2-METHYLTHIO N^6-(Δ^2-ISOPENTENYL) ADENOSINE: A COMPONENT OF E. COLI TYROSINE TRANSFER RNA

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Transfer RNA's (tRNA's) contain a high proportion of modified nucleosides in addition to the four common nucleosides. These modified nucleosides are mostly distributed in the looped or single-stranded regions in the "clover-leaf model" and some of the modified nucleosides occupy specific positions on the tRNA (For a review see Madison, 1968). The role of these modified nucleosides for the overall function of tRNA's is, however, not clearly understood. For several reasons, we have been interested in the nucleotide sequence of E. coli tyrosine tRNA. In this report, we describe briefly the characterization of a modified nucleoside in E. coli tyrosine tRNA as 2-methylthio,N^6-isopentenyl adenosine (2MeSN^6isoPeA). The structure of this nucleoside is of particular interest because of its location next to the anticodon in E. coli tyrosine tRNA. This nucleoside was previously designated as A^* by Goodman et al. (1968), who have also reported the total nucleotide sequence of E. coli tyrosine tRNA.

This nucleoside has recently been independently isolated and identified as one of the cytokinin active components from the total mixture of E. coli tRNA's (Burrows et al., 1968).

MATERIALS AND METHODS

Paper chromatography. Paper chromatography was carried out on Whatman No. 1

paper using the descending technique. The solvent systems used were: A, 2-propanol - concentrated ammonium hydroxide - water (7:1:2, v/v); B, ethanol - 1 M ammonium acetate pH 7.5 (7:3, v/v); C, 1-butanol - water (86:14, v/v) and D, ethylacetate - 1-propanol - water (4:1:2, v/v; upper layer).

Ultraviolet absorption spectra were measured using a Cary 14 spectrophotometer.

Pancreatic RNase, snake venom phosphodiesterase and E. coli alkaline phosphatase were obtained from Worthington Biochemicals and T1-RNase from Sankyo Chemical Company, T2-RNase was a gift from Dr. Hiroshi Okazaki and E. coli cyclic phosphodiesterase from Dr. Yukio Sugino.

2-methylthio adenosine (2MeSA) was prepared according to Schaeffer and Thomas (1958) and purified by paper chromatography. 2-methylthio N^6-methyl adenosine (2MeSN^6MeA) was a gift from Ajinomoto Co., Inc., and 2-methylthio N^6-isopentenyl adenosine (2MeSN^6isoPeA) was a synthetic sample, kindly provided by Dr. N. J. Leonard.

E. coli Tyrosine tRNA was purified as described previously (Nishimura et al., 1967). Ion exchange chromatography of crude E. coli B tRNA on a column of DEAE-Sephadex yielded two clearly resolved peaks (I and II) of tyrosine acceptor activity; the work described in this report was carried out on Peak II after further purification by reverse phase column chromatography (Kelmers, Novelli and Stulberg, 1965). The tRNA used was 95% pure with respect to tyrosine acceptor activity.

Isolation of the unknown nucleoside: The unknown nucleoside† was present in the tetranucleotide ApNpApΨp obtained by degradation of tRNA (300 O.D. 260 units) with pancreatic RNase (1.2 mg) for 5 hours at 37° followed by chromatography of the mixture on DEAE-Sephadex or DEAE-cellulose. Alternatively the tRNA was first degraded with T1-RNase and the dodecanucleotide containing N was isolated by column chromatography on DEAE-cellulose and then degraded further with pancreatic RNase to yield ApNpApΨp. The isolation of N was then carried out by either of the two methods described below. (1) ApNpApΨp (16 O.D. 260 units) was incubated with T2-RNase (10 units; Takahashi, 1961), for 6 hours at 37° and the products Ap, Np! and Ψp were separated by paper chromatography in solvent B. The band corresponding to Np! (R_f = 0.90) was eluted and the Np! was then converted to the nucleoside N by treatment (in 0.1 ml total value) with E. coli 2',3'-cyclic phosphodiesterase (27 units; Anraku, 1964). The nucleoside N is sparingly soluble in water and under the conditions of incubation readily crystallized out. The crystals were washed with small volumes (4 x 0.1 ml) of ice cold water and collected by centrifugation. (2) ApNpApΨp (18 O.D. 260 units) was incubated in a total volume of 3 ml with E. coli alkaline phosphatase (12.5 µg) and snake venom phosphodiesterase (125 µg) and after concentration the mixture of nucleosides produced were separated by paper chromatography in solvent A. N (R_f = 0.86) was eluted with 1-propanol - water (50:50, v/v) and was either used directly or crystallized from a concentrated solution.

RESULTS

Earlier indication that N was an adenosine derivative which probably contained sulphur and a methyl group was obtained by Goodman et al. (1968), who isolated radioactive N from E. coli tyrosine tRNA when the E. coli was grown

† Designated here as N and corresponding to A* of Goodman et al. (1968).

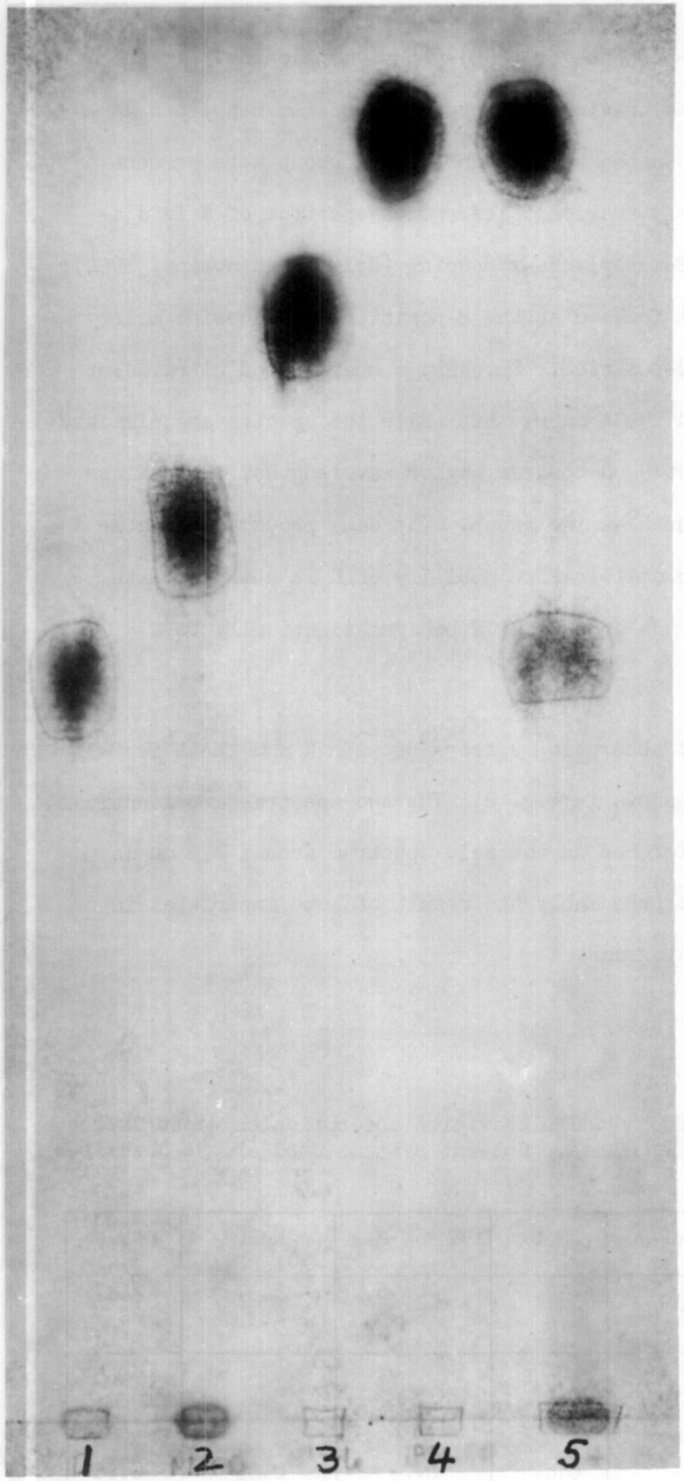

Fig. 1. Paper chromatogram developed in solvent A using 1, A; 2, 2MeSA; 3, 2MeSN^{6}MeA; 4, 2MeSN^{6}isoPeA; 5, unknown nucleoside N to which an internal marker of A was added.

in the presence of either (^{35}S)-sulphate or (^{14}C)-methyl methionine. In the present work, the similarity of ultraviolet absorption spectrum between N and 2MeSA strongly suggested that both the sulphur and methyl group were present in a methylthio group in N. The ultraviolet absorption spectrum of N is distinctly different from that of 8-methylthio-adenosine (Holmes and Robins, 1964); thus the methylthio group is not located at the 8-position of adenosine moiety and is probably located at the 2-position. Further, comparison of ultraviolet absorption spectra between N and 2MeSA shows that while the spectra are similar, the λmax of N is shifted by about 9 mμ towards higher wavelengths, indicating an additional substitution in the N^6-amino group. The data provided in Table I and Fig. 1 in which the paper chromatographic mobility of N is compared with that of various synthetic compounds shows that N behaves identically to 2MeSN^6isoPeA in all systems.

A comparison of ultraviolet absorption spectra between N and 2MeSN^6isoPeA at three different pH values is shown in Fig. 2. The two spectras are identical in all respects, the slight difference in the acid spectrum around 275 mμ is not consistently observed and is presumably the result of some impurities in the N isolated from paper chromatograms.

TABLE I

Paper chromatographic mobility and ultraviolet absorption characteristics of adenosine and substituted adenosines. Solvent systems used are as described in Materials and Methods.

	A	N^6MeA	N^6isoPeA	2MeSA	2MeSN^6MeA	2MeS N^6isoPeA	N
λ_{max}(mμ) at pH 7.0	260	266	269	235, 274	241.5, 280.5	243, 283	243, 283
R_f in Solvent System							
A	0.52	-	-	0.62	0.75	0.86	0.86
B	0.56	-	-	0.67	0.79	0.89	0.89
C	0.23	-	-	0.43	0.63	0.82	0.82
D	0.37	-	-	0.68	0.83	0.92	0.92

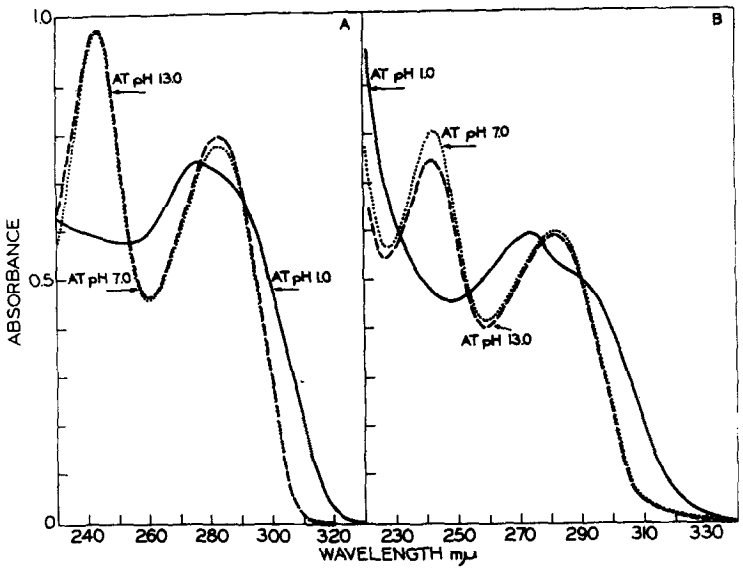

Fig. 2. Ultraviolet absorption spectra of A, 2MeSN^{6}isoPeA; B, N.

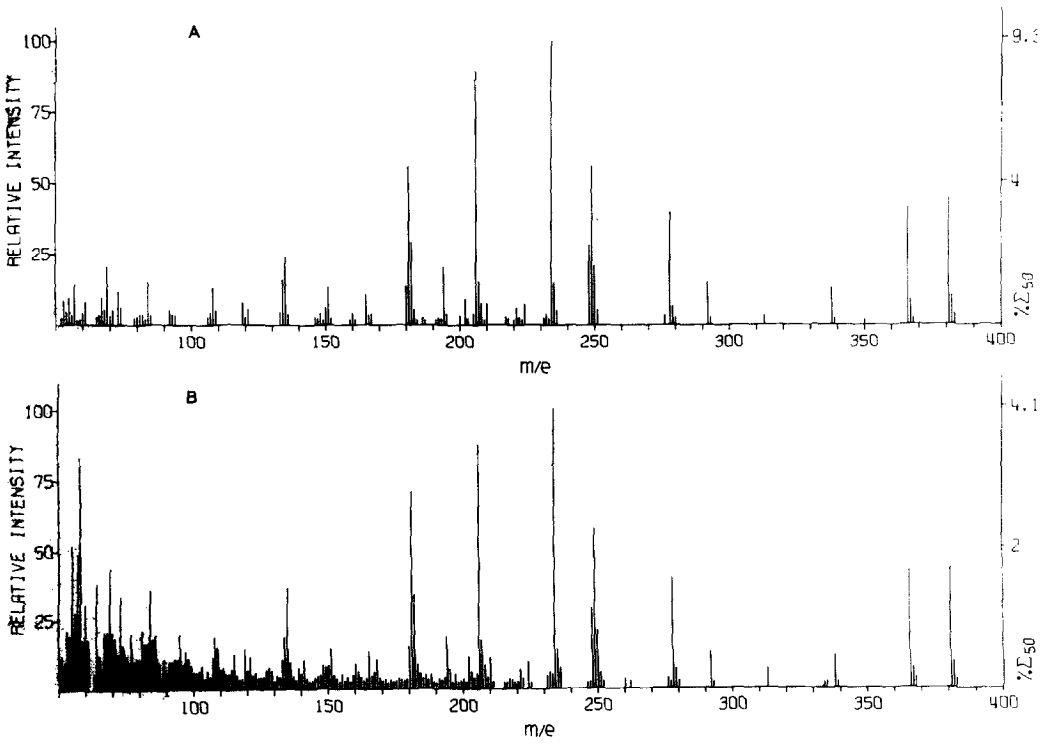

Fig. 3. Low resolution mass spectra of A, 2MeSN^{6}isoPeA; and B, N; carried out at 70 ev using a direct inlet system at a temperature of 140° C on a LKB-mass spectrometer.

Final evidence that N is identical to 2MeSN^{6}isoPeA was provided by the high and low resolution mass spectra of these two compounds. High resolution mass spectrum confirmed the presence of sulphur and showed a molecular ion peak for N at 381.1478, corresponding to the formula $C_{16}H_{23}N_5O_4S$ (calculated 381.1470); under identical conditions 2MeSN^{6}isoPeA showed a molecular ion peak at 381.1471. An examination of the low resolution mass spectra of N and 2MeSN^{6}isoPeA (Fig. 3) shows that the fragmentation patterns of these two compounds are identical, the fragments obtained being the same as those described by Burrows et al. (1968). Furthermore, the fragmentation pattern of N closely parallels that of N^6-isopentenyl adenosine (N^{6}isoPeA)(Biemann et al., 1966; Robins et al., 1967) except that the m/e peaks in case of N are consistently 46 mass units higher and confirms the presence of a methylthio group in N.

DISCUSSION

As a part of our studies on the sequence of E. coli tyrosine tRNA, this paper has reported on the identification of a minor nucleoside (N) adjacent to the anticodon in the tRNA and previously designated as A* (Goodman et al., 1968). This minor nucleoside is shown to be identical to chemically synthesized 2MeSN^{6}isoPeA by comparison of ultraviolet absorption spectra, paper chromatographic mobility in four different solvent systems and high and low resolution mass spectrometry. The isolation of 2MeSN^{6}isoPeA in the present work from E. coli tyrosine tRNA and by Burrows et al. (1968) from total E. coli tRNA represents the existence of methylthio nucleosides in a second class of naturally occurring product, the only previously known example being in analogs of vitamin B_{12} (Friedrich and Bernhauer, 1957).

Biosynthesis of N. The characterization of N as 2MeSN^{6}isoPeA raises the question of the biosynthesis of this nucleoside at the level of tRNA. The observed replacement of this modified nucleoside by A in tyrosine tRNA isolated from starved E. coli cells (Goodman et al., 1968) indicates that A is a precursor of 2MeSN^{6}isoPeA. It is also worth noting that this particular A residue must

have been modified on the tRNA at least twice or even possibly three times. The presence of N^6isoPeA in exactly the same position (next to the anticodon) in yeast tyrosine and serine tRNA's suggests that the biosynthetic pathway is probably A → N^6isoPeA → (2SHN^{6}isoPeA)? → 2MeSN^{6}isoPeA. Since the sequences around the anticodon in yeast serine tRNA, tyrosine tRNA and E. coli tyrosine tRNA's are rather similar, the lack of further modification of N^6isoPeA in the two yeast tRNA's can be attributed either to (a) the lack of enzymes catalyzing these changes in yeast or (b) strict specificity on the part of the modifying enzymes. Use of purified yeast tyrosine or serine tRNA's as substrates for conversion of N^6isoPeA to 2MeSN^{6}isoPeA in conjunction with crude E. coli enzyme systems would be expected to provide an answer to this question and possibly to the biosynthetic steps involved. Experiments along these lines are in progress.

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