Morrison, M., & Hultquist, D. E. (1963) *J. Biol. Chem. 238*, 2847–2849.
Morrison, M., & Bayse, S. B. (1970) *Biochemistry 9*, 2995–3000.
Pon, C. L., & Gualerzi, C. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 4950–4954.
Pon, C. L., & Gualerzi, C. (1976) *Biochemistry 15*, 804–811.
Pon, C. L., & Gualerzi, C. (1979) *Methods Enzymol. 60*, 230–239.
Pon, C. L., Brimacombe, R., & Gualerzi, C. (1977) *Biochemistry 16*, 5681–5686.
Pon, C. L., Wittmann-Liebold, B., & Gualerzi, C. (1979) *FEBS Lett. 101*, 157–160.
Sabol, S., Sillero, M. A. G., Iwasaki, K., & Ochoa, S. (1970) *Nature (London) 228*, 1269–1273.
Seeman, N. C., Rosenberg, J. M., & Rich, A. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 804–808.
Studier, F. W. (1973) *J. Mol. Biol. 79*, 237–248.
Wickstrom, E. (1974) *Biochim. Biophys. Acta 349*, 125–130.
Yaguchi, M., Wittmann, H. G., Câbezon, T., De Wilde, M., Villarroel, R., Herzog, A., & Bollen, A. (1975) *Mol. Gen. Genet. 142*, 35–43.

A Novel Enzymatic Activity of Phenylalanyl Transfer Ribonucleic Acid Synthetase from Baker's Yeast: Zinc Ion Induced Transfer Ribonucleic Acid Independent Hydrolysis of Adenosine Triphosphate†

Gabor L. Igloi, Friedrich von der Haar, and Friedrich Cramer*

ABSTRACT: Phenylalanyl-tRNA synthetase from baker's yeast in the presence of phenylalanine or other amino acids misactivated by the enzyme, ATP, and low concentrations of Zn^{2+} is able to hydrolyze ATP to AMP and PP_i very efficiently. After dialysis of the enzyme against ethylenediaminetetraacetic acid (EDTA), this amino acid dependent but $tRNA^{Phe}$-independent hydrolysis is suppressed to negligible levels. The ATP hydrolysis can be restored by the addition of Zn^{2+} to the EDTA-dialyzed enzyme. During aminoacylation of $tRNA^{Phe}$ the Zn^{2+}-induced ATP hydrolysis parallels the aminoacylation reaction, leading to nonstoichiometric production of AMP. Mechanistically, we conclude that Zn^{2+} can be bound to phenylalanyl-tRNA synthetase and can influence the stability of ATP if an activatable amino acid is present. The influence of Zn^{2+}, if any, on the aminoacylation of $tRNA^{Phe}$ is not known. In practice, this side reaction is of the utmost importance in all cases in which the fate of ATP during aminoacylation is followed, especially if the stoichiometry of ATP consumption in relation to Phe-$tRNA^{Phe}$ formation has to be determined.

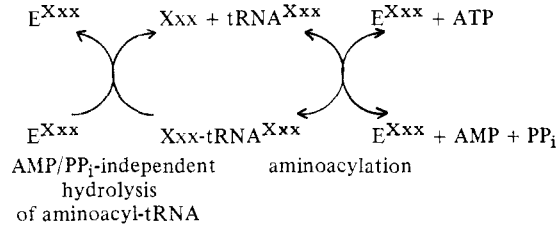
ATP is the essential energy source during the aminoacylation of tRNA by aminoacyl-tRNA synthetases. According to the generally accepted reaction sequence (eq 1; Xxx is the amino acid and E^{Xxx} is the corresponding aminoacyl-tRNA synthetase), one tRNA should be aminoacylated for each ATP consumed.

$$Xxx + ATP + E^{Xxx} \rightleftharpoons [E^{Xxx}\cdot AMP\text{-}Xxx] + PP_i \quad (1)$$

$$[E^{Xxx}\cdot AMP\text{-}Xxx] + tRNA \rightleftharpoons Xxx\text{-}tRNA + E^{Xxx} + AMP$$

The quantitation of this process and the possibility of editing or proofreading processes acting on the product (which would release free tRNA and thereby decrease the stoichiometry) have recently been the subject of some comment (Hopfield et al., 1976; Mulvey & Fersht, 1977). Whereas Hopfield et al. (1976) found a value of 0.65 Ile-$tRNA^{Ile}$/ATP, Mulvey & Fersht (1977) obtained a value of 0.99 for the same process. One adequate explanation of this discrepancy (involving the use of excess ATP over tRNA and thus including in the calculation the contribution of ATP involved in the synthesis of E·Ile-AMP after complete aminoacylation of tRNA) has been given by Mulvey & Fersht (1977). Nevertheless, the importance of a determination of ATP consumption during the overall aminoacylation process (Igloi et al., 1979) makes it imperative for one to be confident that the production of AMP does indeed stem from the aminoacylation and not from other "side" reactions.

The tRNA-dependent continuous ATP hydrolysis during the aminoacylation reaction has been established for several synthetases (von der Haar & Cramer, 1976) and has been called the "AMP/PP_i-independent hydrolysis of aminoacyl-tRNA" to emphasize its interpretation as a *cyclic reaction* (eq 2) and to distinguish it from the AMP/PP_i-dependent deacylation of aminoacylated tRNA.



We would now like to report a novel enzymatic activity of phenylalanyl-tRNA synthetase in which a different but readily interconvertible form of the enzyme brings about ATP hydrolysis in the absence of tRNA. This activity can be manipulated by the removal or addition of Zn^{2+} and leads to the conclusion that phenylalanyl-tRNA synthetase is a metalloprotein.

Materials and Methods

Phenylalanyl-tRNA synthetase (EC 6.1.1.20) was purified to homogeneity from baker's yeast according to von der Haar

† From the Max-Planck-Institut für Experimentelle Medizin, Abteilung Chemie, D-3400 Göttingen, Federal Republic of Germany. *Received August 22, 1979.*

0006-2960/80/0419-1676$01.00/0

(1979). During certain preparations EDTA was omitted from the buffers. $tRNA^{Phe}$ was isolated as described (Schneider et al., 1972). $tRNA^{Lys}$ from *Escherichia coli* was a gift from Dr. M. Sprinzl of this institute. Tyrosyl-tRNA synthetase was a homogeneous preparation and a gift from Dr. H. Faulhammer of this institute. [^{14}C]ATP (specific activity 44 Ci/mol) was from Schwarz (Orangeburg, NY), and PEI-coated TLC sheets were from Macherey & Nagel (Düren, West Germany). The ADP content of [^{14}C]ATP was ~4%. All other materials were of commercial analytical grade quality.

AMP Formation during Aminoacylation (Igloi et al., 1979). The assay mixture contained 150 mM Tris-HCl, pH 7.6, 150 mM KCl, 10 mM $MgSO_4$, 1–5 mM amino acid, 0.5 mM [^{14}C]ATP (with a specific activity of ~45 Ci/mol), and ~5 μM tRNA in a total volume of 50 μL in a 38 × 6 mm test tube. The reaction was initiated by the addition of enzyme to give a 1.5–1.7 μM solution. After the reaction was maintained at 37 °C, 1-μL aliquots were removed and spotted onto plastic-backed poly(ethylenimine) (PEI)–cellulose UV_{254} sheets (5 × 20 cm) (Macherey & Nagel, Düren, West Germany). Sufficient unlabeled ATP, ADP, and AMP for UV detection were applied to the sheets at the origin prior to use. The nucleotides were separated by chromatography with 2-propanol–1 M LiCl in 1 M acetic acid (1:2 v/v). The sheets were dried under IR lamps, the nucleotide spots were located by UV absorption and cut out, and the radioactivity was determined by liquid scintillation counting in a toluene-based scintillator. For removal of metal ions from the enzyme, it was preincubated at a concentration of 20–85 μM at 37 °C for 45 min with 1 mM EDTA in the storage buffer (von der Haar, 1979) and then either used directly, giving a concentration of 20 μM EDTA in the incubation, or dialyzed extensively against a buffer containing 150 mM Tris-HCl, pH 7.6, 100 mM KCl, 10 mM $MgSO_4$, and 50% glycerol. Aminoacylation activity was measured as described (von der Haar & Cramer, 1976).

Results

tRNA-Independent Hydrolysis of ATP. Phenylalanyl-tRNA synthetase from baker's yeast on incubation with ATP alone does not lead to any ATP hydrolysis in all cases tested. In the presence of ATP, $tRNA^{Phe}$, and phenylalanine, two effects can be observed. Firstly, phenylalanyl-tRNA synthetase purified with EDTA present in all buffers catalyzes the nonstoichiometric hydrolysis of ATP to AMP, in a strictly *amino acid and tRNA dependent* manner (eq 2; Figure 1a; von der Haar & Cramer, 1976). Secondly, if, alternatively, a preparation of this enzyme is used in whose purification EDTA had been omitted from all buffers (referred to below as non-EDTA-treated phenylalanyl-tRNA synthetase), the tRNA dependence of the reaction is essentially lost and there is a buildup of AMP (Figure 1b). The level of ADP, which is present in all commercial [^{14}C]ATP samples and which acts as an internal control for contaminating phosphatases, remains unchanged.

It has recently been established that methionyl-tRNA synthetase from *E. coli* exists as a metalloprotein (Posorske et al., 1979). We, therefore, investigated the effect of added Zn^{2+} on the EDTA-treated phenylalanyl-tRNA synthetase and of EDTA on the non-EDTA-treated phenylalanyl-tRNA synthetase. Zn^{2+} stimulated the tRNA-independent ATP hydrolysis of both enzyme preparations (parts a and c of Figure 1) whereas EDTA depressed the effect in the case of non-EDTA-treated phenylalanyl-tRNA synthetase (Figure 1b). The effect of EDTA was mimicked by 2-mercaptoethanol at concentrations which are greater than would be expected for an –S–S– link reduction. The 2-mercaptoethanol, therefore, probably also acts by metal sequestering (Cornell & Crivaro, 1972; Vallee & Wacker, 1970). Preincubation of the non-EDTA-treated phenylalanyl-tRNA synthetase with 140 mM 2-mercaptoethanol at 0 °C for 90 min resulted in an enzyme which, once diluted for assay purposes (×50), still hydrolyzed ATP to a small extent, but if the assay solution also contained 30 mM 2-mercaptoethanol or 1 mM EDTA the absolute tRNA dependence was once more induced (Figure 1b).

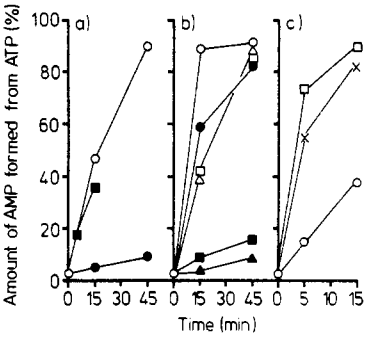


FIGURE 1: (a) ATP hydrolysis in the presence (open symbols) and in the absence (filled symbols) of 5 μM tRNA by EDTA-treated phenylalanyl-tRNA synthetase (○ and ●) and by the same enzyme containing 200 μM Zn^{2+}, 20 μM EDTA, and 8.2 mM mercaptoethanol in the incubation mixture (■). (b) ATP hydrolysis in the presence (open symbols) and in the absence (filled symbols) of 5 μM tRNA by non-EDTA-treated phenylalanyl-tRNA synthetase (○ and ●) and by the same enzyme containing 30 mM mercaptoethanol in the incubation mixture (□ and ■) or 1 mM EDTA in the incubation mixture (△ and ▲). (c) ATP hydrolysis by non-EDTA-treated phenylalanyl-tRNA synthetase simulated by Zn^{2+}. 1.7 μM enzyme was preincubated with 0 (○), 20 (×), and 50 (□) μM Zn^{2+} for 10 min at 37 °C before the reaction was initiated by the addition of phenylalanine to a concentration of 1 mM.

Indications That Zn^{2+} Binds to Phenylalanyl-tRNA Synthetase. The EDTA-treated phenylalanyl-tRNA synthetase shows slightly altered Michaelis–Menten constants for phenylalanine during aminoacylation. For the untreated enzyme, K_m = 6 μM and V = 3 μmol/(min mg); for the EDTA-treated enzyme, K_m = 25 μM and V = 5 μmol/(min mg). The capacity of the EDTA-treated enzyme to misaminoacylate $tRNA^{Phe}$-C-C-A(3′NH_2) with tyrosine (Igloi et al., 1978) was unaltered (data not shown).

The attribution of these effects to phenylalanyl-tRNA synthetase was confirmed by a number of observations. (1) Sodium dodecyl sulfate gel electrophoreses of EDTA-treated and of Zn^{2+}-treated enzyme show no differences (data not shown). (2) The absence of nonspecific, amino acid independent ATPase activity was emphasized by the stability of ATP in the presence of the enzyme (see, e.g., t = 0 value in Figure 1c). (3) "tRNA-independent" ATP hydrolysis by non-EDTA-treated enzyme is specifically stimulated by phenylalanine and only weakly by the misactivatable amino acids (Igloi et al., 1978) tyrosine and methionine (Figure 2a). (4) The theoretical possibility remains that the presence of Zn^{2+} in the assay solution causes an enhanced rate of hydrolysis of Phe-AMP-like intermediates diffusing from the enzyme into free solution. Several pieces of evidence argue against this interpretation. The rate of nonenzymatic hydrolysis of an intermediate should be independent of the source of that intermediate. We, therefore, compared the rate of Zn^{2+}-stimulated ATP hydrolysis in the presence of tyrosine with phenylalanyl-tRNA synthetase and with tyrosyl-tRNA synthetase. In both cases the Tyr-AMP generated should be subject to a hypothetical enzyme-independent, Zn^{2+}-catalyzed hydrolysis. Whereas in the presence of phenylalanyl-tRNA synthetase

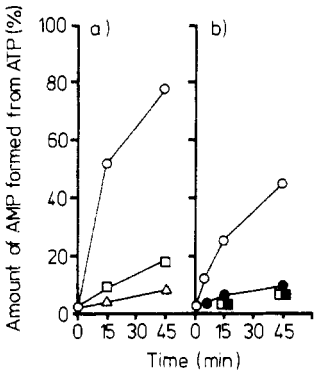


FIGURE 2: (a) ATP hydrolysis in the presence of amino acids by non-EDTA-treated phenylalanyl-tRNA synthetase. The incubation mixture, as described under Materials and Methods, contained 5 mM phenylalanine (○), 5 mM tyrosine (□), or 5 mM methionine (△). (b) ATP hydrolysis in the presence (open symbols) or in the absence (filled symbols) of 100 μM Zn^{2+} by non-EDTA-treated phenylalanyl-tRNA synthetase (○ and ●) and by tyrosyl-tRNA synthetase (□ and ■) in an incubation mixture containing 20 μM EDTA, 3 mM mercaptoethanol, and 6.3 mM tyrosine as the activated amino acid.

such a hydrolysis is observed, ATP is stable in the presence of tyrosyl-tRNA synthetase and tyrosine (Figure 2b). In making this comparison, we must take into account the inactivation of tyrosyl-tRNA synthetase by heavy metals including Zn^{2+} at high Zn^{2+}/enzyme ratios (Faulhammer, 1977). Under the conditions used, we found that the inactivation of aminoacylation activity of tyrosyl-tRNA synthetase was limited to 30%, so that the bulk of the enzyme still had the capacity to bring about Tyr-AMP formation. The difference in the rates of ATP hydrolysis between similarly treated phenylalanine and tyrosine enzymes during the activation of the same amino acid, tyrosine, is much greater than is accountable for by the inactivation of the tyrosine enzyme (Figure 2b) and would seem to reflect a true phenylalanyl-tRNA synthetase dependent reaction.

Another approach which makes the nonenzymatic pathway improbable relies on the literature values for several relevant rate constants. The dissociation rate of a typical aminoacyl-AMP from a synthetase lies in the region of 3×10^{-3} s^{-1} (Mulvey & Fersht, 1977). Once dissociated, this compound decomposes at a rate of $\sim 4 \times 10^{-3}$ s^{-1} (Mulvey & Fersht, 1977). We have observed turnover rates for ATP hydrolysis of 0.05–0.88 s^{-1} (see below). The nonenzymatic stimulation of hydrolysis by Zn^{2+} would therefore have to be at least by a factor of 100. From the literature data of metal-catalyzed breakdown of acyl phosphates (Lipmann & Tuttle, 1944; Koshland, 1952) and considering the low concentrations of Zn^{2+} involved, a stimulation of this magnitude would seem to be extremely unlikely.

Nature of the Zn^{2+} Stimulation and of the EDTA Inhibition. The fact that the inhibition by EDTA is not due to a direct interaction of the chelating agent with a protein side chain was shown by dialyzing an EDTA-treated sample of phenylalanyl-tRNA synthetase against a large volume of buffer. Atomic absorption spectroscopy points to the fact that phenylalanyl-tRNA synthetase retains one, if not more, tightly bound Zn^{2+} ion even after EDTA treatment (H. Sternbach, personal communication). Nevertheless, the resulting metal-depleted and EDTA-free phenylalanyl-tRNA synthetase was devoid of tRNA-independent splitting activity (Figure 3), indicating that this additional Zn^{2+} does not influence the reaction described in this paper. ATP hydrolysis could, however, be reconstituted by treatment with Zn^{2+} (see below). This reconstitution exhibits a strong cooperative Zn^{2+} concentration dependence (Figure 4).

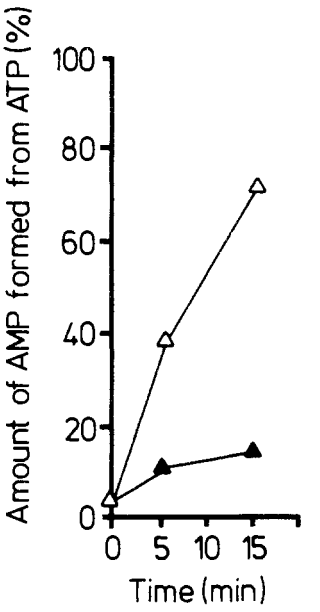


FIGURE 3: ATP hydrolysis in the presence (open symbols) or absence (filled symbols) of 5 μM tRNA by non-EDTA-treated phenylalanyl-tRNA synthetase after incubation with 1 mM EDTA for 45 min at 37 °C and subsequent dialysis twice against a 4000-fold excess of 150 mM Tris, pH 7.6, containing 100 mM KCl and 10 mM Mg^{2+} in 50% glycerol.

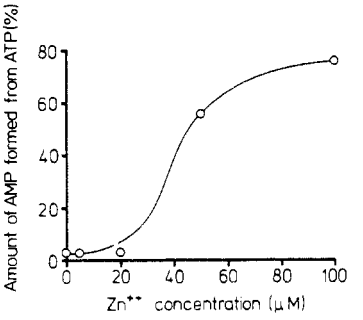


FIGURE 4: Zn^{2+} concentration dependence of the reappearence of ATP hydrolyzing activity in a non-EDTA-treated phenylalanyl-tRNA synthetase which had been incubated and dialyzed as described in Figure 3. The rate of ATP hydrolysis was measured after preincubation of the enzyme (1.7 μM) with the given concentration of Zn^{2+} for 10 min at 37 °C following the reaction for 5 min.

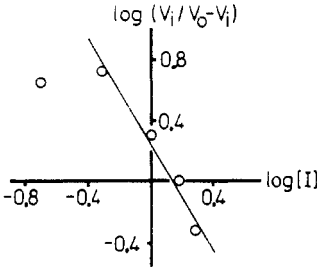


FIGURE 5: Hill plot for the inhibition of ATP splitting by EDTA by using phenylalanyl-tRNA synthetase isolated in the absence of EDTA buffers. The results are treated according to log $[V_i/(V_o - V_i)]$ = log $K_i - n$(log [I]).

The nature of the Zn^{2+} binding and its influence on the activity of phenylalanyl-tRNA synthetase were investigated by using the inhibition of ATP hydrolyzing activity by EDTA. The results of such an EDTA concentration dependence can be analyzed according to a Hill type plot (Segel, 1975) to give a K_i for the binding of the chelating agent and an n for the number of inhibitor molecules bound. Such a treatment of

Table I: Parameters Deduced from the Inhibition of ATP Splitting Activity by EDTA

prepn no.	enzyme prepn	n	K_i (μM)	sp act. [pmol/(min μg of enzyme)] in ATP hydrolysis	sp act. [pmol/(min μg of enzyme)] in amino-acylation
1	Phe-tRNA synthetase isolated in the absence of EDTA	1.7	1.7	34.5	1.31
2	enzyme prepn no. 1 treated with 1 mM EDTA (see legend to Figure 2), dialyzed (see Materials and Methods), treated with 50 μM $ZnSO_4$ for 10 min at room temp, and dialyzed	1.5	0.45	12.6	1.19
3	enzyme prepn no. 1 passed over a DEAE-cellulose column in a further purifn attempt	1.5	11.5	47.9	0.79
4	enzyme prepn no. 3 treated with 1 mM EDTA (see legend to Figure 2), dialyzed, treated with 1 mM $ZnSO_4$ for 10 min at room temp, and dialyzed	nd[a]	40[b]	202.0	0.77

[a] nd = not determined. [b] Calculated from two-point determination by assuming $n = 1.5$.

the data is exemplified in Figure 5 for the case of the phenylalanyl-tRNA synthetase isolated in the absence of EDTA buffers. However, whereas the value of n seems to be independent of the enzyme preparation, K_i values vary substantially (Table I). This variation can be correlated with a concomitant change in the specific activity for the ATP splitting reaction. The changes in the specific activity for aminoacylation are, in comparison, insignificant (Table I). The fact that the activity in question can be stimulated to an extent greater than that obtained for the phenylalanyl-tRNA synthetase isolated in the absence of EDTA is in line with the additional activity of the latter enzyme when further Zn^{2+} is added to the assay solution (Figure 1c).

The strength of the Zn^{2+} binding in the freshly isolated phenylalanyl-tRNA synthetase can be estimated from the fact that, although it appears to be removed by low concentrations of EDTA [$K(Zn^{2+}$–EDTA) = 10^{16} M^{-1}; Martell & Calvin, 1952] in contrast to alkaline phosphatase (von der Haar, 1978), it is not at all or only partially displaced by NH_4^+ ions during salting out chromatography. This phosphatase binds Zn^{2+} with a K of 10^8–10^{10} M^{-1} (Friedberg, 1974). The ATP splitting activity is not inhibited by 4 mM of the so-called metal-directed affinity labeling reagent (*R,S*)-2-bromo-3-(5-imidazoyl)propionic acid (Dahl et al., 1979).

Influence of Zn^{2+} on Misaminoacylation of $tRNA^{Lys}$ from E. coli by Phenylalanyl-tRNA Synthetase from Yeast. $tRNA^{Lys}$ from *E. coli* is readily misaminoacylated by a phenylalanyl-tRNA synthetase from yeast, which has been treated with EDTA and hence lacks the potential to hydrolyze ATP (Figure 6). Induction of ATP hydrolysis by the addition of Zn^{2+} drastically reduces the amount of phenylalanine which can be esterified to $tRNA^{Lys}$ (Figure 6).

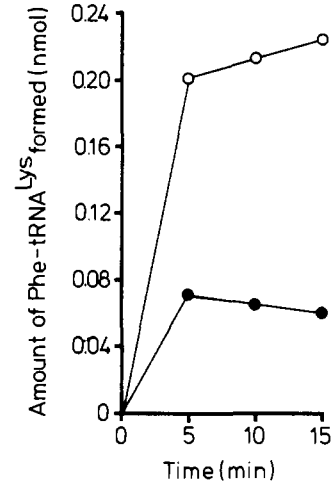


FIGURE 6: Phenylalanylation of $tRNA^{Lys}$ from *E. coli* with non-EDTA-treated phenylalanyl-tRNA synthetase from yeast (filled circles) and with EDTA-treated enzyme (open circles). Reaction conditions were as described (von der Haar & Cramer, 1978) with 0.16 A_{260} unit of $tRNA^{Lys}$ and 17 μg of phenylalanyl-tRNA synthetase in a 100-μL assay. The maximum aminoacylation of $tRNA^{Lys}$ corresponds to 1.4 nmol/A_{260} unit, which is more than 90% of the value obtained during aminoacylation with lysine and lysyl-tRNA synthetase from *E. coli*.

Discussion

The possibility of an involvement of a metal ion in the function of aminoacyl-tRNA synthetases was proposed by Loftfield 10 years ago (Loftfield & Eigner, 1969). This suggestion has been largely ignored and further experimental evidence for it has not accumulated, although some indications have occasionally appeared in the literature (Takeda & Ohnishi, 1975). Recently, Posorske et al. (1979) have shown the methionyl-tRNA synthetase from *E. coli* to be a Zn^{2+} metalloprotein with additional Mn^{2+} binding sites. We have found that a homogeneous preparation of phenylalanyl-tRNA synthetase which has not been in contact with EDTA or other strong chelating agents during its preparation causes a tRNA-independent hydrolysis of ATP in the presence of an activatable amino acid. This activity, which can be suppressed by EDTA or mercaptoethanol—acting as a chelating agent (Vallee & Wacker, 1970)—has, on the basis of Hill plots of this inhibition, been attributed to the cooperative binding of more than one metal ion per a_2b_2 enzyme molecule [cf. one metal ion per dimeric methionyl-tRNA synthetase (Posorske et al., 1979) and one metal ion per monomeric valyl-tRNA synthetase (Loftfield & Eigner, 1969)].

The multitude of different K_i values observed for the inhibition by EDTA may be rationalized if one considers that under conditions where EDTA inhibits strongly (low K_i), a situation exists where a rather loose protein structure allows the EDTA to effectively complex the somewhat exposed Zn^{2+}. The collection of K_i values and the associated specific activities would then be an indication of changes in the structural form of the enzyme after complexation with Zn^{2+}. The weakest inhibition by EDTA (high K_i) corresponds to a tightly integrated metal atom which is inaccessible to the inhibitor and therefore requires larger amounts of chelating agent before inhibition occurs. The facile interconvertibility of two forms of the enzyme under the influence of salt has already been demonstrated (von der Haar, 1976). Such a salt effect may be the cause of the altered parameters following DEAE-cel-

lulose chromatography while the effect of varying the concentration of Zn^{2+} prior to dialysis reflects the cooperative nature of the metal ion binding (Figure 4).

The realization of the occurrence of an ATP hydrolyzing reaction not caused by transfer of the amino acid to tRNA is of some consequence for the determination of aminoacylation stoichiometries. The possibility of such a side reaction which may be easily manipulated by variations in the preparation and assay conditions must be borne in mind during these determinations since, as stated by Mulvey & Fersht (1977), "any hydrolysis of the ATP occurring during storage or in the reaction mixture will lower the stoichiometry". Apparent differences in stoichiometries should, therefore, be interpreted in the light of our findings and with suitable controls. Similarly, the apparent involvement of enzyme-stimulated breakdown of adenylates in fidelity-maintaining processes (Jakubowski, 1978) may need reexamination. The fact that phenylalanyl-tRNA synthetase without the ATP splitting capacity is able to phenylalanylate $tRNA^{Lys}$ from *E. coli* completely whereas the ATP splitting enzyme does not can be explained in the following way. A relatively large amount of enzyme has to be used in order to misaminoacylate the noncognate $tRNA^{Lys}$. Hence, within the time needed for aminoacylation of $tRNA^{Lys}$, a substantial amount of ATP is hydrolyzed and the reaction is terminated because of the lack of ATP. The Phe-$tRNA^{Lys}$ already formed then starts to decompose slowly, both because of its inherent instability and because of the enzyme-catalyzed AMP/PP_i-independent hydrolysis of the ester linkage. One can easily imagine that with a substrate even poorer than $tRNA^{Lys}$, a per se possible misaminoacylation could be completely overlooked. The observation of Godeau & Charlier (1979) on arginyl-tRNA synthetase from *Bacillus subtilis* which acts as an ATPase in the absence of other substrates may underline the importance of considering alternative pathways for ATP hydrolysis by the synthetases. On the other hand, we have previously observed a similar ADP formation in enzyme preparations containing trace amounts of an unidentified contaminating enzyme.

Finally, we have, as part of a general scheme, proposed the use of the AMP/PP_i-independent hydrolysis of aminoacyl-tRNA during aminoacylation as indicative of transfer of an activated amino acid to tRNA (Igloi et al., 1979; Igloi & Cramer, 1978; Cramer et al., 1979). The resulting nonstoichiometric ATP hydrolysis is phenotypically identical with that described in this work and thus is not necessarily sufficient evidence for transfer. However, since we have shown that the metal-catalyzed reaction is also amino acid specific and since all activated amino acids are transferred to tRNA (Igloi et al., 1978), our present results merely require the addition of a cautionary note to our previous suggestions and do not invalidate the general scheme.

References

Cornell, N. W., & Crivaro, K. E. (1972) *Anal. Biochem. 47*, 203–208.

Cramer, F., von der Haar, F., & Igloi, G. L. (1979) *Cold Spring Harbor Symp. Quant. Biol.* (in press).

Dahl, K. H., McKinley-McKee, J. S., Beyerman, H. C., & Noordam, A. (1979) *FEBS Lett. 99*, 308–312.

Faulhammer, H. (1977) Ph.D. Thesis, University of Braunschweig, Federal Republic of Germany.

Friedberg, F. (1974) *Q. Rev. Biophys. 7*, 1–33.

Godeau, J. M., & Charlier, J. (1979) *Biochem. J. 179*, 407–412.

Hopfield, J. J., Yamane, T., Yue, V., & Coutts, S. M. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 1164–1168.

Igloi, G. L., & Cramer, F. (1978) in *Transfer RNA* (Altman, S., Ed.) pp 294–349, MIT Press, Cambridge, MA.

Igloi, G. L., von der Haar, F., & Cramer, F. (1978) *Biochemistry 17*, 3459–3468.

Igloi, G. L., von der Haar, F., & Cramer, F. (1979) *Methods Enzymol. 59*, 282–291.

Jakubowski, H. (1978) *FEBS Lett. 95*, 235–238.

Koshland, D. E. (1952) *J. Am. Chem. Soc. 74*, 2286–2292.

Lipmann, F., & Tuttle, L. C. (1944) *J. Biol. Chem. 153*, 571–582.

Loftfield, R. B., & Eigner, E. A. (1969) *J. Biol. Chem. 244*, 1746–1754.

Martell, A. E., & Calvin, M. (1952) *Chemistry of the Metal Chelate Compounds*, Prentice-Hall, New York.

Mulvey, R. S., & Fersht, A. R. (1977) *Biochemistry 16*, 4731–4737.

Posorske, L. H., Cohn, M., Yanagisawa, N., & Auld, D. S. (1979) *Biochim. Biophys. Acta 576*, 128–133.

Schneider, D., Solfert, R., & von der Haar, F. (1972) *Hoppe-Seyler's Z. Physiol. Chem. 353*, 1330–1336.

Segel, I. H. (1975) *Enzyme Kinetics*, Wiley, New York and London.

Takeda, Y., & Ohnishi, T. (1975) *J. Biol. Chem. 250*, 3878–3882.

Vallee, B. L., & Wacker, W. E. C. (1970) *Proteins, 2nd Ed. 5*.

von der Haar, F. (1976) *Eur. J. Biochem. 64*, 395–398.

von der Haar, F. (1978) in *Theory and Practice in Affinity Techniques* (Sundaram, P. V., & Eckstein, F., Eds.) pp 1–13, Academic Press, London, New York, and San Francisco.

von der Haar, F. (1979) *Methods Enzymol. 59*, 257–267.

von der Haar, F., & Cramer, F. (1976) *Biochemistry 15*, 4131–4138.

von der Haar, F., & Cramer, F. (1978) *Biochemistry 17*, 4509–4514.