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Purification and characterization of two serine isoacceptor tRNAs from bovine mitochondria by using a hybridization assay method

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ABSTRACT

For large scale preparation of mitochondrial tRNAs, a new hybridization assay method using synthetic oligodeoxyribonucleotide probes (16-17mer) complementary to individual tRNA sequences was developed and applied for the purification of two serine isoacceptor tRNAs ($tRNA^{Ser}_{AGY}$ and $tRNA^{Ser}_{UCN}$) from bovine mitochondria. It is about 100 times more sensitive than the conventional aminoacylation assay method. 2-4 A_{260} units each of both $tRNA^{Ser}$ isoacceptors were purified from 17.5 kg of bovine liver, and they were characterized by means of nuclease digestion, melting profiles and aminoacylation activity. It is suggested that $tRNA^{Ser}_{UCN}$ possesses the D loop/T loop interaction like usual L-shaped tRNAs, and that $tRNA^{Ser}_{AGY}$ lacking almost an entire D arm is aminoacylated with an efficiency not very much lower than that of $tRNA^{Ser}_{UCN}$.

INTRODUCTION

Most tRNAs of animal mitochondria are known to possess unusual secondary structures ; many tRNAs lack invariant GG and TΨC sequences in the D and T loops, respectively (1,2), suggesting no interaction of D and T loops which is necessary for forming the common L-shaped tertiary structure. $tRNA^{Ser}_{AGY}$s of both vertebrate and invertebrate lack the normal D arm (3-12) and most mitochondrial (mt) tRNAs from nematoda worms lack the T arm (13).

However these facts were mainly deduced from the nucleotide sequence of tRNA genes, and studies on the structure-function relationship of unusual mt tRNAs have not progressed yet because of many difficulties in preparing them from animal tissues in sufficient amounts for biochemical studies (14,15). Another reason is that there has been no efficient method of detecting mt tRNA. The conventional aminoacylation assay method (16) has the

following disadvantages for the purification of mt tRNAs. First, the sensitivity (hence the specific activity of labeled amino acids) is insufficient for detecting a limited amount of mt tRNA out of a much greater amount of cytosolic (cyt) tRNA; second, it is impossible to discriminate mt tRNA from cyt tRNA, because mt synthetase aminoacylates both mt and cyt tRNAs (17 and Kumazawa et al, submitted); and third, it is also impossible to discriminate isoacceptor tRNAs.

To overcome these disadvantages we adopted a hybridization assay method using oligodeoxyribonucleotides as probes, which are complementary to particular regions of tRNA. By using this method two serine isoacceptor tRNAs could be purified, and the higher-ordered structures and aminoacylation activities of these two structurally variant tRNAs were compared. The new method proved to be very useful for the purification of not only mt tRNAs but also other bacterial and cytosolic tRNAs.

MATERIALS AND METHODS

Preparation of mt tRNAs

Mt bulk tRNAs ($tRNA^{Mix}$) and partially purified seryl-tRNA synthetase were obtained from digitonin-treated mitochondria (mitoplasts) which were prepared from bovine liver according to the methods of Eberly et al (18) and de Vries and van der Koogh-Schuuring (19). 5 kg of bovine liver was minced and suspended in 5 l of ST buffer (0.25 M sucrose and 2mM TES-KOH pH 7.4), which was then homogenized with a tefron homogenizer. The homogenate was centrifuged at 700 x g for 10 min to remove nuclei etc. and then the supernatant was centrifuged down at 7,000 x g for 10 min to obtain the mitochondrial fraction. It was resuspended into ST buffer and washed by repeating the centrifugation. The resulting crude mt fraction containing about 100 g of mt proteins was diluted with ST buffer to a final concentration of 20 g of mt proteins/l, into which 12 g of digitonin , which had been dissolved in hot ST buffer (12 ml) followed by chilling to $0^{o}C$ was added slowly. The mixture was stirred for 15 min and centrifuged at 7,000 x g to obtain the mitochondria as a pellet. Remaining digitonin was washed out by repeating this procedure

several times. About 350 g (wet weight) of digitonin-treated mitochondria (mitoplasts) was finally recovered through this process, which was repeated a number of times to obtain an adequate amount of mitoplasts.

To 450 g of mitoplast suspension, 2 l of 50 % phenol solution containing 30 mM NaOAc, 5 mM $MgCl_2$, 0.1 mM EDTA and 1.2 % diethylpyrocarbonate was added. After shaking at room temperature for 11 hrs, the sup fraction was recovered by centrifugation at 10,000 x g for 15 min. The precipitate was resuspended into the above solution without phenol and the mixture was shaken, centrifuged as above and the resulting sup fraction was combined with the first sup fraction. After treatment with chloroform to remove contaminating phenol followed by ethanol precipitation, the precipitate was suspended with 1 M NaCl at $0^{o}C$ and centrifuged in order to remove the ribosomal RNA fraction as a precipitate. Mitochondrial $tRNA^{Mix}$ was recovered as the sup fraction with a yield of 2,240 A_{260} units. It was sometimes subjected further to DEAE-cellulose chromatography and the purified mt $tRNA^{Mix}$ was finally recovered with about a 40 % yield from 1 M NaCl eluate containing 20 mM Tris-HCl (pH 7.5) and 10 mM $MgCl_2$.

Preparation of mt seryl-tRNA synthetase

To 190 g of mitoplasts obtained as above, Buffer A [30 mM Tris-HCl pH 7.5, 5 mM $MgCl_2$, 6 mM β-mercaptoethanol, 10 % glycerol and 10 μM phenylmethylsulfonylfluoride (PMSF)] was added up to a total volume of 360 ml. The suspension was sonicated (2 min each, 6 times)in ice using a Branson cell disruptor 200, and was then centrifuged at 10,5000 x g for 3 hrs to obtain the S 100 fraction as the supernatant. The S 100 fraction was precipitated with 70 % saturated ammonium sulfate, dialyzed against Buffer A and adsorbed onto a DEAE-cellulose column (Whatman DE 52, ø 2.4 x 35 cm). The seryl-tRNA synthetase fraction was eluted at a NaCl concentration of 0.15-0.2 M in Buffer A. The fraction was precipitated with 75 % saturated ammonium sulfate and dialyzed against Buffer B [20 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$, 6 mM β-mercaptoethanol, 10 % glycerol and 10 uM PMSF] which was then subjected to Sephacryl S-300 column chromatography to remove any nuclease contamination. The enzyme recovered from the seryl-tRNA

synthetase activity-enriched fractions showed a specific activity 3-6 times higher than that of the S 100 fraction (total yield was 90 mg protein) and was stable for at least a month when stored at $-80^{o}C$.

Hybridization probes

The deoxyribonucleotide oligomers used for hybridization probes as shown in Fig. 1 were automatically synthesized with a DNA synthesizer (Applied Biosystems Inc. Model 381A) and purified by the conventional procedures. The oligomers were labeled at the 5'-ends with γ-^{32}P-ATP and T_4 polynucleotide kinase (Boehringer Co.).

Dot blot hybridization

The hybridization technique was essentially based on the method of Thomas (20,21), modified so as to be applicable for the probes of oligodeoxiribonucleotides with short chain length, as follows; tRNA solution was successively diluted by 1/10 and 1 ul each was spotted onto a nylon membrane filter (PALL BIODYNE^{TM}) and dried. After tRNA was made to cross-link to the filter by irradiating UV light at 254 nm with a Transilluminator Model TS-254 (Spectroline Co.) for 2 min, the membrane was preincubated for 1 hr in 5X Denhardt's solution (22) [0.1 % (W/V) Ficoll 400 (Pharmacia, Co.), 0.1 % Polyvinylpyrrolidone (Wako Pharmaceutical CO.) and 0.1 % Bovine serum alubumin (Nakarai Chemical Co.)], 5X SSPE buffer [900 mM NaCl, 50 mM NaH_2PO_4 and 5 mM EDTA (pH 7.4)], and 0.1 % SDS, and then incubated overnight in 900 mM NaCl, 6 mM EDTA, 90 mM Tris-HCl (pH 8.0), 0.3 % SDS, and 0.1 ~ 0.3 pmoles of 5'[^{32}P]-labeled AGY- or UCN-probe. The incubation temperature was set according to the GC content of the probes as proposed in the literature (23). After incubation, the filter was washed with 3X SSC [450 mM NaCl and 45 mM Na-citrate (pH 7.0)] at room temperature for 30 min. This procedure was repeated several times and the filter was dried. It was autoradiographed overnight using a RX 50 X-ray film (Fuji Film Co.) with an intensifying screen.

BD-cellulose column chromatography

2,240 A_{260} units of mt $tRNA^{Mix}$ were applied onto a column (ø 1.5 x 82 cm). Elution was performed at 4^{o} C by a linear gradient (1.6 l) of NaCl (0.2 M - 2M) in 10 mM NaOAc buffer pH

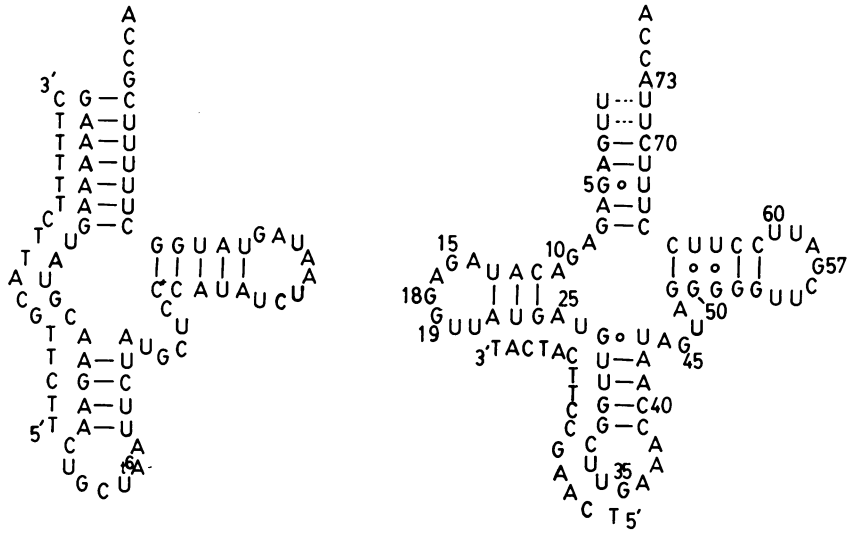

Fig. 1. Clover-leaf structures of mt tRNA^{Ser} isoacceptors, tRNA$^{Ser}_{AGY}$ and tRNA$^{Ser}_{UCN}$ together with oligodeoxyribonucleotides complementary to particular regions of tRNAs used for hybridization probes. C^{+} in the T stem of tRNA$^{Ser}_{AGY}$ means that C is partially substituted by 5-methyl C (14). The tRNA$^{Ser}_{UCN}$ sequence was deduced from the DNA sequence (4) so that modified residues have not yet been assigned.

6.0, followed by a linear gradient (500 ml) of ethanol (10 - 20 %) in the same buffer (10 mM NaOAc + 2M NaCl).

Digestion conditions for tRNA$^{Ser}_{UCN}$ with nuclease S_1, RNase T_1 and alkali

3'[^{32}P]-labeled tRNA$^{Ser}_{UCN}$ (containing 83 pmol tRNA) was digested at 37° C for 10 min either with 1.77 units and 17.7 units of RNase S_1 (Takara Shuzo) in 0.28 M NaCl, 50 mM NaOAc (pH 4.6) and 4.5 mM $ZnSO_4$, or with 0.1 unit and 1 unit of RNase T_1 (Pharmacia) in 50 mM Tris-HCl (pH 7.5) and 10 mM $MgCl_2$. An extensive digestion with RNase T_1 was carried out at 37° C for 10 min with 1 unit RNase T_1 in 50 mM Tris-HCl(pH 7.5). Alkaline digestion was performed at 90° C for 15 min in 50 mM Na_2CO_3 (pH 9.0) and 1 mM EDTA.

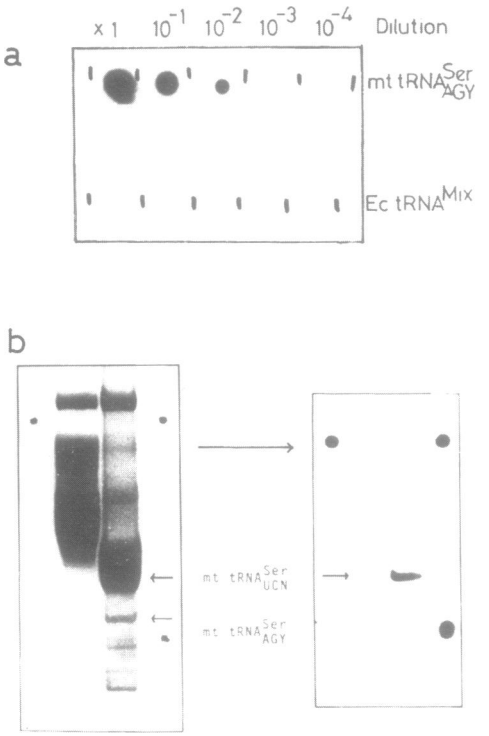

Fig. 2a. Dot hybridization of mt. tRNA$^{Ser}_{AGY}$ with [^{32}P]-labeled AGY-probe. 4 A_{260} units/ml tRNA$^{Ser}_{AGY}$ (upper part) and 200 A_{260} units/ml E. coli unfractionated tRNA as the control (lower part) were used as samples.

2b. Northern hybridization of cyt (left lane of left figure) and mt (right lane) unfractionated tRNAs with UCN-probe. Both tRNAs (3 A_{260} units each) were electrophoresed on 15% polyacrylamide gel (160 x 180 x 1 mm) with 7M urea and stained with toluidine blue (left figure). The materials on the gel were electrophoretically transfered to a nylon membrane , which was then hybridized with the UCN-probe and autoradiographed as described above(right figure).

Measurements of melting profiles

Melting profiles and their first derivatives were measured automatically with a Gilford Response II spectrophtometer. The conditions were the same as reported previously (24).

Aminoacylation assay

The aminoacylation assay of two serine tRNA isoacceptors was carried out at 37°C in a reaction mixture (total 100 µl) containing 100 mM Tris-HCl pH 8.0, 5 mM $MgCl_2$, 10 mM KCl, 2 mM

ATP, 46 μM ^{14}C uniformly labeled serine (173 Ci/mol, Amersham Co.) and 5 μl of the seryl-tRNA synthetase fraction (18 mg/ml). The concentration of $tRNA^{Ser}$ was varied in five steps from 0.008 μM to 0.3 μM for $tRNA^{Ser}_{UCN}$, and from 0.16 μM to 0.6 μM for $tRNA^{Ser}_{AGY}$. Aliquotes (20 μl each) were withdrown from the reaction mixture at appropriate time for assay, and the initial rate of aminoacylation was measured, from which the Km and Vmax values were deduced.

RESULTS

Hybridization assay of mt $tRNA^{Ser}_{AGY}$ with a deoxyribonucleotide oligomer

Bovine mt $tRNA^{Ser}_{AGY}$ was at first used for a trial sample because its structure had been well characterized (14,15). A synthetic oligodeoxyribonucleotide 5'-TTCTTGCATTCTTTTTC-3' complementary to the 17 nucleotides from the 5'-end of mt $tRNA^{Ser}_{AGY}$ was chosen as a DNA-probe for hybridization (it was named the AGY-probe, Fig. 1) which had been labeled at the 5'-end with ^{32}P. $tRNA^{Ser}_{AGY}$ was spotted onto a nylon membrane filter by diluting every 1/10 time and hybridized with the ^{32}P-AGY probe, as described in Materials and Methods. As shown in Fig. 2a, the detection limit was found to be 1 ng. *E. coli* $tRNA^{Mix}$ used as a control, even at an amount of 10 μg, could not be hybridized with the AGY-probe, indicating a high specificity of this hybridization technique. When each spot was excised and its radioactivity measured by the Cherenkov method, the radioactivities of the spots in the range from several ten to several thousand cpm correlated well with the amounts of tRNA samples in the range from 1 ng to 1 μg (data not shown). This demonstrates that the amount of a specific tRNA in any solution can be quantitatively estimated by this procedure, the detection limit being 1 ng tRNA.

Treatment with glyoxal (25) and/or formamide (26,27) to denature the higher-ordered structure of tRNA during hybridization unexpectedly decreased the sensitivity (data not shown). No hybridization occurred with glyoxal treatment in the range of 1 ng ~ 1 μg of $tRNA^{Ser}_{AGY}$, and formamide treatment decreased the sensitivity to 1/10. The same tendency was also

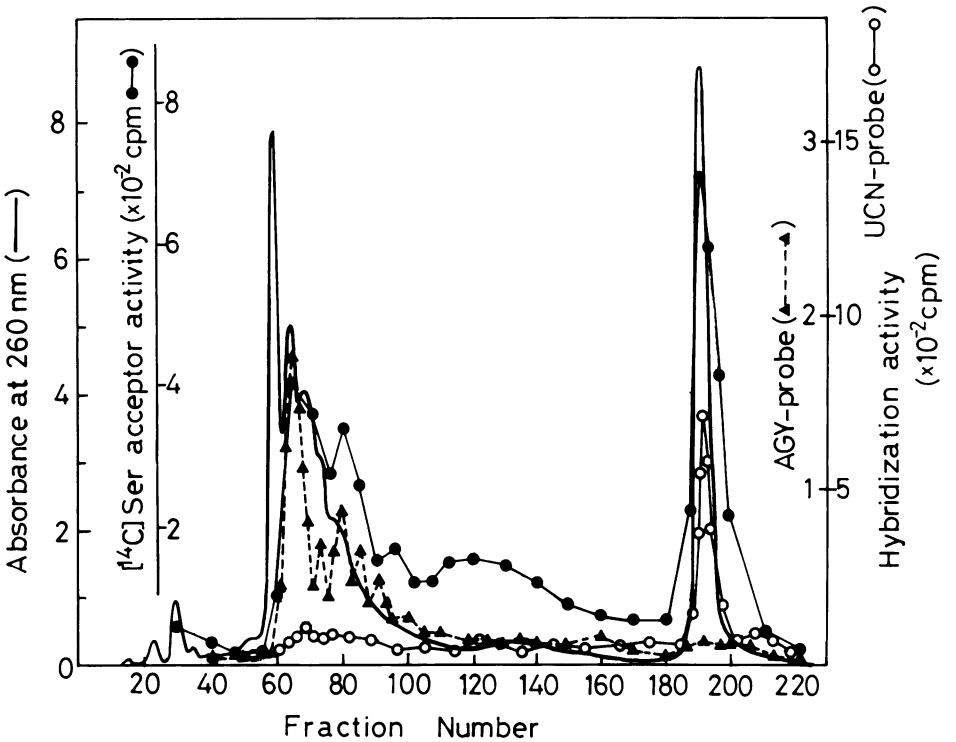

Fig. 3. BD-cellulose column chromatography of bovine liver mt tRNA. The solid line indicates the absorbance monitored at 260 nm, and the line with closed circles indicates [^{14}C]serine-acceptor activity by partially purified mt seryl-tRNA synthetase. For notations of the other plotted lines, see Fig. 4 caption.

observed for $tRNA^{Ser}_{UCN}$, which seems to take on a usual tertiary structure (28, and see Fig. 1).

Detection and purification of $tRNA^{Ser}_{UCN}$ from bovine mitochondria

We attempted to apply this method to isolating $tRNA^{Ser}_{UCN}$ from bovine mitochondria using a UCN-probe (5'-TCAAGCCTTCATCAT-3') complementary to the region from the D stem to the anticodon loop of $tRNA^{Ser}_{UCN}$ (Fig. 1). Northern blot hybridization using unfractionated mt tRNA ($tRNA^{Mix}$) as an RNA source gave only one band located at a slower-migrating position than the position of $tRNA^{Ser}_{AGY}$ (Fig. 2b). No band was detected in the lane of cytoplasmic $tRNA^{Mix}$ which had been run in parallel with the mt $tRNA^{Mix}$. Thus it was made clear that $tRNA^{Ser}_{UCN}$ in fact exists in mt $tRNA^{Mix}$.

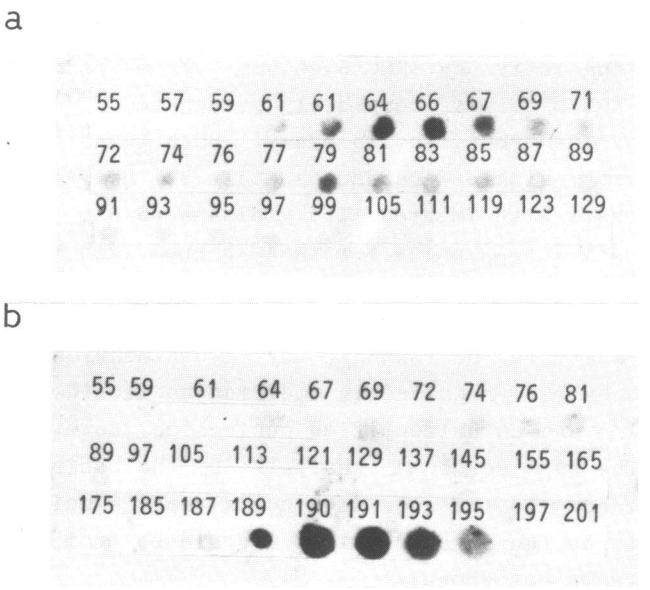

Fig. 4. Hybridization assay using the AGY- (a) and UCN- probes (b). The counts of spots from the column eluates (numbered above each spot) were measured by the Cherenkov method and plotted by the closed triangles and open circles for the AGY- and UCN-probes, respectively, in Fig. 3.

Next, large scale isolation of $tRNA^{Ser}_{UCN}$ together with $tRNA^{Ser}_{AGY}$ from mt $tRNA^{Mix}$ was attempted by conventional procedures, successive column chromatographies of BD-cellulose and RPC-5, and gel electrophoresis. Fig. 3 shows the elution profiles by BD-cellulose column chromatography. Three main peaks were obtained by [^{14}C]-serine charging assay, the first two peaks overlapping with the peaks detected by the hybridization assay with the AGY-probe (Fig. 4a and the line with closed triangles in Fig. 3) and the third peak (ethanol region) overlapping with that of the UCN-probe (Fig. 4b and the line with open circles in Fig. 3). No cross hybridization occurred between the first two peaks and the third peak. Thus, $tRNA^{Ser}_{AGY}$ was mainly contained in the first peak fraction [this is consistent with the previous results (15)], whereas $tRNA^{Ser}_{UCN}$ was contained in the last peak fraction in which mt $tRNA^{Phe}$ was also included as monitored by hybridization with a probe for $tRNA^{Phe}$ (Kumazawa et al, submitted).

The $tRNA^{Ser}_{AGY}$- and $tRNA^{Ser}_{UCN}$-rich fractions were further purified separately by RPC-5 column chromatography. In this procedure $tRNA^{Ser}_{UCN}$ was completely separated from $tRNA^{Phe}$ as monitored by the hybridization assay. Both $tRNA^{Ser}$s were finally purified to single bands by 15 % polyacrylamide gel electrophoresis with 7M urea. The yields were 5.0 A_{260} units for $tRNA^{Ser}_{AGY}$ and 1.7 A_{260} units for $tRNA^{Ser}_{UCN}$ from 2240 A_{260} units of unfractionated mt tRNA isolated from 17.5 Kg bovine liver.

Structural Comparison of two serine isoacceptor tRNAs

Base analysis of $tRNA^{Ser}_{UCN}$ by two-dimensional thin-layer chromatography (29) resulted in modified nucleotides of pΨ, pm^1A, pm^5C, pms^2i^6A and an unidentified nucleoside, however pT was not detected. The base sequence analysis by the Donis-Keller method (30) coincided with the gene sequence already elucidated (4), at least as far as the arrangement of G residues from G3 to G57 was concerned (data not shown).

The higher-ordered structure of $tRNA^{Ser}_{UCN}$ was examined by limited digestions with nuclease S_1 and RNase T_1 at various concentrations (Fig. 5); nuclease S_1 cut both the 3' end and the anticodon loop preferentially, whereas RNase T_1 cut G35 in the anticodon loop preferentially, and G45 and G57 slightly at a low concentration of RNase T_1 [the base numbering conforms to the literature (28), see Fig. 1]. At a high concentration of RNase T_1, cleavage at G57, G45 and G50 became prominent. Cleavage at G18 and G19 was also observed at a low concentration of RNase T_1, but the extent was very low. It dissapeared at a high concentration of RNase T_1, because more cleavage occurred at the 3'-half region of the tRNA. These results suggest that $tRNA^{Ser}_{UCN}$ possesses the T loop / D loop interaction like usual tRNAs with a normal L-shaped tertiary structure.

This was also supported by the melting profiles of these $tRNA^{Ser}$s, as shown in Fig. 6. Whereas $tRNA^{Ser}_{AGY}$ showed a single-step melting as reported previously (15), $tRNA^{Ser}_{UCN}$ showed two-step melting in the absence of Mg^{2+} and at 1mM Mg^{2+}. This seems to be a prominent feature of L-shaped tRNA with the T loop/D loop interaction (24). It is intriguing that the first transition point of $tRNA^{Ser}_{UCN}$ is always slightly lower than that of $tRNA^{Ser}_{AGY}$,

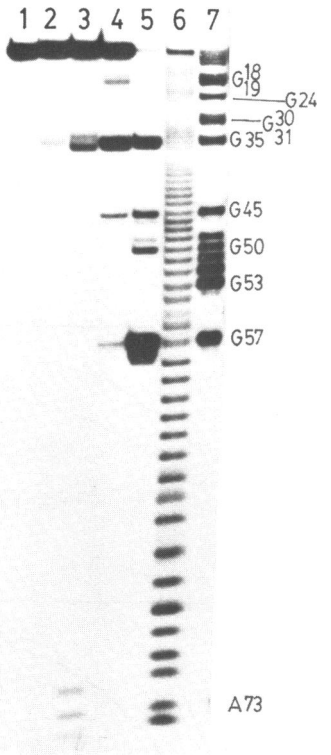

Fig. 5. Limited digestion patterns of mt $tRNA^{Ser}_{UCN}$ with nuclease S_1 and RNase T_1. Lane 1, tRNA only; lane 2, nuclase S_1 1.77 units; lane 3, nuclease S_1 17.7 units; lane 4, RNase T_1 0.1 unit; lane 5, RNase T_1 1 unit; lane 6, alkaline digestion; ; lane 7, an extensive digestion with RNase T_1.

even at 10 mM Mg^{2+}. This means that the secondary as well as the tertiary structures of $tRNA^{Ser}_{UCN}$ are slightly weaker than those of $tRNA^{Ser}_{AGY}$, probably due to less stable stem structures of $tRNA^{Ser}_{UCN}$ containing four U-G base pairs in a whole tRNA molecule and two unusual U-U pairs at the end of the amino acid stem region (Fig. 1). Moreover $tRNA^{Ser}_{UCN}$ seems to take a slightly loose tertiary structure, because its hyperchromicity was about only two-thirds those of the usual tRNAs (24).

Aminoacylation activities

Finally the aminoacylation activity of $tRNA^{Ser}_{UCN}$ was compared

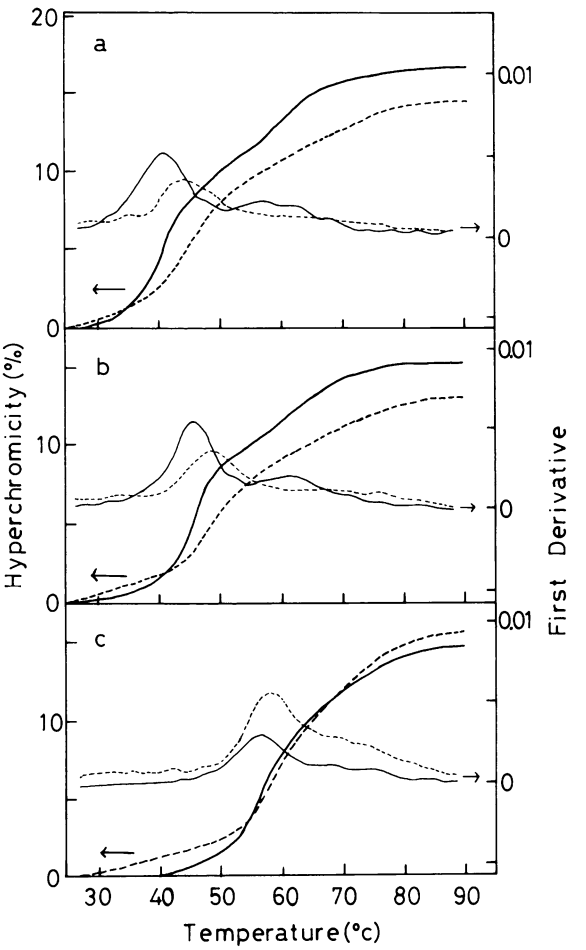

Fig. 6. Melting profiles (bold lines) and their first derivatives (fine lines) of mt $tRNA^{Ser}_{UCN}$ (solid line) and $tRNA^{Ser}_{AGY}$ (dotted line) at various Mg^{2+} concentrations [a, no addition; b, 1 mM; c, 10 mM]. The buffer used was 10 mM Tris-HCl pH 7.5 and 0.2 N NaCl with or without $MgCl_2$.

with that of $tRNA^{Ser}_{AGY}$ using the mt seryl-tRNA synthetase fraction. Although the Km value for $tRNA^{Ser}_{UCN}$ was about 1/4 of that for $tRNA^{Ser}_{AGY}$, the relative Vmax value for $tRNA^{Ser}_{UCN}$ was 2/3 of that for $tRNA^{Ser}_{AGY}$ (Table 1). The Km value for $tRNA^{Ser}_{UCN}$ (0.046 μM) showed the similar value with that obtained for a hen liver cyt $tRNA^{Ser}$-SerRS system (0.03 μM; 31), suggesting the validity of the

Table 1. Kinetic parameters for aminoacylation reaction of mt $tRNA^{Ser}$ isoacceptors by mt seryl-tRNA synthetase fraction

tRNA	Km(μM)	Vmax*(pmole/min)	Vmax*/Km
$tRNA^{Ser}_{AGY}$	0.178	1.8	10
$tRNA^{Ser}_{UCN}$	0.046	1.2	26

*Relative values because the seryl-tRNA synthetase fraction used consisted of a partially purified nuclease-free fraction, thus the enzyme concentration could not be determined precisely.

present experiment. Thus it turned out that two mt isoacceptor $tRNA^{Ser}$s having quite different secondary structures are able to be aminoacylated with not very different efficiency.

DISCUSSION

There have so far been a few reports concerning the structural study of mt tRNAs: de Bruijn and Klug (14) proposed a truncated tertiary structural model of $tRNA^{Ser}_{AGY}$s isolated from bovine and human mitochondria from chemical probing data. We once prepared 5 A_{260} units of $tRNA^{Ser}_{AGY}$ from bovine heart mitochondria and examined its secondary structure by nuclease digestions and melting profiles (15). Gebhardt-Singh and Sprinzl (32) reported that both $tRNA^{Ser}_{AGY}$ and $tRNA^{Ser}_{UCN}$ of bovine heart mitochondria can form a ternary complex with bacterial elongation factor Tu and GTP.

Thus the mt tRNAs so far studied have been mostly restricted to $tRNA^{Ser}_{AGY}$, because its extraordinarily short chain length enables it to be easily isolated from other mt tRNAs by gel electrophoresis (15). In searching for a detection method for other mt tRNAs, as well as isoacceptor tRNAs, we developed the hybridization assay method as described above. The sensitivity was found to be more than 100 times higher than that of the conventional aminoacylation method. By using both methods together, we could purify two serine isoacceptor tRNAs in this study and $tRNA^{Phe}$ as well (Kumazawa et al., submitted) from

bovine liver.

The hybridization method described above is also applicable to bacterial and cyt tRNAs, suggesting a possibility that this method may be generally usable for the purification and identification of tRNAs and their precursors (in preparation).

The two serine isoacceptor tRNAs showed quite different sensitivities towards RNase T_1 and nuclease S_1; in $tRNA^{Ser}_{AGY}$ the T loop was also sensitive to nucleases, besides the anticodon loop (15). However, the T and D loops of $tRNA^{Ser}_{UCN}$ were much less sensitive than the anticodon and extra loops. This suggests that the T loop of $tRNA^{Ser}_{AGY}$ is rather exposed, forming no interaction with other regions [although this is not consistent with the results of de Bruijn and Klug (14), we regard this as being due to a difference of the conditions under which the tRNA is laid, see ref.15.] whereas that of $tRNA^{Ser}_{UCN}$ is rather buried in the higher-ordered structure of tRNA, probably through T loop/D loop interaction.

The assumption that the secondary structure of $tRNA^{Ser}_{UCN}$ is rather weak because it possesses four G-U pairs and two non-base-paired U-U pairs at the aminoacyl stem end was clearly supported by the differential melting profiles as shown in Fig. 6. The first transition points of $tRNA^{Ser}_{UCN}$ were 2-3^{o} C lower than that of $tRNA^{Ser}_{AGY}$ at three different concentrations of Mg^{+2}.

Contrary to this observation, the aminoacylation activity of $tRNA^{Ser}_{UCN}$ was about three times more efficient than that of $tRNA^{Ser}_{AGY}$, mainly due to the difference in the Km value. This may reflect the presence of the D stem region in $tRNA^{Ser}_{UCN}$, because in bacterial and cytoplasmic systems the recognition region of tRNA by the cognate aminoacyl-tRNA synthetase has been regarded as being located inside the L-shaped tertiary structure, including the D stem (33). Therefore it seems rather interesting that $tRNA^{Ser}_{AGY}$ lacking the entire D arm is able to accept its cognate amino acid. With respect to a problem of tRNA identity which has recently been raised (34,35), it would be intriguing to determine which parts in each $tRNA^{Ser}$s with quite different secondary structure are recognized with seryl-tRNA synthetase, which is the next problem to be solved.

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