URIDIN-5-OXY ACETIC ACID: A NEW MINOR CONSTITUENT FROM *E. coli* VALINE TRANSFER RNA I

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The primary sequence of *E. coli* $tRNA_I^{Val}$ was recently established (1,2), and it was found that an unidentified minor component designated as V was located in the first position of the anticodon of this tRNA (1-3). The unique structure of V is of particular interest, since it must participate directly in codon-anticodon base pairing in the decoding process in protein synthesis. This report describes the characterization of this minor nucleoside as uridin-5-oxy acetic acid (Fig. 1).

MATERIALS AND METHODS

Materials: Unfractionated *E. coli* tRNA was prepared as described by Zubay (4) with a slight modification (5). *E. coli* $tRNA_I^{Val}$ was prepared as described previously (6,7). Bovine pancreatic RNase (1-A) was purchased from Worthington Biochemical Co. DEAE-Sephadex A-25 (capacity, 3.5 ± 0.5 mequiv/g; particle size, 40-120 μ) was obtained from Pharmacia Fine Chemicals. A synthetic sample of uridin-5-oxy acetic acid was prepared by carboxymethylation of 5-hydroxyuridine. Details of this synthesis will be published later. 5-Carboxyuridine was a gift from Dr. K. Imai of the Research Laboratories, Takeda Chemical Industries, Ltd.

Thin-layer chromatography and paper electrophoresis: Thin-layer chromatography was carried out by the ascending technique using Avicel SF plates (10 x 10 cm). The solvent systems used were: A, isopropanol-1 % ammonium sulfate (2:1, v/v); B, ethanol-1 M ammonium acetate, pH 7.5 (7:3, v/v); C, n-propanol-conc. NH_4OH-water (55:10:35, v/v/v); D, isopropanol-conc. NH_4OH-water (7:1:2, v/v/v); E, n-butanol-acetic acid-water (4:1:2, v/v/v); F, isobutyric acid-0.5 M NH_4OH (5:3, v/v); G, isopropanol-conc. HCl-water (70:15:15, v/v/v). Paper electrophoresis was carried out in 0.05 M triethylammonium bicarbonate buffer, pH 7.5 on Toyo-Roshi No 51A paper at 20 V/cm for 1 hour.

Nuclear magnetic resonance (NMR) spectral measurement: NMR spectra were measured in Model JNN-3H-60 or Model JNM-4H-100 spectrometer from Japan Electron Optics Laboratory Co. Chemical shifts were expressed relative to that of t-butanol used as

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Abbreviations: V, unknown nucleoside corresponding to X of Yaniv and Barrell (2); Vp, 3'-nucleotide of V.

an external standard at τ 8.72.

Isolation of V: E. coli $tRNA_1^{Val}$ (500 OD units) was hydrolyzed extensively with pancreatic RNase and chromatographed on a DEAE-Sephadex A-25 column (0.5 x 150 cm) with a linear gradient of NaCl in 7 M urea, as described previously (8). Vp was eluted between the fractions of dinucleotide and trinucleotide as a single component. A large amount of V was also isolated from unfractionated E. coli tRNA. Five grams of tRNA were hydrolyzed with 100 mg of pancreatic RNase. The digest was loaded on a column (5 x 90 cm) of DEAE-Sephadex A-25 equilibrated with buffer containing 0.14 M NaCl, 0.02 M Tris-HCl (pH 7.5) and 7 M urea. Then, the column was washed with the same buffer. Vp was eluted as a small peak after most dinucleotides had been eluted. To remove contaminating dinucleotides, the Vp fraction was treated with RNase T_2 and rechromatographed on a DEAE-cellulose column. The Vp obtained was hydrolyzed with E. coli alkaline phosphomonoesterase and the resulting nucleoside, V was purified by Dowex 1 (x 8, formate form) column chromatography. The yield of V was approximately 100 OD^{275} mμ units. The V isolated from unfractionated tRNA had identical characteristics with the V isolated from the $tRNA_1^{Val}$ with respect to its ultraviolet absorption spectrum, paper chromatographic mobilities and paper electrophoretic mobility.

RESULTS

From previous studies it was concluded that V was a derivative of uridine (2,3). This was concluded from its ultraviolet absorption spectra (2,3), paper chromatographic mobilities (5), and the fact that the phosphodiester bond of V was hydrolyzed by pancreatic RNase, but not by RNase T_1 (2,3). V seemed to have one negative charge, since Vp was eluted after dinucleotide from a column of DEAE-Sephadex A-25, and V moved the same distance to the anode as uridine 2',3'-cyclic phosphate on paper electrophoresis at pH 7.5. The pKa value of V was 2.9, suggesting that the carboxyl group is probably the acidic group in V. The NMR spectrum (τ)(in D_2O) showed bands at 2.43 (1 H, singlet; H-6), 3.97 (1 H, doublet; H-1'), 5.60 (2 H, singlet; probably methylene) and 6.08 (2 H, doublet; H-5'). The absence of an H-5 proton and the presence of a singlet H-6 proton suggested that V may be a 5-substituted uridine derivative. From comparison of the ultraviolet absorption of V with those of several 5-substituted uridine derivatives, such as 5-carboxyuridine [$\lambda_{max}^{H_2O}$, 275 mμ; Imai and Honjo (9)], 5-carboxymethyluridine [$\lambda_{max}^{pH\ 7.0}$, 266.5 mμ; Gray and Lane (10)], 5-acetyluridylate [$\lambda_{max}^{H_2O}$, 283-284 mμ; Carrington et al. (11)] and 5-hydroxyuridine [$\lambda_{max}^{H_2O}$, 276 mμ; Ueda (12)], it is concluded that a carboxyl group attached to the base is linked to the 5 position of the ring through a $-COCH_2-$ or $-OCH_2-$ chain. The presence of the $-COCH_2-$ linkage in the structure of V was

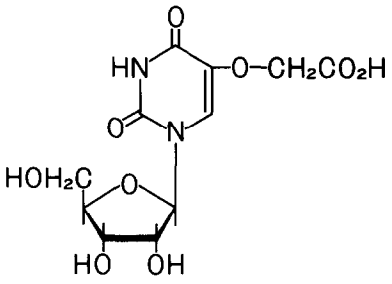

Fig. 1: Structural formula of uridin-5-oxy acetic acid

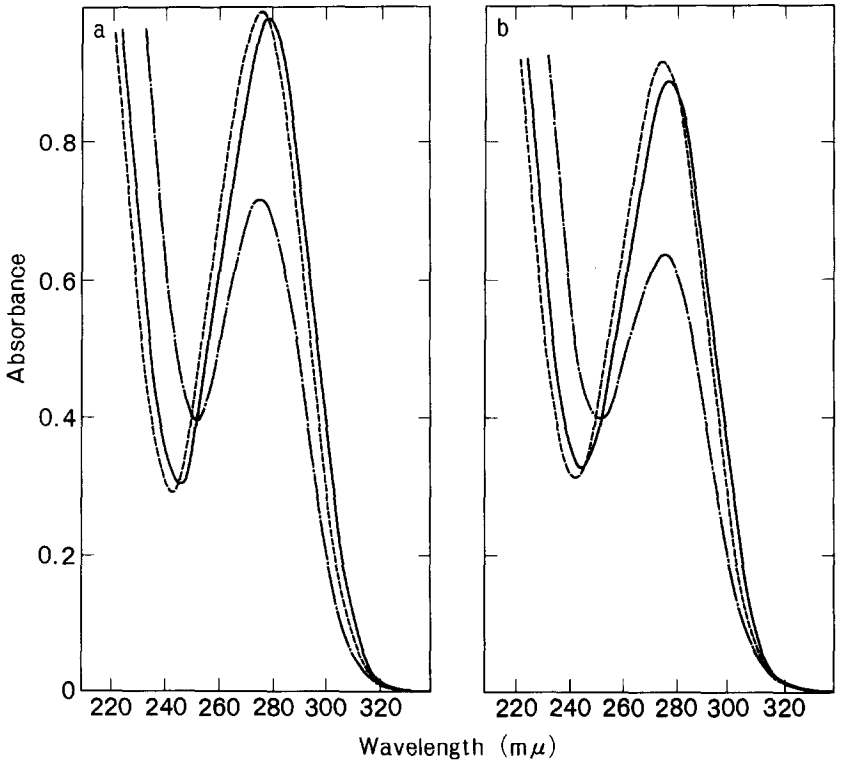

Fig. 2: Ultraviolet absorption spectra of (a) V and (b) uridin-5-oxy acetic acid. pH 7.0, ——— ; pH 2.0, - - - - - ; pH 12.0, ------.

excluded from the observation that neither an active methylene reagent, such as trinitrobenzene (13), nor a water-soluble carbonyl reagent, such as Girard T reagent (14), reacted with V. Thus the most probable structure of V is considered to be uridin-5-oxy acetic acid (Fig. 1). To obtain direct proof of this, uridin-

5-oxy acetic acid was synthesized chemically and compared with V. Fig. 2 shows a comparison of the ultraviolet absorption spectra of V and synthetic uridin-5-oxy acetic acid at three different pH values. The two spectra are identical in all respects, strongly suggesting that V is uridin-5-oxy acetic acid. Further proof that V is identical with uridin-5-oxy acetic acid was obtained from the Rf values of the two materials on thin-layer chromatography with seven different solvent systems and from their relative electrophoretic mobilities as shown in Table I. The data clearly demonstrate that V is uridin-5-oxy acetic acid, since V behaved identically with the synthetic material in all systems. The identity of V with uridine-5-oxy acetic acid was confirmed by comparison of the NMR spectra of these two compounds. The singlet methylene signal (τ 5.60) and other signals of the NMR spectrum of V completely coincided with those of authentic uridin-5-oxy acetic acid. Finally, mass spectral analysis of V-TMS-TFA (modified V prepared by trifluoroacetylation followed by trimethylsilylation) showed two molecular ions: m/e 630 and 654. A sample of synthetic uridin-5-oxy acetic acid treated in the same way gave the same molecular ions: m/e 630 and 654. Thus mass spectral evidence also gave overwhelming evidence for the identity of V with uridin-5-oxy acetic acid.

Table I

Relative chromatographic mobilities and electrophoretic mobilities of uridine and substituted uridines. The solvent systems used are described in the text.

	Thin-layer chromatography Rf in solvent system							Electrophoresis R2',(3')UMP
	A	B	C	D	E	F	G	
Nucleoside V	0.43	0.26	0.52	0.33	0.30	0.32	0.48	0.61
Uridin-5-oxy acetic acid	0.43	0.26	0.52	0.33	0.30	0.32	0.48	0.61
5-Carboxyuridine	0.43	0.25	0.46	0.32	0.37	0.40	0.47	0.56
Uridine	0.58	0.71	0.72	0.49	0.42	0.50	0.52	0

DISCUSSION

A minor nucleoside, V, located in the first position of the anticodon of E. coli $tRNA_I^{Val}$ was throughly characterized as uridin-5-oxy acetic acid. This newly identified minor component was not previously isolated from all types of nucleic

acid as well as from all species of transfer RNA. The existence of a $-OCH_2COOH$ group in the nucleoside is unique, and no nucleoside with this side group has previously been found in material from natural sources.

E. coli $tRNA_I^{Val}$ was recognized by GUU with 20 % efficiency as compared with GUA and GUG, when tested in an experiment on tRNA-ribosome binding (6,15). It is not clear yet whether the particular wobbling characteristics of *E. coli* $tRNA_I^{Val}$ are due to an artifact or whether they are actually involved in protein synthesis *in vivo*. In any case, uridin-5-oxy acetic acid must have a specific function to facilitate precise matching of $tRNA_I^{Val}$ with corresponding codon sequences.

The discovery of this unique nucleoside in a specific location in tRNA raises several interesting questions. Namely (a) What steps are involved in biosynthesis of uridin-5-oxy acetic acid ? (b) Is uridin-5-oxy acetic acid also present in other species of tRNA which have similar codon recognition properties to *E. coli* $tRNA_I^{Val}$? and (c) What are the physicochemical properties of the interaction of uridin-5-oxy acetic acid with other nucleic acid components ? Experiments on these problems are in progress.

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