels were done similarly.

Heat inactivations referred to in the text were at 42.5° in 0.01 M potassium phosphate (pH 6.8)–12.5% glycerol–1 mM dithiothreitol.

Thin-layer chromatography has been described (1). Deacylation for chromatography was not performed under standard deacylation conditions, but rather in 0.01 M Mg(OAc)$_2$ (pH 7) in order to minimize the disturbance of chromatography.

RESULTS

The purification of Phe-tRNA synthetase is outlined in Table 1; an overall purification of 1450-fold was achieved. An aliquot of this preparation, subjected to acrylamide gel electrophoresis in the presence of sodium dodecyl sulfate and 2-mercaptoethanol (10), gave only one major stained band; this band was located at the place expected of the individual polypeptide chains that comprise Phe-tRNA synthetase (11), and included about 70% of the total material.

Assays for fourteen other enzymes were conducted. Val-tRNA synthetase (EC 6.1.1.9) activity was present at $\leq$1/25,600 that for phenylalanine; for alanine, aspartic acid, arginine, glutamic acid, leucine, and lysine, the activity was <1/12,650 that for phenylalanine; for histidine, 1/2650; for threonine, 1/1600; and for glycine and isoleucine, 1/1000. These activities are all present in comparable amounts in the crude extract (e.g., see ref. 5) and thus, these results confirm the figure for overall purification. In addition, no release of radioactive nucleotide from [^{3}H]poly(U) or [^{3}H]-poly(A) was detected (see *Methods*). Expressed in the same units, RNase activity was therefore <1/1500 that of Phe-tRNA synthetase activity.

The essential observation of this work is presented in Fig. 1. Phe-tRNA synthetase catalyzes the rapid degradation of Ile-tRNA^{Phe} (*E. coli*), although Phe-tRNA^{Phe} in the same reaction is stable. Ile-tRNA^{Ile}, as well as several other aminoacyl-tRNAs do not decay at a rate exceeding that due to the intrinsic instability of the aminoacyl ester.

The lack of activity toward Phe-tRNA^{Phe}, present in the same reaction mixture as the Ile-tRNA^{Phe} (Fig. 1), rules out the possibility that this reaction depends on contamination by AMP and PP$_i$; that is, the degradation of the Ile-tRNA^{Phe} occurs simply by the reverse of the aminoacyl transfer re-

action:

aminoacyl-tRNA^{Phe} + AMP + PP_i → ATP + amino acid + tRNA^{Phe}.

Thus, the data of Fig. 1 suggest a new activity for Phe-tRNA synthetase, an activity which is not implied by its role in the catalysis of the Phe-tRNA^{Phe} synthesis.

The stability of Ile-tRNA^{Ile}, in contrast to Ile-tRNA^{Phe} (Fig. 1), indicates that the reaction requires more than an isoleucine residue; the stability of the six other aminoacyl-tRNAs besides Ile-tRNA^{Phe}, taken together, provides a compelling argument against a nonspecific deacylase or RNase (see below). The fact that the deacylase activity is limited to a derivative of tRNA^{Phe} is an argument that Phe-tRNA synthetase itself, and not a contaminant of the preparation, is responsible for the reaction.

What is the nature of the net reaction? Figs. 2 and 3 show that the overall reaction is a hydrolysis; the elements of water are inserted at the Ile-tRNA^{Phe} ester linkage.

If ATP and [^{3}H]phenylalanine are added to the reaction mixtures containing Ile-tRNA^{Phe} and Phe-tRNA synthetase, the release of [^{14}C]isoleucine occurs simultaneously with its replacement by [^{3}H]phenylalanine (Fig. 2). The two processes occur to equal extents and the final amount of [^{3}H]Phe-tRNA obtained is the same as the original phenylalanine-acceptor capacity of this preparation (6.5 nmol/10 A_{260}). Thus, since over half of the available chains for [^{3}H]phenylalanine were occupied by isoleucine at the beginning of the experiment, the tRNA^{Phe} released from isoleucine during incubation must have been capable of accepting phenylalanine. The same result has been obtained (data not shown) by starting with [^{14}C]Ile-tRNA^{Phe} treated with periodate, and performing the removal of isoleucine and reacylation with phenylalanine as separate, successive steps. Thus, Phe-tRNA synthetase, acting on Ile-tRNA^{Phe}, releases tRNA^{Phe}.

Fig. 3 shows the chromatographic behavior of the radiocarbon released from [^{14}C]Ile-tRNA^{Phe}. The slow migration of the markers suggests that, if even a nucleoside were attached to [^{14}C]isoleucine, a difference in chromatographic behavior would have been evident. The radioactivity at the origin in the control, incubated without enzyme, shows that an aminoacyl-nucleoside or -nucleotide linkage would have been stable. Thus, the product behaves as free [^{14}C]isoleucine. The same conclusion has been reached from an experiment demonstrating that the released radioactive isoleucine can be reincorporated into [^{14}C]Ile-tRNA^{Ile} (data not shown). Thus, the freed isoleucine must be in a form recognizable to Ile-tRNA synthetase as a substrate. I conclude that the net reaction catalyzed by Phe-tRNA synthetase in the presence of [^{14}C]Ile-tRNA^{Phe} is hydrolysis of the aminoacyl ester.

The kinetics of disappearance of [^{14}C]Ile-tRNA^{Phe}, shown in Fig. 2, suggest that there is a fraction (8–25%) of the radioactivity resistant to hydrolysis (see Fig. 1). This fraction is independent of the input concentration of [^{14}C]Ile-tRNA^{Phe}, and increases slowly with the age of a preparation. By hydrolysis to a limit with Phe-tRNA synthetase, the residual fraction may be isolated and studied. It has the following characteristics: (*a*) sensitivity to RNase, (*b*) sensitivity to 0.3 N NaOH, and (*c*) the same sensitivity to 1 M hydroxylamine (pH 7) as Ile-tRNA^{Ile}. It is, therefore, probably an Ile-tRNA. However, the resistant material is not removed when (*d*) more Phe-tRNA synthetase is added or (*e*) AMP and PP_i are added, in addition to Phe-tRNA synthetase. Less than 1 chain in 20,000 of this tRNA^{Phe} preparation is tRNA^{Ile} (1); further, the addition of purified Ile-tRNA synthetase, in addition to AMP and PP_i, does not remove this acid-precipitable radioactivity. Thus, the residual fraction is not Ile-tRNA^{Ile}, but is probably Ile-tRNA^{Phe} in which the tRNA has become inactivated. The inactivation probably is unrelated to misacylation; a similar fraction of [^{3}H]Phe-tRNA^{Phe} becomes insensitive to deacylation (Fig. 5, below).

During interaction with Phe-tRNA synthetase, isoleucine in Ile-tRNA^{Phe} may be near the normal site for phenylalanine. I have therefore attempted to get an indication of the role of this site in the hydrolysis of Ile-tRNA^{Phe} by measurement of the velocity of the reaction in the presence of normal substrates of Phe-tRNA synthetase; phenylalanine, ATP, AMP, and PP_i (Table 2). It appears that the reaction is quite resistant to inhibition by high concentrations of phenylala-

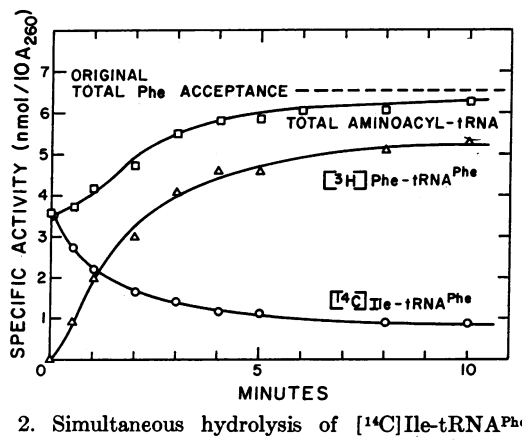

FIG. 2. Simultaneous hydrolysis of [^{14}C]Ile-tRNA^{Phe} and reacylation with [^{3}H]phenylalanine. The reaction contained, in 100 μl, 15.6 pmol [^{14}C]Ile-tRNA^{Phe} and 0.7 aminoacylation units of Phe-tRNA synthetase. ATP and [^{3}H]phenylalanine (1.65 Ci/nmol) were also present to a final concentration of 1.6 mM and 0.61 mM, respectively. Aliquots of 10 μl were acid-precipitated at intervals.

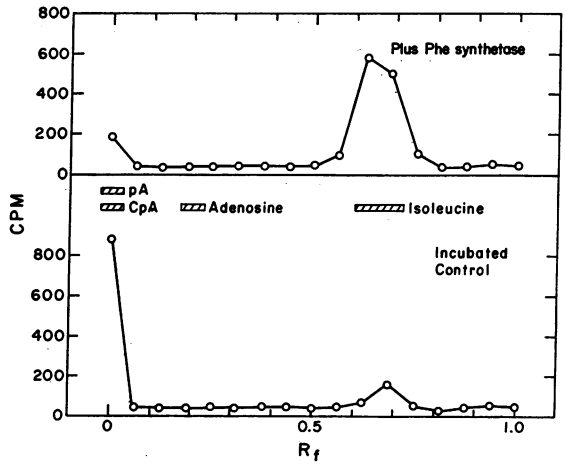

FIG. 3. A chromatographic identification of the radiocarbon released by Phe-tRNA synthetase. The control was incubated at 37° without enzyme. Each point is plotted at the center of the section of the thin-layer chromatogram, which was counted to obtain it. The position of the reference compounds is represented by *hatched bars;* the nucleosides and nucleotides were detected as fluorescence-quenched areas in ultraviolet light; the isoleucine marker, was a ninhydrin-positive spot.

TABLE 2. *Effects* of amino acids and nucleotides on the rate of Ile-tRNA^{Phe} hydrolysis*

Additions	Concentrations (mM)	% Uninhibited velocity
None	—	100
Isoleucine	0.7	103
Lysine	0.5	101
Phenylalanine	0.5	97
ATP + isoleucine	0.85 + 0.7	104
ATP + lysine	0.85 + 0.5	99
ATP + phenylalanine	0.85 + 0.5	83
ATP† + phenylalanine	1.7 + 1.0	66
ATP + phenylalanine	4.3 + 2.5	61
ATP + phenylalanine	8.5 + 5.0	54
AMP	0.8	100
AMP + PP_i	0.8 + 0.5	110

* Reactions contained 0.6 aminoacylation units of Phe-tRNA synthetase and 19 nM [^{14}C]Ile-tRNA^{Phe} in 100 μl. Uninhibited velocity is 1.52 pmol/min.

† The concentration of Mg^{2+} was maintained at 2 mM in excess of the ATP concentration; otherwise the reaction stops for lack of Mg^{2+}.

nine. Only the combination ATP plus phenylalanine is inhibitory, and even then, the inhibition increases slowly with concentration and may go to a limit of <100%. In addition, the reaction is not stimulated by AMP, and only slightly by AMP and PP_i together. The small effects observed correspond to effects to be expected from the synthesis of an aminoacyladenylate by Phe-tRNA synthetase. But the small magnitude of the inhibition by ATP and phenylalanine, and stimulation by AMP and PP_i, considered together, suggest that the usual phenylalanine site (i.e., that occupied by Phe-AMP) is not coextensive with the site of hydrolysis of Ile-tRNA^{Phe}.

Using the assay for release of soluble radioactivity (see *Methods*), I have measured the kinetic constants of the reaction. The results are shown in Fig. 4†. The K_m (Michaelis constant) is in the normal range for the interaction of a tRNA and its aminoacyl-tRNA synthetase. Since the Phe-tRNA synthetase added to the reactions of Fig. 4 was capable of transferring 7.3 pmol/min of phenylalanine to tRNA^{Phe} under these same conditions (see *Methods*), the maximal velocity for the hydrolysis of Ile-tRNA^{Phe} is close† to the maximal velocity for synthesis of Phe-tRNA^{Phe}. The new reaction is therefore not a quantitatively minor activity of Phe-tRNA synthetase.

Will Phe-tRNA synthetase remove any acyl group sufficiently unlike phenylalanine? To test this, I attempted to make [^{14}C]Ile-tRNA^{Phe} even more unlike Phe-tRNA^{Phe}; this was done by brief treatment with nitrous acid, which oxidatively deaminates the amino acid before a significant reaction occurs with the tRNA (3). After treatment of Ile-tRNA^{Phe} with nitrous acid, HOMeVal-tRNA^{Phe} is formed. The survival of the tRNA has been shown by three controls: (*a*) unacylated tRNA^{Phe} treated with nitrous acid still accepts 80% of the normal amount of phenylalanine, (*b*) the free tRNA^{Phe} chains in the preparation of [^{14}C]Ile-tRNA^{Phe} used still accept phenylalanine after treatment with nitrous acid, and (*c*) the tRNAs carrying the HOMeVal residue, which can be recovered after removal of HOMeVal at pH 10.4 in 0.05 M Na_2CO_3, are still acceptors of phenylalanine.

Fig. 5 shows that HOMeVal-tRNA^{Phe} is not hydrolyzed by Phe-tRNA synthetase, either with or without addition of AMP and PP_i. Under these same conditions, [^{3}H]Phe-tRNA^{Phe} is rapidly deacylated (in the presence of AMP and PP_i), as is untreated Ile-tRNA^{Phe}.

Thus, tRNA^{Phe} carrying an incorrect acyl group is not necessarily hydrolyzed; an intact α-NH_2 group is required. To put it another way, Phe-tRNA synthetase requires certain features of the acyl group besides the ester linkage in order to hydrolyze it. The absolute requirement for an α-NH_2 group is consistent with the proposal that the hydrolysis mechanism may have evolved to destroy incorrectly acylated tRNAs.

DISCUSSION

Phe-tRNA synthetase is the protein responsible for hydrolysis of Ile-tRNA^{Phe}: (*a*) the two activities remain together through a 1450-fold purification (Table 1), (*b*) the selectivity among tRNAs is that expected of Phe-tRNA synthetase (Fig. 1), (*c*) the slight effects of small molecule substrates are also in accord with this idea (Table 2), (*d*) the heat sensitivities of the two activities ($T_{1/2}$ = 30 min at 42.5°, see *Methods*) are the same, within experimental error, and (*e*) the hydrolytic and

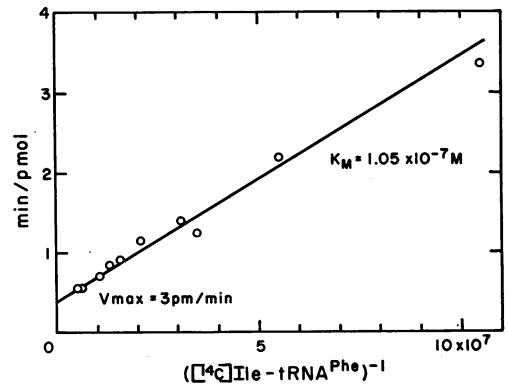

FIG. 4. Double-reciprocal kinetic plot for the hydrolysis of [^{14}C]Ile-tRNA^{Phe} by Phe-tRNA synthetase.† Reactions of 100 μl maintained at 37° contained 0.11 aminoacylation unit of Phe-tRNA synthetase; that is, sufficient Phe-tRNA synthetase to aminoacylate 7.3 pmol of tRNA^{Phe} per min. The cetyltrimethylammoniumbromide method was used to measure reaction velocities (see *Methods*).

† The values shown in Fig. 4 require correction. There is an intrinsic competitive inhibitor in these reactions in the form of tRNA^{Phe}, which is present because only about half the chains that could otherwise bear phenylalanine have been acylated with [^{14}C]isoleucine. Other experiments do show that added tRNA^{Phe} is inhibitory. Thus, these kinetics correspond to an inhibited reaction, in which the ratio of inhibitor to substrate is constant. This implies, if one assumes that $K_M \sim K_I$ and that $^1/_2$ tRNA^{Phe} carries isoleucine, that the corrected values are

$$K_M = \left\{1 + \frac{(I)}{(S)}\right\} K_{M,APP} = 0.21\ \mu M, \text{ and } V_{max} = \left\{1 + \frac{K_m(I)}{K_I(S)}\right\} V_{max,APP} = 6 \text{ pmol/min},$$

where quantities subscripted APP are those apparent in Fig. 4. These factor-of-two corrections do not affect the argument in the text.

Phe-tRNA synthetase activities move together in gel filtration (P200, BioRad Laboratories), suggesting the same large molecular weight of about 190,000.

The suggestion that hydrolysis may occur at another amino-acid site (Table 2 and Fig. 5) than that which is used for Phe-AMP is not unprecedented. When Ile-tRNA is bound to Ile-tRNA synthetase, isoleucine is not located at the same site it occupies in Ile-AMP (3). More direct information is needed, however, to define the locus and specificity of the hydrolysis reaction.

What is the purpose and generality of this type of activity? It has been shown that Ile-tRNA^{Phe} (*E. coli*) is synthesized by Ile-tRNA synthetase from *E. coli* under normal *in vitro* conditions, albeit at a maximal rate only several tenths of a percent that of Ile-tRNA^{Ile} synthesis (1). Quite possibly Phe-tRNA synthetase possesses the hydrolytic activity in order to correct this mistake in aminoacylation. The hydrolysis reaction would be an appealingly economical addition to the current account of the specificity of the code, since it exploits the known selectivity of the aminoacyl tRNA synthetases for tRNAs and amino acids and returns the tRNA and amino acid to the cell in usable form.

Schreier and Schimmel (12) have found that Ile-tRNA synthetase from *E. coli* B slowly hydrolyzes Ile-tRNA^{Ile} (50–100 times slower than the rate of Ile-tRNA^{Ile} synthesis). This reaction is also inhibited only partially by ATP and isoleucine, releases unaltered tRNA^{Ile}, and is specific to tRNA^{Ile}. The reaction, though slower when directed against a properly acylated tRNA, is probably the same as the one reported here, and thus Phe-tRNA synthetase may not be unique in this respect.

It is conceivable also that the destruction of Ile-tRNA synthetase (AMP-Val) in the presence of tRNA^{Ile}, discovered by Baldwin and Berg (18), occurs by formation of Val-tRNA^{Ile}, followed by the hydrolysis of the aminoacyl-tRNA linkage in this misacylated tRNA by the mechanism revealed in these studies.

Some previous experiments with tRNAs carrying unusual acyl groups that have shown no signs of destruction of misaminoacylated tRNA (13–17) have been performed in interspecific systems (13, 15–17). Thus, it is not clear that misaminoacylated tRNA (*E. coli*) could have been hydrolyzed by the corresponding rabbit (13, 15) or yeast (16, 17) enzymes, even if the relevant enzymes were present. In one experiment, however, Chapeville *et al.* (14) demonstrated that Ala-tRNA^{Cys}, chemically converted from Cys-tRNA^{Cys} (*E. coli*), was incorporated into oligopeptide as well as the original Cys-tRNA in a crude system composed entirely of *E. coli* components. I conclude that, while there are preliminary indications that the hydrolysis of aminoacyl-tRNAs may be a more general phenomenon in *E. coli*, it may not be universal. A more specific conclusion must await more specific experiments.

This is particularly true, since it is likely that published data are highly selected. Only successful cases of misaminoacylation and cases in which misaminoacylated tRNA survives to express its biological activity are reported. Attempts to synthesize or assay for misaminoacylated tRNAs must be conducted with highly purified synthetases in order to be at all conclusive.

Finally, perhaps a comment is in order about the general conclusions that emerge from this paper. Ile-tRNA synthetase, while quite selective among tRNAs, is not nearly so specific as has sometimes been supposed (1). We have found that it is capable of other misaminoacylations besides the synthesis of Ile-tRNA^{Phe} (Mertes, M., Peters, M., Mahoney, W. & Yarus, M., submitted to *J. Mol. Biol.*). Accordingly, novel mechanisms exist for ensuring the correctness of the catalogue of aminoacyl-tRNAs.

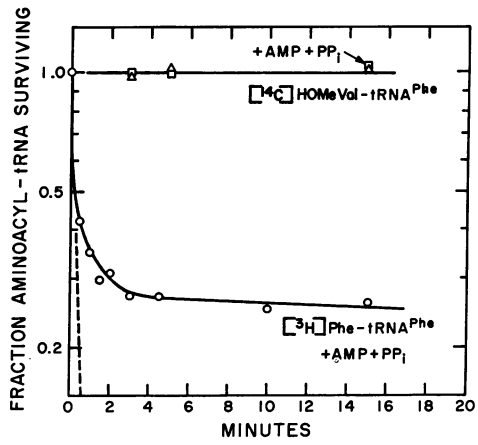

FIG 5. The resistance of [^{14}C]HoMeVal-tRNA^{Phe} to Phe-tRNA synthetase. Reactions of 100 μl contained 0.6 pmol [^{14}C]-HoMeVal-tRNA^{Phe} and 0.3 aminoacylation units of Phe-tRNA synthetase; *triangles* represent data obtained without other additions; *squares* represent data obtained when 0.4 mM AMP and 0.5 mM PP_i were also added. *Circles* represent an experiment in which 0.73 pmol of [^{3}H]Phe-tRNA^{Phe} replaced [^{14}C]Ile-tRNA^{Phe}. The *dashed line* represents the kinetics in this last case after subtraction of the resistant fraction.

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