ATP and Mg^{2+} Ions Regulate Both Conformation and Aminoacylation Capacity of Transfer RNA

ATP と Mg^{2+} イオンが転移 RNA のコンフォメーションと
アミノ酸受容能を共に調節する

Takashi OHYAMA¹, Shosuke TAKEMURA and Kazuya NISHIKAWA²
Institute of Molecular Biology, School of Science, Nagoya University,
Furo-cho, Chikusa-ku, Nagoya 464, JAPAN

¹ Present address: Division of Molecular Biology, Meiji Institute of Health Science,
540 Naruda, Odawara 250, JAPAN

² Present address: Department of Biological Sciences, Faculty of Bioscience and Biotechnology,
Tokyo Institute of Technology, 4259 Nagatsuta, Midori-ku, Yokohama 227, JAPAN
TEL : 81-45-922-1111, ext. 2470, · FAX : 81-45-923-1136

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ABSTRACT

It has been known that transfer RNA assumes a functional conformation in the presence of certain metal ions, most commonly magnesium ions. Several Mg^{2+} -binding sites of high affinity have been revealed by X-ray crystallographic analyses of yeast tRNA^{Phe}. On the other hand, ATP, a naturally occurring high-energy compound and which is one of the substrates in aminoacylation of tRNA, is known to have ability to chelate Mg^{2+} . We therefore examined the possibility that the conformation of tRNA is regulated by the quantitative balance between Mg^{2+} and ATP, and consequently biological activity of tRNA is modulated as well. Structural transition of tRNA was monitored by measuring the melting profiles of yeast tRNA^{Tyr} in the presence of Mg^{2+} and/or ATP at various [ATP]/[Mg^{2+}] ratios. Tyrosine-accepting activity of yeast tRNA^{Tyr} was also measured under the similar solution conditions. These results clearly indicated that conformation of tRNA and its aminoacylation capacity can be easily modulated by changing the relative concentration ratio of Mg^{2+} and ATP.

Key words: Transfer RNA Conformation, Tertiary Structure, Adenosine Triphosphate, Melting Temperature, Magnesium Ion, Aminoacylation.

1. INTRODUCTION

Transfer RNA (tRNA) plays a key role in protein synthesis by providing the crucial link between the nucleotide sequence of a triplet codon and a specific amino acid which is inserted into a growing polypeptide chain. The biological function of tRNA has been discussed on the basis of the "L-shaped" tertiary structure of the molecule since the three-dimensional structure of yeast tRNA^{Phe} was revealed by X-ray crystallographic analyses.^{1,2} There is growing evidence to show that the L-shaped structure is essential

for biological activity of tRNA, such as aminoacylation and peptide formation on ribosomes. We have previously reported that the interaction between the D-loop and the TΨC-loop of tRNA is important both for the maintenance of the L-shaped tertiary structure and for amino-acylation capacity by our *in vitro* experiments using enzymatically reconstructed tRNA variants. We have also pointed out that the Mg^{2+} concentration in the tRNA solution determines the stability of the L-shaped structure.³⁻⁶ However, whether tRNA functioning *in vivo* still maintains the L-

shaped structure or not is an open question. Enormous variety of low-molecular-weight compounds and macromolecules are contained in the cellular matrix. And some of them, citrate and adenosine triphosphate (ATP) for example, have ability to chelate Mg^{2+} ions. What if these compounds competed with tRNA for Mg^{2+} ions available in the cellular matrix? Is it possible for these compounds to modulate tRNA conformation, and activity as well, by depriving tRNA of bound Mg^{2+} ions?

In this paper, we describe results of two lines of *in vitro* experiments attempted to answer the questions above. First, the melting profile of yeast tRNA^{Tyr} was measured in the presence of Mg^{2+} and/or ATP at various $[ATP]/[Mg^{2+}]$ ratios. Melting profile has been used as a simple and powerful tool in studying the conformational states of the macromolecules. Although several reports have been published on the melting transition of tRNA in the absence or presence of Mg^{2+} ions,⁷⁻¹⁰ effect of ATP on the melting profile of tRNA has not been investigated so far. Second, Tyrosine-accepting activity of yeast tRNA^{Tyr} was measured under the conditions containing both of Mg^{2+} and ATP at various $[ATP]/[Mg^{2+}]$ ratios. These results indicated that conformation of tRNA and its aminoacylation capacity can be easily modulated by changing the relative concentration ratio of Mg^{2+} and ATP.

2. EXPERIMENTAL

Yeast tRNA^{Tyr} and tyrosyl-tRNA synthetase (TyrRS) were purified from *Torulopsis utilis* cells by several steps of column chromatographies, as reported previously.⁵ This preparation of tRNA^{Tyr} accepted 1.8 nmol tyrosine/A₂₆₀ unit. Melting profiles of tRNA were recorded automatically by using a UVIDEC 650 spectrophotometer (JASCO) equipped with a microcuvette holder, whose temperature was controlled by circulating hot water with the aid of refrigerated circulating bath RTE-5 and temperature programmer ETP-3 (NESLAB). The temperature increase rate was 2°C/min. 3-(*N*-morpholino) propanesulfonic acid (MOPS)-NaOH buffer

(pH 7.4) or *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES)-NaOH buffer (pH 7.6) was used to minimize the pH change following the temperature increase. Since the concentration of ATP used in this experiment was about 10³-fold higher than that of sample tRNA, the melting transition of tRNA was monitored at 290 nm rather than 260 nm, in order to obtain better S/N ratio (Fig. 1). The increase of Absorbance at 290 nm (Hyperchromicity) reflects the unfolding of tRNA structure. The melting temperature (*T*_m) was determined from the differential melting profile which was computed by a built-in program within the spectrophotometer.

Fig. 1. UV-absorption spectra of 66.7 μ M ATP(A) and 1.8 μ M yeast tRNA^{Tyr}(B) at pH 7.6.

Aminoacylation (tyrosine-acceptance) assay was performed as follows. Reaction mixtures (150 μ l) containing about 1.6 μ M tRNA^{Tyr}, 100 mM MOPS-NaOH (pH 7.4), 40 mM KCl, 25 mM L-[¹⁴C]-tyrosine (50 mCi/mmol, obtained from Amersham), 3.6 μ g/ml TyrRS and variable concentrations (indicated in the Figure) of $MgCl_2$ and ATP were incubated at 30°C. Aliquots (25 μ l each) were removed at pre-determined time intervals and spotted on small pieces of Whatman 3MM filters, and then the filters were immersed into cold 0.1 N HCl. The filters were washed twice with 0.1 N HCl, once with ethanol and dried. The radioactivities on the filters were counted in a liquid scintillation counter.

3. RESULTS AND DISCUSSION

At first, the effect of Mg^{2+} on the melting profiles of *T. utilis* tRNA^{Tyr} was investigated. As shown in Fig. 2, this tRNA showed a very steep transition with $T_m=80^\circ C$ at Mg^{2+} concentrations higher than 1 mM (curves C, D). This is in good agreement with the results of earlier studies,^{3,10} in which T_m values of $78-81^\circ C$ for native tRNA^{Tyr} were obtained under similar conditions containing 10 mM $MgCl_2$. Concentration of 1mM Mg^{2+} seems to be enough for maintaining the rigid (L-shaped) structure of tRNA^{Tyr}. At lower Mg^{2+} concentrations, transition temperature gradually shifted to lower temperatures ($T_m=73^\circ C$ at 200 μM Mg^{2+} , and $T_m=64^\circ C$ at 50 μM Mg^{2+}). Therefore Mg^{2+} concentration was fixed at 1 mM in the following experiments for measuring melting profiles in the presence of ATP. The effect of addition of various concentrations of ATP on the melting

Fig. 2. Effect of Mg^{2+} ion on the melting profile of yeast tRNA^{Tyr}. The measurement was performed with 1.7 μM tRNA in 20 mM MOPS-NaOH (pH 7.4) containing (A) 50 μM , (B) 200 μM , (C) 1 mM, or (D) 10 mM $MgCl_2$, respectively.

profile of tRNA^{Tyr} was examined in the presence of 1 mM Mg^{2+} (Fig. 3). As seen in the figure, ATP can drastically lower the melting temperature of tRNA. When 1 mM ATP was added in the tRNA solution ($[ATP]/[Mg^{2+}]=1$, curve C), melting temperature (T_m) was lowered by $23^\circ C$ (from 80 to $57^\circ C$). Furthermore, when the ratio of $[ATP]/[Mg^{2+}]$ was raised to 2.5 (curve E), the

T_m drop was $38^\circ C$ (80 to $42^\circ C$). In this case, it should also be mentioned that, melting of tRNA was observed to begin already at about $25^\circ C$ (not shown in Fig. 3, since the melting curves are "normalized" to show the relative Absorbance). This observation suggests that tRNA can be melted or "loosened" even at room temperature if the total concentration of ATP and compounds which can chelate Mg^{2+} (such as citrate, phosphate, and so on) is comparable to or higher than the Mg^{2+} concentration.

Fig. 3. Effect of ATP on the melting profile of yeast tRNA^{Tyr}. The measurement was performed with 1.7 μM tRNA in 20 mM MOPS-NaOH (pH 7.4) containing 1 mM $MgCl_2$ and various concentrations of ATP; (A) no ATP, (B) 0.5 mM, (C) 1 mM, (D) 1.5 mM, (E) 2.5 mM.

Thus, it was shown that ATP has ability to induce tRNA melting at relatively low temperature. In order to verify that this effect is brought about by the Mg^{2+} -chelating ability of ATP, adenosine (Ado) or adenosine diphosphate (ADP) was used instead of ATP in the measurement of melting profile. As shown in the Fig. 4, Ado had no effect at all and the T_m was $80^\circ C$, which is the same value as that obtained in the absence of ATP (Fig. 2C). However, ADP was moderately effective in lowering the T_m of tRNA (from 80 to $62^\circ C$). Considering that 1.5 mM ATP lowered the T_m by $35^\circ C$ (from 80 to $45^\circ C$), ADP is about half as much active as ATP in lowering the T_m . These result corresponds well with the fact that phosphate groups of ATP play an important role in chelating Mg^{2+} .

Fig. 4. ADP can also lower the T_m of tRNA but Adenosine cannot. Melting profiles were measured in 20 mM HEPES-NaOH (pH 7.6) containing 1 mM $MgCl_2$ and 1.5 mM each of (A) ATP, (B) ADP, or (C) Adenosine.

Tyrosine-acceptance under various combinations of Mg^{2+} and ATP concentrations is shown in Fig. 5. With $[Mg^{2+}] = 2$ mM and

Fig. 5. Effect of Mg^{2+} and ATP concentrations on tyrosine acceptance of $tRNA^{Tyr}$. Reaction mixtures (150 μ l) containing about 1.6 μ M $tRNA^{Tyr}$, 100 mM MOPS-NaOH (pH 7.4), 40 mM KCl, 25 μ M L- $[^{14}C]$ -tyrosine (50 mCi/mmol), 3.6 μ g/ml TyrRS and variable concentrations of $MgCl_2$ and ATP were incubated at 30°C. (A) $[Mg^{2+}] = 2$ mM and $[ATP] = 1.5$ mM, (B) same as (A), but ATP was added at 1 min (open arrow), (C) $[Mg^{2+}] = 2$ mM and $[ATP] = 3.5$ mM, (D) same as (C), but Mg^{2+} was added at 2 min (closed arrow). Aliquots (25 μ l each) were withdrawn at time intervals shown in the Figure.

$[ATP] = 1.5$ mM, tyrosine is accepted normally by the tRNA (curve A). However, when ATP is added at 1 min to the final concentration of 3.5 mM ($[ATP] > [Mg^{2+}]$), the reaction immediately stops (curve B). On the other hand, reaction which did not proceed significantly with $[Mg^{2+}] = 2$ mM and $[ATP] = 3.5$ mM (curve C) resumes as soon as Mg^{2+} is added to the final concentration of 4 mM ($[ATP] < [Mg^{2+}]$, curve D). These results clearly indicate that the aminoacylation capacity of $tRNA^{Tyr}$ is totally modulated by the $[ATP]/[Mg^{2+}]$ ratio in the assay system. Since these effects were observed in the range of physiologically feasible concentration for ATP or Mg^{2+} , regulation of tRNA conformation and function by $[ATP]/[Mg^{2+}]$ ratio may take place also *in vivo*.

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