81. Structural Features of Bovine Mitochondrial tRNA$^{\text{Ser}}_{\text{AGY}}$ Lacking the D Arm

By Takuya UEDA, Kimitsuna WATANABE, and Takahisa OHTA
Department of Agricultural Chemistry, Faculty of Agriculture, University of Tokyo, Bunkyo-ku, Tokyo, 113

(Communicated by Masao KOTANI, M. J. A., Dec. 12, 1983)

Most of mammalian mitochondrial tRNAs have unique structural characteristics; they lack invariant or semi-invariant sequences such as GG in the D loop and TGC in the T loop and there are considerable amount of non-Watson-Crick base pairing, in addition to very high A-U pair content in the stem regions.\(^1\)-\(^5\) Above all, tRNA$^{\text{Ser}}_{\text{AGY}}$ is the strangest species, since it lacks the D stem,\(^4\)\(^,\)\(^5\) which has been regarded to be important for the recognition of tRNA by aminoacyl-tRNA synthetase in the non-mitochondrial systems,\(^6\) as well as for the maintenance of the L-shaped structure of tRNA by tertiary interaction between the D and T loops.\(^7\)

From these points of view, it is of interest to investigate the structure-function relationship of this unique species, however, there have been considerable difficulties in preparing generally mammalian mitochondrial tRNAs in amount enough for biochemical studies.\(^8\) In addition, it is said that nobody has so far succeeded to charge a purified tRNA species in \textit{in vitro} systems.\(^5\) de Bruijn and Klug examined the tertiary structure of tRNA$^{\text{Ser}}_{\text{AGY}}$ by chemical probing.\(^5\) Recently we reported a method for large scale (milligram order) preparation and some properties of tRNA$^{\text{Ser}}_{\text{AGY}}$ from bovine heart mitochondria. The tRNA$^{\text{Ser}}_{\text{AGY}}$ was still active in serine acceptor activity \textit{in vitro}.\(^9\) We will describe here more detailed structural features of this species.

Materials and methods. tRNA$^{\text{Ser}}_{\text{AGY}}$ was prepared as described previously.\(^9\) From 6.3 kg of bovine hearts, about 5 A$_{260}$ units of tRNA$^{\text{Ser}}_{\text{AGY}}$ was finally obtained and the sample was active in \textsuperscript{14}C-serine acceptance (502 pmoles/A$_{260}$) by mitochondrial S-100 fraction prepared by the method of Lynch and Attardi.\(^10\) Melting profiles and CD spectra of tRNAs were obtained as described previously.\(^11\),\(^12\) Molar extinction coefficients for active and inactive forms of tRNA$^{\text{Ser}}_{\text{AGY}}$ were determined as described.\(^13\) Limited hydrolysis of tRNA by nucleases was carried out according to Wrede \textit{et al.}\(^14\)

Results and discussion. Fig. 1 shows the melting profiles of tRNA$^{\text{Ser}}_{\text{AGY}}$ in the presence and absence of Mg$^{2+}$, with yeast tRNA$^{\text{ns}}$.
as a reference. Whereas yeast tRNA^{Phe} showed normal melting profiles in both the presence and absence of Mg^{2+}, those of tRNA^{Ser}_{AGY} were quite unique. In the absence of Mg^{2+}, the absorbance began to increase at 25°C, with the midpoint at around 40°C. This is consistent with the results of de Bruijn and Klug. The melting started

Fig. 1. Melting profiles of tRNA^{Ser}_{AGY} (solid line) and yeast tRNA^{Phe} (dotted line) in the standard buffer without (A) and with Mg^{2+} (B). 0.1 A_{260} unit/ml tRNA (0.35 ml) was measured in the cuvette of 1 cm path length.

Fig. 2. a: Autoradiograph of partial digestion of 3'-32P-labeled tRNA^{Ser}_{AGY} with alkali (lane 5), RNase T1 (lane 1, 0.005 U; 2, 0.01 U; 3, 0.02 U; 4, 0.01 U plus EDTA) and nuclease S1 (lane 6, 0.1 U and 7, 0.2 U) as reported. b: Secondary structure of tRNA^{Ser}_{AGY} as supposed by this work. The arrows show the sensitive sites toward nuclease S1.

at 50°C in the presence of Mg^{2+} with the midpoint at 56°C. These results also suggest the unusual secondary or tertiary structure of tRNA^{Ser}_{AGY} with high A-U pairs.

Then, the tertiary structure of tRNA^{Ser}_{AGY} was examined by limited hydrolysis with RNase T1 and/or nuclease S1, of tRNA^{Ser}_{AGY} labeled with 32P-pCp at the 3'-end. As Fig. 2a shows, G20 is the most sensitive site toward RNase T1 and all the other G residues can be cleaved as the amount of the enzyme is increased. Nuclease S1, on the other hand, which attacks only the residues within the single-stranded regions, cleaves U42, U45, and U48 in the T loop, C61 and C62 at the CCA end, U8 in the irregular D-loop, G20 in the anticodon loop and U28 in the anticodon stem. Little band was
observed for the residues in the D loop except for U8, and those in the extra loop. These results strongly suggest that some extra base-pairings can occur between the D and extra loops, namely, G11-C32, C12-G31 and A13-U30. The base pairs should be formed as shown by the dotted lines in Fig. 2b.

These findings coincide well with the conclusion by de Bruijn and Klug. However, the possible base-pairings of U8-A46, A9-U45, and U10-A43, also suggested by these authors seem improbable from our results, because U8 and three U residues in the T-loop are very sensitive toward nuclease S1 as mentioned above.

From these results, it may be supposed that in the tertiary structure of tRNA the T loop is protruded from the double helix formed by amino acid acceptor stem and T stem, and the anticodon stem is prolonged by the additional three base pairs. Thus the whole tertiary structure may be as T-shaped rather than L-shaped.

The fact that the 14C-serine acceptor activity was completely lost at a certain step was observed during the purification process of tRNA. It turned out that the RPC-5 column chromatographic step at the acidic pH was the most responsible for the activity loss. Thus it seems that there exist at least two forms for tRNA; active and inactive forms.

The inactive form was obtained by purifying the tRNA-rich fraction through the RPC-5 column at pH 4.35, followed by 15% polyacrylamide gel electrophoresis with 7 M urea. This form could not be renatured by conventional renaturation procedures, such as heating with Mg2+ or addition of spermine. It was renatured...
when it was incubated at 55°C in 50% formamide. These results suggest that a great structural rearrangement should occur between active and inactive forms, involving the formation and breakage of several base pairs.

On the basis of these findings an attempt was made to clarify the structural difference between these two forms. The molar extinction coefficients of the two forms determined by the alkaline hydrolysis method,\(^1\) were 8,600 for the active form and 8,300 for the inactive form, in the standard buffer consisting of 10 mM Tris-HCl (pH 7.5), 10 mM MgCl\(_2\) and 0.2 M NaCl. Both values are about 10% higher than those of the normal tRNAs (7,000–7,500),\(^1\) probably indicating that the tertiary structure of these two forms are less compact than those of the normal L-shaped tRNAs.\(^1\)

The CD spectra of the two forms were also obtained as shown in Fig. 3. The molecular ellipticity coefficient [\(\Theta\)] of the inactive form was reduced by 4,000 at the main peak and the peak shifted to the longer wavelength side by 2 nm, as compared with those of the active form. This suggests that the inactive form has less A-U pairs than does the active form.\(^1\) In addition, the inactive form is difficult to be labeled at the 3'-end with \(^{32}\)P-pCp and RNA ligase, this implying that the 3'-end must be masked by the base pairing. This is one of the reason why the inactive form does not possess amino acid acceptor activity any more. Although the inactive form might include several conformers, one probable structure may be supposed as shown in Fig. 4b. Thus, it may not be difficult to suppose that a great structural rearrangement is necessary for trans-conformation between the active and inactive forms.

References

13) Blum, A. D. et al. (1972) : ibid., 11, 3248–3256.