Articles

A Fluorescence Spectroscopic Study of Substrate-Induced Conformational Changes in Glutaminyl-tRNA Synthetase†

Tista Bhattacharyya and Siddhartha Roy*

Department of Biophysics, Bose Institute, P-1/12 CIT Scheme VII M, Calcutta 700 054, India

Received October 19, 1992; Revised Manuscript Received February 15, 1993

ABSTRACT: Glutaminyl-tRNA synthetase from *Escherichia coli* is a member of a subgroup of aminoacyl-tRNA synthetases that do not catalyze ATP–PP_i exchange in the absence of the cognate tRNA. Such behavior suggests conformational changes upon substrate binding. Two different fluorescent probes, pyrenylmaleimide and acrylodan, were used to specifically label a nonessential sulfhydryl group of GlnRS. Conformational changes induced by substrates were studied using glutaminyl-tRNA synthetase labeled with these two environment-sensitive probes. ATP was shown to cause a significant conformational change that alters the mode of binding to $tRNA^{Gln}$ to GlnRS. The alteration of the salt sensitivity pattern of $tRNA^{Gln}$ binding to GlnRS by ATP supports this. Binding of $tRNA^{Gln}$ causes a conformational change that may be different in nature for the ATP/GlnRS complex and free GlnRS. Hydrodynamic parameters deduced from fluorescence polarization studies and the use of a noncovalent probe indicate that the ATP-induced conformational change may not be global in character.

Aminoacyl-tRNA synthetases catalyze reactions of the same chemical nature as shown below:

ATP + amino acid + tRNA → aminoacyl-tRNA + AMP + PP_i

Their sequence and structure, however, vary widely (Schimmel, 1987). Recent structure and sequence analysis has indicated that they may be classified into two classes and possibly even subclasses (Eriani et al., 1990; Nagel & Dolittle, 1991). The two major classes differ in the site of aminoacylation (2′-OH vs 3′-OH) and the presence of Rossman fold and some signature sequences such as HIGH and KMSKS. The existence of such classes and subclasses may indicate evolutionary relationships among the aminoacyl-tRNA synthetases and may be a key to understanding the evolution of the genetic code and protein synthesis. Apart from the sequence and structural homologies, the unity of enzyme mechanisms and associated structural and conformational changes could be of great importance in understanding interrelationships of aminoacyl-tRNA synthetases.

Most aminoacyl-tRNA synethetases catalyze the partial reaction, ie., tRNA-independent ATP–PP_i exchange. A subgroup of *Escherichia coli* aminoacyl-tRNA synthetases, glutamyl-, glutaminyl-, and arginyl-tRNA synthetase do not catalyze the tRNA-independent ATP–PP_i exchange. ATP–PP_i exchange, however, is known to occur in the presence of the cognate tRNA (Ravel et al., 1965). These aminoacyl-tRNA synthetases are all small monomeric proteins. A high degree of sequence homology between glutamyl- and glutaminyl-tRNA synthetases (Breton et al., 1986) is also indicative of similarity within this group.

We have chosen to study glutaminyl-tRNA synthetase as a representative of this subclass of enzyme. The enzyme is a monomer of molecular mass 63 kDa (550 amino acids). The primary sequence of glutaminyl-tRNA synthetase (GlnRS)[1] is known (Yamao et al., 1982), and the crystal structure of its complex with ATP/$tRNA^{Gln}$ is also known (Rould et al., 1990). The importance of $tRNA^{Gln}$ in promoting ATP–PP_i exchange may suggest substrate-induced conformational changes during the catalytic cycle. Substrate-induced conformational changes in many proteins are well-known. Such conformational changes are of fundamental importance to the functioning of proteins and are known to mediate allosteric effects, enhancement of substrate specificity (Anderson et al., 1979), etc. tRNA-induced conformational changes had been reported in some aminoacyl-tRNA synthetases, although their functional relevance is unclear at this moment (Ferguson & Yang, 1986).

In a previous study (Bhattacharyya et al., 1991), we have shown that the binding of $tRNA^{Gln}$ does not affect binding constants of other substrates, to any significant extent. We have also reported that binding of $tRNA^{Gln}$ alone does not alter the structure of the enzyme in a significant way. In this study, we have shown that ATP induces a significant conformational change in GlnRS which changes the mode of interaction of $tRNA^{Gln}$ with GlnRS.

MATERIALS AND METHODS

Materials. Pyrenylmaleimide, tempol, and acrylodan were purchased from Molecular Probes Inc. (Eugene, OR). Sephadex G-25 was from Pharmacia United (Sweden). All other reagents were of analytical grade.

Enzyme and $tRNA^{Gln}$ Purification. Glutaminyl-tRNA synthetase and $tRNA^{Gln}$ were purified according to Bhattacharyya et al., 1991). Enzyme assays are also described in the same paper.

† Supported by the Council of Scientific and Industrial Research (India).

* To whom correspondence should be addressed.

[1] Abbreviations: GlnRS, glutaminyl-tRNA synthetase; TyrRS, tyrosyl-tRNA synthetase; PM, pyrenylmaleimide; AC, acrylodan; DTNB, dithiobis(2-nitrobenzoic acid); DMF, *N*,*N*-dimethylformamide; tempol, 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl.

0006-2960/93/0432-9268$04.00/0

Fluorescence Methods. All spectra were recorded in a Hitachi F 3000 spectrofluorometer. Excitation and emission band-passes were 5 nm unless mentioned otherwise. Spectra of appropriate buffers were always subtracted from fluorescence spectra. Corrections due to the inner filter effect were done according to the formula:

$$F = F_{obs}\,\text{antilog}[(A_{ex} + A_{em})/2]$$

where A_{ex} is the absorbance at the excitation wavelength and A_{em} is the absorbance at the emission wavelength. Binding of $tRNA^{Gln}$ was determined from quenching of tryptophan fluorescence according to Bhattacharyya et al. (1991). The binding experiments were completed within 30 min. Under these conditions, no significant quenching of the tryptophan fluorescence of the free GlnRS occurs upon addition of an equivalent amount of buffer. The quenching data were fitted to a single binding site equation by a nonlinear least-squares fit procedure. In this procedure, the initial fluorescence, the fluorescence at infinite ligand concentration, and the dissociation constant were systematically varied within a given range. The set of values that gave the lowest χ^2 was chosen as the best-fit curve.

Fluorescence lifetimes were determined on an Applied Photophysics single photon counting apparatus using a nitrogen lamp as an excitation source. The output was deconvoluted and fitted to a double-exponential function. The reduced χ^2 values obtained for the best-fit curves were close to 1, indicating a good fit. The experiments were conducted in 0.1 M Tris-HCl, pH 7.5, containing 15 mM $MgCl_2$ at 25 °C. The excitation wavelength was 337 nm, and the emission was at 373 nm.

Chemical Modifications. DTNB modification of GlnRS was carried out in 0.1 M potassium phosphate buffer, pH 8.0, at 25 °C; 5 μM GlnRS was incubated with 0.5 mM DTNB, and the absorbance at 412 nm was monitored. Pyrene modification of GlnRS was done according to Bhattacharyya et al. (1991). For acrylodan labeling, 5 μM GlnRS was mixed with a DMF solution of acrylodan to a final concentration of 50 μM acrylodan, so as to keep the final DMF concentration below 1.5%. After appropriate times, the reaction was quenched with 2-mercaptoethanol to a final concentration of 1 mM and dialyzed extensively. The incorporation ratio was calculated using a molar extinction coefficient of 16 400 M^{-1} cm^{-1} (Haugland, 1989). The protein was estimated by the Bio-Rad protein assay using unmodified GlnRS as the standard (Bradford, 1976). For binding studies, GlnRS was mixed with acrylodan or pyrenylmaleimide in a 1:1 molar ratio (typically at 35 μM). After 30 (AC) or 15 (PM) min of reaction, the mixture was put on a Sephadex G-25 column (1.4 × 14 cms), equilibrated in 0.1 M Tris-HCl, pH 7.5, containing 15 mM $MgCl_2$, and eluted with the same buffer.

Perrin Plot. Correlation times and limiting anisotropies were determined from isothermal Perrin plots. The experiment was performed using 0.5 μM pyrene-labeled GlnRS in the absence of ATP or in the presence of 2 mM ATP. The viscosity of the solution was varied by inclusion of sucrose in the range of 9–50% in 0.1 M Tris-HCl, pH 7.5, containing 15 mM $MgCl_2$. The viscosity values were obtained from Weast (1977). The correlation times were calculated using a second-order average lifetime, $\langle\tau_{av}\rangle = (\alpha_1\tau_1^2 + \alpha_2\tau_2^2)/(\alpha_1\tau_1 + \alpha_2\tau_2)$, of the biexponential fluorescence decay (Benecky et al., 1990).

RESULTS

Sulfhydryl groups of proteins are favorite attachement points for external covalent probes (e.g., fluorescence labels, spin-labels, etc.) because of the high degree of group specificity exhibited by sulfhydryl reagents. Nonessential sulfhydryl groups are ideal for such purposes. GlnRS has 10 cysteine residues, 2 of which are probably involved in a disulfide linkage (Rould et al., 1990). We have measured the cysteine reactivities of GlnRS toward DTNB. The absorbance increase was followed at 412 nm upon incubation of free GlnRS with DTNB and when saturating amounts of the substrates ATP, glutamine, and $tRNA^{Gln}$ were present. The profiles of absorbance increase are similar and asymptotically approach a value approximately equivalent to three sulfhydryl groups. On the other hand, eight sulfhydryl groups become totally exposed at 8 M urea (data not shown), indicating the presence of a disulfide bond. Assuming that in the native protein only three sulfhydryl groups react at infinite time, a plot of $\ln[([SH]_\infty - [SH]_t)/[SH]_\infty]$ versus time yields a triphasic curve with half-lives on the order of a few seconds, 2 min, and 12 min (Figure 1). The inclusion of saturating amounts of ATP, glutamine, and $tRNA^{Gln}$ in the DTNB reaction does not alter the basic character of the profile. The half-life of the slowest phase of the reaction is, however, slightly longer in the presence of substrates, indicating some influence of substrate binding on the reactivity of at least one of the cysteines. Thus, it appears that the reactive sulfhydryls are not directly involved in the active site.

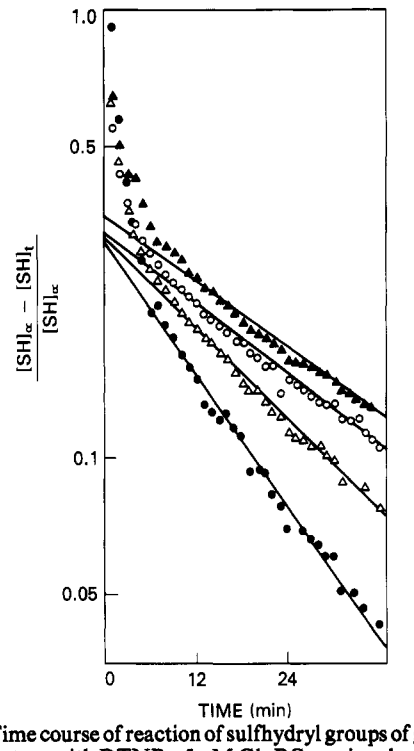

FIGURE 1: Time course of reaction of sulfhydryl groups of glutaminyl-tRNA synthetase with DTNB. 5 μM GlnRS was incubated with 0.5 mM DTNB in 0.1 M potassium phosphate buffer, pH 8.0. (●) represents free GlnRS, (△) represents reaction containing 10 mM ATP, (▲) represents reaction containing 10 mM glutamine, and (○) represents reaction containing 2.5 A_{260} units/mL $tRNA^{Gln}$.

Acrylodan is a sulfhydryl-specific fluorescent probe whose emission maximum is very sensitive to environmental polarity. Thus, acrylodan may be used for detecting substrate-induced conformational chantes in GlnRS. One mole of acrylodan gets incorporated into 1 mol of GlnRS rapidly, followed by very slow further incorporation. The enzyme activity is not significantly affected by covalent modification with acrylodan (approximately 80% or more of the initial activity remains upon modification with acrylodan) (data not shown). Thus, we may conclude that acrylodan modifies one sulfhydryl group

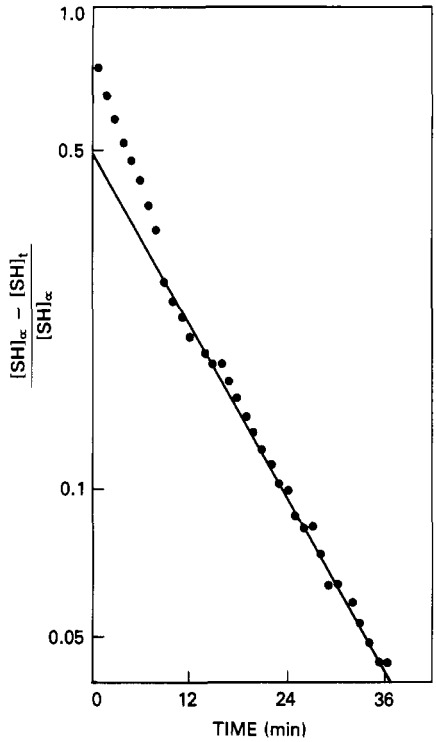

FIGURE 2: Time course of reaction of sulfhydryl groups of acrylodan-modified GlnRS (0.95 mol of acrylodan/mol of GlnRS) with DTNB. Solution conditions were the same as in Figure 1.

in GlnRS which is not essential for activity. Figure 2 shows the DTNB reaction with the acrylodan-modified GlnRS (acrylodan incorporation 0.95 mol/mol of GlnRS). The absorbance at 412 nm asymptotically approaches a value equivalent to 2.1 mol of sulfhydryl group per mole of enzyme (data not shown). A plot of $\ln[([SH]_\infty - [SH]_t)/[SH]_\infty]$ versus time can be fitted to two approximately equal phases with half-lives of approximately 1–2 and 10 min. This indicates that the fast-reacting sulfhydryl group is the only sulfhydryl modified by acrylodan and that it is not essential for the enzymatic activity of GlnRS.

Figure 3A, B shows the effect of binding of ATP, glutamine, and $tRNA^{Gln}$ to the acrylodan-modified glutaminyl-tRNA synthetase. The binding of substrates leads to changes in the fluorescence spectrum of the acrylodan-modified GlnRS that are qualitatively and/or quantitatively different in each case. The most dramatic effect is seen with ATP. The binding of ATP shifts the emission maximum of acrylodan from 494.5 to 483 nm, accompanied by almost 30% quenching of fluorescence. This indicates a change of environment of acrylodan to a significantly less polar environment. Binding of glutamine, on the other hand, leads to only about 8% quenching of fluorescence, and the emission maximum shifts 2 nm to the red. Binding of $tRNA^{Gln}$ leads to a small enhancement and less than 2-nm blue shift. Nonlinear least-squares fit of the data yielded K_d values which are not very different from the values for the unmodified enzyme (dissociation constants: ATP, 580 μM; glutamine, 140 μM; $tRNA^{Gln}$, 0.7 μM; ATP in the presence of $tRNA^{Gln}$, 240 μM; $tRNA_{Gln}$ in the presence of ATP, 0.4 μM).

A second fluorescent probe was used to verify the large change that was seen upon ATP binding. Previously, it was shown that pyrenylmaleimide also modified one sulfhydryl group without significant loss of enzymatic activity. When ATP was used to titrate PM-GlnRS, a large enhancement of fluorescence occurred, as shown in the inset of Figure 4. Figure 4 shows the real-time decay of fluorescence of pyrenylmaleimide-labeled GlnRS in the absence and in the presence of ATP. A dramatic difference is seen between free PM-GlnRS and ATP/PM-GlnRS complex. In most cases, the fluorescence decay of pyrenes is biexponential (Rao et al., 1979; Howlaka & Hammes, 1977). Our results can also be fitted to a biexponential function (Table I). In the free PM-GlnRS, the shorter lifetime component predominates, whereas in the ATP complex the longer component predominates. In addition, both components are lengthened significantly in the ATP/GlnRS complex. This has resulted in an increase of the second-order average lifetime from 36 to 63 ns upon binding of ATP to PM-GlnRS. The results clearly indicate that ATP binding to PM-GlnRS alters the environment of pyrene.

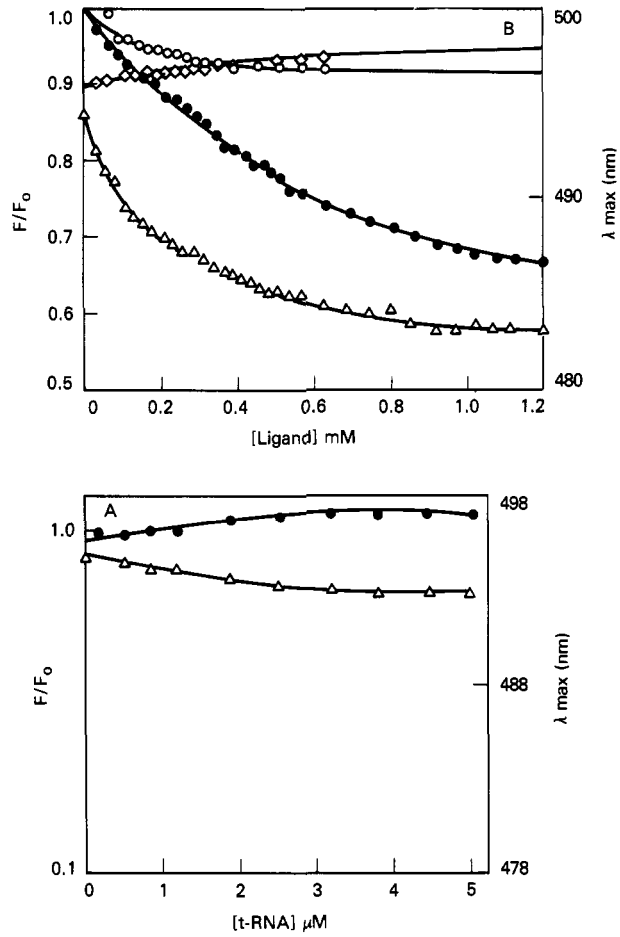

FIGURE 3: Effect of substrate binding on the acrylodan emission spectrum of acrylodan-modified GlnRS. The solution conditions were 0.1 M Tris-HCl, pH 7.5, containing 15 mM $MgCl_2$. The excitation wavelength was 387 nm, and the emission wavelength was 500 nm. (A) Titration with $tRNA^{Gln}$: (●) represents enhancement, and (△) represents the shift of emission maximum. (B) Titration with glutamine and ATP: (○) represents the quenching and (◇) represents the emission maximum shift that occurs upon glutamine binding: (●) represents the quenching and (△) represents the emission maximum shift that occurs upon ATP binding.

The increase of the fluorescence lifetime of PM-GlnRS upon ATP binding may involve movement of the probe to a less accessible environment. Thus, we have used tempol quenching to probe the accessibility of pyrene in PM-GlnRS in the presence and in the absence of ATP. Figure 5 shows the Lehrer plot of tempol quenching of the pyrene fluorescence of PM-GlnRS (Lehrer, 1971). The intercept of the Lehrer plot of the free enzyme is close to 1 with a Stern–Volmer constant of 26.8 M^{-1}. In the presence of excess ATP, the intercept is 2.9, whereas the Stern–Volmer constant is 39.4 M^{-1}. This indicates that approximately 67% of the initial fluorescence is not quenchable by low concentrations of tempol

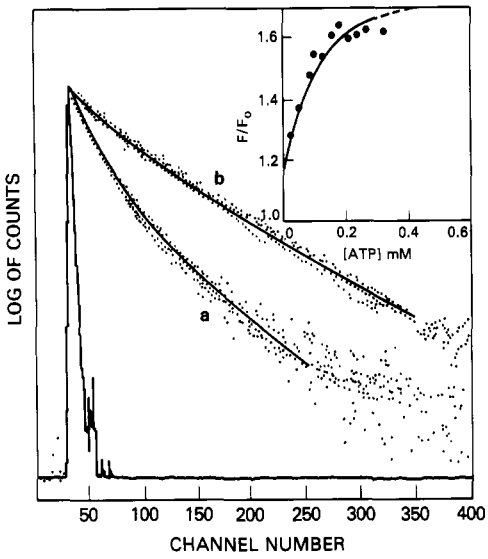

FIGURE 4: Time decay of pyrene fluorescence of PM-GlnRS. Each channel is 0.667 ns. The excitation wavelength was 337 nm, and the emission wavelength was 377 nm. The solution conditions were 0.1 M Tris-HCl, pH 7.5, containing 15 mM $MgCl_2$. Protein concentration was 11.55 μM. The lines in the figure are the best-fit biexponential lines. (a) Free PM-GlnRS; (b) PM-GlnRS with 1 mM ATP. The inset shows titration of the PM-GlnRS complex.

in the ATP complex. At 2 mM ATP, approximately 90% GlnRS should be as ATP/GlnRS complex. Since the total incorporated pyrene may contain some labeling at other sites which may be totally exposed to tempol in the ATP complex, it may not be unreasonable to assume that the nonquenchable part originates from the ATP/GlnRS complex. This indicates that in the ATP/GlnRS complex, pyrene is less accessible toward the external collisional quenchers, such as tempol.

The conformational change that occurs upon ATP binding may be of local nature, such as loop movement etc., or may involve substantial change in the global conformation. A global conformational change may affect the hydrodynamic properties. We have investigated the hydrodynamic properties of the free enzyme and ATP/GlnRS complex by Perrin plot. Figure 6 shows the isothermal Perrin plot for free GlnRS and ATP/GlnRS complex. The intercept of the plot on the $1/A$ axis is the inverse of the limiting anisotropy (A_0). For many probes, the theoretical limiting anisotrophy is 0.4, which is almost never reached due to limited probe motion. In many cases, a value of 0.2 is common (Yguerabide, 1972). The values obtained here (0.28 and 0.3 for free GlnRS and ATP/GlnRS, respectively) are high when compared to other labeled proteins, indicating little segmental fast motion of the probe. The attached pyrene probe is thus in a near-rigid state. The correlation times are calculated from slope and intercept values obtained from the Perrin plot using a second-order mean lifetime (Benecky et al., 1990; Brochon & Wahl, 1972). The correlation times are 36 and 34 ns for the free and the ATP complex of GlnRS, respectively, indicating that no major shape and size change occurs upon ATP binding (the large change in the slope in the presence of ATP is a result of the greatly increased fluorescence lifetime).

Bis-ANS is an environmentally sensitive, noncovalent fluorescence probe which often binds to hydrophobic regions of proteins. In many proteins, it has been shown to be a good probe for detecting conformational changes. We have investigated if ATP binding alters the bis-ANS binding to GlnRS (Bhattacharyya et al., 1991). ATP binding has very little effect on binding of bis-ANS to GlnRS (data not shown). This supports the idea that the conformational change that occurs upon ATP binding is not global in nature.

We have looked into the possibility that this ATP-induced conformational change may play a crucial role in the enzymatic mechanism. This conformational change alone is not sufficient for inducing ATP–PP_i exchange. The presence of $tRNA^{Gln}$ is required. Thus, we have investigated the influence of ATP on various aspects of $tRNA^{Gln}$ binding to GlnRS. Figure 7 shows the titration of acrylodan-labeled GlnRS with $tRNA^{Gln}$ in the presence of excess ATP and titration with ATP in the presence of excess $tRNA^{Gln}$. The binding of $tRNA^{Gln}$ in the presence of a saturating amount of ATP leads to approximately 12% quenching of fluorescence. This is in contrast to approximately 5% enhancement of fluorescence in the absence of ATP (Figure 3A). Binding also leads to an approximately 5-nm blue shift of the emission maximum, which is substantially higher than the 2-nm blue shift seen in the absence of ATP. The binding of ATP in the presence of a saturating amount of $tRNA^{Gln}$ causes a quenching of approximately 30% and a blue shift of the emission maximum of approximately 11 nm. These values are somewhat larger than when compared to values without ATP, but close to it. This indicates that in the presence of ATP, binding of $tRNA^{Gln}$ to GlnRS may be substantially different.

Salt effects on polynucleotide binding, particularly of double-stranded DNA, to proteins are well-known and have been analyzed in detail. In many cases, the salt effect has been used to differentiate nonspecific binding of polynucleotides from specific binding. Nonspecific binding is characterized by a predominantly electrostatic interaction which is highly sensitive to increased ionic strength. The specific binding, on the other hand, consists of other types of interactions, which are much less sensitive to ionic strength (deHaseth et al., 1977; Record et al., 1977). In general, the binding affinities of polynucleotides decrease with increasing concentrations of salt. ln K_a versus Ln $[M^+]$ plots are usually straight lines but in some cases may be curved. A change in the slope or the nature of the plot (i.e., straight line versus curve) is indicative of a change in interaction. Figure 8 shows the quenching profile of tryptophan fluorescence of GlnRS upon titration with $tRNA^{Gln}$ in the presence and in the absence of ATP. The line is the best-fit line obtained from nonlinear least-squares fit. The inset shows the ln K_a versus ln $[Na^+]$ plot for the $tRNA^{Gln}$/GlnRS interaction in the presence and in the absence of ATP. In the absence of ATP, the plot is approximately a straight line. In the presence of ATP, the plot is distinctly curved, with K_a values increasing at salt concentrations beyond 0.2 M. Such curvatures may occur due to anion release or the presence of strong hydrophobic interactions (Koblans & Ackers, 1991). Whatever may be the cause, it is clear, however, that the presence of ATP significantly alters the nature of the interaction of $tRNA^{Gln}$ with GlnRS. Florentz et al. (1990) have reported that ammonium sulfate inhibits the aminoacylation of AspRS but at higher concentrations causes activation. Whether this effect is related to enhancement of tRNA binding at higher ionic strengths is not known.

DISCUSSION

Ligand-induced conformational changes in proteins are well-known. Some of them are well characterized down to molecular levels and linked to specific events in the functional cycle. Some conformational changes transmit allosteric effects, e.g., in hemoglobin and aspartate transcarbamylase. Some others switch the proteins to the active conformation (e.g., induced fit) such as hexokinase (Anderson et al., 1979).

Table I: Lifetimes and Correlation Times of PM-GlnRS[a]

sample	α_1	α_2	τ_1 (ns)	τ_2 (ns)	τ_{av}[b] (ns)	τ_c (ns)
PM-GlnRS	0.25	0.11	12.2	49.4	36	36
PM-GlnRS + ATP[c]	0.09	0.25	17.6	67.2	63	34

[a] Solution conditions are given in the legends of Figures 4 and 5. [b] Represents second-order average. [c] ATP concentration was 1 mM.

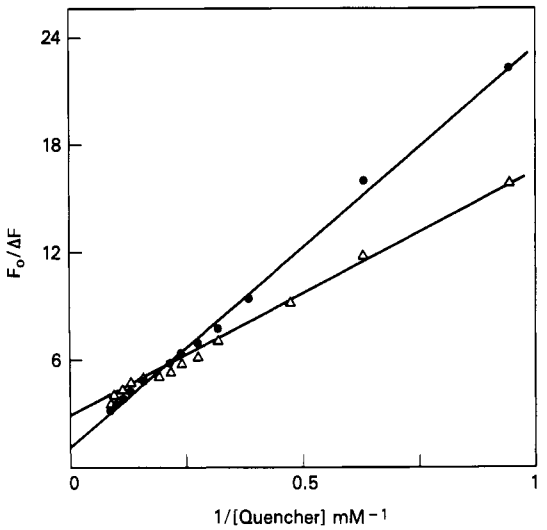

FIGURE 5: Lehrer plot of tempol quenching of PM-GlnRS in the absence (●) and in the presence of 2 mM ATP (△). The solution conditions were 0.1 M Tris-HCl, pH 7.5, containing 15 mM $MgCl_2$. The excitation wavelength was 343 nm, and the emission wavelength was 377 nm.

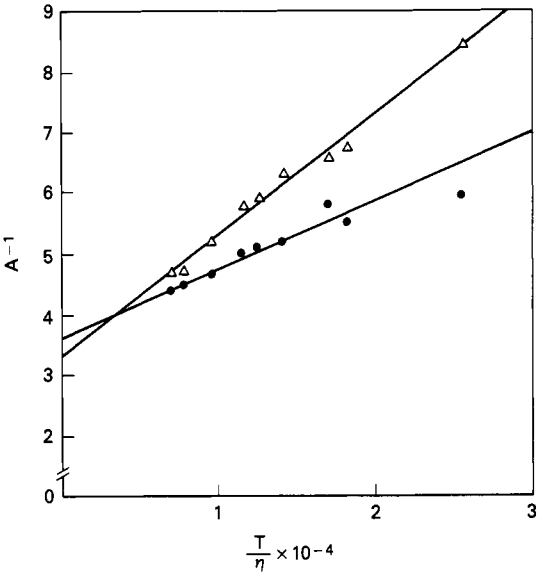

FIGURE 6: Perrin plot for PM-GlnRS in the absence (●) and in the presence of 2 mM ATP (△). The solution conditions were 0.1 M Tris-HCl, pH 7.5, containing 15 mM $MgCl_2$. GlnRS concentration was 0.5 μM. The excitation wavelength was 343 nm, and the emission wavelength was 377 nm.

Many conformational changes are still not well characterized, and their significance is unknown.

Although the mechanisms of some aminoacyl-tRNA synthetases have been explored to a moderate degree, with the possible exception of tyrosyl-tRNA synthetase of *E. coli*, details of catalytic mechanisms have not been well studied. Substrate-induced conformational changes have been reported for some aminoacyl-tRNA synthetases (Ferguson & Yang, 1986), but lack of structural information, as well as detailed mechanistic studies, has prevented characterization of these conformational changes. The availability of the crystal structure of ATP/

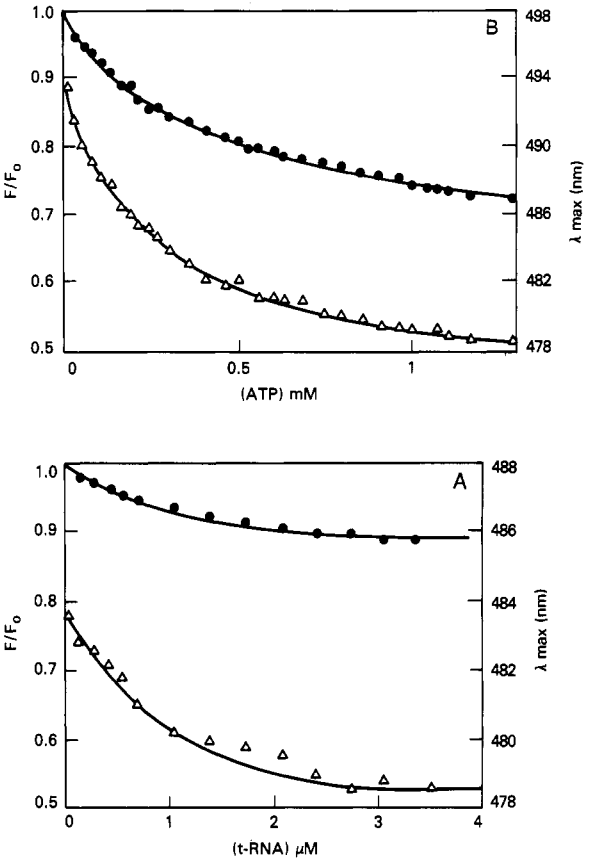

FIGURE 7: Effect of ligand binding on the acrylodan spectra of AC-GlnRS in the presence of other ligands. Solution conditions were the same as in Figure 3. The excitation wavelength was 387 nm, and the emission wavelength was 500 nm. Protein concentrations were 1 μM. (A) $tRNA^{Gln}$ titration was done in the presence of 1 mM ATP: (●) represents the quenching, and (△) represents the emission maximum shift. (B) ATP titration in the presence of 2 μM $tRNA^{Gln}$: (●) represents the quenching, and (△) represents the emission maximum shift.

$tRNA^{Gln}$/GlnRS may provide a link between mechanistic and structural studies in this enzyme (Rould et al., 1990).

The ATP-liduced conformational change reported here changes the enzyme in a way that alters $tRNA^{Gln}$ binding to GlnRS. The binding of $tRNA^{Gln}$ to the ATP/GlnRS complex causes a conformational change that may be of a different nature than that caused by the binding of $tRNA^{Gln}$ to free GlnRS.

ATP is known to induce loop movements in tyrosyl-tRNA synthetase, bringing essential groups in proper positions for transition-state stabilization (Fersht et al., 1988). The effect of ATP on the mode of tRNA binding has previously been reported for yeast tyrosyl-tRNA synthetase. Two types of complexes were detected in the gel retardation assay, and ATP favors the 1:1 complex (presumably the catalytically active one). Rublej et al. (1990) hypothesized that the ATP-bound conformation of TyrRS is substantially different from free TyrRS. An ATP-mediated conformational change has also been reported in methionyl-tRNA synthetase from *E. coli* (Brunie et al., 1990).

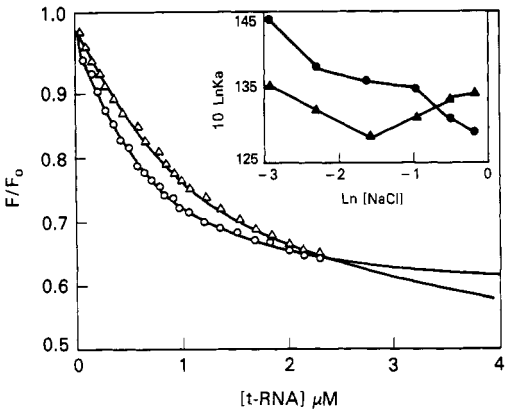

FIGURE 8: Quenching of tryptophan fluorescence upon binding of $tRNA^{Gln}$ in the presence and in the absence of 1.5 mM ATP. The lines are the best-fit curves. Solution conditions were the same as in Figure 3. The excitation wavelength was 295 nm, and the emission wavelength was 340 nm. Protein concentrations were 0.2 μM. The inset shows the salt concentration dependence of the association constants of $tRNA^{Gln}$ binding in the presence (▲) and in the absence (●) of ATP.

A strong effect of ATP on $tRNA^{Gln}$ binding to GlnRS may result in at least a partially ordered kinetic mechanism. Although the kinetic mechanism of GlnRS is not known, we note that many aminoacyl-tRNA synthetases have bi-uni uni-bi ping-pong mechanisms in which the tRNA binds after binding of the first two substrates (Friest & Sternbach, 1988). Obligatory ordered substrate bindings are commonplace in dehydrogenases and in many cases involve major conformational change after binding of the first substrate. At least in one case, alcohol dehydrogenase, it is suggested that the conformational change may put in place a catalytically important proton relay system, involving the 2′-OH group of the coenzyme. This allows the second substrate, the alcohol, to bind in correct orientation with the proton relay in place (Andersson et al., 1981; Fersht, 1985). Whether ATP plays a similar role in GlnRS in realigning the catalytic groups in the active site remains to be seen.

Correct recognition of tRNAs is of great importance in protein synthesis. The two different modes of binding of $tRNA^{Gln}$ seen in this study (in the presence and in the absence of ATP) point toward a crucial role of ATP in the correct recognition of $tRNA^{Gln}$ by GlnRS.

ACKNOWLEDGMENT

We thank Dr. Kankan Bhattacharyya for helping us with the lifetime measurements and Prof. B. Bhattacharyya for helpful discussions.

REFERENCES

Anderson, C. M., Zucker, F. H., & Steitz, T. A. (1979) *Science 204*, 375–380.

Andersson, P., Krassman, J., Linstorm, A., Olden, B., & Pelterson, G. (1981) *Eur. J. Biochem. 113*, 425–433.

Benecky, M. J., Kolvenbach, C. G., Wine, R. W., DiOrio, J. P., & Mosesson, M. W. (1990) *Biochemistry 29*, 3082–3091.

Bhattacharyya, T., Bhattacharyya, A., & Roy, S. (1991) *Eur. J. Biochem. 200*, 729–745.

Bradford, M. M. (1976) *Anal. Biochem. 72*, 248–254.

Breton, R., Sanfacon, H., Papayannopoulos, I., Biemann, K., & Lapointe, J. (1986) *J. Biol. Chem. 261*, 10610–10617.

Brochon, J. C., & Wahl, P. (1972) *Eur. J. Biochem. 25*, 20–32.

Brunie, S., Zelwar, C., & Risler, J. L. (1990) *J. Mol. Biol. 216*, 411–424.

Dehaseth, P. L., Lohman, T. M., & Record, M. T. (1977) *Biochemistry 16*, 4783–4790.

Erianni, G., Delarue, M., Poch, O., Gangloff, J., & Moras, D. (1990) *Nature 347*, 203–206.

Ferguson, B. Q., & Yang, D. C. H. (1986) *Biochemistry 25*, 2743–2748.

Fersht, A. R. (1985) *Enzyme Structure and Mechanism*, W. H. Freeman & Co., New York.

Fersht, A. R., Knill-Jones, J. W., Bedouelle, H., & Winter, G. (1988) *Biochemistry 27*, 1581–1587.

Florentz, C., Kern, D., & Giege, R. (1990) *FEBS Lett. 261*, 335–338.

Friest, W., & Sternbach, H. (1988) *Eur. J. Biochem. 177*, 425–433.

Haugland, R. P. (1985) *Handbook of Fluorescent Probes and research Chemicals*, Molecular Probes, Molecular Probes Inc., Eugene, OR.

Howlaka, D. A., & Hammes, G. G. (1977) *Biochemistry 16*, 5538–5545.

Koblans, K. S., & Ackers, G. K. (1991) *Biochemistry 30*, 7822–7827.

Lehrer, S. S. (1971) *Biochemistry 10*, 3254–3263.

Nagel, G. M., & Doolittle, R. F. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 8121–8125.

Rao, A., Martin, P., Reithmeier, R. A. F., & Cantley, L. C. (1979) *Biochemistry 18*, 4505–4516.

Ravel, J. M., Wang, S., Heinemeyer, C., & Shive, W. (1965) *J. Biol. Chem. 240*, 432–438.

Record, M. T., Dehaseth, P. L., & Lohman, T. M. (1977) *Biochemistry 16*, 4791–4796.

Rould, M. A., Perona, J. J., Soll, D., & Steitz, T. A. (1989) *Science 246*, 1135–1142.

Rublej, I., Weygand-Durasevic, I., & Kucan, Z. (1990) *Eur. J. Biochem. 193*, 783–788.

Schimmel, P. (1987) *Annu. Rev. Biochem. 56*, 125–128.

Weast, R. C. (1977) *CRC Handbook of Chemistry and Physics*, 57th ed., CRC Press, Boca Raton, FL.

Yamao, F., Inokuchi, H., Cheung, A., Ozeki, H., & Soll, D. (1982) *J. Biol. Chem. 257*, 11639–11643.

Yguerabide, J. (1972) *Methods Enzymol. 26*, 498–578.