Conformation Change of $tRNA^{Glu}$ in the Complex with Glutamyl-tRNA Synthetase Is Required for the Specific Binding of L-Glutamate†

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ABSTRACT: The binding of *Thermus thermophilus* glutamyl-tRNA synthetase (GluRS) with *T. thermophilus* $tRNA^{Glu}$, *Escherichia coli* $tRNA^{Glu}$, and amino acids was studied by fluorescence measurements. In the absence of $tRNA^{Glu}$, GluRS binds with D-glutamate as well as L-glutamate. However, in the presence of *E. coli* $tRNA^{Glu}$, GluRS binds specifically with L-glutamate. The KCl effects on the Michaelis constants (K_m) for $tRNA^{Glu}$, L-glutamate, and ATP were studied for the aminoacylation of the homologous $tRNA^{Glu}$ and heterologous $tRNA^{Glu}$ species. As the KCl concentration is raised from 0 to 100 mM, the K_m value for L-glutamate in the heterologous system is remarkably increased whereas the K_m value for L-glutamate in the homologous system is only slightly increased. The circular dichroism analyses were made mainly of the bands due to the 2-thiouridine derivatives of $tRNA^{Glu}$ in the complex. The conformation change of *T. thermophilus* $tRNA^{Glu}$ upon complex formation with GluRS is not affected by addition of KCl. In contrast, the heterologous $tRNA^{Glu}$·GluRS complex is in an equilibrium of two forms that depends on KCl concentration. The predominant form at low KCl concentration is closely related to the small K_m value for L-glutamate. In this form of the complex, the conformation of $tRNA^{Glu}$ is appreciably different from that of free molecule. Accordingly, such a conformation change of $tRNA^{Glu}$ in the complex with GluRS is required for the specific binding of L-glutamate as the substrate.

Aminoacyl-tRNA synthetases are a group of enzymes that charge cognate tRNA species with cognate amino acids. The strict recognition of tRNA species by the cognate aminoacyl-tRNA synthetases plays essential roles in correct translation of the genetic code. The conformations of such tRNA species and aminoacyl-tRNA synthetase have been found to change upon the cognate complex formation, by the methods of *T*-jump (Riesner et al., 1976; Krauss et al., 1976), nuclease digestion (Yamashiro-Matsumura & Kawata, 1981), circular dichroism (CD)[1] (Willick & Kay, 1976), and fluorescence (Favre et al., 1979; Lefevre et al., 1980; Ehrlich et al., 1980). Such conformation changes have not been observed upon the formation of complexes, if any, of aminoacyl-tRNA synthetases with noncognate tRNA species (Krauss et al., 1976; Favre et al., 1979; Yamashiro-Matsumura & Kawata, 1981). The mutual adaptation of the cognate tRNA and aminoacyl-tRNA synthetase has been suggested to be related to the activation of catalytic site of the enzyme (Renaud et al., 1981; Bacha et al., 1982). Thus, the conformation change upon the formation of cognate complex seems to be important for the specific aminoacylation process.

We have taken up a system of glutamyl-tRNA synthetase (GluRS) from an extreme thermophile, *Thermus thermophilus* HB8, in combination with the $tRNA^{Glu}$ species from *T. thermophilus* and *Escherichia coli*. We have already found that GluRS from *T. thermophilus* is remarkably thermostable and is suitable for a variety of physicochemical measurements. This GluRS catalyzes the aminoacylation of heterologous $tRNA^{Glu}$ from *E. coli*, as well as the homologous $tRNA^{Glu}$ from *T. thermophilus* in the presence of 50 mM KCl and 10 mM $MgCl_2$ at pH 8.0 and 65 °C (Hara-Yokoyama et al., 1984). In the present study, we have analyzed the conformation change of $tRNA^{Glu}$ upon the formation of the complex with GluRS by the observation of circular dichroism (CD) and fluorescence and have found a conformation change of the $tRNA^{Glu}$·GluRS complex that allows the specific binding of L-glutamate.

Experimental Procedures

Materials. L-[U-^{14}C]Glutamic acid was purchased from Amersham International. ATP (dipotassium salt) was obtained from Sigma. L-Glutamic acid, L-tryptophan, and quinine sulfate dihydrate were obtained from Wako Pure Chemical Industries, D-glutamic acid was purchased from Nakarai Chemicals, and L-glutamine, L-aspartic acid, and L-alanine were obtained from Kyowa Hakko Co.

T. thermophilus Cells. T. thermophilus HB8 strain was kindly provided by Prof. T. Oshima. Cells were grown at 65 °C and then harvested by centrifugation near the end of the logarithmic phase of growth and stored at −80 °C.

Purification of GluRS from T. thermophilus. GluRS was purified to homogeneity from *T. thermophilus* cells as described previously (Hara-Yokoyama et al., 1984). In SDS–polyacrylamide gel electrophoresis, the final preparation of GluRS was found to migrate as a single polypeptide chain with a molecular weight of 50 000. The extinction coefficient (at 280 nm) of GluRS from *T. thermophilus* HB8 was found to be $E^{0.1\%} = 2.05$ mL mg^{-1} cm^{-1}.

Purification of $tRNA^{Glu}$ Species from E. coli and from T. thermophilus. E. coli $tRNA_2^{Glu}$ was purified according to the method of Ohashi and co-workers (1972), from the phenol extract kindly provided by Dr. Nishimura. The concentration

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[1] Abbreviations: ATP, adenosine 5′-triphosphate; CD, circular dichroism; GluRS, glutamyl-tRNA synthetase; mnm^{5}s^{2}U, 5-[(methylamino)methyl]-2-thiouridine; s^{2}T, 2-thioribothymidine; SDS, sodium dodecyl sulfate; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

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of this tRNA was determined with the molecular weight of 26 300 and the extinction coefficient (at 260 nm) of $E^{0.1\%}$ = 23.4 mL mg^{-1} cm^{-1} (Willick et al., 1973). *T. thermophilus* $tRNA_2^{Glu}$ was purified as reported previously (Hara-Yokoyama et al., 1984). The purified preparation of $tRNA_2^{Glu}$ was found to migrate as a single band in polyacrylamide gel electrophoresis under three different conditions (10% and 20% polyacrylamide gel in the absence of urea and 15% polyacrylamide gel in the presence of 7 M urea). The nucleotide sequence of *T. thermophilus* $tRNA_2^{Glu}$ was analyzed according to the procedure of Nishimura and Kuchino (1983); this tRNA species had 2-thioribothymidine (s^2T) instead of ribothymidine in position 54. One A_{260} unit of this $tRNA_2^{Glu}$ preparation accepted 1410 nmol of L-glutamate. The concentration of this $tRNA_2^{Glu}$ was obtained with the extinction coefficient ($E^{0.1\%}$) of *E. coli* $tRNA_2^{Glu}$.

Aminoacylation Reactions. All substrates, except one whose concentration was variable, were present in saturating concentrations, namely 4 mM ATP, 2 mM L-[U-^{14}C]glutamate (7 mCi/mmol), and 10 μM *E. coli* $tRNA^{Glu}$ or 5 μM *T. thermophilus* $tRNA^{Glu}$. The other components of the reaction mixtures for the aminoacylation reaction were 10 mM Tris-HCl (pH 8.0 at 65 °C), 10 mM $MgCl_2$, and 0–100 mM KCl. After various times of incubation at 65 °C, the aminoacylation product [^{14}C]Glu-tRNA was assayed as described (Hara-Yokoyama et al., 1984). The kinetic data were analyzed by a Lineweaver–Burk plot (Lineweaver & Burk, 1934).

Fluorescence Measurements. The fluorescence of GluRS was measured with a Hitachi 850 fluorescence spectrophotometer, where the cell temperature was controlled at 60 °C. The fluorescence was excited at 290 nm and was observed at 350 nm to avoid Raman scattering of water molecules. The fluctuations in source intensity was found to be negligible with 0.2 μM quinine sulfate as the fluorescence standard. For the correction of the inner filter effect, observed fluorescence intensities were multiplied by $[A(1 - 10^{-A_0})]/[A_0(1 - 10^{-A})]$, where A_0 is the absorbance of the enzyme alone at the exciting wavelength and A is the total absorbance at this wavelength after the addition of tRNA (Hélène et al., 1971). The validity of this correction was confirmed by using tryptophan and ATP. The sample solution contained 10 mM Tris-HCl (pH 7.0 at 60 °C), 10 mM $MgCl_2$, 5 mM 2-mercaptoethanol, specified amounts of *T. thermophilus* GluRS, *T. thermophilus* $tRNA^{Glu}$, or *E. coli* $tRNA^{Glu}$, and glutamate. For the curve fitting of experimental data, the method of nonlinear least squares was used with variable parameters including the association constant and the initial and final quenching levels.

CD Measurements. The CD spectra of *T. thermophilus* $tRNA^{Glu}$ (30 μM), *E. coli* $tRNA^{Glu}$ (30 μM), and/or *T. thermophilus* GluRS (30 μM) were recorded on a Jasco J-40 spectropolarimeter, where the cell temperature was controlled at 60 °C. The sample solution contained 10 mM Tris-HCl (pH 7.0 at 60 °C), 10 mM $MgCl_2$, 5 mM 2-mercaptoethanol, and 50% (v/v) glycerol, in addition to $tRNA^{Glu}$ and/or GluRS. CD spectra are presented on the basis of the molar concentration of the mnm^5s^2U or s^2T residue.

Results and Discussion

Binding of T. thermophilus $tRNA^{Glu}$ and E. coli $tRNA^{Glu}$ to T. thermophilus GluRS. The association constants for the binding of these two $tRNA^{Glu}$ species with *T. thermophilus* GluRS were obtained from the observation of the fluorescence due to the tryptophan residues (Hélène et al., 1971). As shown in Figure 1, the fluorescence intensity of GluRS at 350 nm was decreased upon the addition of $tRNA^{Glu}$ species. The observed dependences of fluorescence intensities on the tRNA

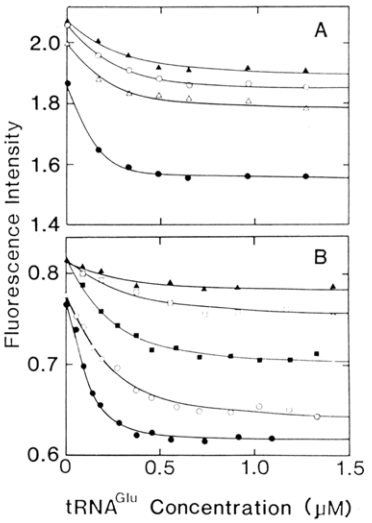

FIGURE 1: Dependence of fluorescence intensity (pH 7.0, 60 °C, 10 mM $MgCl_2$) of *T. thermophilus* GluRS [0.2 μM for (A), 0.1 μM for (B)] on the concentration of *T. thermophilus* $tRNA^{Glu}$ (A) and *E. coli* $tRNA^{Glu}$ (B), in the presence of KCl at various concentrations. The solid lines show the simulated fluorescence intensity curves as obtained by the nonlinear least-squares method. The association constants (in 10^6 M^{-1}) for *T. thermophilus* $tRNA^{Glu}$ (A) are thus estimated as 75 ± 21 for 0 mM KCl (●), 22 ± 5 for 50 mM KCl (△), 12 ± 2 for 100 mM KCl (○), and 8 ± 4 for 150 mM KCl (▲). The association constants (in 10^6 M^{-1}) for *E. coli* $tRNA^{Glu}$ (B) are obtained as 81 ± 27 for 0 mM KCl (●), 9 ± 2 for 30 mM KCl (○), 10 ± 2 for 60 mM KCl (■), 6 ± 2 for 100 mM KCl (□), and 7 ± 5 for 150 mM KCl (▲).

concentration were closely simulated, by the method of least squares, with 1:1 stoichiometry for the complex of GluRS and tRNA (Figure 1) rather than with 1:2 stoichiometry. The association constants thus obtained are listed in the legend of Figure 1. As the concentration of KCl is raised, the association constant for the complex of *T. thermophilus* $tRNA^{Glu}$ and GluRS and the association constant for the complex of *E. coli* $tRNA^{Glu}$ and *T. thermophilus* $tRNA^{Glu}$ are both decreased in a manner similar to each other. Therefore, at various concentrations of KCl (0–150 mM), the affinity of binding with GluRS is not much different between the homologous $tRNA^{Glu}$ species of *T. thermophilus* and the heterologous $tRNA^{Glu}$ species of *E. coli*.

k_{cat} and K_m Values for the Aminoacylation of T. thermophilus $tRNA^{Glu}$ and E. coli $tRNA^{Glu}$ Species. The aminoacylation reactions of the homologous and heterologous $tRNA^{Glu}$ species by *T. thermophilus* GluRS were compared at various concentrations of KCl. The dependences of catalytic constants (k_{cat}) on KCl concentration are shown in Figure 2. As for the aminoacylation of the homologous $tRNA^{Glu}$, the k_{cat} value is drastically increased as the KCl concentration is raised from 0 mM to the optimum KCl concentration of 100 mM and then is decreased as the KCl concentration is raised to 200 mM. In contrast, for the aminoacylation of the heterologous $tRNA^{Glu}$ species, the k_{cat} value is slightly increased as the KCl concentration is raised from 0 mM to the optimum concentration of 30 mM and then is remarkably decreased as the KCl concentration is raised to 200 mM. Such a remarkable difference in the salt effect suggests that the properties of the Michaelis complexes are significantly different between the homologous complex and heterologous complex.

Furthermore, the KCl effects on Michaelis constants (K_m) were examined for the aminoacylation of *T. thermophilus* $tRNA^{Glu}$ and *E. coli* $tRNA^{Glu}$ (Figure 3). For ATP as a substrate, the K_m values are not affected by the addition of KCl in the aminoacylation of the homologous *T. thermophilus*

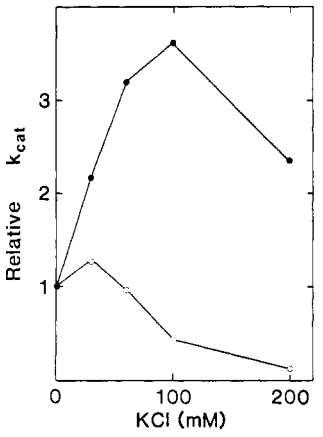

FIGURE 2: Relative k_{cat} values for the aminoacylation of *T. thermophilus* $tRNA^{Glu}$ (●) and *E. coli* $tRNA^{Glu}$ (○) by *T. thermophilus* GluRS (pH 8.0 at 65 °C, 10 mM $MgCl_2$), in the presence of KCl at various concentrations. At the KCl concentration of 50 mM, the k_{cat} values for the aminoacylation of *T. thermophilus* $tRNA^{Glu}$ and *E. coli* $tRNA^{Glu}$ have been found to be 3.0 and 2.9 s^{-1}, respectively (Hara-Yokoyama et al., 1984).

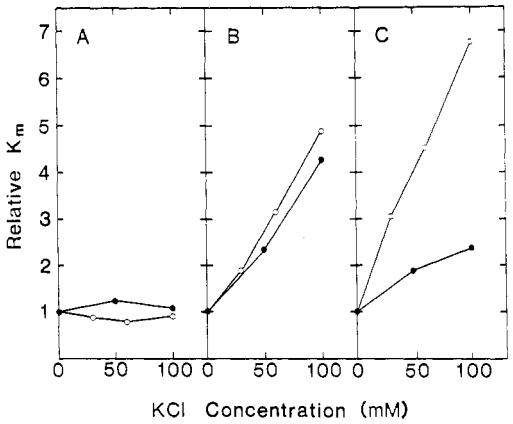

FIGURE 3: Relative K_m values for ATP (A), $tRNA^{Glu}$ (B), and L-glutamate (C) in the aminoacylation of *T. thermophilus* $tRNA^{Glu}$ (●) and *E. coli* $tRNA^{Glu}$ (○) by *T. thermophilus* GluRS (pH 8.0 at 65 °C, 10 mM $MgCl_2$), in the presence of KCl at various concentrations. For the measurements of K_m values for ATP, 2 mM L-[U-^{14}C]glutamate (7 mCi/mmol), 10 μM *E. coli* $tRNA^{Glu}$ (○) or 5 μM *T. thermophilus* $tRNA^{Glu}$ (●), and various concentrations of ATP were used. For the measurements of K_m values for $tRNA^{Glu}$, 4 mM ATP, 2 mM L-[U-^{14}C]glutamate (7 mCi/mmol), and various concentrations of *E. coli* (○) or *T. thermophilus* $tRNA^{Glu}$ (●) were used. For the measurements of K_m values for L-glutamate, 4 mM ATP, 10 μM *E. coli* $tRNA^{Glu}$ (○) or 5 μM *T. thermophilus* $tRNA^{Glu}$ (●), and various concentrations of L-[U-^{14}C]glutamate were used. At the KCl concentration of 50 mM, the K_m values for ATP, $tRNA^{Glu}$, and L-glutamate have been found to be 230 μM, 0.65 μM, and 70 μM, respectively, for homologous aminoacylation, and 300 μM, 0.60 μM, and 80 μM, respectively, for heterologous aminoacylation (Hara-Yokoyama et al., 1984).

$tRNA^{Glu}$ or heterologous *E. coli* $tRNA^{Glu}$ species (Figure 3A). However, the K_m values for the homologous *T. thermophilus* $tRNA^{Glu}$ and the heterologous *E. coli* $tRNA^{Glu}$ species are both increased 4–5-fold on addition of KCl up to 100 mM (Figure 3B). This is consistent with the finding from the fluorescence analysis that the binding of $tRNA^{Glu}$ with GluRS is weakened on addition of KCl (Figure 1). In contrast, the KCl effect on the K_m value of L-glutamate in the aminoacylation of *T. thermophilus* $tRNA^{Glu}$ is clearly different from that in the aminoacylation of *E. coli* $tRNA^{Glu}$ (Figure 3C). On addition of KCl up to 100 mM, the K_m value for L-glutamate is slightly increased (only 2-fold) in the aminoacylation of homologous *T. thermophilus* $tRNA^{Glu}$ species, while the K_m value for L-glutamate is remarkably increased

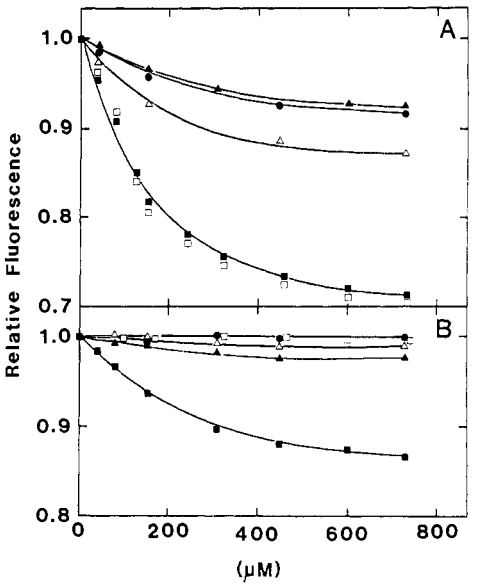

FIGURE 4: Dependence of the fluorescence intensity of *T. thermophilus* GluRS (0.2 μM, pH 7.0 at 60 °C, 10 mM $MgCl_2$) on the concentration of L-glutamate (■), D-glutamate (□), L-glutamine (▲), L-aspartate (△), or L-alanine (●) in the absence (A) of and in the presence (B) of *E. coli* $tRNA^{Glu}$ (2 μM).

(7-fold) in the aminoacylation of heterologous *E. coli* $tRNA^{Glu}$ species.

Binding of L-*Glutamate or* D-*Glutamate to T. thermophilus GluRS.* Then the interaction of L-glutamate or D-glutamate with GluRS was studied, by fluorescence observation, in the absence of and in the presence of *E. coli* $tRNA^{Glu}$ species. In the absence of $tRNA^{Glu}$ (Figure 4A), the fluorescence intensity (350 nm) of *T. thermophilus* GluRS is decreased as the glutamate concentration is raised from 0 to 730 μM. The quenching curves for L-glutamate and D-glutamate are practically the same as each other. In comparison, the fluorescence quenching by the addition of L-glutamine is much weaker than that of glutamate (Figure 4A). Accordingly, such a fluorescence quenching of GluRS in the absence of $tRNA^{Glu}$ is probably due to a largely electrostatic interaction between the negatively charged carboxylate group in the side chain of glutamate and positively charged group(s) in the binding site for L-glutamate and/or $tRNA^{Glu}$ of GluRS. The quenching by L-aspartate is much weaker than that of L-glutamate but is still appreciably stronger than that of L-alanine, probably because of the effect from the carboxylate group in the side chain of L-aspartate.

In the presence of $tRNA^{Glu}$ (Figure 4B), the fluorescence intensity of GluRS is also decreased as the concentration of L-glutamate is raised from 0 to 730 μM (Figure 4B). By contrast, the addition of D-glutamate, L-glutamine, L-aspartate, or L-alanine up to 730 μM does not affect the fluorescence intensity of GluRS (Figure 4B). This indicates that noncognate amino acids interact with GluRS in the absence of $tRNA^{Glu}$ but essentially not in the presence of $tRNA^{Glu}$. Thus, the specific interaction of L-glutamate, rather than noncognate amino acids (including D-glutamate), to GluRS occurs only in the presence of $tRNA^{Glu}$. This is consistent with the previous report that L-glutamate binds to *E. coli* GluRS only in the presence of the cognate $tRNA^{Glu}$ (Kern & Lapointe, 1979). Thus, the binding of $tRNA^{Glu}$ with GluRS induces a conformation change in GluRS, allowing the specific interaction with L-glutamate rather than D-glutamate. Therefore, the difference in the KCl effect on the K_m value of L-glutamate (Figure 3B) may now be ascribed to the difference in the interaction of *T. thermophilus* GluRS with the homologous

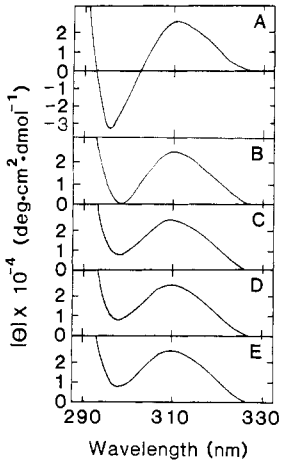

FIGURE 5: CD spectra of *T. thermophilus* $tRNA^{Glu}$ at 22 (A) and 60 (B) °C and of the complex of *T. thermophilus* $tRNA^{Glu}$ with GluRS in the presence of KCl at the concentration of 0 (C), 50 (D), and 100 (E) mM at 60 °C (pH 7.0, 10 mM $MgCl_2$).

$tRNA^{Glu}$ species (*T. thermophilus*) and the heterologous $tRNA^{Glu}$ species (*E. coli*).

CD Spectra of T. thermophilus $tRNA^{Glu}$ and E. coli $tRNA^{Glu}$ Species. The CD spectrum (290–330 nm) of *T. thermophilus* $tRNA^{Glu}$ at 22 °C is shown in Figure 5A. The positive CD band at ~310 nm is due to the 2-thioribothymidine (s^2T) residue in position 54 (the sequence data not shown). The intensity and position of this CD band are not affected by raising the temperature from 22 to 60 °C (Figure 5B). However, the negative CD band as observed at 295 nm at 22 °C is shifted to 298 nm, with a significant intensity decrease as the temperature is raised from 22 to 60 °C (Figure 5B).

The CD spectra (300–360 nm) of *E. coli* $tRNA^{Glu}$ at 23 and 60 °C are shown in parts A and B of Figure 6, respectively. The negative CD band at 335 nm is due to the 5-(methylamino)methyl-2-thiouridine (mnm^5s^2U) residue in the first position of the anticodon (Willick & Kay, 1976). Another negative CD band is observed at 305 nm at 23 °C and at 315 nm at 60 °C. On the other hand, *T. thermophilus* GluRS (at the concentration of 30 μM) does not exhibit any CD bands in the wavelength region longer than 290 nm (data not shown). Therefore, the CD bands (300–360 nm) of $tRNA^{Glu}$ species may be used for studying the conformation changes of $tRNA^{Glu}$ upon the formation of complexes with *T. thermophilus* GluRS.

CD Spectra and Conformation of T. thermophilus $tRNA^{Glu}$ in the Complex with T. thermophilus GluRS. The CD spectrum of *T. thermophilus* $tRNA^{Glu}$ (30 μM) in the presence of *T. thermophilus* GluRS at 60 °C is shown in Figure 5C. In this solution, more than 98% of $tRNA^{Glu}$ is bound to GluRS, as estimated from the association constant (legend to Figure 1A). Accordingly, the CD curve in Figure 5C is predominantly due to the $tRNA^{Glu}$·GluRS complex.

T. thermophilus tRNA species commonly have the s^2T residue in place of the ribothymidine residue in position 54 (Watanabe et al., 1976). The s^2T residue exhibits the positive CD band at ~310 nm in the native conformation of tRNA. However, upon melting of tRNA, the T-loop no longer interacts with the D-loop, and then the $s^2T(54)$ residue exhibits the negative CD band at 330 nm, exactly the same as that of the s^2T monomer (Watanabe et al., 1976). Thus, the CD band due to the s^2T residue reflects the tertiary interaction involving this residue in the tRNA molecule. As for *T. thermophilus* $tRNA^{Glu}$, the CD band (at 310 nm) due to the s^2T residue is

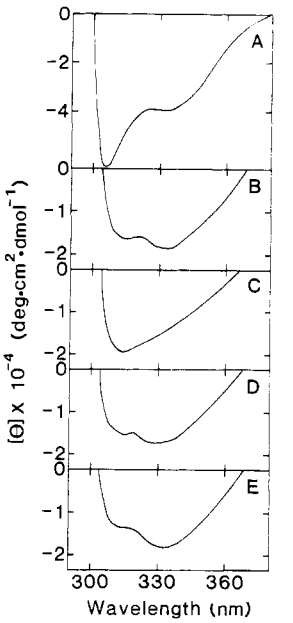

FIGURE 6: CD spectra of *E. coli* $tRNA^{Glu}$ at 23 (A) and 60 (B) °C and of the complex of *E. coli* $tRNA^{Glu}$ with *T. thermophilus* GluRS in the presence of KCl at the concentration of 0 (C), 50 (D), and 100 (E) mM at 60 °C (pH 7.0, 10 mM $MgCl_2$).

affected little by the complex formation with *T. thermophilus* GluRS at 60 °C (Figure 5B,C), indicating that the intramolecular interaction between the T-loop and D-loop of $tRNA^{Glu}$ is not significantly altered.

In addition to the positive CD band at 310 nm, *T. thermophilus* $tRNA^{Glu}$ at 22 °C exhibits a negative CD band at 295 nm, which is shifted to 298 nm at 60 °C (Figure 5A,B). This negative CD band at ~295 nm has been found to be due to tertiary interactions (Willick et al., 1973). As for *T. thermophilus* $tRNA^{Glu}$, the intensity of the negative CD band at 298 nm appreciably changes upon the formation of complex with GluRS (Figure 5C). This indicates that the conformation of *T. thermophilus* $tRNA^{Glu}$ species appreciably changes upon the formation of complex with *T. thermophilus* GluRS.

Conformation Change of E. coli $tRNA^{Glu}$ upon Complex Formation with T. thermophilus GluRS. The CD spectrum of *E. coli* $tRNA^{Glu}$ (30 μM), in the presence of *T. thermophilus* GluRS (30 μM) at 60 °C, is shown in Figure 6C. In this solution, more than 98% of $tRNA^{Glu}$ is bound to GluRS, as estimated from association constants (legend to Figure 1). Accordingly, the CD curve in Figure 6C is predominantly due to the $tRNA^{Glu}$·GluRS complex. As for this complex, the intensity of the negative CD band at 315 nm is significantly increased while the intensity of the negative CD band (335 nm, due to mnm^5s^2U) is decreased (Figure 6C), as compared with the CD bands of free *E. coli* $tRNA^{Glu}$ (Figure 6B). Similar CD changes have been reported for the complex formation of *E. coli* $tRNA^{Glu}$ with *E. coli* GluRS (Willick & Kay, 1976). These indicate that the conformation of *E. coli* $tRNA^{Glu}$ also changes appreciably upon complex formation with *T. thermophilus* GluRS at 65 °C.

KCl Effect on CD Spectra of Heterologous Complex of E. coli $tRNA^{Glu}$ and T. thermophilus GluRS. The CD spectrum of *T. thermophilus* $tRNA^{Glu}$ as bound to GluRS (60 °C; Figure 5C) is not affected by the addition of KCl up to the concentration of 50 (Figure 5D) or 100 mM (Figure 5E). By contrast, the CD spectrum of *E. coli* $tRNA^{Glu}$ as bound to *T. thermophilus* GluRS (Figure 6C) remarkably changes on addition of KCl up to the concentration of 50 (Figure 6D) and 100 mM (Figure 6E). In particular, in the presence of 100

mM KCl, the negative CD band (335 nm) of *E. coli* $tRNA^{Glu}$ as bound to GluRS is as strong as that of *E. coli* $tRNA^{Glu}$ itself. It should be emphasized here that, in the experimental condition of this CD measurement, more than 90% of *E. coli* $tRNA^{Glu}$ is bound to GluRS, as estimated from the association constant (legend to Figure 1). Accordingly, the intensity change of the negative CD band (335 nm) of *E. coli* $tRNA^{Glu}$ is not due to partial dissociation of the $tRNA^{Glu}$·GluRS complex. On the other hand, in the absence of *T. thermophilus* GluRS, the CD spectrum of *E. coli* (or *T. thermophilus*) $tRNA^{Glu}$ is not affected by the addition of KCl up to the concentration of 100 mM (data not shown). All these observations indicate that the conformation change of *E. coli* $tRNA^{Glu}$ upon complex formation with *T. thermophilus* GluRS is largely suppressed by the addition of KCl up to 100 mM.

Conformation Equilibrium of $tRNA^{Glu}$·GluRS Complex. As for the binding of aminoacyl-tRNA synthetase with the cognate tRNA species, a two-step mechanism has been suggested (Schimmel & Söll, 1979). The enzyme and tRNA form an initial complex (form I) in which the conformations of constituent molecules are much the same as those in the free state. Subsequently, the conformation of the complex changes from form I to another form (form II). In the present case of the heterologous complex of *E. coli* $tRNA^{Glu}$ and *T. thermophilus* GluRS, an apparent isosbestic point was found at about 325 nm in the CD spectra for the KCl concentration range of 0–100 mM (Figure 6). This shows that the heterologous $tRNA^{Glu}$·GluRS complex is, in fact, in an equilibrium of two forms (forms I and II), which is now found to depend on the KCl concentration. The conformation of $tRNA^{Glu}$ in the complex at high KCl concentration (form I) is similar to that of free $tRNA^{Glu}$, while the conformation of $tRNA^{Glu}$ in the complex at low KCl concentration (form II) is significantly different from that of free $tRNA^{Glu}$ (Figure 6). This KCl-dependent conformation equilibrum is found to correlate with the enzyme activity of *T. thermophilus* GluRS as described below.

Conformation Change of $tRNA^{Glu}$·GluRS Complex Is Required for Specific Binding of L-*Glutamate.* In relation to the KCl effect on the conformation equilibrium of the heterologous complex, the KCl effect on the Michaelis constants for the three substrates will now be discussed. The K_m value for ATP is not affected by the addition of KCl in homologous aminoacylation or in heterologous aminoacylation (Figure 3A). Thus, the binding of ATP to *T. thermophilus* GluRS is not related to the conformation change of the complex. On the other hand, the K_m values for *T. thermophilus* $tRNA^{Glu}$ and *E. coli* $tRNA^{Glu}$ species are increased 4–5-fold by the addition of KCl up to 100 mM (Figure 3B). The addition of KCl will reduce the electrostatic interaction between $tRNA^{Glu}$ and GluRS. However, such KCl effects on the K_m values as found for both $tRNA^{Glu}$ species are not correlated to the KCl effect on the conformation as found only for *E. coli* $tRNA^{Glu}$ in the complex with GluRS.

By contrast, the KCl effect on the K_m value for L-glutamate in homologous aminoacylation is significantly different from that in heterologous aminoacylation (Figure 3C). In the homologous system, the K_m value of L-glutamate is increased only 2-fold on the addition of KCl up to the concentration of 100 mM and the conformation of $tRNA^{Glu}$ as bound to GluRS is not affected (Figure 5). By contrast, in the heterologous system, the K_m value of L-glutamate is increased as much as 7-fold on addition of KCl up to the concentration of 100 mM, in close correspondence to the conformation change of the complex as found by the CD analysis (Figure 6). These observations indicate that, for the heterologous complex of *E. coli* $tRNA^{Glu}$ and *T. thermophilus* GluRS, the conformation change of the complex from form I to form II is closely related to L-glutamate binding with GluRS.

Furthermore, the fluorescence quenching of the heterologous $tRNA^{Glu}$·GluRS complex by L-glutamate is much weaker in the presence of 100 mM KCl (data not shown) than in the absence of KCl (Figure 4B). Thus the $tRNA^{Glu}$·GluRS complex in form I binds only weakly with L-glutamate while the complex in form II strongly binds with L-glutamate, discriminating L-glutamate from noncognate amino acids including D-glutamate. These observations allow us to conclude that the conformation change of the $tRNA^{Glu}$·GluRS complex from form I to form II is required for specific binding with L-glutamate as the substrate.

Structure Difference between T. thermophilus $tRNA^{Glu}$ and E. coli $tRNA^{Glu}$. The KCl-dependent conformation equilibrium is thus observed for the complex of *T. thermophilus* GluRS with *E. coli* $tRNA^{Glu}$ rather than with *T. thermophilus* $tRNA^{Glu}$. This should be ascribed to the structure difference between the two $tRNA^{Glu}$ species. There are at least two major differences in the primary structure between these $tRNA^{Glu}$. Firstly, in the variable loop, *E. coli* $tRNA^{Glu}$ has four nucleotides (Ohashi et al., 1972; Munninger & Chang, 1972), while *T. thermophilus* $tRNA^{Glu}$ has five nucleotides (Hara-Yokoyama et al., unpublished experiments). From the crystal analyses of yeast $tRNA^{Phe}$ and $tRNA^{Asp}$ species, the number of nucleotides in the variable loop has been found to affect the tertiary interaction between the D-loop and the variable loop (Giegé et al., 1984). Such an interaction is probably different between *E. coli* $tRNA^{Glu}$ and *T. thermophilus* $tRNA^{Glu}$. Secondly, in the first position of the anticodon, *E. coli* $tRNA^{Glu}$ has mnm^5s^2U (Ohashi et al., 1972), while *T. thermophilus* $tRNA^{Glu}$ has cytidine (Hara-Yokoyama et al., 1986). The electrostatic interaction, between the positively charged 5 substituent of mnm^5s^2U of $tRNA^{Glu}$ and negatively charged residue(s), if any, of GluRS will be weakened on addition of KCl.

Concluding Remarks. The extremely low error rate of the aminoacylation reaction may not be explained only by the difference in the affinity constants between the cognate tRNA and noncognate tRNA species (Ebel et al., 1973). In fact, the specific aminoacylation reaction can be attained only after the catalytic site of the aminoacyl-tRNA synthetase is formed by the binding with the cognate tRNA (Renaud et al., 1981). In the present study, the KCl-dependent conformation equilibrium is found for the complex of *T. thermophilus* GluRS and *E. coli* $tRNA^{Glu}$ species. As for the specific binding of GluRS with L-glutamate, the active form is dominant at low KCl concentration while the inactive form is dominant at high KCl concentration. Accordingly, proton NMR analyses on this heterologous complex are now in progress in our laboratory (to be reported separately), in the hope of elucidating the structural difference between the active form and the inactive form of the complex.

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Registry No. GluRS, 9068-76-2; ATP, 56-65-5; D-Glu, 6893-26-1; L-Glu, 56-86-0.

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Effect of Al^{3+} plus F^- on the Catecholamine-Stimulated GTPase Activity of Purified and Reconstituted G_s[†]

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ABSTRACT: The effects of Al^{3+} and F^- on the catecholamine-stimulated GTPase cycle were studied by using reconstituted phospholipid vesicles that contained purified β-adrenergic receptor and the stimulatory GTP-binding protein of the adenylate cyclase system, G_s. Al^{3+}/F^- activated reconstituted G_s to levels previously reported for detergent-solubilized, purified G_s, although both activation and deactivation were faster in the reconstituted preparation. Under these conditions, Al^{3+}/F^- did not inhibit by more than 15% the β-adrenergic agonist-stimulated GTPase activity of the vesicles nor did it significantly inhibit the rates of GTP binding, GTP hydrolysis, or GDP release. When Mg^{2+} (50 mM) was used instead of agonist to promote GTP hydrolysis in the receptor-G_s vesicles, Al^{3+}/F^- was found to inhibit GTPγS binding, GDP release, and steady-state GTPase activity to unstimulated levels. These data can be interpreted as indicating that the receptor catalyzes nucleotide exchange by G_s faster or more efficiently than does Mg^{2+}.

Although stimulation of adenylate cyclase activity by fluoride was noted in the initial description of the enzyme by Rall and Sutherland (1958), little was known about its mechanism of stimulation for over a decade. Ross and Gilman (1977) showed that fluoride acts on a GTP-binding, regulatory protein (G_s)[1] that is distinct from adenylate cyclase itself and acts as an intermediary regulator between receptor and the cyclase [see Ross and Gilman (1980)]. Sternweis and Gilman (1982) later showed that Al^{3+} or Be^{2+} was also required for stimulation of G_s by F^- and suggested that AlF_4^- or FeF_3^- might be the activating species.[2] The mode of action of

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[1] Abbreviations: DTT, dithiothreitol; G_s, stimulatory, GTP-binding protein of the adenylate cyclase system; GTPγS, guanosine 5′-*O*-(3-thiotriphosphate).

[2] Although it is likely that the AlF_4^- anion is the relevant species, we use Al^{3+}/F^- to refer to the mixture of $AlCl_3$ and NaF, usually 0.1 and 20 mM, respectively, that was used.

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