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Phenylalanyl-tRNA Synthetase of Baker's Yeast. Modulation of Adenosine Triphosphate–Pyrophosphate Exchange by Transfer Ribonucleic Acid†

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ABSTRACT: Native and modified phenylalanine transfer ribonucleic acid ($tRNA^{Phe}$) can modulate phenylalanine-dependent adenosine triphosphate–inorganic [^{32}P]pyrophosphate (ATP–[^{32}P]PP_i) exchange activity via inhibition of adenylate synthesis. Inhibition is visualized if concentrations of L-phenylalanine, ATP, and pyrophosphate are subsaturating. In the proposed mechanism, $tRNA^{Phe}$ is a noncompetitive inhibitor at conditions where only one of the two active sites per molecule of enzyme is occupied by L-phenylalanine, ATP, and pyrophosphate. At saturating concentrations of these reactants, both active sites are occupied and, according to the model, inhibition is eliminated. Occupation by these reactants is assumed to follow homotropic negative cooperativity. The type of effects depends on modification of $tRNA^{Phe}$. Native $tRNA^{Phe}$, $tRNA^{Phe}_{2'\text{-dA}}$, and $tRNA^{Phe}_{oxi\text{-}red}$ are inhibitors, $tRNA^{Phe}$pCpC has no effect, and $tRNA^{Phe}_{ox}$ is an activator. Kinetics of activation by $tRNA^{Phe}_{ox}$ are slow, following the time course of Schiff base formation and subsequent reduction by added cyanoborohydride. Besides showing that a putative enzyme amino group is nonessential for substrate binding and adenylate synthesis, this result may suggest that an enzyme amino group could interact with the 3′-terminal adenyl group of cognate tRNA. In the case of asymmetrical occupation of the enzyme active sites by all of the small reactants ATP, L-phenylalanine, and pyrophosphate, the interaction with the amino group might trigger the observed noncompetitive inhibition of the pyrophosphate exchange by $tRNA^{Phe}$.

Yeast phenylalanine-tRNA synthetase has been demonstrated to bind L-phenylalanine in an anticooperative fashion to its two active sites (Berther et al., 1974; Fasiolo et al., 1977; Fasiolo & Fersht, 1978). The same enzyme from *Escherichia coli* was found to exhibit anticooperativity for both the small substrates, L-phenylalanine and ATP (Pimmer & Holler, 1979). Occupancy of a single site by one of these substrates and presumably also pyrophosphate may be expected to induce asymmetry within the enzyme molecule. An effect of asymmetry can manifest itself as a change in pyrophosphate exchange activity as is reported in the present investigation. If L-phenylalanine, ATP, and pyrophosphate each bind anticooperatively to the enzyme in the absence of the other substrates, it is of interest to see whether the other added substrates can modify this phenomenon. The experiment is to measure the saturation dependence of the pyrophosphate exchange for each substrate varied in the presence of saturating and nonsaturating concentrations of the other substrates. It will be shown that asymmetry is displayed in the presence of cognate tRNA and that asymmetry is eliminated after saturation by any of the substrates L-phenylalanine, ATP, and pyrophosphate.

Materials and Methods

Phenylalanyl-tRNA synthetase from baker's yeast (EC 6.1.1.20) was purified to homogeneity as described previously (Fasiolo & Ebel, 1974). $tRNA^{Phe}$ was purified in our laboratory from total brewer's yeast (Boehringer, Mannheim) by the countercurrent distribution technique. Na[^{32}P]PP_i was a product of the Radiochemical Center (Amersham). [^{14}C]Phenylalanine was from CEA (France). All other reagents were of highest commercially available purity.

ATP–PP_i Exchange Reaction. All the experiments described have been performed at 25 °C in a standard buffer containing 144 mM Tris-HCl, pH 7.8, 10 mM 2-mercaptoethanol, 10 mM $MgCl_2$, and 0.1 mM phenylmethanesulfonyl fluoride. Final concentrations for the ATP–PP_i exchange assay are specified in the figure legends. The initial rates of exchange

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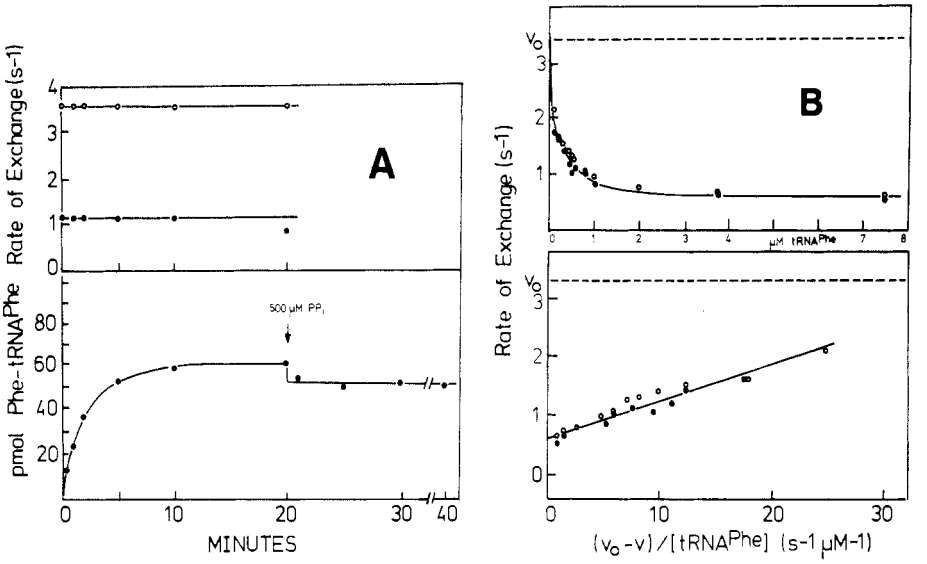

FIGURE 1: Pyrophosphate exchange activity in the absence (A, upper panel, O) and presence of 1 μM tRNA^{Phe} (A, upper panel, ●), aminoacylation in the absence and presence of added pyrophosphate (A, arrow in lower panel), and initial rate of pyrophosphate exchange as a function of tRNA^{Phe} concentration (B). In one case, exchange rates were measured immediately after addition of tRNA^{Phe} to the enzyme (B, O) and in the other case after a 40-min preincubation of enzyme and tRNA^{Phe} (B, O). Initial rates were used to analyze interaction constants according to eq 1 in the lower panel of (B). Experimental details are as follows. (A) Three separate reaction pools contained each 100 μM ATP, 10 mM $MgCl_2$, 144 mM Tris-HCl (pH 7.5), and 5.6 nM enzyme; one pool contained in addition only pyrophosphate (0.5 mM) and L-phenylalanine (0.1 mM) (O), and the second pool contained in addition L-phenylalanine (0.1 mM), tRNA^{Phe} (1 μM), and pyrophosphate (0.5 mM). [^{32}P]Pyrophosphate was added after variable preincubation times in order to measure exchange rates (●). A third pool contained in addition L-[^{14}C]phenylalanine (0.1 mM; 50 μCi/μmol) and tRNA^{Phe} but no pyrophosphate in order to measure the extent of aminoacylation (A, lower panel). (B) Two separate reaction pools contained 100 μM ATP, 100 μM L-phenylalanine, 10 mM $MgCl_2$, 144 mM Tris-HCl, and 5.6 nM enzyme. One contained 0.5 mM [^{32}P]pyrophosphate and tRNA^{Phe} in order to measure the rate of exchange immediately after the addition of the enzyme (O). The other pool was preincubated with enzyme and tRNA^{Phe} for 40 min before [^{32}P]pyrophosphate (0.5 mM) was added and exchange measured (●).

were measured by removing at 4, 8, and 12 min 80–100-μL aliquots from the assay solution and counting the ^{32}P radioactivity incorporated into ATP as previously described (Fasiolo & Fersht, 1978). Furthermore, the ATP–PP_i exchange assays were monitored after variable times of preincubation of the tRNA solution containing phenylalanine, ATP, and catalytic amounts of enzyme (0.2 mL). The exchange reaction was promoted by adding 50 μL of [^{32}P]PP_i solution. The final substrate concentrations, which are indicated in the figure legends, correspond to final concentrations in the isotopic exchange.

Aminoacylation of tRNA^{Phe}. The assay was performed in the standard buffer described above. The final concentrations of the substrates were 10 μM [^{14}C]phenylalanine, 2 mM ATP, 240 μM total tRNA, and 3 mM enzyme. After 1, 2, 3, and 5 min, 40-μL aliquots were spotted onto Whatman 3MM filter papers. The washed trichloroacetic acid precipitates of Phe-tRNA^{Phe} were counted by liquid scintillation.

Modified tRNA^{Phe}. tRNA^{Phe} (160 μM) which had an amino acid acceptance of 1.26 nmol (73%)/A_{260} unit was subjected to mild periodate oxidation (1 mM $NaIO_4$ for 5 min). After this treatment, the phenylalanine acceptance activity disappeared completely. Removal of the 3′-terminal adenosine and of the exposed 3′-phosphate group yields the tRNA^{Phe}pCpC[1] (Khym & Uziel, 1968). Exposure of tRNA^{Phe}pCpC to nucleotidyltransferase in the presence of either [^{3}H]ATP or 2′-[^{3}H]ATP ensured incorporation of 1.56 nmol of nucleotide/A_{260} unit of tRNA. The resulting tRNA^{Phe}pCpCp[^{3}H]A and tRNA^{Phe}pCpCp[^{3}H]2′-dA forms could respectively incorporate 1.26 and 0.05 nmol of phenylalanine/A_{260} unit of tRNA. Reaction of tRNA$^{Phe}_{ox}$ by sodium borohydride yielded the tRNA$^{Phe}_{oxi\text{-}red}$ form which incorporated 1.13 nmol of phenylalanine/A_{260} unit of tRNA (66%).

Schiff Base Formation between tRNA$^{Phe}_{ox}$ and Phenylalanyl-tRNA Synthetase. The covalent incorporation of oxidized tRNA^{Phe} into phenylalanyl-tRNA synthetase was measured as described by Baltzinger et al. (1979).

Results

Inhibition of ATP–[^{32}P]PP_i Exchange Activity by tRNA^{Phe}. When tRNA^{Phe} was added to an ATP–[^{32}P]PP_i exchanging mixture, the rate of exchange dropped from 3.6 to 1.2 s^{-1} (Figure 1A). Exchange rates after addition were independent of time. They did not correlate with the progress curve of phenylalanylation (Figure 1A). Results for freshly added tRNA^{Phe} and for Phe-tRNA^{Phe} synthesized in situ were identical (Figure 1). It was verified that added pyrophosphate at concentrations present in the exchange mixture had only a marginal effect on the equilibrium level of aminoacylation (10% less Phe-tRNA^{Phe} at 0.43 mM PP_i in comparison to zero added PP_i).

The depression of the exchange activity was a function of tRNA^{Phe} concentration (Figure 1B). The concentration dependence was hyperbolical and could be used to determine an inhibition constant for tRNA^{Phe} according to linearization of data points via eq 1 (Jacques & Blanquet, 1977), where v =

$$v = v_{tRNA} + K_{tRNA}(app)\frac{v_0 - v}{[tRNA]} \quad (1)$$

the observed exchange rate, v_0 = the observed exchange rate in the absence of tRNA, v_{tRNA} = the observed (or extrapolated) exchange rate in the presence of saturating tRNA, and K_{tRNA}(app) = the inhibition constant. Values for v_{tRNA} and

[1] Abbreviations used: EDTA, ethylenediaminetetraacetic acid; tRNA^{Phe}pCpC, tRNA^{Phe} lacking the 3′-terminal AMP; tRNA$^{Phe}_{2'\text{-}dA}$, tRNA^{Phe} with 3′-terminal 2′-deoxyadenosine; tRNA$^{Phe}_{ox}$, tRNA^{Phe} with the 3′-terminal *cis*-diol group oxidized by periodate to yield the dialdehyde moiety; tRNA$^{Phe}_{oxi\text{-}red}$, tRNA$^{Phe}_{ox}$ subsequently reduced by borohydride to two alcohol groups; Phe-tRNA^{Phe}, tRNA^{Phe} phenylalanylated at the 3′ terminus.

Table I: Kinetic Parameters of ATP-[^{32}P]PP_i Exchange

substrate varied (mM)	substrates fixed (mM)	$tRNA^{Phe}$ (μM)	v_{max} (s^{-1})	K_m (mM)
Phe (0.02-2)	subsaturating, ATP (1), PP_i (0.2)	none	46 ± 3	0.05 ± 0.01
		6	12 (I), 46 ± 6 (II)	0.05 (I), 0.5 ± 0.1 (II)
	saturating, ATP (4), PP_i (2)	none	56 ± 6	0.03 ± 0.01
		1	58 ± 6	0.06 ± 0.01
ATP (0.1-4)	subsaturating, Phe (0.03), PP_i (0.2)	none	62 ± 4	1.0 ± 0.1
		6	8 ± 2 (I), 44 (II)	0.6 ± 0.2 (I), 9 (II)
	saturating, Phe (2), PP_i (2)	none	46 ± 2	0.8 ± 0.1
		6	58 ± 8	1.1 ± 0.2
PP_i (0.06-2)	subsaturating, Phe (0.03), ATP (1)	none	24 ± 4	0.05 ± 0.03
		6	6 ± 1 (I), 24 (II)	0.09 ± 0.01 (I), 6 (II)
	saturating, Phe (2), ATP (4)	none	31 ± 3	0.08 ± 0.01
		6	21 ± 3	0.20 ± 0.03

K_{tRNA}(app) are determined from intercept and slope, respectively. In the case of Figure 1B, they were $v_{tRNA}/v_0 = 0.2$ and K_{tRNA}(app) = 0.15 μM.

Effects of $tRNA^{Phe}$ on Kinetic Parameters of the ATP–[^{32}P]PP_i Exchange. Exchange activity was measured as a function of one of the reactants (Phe, ATP, PP_i) at varied concentrations and in the absence or presence of saturating concentrations of $tRNA^{Phe}$ (>1 μM). In one set of experiments, the fixed concentrations of the small reactants are at subsaturating concentrations, in another set at almost saturating concentrations. Representative concentration dependencies are shown in Figure 2. Results in terms of kinetic parameters are listed in Table I.

Within limits of experimental accuracy, the following conclusions may be drawn from Figure 2 and Table I. (1) In the concentration range investigated, Eadie (1942) plots were linear in the absence of tRNA. (2) In the presence of $tRNA^{Phe}$, Eadie plots were biphasic in the presence of low concentrations of the fixed substrates and monophasic in the presence of high concentrations. (3) At subsaturating concentrations and tRNA present, values of Michaelis–Menten constants of the low concentration phase of varied substrate were the same as those measured in the absence of tRNA. (4) Values of Michaelis–Menten constants at high concentrations of substrate varied (subsaturating concentrations of other substrates and tRNA present) were 10–15-fold higher than those of the low concentration phase of the varied substrate. An exception was pyrophosphate, with an approximately 90-fold higher value for the high concentration phase. (5) Maximal reaction rates of high concentration phases (subsaturating concentrations of fixed substrates and tRNA present) were 4–6 times the maximal rates of the low concentration phases of the varied substrate. Furthermore, they were the same (within experimental limits) as those measured in the absence of tRNA. (6) At (almost) saturating concentrations of fixed substrates and tRNA present, Eadie plots for the varied substrate became linear. Values of Michaelis–Menten constants and of maximal reaction rates became (almost) identical with those measured in the absence of tRNA.

Effects after Ring Opening as in $tRNA^{Phe}_{oxi\text{-}red}$ and $tRNA^{Phe}_{ox}$. Addition of $tRNA^{Phe}_{oxi\text{-}red}$ to an ATP–[^{32}P]PP_i exchanging mixture caused a time-dependent decrease of activity (Figure 3A). Kinetics did not follow the level of aminoacylation of

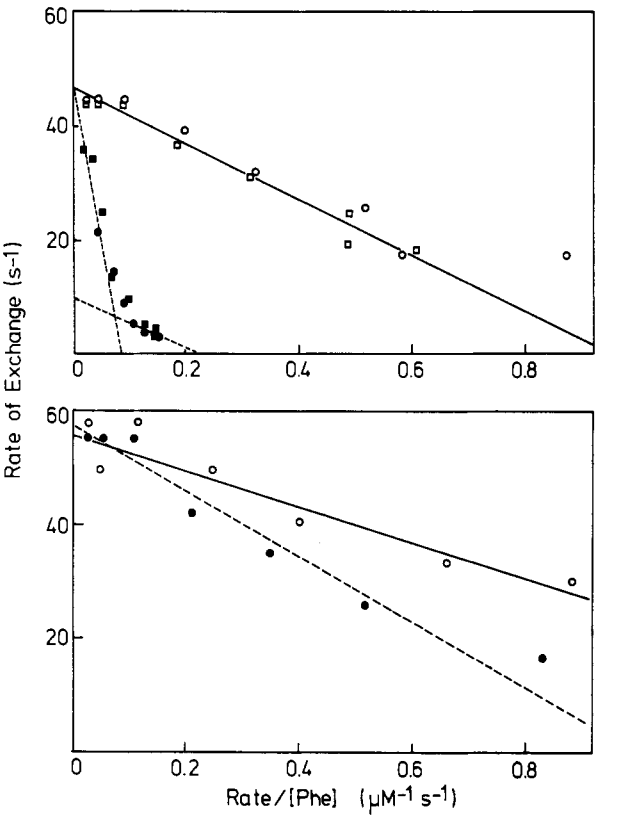

FIGURE 2: Rate of ATP–[^{32}P]PP_i exchange as a function of L-phenylalanine concentration at saturating (lower) and nonsaturating (upper) concentrations of ATP and pyrophosphate. Conditions were 0.144 M Tris-HCl buffer (pH 7.5), 10 mM $MgCl_2$, 6 nM phenylalanyl-tRNA synthetase, and 1 mM ATP and 0.2 mM pyrophosphate with 6 μM $tRNA^{Phe}$ (upper, filled symbols) or without $tRNA^{Phe}$ (upper, open symbols) or 4 mM ATP (as MgATP), 2 mM pyrophosphate, and 1 μM $tRNA^{Phe}$ (lower, filled circles) or without $tRNA^{Phe}$ (lower, open circles). Circles and squares in the upper panel refer to two separate experiments.

the same tRNA (Figure 3A). The slow dependence is in contrast with the instantaneous inhibition by native $tRNA^{Phe}$.

Addition of saturating amounts of $tRNA^{Phe}_{ox}$ (6–8 μM) to the reaction mixture caused the rate of exchange to increase (Figure 3B). Similarly as observed for $tRNA^{Phe}_{oxi\text{-}red}$, this was a time-dependent effect. Initial rates of the exchange (observed during the small lag phase in Figure 3B) were approximately 20% below rates observed in the absence of modified tRNA. Effects of $tRNA^{Phe}_{ox}$ on aminoacylation activity indicated competitive inhibition with respect to varied $tRNA^{Phe}$ concentration (K_i = 0.23 μM). We conclude that $tRNA^{Phe}_{ox}$, although binding to the enzyme, is less inhibitory than the other forms of $tRNA^{Phe}$ but is an activator as a result of prolonged incubation.

Aminoacylation of $tRNA^{Phe}$ was repeated at conditions, where the incubation mixture of enzyme and $tRNA^{Phe}_{ox}$ (both in the micromolar range) had been 1000-fold diluted into the assay (Figure 3C). Under this condition, which avoided competitive inhibition by $tRNA^{Phe}_{ox}$ in the reaction mixture, slow kinetics of inhibition of aminoacylation activity were observed (Figure 3C). The kinetics seemed to coincide with those of activation of the exchange activity also at dilution condition (Figure 3C). A following experiment established the correlation between inhibition and stoichiometry of Schiff base formation (Figure 3D) that is known to occur between an amino group of the enzyme and $tRNA^{Phe}_{ox}$ (Baltzinger et al., 1979). It is seen from Figure 3D that both inhibition of aminoacylation and activation of pyrophosphate exchange follow the incorporation of two covalently bound tRNA

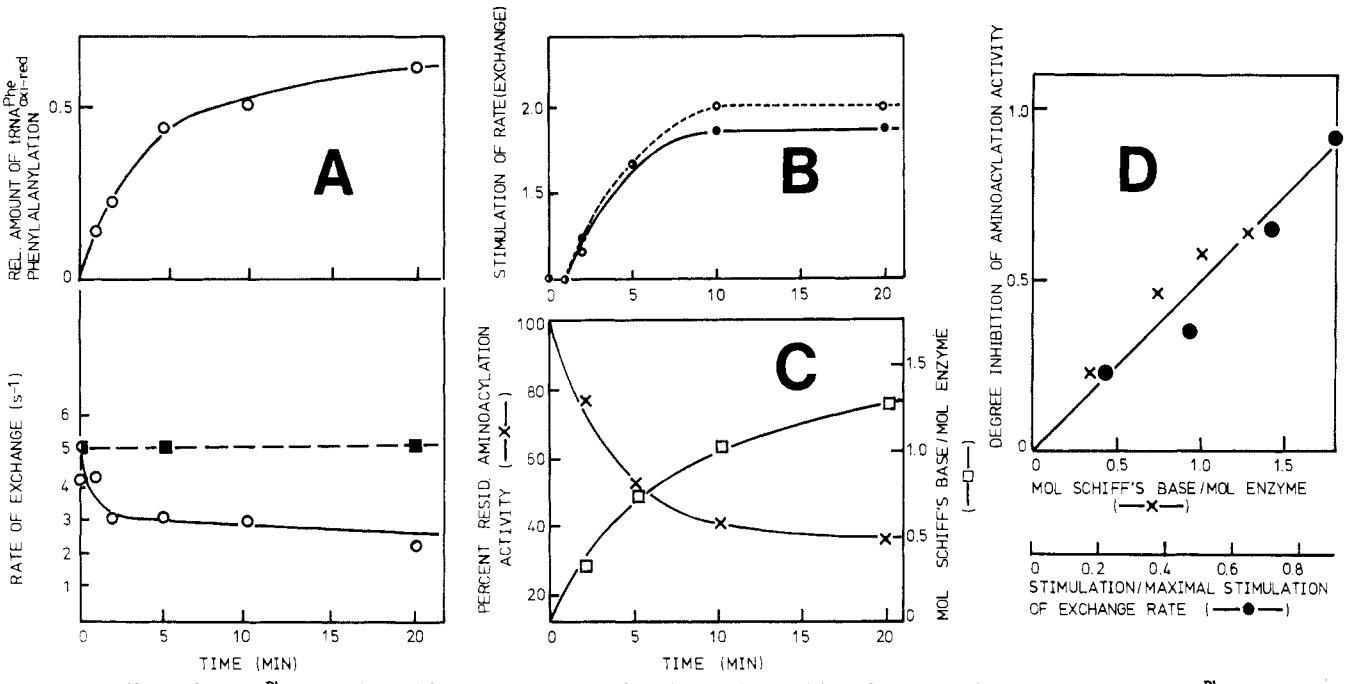

FIGURE 3: Effects of $tRNA^{Phe}$ derivatives with open 3'-terminal adenosine. (A) Inhibition of pyrophosphate exchange by $tRNA^{Phe}_{oxi-red}$: (upper) time dependence of aminoacylation of this tRNA; (lower) time dependence of inhibition of pyrophosphate exchange activity. (■) Exchange rate in the absence of tRNA; 0.144 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 0.1 mM ATP, 0.2 mM PP_i, 0.1 mM [^{14}C]Phe, 11 nM enzyme, and 7.3 μM $tRNA^{Phe}_{oxi-red}$. (B) Stimulation of pyrophosphate exchange by $tRNA^{Phe}_{ox}$ (6 μM) after cyanoborohydride reduction. (○) Nonsaturating substrate concentrations, 0.1 mM ATP, 0.4 mM PP_i and 0.1 mM Phe; (●) saturating substrate concentrations, 2 mM ATP, 4 mM PP_i, 2 mM Phe, 10 mM free $MgCl_2$, and 10 nM enzyme. (C) Correlation of Schiff base formation and inhibition of aminoacylation activity. The Schiff base was formed in the presence of 0.144 M Tris-HCl (pH 7.8), 0.1 mM phenylmethanesulfonyl fluoride, 5 mM cyanoborohydride, 10 mM $MgCl_2$, 0.1 mM ATP, 0.1 mM Phe, 3 μM enzyme, and 7.2 μM $tRNA^{Phe}_{ox}$. At the times indicated, 2-μL samples were drawn and diluted 1000-fold into the aminoacylation reaction mixture. (D) Correlation between Schiff base formation, degree of inhibition of aminoacylation activity, and stimulation of pyrophosphate exchange activity. The reaction mixture was the same as under (C) except that 0.42 mM PP_i had been added and enzyme was 10 nM. Aliquots were drawn as a function of time and assayed for pyrophosphate exchange and aminoacylation activity, respectively.

molecules. This correlation is considered as evidence that activation is a consequence of Schiff base formation. The conclusion is supported by effects of pH and concentration of 2-mercaptoethanol on the kinetics of appearance of the pyrophosphate exchange activation (results not shown), in agreement with high pH favoring Schiff base formation and the presence of 2-mercaptoethanol disfavoring it.

Effects of Other Modified tRNA. $tRNA^{Phe}_{2'-dA}$ inhibits the exchange activity in a fashion independent of time similarly as did native $tRNA^{Phe}$ (data not shown). Evaluation of parameters according to eq 1 leads to $v_{tRNA}/v_0 = 0.1$ and $K_{tRNA}(app) = 0.06$ μM. $tRNA^{Phe}$pCpC (at concentrations up to 5 μM) and $tRNA^{Val}$ (1 μM) were without effect on the exchange activity.

Discussion

Toward a Kinetic Model To Account for $tRNA^{Phe}$ Inhibition. Modulation of ATP–[^{32}P]PP_i exchange activity by $tRNA^{Phe}$ as manifested in biphasic concentration dependence for L-phenylalanine, ATP, or PP_i requires that concentrations of fixed substrates are subsaturating (Figure 2). Inhibition and biphasic behavior are overcome in the presence of saturating concentrations of fixed substrates. These observations will be explained on the basis of homotropic negative cooperativity (anticooperativity among alike ligands) and tRNA-induced (conformational) modulation of the active site as follows.

At first, the following naïve calculation will be used to estimate the degree of inhibition expected by the fact that tRNA acylation competes with pyrophosphorolysis in the consumption of the adenylate intermediate. Assume apparent steady-state rate constants k_s, k_p, and k_a for adenylate synthesis, pyrophosphorolysis, and aminoacylation, respectively, at a given set of concentrations of ATP, PP_i, Phe, and $tRNA^{Phe}$ at chemical equilibrium. In order to simplify calculation, the above rate constants are thought to be dependent on substrate concentrations such that each set of ATP and Phe concentration has its own k_s, each set of PP_i concentration its k_p, and each set of $tRNA^{Phe}$ concentration its k_a. Coupling between binding sites of different substrates is assumed to be negligible. With these assumptions, the equilibrium concentration of enzyme·adenylate is [E·Phe~AMP] = k_s/k_p in the absence and $k_s/(k_p + k_a)$ in the presence of $tRNA^{Phe}$ (and aminoacylation). Since the initial rate of ATP–[^{32}P]PP_i exchange is $v = k_p$[E·Phe~AMP], one obtains $v_0 = k_s$ in the absence and $v = k_p k_s/(k_p + k_a)$ in the presence of $tRNA^{Phe}$ (and aminoacylation). In a first approximation, we can express the concentration dependence for pyrophosphorolysis by $k_p = k_p'[PP_i]/(K_{PP} + [PP_i])$, where k_p' is the (true) rate constant. Values of k_p' have been reported to be in the range 30–1000 s^{-1} (Holler, 1978). Under the conditions of inhibition (Figure 2, upper), pyrophosphate (0.2 mM) is almost saturating (K_m = 50 μM); thus $k_p \cong k_p'$. In order to account for competition by tRNA aminoacylation, a steady-state rate constant ($tRNA^{Phe}$ is saturating) $k_a = 6$ s^{-1} is assumed (Fasiolo & Fersht, 1978). Initial rates of ATP–[^{32}P]PP_i exchange in the presence of steady-state tRNA aminoacylation are then 80%–99% k_s, i.e., almost within experimental error lower than initial rates in the absence of tRNA. The observed effect for $tRNA^{Phe}$ in Figure 2 (upper) and Table I is much larger and, hence, cannot be accounted for by "kinetic" competition.

It has been shown that binding of $tRNA^{Phe}$ to enzyme·phenylalanyladenylate stimulates hydrolysis of the intermediate (Baltzinger & Remy, 1977). For reasons similar to those above, hydrolysis should be rapid compared to pyro-

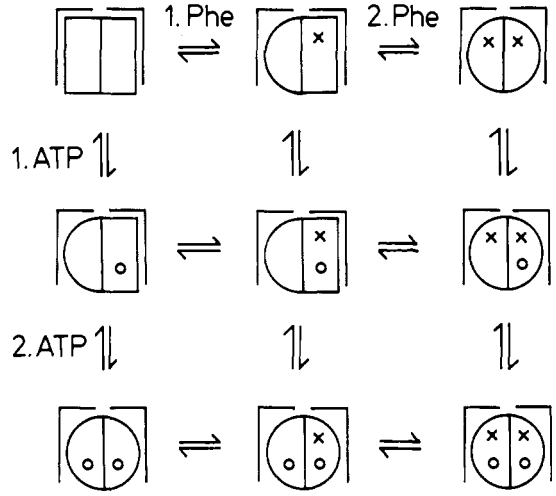

FIGURE 4: Minimal model to account for substrate-dependent inhibition of ATP–[^{32}P]PP_i exchange by $tRNA^{Phe}$. The model is illustrated for substrates ATP and L-phenylalanine. The presence of pyrophosphate would require a third dimension. It is assumed that substrates bind to the active sites of the enzyme in an anticooperative fashion. $tRNA^{Phe}$ is always bound at both active sites. The complex in the center has low exchange activity and is seen by the low concentration phase in Figure 2 (upper) in the presence of subsaturating concentrations of fixed substrates. Symbols refer to enzyme in different symmetrical states (two half-circles connected or two rectangles connected are symmetrical with respect to exchange activity; see text) or in asymmetrical state (half-circle connected to rectangle). ⌐ = $tRNA^{Phe}$, × = Phe, ○ = ATP, respectively.

phosphorolysis and large amounts of AMP should be produced besides the amount due to phenylalanylation of $tRNA^{Phe}$. This has not been observed, ruling out tRNA-induced hydrolysis as a possible explanation (F. Fasiolo and A. R. Fersht, unpublished results).

We can conclude that $tRNA^{Phe}$ behaves as a noncompetitive inhibitor of the exchange reaction at low concentrations of substrates. Inhibition disappears by increasing the concentration of the varied substrate (Figure 2, upper, second phase) or by applying saturating concentrations of the fixed substrates (Figure 2, lower). What is the mechanism by which saturation with substrates eliminates tRNA inhibition? The model in Figure 4 depicts a possibility taking into account the $(\alpha\beta)_2$ type of subunit structure (Fasiolo et al., 1970; Schmidt et al., 1971), the existence of two active sites (Fasiolo et al., 1974), and homotropic negative cooperativity of binding of small substrates (Fasiolo et al., 1974, 1977; Berther et al., 1974; Fasiolo & Fersht, 1978). One can assume that the active sites have special conformations depending on whether a single active site (asymmetrical case) or both active sites (symmetrical case) are occupied. The characteristic points are the following: (1) allosterism is expressed via pyrophosphate activity, (2) removal of $tRNA^{Phe}$ inhibition is independent of the kind of substrate, suggesting a general trigger mechanism, and (3) this trigger is inferred by complexation with $tRNA^{Phe}$. A conformational change that might be responsible as a trigger has been observed in terms of a contraction of the enzyme (Fasiolo et al., 1974; Pilz et al., 1979; Ehrlich et al., 1980; Lefevre et al., 1980). More information is required in support and elucidation of the model.

Nature of the Inhibition. Noncompetitive inhibition by $tRNA^{Phe}$ of the ATP–[^{32}P]PP_i exchange activity occurs at the level of the asymmetrical conformation of the enzyme in Figure 4. Heterotropic negative cooperativity (between different ligands) is not considered. It is rather the rate constant of adenylate synthesis, which is decreased by the presence of $tRNA^{Phe}$ under conditions of simultaneous asymmetrical occupation of the two active sites by all small substrates.

The structural requirements for the tRNA shed light on the primary interaction of the nucleic acid that triggers the observed inhibition. (1) Under experimental conditions, $tRNA^{Phe}$pCpC binds efficiently to the synthetase (Krauss et al., 1979; von der Haar & Gaertner, 1975). The absence of effects suggests that the 3′-terminal pA is responsible for triggering the allosteric inhibition by native $tRNA^{Phe}$. (2) An intact ribose seems not be required since $tRNA^{Phe}_{2'\text{-dA}}$ and $tRNA^{Phe}_{oxi\text{-}red}$ are inhibitors. The slowness of inhibition by $tRNA^{Phe}_{oxi\text{-}red}$, however, seems to reflect that the sugar ring is no longer closed. (3) Schiff base formation between the enzyme and $tRNA^{Phe}_{ox}$ reveals an enzyme amino group, probably a lysyl side chain, to be involved in an activation of the exchange activity. The kinetics of activation seem to reflect those of the chemical event but could as well follow a structural rearrangement of the enzyme·$tRNA^{Phe}_{ox}$ complex followed by a comparably rapid Schiff base reaction. If such a rearrangement is involved in the time dependence of inhibition by $tRNA^{Phe}_{oxi\text{-}red}$, similar slow rearrangement kinetics could be responsible for the observed lack of noncompetitive inhibition in the case of $tRNA^{Phe}_{ox}$.

The enzyme·tRNA derivative obtained from the Schiff base adduct by cyanoborohydride reduction might still have the tRNA moiety in exactly the same positions as native tRNA fixed to the enzyme, the only difference being the covalent attachment to the enzyme amino group. It could be speculated that this group is the key to the noncompetitive inhibition in the case of native $tRNA^{Phe}$. The amino group needs to be free in order to provoke inhibition by probably being in contact with the 3′-terminal pA since $tRNA^{Phe}$pCpC is ineffective as an inhibitor. Whether a change in the state of ionization or a salt bridge with the terminal phosphate is involved remains to be established. The fact that Schiff base formation (and subsequent cyanoborohydride reduction) does not inhibit but rather activates the exchange activity establishes that the amino group is neither required for binding of the small substrates L-phenylalanine, ATP, and pyrophosphate nor for the synthesis of adenylate.

Other Aminoacyl-tRNA Synthetases. Inhibition of ATP–[^{32}P]PP_i exchange activity by cognate tRNA has been reported for the methionine-specific system of *E. coli* (Jacques & Blanquet, 1977). In accordance with what has been observed here, binding of $tRNA^{Met}$ acts anticooperatively not on the formation of enzyme complexes with L-methionine and ATP but rather on the rate of adenylate synthesis. The inhibition of the exchange activity due to methionylation of tRNA is, however, not mirrored in the yeast phenylalanine specific system. A possible explanation is that Phe-$tRNA^{Phe}$, in contrast to Met-$tRNA^{Met}$, cannot interact with the ATP–[^{32}P]PP_i exchanging site (a possible consequence of half-of-the-sites reactivity).

An inhibition of the exchange activity has been observed for the *E. coli* phenylalanyl-tRNA synthetase (Santi et al., 1971). Inhibition by $tRNA^{Phe}_{ox}$ was indistinguishable from that by native $tRNA^{Phe}$. Heterotropic negative cooperativity as well as inhibition of adenylate synthesis at suboptimal Mg^{2+} concentration has been reported (Pimmer & Holler, 1979). An absolute requirement of the ATP–[^{32}P]PP_i exchange for tRNA is found for the arginine (Mitra & Mehler, 1967; Fersht et al., 1978) and the glutamine specific systems (Deutscher, 1967; Kern et al., 1979).

From these and other results, the general picture emerges that cognate tRNA induces conformational changes of the corresponding synthetase. These changes lead to phenomena

of positive and negative cooperativity between the tRNA and the small substrates as well as to inhibition or activation of the kinetics of adenylate synthesis. They are almost certainly involved in as yet unrecognized mechanisms of half-of-the-sites mechanisms (Jakes & Fersht, 1975; Fasiolo et al., 1977). The results presented here confirm and extend previous conclusions by von der Haar & Gaertner (1975) on the role of the tRNA amino acid acceptor end in aminoacylation kinetics.

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Tropomyosin Stability: Assignment of Thermally Induced Conformational Transitions to Separate Regions of the Molecule†

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ABSTRACT: Tropomyosin was prepared from rabbit skeletal muscle and studied in a differential scanning calorimeter. The characteristics of the observed endotherms were studied as a function of pH, salt concentration, oxidation state of Cys-190, and concentration of the divalent metal ions Ca^{2+} and Mg^{2+}. The large shifts observed for the T_m values of the components of the endotherms with changing pH and salt concentration are consistent with electrostatic effects being an important determinant of the structural stability of tropomyosin. For reduced tropomyosin or tropomyosin blocked with *N*-ethylmaleimide, two endotherms were observed with T_m values of 41.5 and 52.5 °C at neutral pH in a low-salt buffer. For tropomyosin containing a disulfide link at Cys-190, two endotherms were observed with T_m values of 32 and 52 °C under the same conditions. The endotherm at 52 °C contains contributions from conformational transitions in two independent structural regions. An analysis of the heat-capacity profiles for the two large cyanogen bromide peptides, CN1A and CN1B, enabled the assignment of two components of the endotherms to structural transitions in the C-terminal region which includes Cys-190 and in the N-terminal region. Calcium and magnesium ions in the 1–10 mM range increased the stability of several of the regions of the structure, presumably by binding to localized areas of excess negative charge. Unfolding of tropomyosin in the 20–70 °C range is a multistep process and occurs with an average enthalpy of 4 cal g^{-1}.

The calcium-activated switch of skeletal muscle functions through induced conformational changes which act as signals modulating the interactions between the constituent molecules. Calcium binding to troponin causes a conformational signal to be conveyed to tropomyosin. By alteration of its interaction with actin, tropomyosin allows actin–myosin interaction and tension generation. Although there is some controversy concerning the exact molecular mechanism of calcium activation in skeletal muscle, it is clear that tropomyosin is central to the transmission of the signal initiated by the binding of calcium to troponin. Thus, some knowledge of the conformational states available to tropomyosin should assist us in determining how the intrinsic properties of this structure dictate its function in the calcium switch.

Tropomyosin is an α-helical coiled-coil protein with two identical ($\alpha\alpha$) or very closely related ($\alpha\beta$) subunits of known sequence, each consisting of 284 amino acid residues (Sodek et al., 1978; Stone & Smillie, 1978; Mak et al., 1979). In rabbit skeletal muscle, the ratio of α to β subunits is ~3.5:1 while cardiac muscle contains only the α subunit. The α-

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