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Conformational changes of aminoacyl-tRNA and uncharged tRNA upon complex formation with polypeptide chain elongation factor Tu

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The conformation change of *Thermus thermophilus* $tRNA_1^{Ile}$ upon complex formation with *T. thermophilus* elongation factor Tu (EF-Tu) was studied by analysis of the circular dichroism (CD) bands at 315 nm (due to the 2-thioribothymidine residue in the T-loop) and at 295 nm (due to the core structure of tRNA). Formation of the ternary complex of isoleucyl-$tRNA_1^{Ile}$ and EF-Tu·GTP increased the intensities of these CD bands, indicating stabilization of the association between the T-loop and the D-loop and also a significant conformation change of the core region. Upon complex formation of EF-Tu·GTP and uncharged tRNA, however, the conformation of the core region is not changed, while the association of the two loops is still stabilized. On the other hand, the binding with EF-Tu·GDP does not appreciably affect the conformation of isoleucyl-tRNA or uncharged tRNA. These indicate the importance of the γ-phosphate group of GTP and the aminoacyl group in the formation of the active complex of aminoacyl-tRNA and EF-Tu·GTP.

Elongation factor Tu; Aminoacyl-tRNA; Circular dichroism; Conformation change; (*Thermus thermophilus* HB8)

1. INTRODUCTION

EF-Tu is tightly bound with GTP or GDP. The EF-Tu·GTP complex transfers aminoacyl-tRNA, rather than uncharged tRNA, to the A-site of ribosomes [1]. By contrast, EF-Tu·GDP is not active in the A-site binding of aminoacyl-tRNA [2]. Nevertheless, EF-Tu·GDP forms a weak complex with aminoacyl-tRNA [3]. Further, it has been suggested that EF-Tu·GTP interacts with uncharged tRNA [4]. Therefore, it is interesting to inquire why the complex of EF-Tu·GTP and aminoacyl-tRNA is much more effective in the A-site binding than other EF-Tu·tRNA complexes. Such differences in the binding activity are probably related to conformational differences among EF-Tu·tRNA complexes, because ribonuclease digestion experiments have suggested that the conformation of aminoacyl-tRNA changes upon binding with EF-Tu·GTP [5,6]. Therefore, it is important to compare the conformation changes of tRNAs (aminoacyl-tRNA and uncharged tRNA) upon binding with EF-Tu·GTP or EF-Tu·GDP. *Thermus thermophilus* EF-Tu [7–9] is stable enough for various analyses including NMR spectroscopy [10–12]. We have also found that the CD bands of 2-thioribothymidine residues of *T. thermophilus* tRNAs [13–18] are useful for analyzing interactions between tRNA and protein, for example, $tRNA^{Glu}$ and glutamyl-tRNA synthetase from *T. thermophilus* [17]. Therefore, in the present study, we analyzed the CD spectra of the complexes of *T. thermophilus* $tRNA_1^{Ile}$ and EF-Tu, in order to elucidate how the conformation of tRNA in the complex depends on the bound nucleotide (GTP or GDP) and the state of tRNA (aminoacylated or uncharged).

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Abbreviations: CD, circular dichroism; EF-Ts, polypeptide chain elongation factor Ts; EF-Tu, polypeptide chain elongation factor Tu; Ile-tRNA, isoleucyl-$tRNA_1^{Ile}$; IleRS, isoleucyl-tRNA synthetase; s^2T, 2-thioribothymidine

2. MATERIALS AND METHODS

EF-Tu·GDP and EF-Tu·EF-Ts were purified from an extract of *T. thermophilus* HB8 by successive chromatography on columns of DEAE-Sephadex A-50, DEAE-Toyopearl and Butyl-Toyopearl. EF-Tu·EF-Ts was finally purified by high-performance liquid chromatography on a gel-filtration column of G3000SW. *T. thermophilus* $tRNA_1^{Ile}$ and *Escherichia coli* $tRNA_1^{Ile}$ were prepared as described [16,19]. IleRS was purified from *E. coli* cells harboring the *ileS* gene on a runaway-replication plasmid [20].

The aminoacylation reaction was performed in 100 mM Tris-HCl buffer (pH 7.5) containing 5 mM magnesium acetate, 10 mM KCl, 2 mM ATP, 0.1 mM isoleucine, 1.5 μM IleRS and 17 μM $tRNA_1^{Ile}$ at 37°C for 10 min. Ile-tRNA was isolated by phenol extraction, dialysis against 100 mM magnesium acetate and ethanol precipitation.

For detection of crosslinked ternary complexes, [5′-^{32}P] labeling of *E. coli* $tRNA_1^{Ile}$ was performed as described [21]. [5′-^{32}P]$tRNA_1^{Ile}$ was

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mixed with cold $tRNA_I^{Ile}$ to adjust the specific radioactivity to 37 kBq/mmol. Aminoacylated or uncharged [5′-^{32}P]$tRNA_I^{Ile}$ and GTP-bound or GDP-bound EF-Tu at the same concentrations as in CD measurements were incubated together with 0.7 mM *trans*-diaminedichloro-platinum(II) [22] and subjected to 7.5% polyacrylamide gel electrophoresis and autoradiography.

The CD spectra (290–340 nm) of $tRNA_I^{Ile}$ (33 μM) and/or EF-Tu (50 μM) were recorded on a Jasco J500A spectropolarimeter. The sample solutions contained 50 mM Tris-HCl (pH 7.5 at 30°C), 10 mM magnesium acetate, 150 mM NH_4Cl, and 5 mM 2-mercaptoethanol in addition to $tRNA_I^{Ile}$ and/or EF-Tu. For conversion of EF-Tu·GDP into EF-Tu·GTP, the sample solution was supplemented with 0.75 μM EF-Tu·EF-Ts, 60 μM GTP, 0.8 mM phosphoenolpyruvate, and 0.6 μM pyruvate kinase.

3. RESULTS AND DISCUSSION

3.1. *Crosslinking analysis of ternary complex formation*

For the combination of *T. thermophilus* EF-Tu·GTP and *E. coli* Ile-tRNA, a band (B) migrating slower than that (A) of tRNA was detected (lane 2, Fig. 1), which was assigned to a crosslinked ternary complex. The amount of the Ile-tRNA that was crosslinked to EF-Tu·GTP was estimated from the band densities to be as high as 35% of that of total Ile-tRNA. In the case of EF-Tu·GTP and Phe-tRNA from *E. coli* [22], it has been reported that 35–40% of Phe-tRNA is crosslinked to EF-Tu·GTP when the ternary complex was isolated and then treated with the crosslinking reagent under the same condition as that in the present study. This indicates that *T. thermophilus* EF-Tu·GTP and *E. coli* Ile-tRNA predominantly form a ternary complex in the present experimental conditions. Similarly, for each of the combinations of EF-Tu·GDP and Ile-tRNA (lane 3), EF-Tu·GTP and uncharged tRNA (lane 4), and EF-Tu·GDP and uncharged tRNA (lane 5), formation of a ternary complex was indicated from a retarded band due to the crosslinked complex (band B in Fig. 1). Note that no crosslinking was observed for the combination of tRNA and bovine serum albumin (lane 6) or for tRNA alone (lane 1, Fig. 1).

For the combination of EF-Tu·GTP and uncharged tRNA, the band of the crosslinked complex is weaker (the crosslinking yield is about 10%) than those for the other three combinations (Fig. 1). However, the crosslinking yield possibly depends on the conformation of the complex in addition to the extent of complex formation. In fact, 99% of Ile-$tRNA_I^{Ile}$ and 95% of uncharged $tRNA_I^{Ile}$ form complexes with *T. thermophilus* EF-Tu·GTP in the conditions of the crosslinking experiments, which was estimated from the dissociation constants of these complexes (0.2 μM and 1 μM, respectively) [23]. Thus, the ternary complexes, EF-Tu·GTP·Ile-tRNA, EF-Tu·GDP·Ile-tRNA, EF-Tu·GTP·tRNA, and EF-Tu·GDP·tRNA, are predominantly formed in the present conditions of crosslinking experiments and CD measurements.

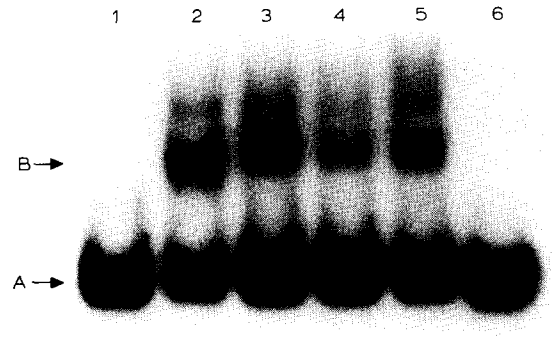

Fig. 1. Detection of the crosslinked complex of [5′-^{32}P]-labeled *E. coli* $tRNA_I^{Ile}$. Samples of (1) uncharged tRNA alone, (2) EF-Tu·GTP and Ile-tRNA, (3) EF-Tu·GDP and Ile-tRNA, (4) EF-Tu·GTP and uncharged tRNA, (5) EF-Tu·GDP and uncharged tRNA, and (6) bovine serum albumin and uncharged tRNA were treated with crosslink reagent and subjected to polyacrylamide gel electrophoresis and autoradiography. Band A is due to the intact tRNA, and band B is assigned to the crosslinked complex.

3.2. *CD spectrum and conformation of* T. thermophilus *$tRNA_I^{Ile}$*

Fig. 2 shows the CD spectra of *T. thermophilus* $tRNA_I^{Ile}$ in the near UV region at various temperatures. A positive band at 315 nm and a weak negative band at 330 nm were observed at 30–65°C. As the temperature was raised up to 95°C, the positive band at 315 nm disappeared and the intensity of the negative band at 330 nm remarkably increased (Fig. 2). In the cases of *T. thermophilus* $tRNA_f^{Met}$ and $tRNA^{Glu}$, these bands at 315 nm and 330 nm have been assigned to the s^2T residue in position 54 of these tRNAs [13,18]. In *T. thermophilus* tRNAs including $tRNA_I^{Ile}$, the ribothymidine (T) residue in the T-loop is further modified to s^2T at high temperatures, which is important for the thermostability of *T. thermophilus* tRNAs [13–18].

As for the negative band of *T. thermophilus* $tRNA_I^{Ile}$ at 330 nm, the wavelength and the intensity at 95°C (Fig. 2) are the same as those of the s^2T monomer [13]. Thus, at this temperature which is higher than the melting temperature of $tRNA_I^{Ile}$ (86.2°C) [16], $s^2T(54)$ is no longer involved in the tertiary structure. This negative band at 330 nm has been ascribed to $s^2T(54)$ in the denatured state in the cases of *T. thermophilus* $tRNA_f^{Met}$ and $tRNA^{Glu}$ ([13,18], unpublished results). Further, in these cases, the positive band at 315 nm has been assigned to $s^2T(54)$ that is involved in the tertiary structure. Similar to T(54), $s^2T(54)$ forms a reversed Hoogsteen base pair with 1-methyladenosine in position 58 and supports the tertiary base pairs joining the T-loop and the D-loop in the native conformations of *T. thermophilus* tRNAs [18]. Therefore, in the case of $tRNA_I^{Ile}$ also, the positive band at 315 nm is due to the formation of the tertiary structure where the T-loop and the D-loop are associated with each other [18].

However, the intensity of the positive band (315 nm) of $tRNA_I^{Ile}$ at 30°C is about 50% of that of $tRNA_f^{Met}$

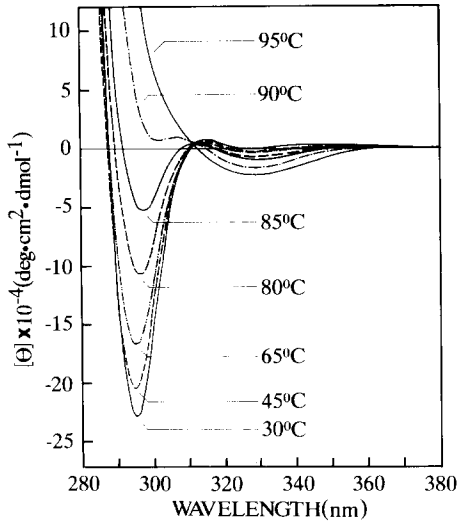

Fig. 2. CD spectra of *T. thermophilus* $tRNA_1^{Ile}$ at various temperatures.

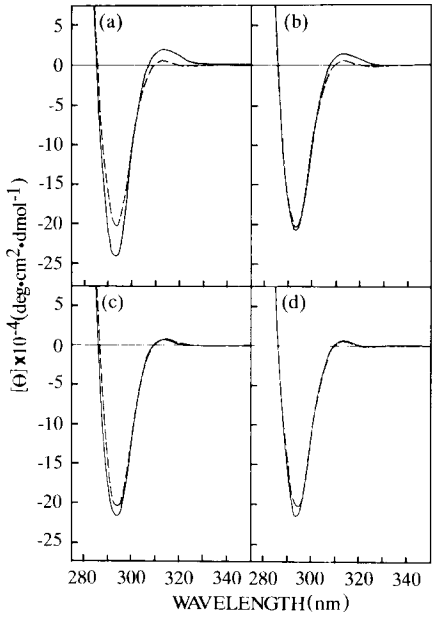

Fig. 3. CD spectra of *T. thermophilus* Ile-tRNA (or $tRNA_1^{Ile}$) (at 30°C) in (a) Ile-tRNA·EF-Tu·GTP, (b) tRNA·EF-Tu·GTP, (c) Ile-tRNA·EF-Tu·GDP, and (d) tRNA·EF-Tu·GDP, from each of which the small CD contribution of EF-Tu·GTP (or GDP) has been subtracted. Broken lines show the CD spectra of Ile-tRNA (or tRNA).

and $tRNA^{Glu}$ [13,17]. Further, the negative band at 330 nm was not observed for $tRNA_f^{Met}$ or $tRNA^{Glu}$ at 25–65°C [13,17], whereas this band was weakly observed for $tRNA_1^{Ile}$ at 30–65°C (Fig. 2). This indicates that the association of the T-loop and the D-loop in the native state of $tRNA_1^{Ile}$ is not as tight as that of $tRNA_f^{Met}$ and $tRNA^{Glu}$. This is probably due to the sequence difference in the T-loops of these tRNAs; $tRNA_1^{Ile}$ has s^2T-Ψ-C-A-m^1A while $tRNA_f^{Met}$ and $tRNA^{Glu}$ have s^2T-Ψ-C-G-m^1A. In yeast $tRNA^{Phe}$ having T-Ψ-C-G-m^1A, the 2-NH_2 group of G(57) forms hydrogen bonds with an oxygen atom of the ribose ring of G(19) and the 2′-OH group of the ribose ring of G(18) in the D-loop [24]. These two hydrogen bonds cannot be formed in tRNAs having A(57) in place of G(57), so that the association of the T-loop and the D-loop is less stable than in tRNAs having G(57).

In addition to the CD bands due to $s^2T(54)$ of *T. thermophilus* $tRNA_1^{Ile}$, a negative band was observed at 295 nm, which disappeared on denaturation at high temperatures (Fig. 2). A similar band has been assigned to the tertiary core structure consisting of the D-arm and the variable loop [13,17,25]. Therefore, this CD band due to the core structure and that due to the $s^2T(54)$ residue reflect the tertiary structure of $tRNA_1^{Ile}$. By contrast, in the CD spectrum of EF-Tu·GTP or EF-Tu·GDP in the absence of (aminoacyl-)tRNA, no CD band was observed in the region between 295 nm and 330 nm. Therefore, the CD bands of $tRNA_1^{Ile}$ in this region are useful for analyzing the conformation changes of this tRNA upon complex formation with EF-Tu.

3.3. *Conformation changes of tRNAs upon ternary complex formation*

The intensity of the positive CD band of Ile-tRNA (315 nm) was enhanced up to about three times upon complex formation with EF-Tu·GTP (Fig. 3a). The intensity of the CD band due to $s^2T(54)$ was also enhanced (two times) upon complex formation of uncharged $tRNA_1^{Ile}$ with EF-Tu·GTP (Fig. 3b). In addition, the weak negative band at 330 nm completely disappeared in these two cases. This indicates that the association of the T-loop and the D-loop of $tRNA_1^{Ile}$ is stabilized in the complex with EF-Tu·GTP as well as in the native conformations of $tRNA_f^{Met}$ and $tRNA^{Glu}$ alone. Such a rigid conformation of tRNA in the complex with EF-Tu·GTP may be required in general for the binding to the A-site of the ribosome. Probably, EF-Tu·GTP binds close to $s^2T(54)$ and stabilizes the tertiary structure around this residue. In fact, it has been shown that EF-Tu·GTP is in contact with the tandem of the aminoacyl-stem and the T-stem of aminoacyl-tRNA [5,6]. It should be noted that this conformational stabilization occurs regardless of the aminoacylation of $tRNA_1^{Ile}$.

In contrast, no significant intensity enhancement of the CD band of $s^2T(54)$ is observed upon complex formation of EF-Tu·GDP and Ile-tRNA or tRNA (Fig. 3c,d). This indicates that the complex formation with EF-Tu·GDP does not affect the association of the T-loop and the D-loop of tRNA. Thus, the γ-phosphate group of GTP bound to EF-Tu is important for the interaction of EF-Tu with the T-arm of tRNA that stabilizes the association of the T-loop and the D-loop.

The T-loop and the D-loop of aminoacyl-tRNA have been found to be dissociated upon EF-Tu-dependent binding to the A-site of ribosomes, and then the conserved GTΨC sequence in the T-arm forms base pairs with the complementary sequence of ribosomal RNA [26,27]. Our present results indicate that the dissociation of the T-loop and the D-loop does not occur before the A-site binding.

The intensity of the CD band at 295 nm reflects the tertiary structure of the core region of tRNA composed of the D-arm and the variable loop. Upon complex formation with EF-Tu·GTP, the band intensity of Ile-$tRNA_1^{Ile}$ is significantly enhanced, whereas that of uncharged $tRNA_1^{Ile}$ is not affected (Fig. 3a,b). In contrast, the intensity change of this CD band of $tRNA_1^{Ile}$ upon complex formation with EF-Tu·GDP is small, regardless of the aminoacylation of the tRNA (Fig. 3c,d). This indicates that the aminoacyl group of Ile-tRNA allows an additional conformation change in the core region of the tRNA upon complex formation with EF-Tu·GTP. This is consistent with the previous findings on *E. coli* phenylalanyl-tRNA that the fluorescence intensity of a label at s^4U (near the D-arm) changes [28] and further the D-loop becomes more susceptible to ribonuclease digestion [6] upon complex formation with EF-Tu·GTP.

The CD analyses in the present study clearly indicate that the γ-phosphate group of GTP bound to EF-Tu is essential for stabilization of the association of the T-loop and the D-loop of tRNA. Further, the interaction of the aminoacyl group of aminoacyl-tRNA with EF-Tu·GTP induces an additional change in the tertiary core structure of tRNA. These conformational features of aminoacyl-tRNA are likely to be important for the A-site binding. In this context, EF-Tu·GTP has been found to bind specifically the 3′-isomer rather than the 2′-isomer of aminoacyl-tRNA [29], which is required for the A-site binding and the peptidyl transferase reaction [30–33]. Consequently, EF-Tu·GTP plays an important role in providing aminoacyl-tRNA with the structural features that are essential for the A-site binding.

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REFERENCES

[1] Kaziro, Y. (1978) Biochim. Biophys. Acta 505, 95–127.
[2] Lucas-Lenard, J. and Haenni, A.-L. (1968) Proc. Natl. Acad. Sci. USA 59, 554–560.
[3] Pingoud, A., Block, W., Wittinghofer, A., Wolf, H. and Fisher, E. (1982) J. Biol. Chem. 257, 11261–11267.
[4] Picone, D. and Parmeggiani, A. (1983) Biochemistry 22, 4400–4405.
[5] Boutrin, A.S., Clark, B.F.C., Ebel, J.P., Kruse, T.A., Petersen, H.U., Remy, P. and Vassilenko, S. (1981) J. Mol. Biol. 152, 593–608.
[6] Wikman, F.P., Siboska, G.E., Petersen, H.U. and Clark, B.F.C. (1982) EMBO J. 9, 1095–1100.
[7] Arai, K., Ota, Y., Arai, N., Nakamura, S., Henneke, C., Oshima, T. and Kaziro, Y. (1978) Eur. J. Biochem. 92, 509–519.
[8] Nakamura, S., Ohta, S., Arai, K., Arai, N., Oshima, T. and Kaziro, Y. (1978) Eur. J. Biochem. 92, 533–543.
[9] Gulewicz, K., Faulhammer, H.G. and Sprinzl, M. (1981) Eur. J. Biochem. 121, 155–162.
[10] Nakano, A., Miyazawa, T., Nakamura, S. and Kaziro, Y. (1979) Arch. Biochem. Biophys. 196, 233–238.
[11] Nakano, A., Miyazawa, T., Nakamura, S. and Kaziro, Y. (1980) Biochemistry 19, 2209–2215.
[12] Fischer, W., Derwenskus, K.-H. and Sprinzl, M. (1982) Eur. J. Biochem. 125, 143–149.
[13] Watanabe, K., Oshima, T. and Nishimura, S. (1976) Nucleic Acids Res. 3, 1703–1713.
[14] Watanabe, K., Kuchino, T., Yamaizumi, Z., Kato, M., Oshima, T. and Nishimura, S. (1979) J. Biochem. (Tokyo) 86, 893–905.
[15] Watanabe, K., Oshima, T., Iijima, K., Yamaizumi, Z. and Nishimura, S. (1980) J. Biochem. (Tokyo) 87, 1–13.
[16] Horie, N., Hara-Yokoyama, M., Yokoyama, S., Watanabe, K., Kuchino, Y., Nishimura, S. and Miyazawa, T. (1985) Biochemistry 24, 5711–5715.
[17] Hara-Yokoyama, M., Yokoyama, S. and Miyazawa, T. (1986) Biochemistry 25, 7031–7036.
[18] Yokoyama, S., Watanabe, K. and Miyazawa, T. (1987) Adv. Biophys. 23, 115–147.
[19] Yarus, M. and Barrell, B.G. (1971) Biochem. Biophys. Res. Commun. 43, 729–734.
[20] Kawakami, M., Miyazaki, M., Yamada, H. and Mizushima, S. (1985) FEBS Lett. 185, 162–164.
[21] Donis-Keller, H., Maxam, A.M. and Gilbert, W. (1977) Nucleic Acids Res. 4, 2527–2538.
[22] Wikman, F.P., Romby, P., Metz, M.H., Reinbolt, J., Clark, B.F.C., Ebel, J.P., Ehresmann, C. and Ehresmann, B. (1987) Nucleic Acids Res. 15, 5787–5801.
[23] Kohno, T., Kohda, D., Haruki, M., Yokoyama, S. and Miyazawa, T. (1990) J. Biol. Chem., in press.
[24] Quigley, G.J. and Rich, A. (1976) Science 194, 796–806.
[25] Willik, G., Oikawa, K. and Kay, C.M. (1973) Biochemistry 12, 899–904.
[26] Schwarz, U., Menzel, H.M. and Gassen, H.G. (1976) Biochemistry 15, 2484–2490.
[27] Sprinzl, M., Wagner, T., Lorenz, S. and Erdmann, V.A. (1976) Biochemistry 15, 3031–3039.
[28] Adkins, H.J., Miller, D.L. and Johnson, A.E. (1983) Biochemistry 22, 1208–1217.
[29] Taiji, M., Yokoyama, S. and Miyazawa, T. (1985) J. Biochem. (Tokyo) 98, 1447–1453.
[30] Sprinzl, M. and Wagner, T. (1979) in: Transfer RNA: Structure, Properties, and Recognition (Schimmel, P.R., Söll, D. and Abelson, J.N. eds) pp. 473–485, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
[31] Taiji, M., Yokoyama, S., Higuchi, S. and Miyazawa, T. (1981) J. Biochem. (Tokyo) 90, 885–888.
[32] Taiji, M., Yokoyama, S. and Miyazawa, T. (1983) Biochemistry 22, 3220–3225.
[33] Taiji, M., Yokoyama, S. and Miyazawa, T. (1985) Biochemistry 24, 5776–5780.