TERTIARY STRUCTURES OF *ESCHERICHIA COLI* tRNA AS STUDIED BY NMR SPECTROSCOPY WITH ^{13}C-LABELING METHOD

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1. Introduction

Biological functions of tRNA in protein biosyntheses are closely related with the tertiary structures of the complex of tRNA with other types of RNA and/or specific proteins. Nuclear magnetic resonance (NMR) spectroscopy, especially in combination with specific labeling of tRNA with ^{13}C nuclei, is useful for analyses of the conformational changes, if any, of tRNA upon complex formation with other biomolecules. [4-^{13}C]Uracil [1,2], [2-^{13}C]uracil and [2-^{13}C]-adenine [3] have been incorporated in tRNA in vivo, and some ^{13}C resonances of modified uracil bases were assigned [2]. The methyl groups of modified nucleosides at specific sites in tRNAs were labeled in vivo, by the use of [*methyl*-^{13}C]methionine as the ^{13}C source [3–5]. The ^{13}C NMR spectra of ^{13}C-labeled and fractionated tRNA^{Phe}, tRNA^{Tyr} and tRNA^{Cys} from an *Escherichia coli* strain auxotrophic for methionine were reported [6]. By the comparison with the primary structures of these tRNAs, unambiguous assignments were made of the methyl carbons of ribothymidine (rT), 2-methylthio-N^6-(Δ^2-isopentenyl) adenosine (ms^{2}i^{6}A) and 7-methylguanosine (m^{7}G).

Here, by a similar method, ^{13}C-labeled and fractionated preparations of 8 tRNAs were obtained from *E. coli* and the methyl-^{13}C resonances of 8 modified nucleosides in these tRNAs were unambiguously assigned. Tertiary structures around rT, m^{7}G, 5-methylaminomethyl-2-thiouridine (mnm^{5}s^{2}U) and 2-methyladenosine (m^{2}A) were shown to be much the same among these tRNA species. However, as for the tertiary structure around 2′-*O*-methylguanosine (Gm) in the D loop, tRNA$_m^{Met}$ and tRNA^{Tyr} were found to be different from each other.

2. Materials and methods

Escherichia coli met$^-$ *rel*$^-$ strain was grown in 2501 medium containing [*methyl*-^{13}C]methionine (8 μg/ml). tRNA (100 000 A_{260} units) was extracted by Zubay's method. By chromatography on columns of DEAE–Sephadex A-50, BD-cellulose, Sepharose 4B and RPC-5, 7 fractions (table 1) containing 1 or 2 tRNA species were obtained.

For NMR measurements, 1.5 ml ^2H_2O solution of tRNA (300–900 A_{260} units), $MgCl_2$ (10 mM) and NaCl (100 mM) at neutral pH was prepared. ^{13}C NMR spectra (67.9 MHz) were recorded on a Bruker WH-270 spectrometer with proton noise decoupling. Probe temperature was kept at 30°C. Dioxane (67.4 ppm from tetramethylsilane) was used as the internal standard for chemical shifts.

3. Results and discussion

In the NMR spectra of ^{13}C-labeled tRNAs, two or more prominent ^{13}C signals are observed in the 70–10 ppm region as shown in fig.1. These signals were classified into 8 groups, each with chemical shifts within 0.1–0.6 ppm (table 1). By the comparison with the primary structures of tRNAs [7], these groups of signals are readily assigned to specific carbon nuclei of modified nucleosides as synthesized in vivo from methionine through *S*-adenosylmethionine (SAM). In all the spectra (fraction 1–7), a prominent signal is

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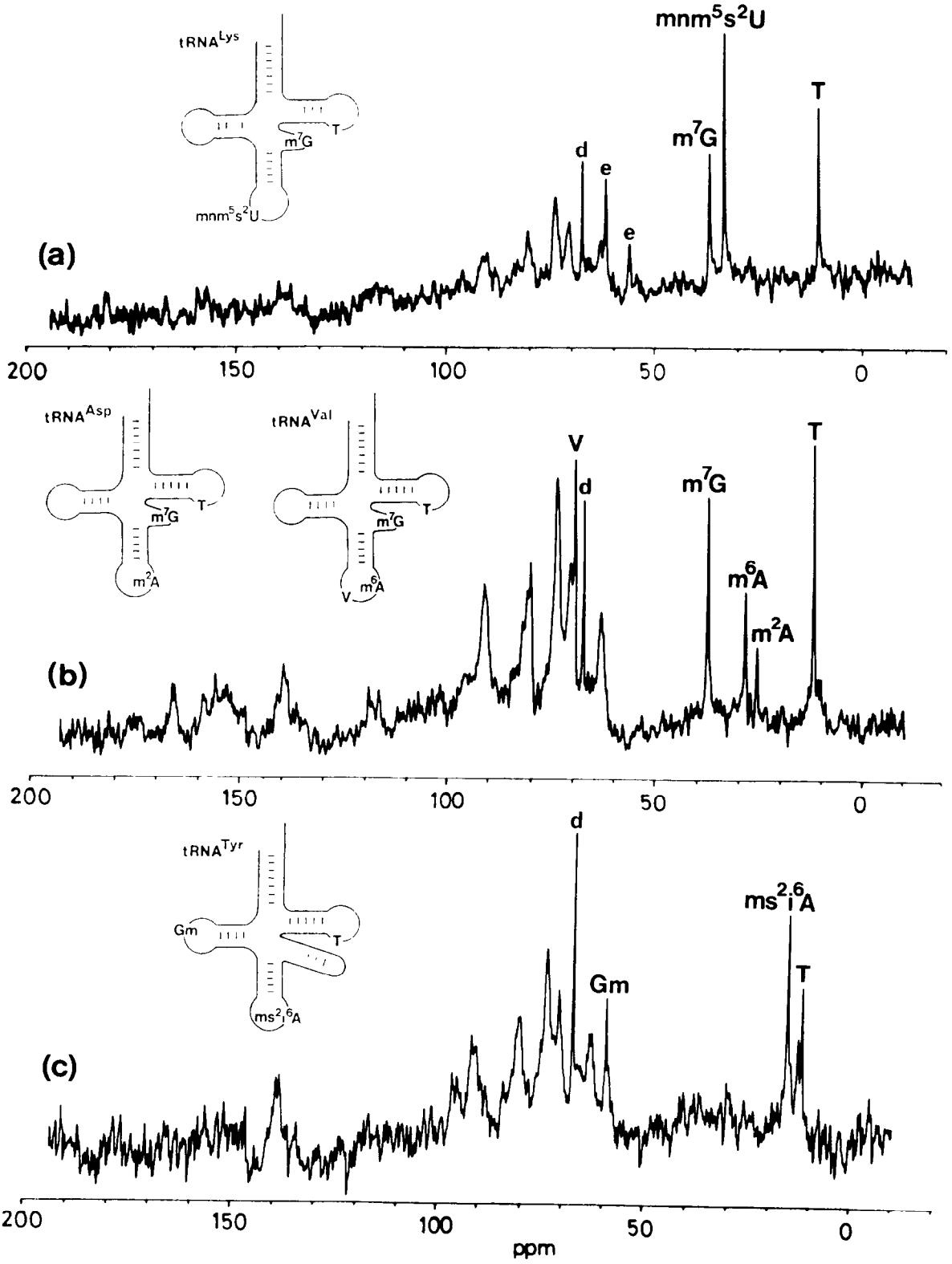

Fig.1. ^{13}C NMR spectra at 67.9 MHz of ^{13}C-labeled *E coli* tRNA: (a) fraction 4 (40 000 transients); (b) fraction 5 (86 000 transients); (c) fraction 7 (80 000 transients). 'd' denotes dioxane and 'e' denotes contaminating EDTA.

Table 1
Chemical shifts (ppm) of methyl-^{13}C resonances

Fraction	V	Gm	mnm^{5}s^{2}U	m^{6}A	m^{2}A	ms^{2}i^{6}A	m^{7}G	rT
1 $tRNA_m^{Met}$		58.4						
$tRNA^{Ile}$							36.9	11.2
2 $tRNA^{Arg}$					24.8		36.8	11.2
$tRNA^{Ile}$								
3 $tRNA^{Lys}$			33.5				36.9	11.2
$tRNA^{Ile}$								
4 $tRNA^{Lys}$			33.6				37.0	11.1
5 $tRNA^{Asp}$					24.7			
$tRNA^{Val}$	69.5[a]			27.7			36.9	11.2
6 $tRNA_2^{Glu}$			33.6		24.7			11.2
7 $tRNA^{Tyr}$		59.0				14.7		11.1

[a] This is due to the [^{13}C]methylene group of 5-substituent

observed at 11.2–11.1 ppm (table 1). This signal is assigned to the methyl carbon of rT which is common to all the tRNAs treated here. In the spectra of fractions ($tRNA^{Glu}$) and 7 ($tRNA^{Tyr}$) (fig.1(c)), weak signals are observed at 12.0 and 11.9 ppm, respectively, and are assigned to the methyl carbon of rT in partially denatured tRNAs. $tRNA_2^{Glu}$ and $tRNA^{Tyr}$ are known not to have m^{7}G in the extra loop [7]. Accordingly, the ^{13}C signals observed at 37.0–36.8 ppm for Fractions 1–5 are assigned to the methyl carbon nuclei of m^{7}G.

$tRNA^{Lys}$ and $tRNA_2^{Glu}$ have mnm^{5}s^{2}U in the first position of anticodons [7]. The NMR spectra of fractions 3,4 (fig.1(a)), and 6 exhibit ^{13}C signals at 33.6–33.5 ppm. Therefore these signals are assigned to the methyl carbon nuclei of mum^{5}s^{2}U. $tRNA^{Arg}$, $tRNA^{Asp}$, and $tRNA_2^{Glu}$ commonly have m^{2}A in the anticodon loop and accordingly the ^{13}C signals observed at 24.8–24.7 ppm for fractions 2,5 (fig.1(b)), and 6 are assigned to the methyl carbon nuclei of m^{2}A. $tRNA_m^{Met}$ and $tRNA^{Tyr}$ have Gm in the D loop [7]. For the methyl carbon of Gm, the ^{13}C signal of fraction 1 (including $tRNA_m^{Met}$) at 58.4 ppm and the signal of fraction 7 ($tRNA^{Tyr}$) are assigned. For $tRNA^{Tyr}$ (fraction 7, fig.1(c)), the remaining signal at 14.7 ppm is now assigned to the methyl carbon of ms^{2}i^{6}A in the anticodon loop. $tRNA^{Val}$ (in fraction 5) has 6-methyladenosine (m^{6}A) in the anticodon loop and uridine-5-oxyacetic acid (V) in the first position of the anticodon [7]. The methylene carbon in the 5-substituent of V is also introduced from the methyl carbon of SAM [8]. In the spectrum of fraction 5 (including $tRNA^{Val}$) (fig.1(b)), the ^{13}C signal (27.7 ppm) in the methyl region is assigned to the methyl carbon of m^{6}A, and then the signal (69.5 ppm) in the methylene region is assigned to the methylene carbon of V in $tRNA^{Val}$. Thus, all the prominent ^{13}C signals were unambiguously assigned to specific carbon nuclei of modified nucleosides in tRNAs.

Tertiary structures may now be compared among tRNA species by the use of ^{13}C chemical shifts of modified nucleosides. The chemical shifts of methyl-^{13}C signals of rT in all the spectra are in the narrow range from 11.2–11.1 ppm. These signals are upfield shifted more in the native form than in the denatured form, probably due to the ring current effect of guanosine commonly located next to the 5′-side of rT. Therefore, the tertiary structures around rT appear to be similar among tRNA species in the native form. The narrow range of chemical shifts (37.0–36.8 ppm) of the methyl-^{13}C signals of m^{7}G also indicates the similarity of the tertiary structures around m^{7}G among tRNA species with short extra loop.

The tertiary structures of anticodon loops as monitored by the chemical shifts of the methyl signal of m^{2}A are nearly the same among $tRNA^{Arg}$, $tRNA^{Asp}$, and $tRNA_2^{Glu}$. The local conformations of the 5-substituents in mnm^{5}s^{2}U of $tRNA_2^{Glu}$ and $tRNA^{Lys}$ are also found to be much the same (the strong intensity of the methyl-^{13}C-signal of mnm^{5}s^{2}U relative to that of rT indicates the higher mobility of the methyl group in mnm^{5}s^{2}U). On the other hand, the chemical shifts of Gm in $tRNA_m^{Met}$ and $tRNA^{Tyr}$ are different by 0.6 ppm from each other. This observation indicates that the tertiary structures around Gm in the D loop are significantly different between these tRNAs, pos-

sibly depending on the larger extra loop in $tRNA^{Tyr}$. Such methyl-^{13}C resonances will be important for further analyses of the interactions of tRNAs with other types of RNAs and/or specific proteins.

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