THE CONFORMATION DIFFERENCE BETWEEN $tRNA_f^{met}$ AND FORMYLMETHIONYL-$tRNA_f^{met}$ FROM E. COLI

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Summary : The local conformation around 4-thiouracil (4TU) base in fmet-$tRNA_f^{met}$ and $tRNA_f^{met}$ were studied by CD spectra in near ultraviolet region as well as by the hyperchromicity at 330 nm. The presence and absence of the negative CD band at 360 nm observed for $tRNA_f^{met}$ and fmet-$tRNA_f^{met}$ respectively, indicated that 4TU base has strong interaction with the other bases in the former but not in the latter. This was confirmed by the hyperchromicity at 330 nm accompanied by an elevation of temperature in the former but absent in the latter. The tertiary structural difference between these two tRNAs is discussed.

Introduction : Aminoacyl tRNA and stripped tRNA behaves quite differently in their interaction with aminoacyl RNA synthetase[1], Tu-factor[2] or initiation factor f2[3]. Since their primary structures are the same except for the terminal amino acid, it can be deduced that their functional difference should exist in their variant conformations. However, any physicochemical properties reflecting their secondary structures have failed to differentiate them distinctly.[4)-7)] Thus it will be suggested that some difference in their tertiary structure would allow explanation for the functional dissimilarity.[8),9)]

In order to investigate the tertiary structural differences between acylated and deacylated tRNAs, we focused our attention on the 4-thiouracil group (4TU), which exists at the intermediate position between the CCA arm and dihydrouracil (DiHU) arm of most tRNAs derived from E.coli. If the clover-leaf structure could be folded into some tertiary structure then the 4TU group should correspond to the hinge of those two arms, and it can be expected that its conformation would vary depending upon the tertiary structure of tRNA.

In this communication we shall report the circular dichroism and hyperchroism of the 4TU group in formyl-methionyl-tRNA (fmet-$tRNA_f^{met}$) and $tRNA_f^{met}$ and shall show that these two optical properties are quite different between the two tRNAs. Since 4TU has its absorption band at around 330 nm[10),11)], optical properties mentioned above cannot be overshaded by those of other bases.[12),13)]

Materials and Methods : Aminoacylation and formylation of $tRNA_f^{met}$ were carried out as described.[14)] Unreacted amino acid and ATP were removed chromatographically on a Sephadex G-25 column. The tRNA fraction thus obtained was further fractionated into stripped, methionyl and formylmethionyl tRNAs on a benzoylated DEAE-cellulose column. The details of this procedure are to be published later.[15)] The chromatographic pattern gave three peaks ; the two retarded peaks were radioactive. From the nature of BD-cellulose, it was deduced that the first peak corresponded to uncharged $tRNA_f^{met}$, the second to met-$tRNA_f^{met}$ and the last to fmet-$tRNA_f^{met}$. The extent of aminoacylation of fmet-tRNA was estimated to be more than 70 %, being based on 5 % cold TCA-precipitable ^{3}H-met activity and optical density. The possibility of deacylation was cancelled by counting the specific activity of fmet-tRNA before and after each measurement. ^{3}H-met activity was monitored by a HORIBA Liquid Scintillation Spectrometer LS-500. The hyperchromicity of tRNA on heating was obtained on a Gilford spectrophotometer model 240. The concentration of base necessary for the determination of the molecular ellipticity coefficient (θ) was obtained by phosphate assay.[16)] UV and CD spectra were obtained on a JASCO ORD/UV-5 spectropolarimeter with CD attachment. All the experiments were carried out at 25° except otherwise mentioned.

Results and Discussion : Fig.1(a) shows CD spectra of $tRNA_f^{met}$ both in the presence and absence of 5 mM Mg^{2+}. When Mg^{2+} was omitted from the solution the CD spectrum gave one broad positive band with a peak

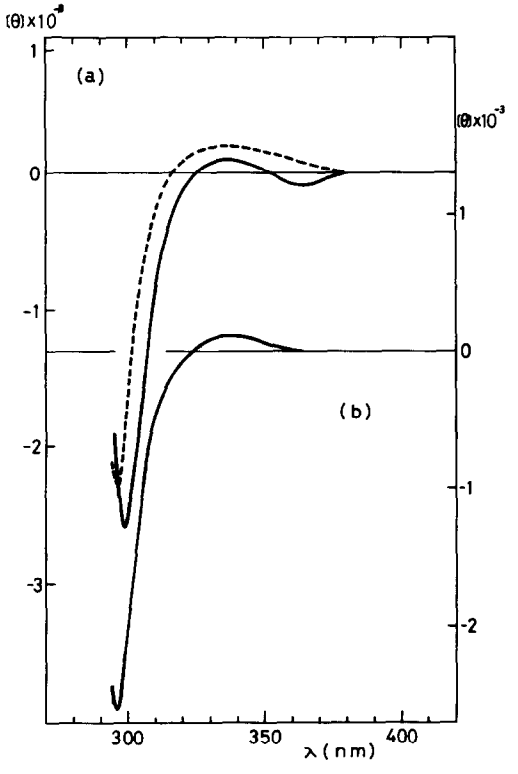

Figure 1 (a) CD spectra of $tRNA_f^{met}$ in the presence (solid line) and absence (dotted line) of $Mg(OAc)_2$. Solvent contained 0.2 M NaCl, 0.02 M NaOAc (pH 5.0) with or without 5 mM $Mg(OAc)_2$ In the following figures solvent used was the same as in this figure.

Figure 1 (b) CD spectrum of fmet-$tRNA_f^{met}$ in the presence of Mg^{2+}.

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around 337 nm[12] and a large and sharp negative band at 296 nm. However, when 5 mM Mg^{2+} was added the CD spectrum produced small negative and positive bands at 364 and 335 nm, respectively[13], besides a large and sharp negative band at 298 nm. The band around 340 nm could be attributed to the 4TU group since both 4-thiouridylic acid and 4-thiouridine gave UV and CD bands at about this wavelength.[11] The band at 365 nm is difficult to assess. However, as can be shown in Fig.2 the UV spectrum of $tRNA_f^{met}$ revealed a shoulder around 360 nm in the presence of 5 mM Mg^{2+} but not in the absence of Mg^{2+}. It is evident from the figure that the addition of Mg^{2+} resulted in hypochromicity of the 340 nm absorption band. The appear-

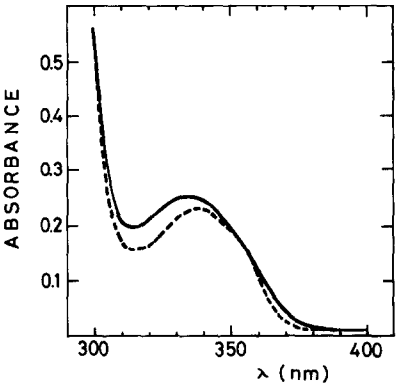

Figure 2 Near ultraviolet absorption spectra of $tRNA_f^{met}$ in the presence (dotted line) and absence (solid line) of Mg^{2+}.

ance of UV and CD bands at 360 nm and hypochromicity cannot be due to the binding of Mg^{2+} to the 4TU group since the addition of Mg^{2+} to the solution of 4-thiouridylic acid failed to result in such effects. Thus, it is suggested that in the presence of Mg^{2+} the 4TU base of $tRNA_f^{met}$ interacts strongly with other bases but little interaction exists when Mg^{2+} was omitted. Such interaction is probably strong enough to split the π-π^* transition band at 340 nm or allow n-π^* transition at 360 nm, the latter of which was originally forbidden. Fig.1 (b) indicates that the CD spectrum of fmet-$tRNA_f^{met}$ with a small positive band at 338 nm and a large and sharp negative band at 296 nm is similar to that of $tRNA_f^{met}$ in Mg^{2+} free solution. It should be stated that the CD spectrum of fmet-$tRNA_f^{met}$ was obtained in the presence of 5 mM Mg^{2+}. Thus it is suggested that the 4TU group of fmet-$tRNA_f^{met}$ does not interact with other bases even in the presence of Mg^{2+}.

The difference in local conformations around the 4TU group of each of the two tRNAs can be proved by the melting of these conformations being traced by the hyperchromicity at 330 nm.[17] The results are shown in Fig.3(a). A sharp increase in the optical density at 330 nm was observed when uncharged $tRNA_f^{met}$ was heated above 70°. This melting curve runs almost in parallel with that traced by the hyperchromicity at 260 nm, which is shown in Fig. 3 (b). This indicates that the local conformation around 4TU

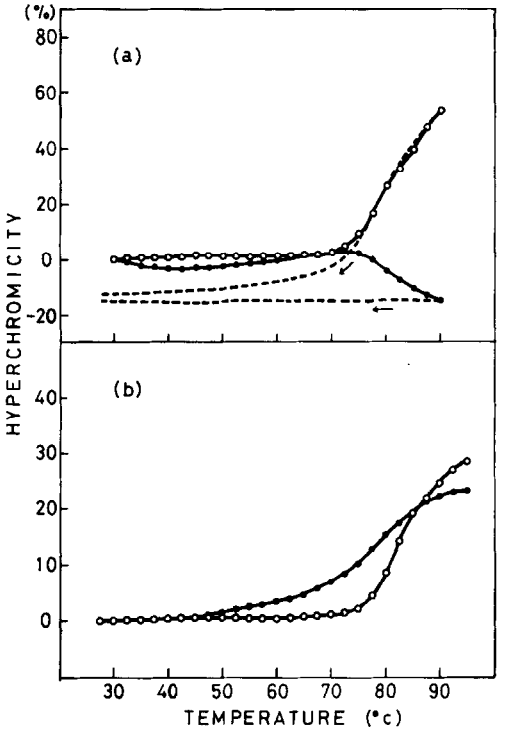

Figure 3 (a) Thermal denaturation profiles of $tRNA_f^{met}$ (—o—o—) and fmet-$tRNA_f^{met}$ (—●—●—) monitored by the absorbance at 330 nm in the presence of Mg^{2+}.

Figure 3 (b) Thermal denaturation profiles of $tRNA_f^{met}$ (—o—o—) and fmet-$tRNA_f^{met}$ (—●—●—) monitored by the absorbance at 260 nm in the presence of Mg^{2+}.

breaks down almost in parallel with the rest of the molecule. Vividly in contrast, the optical density at 330 nm did not increase but rather decreased when fmet-$tRNA_f^{met}$ was heated. However, a normal melting curve could be obtained when the increase in optical density was traced at 260 nm. These results clearly indicate that there exist a remarkable difference in the local conformations around 4TU group of two tRNAs.

The decrease in optical density observed in Fig. 3 (a) can be interpreted as the conversion of 4TU into uracil since cooling curves of two samples converged to almost the same point as indicated in Fig.3 (a). It has been reported that 4TU can be easily converted to uracil by heat treatment in acid solution.[10] It should be added that the above results are repeatable

and when fmet-tRNA was discharged by alkaline treatment it gave completely the same results with uncharged tRNA.

By summarizing all the results mentioned above we can conclude the following. The local conformation around the 4TU group of acylated and deacylated $tRNA_f^{met}$, which are dissolved in solution containing 5 mM Mg^{2+}, are different from each other. In deacylated $tRNA_f^{met}$, the 4TU base interacts or stacks with other bases, while in fmet-$tRNA_f^{met}$, it has little interaction or stacking with other bases. On the other hand, little difference could be observed between acylated and deacylated tRNA in their secondary structures of base paired regions being judged from the previous CD and hyperchromicity studies.[5)-7)] If we consider that the 4TU group locates itself at the hinge of the CCA arm and DiHU arm of the clover-leaf structure, it can be deduced that the difference in local conformations around the 4TU group reflects the difference of the tertiary structures of acylated and deacylated tRNA. Probably in deacylated tRNA the four arms of the clover-leaf structure would be packed into a tight tertiary structure, but in fmet-$tRNA_f^{met}$, the four arms would stick out rather freely with little interaction between them. The less cooperative melting curve with a lower Tm value obtained for fmet-$tRNA_f^{met}$ monitored by the absorbance at 260 nm supports this conclusion.

In any case the difference of tertiary structures observed for acylated and deacylated tRNA would have a significant meaning in their functions.

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