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Assignment of Imino Proton Signals of G-C Base Pairs and Magnesium Ion Binding: An NMR Study of Bovine Mitochondrial $tRNA^{Ser}_{GCU}$ Lacking the Entire D Arm[1]

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The mammalian mitochondrial $tRNA^{Ser}_{GCU}$ (mt $tRNA^{Ser}_{GCU}$) has a unique structure in that it lacks the whole D arm. To elucidate its higher-order structure, we synthesized unmodified bovine mt $tRNA^{Ser}_{GCU}$ using T7 RNA polymerase and measured its ^{1}H-NMR spectrum in the imino proton region. Although the imino proton signals heavily overlapped, we succeeded in assigning all the seven imino proton signals of the G-C base pairs by a combination of base replacement and ^{15}N-labeling of the G residues of a whole tRNA molecule or of the 3′-half fragment. The results indicate that the tRNA possesses the secondary structure that has been supposed on the basis of biochemical studies. Analysis of the effect of the magnesium concentration on the G-C pairs suggests that the acceptor and T stems do not form a co-axial helix, and that the core region of the tRNA does not interact with magnesium ions. These features are significantly different from those of canonical tRNAs. Despite this, it is very likely that the tRNA as a whole takes a nearly L-shape tertiary structure.

Key words: assignment of imino proton signals, ^{1}H-NMR, magnesium effect, mitochondrial tRNA, ^{15}N-labeling.

Many animal mitochondrial (mt) tRNAs are thought to have unusual secondary structures as inferred from their gene sequences; they seem to lack the interaction between the D and T arms that usually occurs in tRNAs (*1, 2*). Serine tRNA specific for codon AGY (Y; C or U, $tRNA^{Ser}_{GCU}$) has the most unique secondary structure in that it lacks the whole D arm (*3, 4*) but still has serine-accepting activity *in vitro* (*5*). The codon AGY has been found to be used as serine in almost all the protein-encoding genes so far identified in mammalian mitochondria (*4*). Thus, $tRNA^{Ser}_{GCU}$, possessing the truncated cloverleaf, is presumed to function in the mt protein synthesis system in a same way as other mt tRNAs possessing both the D and T arms.

If this presumption is correct, all mt tRNAs would share common structural features to work on the mt ribosomes (*6, 7*). An early work proposing a tertiary structural model for mt $tRNA^{Ser}_{GCU}$ on the basis of chemical probing suggested that the tRNA also possesses an L-shape-like structure (*8*) but with a slightly smaller dimension than those of other canonical tRNAs (*9*). Recently, Steinberg *et al.* (*6*) carried out computer modeling of the tRNA under the condition that the distance and orientation between the anticodon and the CCA-3′ end have to be kept constant in order that the tRNA can participate in the mt protein synthesis system (*7*), which resulted in a model with a "boomerang" shape rather than an "L" shape. However, no concrete experimental analysis of the tertiary structure of mt tRNAs has so far been reported. Our goal is to elucidate the detailed tertiary structure of $tRNA^{Ser}_{GCU}$, mainly by means of NMR spectroscopy.

To analyze the structure of tRNA molecules by NMR, attention should first be directed to assigning imino proton signals, because they supply information on the base pairs in the helical stems as well as on the tertiary interactions (*10-14*). However, the limitation in this analysis lies in the difficulty of signal assignment, because in many cases signals heavily overlap. $tRNA^{Ser}_{GCU}$ is just one such case, mainly because the tRNA contains many more A-U pairs than other cytosolic tRNAs in the stem regions (*1*).

We succeeded in completing the assignment of the imino protons of all seven G-C pairs in $tRNA^{Ser}_{GCU}$ by base replacement and ^{15}N-labeling of guanine residues or of a specific fragment. The samples supplied for NMR measurement were mainly synthesized *in vitro* using T7 RNA polymerase (*15*). By means of these techniques, we were able to confirm the secondary structure of mt $tRNA^{Ser}_{GCU}$ which had been assumed from biochemical studies (*5, 8, 16*). We also examined the effect of the magnesium ion concentration on the chemical shifts of ^{15}N-guanine-labeled tRNA. Although the assignment of all the imino protons has

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not been completed, magnesium titration experiments demonstrated that, unlike the canonical tRNAs examined so far, $tRNA^{Ser}_{GCU}$ possesses a characteristic dependence on the magnesium ion.

MATERIALS AND METHODS

In Vitro Synthesis and Purification of tRNA Transcript—A DNA template for the *in vitro* transcription reaction was constructed by using T7 RNA polymerase as described previously (*16*). For 20 ml reaction mixture, 2 mg of linearized DNA was used as described previously (*17*) with 2 mM each of non-labeled NTPs and 10 mM 5′-GMP. The ^{15}N-labeled tRNA was prepared almost as above, using 2 mM ^{15}N-labeled UTP or GTP (Nippon Sanso, Tokyo) instead of the corresponding unlabeled NTP and without 5′-GMP. The reaction was performed at 42°C for 4 h. In order to separate the RNA and DNA, free nucleotides, and short abortive fragments, the reaction mixture was then subjected to DEAE-Toyopearl 650M column chromatography (10 mm $\phi \times 10$ cm). A linear gradient of NaCl from 0.2 to 0.4 M was used for the elution buffer containing 20 mM HEPES (pH 8) and 10 mM $MgCl_2$. The tRNA fractions were collected and precipitated overnight with 2.5 volumes of ethanol at −80°C, centrifuged, and washed with ethanol. The tRNA fraction was further purified by 12% polyacrylamide–7 M urea gel electrophoresis in TBE buffer (denatured PAGE). The final yield of the $tRNA^{Ser}_{GCU}$ transcript was 4 mg on average from 20 ml of the reaction mixture.

tRNA Fragment Labeling—Limited digestion was performed with the ^{15}N-labeled transcript using 50 mM Tris-HCl (pH 7.5) and 150 mM $MgCl_2$. The reaction mixture was cooled on ice for over 30 min before the reaction and then RNase T1 (80 units/ml, Sigma) was added to the mixture. The reaction was continued at 0°C for 30 min. After the reaction, the RNA fraction was extracted with phenol twice as usual, and precipitated with ethanol. The product RNA was purified by 8% denatured PAGE. The kination reaction of the ^{15}N-labeled 3′-half fragment was done with 2 mM ATP using T4 polynucleotide kinase (Toyobo). After the removal of ATP by passage through 1 ml DEAE-Toyopearl 650M, the ^{15}N-labeled 3′-half fragment was precipitated with ethanol and then two-times the amount of the unlabeled synthetic 5′-half fragment was added. The mixture of both fragments was heated to 65°C for 5 min and then slowly cooled to 37°C in 50 mM Tris-HCl (pH 7.5) and 15 mM $MgCl_2$. The ligation reaction mixture included ~2 μM annealed complex, 250 mM Tris-HCl (pH 7.5), 15 mM $MgCl_2$, 21 mM DTT, 60 μg/ml BSA, and 500 units/ml T4 RNA ligase (Takara Shuzo). The mixture was incubated at 37°C for 40 min. The chimeric tRNA was purified by 8% denaturing PAGE.

NMR Spectroscopy—The tRNAs recovered by lyophilization were dissolved in 1 ml buffer consisting of 10 mM sodium cacodylate (pH 6.5), 10 mM $MgCl_2$, and 150 mM NaCl and concentrated to 180 μl by Centricon-10 (Amicon), which was used for NMR measurement. In the experiments to examine the effect of magnesium, the tRNA sample was dissolved in a buffer consisting of 10 mM Na-cacodylate (pH 6.5), 10 mM EDTA, and 150 mM NaCl. The tRNA solution was then incubated at 37°C for 2 h to remove the remaining magnesium ions tightly bound to the tRNA. After the incubation, the solution was concentrated and washed several times by a final buffer containing 10 mM Na-cacodylate (pH 6.5), 0.1 mM EDTA, and 150 mM NaCl with Centricon-10. To 180 μl sample solution, 10 μl of D_2O was added. NMR spectra were recorded on a Bruker AMX-500 spectrometer. A jump-and-return pulse was used to observe the exchangeable imino protons in H_2O (*18*) in all experiments described below. A sweep width of 15,152 Hz and a data point of 16 K were used for one-dimensional (1D) ^1H experiments. 1D NOE experiment were carried out with 0.5 s presaturation of each signal with 58 dB below 0.5 W power. All two-dimensional (2D) NMR spectra were recorded in the phase-sensitive mode using the TPPI method (*19*). NOESY spectra were recorded at 150 ms mixing time by using a standard pulse sequence (*20*) with data points of 4 K and 512 for the t2 and t1 dimensions, respectively. For 2D ^{15}N-^1H heteronuclear multiple quantum coherence (HMQC) spectra, a standard pulse sequence (*21*) was used with data points of 4 K and 64 for the t2 and t1 dimensions, respectively. One-dimensional ^{15}N-edited ^1H-NMR spectra (1D HMQC spectra) were observed with the same pulse sequence as that used in the 2D HMQC experiment except for t1 incrementation. For 1D and 2D HMQC experiments, GARP decoupling (*22*) was used for ^{15}N decoupling.

RESULTS

Preparation of Bovine mt $tRNA^{Ser}_{GCU}$ Transcript—The secondary structure of bovine mt $tRNA^{Ser}_{GCU}$ is shown in Fig. 1. Since this tRNA has only one modified nucleotide in its anticodon loop (*16*), there appears to be no appreciable structural difference between the native tRNA and its unmodified transcript.

The amino acid acceptance (*16*) as well as the melting profiles (data not shown) of the native tRNA and its

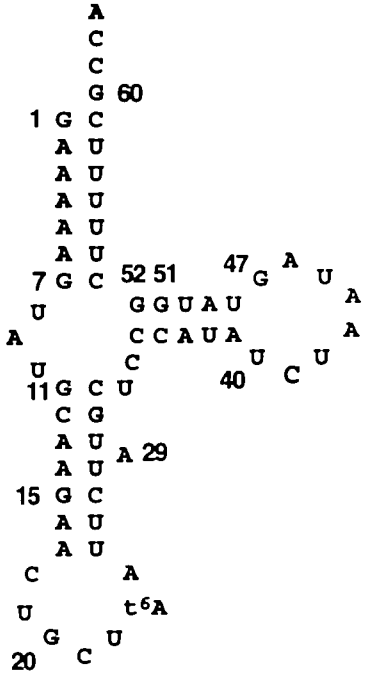

Fig. 1. **Cloverleaf structure of bovine mt $tRNA^{Ser}_{GCU}$.**

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transcript are also almost identical. It was thus concluded that the tRNA transcript was fully employable as a substitute for the native tRNA$^{Ser}_{GCU}$. By optimizing the transcription conditions, the tRNA transcript could be prepared in a quantity sufficient for NMR analysis (see "MATERIALS AND METHODS").

Acceptor Stem Base Replacement—Imino protons involved in base-paired hydrogen bonds in the stem regions or in tertiary interactions are protected from exchange by a solvent (*23*). These imino protons are thus visible in the downfield region of the ^{1}H-NMR spectrum between 11 and 15 ppm.

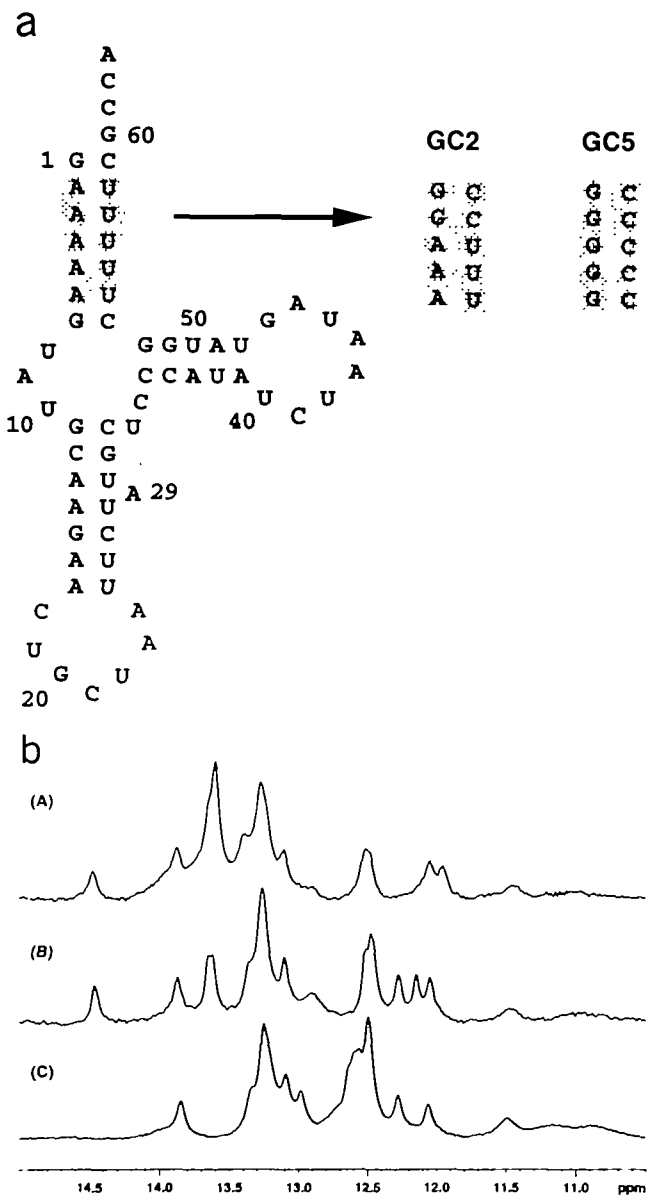

Fig. 2. *(a) Secondary structure of the bovine mt* tRNA$^{Ser}_{GCU}$ **transcript and its mutants GC2 and GC5 in which a stretch of 5 A-U pairs is replaced by G-C and/or A-U pairs (shown by shading). (b)** ^{1}**H-NMR spectra of the mt** tRNA$^{Ser}_{GCU}$ **transcript (A) and its mutants GC2 (B) and GC5 (C) in the imino proton region in** H_2O**.** The solvent was H_2O (10% D_2O) with 10 mM sodium cacodylate (pH 6.5), 10 mM $MgCl_2$, and 150 mM NaCl.

Figure 2b (spectrum A) shows the presence of extensive spectral overlap in the 1D spectrum, which is due to the richness of A-U pairs in the molecule. Since, on the basis of chemical and enzymatic probing, the tRNA is assumed to possess the normal acceptor and T stems (*5, 8*), it was expected that replacing a stretch of A-U pairs in the acceptor stem by G-C pairs would be useful for assigning A-U pairs in other regions. This approach is also rationalized by the observation of Ueda *et al.* (*16*) that the acceptor stem is not the main recognition site of mt seryl-tRNA synthetase, suggesting that base pair conversion in the acceptor stem causes no appreciable comformational change in the whole tRNA structure.

For the assignment of A-U pairs, we prepared two tRNA derivatives, GC2 and GC5, with base pair replacements in the acceptor stem, as shown in Fig. 2a. The 1D spectra of the imino proton region of the native tRNA transcript and the two derivatives are shown in Fig. 2b. Extensive differences are seen in the region between 13.5 and 14.5 ppm. In the spectrum of GC5 (spectrum C), the signal overlap observed in the wild type between 13.5 and 13.8

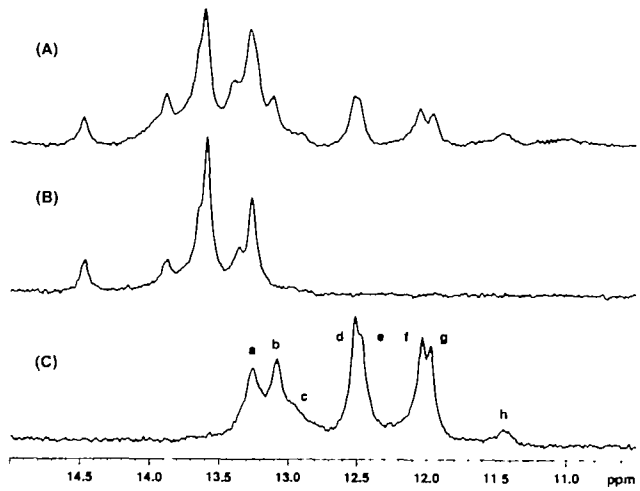

Fig. 3. ^{1}**H-NMR spectra of the mt** tRNA$^{Ser}_{GCU}$ **transcript in the *imino proton region*, *along with transcripts labeled by* ^{15}N uridine or guanine residues.** (A) Non-labeled mt tRNA$^{Ser}_{GCU}$ transcript (0.51 mM); (B) ^{15}N-U-labeled transcript (1.0 mM); (C) ^{15}N-G-labeled transcript (0.95 mM). The 1D spectra are 1D HMQC spectra with a ^{15}N filter. Each peak derived from G-C base pairs is named in alphabetical order from left to right.

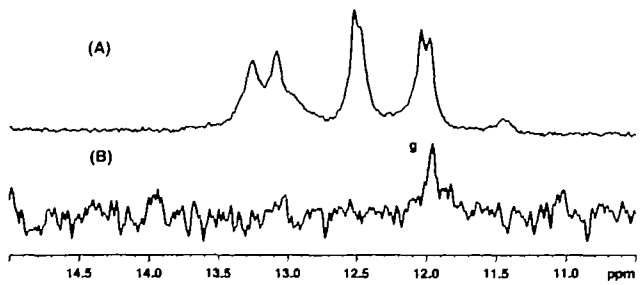

Fig. 4. **1D HMQC spectrum of bovine mt** tRNA$^{Ser}_{GCU}$ **transcript whose first position (G1) was labeled with ^{15}N-GMP (B) in comparison with the reference spectrum of ^{15}N-G-labeled tRNA transcript (A).** The conditions were the same as those in Fig. 2. The concentration of the G1-labeled tRNA was 0.05 mM.

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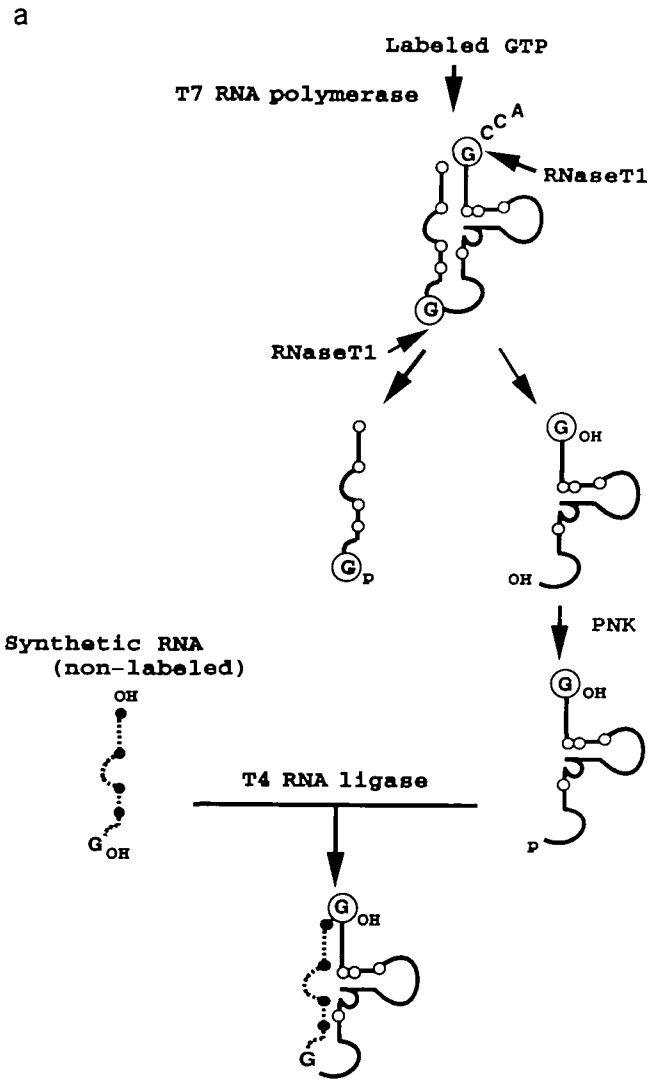

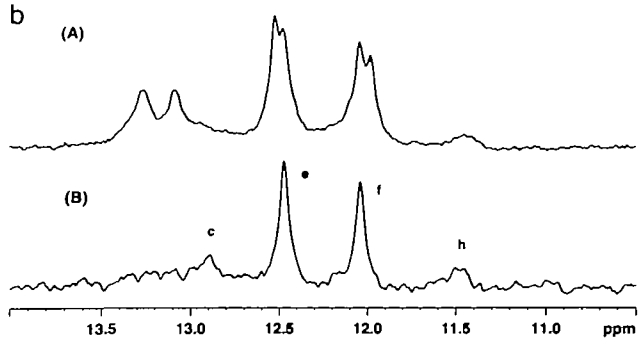

Fig. 5. **(a) Schematic depiction of the scheme for synthesizing chimeric $tRNA^{Ser}_{GCU}$ in which the 3′-half fragment is labeled with ^{15}N-G residues.** The open and filled circles indicate the ^{15}N-labeled and non-labeled G-residues, respectively. **(b) 1D ^{1}H-^{15}N HMQC of chimeric $tRNA^{Ser}_{GCU}$ (A) and the ^{15}N-edited spectrum of the tRNA transcript (B).** Four G-residue signals from the 3′-half fragment are observed in this spectrum. Each signal is named as in Fig. 3 (C).

ppm is moved to the region between 12.4 and 12.8 ppm; the signals between 13.5 and 13.8 ppm can thus be assigned to the stretch of A-U pairs in the acceptor stem. The one exceptional signal is that at 14.45 ppm observed in the wild type (spectrum A). Conversion of all five base pairs in the acceptor stem results in disappearance of the signal (spectrum C), but it remains in GC2 (spectrum B). It is thus highly possible that the A-U pair at 14.45 ppm is one of the base pairs in the acceptor stem region. It is thought this signal may be derived from either U54 or U55, because the signal of U56 must be greatly influenced by conversion of the nearest neighbor base pair from A3-U57 to G-C in GC2.

^{15}N-^{1}H NMR Spectroscopy—^{15}N-Edited spectra of the ^{15}N-labeled tRNAs are shown in Fig. 3. Heavily overlapped signals are observed in the spectrum of the ^{15}N-U-labeled tRNA transcript in which the uridine (U) residues are selectively labeled with ^{15}N-U [spectrum (B) of Fig. 3]; the overlap was not resolved even in the 2D ^{15}N-^{1}H HMQC spectrum (data not shown). In contrast, because of fewer G-C pairs in the molecule, all the seven G-C pairs with a broadening peak (c) are identified by selectively introducing ^{15}N-labeled guanine residues into the transcript (^{15}N-G-labeled tRNA). The resolved peaks are marked in alphabetical order from left to right [a to g in spectrum (C) of Fig. 3; for peak h, see below]. As the number of the G-C pairs thus obtained is consistent with that already supposed from chemical probing data (*8*), $tRNA^{Ser}_{GCU}$ seems to possess the cloverleaf-like secondary structure, but without the D arm.

Selective Labeling of G1—It is known that if an excess amount of 5′-GMP is added in the *in vitro* transcription reaction, this is incorporated into the 5′-end of the transcript instead of GTP (*17*). As ^{15}N-labeled 5′-GMP was added at 5 times the amount of unlabeled GTP, the 5′ terminus of the transcript was adequately labeled with ^{15}N-G, as shown in Fig. 4. From the ^{15}N-edited spectrum [spectrum (B)], the resonance at the high field of the imino proton region (at 12.0 ppm, peak g) was thus assigned to G1.

Specific Labeling of 3′-Half Fragment with ^{15}N-G Residues—Even though the signals of the G-C pairs could be separated by introducing ^{15}N-G into the transcript, their continuous NOE assignment was nearly impossible because most signals of the A-U pairs overlap at similar chemical shifts. In this situation, selective labeling of each residue of a whole tRNA molecule or of a certain fragment will facilitate resonance assignment. A flow chart of the method used to label the 3′-half fragment of the transcript with ^{15}N-G using RNase T1 and T4 RNA ligase is shown in Fig. 5a. When the target tRNA has G residues in the loop region, RNase T1 can cleave the loop specifically under the conditions of a low temperature and high magnesium concentration, while the other G residues in the stem regions remain uncleaved. Using the ^{15}N-G-labeled transcript as the starting material, we were able to pick up the resonances of only the G residue derived from the G-C pairs in the 3′-half fragment when it was combined with the corresponding unlabeled 5′-half fragment, as is clearly demonstrated by the scheme in Fig. 5a.

The ^{15}N-edited spectrum of the ^{15}N-G-labeled chimeric tRNA is shown in Fig. 5b. Judging from the secondary structure of $tRNA^{Ser}_{GCU}$ (Fig. 2a), G31, G47, G51, and G52

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were expected to give signals in this 1D HMQC spectrum of the chimeric tRNA, and four signals can, in fact, be observed in the imino proton region, two being sharp (e and f) and two broad (c and h).

Assignment of G-C Pairs of tRNA$^{Ser}_{GCU}$ *Transcript*—Although each signal of the ^{15}N-edited spectrum of the ^{15}N-G-labeled tRNA transcript could be separated, their continuous assignment using NOEs was still difficult. To distinguish the NOEs in the peaks and determine which was from a G-C pair and which from an A-U pair, we measured the NOESY spectra of non-labeled and ^{15}N-U-labeled tRNA transcripts without decoupling [Fig. 6, a and b]. In the NOESY spectrum of ^{15}N-U-labeled tRNA, the NOE of A-U pairs will split, whereas that of G-C pairs will not.

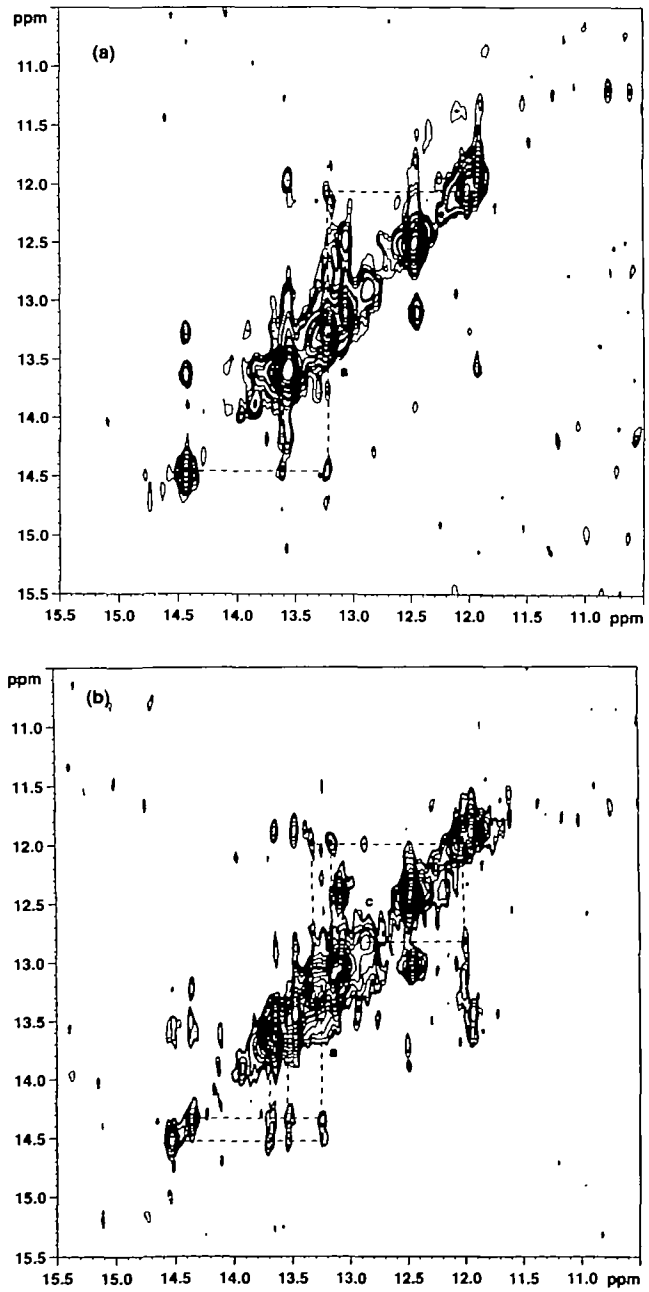

Fig. 6. **NOESY spectra of the non-labeled (a) and ^{15}N-U-labeled (b) tRNA**$^{Ser}_{GCU}$ **transcript.** The connectivities derived from G-C base pairs are shown by dashed lines.

The signal at 14.45 ppm in the spectrum shown in Fig. 6a, which splits into signals at 14.54 and 14.36 ppm in the spectrum in Fig. 6b, has two NOEs—at 13.58 and 13.25 ppm—in the spectrum in Fig. 6a. In the NOESY spectrum of the ^{15}N-U-labeled tRNA transcript, the former NOE at 13.58 ppm splits into four, whereas the latter NOE at 13.25 ppm splits into two. Thus, it is evident that the NOE at 13.58 ppm is that of the A-U pair, while the NOE at 13.25 ppm is that of the G-C pair. As already described, the A-U pair at the lowest position was assumed to be U54 or U55. These observations thus clearly show that the signal at 14.45 ppm comes from U54 and the NOE at 13.25 ppm from G7 [peak a in spectrum (C) of Fig. 3].

Furthermore, we could observe the NOE connectivities from the G-C at 12.90 ppm (peak c) to the G-C at 12.05 ppm (peak f), and to the peak at 13.25 ppm (Fig. 6b). The fact that the NOE splits into two proves that the peak at 13.25 ppm is an A-U pair (Fig. 6b). The 1D HMQC spectrum of the 3′-half fragment-labeled tRNA [spectrum (B) of Fig. 5b] shows that the two peaks c and f came from the ^{15}N-labeled 3′-half fragment. As the continuous G residues in the 3′-half fragment exist only in the T stem region in the secondary structure of tRNA$^{Ser}_{GCU}$, the three peaks observed in the above NOE experiment should be assignable to G52, G51, and U50.

Other assignments were done by 1D NOE experiments. Peak b gives an NOE to peak e, which gives an NOE to the signal at 13.90 ppm in spectra (A) and (B) of Fig. 3 (data not shown). The fragment-labeled tRNA showed that peaks b and e were derived from 5′- and 3′-half fragments, respectively, which suggests that the signals of G11, G31, and U30 correspond to the continuous G-C, C-G, and A-U pairs of peaks b and e, respectively. The remaining peak d, which comes from the 5′-half fragment should, therefore, be G15. Two guanosine residues, G47 and G60, remain as candidates for peak h. Since the chemical shift of peak h did not change [*cf.*, spectra (A) and (B) of Fig. 5b] before and after digestion with RNase T1, which should have cut off the CCA-3′ end at G60 in the chimeric tRNA, peak h was assigned to G47. The peak can be observed exceptionally in the highest field due to the stacking force of the T stem, since G47 is positioned at the end of the T loop, in the face of the T helix (*24*). The above assignments are summarized in Table I.

Effect of Magnesium Concentration—The effect of magnesium on the low field ^{1}H-NMR spectra ascribed to the G-C pairs of tRNA$^{Ser}_{GCU}$ transcript was measured at 293 K under various concentrations of magnesium and the results were confirmed by the ^{1}H-^{15}N HMQC spectra. The effect on the shift is shown in Fig. 7a. Considerable shifts occurred in

TABLE I. **Summary of guanine signal assignments.**

Peak	Chemical shift (ppm)	Assignment	Fragment (5′- or 3′-half)	NOE
a	13.25	G7	5′	14.45 (U54)
b	13.07	G11	5′	e
c	12.85	G52	3′	f
d	12.55	G15	5′	
e	12.45	G31	3′	b, 13.90 (U13)
f	12.05	G51	3′	c, 13.25 (U50)
g	12.00	G1	5′	(specific labeling)
h	11.45	G47	3′	

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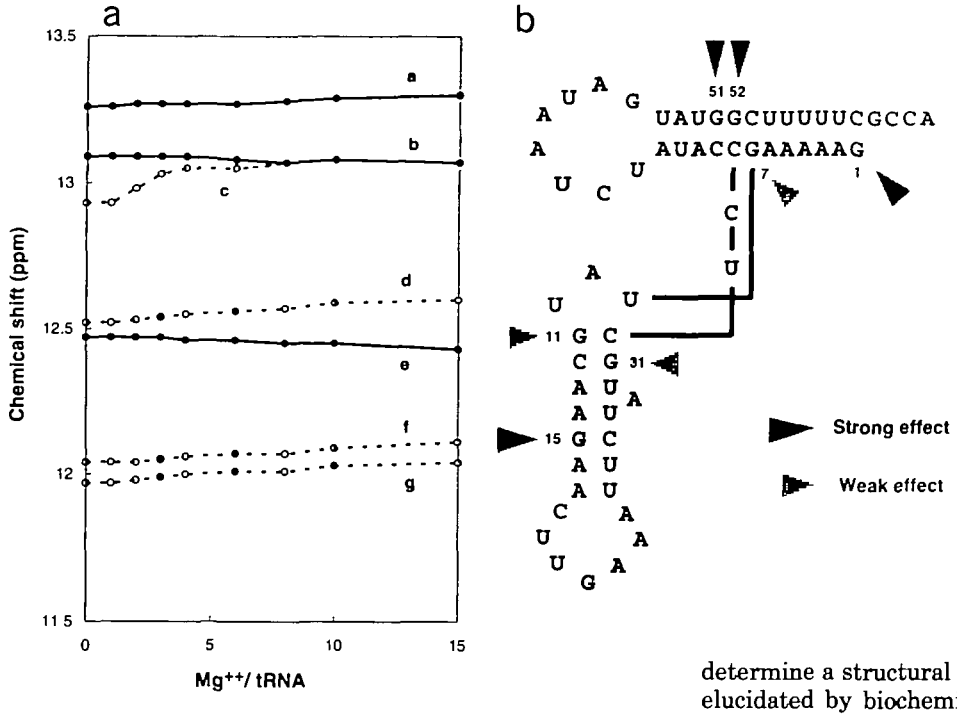

Fig. 7. **Effect of magnesium ions on the imino proton region of the tRNA$^{Ser}_{GCU}$ transcript.** (a) Dependence of ^{1}H chemical shift of guanine residues on the magnesium concentration. Each signal is named as in Fig. 3c. The broken lines depict signals strongly affected (>0.05 ppm) by the increasing magnesium concentration. (b) Secondary structure of the tRNA$^{Ser}_{GCU}$ transcript in which the effect on the residues of magnesium is shown by arrows.

peaks c, d, f, and g (assigned to G52, G15, G51, and G1, respectively). In particular, G52, which exhibited the most broadening among the seven G-C pairs, shifted to a lower field and the signal was further broadened with increased magnesium concentration. G1 also showed a large chemical shift, probably because of the non-specific interaction between magnesium ions and the three phosphates attached to the G1 residue (because no 5′-GMP was added to the transcription reaction in this case). Unlike the other signals, peaks b and e (assigned to G11 and G31, respectively) scarcely showed chemical shift changes during titration. It is intriguing that the core region of tRNA$^{Ser}_{GCU}$ containing these residues did not respond to magnesium ions so much as expected from the responses of other canonical tRNAs (see "DISCUSSION"). These results are summarized in Fig. 7b.

DISCUSSION

We measured the imino proton spectrum of bovine mt tRNA$^{Ser}_{GCU}$, assigned the signals from the G-C pairs, and analyzed the effects of magnesium ions on the tRNA. In NMR spectroscopy of RNA molecules consisting of more than 40 bases, signal overlapping is a serious problem that is yet to be solved. Here, we partially solved the problem by base replacement and labeling the G residues with ^{15}N in a whole tRNA molecule or the 3′-half fragment, although this method is restricted to using RNase T1. Recently, Lapham and Crothers (*25*) reported a similar method with less restriction, which uses RNase H and T4 DNA ligase.

Our NMR spectroscopy results confirmed the secondary structure of bovine mt tRNA$^{Ser}_{GCU}$ which had previously been postulated on the basis of chemical and RNase probing (*5, 8, 16*). Seven peaks belonging to guanine residues were observed in the ^{1}H-^{15}N HMQC spectrum of ^{15}N-guanine labeled tRNA. By means of NMR analysis, we could determine a structural feature of mt tRNA never hitherto elucidated by biochemical analysis: the base pair of the midpoint of the acceptor-T stem (C35-G52) exhibited a broad peak, indicating it forms a slightly loosened base pair, when exposed to the solvent (*26*). This signal (c) in spectrum (*C*) of Fig. 3, assigned to G52, has no NOE to the signal assigned to G7 (signal a). In all canonical tRNAs so far assigned, NOE are observed between the base pair at the end of the acceptor stem and that of the T stem, showing that those two stems co-stack together (*27*). Our observations clearly show that the acceptor stem and the T stem of mt tRNA$^{Ser}_{GCU}$ do not form a regular co-axial helix, which may be related to some form of distortion in the normal L-shape of the tRNA caused by the lack of the D arm.

tRNA conformational changes resulting from the binding of magnesium ions have been well studied using NMR spectroscopy in comparison with the tRNA structure determined by X-ray crystallography (*27-29*). Four strong Mg^{2+} binding sites have been determined in the crystal structure of yeast tRNA^{Phe} (*30-32*); two sites in the D loop, one near the D arm, and one in the anticodon loop. In our magnesium ion binding experiment, though the G15 signal (peak d) near the anticodon loop showed good agreement with that of canonical tRNAs, other signals were quite different; in particular, large changes are seen in the chemical shifts of peaks c (G52) and f (G51), whose G residues are placed at the junction of the acceptor and T stems. By binding magnesium ions to this conformationally distorted region, tRNA$^{Ser}_{GCU}$ could be stabilized so as to preserve the tertiary interaction. The most significant difference from canonical tRNAs lies in the chemical shifts of peaks b (G11) and e (G31), which show almost no changes in tRNA$^{Ser}_{GCU}$ compared with the drastic changes that are usually seen around the core region in the canonical tRNAs. This finding suggests that mt tRNA$^{Ser}_{GCU}$ may have lost magnesium binding sites around the D arm because it is lacking in this tRNA. It is intriguing that the same sort of phenomenon can be seen in another serine tRNA from bovine mitochondria, tRNA$^{Ser}_{UGA}$, which possesses a slightly altered cloverleaf structure; in this tRNA, two magne-

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sium binding sites were also found to be lost when the effect of magnesium was investigated (our unpublished data). These two serine tRNAs possess similar low melting temperatures (*5, 16*), suggesting that their similar conformational instability may stem from the elimination of magnesium binding sites in the core region, which could be brought about by either the lack of a D arm (in tRNA$^{Ser}_{GCU}$) or structural compensation between the lack of several common residues and the addition of one extra base pair into the anticodon stem (in tRNA$^{Ser}_{UGA}$; *7*). The precise conformations of the magnesium binding sites and the structural similarity of these tRNAs require further elucidation.

Recently, a tertiary structural model for mammalian mt tRNA$^{Ser}_{GCU}$ has been proposed based on a theoretical estimation of the universal constraints on tRNAs (*6*). This proposes that the 3′ region of the T loop interacts with the two unpaired regions flanking the anticodon stem by forming a base-triple of A44, U10, and U33, and that the central part of the T loop interacts with the central part of the anticodon stem, the bulged A29 being involved in a base pair with U42. We prepared a derivative of tRNA$^{Ser}_{GCU}$ in which A29 was replaced by G29 to measure the serine acceptance and NMR spectrum in the imino proton region (data not shown). The results were all the same as those observed with the wild-type transcript, suggesting that the A29 residue does not interact with another residue. Taking this finding together with the broadening signal of G52, we came to the conclusion that tRNA$^{Ser}_{GCU}$ possesses an L-shape-like structure rather than the "boomerang" model of Steinberg *et al.* (*6*). The broadning signal of G52 strongly suggests a distortion of the T stem, leading to the assumption that the T stem does not form a co-axial helix with the acceptor stem, and hence makes close contact with the anticodon stem. Thus, tRNA$^{Ser}_{GCU}$ may take an L-shape-like structure, preserved by several tertiary interactions. To clarify this assumption, the tertiary structure of mt tRNA$^{Ser}_{GCU}$ needs to be fully elucidated.

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