Glutamyl Transfer Ribonucleic Acid
Synthetase of Escherichia coli

II. INTERACTION WITH INTACT GLUTAMYL TRANSFER RIBONUCLEIC ACID*

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SUMMARY

Escherichia coli glutamyl transfer ribonucleic acid synthetase acylates the three homologous tRNA$_{Glu}$ isoacceptors with very similar $K_m$ values (2.4 to $4.6 \times 10^{-7}$ M). The pure enzyme forms a 1:1 complex with its cognate tRNA as judged by gradient centrifugation and fluorescence-quenching studies. The biological specificity of complex formation is not strictly observed in vitro since fluorescence-quenching studies demonstrate complexes of this enzyme with Escherichia coli tRNA$_{Val}$ and tRNA$_{Leu}$. Studies on the protection by tRNA$_{Glu}$ against heat inactivation of the enzyme give a binding constant of $3.6 \times 10^{-7}$ M for this interaction. Experiments performed in this paper indicate a concerted mechanism of glutamyl-tRNA formation.

Specific complexes between aminoacyl-tRNA synthetases and their cognate tRNA substrates have been observed in many cases (1, 2). They have been detected by the increased heat stability of these enzymes in the presence of their cognate tRNAs (3), or by changes in the circular dichroism (4) and in the fluorescence properties of the enzymes in the presence of tRNA (5-10). They also have been isolated by sedimentation on density gradients, by gel filtration, or by adsorption to nitrocellulose filters. Usually tRNA and its cognate aminoacyl-tRNA synthetase form a 1:1 complex, the dissociation constant of which is approximately $10^{-4}$ M (see, e.g. 9).

The mechanism of aminoacyl-transfer RNA formation is thought to be a two step process involving, successively, the formation and transfer of the amino acid. In this scheme the product of the first step is the enzyme-bound aminoacyl-adenylate from which the activated amino acid is transferred onto tRNA (1, 2). Escherichia coli glutamyl-tRNA synthetase requires its cognate tRNA for the activation step as shown by the requirement for tRNA$_{Glu}$ in the ATP-PP$_i$ exchange reaction. This observation suggests that in this case the aminoacylation reaction may not be a two step process but a concerted mechanism.

In the preceding paper we have shown that the E. coli GluRS$_1$ consists of two polypeptide chains, only one of which is responsible for the catalytic activity (11). In this study the interaction between GluRS and intact tRNA$_{Glu}$ is investigated in order to determine which subunit of GluRS can bind the cognate tRNA. An extension of these experiments has produced some evidence that tRNA$_{Glu}$ must have some elements of tertiary structure intact in order to be recognized by GluRS. The experimental results of this study strongly suggest that glutamyl-tRNA is formed by a concerted mechanism.

MATERIALS AND METHODS

General—Sodium$_{32}$P$_3$pyrophosphate and uniformly labeled L-[14C]glutamic acid (specific activity 189 mCi per mmole) were obtained from New England Nuclear. Crystalline bovine plasma albumin was obtained from Pentex. Protein was determined according to Lowry et al. (12) with crystalline bovine plasma albumin as standard.

References to the preparation of unfractionated E. coli K12 (strain CA244) tRNA and of purified tRNA$_{Val}$, tRNA$_{Ser}$, and tRNA$_{Leu}$ species and valyl-tRNA synthetase from the same strain are given in the preceding paper (11). Purified E. coli K12 tRNA$_{Glu}$ with a specific activity of 1450 pmoles per A$_{260}$ unit was obtained from Dr. A. D. Kelmers of the Oak Ridge National Laboratory. This tRNA is the major species of tRNA$_{Glu}$ in E. coli and is designated tRNA$_{Glu}$. Two other isoacceptors were obtained in lower purity by RPC-5 chromatography (13) of E. coli K12 (CA244) tRNA. The samples were designated tRNA$_{Glu}^1$ (specific activity of 1030 pmoles per A$_{260}$ unit) and tRNA$_{Glu}^2$ (specific activity of 107 pmoles per A$_{260}$ unit).

Assay for GluRS activity in the aminoclaylation or ATP-PP$_i$ exchange reaction was performed as described previously (11). Measurement of $K_m$ and $V_{max}$—The initial velocity of the reaction catalyzed by a fixed amount of enzyme in the presence of variable amounts of one of the substrates at saturating concentrations (above the $K_m$ value) of all the other substrates was calculated from the amount of glutamyl-tRNA formed during

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at least three different reaction times. The reactions were carried out in duplicate. The assay mixture containing all the substrates but one was pre-equilibrated at 30°. At time zero, the missing substrate (also kept in solution at 30°) was added with mixing. The kinetic data were analyzed according to Lineweaver and Burk (11).

The concentrations of Mg++ and ATP in the assay mixture were chosen to optimize the initial velocity of the reaction in the presence of $3 \times 10^{-8}$ M $tRNA^{Glu}$ (about 10 times the $K_m$ of GluRS for $tRNA^{Glu}$ measured in a preliminary experiment) present in unfraccionated *E. coli* tRNA$^3$. Keeping the ATP concentration fixed at 0.002 M, an increase in the Mg++ concentration increases the initial velocity until a plateau is reached. A value of 0.015 M Mg++ (on the plateau) was chosen. The experimental conditions were: 0.10 M sodium Hepes (pH 7.2), 0.01 M KCl-0.002 M MgCl$_2$-0.002 M 2-mercaptoethanol-6 $\times 10^{-4}$ M glutamate. The concentration of 2-mercaptoethanol in the reaction mixture was sufficient to protect the enzyme for the duration of the reaction as judged by the linearity of the formation of glutamyl-tRNA as a function of time. KCl, 10 mM, was included in all the enzyme assays because potassium has been observed to have a stimulating effect on the activity of some aminocyl-tRNA synthetases (see e.g. 15).

**ATP Cleavage Reaction**—The cleavage of [γ-32P]ATP was determined under the conditions of the aminocyclation reaction in the presence and absence of tRNA. Residual labeled ATP was measured after adsorption to charcoal as described for the ATP-PP$_i$ exchange reaction or by paper chromatography of the reaction mixture in methyl ethyl ketone-methanol-water-concentration HCl (40:20:20:1).

**Fluorescence Measurements**—These were carried out with a spectrofluorimeter built by Dr. Juan Yaguezabide. The solution was in a 1 cm quartz cuvette and the absorption was less than 10% at the excitation wavelength of 295 nm at tRNA concentrations up to $8 \times 10^{-4}$ M. The intensity of the light emitted in the direction perpendicular to that of the exciting beam was measured with a spectrophotometer from 300 to 500 nm and plotted with a Hewlett-Packard X-Y recorder 7004A. All measurements were made at 20° in presence of 0.002 M 2-mercaptoethanol.

**RESULTS**

### $K_m$ Values for Three tRNA$^{Glu}$ Species

When $K_m$ values for isoacceptor tRNAs are determined in the aminocyclation reaction with their cognate aminocyl-tRNA synthetases, a remarkable similarity in these constants is seen. *E. coli* tRNA$^{Met}$ and tRNA$^{Met}_{\text{leu}}$ (6), and the *E. coli* leucine, serine, and valine isoacceptor RNA species (16) show very similar $K_m$ values within each group. The only marked difference reported was for an *E. coli* missense suppressor tRNA$^{Glu}$; it is acylated by glycyyl-tRNA synthetase about $10^4$ times more slowly than is the normal tRNA$^{Glu}$ (17).

Reversed phase chromatography of *E. coli* tRNA resolves three tRNA$^{Glu}$ peaks. Although we have no sequence information to indicate that the tRNA$^{Glu}$ in these peaks represents different species this conclusion is made more likely since they could also be separated by benzoylated DEAE-cellulose chromatography. The $K_m$ and relative $V_{max}$ values of the pure GluRS for the tRNA$^{Glu}$ contained in these three peaks were determined. As seen in Table I the $K_m$ values for the isoacceptors were fairly similar when measured in the aminocyclation reaction.

**GluRS-tRNA$^{Glu}$ Interactions**

**Isolation of a Specific Complex by Density Gradient Centrifugation**—In order to find out the affinity of the Fraction V GluRS and of the isolated 46K and 56K proteins for tRNA, we studied their ability to form complexes with tRNA$^{Glu}$ by gradient centrifugation. When a mixture of GluRS and unfraccionated *E. coli* tRNA was layered on the top of a water-deuterium oxide density gradient and centrifuged, a peak of GluRS activity together with tRNA$^{Glu}$ was sedimented faster than either GluRS or tRNA$^{Glu}$ alone. A similar experiment was then conducted with GluRS and the pure major tRN$^{Glu}$ isoacceptor. In neutral (pH 7.2) or acid (pH 5.8) solution a peak of GluRS activity and tRNA$^{Glu}$ co-sedimented about 1.5 times as fast as the free enzyme alone. The specificity of this association was verified by sedimenting a mixture of pure GluRS, pure *E. coli* valyl-tRNA synthetase, pure tRNA$^{Val}$ and pure tRNA$^{Val}$, Fig. 1 shows that tRNA$^{Glu}$ formed a complex only with GluRS, and that tRNA$^{Val}$ does so only with valyl-tRNA synthetase. This result allows us to measure an S value of about 5.7 for the GluRS-tRNA$^{Glu}$ complex by comparison with the value of 7.3 known for the tRNA$^{Val}$-valyl-tRNA synthetase complex formed at the same pH (18). A comparison of the sedimentation behavior of the tRNA$^{Val}$-GluRS complex (Fig. 1) to that of the well studied tRNA$^{Val}$-valyl-tRNA synthetase complex indicated only one tRNA$^{Glu}$ molecule to be present in the former complex, for a larger change in the sedimentation constant compared to the free enzyme would otherwise have been expected.

When a centrifugation experiment similar to Fig. 1 was carried out with tRNA$^{Glu}$ (at $3 \mu M$ concentration) throughout the gradient no shift in the position of the enzyme-tRNA complex was observed. This indicated that the stoichiometry of the complex is not influenced by the addition of tRNA in contrast to the finding of Muench et al. (3) for *E. coli* tryptophanyl-tRNA synthetases.

In addition, these results suggested that the tRNA$^{Glu}$-GluRS complex does not contain both subunits of GluRS. Therefore the individual fractions collected after gradient centrifugation were examined by SDS-polyacrylamide gel electrophoresis for the presence and relative amounts of both polypeptide chains of GluRS. In the experiment with Fraction V GluRS in the absence of tRNA we observed that the 56K protein sedimented slightly faster than the 46K chain, and coincided with both GluRS activities (acylation of tRNA$^{Glu}$ and ATP-PP exchange). When Fraction V GluRS was sedimented in presence of tRNA$^{Glu}$, both 56K and 46K proteins had a higher S value than in the previous case. However, the 56K protein still moved

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1. K. H. Muench, personal communication.

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**Table I**  

<table>
<thead>
<tr>
<th>tRNA$^{Glu}$</th>
<th>$K_m \times 10^5$</th>
<th>Relative $V_{max}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>tRNA$^{Glu}$</td>
<td>4.2</td>
<td>1.15</td>
</tr>
<tr>
<td>tRNA$^{Glu}$</td>
<td>4.6</td>
<td>0.87</td>
</tr>
<tr>
<td>tRNA$^{Glu}$</td>
<td>2.4</td>
<td>1.0</td>
</tr>
</tbody>
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ahead of the 46K protein band and its position was again coincident with the GluRS activities and the peak of bound tRNA\textsuperscript{Glu}. The coincidence of the 56K chain and of the GluRS activity in these two experiments supports the results of the preceding paper (11) that 56K subunit is the catalytic part of GluRS.

In order to confirm the binding of tRNA\textsuperscript{Glu} to the 56K GluRS, gradient centrifugation with the isolated subunits was done. As seen in Fig. 2, tRNA\textsuperscript{Glu} sedimented faster in the presence of the 56K enzyme than in its absence indicating the existence of a complex between the two. Since the 46K protein has no GluRS activity, its ability to bind tRNA was judged by a possible change in the sedimentation behavior of tRNA\textsuperscript{Glu}. Under identical conditions to the ones described for Fig. 2 no shift in the sedimentation position of tRNA\textsuperscript{Glu} was seen in the presence of 46K protein indicating the absence of a complex between the two macromolecules.

**Quenching of the Fluorescence of GluRS by tRNA\textsuperscript{Glu}**—Quenching of the fluorescence of aminoacyl-tRNA synthetase by tRNA has been used to study the detailed interaction of some of these enzymes with their substrates (5-10). In this study we investigated the fluorescence properties of GluRS in order to find the stoichiometry and specificity of its interaction with tRNA.

When a solution of GluRS was excited at 295 nm, the spectrum of its fluorescence showed a peak of intensity centered around 340 nm, probably due to the tryptophan residues of the enzyme. Since some properties of GluRS (e.g. ATP-PP\textsubscript{i} exchange and heat stability) are markedly influenced by changes of pH, the influence of this variable on the fluorescence of GluRS was investigated. The intensity of the fluorescence was assumed to be proportional to its peak value around 340 nm and was estimated by taking an average of the light emitted by the sample between 340 and 345 nm. Fig. 3 shows that the quantum yield of a solution of GluRS increased by about 35% with a sigmoid dependence on pH, when the pH was varied from 5.5 to 8.5. Similar relations have been observed for the valyl- and for the phenylalanyl-tRNA synthetase from E. coli (8, 9).

Next, the quenching of the enzyme fluorescence by tRNA was examined. Since it was shown for E. coli phenylalanyl-tRNA synthetase that complex formation with tRNA occurred only in acidic solutions, we compared three different pH values. When increasing amounts of pure E. coli tRNA\textsubscript{Glu} were added to a solution of GluRS at pH 5.5 the fluorescence intensity decreases rapidly at first and then tends gradually towards a constant value (Fig. 4B). To our surprise E. coli tRNA\textsubscript{Val} (at a concentration comparable to that of tRNA\textsubscript{Glu}) brought about a similar quenching (Fig. 4B) while E. coli tRNA\textsubscript{Leu} had a weaker effect (Fig. 4B). The significance of this unspecific interaction is not
clear since no tRNA\(^{\text{Glu}}\)–GluRS complex could be isolated following sedimentation on a density gradient at pH 5.8. However, it is not unique since yeast tRNA\(^{\text{Tyr}}\) has been shown to quench very efficiently the fluorescence of the seryl-tRNA synthetase from the same organism (7). This nonspecific binding was reduced, but still present at pH 7.0 (Fig. 4A) under the conditions studied. tRNA\(^{\text{Glu}}\) still quenched appreciably, albeit less than at pH 5.5. At pH 8.0 no significant quenching by tRNA\(^{\text{Glu}}\) was observed.

Then the fluorescence quenching of the isolated subunits of GluRS was measured at pH 7.0 under the conditions of Fig. 4A. The fluorescence intensity of the 56K enzyme was lowered by 22% upon the addition of tRNA\(^{\text{Glu}}\) while no quenching was observed with the 46K protein.

In order to study separately the influence of Mg\(^{++}\) and of tRNA\(^{\text{Glu}}\) on the fluorescence of GluRS, both enzyme and tRNA were extensively dialyzed against buffers containing EDTA. The enzyme retained all GluRS activity during this step. When increasing amounts of tRNA\(^{\text{Glu}}\) were added to a solution of GluRS the fluorescence intensity decreases rapidly at first and then tends gradually towards a constant value (Fig. 5A). This indicates complex formation in the absence of Mg\(^{++}\) as was also found for isoleucyl-tRNA synthetase and its cognate tRNA (19). The stoichiometry of the complex can be obtained from the intersection of the extrapolate of the initial slope of the quenching curve with the baseline reached at high tRNA\(^{\text{Glu}}\) concentration (6). Fig. 5 shows this intersection at about 8 \(\times\) \(10^{-6}\) M tRNA\(^{\text{Glu}}\) giving a value of about 0.8 molecule of tRNA\(^{\text{Glu}}\) bound to 1 molecule of GluRS. When the data in Fig. 4A were treated this way a value of 1.1 tRNA molecule per GluRS molecule is obtained. This calculation was based on the assumption that all GluRS molecules present in the enzyme preparation are active. There are indications that the proportion of denatured enzyme molecules in this GluRS preparation is negligible, since upon gradient centrifugation of GluRS with excess tRNA\(^{\text{Glu}}\) no GluRS activity was detected outside the fractions containing the enzyme–tRNA complex. The results described above on the quenching of the fluorescence of GluRS by tRNA\(^{\text{Glu}}\) are consistent with the conclusion obtained from the relative S values of the free GluRS and of its complex with tRNA\(^{\text{Glu}}\) that this is a 1:1 complex. Moreover, the extent of the quenching indicates that at least two of the eight tryptophanyl residues of GluRS are affected by the binding of tRNA\(^{\text{Glu}}\).

Mg\(^{++}\) alone does quench the fluorescence of GluRS. Analysis of the exponential quenching curve given in Fig. 5B reveals the presence of a few sites on the enzyme able to bind Mg\(^{++}\) very strongly (the \(K_d\) is about \(10^{-6}\) M). It is interesting to correlate the existence of these sites with the observation that Mg\(^{++}\) protects GluRS very effectively against heat-inactivation (Fig. 8).

Helene et al. (9) reported a much weaker binding of Mg\(^{++}\) to the E. coli valyl-tRNA synthetase; they measured a \(K_d\) value of \(10^{-7}\) M and found that only 5 to 6% of the total fluorescence of this enzyme could be quenched by Mg\(^{++}\). We also have observed such a small quenching (less than 10%) of the fluorescence of GluRS by Mg\(^{++}\) when the enzyme had been dialyzed previously against Mg\(^{++}\)-free buffer containing no EDTA. However, when GluRS had been dialyzed against EDTA, the strong quenching by Mg\(^{++}\) (Fig. 5B) was still seen.

Protection of GluRS against Heat Inactivation by tRNA—Many...
enzymes are known to be stabilized by their substrates against various inactivating agents. This behavior has been observed for a number of aminoacyl-tRNA synthetases and used to study the specific interaction of certain parts of the tRNA molecule with the enzyme (20). We have tried to check whether this protection occurs in the case of GluRS and whether it is specific for the cognate tRNA<sup>Glu</sup>. As is clearly seen in Fig. 6 tRNA<sup>Glu</sup> in the presence of Mg<sup>2+</sup> specifically protected the enzyme from heat inactivation. Mg<sup>2+</sup> was crucial for this protection and E. coli tRNA<sup>Glu</sup> could not substitute for the cognate tRNA. If various inactivating agents. This behavior has been observed against heat inactivation of GluRS. Glutamyl-tRNA synthetase. In the experiments outlined below we shall consider conformational states of tRNA brought about by variation of the Mg<sup>2+</sup> concentration and their effect on the protection against heat inactivation of GluRS. Glutamyl-tRNA formation or the ATP-PP<sub>i</sub> exchange cannot be used to obtain evidence that the enzyme was in its native state in the absence of Mg<sup>2+</sup>, since both reactions require Mg<sup>2+</sup>. The fluorescence studies performed (see Fig. 5A) indicate formation of a GluRS-tRNA<sup>Glu</sup> complex at 20° even in the absence of Mg<sup>2+</sup>. Thus, it is fair to assume that in the absence of Mg<sup>2+</sup>, GluRS is in its native state under these conditions. The conformation of tRNA<sup>Glu</sup> was monitored by its thermal denaturation profiles in the presence and absence of Mg<sup>2+</sup> in the buffer used in the heat protection studies (Fig. 7). In the presence of Mg<sup>2+</sup> the melting took place in one sharp transition whose <i>T<sub>m</sub></i> is about 90°. This high value is in good agreement with the very high proportion of G-C base pairs (80%) in the clover-leaf model of secondary structure of this tRNA<sup>Glu</sup> (24). In the absence of Mg<sup>2+</sup>, a bimodal melting was observed. The first step which accounted for a loss of only about 10% of the total hypochromicity and was completed at 40° probably reflects the melting of the tertiary structure (24). The second step was broader than the monophasic transition observed in presence of Mg<sup>2+</sup>, and had a <i>T<sub>m</sub></i> of about 60°. Thus, Mg<sup>2+</sup> dramatically stabilizes tRNA<sup>Glu</sup>. It shifts the major tRNA transitions from 60°-90° and prevents a minor unfolding occurring below 40° in its absence.

In order to see the influence of the conformation of tRNA<sup>Glu</sup> on its ability to interact with GluRS, a heat protection experiment was conducted in the absence of Mg<sup>2+</sup> at 40°. The results, summarized in Fig. 8, showed that in the absence of Mg<sup>2+</sup>, tRNA<sup>Glu</sup> gave no protection to GluRS against heat inactivation. However, the addition of Mg<sup>2+</sup> to the incubation mixture led to a marked increase in the stability of GluRS. A control showed that Mg<sup>2+</sup> alone protected the enzyme, but much less so than tRNA and Mg<sup>2+</sup> combined. This experiment shows that tRNA<sup>Glu</sup> must have at least some secondary or tertiary structure in order to interact with its cognate enzyme.

**Mechanism**

As was shown previously (11) GluRS has an absolute requirement for tRNA<sup>Glu</sup> in the ATP-PP<sub>i</sub> exchange reaction. It is conceivable that the tRNA in the ATP-PP<sub>i</sub> exchange is required for forming Glu-tRNA which is then cleaved to yield radioactive ATP, rather than facilitating an earlier step not involving aminoacyl-tRNA formation. Moreover, there is some evidence that...
of glutamate as high as 0.1 M. Upon addition of tRNAaG', the cleavage of ATP was detectable (Fig. 9) even at concentrations 50-fold excess of enzyme over ATP. In the absence of tRNA no t,RNh, and at an ATP concentration such that there was a 70-

for at least a few aminoacyl-tRNA synthetases, the ATP-PPi exchange reaction might not be the reverse of the aminolytic action of tRNA (25).

Consequently, in order to find if GluRS can react with ATP and glutamate in the absence of tRNA, we followed the forward reaction measuring the cleavage of [γ-32P]ATP by GluRS in the absence of added pyrophosphate in presence and absence of tRNA, and at an ATP concentration such that there was a 70-fold excess of enzyme over ATP. In the absence of tRNA no cleavage of ATP was detectable (Fig. 9) even at concentrations of glutamate as high as 0.1 M. Upon addition of tRNAaG', the 32P-label was gradually released from the ATP fraction. These results indicate that not even an amount of ATP equimolar to the GluRS concentration is cleaved in the absence of tRNA. This absolute requirement for tRNA in the enzymatic cleavage of ATP by GluRS supports the idea of a concerted mechanism for GluRS.

**DISCUSSION**

*E. coli* GluRS forms a complex with its cognate tRNA as was shown by gradient centrifugation, fluorescence quenching, and heat inactivation studies. With the first two methods, the stoichiometry of the complex was determined to be approximately 1:1. The latter method was used to calculate a binding constant \(5.6 \times 10^{-7} \text{M}^{-1}\) for tRNA. This value was very similar to the \(K_a\) values for tRNAaG1U in the aminolytic and the ATP-PPi exchange reactions determined from kinetic experiments.

An interesting observation was the lack of specificity in the tRNA-enzyme interactions. This fact was detected only by the most sensitive assay for complex formation, namely fluorescence quenching. At pH 5.5 the titration curve for tRNAa Val was very similar to that for tRNAaGlu (Fig. 5B) indicating a complex of similar stoichiometry and with a similar binding constant.

Possibly varying the ionic strength in these experiments may yield conditions in which there is discrimination between the cognate and noncognate interactions. However one must ask why these nonspecific interactions are not observed in the gradient centrifugation or the heat inactivation studies performed under similar conditions. Gradient centrifugation of GluRS and pure valyl-tRNA synthetase in presence of excess amounts of pure tRNAaGlu and tRNAaVal (Fig. 1) revealed the formation of only specific complexes. However, the presence in this experiment of a cognate tRNA for each enzyme might have prevented the expression of anomalous interactions because of competition by the cognate tRNA. Possibly the pressure effect during the ultracentrifugation helps to prevent detectable nonspecific complex formation. If there are overlapping sites for the various tRNA species on the enzyme as indicated by fluorescence quenching using successively several tRNA species in one experiment, then a nonspecific complex may exist in the heat inactivation experiment. But the geometry of such a complex may not be the correct one for protecting the enzyme against inactivation. The increasing degree of nonspecificity at lower pH may be correlated to a large transition in tertiary structure of tRNA at this pH. Such a change in physical structure has been observed with E. coli tRNA Tyr. The fact that noncognate tRNAs also show some interaction with aminoacyl-tRNA synthetases (see also 7) is less surprising when one considers that a significant part of the energy of complex formation is due to ionic interactions (16). Thus, claims for specificity of complex formation as evidenced by control experiments with only one noncognate tRNA may have to be interpreted cautiously (cf. 8).

No anomalous interaction was observed in studies of the protection of GluRS by tRNA against heat inactivation (Fig. 6). It thus seems that this approach permits one to measure more...
exclusively the part of the tRNA Glu-GluRS interaction which cannot take place in an anomalous interaction; possibly, the heat lability of GluRS might be due mostly to the instability of a chemical group or of a structural conformation in the active site of the enzyme, with which only the cognate tRNA could interact efficiently. In view of this specificity and of the observation that the binding constants calculated from heat protection experiments are very similar to the $K_m$ values, this method is a promising one to use in studying the interaction of modified tRNA Glu or of fragments of tRNA Glu with GluRS.

The fact that GluRS could be dialyzed against EDTA without loss of activity allowed us to perform the heat inactivation experiment described above in order to investigate the tertiary structure requirement of tRNA Glu for its proper interaction with GluRS. The results indicated that some element of tertiary structure is required to protect GluRS from inactivation. It would be interesting to find out how the presence of the enzyme influences the unfolding of tRNA structure. The requirement of some secondary and tertiary structure for aminoacylation of tRNA was also found in the studies on the temperature dependence of this reaction with enzymes from a thermophilic organism (26). Although the sequence of tRNA Glu is known, we cannot suggest any region of it as the site of interaction with the enzyme. At the present time, physical techniques do not permit us to define which parts of the primary structure of a tRNA molecule play a role in maintaining the native conformation of its tertiary structure.

Glutamyl-tRNA formation appears to follow a one step concerted mechanism as indicated by all the experiments we have performed. The striking similarity of the $K_m$ values of GluRS for tRNA Glu in the ATP-PPi exchange and the aminoacylation reactions and the absolute requirement for tRNA Glu in the ATP-PPi exchange catalyzed by GluRS strongly suggest that this reaction is the true reversal of the aminoacylation reaction. The fact that [y-32P]ATP is not cleaved in the presence of glutamate and an excess of enzyme unless tRNA Glu is added provides additional evidence for a concerted mechanism.

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