5-METHYL-2-THIOURIDINE: A NEW SULFUR-CONTAINING MINOR CONSTITUENT FROM RAT LIVER GLUTAMIC ACID AND LYSINE tRNAs

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1. Introduction

2-Thiouridine-5-acetic acid methyl ester and 5-methylaminomethyl-2-thiouridine were found in tRNA from yeast and *E. coli*, respectively [1, 2]. Recently, it was shown that these 2-thiouridine derivatives were possibly located in the first position of the anticodon of the glutamic acid tRNAs and had a specific role for codon recognition [3, 4]. Since a 2-thiouridine derivative is present in tRNA specific for a particular amino acid in both yeast and *E. coli*, it was of interest to investigate whether mammalian tRNAs also contain a 2-thiouridine derivative, and if so, what its structure is. This communication reports that rat liver glutamic acid and lysine tRNAs contain a 2-thiouridine derivative. It is neither 5-methylaminomethyl-2-thiouridine nor 2-thiouridine-5-acetic acid methyl ester. This new minor nucleoside found in the rat liver tRNAs was fully characterized as 5-methyl-2-thiouridine. This is the first report of the characterization of a sulfur-containing minor constituent from mammalian tRNA.

2. Materials and methods

Rat liver $tRNA_3^{Glu}$ and $tRNA_2^{Lys}$ were obtained by successive column chromatographies on DEAE-Sephadex A-50 [5, 6], and benzoylated DEAE-cellulose [7]. Details of the purification procedure will be published elsewhere [8]. A synthetic 2-thiouridine was prepared as described by Brown et al. [9]. Authentic 5-methyl-2-thiouridine was prepared from 2′,3′,5′-tri-*O*-benzoyl-5-methyl-2-thiouridine which was kindly supplied from Dr. M. Sano of Central Research Laboratory, Daiichi Seiyaku Co., Ltd. [10], by alkaline debenzoylation and was purified by paper chromatography. Synthetic 2-thiouridine-5-acetic acid methyl ester was a gift from Dr. R.H. Hall of McMaster University. RNase T_2 was purchased from Sankyo Co. *E. coli* alkaline phosphomonoesterase was obtained from Worthington Biochemical Co. Thin-layer glass plates (10 cm × 10 cm) coated with Avicel SF cellulose were products from Funakoshi Pharmaceutical Co., Tokyo.

To test for the presence of new minor constituents in rat liver tRNAs, 2 absorbance units of the dialyzed tRNAs were extensively hydrolyzed with RNase T_2, and subjected to two dimensional thin-layer chromatography using the solvent system described previously [11]. For isolation of 5-methyl-2-thiouridine 3′-phosphate from rat liver tRNAs on a relatively large scale, the RNase T_2 digest of the tRNA (30 absorbance units) was subjected to two dimensional paper chromatography (Toyo-Roshi No. 51A paper, 30 cm × 30 cm) using the same solvent system as for thin-layer chromatography. The spot containing 5-methyl-2-thiouridine 3′-phosphate was cut off and eluted with water. It was then converted to the corresponding nucleoside by treatment with *E. coli* alkaline phosphomonoesterase.

Abbreviations:
m^1Ap: 1-methyladenosine 3′-phosphate
m^1Gp: 1-methylguanosine 3′-phosphate
m^7Gp: N^7-methylguanosine 3′-phosphate
m^5Cp: 5-methylcytidine 3′-phosphate
N_1p, N_2p, N_3p, N_4p, N_5p: 3′-phosphate of unknown nucleosides

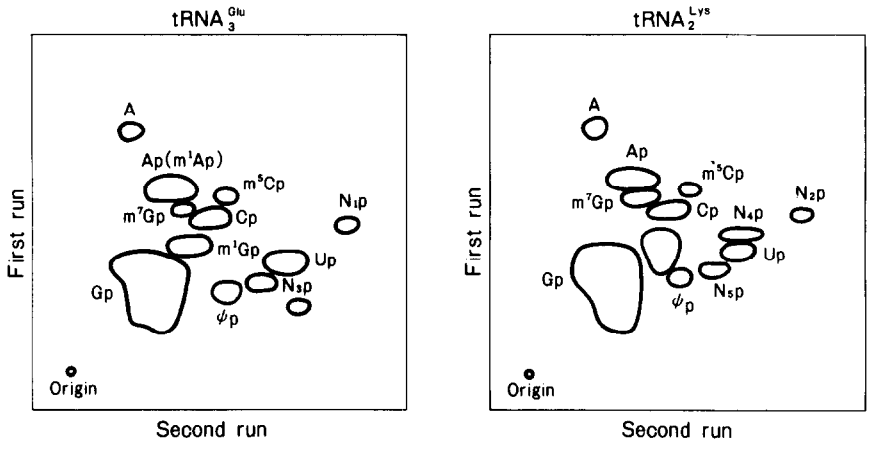

Fig. 1. Two dimensional thin-layer chromatography of RNase T_2 digests of rat liver $tRNA_3^{Glu}$ and $tRNA_2^{Lys}$.

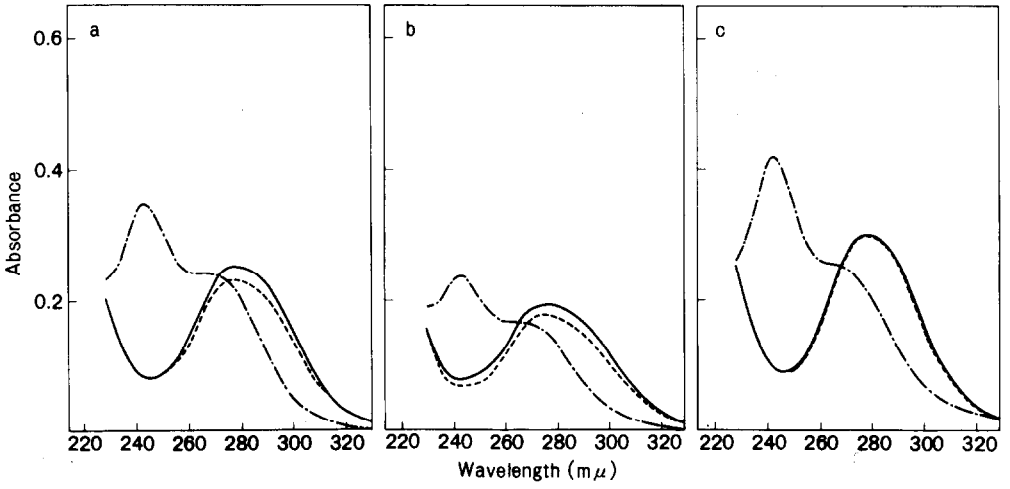

Fig. 2. Ultraviolet absorption spectra of (a) N_1, (b) N_2 and (c) 5-methyl-2-thiouridine. pH 7.0, ———— ; pH 2.0, - - - - - - - - ; pH 12.0, – – – – – – – .

Desulfurization of 5-methyl-2-thiouridine and related compounds was carried out by a similar procedure to that described previously [4, 12] for desulfurization of 4-thiouridine and 5-methylaminomethyl-2-thiouridine. The sample was dissolved in 0.05 ml of 0.1 M sodium carbonate–sodium bicarbonate buffer (pH 8.9), and 0.005 ml of a 2% aqueous solution of cyanogen bromide was added. The mixture was kept at room temperature for 10 min and then 0.001 ml of 10 M NaOH was added, and the mixture was heated at 50° for 1 hr. The alkaline solution was neutralized with Dowex-50 resin, and lyophilized for analysis by thin-layer chromatography and electrophoresis.

3. Results

As shown in fig. 1, several minor components were detected in two dimensional chromatograms of RNase T_2 digests of rat liver $tRNA_3^{Glu}$ and $tRNA_2^{Lys}$, respec-

Table 1
Relative chromatographic mobilities and electrophoretic mobilities of N_1, N_2, 5-methyl-2-thiouridine and related compounds.

	Thin-layer chromatography R_f in solvent system			Electrophoresis R 2′, (3′) UPM
	A	B	C	
Nucleoside N_1	0.54	0.56	0.46	0.28
Nucleoside N_2	0.54	0.56	0.46	0.28
5-Methyl-2-thiouridine	0.54	0.56	0.46	0.28
2-Thiouridine-5-acetic acid methyl ester	0.59	0.60	0.51	0.36
2-Thiouridine	0.48	0.47		0.41
Uridine	0.48	0.40		0.00

The solvent systems used were: A) isobutyric acid–0.5 M NH_4OH (5:3, v/v); B) butanol–acetic acid–H_2O (5:3:2, v/v/v; C) 2-propanol–conc. NH_4OH–H_2O (7:1:2, v/v/v). Thin-layer electrophoresis was carried out using a glass plate coated with Avicel SF cellulose in 0.05 M triethylammonium bicarbonate buffer, pH 7.5.

Table 2
Relative chromatographic mobilities and electrophoretic mobilities of desulfurized nucleosides, ribothymidine and related compounds.

	Thin-layer chromatography R_f in solvent system D	Electrophoresis R 2′, (3′) UMP
Desulfurized N_1	0.55	0.00
Desulfurized N_2	0.55	0.00
Ribothymidine	0.55	0.00
Desulfurized 5-methyl 2-thiouridine	0.55	0.00
Desulfurized 2-thiouridine 5-acetic acid methyl ester	0.29	0.53
5-Methyl-2-thiouridine		0.24
2-Thiouridine 5-acetic acid methyl ester		0.31

The solvent system used was: D) 95% ethanol–H_2O (4:1, v/v). Paper electrophoresis was carried out as described in table 1.

tively. In addition to m^1Ap, m^1Gp, m^5Cp, m^7Gp and Ψp, two unknown minor nucleotides were detected in the digest of $tRNA_3^{Glu}$ and these were designated as N_1p and N_3p, respectively. In the case of $tRNA_2^{Lys}$, three unknown nucleotides, designated as N_2p, N_4p and N_5p, were detected together with m^5Cp, m^7Gp and Ψp. The present communication only deals with the characterization of N_1 and N_2 as 5-methyl-2-thiouridine. No ribothymidine 3′-phosphate was detected at all in these two tRNAs. This is the first observation of the absence of ribothymidine in a pure species of tRNA from eukaryotic cells, and its absence is of interest in understanding the function of ribothymidine in tRNA.

Fig. 2 shows a comparison of the ultraviolet (UV) absorption spectra of the nucleoside preparations, N_1 from $tRNA_3^{Glu}$ and N_2 from $tRNA_2^{Lys}$, with those of synthetic 5-methyl-2-thiouridine. The three spectra were identical in all respects at three different pH values, indicating that both N_1 and N_2 are either 2-thiouridine or a derivative of it. Table 1 shows the R_f values of N_1 and N_2 on thin-layer chromatography and their relative electrophoretic mobilities, as compared with those of 5-methyl-2-thiouridine and related compounds. The mobilities of the nucleoside preparations, N_1 and N_2 were the same as these of synthetic 5-methyl-2-thiouridine in all systems tested, but different from these of 2-thiouridine and 2-thiouridine-5-acetic acid methyl ester. These results strongly suggest that N_1 and N_2 are 5-methyl-2-thiouridine. The lack of identity of N_1 and N_2 with 2-thiouridine-5-acetic acid methyl ester was confirmed by chromatography and electrophoresis of N_1 and N_2 after alkaline treatment [1]. Treatment of N_1 and N_2 with 0.2 M NaOH for 1 hr at 100° did not cause a change in their chromatographic and electrophoretic mobilities.

Further proof that N_1 and N_2 are identical with 5-methyl-2-thiouridine was obtained on desulfurization of N_1 and N_2 to ribothymidine. As shown in table 2, the chromatographic and electrophoretic mobilities of desulfurized N_1 and N_2 were the same as those of authentic ribothymidine and desulfirized 5-methyl-2-thiouridine. Although not shown in the figure, the UV absorption spectra of desulfurized N_1 and N_2 were identical with those of a specimen of ribothymidine.

4. Discussion

A new minor nucleoside isolated from rat liver $tRNA_3^{Glu}$ and $tRNA_2^{Lys}$ was thoroughly characterized

as 5-methyl-2-thiouridine. 5-Methyl-2-thiouridine has not previously been isolated from any type of nucleic acid or tRNA. Moreover, there is no previous report of the isolation and characterization of a sulfur-containing minor nucleoside from mammalian tRNA. Eliceiri recently reported that ^{35}S from ^{35}S-cysteine was incorporated into the 4S fraction of mouse lymphoma cells [13]. He concluded that ^{35}S was incorporated into a fraction other than the 4-thiouridine moiety. Moreover, Carbon et al. showed that the chromatographic profile of one of the isoaccepting lysine tRNAs from rabbit liver is reversibly altered by iodine treatment [14]. These indirect pieces of evidence suggesting the presence of a sulfur containing nucleoside can now be explained by the existence of 5-methyl-2-thiouridine in these mammalian tRNAs.

Based on the assumption that 1 absorbance unit of tRNA is equal to 1.66 mμmole [15], the amounts of 5-methyl-2-thiouridine in $tRNA_3^{Glu}$ and $tRNA_2^{Lys}$ were estimated to be approximately 1 mole per tRNA. As reported in a separate paper, $tRNA_3^{Glu}$ and $tRNA_2^{Lys}$ were preferentially recognized by GAA and AAA, respectively [8]. It was previously shown that there is a correlation between the existence of a 2-thiouridine derivative and preferential recognition of A in the third position of the anticodon in both yeast and *E. coli* $tRNA^{Glu}$ [3, 4]. From the above results and those reported here, it seems likely that 5-methyl-2-thiouridine is also located in the first position of the anticodon of rat liver $tRNA_3^{Glu}$ and $tRNA_2^{Lys}$. The existence of 2-thiouridine derivatives in particular tRNAs of such widely different types as mammalian and bacterial cells suggests that the 2-thiouridine moiety in tRNA is essential for the fundamental process of protein synthesis in cells. It is possibly important for precise codon recognition of tRNA, as discussed previously [4].

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