Protein Science (1998), 7:1046–1051. Cambridge University Press. Printed in the USA.

A cognate tRNA specific conformational change in glutaminyl-tRNA synthetase and its implication for specificity

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(RECEIVED November 11, 1997; ACCEPTED January 22, 1998)

Abstract

Conformational changes that occur upon substrate binding are known to play crucial roles in the recognition and specific aminoacylation of cognate tRNA by glutaminyl-tRNA synthetase. In a previous study we had shown that glutaminyl-tRNA synthetase labeled selectively in a nonessential sulfhydryl residue by an environment sensitive probe, acrylodan, monitors many of the conformational changes that occur upon substrate binding. In this article we have shown that the conformational change that occurs upon $tRNA^{Gln}$ binding to glnRS/ATP complex is absent in a noncognate tRNA $tRNA^{Glu}$–glnRS/ATP complex. CD spectroscopy indicates that this cognate $tRNA^{Gln}$-induced conformational change may involve only a small change in secondary structure. The Van't Hoff plot of cognate and noncognate tRNA binding in the presence of ATP is similar, suggesting similar modes of interaction. It was concluded that the cognate tRNA induces a local conformational change in the synthetase that may be one of the critical elements that causes enhanced aminoacylation of the cognate tRNA over the noncognate ones.

Keywords: conformational change; discrimination; fluorescence; synthetase; tRNA

Translation of nucleotide sequences in mRNAs to amino acid sequences in proteins is characterized by high degree of accuracy. This level of accuracy is primarily determined at the level of aminoacylation of tRNAs by aminoacyl-tRNA synthetases (Schimmel & Soll, 1979; Schimmel, 1987; Carter, 1993). The correct aminoacylation of all the tRNAs involves positive recognition of the cognate tRNAs (McClain, 1993) and negative discrimination of the noncognate tRNAs (Schmitt et al., 1993). Recognition of cognate tRNAs has been studied intensively, and structural elements that are responsible for recognition have now been mapped for many systems (Normanly & Abelson, 1989; Mechulam et al., 1995). Very little is known, however, about the factors that are responsible for negative discrimination.

Even in the case of recognition of cognate tRNAs where the identity elements have been mapped, it is not clear exactly how this elements influence enzymatic properties and translates recognition into catalytic competence. Due to pioneering work by Soll and co-workers, glutaminyl-tRNA synthetase has emerged as one of the best systems to study the recognition and the consequent catalytic activation process. Jahn et al. (1991) and Ibba et al. (1996) have shown that the correct recognition of anti-codon bases increases k_{cat} of the enzyme glutaminyl-tRNA synthetase of *Escherichia coli* significantly. Because the anti-codon binding pocket is approximately 35 Å away from the active site, a protein conformational change or a tRNA conformational change or both are responsible for transmitting the anti-codon binding information to the active site. In subsequent studies, attempts have been made to map the pathway of this information transfer through analysis of mutant proteins. It has been demonstrated that at least two pathways may exist, which carry this information (McClain et al., 1993; Rogers et al., 1994). Clearly, the substrate-induced conformational changes, particularly those induced by the cognate tRNAs, play a major role in achieving high fidelity of aminoacylation.

We have followed a different path in an attempt to understand the conformational changes that accompanies specific aminoacylation of cognate tRNA and rejection of noncognate tRNAs by glutaminyl-tRNA synthetase. We have shown that glutaminyl-tRNA synthetase can be labeled selectively at one sulfhydryl group without the loss of biological activity (Bhattacharyya & Roy, 1993). This labeled enzyme has been used to show that ATP binding induces a conformational change that changes the interaction of the synthetase with the cognate tRNA, as evident by the change of salt effect on $tRNA^{Gln}$ binding. This effect of ATP on the binding

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Abbreviations: GlnRS, glutaminyl-tRNA synthetase; acrylodan, 6-acryloyl-2-dimethyl aminonaphthalene; CD, circular dichroism; tempol, (4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl); GluRS, glutamyl-tRNA synthetase.

of the other substrates is supported by Lloyd et al. (1995). The $tRNA^{Gln}$ binding to ATP/GlnRS complex also induces another conformational change that is not evident in the absence of ATP. In this article we have explored the nature of these conformational changes and its implications for discrimination of cognate and noncognate tRNAs.

Results

From the early days, it was known that substrate-induced conformational changes play crucial roles in functioning of many proteins, particularly enzymes. Even in the case of aminoacyl-tRNA synthetases examples are known (Ferguson & Yang, 1986), but the nature of such conformational changes and its precise significance still eludes us. Previously, we have shown that binding of $tRNA^{Gln}$ to GlnRS, in the presence of ATP, causes a conformational change that can be detected by a fluorescence probe attached to a single reactive nonessential sulfhydryl residue. Because in vivo the discrimination between the cognate and noncognate tRNAs is a major function of this enzyme, we have explored the effect of binding of a noncognate tRNA, $tRNA^{Glu}$, to GlnRS. Figures 1 and 2 show the quenching and the emission maxima shift of the acrylodan labeled GlnRS, upon binding of $tRNA^{Gln}$ and $tRNA^{Glu}$, in the presence of ATP. $tRNA^{Gln}$ causes a quenching of around 15% and an emission maximum shift to the blue in excess of 3.5 nm. In contrast, binding of $tRNA^{Glu}$ causes a quenching of around 4%, and no detectable emission maximum shift. These values are average of several independent titrations. This clearly suggests that the binding of a noncognate tRNA cannot induce the same conformational change as that of the cognate tRNA.

One possible reason for lack of a demonstrable conformational change upon noncognate $tRNA^{Glu}$ binding could be the weak binding of $tRNA^{Glu}$. Thus, we have measured $tRNA^{Glu}$ binding to GlnRS by quenching of the tryptophan fluorescence. Previously we have shown that binding of $tRNA^{Gln}$ leads to significant quenching of tryptophan fluorescence (Bhattacharyya et al., 1991). Figure 3 shows the tryptophan fluorescence of GlnRS upon binding of $tRNA^{Glu}$ in the presence and in the absence of ATP. The plot shows saturation behavior. Clearly, significant noncognate tRNA complex formation occurs below 2.5 μM, and lack of binding cannot be the cause of absence of the conformational change. No change in emission maximum is seen even at $tRNA^{Glu}$ concentration of 6 μM (data not shown). Similar binding of $tRNA^{Glu}$ and $tRNA^{Gln}$

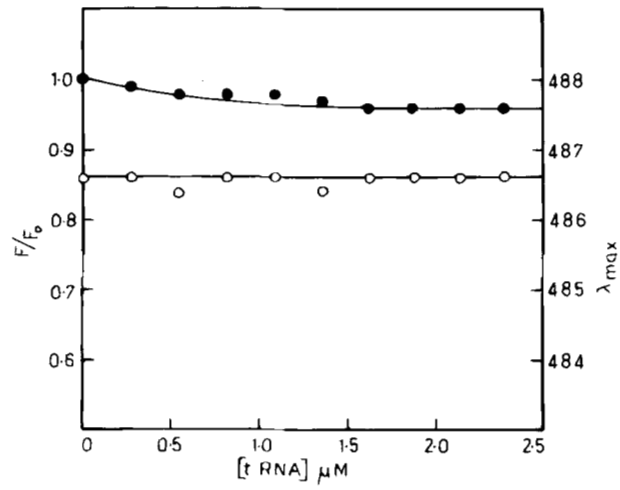

Fig. 2. Quenching (filled circles) and shift of emission maxima shift (open circles) of acrylodan fluorescence upon titration of $tRNA^{glu}$. The experimental conditions were same as in Figure 1.

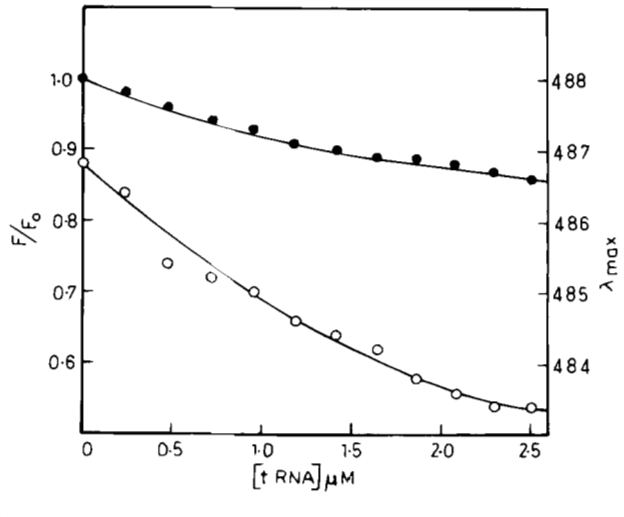

Fig. 1. Quenching and emission maxima shift acrylodan fluorescence upon binding of $tRNA^{Gln}$ in the presence of ATP. Shift of emission maxima (open circles) and quenching (filled circles) of acrylodan fluorescence upon titration of $tRNA^{gln}$. The experiments were conducted in 0.1 M Tris-HCl, pH 7.5, containing 15 mM $MgCl_2$ and 1 mM ATP at 25 °C. The excitation and emission wavelengths were 387 and 500 nm, and the bandpasses were 5 nm each. Glutaminyl-tRNA synthetase concentration was 0.75 μM.

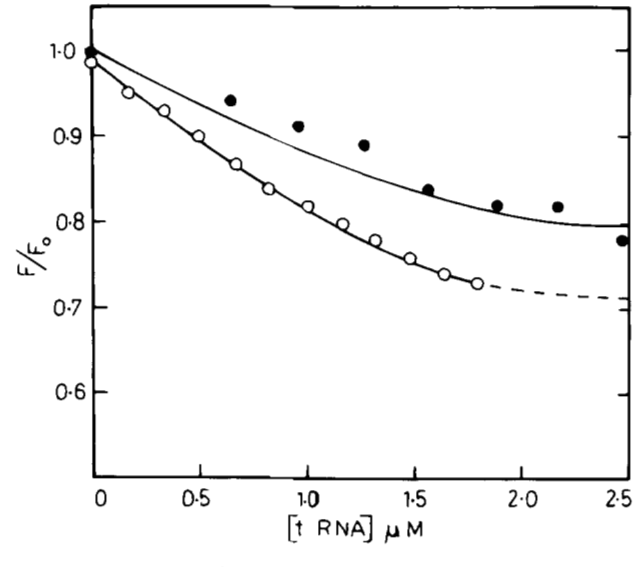

Fig. 3. Binding of $tRNA^{Glu}$ to glutaminyl-tRNA synthetase in the presence (filled circles) and in the absence (open circles) of 1.5 mM ATP as measured by quenching of tryptophan fluorescence. Excitation and emission wavelengths were 295 and 340 nm, respectively. Excitation and emission band passes were 1.5 and 10 nm, respectively. Solution conditions were 0.1 M Tris-HCl buffer pH 7.5 containing 15 mM $MgCl_2$. Protein concentration was 0.2 μM.

to the GlnRS/ATP complex reported here is in contrast to approximately 300-fold difference in binding constant in the absence of ATP reported by Weygand-Durasevic et al. (1993), suggesting a strong ATP effect and possibly ordered substrate binding.

Collisional quenchers have been widely used to determine accessibility of fluorescence probes in proteins. We have used tempol as a quencher of acrylodan fluorescence to determine the accessibility of the acrylodan probe in the AC-glnRS/ATP/tRNA^{Gln} and AC-glnRS/ATP/tRNA^{Glu} complex. Figure 4 shows the Stern-Volmer plot of tempol quenching of the AC-glnRS/ATP/tRNA^{Gln} and AC-glnRS/ATP/tRNA^{Glu} complex. Both plots are linear with Stern-Volmer constants (K_{sv}) of 10 and 17.5 M^{-1}, for tRNA^{Gln} and tRNA^{Glu}, respectively. This suggests that acrylodan probe is significantly more shielded in the cognate tRNA complex than in the noncognate tRNA complex.

Elucidation of the nature of this cognate tRNA-induced conformational change is an important challenge because this may shed light on underlying structural reasons for high specificity of aminoacylation. One of the major tools that can be used to investigate structural changes is circular dichroism. One of the major problems in analyzing changes in circular dichroism spectra of proteins in presence of nucleic acid and other ligands is the possibility of change of both the spectra upon complex formation when spectral overlap occurs. There is no a priori way of knowing whether the changes, if any, is coming from the protein component or the nucleic acid component. Nucleic acids have CD bands throughout the range of 300–200 nm. The proteins, however, have no significant CD bands in the 260 nm range. The intensity of the side chain CD is negligible compared to the nucleic acid bands, at comparable concentrations. We make the reasonable assumption that if no change of CD of nucleic acid occurs in the 260–280 nm band, then it may be assumed that no changes in the nucleic acid spectra occurs in the range 200–220 nm. Hence, any change in the spectra of the complex in the range of 220–200 nm may be taken as change in the protein spectra. Figure 5 shows the CD spectra of free tRNA^{Gln} and in the protein complex, in the presence of saturating concentrations of ATP and in its absence. In neither case is there a significant change in the CD spectra. This is consistent with the conclusions drawn from X-ray crystallographic studies, where only significant distortion seen in the bound tRNA structure from the presumed free tRNA structure is in the acceptor end and anticodon loops, and modest distortions are seen elsewhere (Rould et al., 1989).

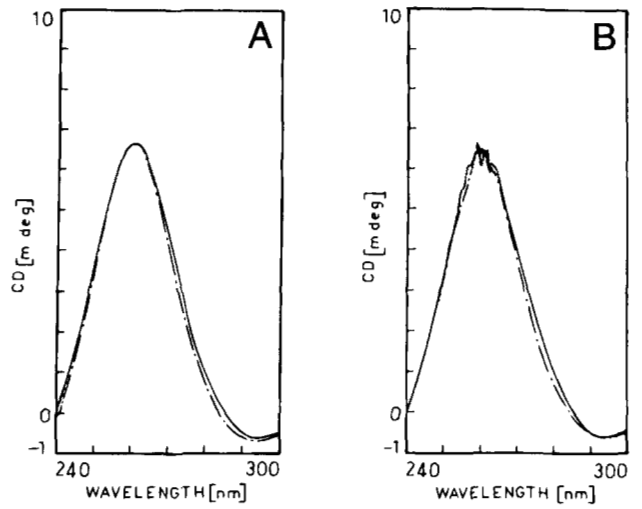

Fig. 5. *Comparison of near UV circular dichroism spectra of* tRNA^{Gln} *under different conditions.* **A:** Spectra in the absence of ATP. (—) Spectra of 4 μM free tRNA^{Gln} and (— · — · —) in the glnRS/tRNA^{Gln} complex (4:4 μM). **B:** In the presence of 1.5 mM ATP. (—) Spectra of 4 μM free tRNA^{Gln} and (— · — · —) spectra of tRNA^{Gln} in the ATP/glnRS complex (4:4 μM). The solution conditions were 0.1 M Tris-HCl buffer, pH 7.5 containing 15 mM $MgCl_2$.

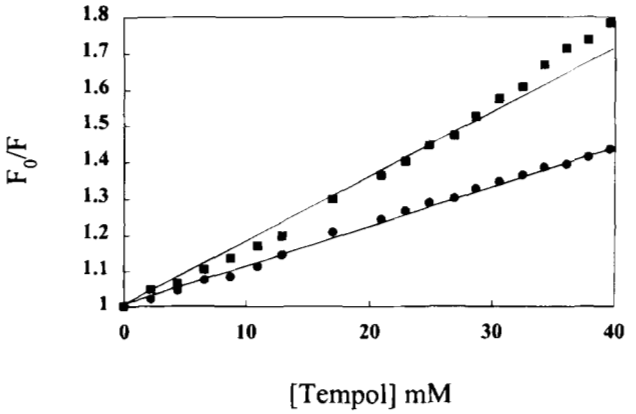

Fig. 4. The Stern-Volmer plot of tempol quenching of acrylodan fluorescence in the presence of 1 mM ATP and 6 μM (filled circles) tRNA^{Gln} and (filled squares) tRNA^{Glu}. The solution conditions were 0.1 M Tris-HCl buffer, pH 7.5 containing 15 mM $MgCl_2$. The excitation and emission wavelengths were 387 and 500 nm, and the bandpasses were 5 nm each. Glutaminyl-tRNA synthetase concentration was 1 μM.

Figure 6 shows the far UV-CD spectra of the protein in a complex with ATP and tRNA^{Gln} and with ATP alone. The upper part of the figure shows the average percentage difference at some representative wavelengths and the standard deviations. This is a result of five independent measurements carried out on different days using the weighing protocols described in Materials and methods. It is clear that small but significant increase in CD spectra occurs upon binding of tRNA^{Gln} to the protein–ATP complex. Lack of any extensive change is consistent with the hydrodynamic measurements reported before (Bhattacharyya & Roy, 1993), although this does not preclude small changes at several distant sites.

It is clear from the above results and that of in the previous study (Bhattacharyya & Roy, 1993) the cognate tRNA^{Gln} induces a conformational change in the protein that the noncognate tRNA^{Glu} is unable to induce. This difference may be due to fundamentally different modes of interaction of the cognate and noncognate tRNA. Variation of dissociation constant with temperature often gives clues to the nature of forces involved in the interaction. Figure 7 shows the Van't Hoff plot of tRNA^{Gln} and tRNA^{Glu} binding to GlnRS in the presence of ATP. Both the plots are parallel to the temperature axis, suggesting little change in enthalpy. Thus, both the binding is strongly entropy driven, with only a modest difference in ΔS. Such entropy driven interaction may originate from ion and water release upon complex formation (Ha et al., 1992). This suggests that the difference in interaction between the cognate and the non-cognate tRNAs may be confined to a relatively small part of the total interaction.

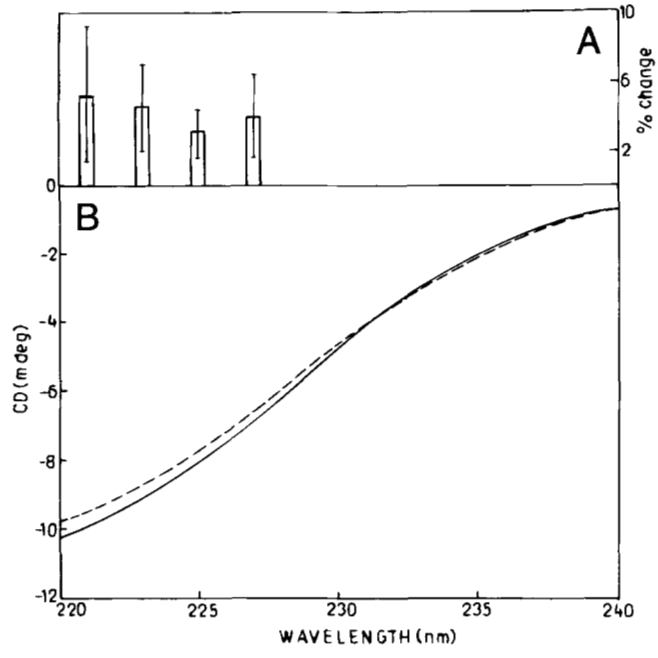

Fig. 6. Far UV circular dichroism spectra of the glutaminyl-tRNA synthetase in the ATP/glnRS/tRNA^{Gln} complex (—) and ATP/glnRS complex (— — — —). The concentration of ATP was 1.5 mM, the concentration of glnRS was 4 μM, and the concentration of tRNA^{gln}, when present, was 4 μM. The solution conditions were same as in Figure 5.

Discussion

The discrimination between cognate and noncognate tRNAs by aminoacyl-tRNA synthetases probably occurs at several levels. Structural and molecular basis of discrimination is not well understood. In this article we have shown that a noncognate tRNA^{Glu} binds to GlnRS with affinity that is not greatly different from that of the cognate tRNA. The Van't Hoff plot suggests that the major part of the tRNA/synthetase interaction remains similar in cognate and noncognate complexes. Thus, the difference in aminoacylation rate must happen in a post-binding step, and this rate is different by three orders of magnitude (Rogers & Soll, 1993; Weygand-Durasevic et al., 1993). As shown in Results, the noncognate tRNA^{Glu} is unable to induce the conformational change that is induced by tRNA^{Gln} as judged by fluorescence of Acrylodan labeled GlnRS.

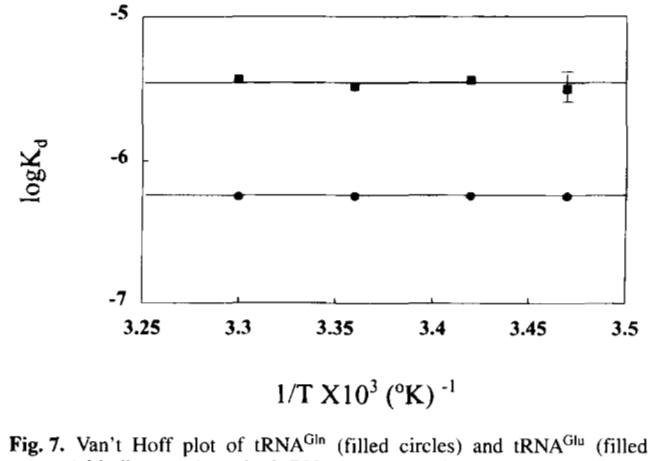

Fig. 7. Van't Hoff plot of tRNA^{Gln} (filled circles) and tRNA^{Glu} (filled squares) binding to glutaminyl-tRNA synthetase, in the presence of ATP. The dissociation constants at four different temperatures, 15, 20, 25, and 30 °C, were determined from tryptophan fluorescence quenching data. The protein concentration was 0.2 μM, and the ATP concentration was 1.5 mM. The experiments were conducted in 0.1 M Tris-HCl buffer, pH 7.5 containing 15 mM $MgCl_2$. Excitation and emission wavelengths were 295 and 340 nm, and bandpasses were 1.5 and 10 nm, respectively. The error bar shown was the maximum among all the eight points.

The CD evidence presented here suggests a limited change in secondary structure. Previously, we have shown that although ATP binding causes a major change in the environment of the fluorescent probe, it does not lead to a significant change in the Stokes radius (Bhattacharyya & Roy, 1993). Hydrodynamic properties of the protein, of course, were not measurable in the tRNA complex due to the large size of the tRNA. But the comparison with the X-ray structure suggests no major change in shape and size of the protein upon cognate tRNA binding. Lack of any major change in secondary structure and hydrodynamic properties, upon binding of the tRNA, suggests a limited and local conformational change.

We have shown previously that the ATP induced conformational change is necessary for correct recognition of the tRNA. The role played by ATP is suggestive that the conformational change detected by the acrylodan fluorescence upon tRNA^{Gln} binding is related to generation of catalytic competence. Recently, we have measured the shift in acrylodan emission maximum at low temperatures (no detectable catalytic activity in standard assay) upon immediate addition of all three substrates. Under these conditions it was calculated that the predominant species may be the catalytically competent quaternary complex. It was observed that the acrylodan emission maximum is about 4 nm blue-shifted in the ATP/gln/tRNA^{Gln} mixture compared to the ATP/gln/tRNA^{Glu} mixture, suggesting a relationship of the conformational change with the generation of catalytic competence (Mandal & Roy, unpubl. obs.). Many years ago Koshland postulated that one of the ways an enzyme can enhance discrimination between a "good" and a "bad" substrate is to allow the good substrate to induce a catalytically competent conformation that cannot be induced by the bad substrate (Koshland, 1958). He termed this mechanism–"induced fit." The induced fit, of course, is paid for by the substrate binding energy. This concept, however, was challenged by Fersht (1985), who showed that as long as the good and the bad substrate is inducing the same catalytically competent conformation, although to a different extent, the discrimination factor between the good and the bad substrate remains the same. The derived equations suggest that k_{cat}/K_M is affected to the same extent for all substrates. Recently, Post and Ray (1995) suggested that if the good and the bad substrate induces conformations of different catalytic competence, induced fit may lead to enhancement of discrimination. In the case of GlnRS, we have seen that cognate and noncognate tRNA, the good and the bad substrate, respectively, binding leads to two different conformations. This clearly suggests that induced fit may be a major mechanism for enhancement of discrimination between cognate and noncognate tRNAs provided the conformational change gives rise to an active site with greater complimentarity to the transition state. Such a mechanism has been suggested before for other aminoacyl-tRNA synthetases (Cusack et al., 1996). We thus conclude that the binding interactions of the cognate and the noncognate tRNAs are similar. The tRNA^{Gln} induces a local conformational change that may contribute substantially to the discrimination. This tRNA^{Glu}-induced conformational change is

likely to be related to the conformational changes implicated in the studies of Soll and co-workers (Jahn et al., 1991; Sherman et al., 1996), although the precise relationships remains to be elucidated. Although we suggest that this conformation change is important in achieving discrimination, it is likely that other factors are operative as well. It appears that the discrimination process between cognate and noncognate tRNAs is a dynamic one involving conformational changes, substrate binding linkages (Bhattacharyya & Roy, 1993; Hong et al., 1996) and optimization of several functions (Sherman & Soll, 1996).

Materials and methods

Materials

$tRNA^{Glu}$, ATP, Sephadex G-25 were purchased from Sigma Chemical Co. (St. Louis, Missouri). The measured specific activity of $tRNA^{Glu}$ was around 1.5 nmol/A_{260} and was used without further purification. Acrylodan was purchased from Molecular Probe Inc. (Eugene, Oregon). All other chemicals used were of analytical grade.

Enzyme purification

Glutaminyl-tRNA synthetase was purified according to Hoben et al. (1982) with some minor modifications as described by Bhattacharyya et al. (1991). Enzyme assays are also described in the same article. The protein preparation showed a single band on SDS polyacrylamide gel and had specific activity comparable to those reported in Bhattacharyya et al. (1991).

$tRNA^{Gln}$ purification

Cells harboring pRS3 were grown according to the method described by Perona et al. (1988), and $tRNA^{Gln}$ was purified from harvested cells using the methodology described in Bhattacharyya et al. (1991). The specific activities of around 2 nmol/A_{260} units were obtained.

Chemical modifications

Acrylodan-modified glnRS was prepared by mixing 1:1 molar ratio (typically at 35 μM) of protein and acrylodan in 0.1 M potassium phosphate buffer, pH 8.0. After 30 min incubation at 0 °C, the labeled protein was separated from the free acrylodan over a Sephadex G-25 column (22 × 1.2 cm) equilibrated with 0.1 M Tris-HCl buffer, pH 7.5, containing 15 mM $MgCl_2$. The protein concentration was measured by the method of Bradford (1976), and the incorporation ratio was determined according to (Bhattacharyya & Roy, 1993).

Fluorescence methods

Steady-state fluorescence was measured in a Hitachi F3010 spectrofluorometer. The temperature was controlled by circulating water at appropriate temperatures through the chamber and the cuvette holder. The excitation and emission band passes were 5 nm, unless stated otherwise. Spectra of appropriate buffers were always subtracted from the fluorescence spectra.

Van't Hoff plot

Binding of $tRNA^{Gln}$ and $tRNA^{Glu}$ in the presence of ATP were determined from quenching of tryptophan fluorescence. We follow a slightly different methodology of tRNA binding from that of Bhattacharyya and Roy (1993). Because the Van't Hoff plot requires determination of binding constants at different temperatures, we were concerned that consecutive additions of tRNA to protein might introduce some systematic temperature-dependent errors if there are small changes in fluorescence during the time of incubation. This has led us to do each concentration point separately. Thus, the fluorescence of 0.2 μM glutaminyl-tRNA synthetase fluorescence was determined with excitation at 295 nm and emission at 340 nm after incubation at a given temperature. Immediately, an aliquot of $tRNA^{Gln}$ was added and incubated at the same temperature for 2 min, and then the fluorescence values were recorded. The ratio of the two values, after the correction for dilution and inner filter effect, was taken as the degree of quenching. The inner filter effect was corrected using the following formula:

$$F_{corr} = F_{obs} \cdot \text{antilog}[(A_{ex} + A_{em})/2].$$

This ratio at different $tRNA^{Gln}$ concentrations were determined and was fitted to a single-site binding equation using a nonlinear least-squares fit program. In this method, the data were fitted using three variable parameters, K_d, initial fluorescence value F_o, and the fluorescence value at infinite concentration, F_∞. The parameters were varied systematically within a given broad range, until the minimum chi-square value was obtained. The parameters that gave the minimum chi-square value was chosen as the best-fit parameters. At least four experiments were done at all temperatures and average values and standard deviations were used to construct the Van't Hoff plot. All the titrations were carried out in 0.1 M Tris-HCl, pH 7.5 containing 1.5 mM ATP and 15 mM $MgCl_2$.

Binding of tRNA to acrylodan-labeled GlnRS

Binding of tRNAs to acrylodan modified GlnRS was carried out at 0.75 μM protein concentration at 25 ± 1 °C, in 0.1 M Tris-HCl buffer, pH 7.5 containing 15 mM $MgCl_2$. The excitation wavelength was 387 nm and emission intensities at various wavelengths were determined. The excitation and emission band passes were 5 nm each.

Tempol quenching

Tempol quenching was carried out in 0.1 M Tris-HCl buffer, pH 7.5 containing 15 mM $MgCl_2$. ATP concentration was 1 mM and the tRNA concentrations were 6 μM. Acrylodan labeled glnRS concentration was 1 μM. The excitation wavelength was 387 nm and the emission wavelength was 500 nm.

Circular dichroism spectroscopy

Circular dichroism spectra were taken in a JASCO J-600 spectropolarimeter at controlled ambient temperatures, which were 24 ± 1 °C. Scan speed were set at 50 nm/min. Ten spectra were signal averaged for the enhancement of signal-to-noise ratio. Because in most cases we are measuring a small difference (around 5%), we adopted a protocol in which pipetting errors were kept low. This

was achieved by making 3X stock solutions of $tRNA^{gln}$, GlnRS, and ATP in the same buffer and mixing them in different proportions by weight, in a Sartorious micro-balance. For example, a 1:1:1 mixture of the above-mentioned solutions was used for the ternary complex spectra. The Sartorious balance used was capable of 0.01 mg accuracy.

Acknowledgments

We acknowledge financial support from Department of Science and Technology, Government of India. We also acknowledge CSIR for a fellowship to Amit Kumar Mandal and Department of Biotechnology for postdoctoral fellowship to Subhra Bhattacharyya. We also thank Distributed Information Center (DIC), and Jaganmoy Guin (PD Laboratory) for helping us with computation and CD work, respectively.

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