Volume 2 number 10 October 1975 Nucleic Acids Research

Distribution of the modified nucleoside Q and its derivatives in animal and plant transfer RNA's

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Received 4 September 1975

ABSTRACT

The modified nucleoside, 7-(4,5-*cis*-dihydroxy-1-cyclopenten-3-yl-aminomethyl)-7-deazaguanosine, designated as Q, and its derivative, Q^*, were found in tRNA's from various organisms, including several mammalian tissues, other animals such as starfish, lingula and hagfish, and wheat germ. Q isolated from rat liver tRNA was found to be identical with *E. coli* Q by mass spectrometry and thin-layer chromatography. Thus the rare modified nucleoside Q originally isolated from *E. coli* tRNA, is widely distributed in various organisms. Analysis of the mass spectrum of Q^* suggested that it has a different side chain from Q.

INTRODUCTION

The modified nucleoside designated as Q was previously found in the first position of the anticodon of *E. coli* $tRNA^{Tyr}$, $tRNA^{His}$, $tRNA^{Asn}$ and $tRNA^{Asp}$ (1). This nucleoside was recently characterized as 7-(4,5-*cis*-dihydroxy-1-cyclopenten-3-yl-aminomethyl)-7-deazaguanosine (2). It is a very unique molecule having a 7-deazaguanosine nucleus and cyclopentenediol as a side chain. Q or a Q-like compound was also found in *Drosophila* tRNA (3) and rat liver tRNA (4), so we became interested in examining whether Q nucleoside is present in other organisms, and if so whether the structure of Q isolated from other cells is the same as that of *E. coli* Q. For this purpose we developed a simple method to isolate Q from unfractionated tRNA. Results showed that Q with the same structure is present in tRNA's from various sources, including starfish, lingula, hagfish and wheat germ, indicating that it is commonly present in tRNA in the animal kingdom, and also even in a plant. A derivative of Q, designated as Q^*, the structure of which is still unknown was also found in tRNA's from animals.

MATERIALS AND METHODS

Preparation of unfractionated tRNA: A slight modification of the

procedure of Brunngraber (5) was used for isolation of tRNA. Cells or tissues [hagfish (*Eptatretus burgeri*) liver, starfish (*Asterias amurensis*) liver, testis and ovary, lingula (*Lingula unguis*), and silkworm larvae] were homogenized with 1.5 volume of 88% phenol and 1.5 volume of 1 M NaCl—0.5 mM EDTA—0.02 M Tris-HCl (pH 7.5)—0.01 M $MgCl_2$ in a Waring blender. The homogenate was shaken for 2 hr at room temperature and then centrifuged for 15 min. at 9000 rpm. The upper aqueous layer was collected and mixed with 2 volumes of cold ethanol. The precipitate was collected by centrifugation and homogenized with an equal volume of cold 1 M NaCl in a mixer. The suspension was centrifuged at 9000 rpm for 15 min. The precipitate was resuspended in a half volume of 1 M NaCl and centrifuged. The supernatants were combined and mixed with 2 volumes of cold ethanol to precipitate tRNA and DNA. The precipitate was dissolved in 0.02 M Tris-HCl buffer (pH 7.5) and loaded on a column of DEAE-cellulose, previously equilibrated with the same buffer. The column was washed with 0.2 M NaCl—0.02 M Tris-HCl (pH 7.5). Then tRNA was eluted with 1 M NaCl—0.02 M Tris-HCl (pH 7.5). The tRNA in the eluate was precipitated by adding 2.5 volumes of ethanol and the precipitate was collected by centrifugation and dissolved in 0.01 M Tris-HCl (pH 7.5)—6 mM β-mercaptoethanol.

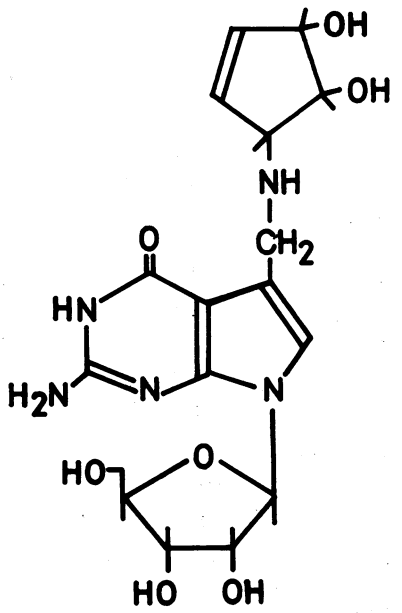
tRNA's from rat liver, rat kidney and rat ascites hepatoma were prepared as described previouslv (6). The procedure used for isolation of baker's yeast tRNA was also described previously (7). *E. coli* tRNA and *Thermus thermophilus* tRNA were prepared by Zubay's procedure (8). Rabbit liver tRNA and wheat germ tRNA were gifts from Drs. H. J. Gross and Y. Lapidot, respectively. To check the purity of tRNA samples, they were analyzed by polyacrylamide gel electrophoresis as described by Peacock and Dingman, using 10% gel in the presence of 7 M urea (9). It was shown that the preparations all consisted mainly of 4S and 5S species, and that the 5S content was less than 5% of the total tRNA content.

Isolation of Q and Q* from alkaline hydrolyzates of unfractionated tRNA: Unfractionated tRNA (300-1000 A_{260} units) was dissolved in 0.3 M KOH (2-10 ml), and incubated for 15 hr at 37°C. The hydrolyzate was adjusted to pH 8 with Dowex 50 [H^+ form] resin, and applied to a column of Dowex 1 [formate form] (0.3 x 30 cm). Elution was performed with a linear gradient of 0 to 0.05 M formic acid. Typical results on the fractionation of rat liver tRNA hydrolyzate by this procedure are shown in Figure 1. The sharp peak eluted just before the Cp fraction contained Qp and Q*p. The fraction containing Qp and Q*p was evaporated to dryness, dissolved in a small volume of water, and

applied to paper (Toyo-Roshi No. 51A or Whatman No. 1) or a thin-layer cellulose plate for two-dimensional paper chromatography. Qp and Q*p were clearly separated from each other by two-dimensional chromatography, as shown in Figure 2. Corresponding nucleosides were obtained by treatment of the nucleotides with *E. coli* alkaline phosphomonoesterase.

Mass spectrometry of trimethylsilyl derivatives of Q and Q*: The conversions of Q and Q* to the trimethylsilyl derivatives were carried out as described previously (2). A dried sample of 0.2 A_{260} units of the nucleoside was dissolved in 10 µl of N,O-bis(trimethylsilyl)trifluoroacetamide-pyridine-trimethylsilylchlorosilane (100:10:1, v/v/v), and heated for 1 hr at 100°C. Mass spectra were recorded using an LKB 9000 instrument, with an ionizing electron energy of 20 eV and an ion source temperature of 250°C. The sample was introduced by direct probe after removal of silylation reagents under vacuum.

Solvent systems for thin-layer chromatography: The solvents used were as follows: solvent A, isobutyric acid–0.5 N NH_4OH (5:3, v/v); Solvent B, 2-propanol-concentrated HCl–H_2O (70:15:15, v/v/v); Solvent C, n-butanol–acetic acid–H_2O (4:1:2, v/v/v); Solvent D, n-propanol–concentrated NH_4OH–H_2O (55:10:35, v/v/v). Solvents A and B were used respectively for the first and second dimensions in two-dimensional thin-layer chromatography.



Structure of Q

RESULTS AND DISCUSSION

The modified nucleoside Q has one positive charge at below pH 8, due to the presence of a secondary amine in the molecule (see structure of Q). This characteristic property of Q was effectively utilized previously for isolation of Q from *E. coli* tRNA (2). When a pancreatic RNase digest of *E. coli* tRNA was fractionated by DEAE-Sephadex A-25 column chromatography in the presence of 7 M urea, QpUp was eluted as a single peak before the mononucleotide fraction. To isolate Q more easily, an alkaline hydrolyzate of tRNA was fractionated by Dowex 1 column chromatography in this work. Alkaline hydrolysis was used instead of enzymatic digestion, since Q is known to be stable under alkaline conditions. Figure 1 shows the chromatographic profile of an alkaline hydrolyzate of rat liver tRNA as a typical example. A small sharp peak is seen before the Cp fraction. The ultraviolet absorption spectrum of this peak was found to be the same as that of Q (*E. coli*). The fraction was evaporated to dryness, and subjected to two-dimensional thin-layer chromatography. It was found that the material in the fraction was resolved into two components. One with the same chromatographic mobility

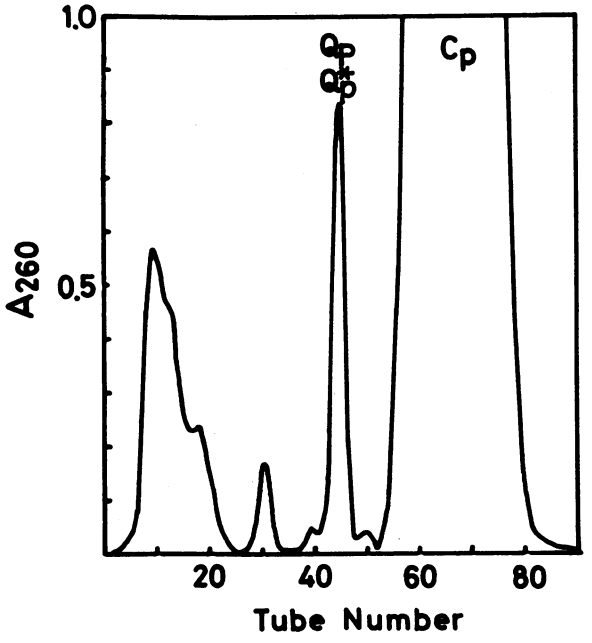


Figure 1. Isolation of Qp and Q*p from an alkaline digest of rat liver tRNA by Dowex 1 column chromatography. Elution was performed with a linear gradient obtained by placing 50 ml of water in the mixing chamber and 50 ml of 0.05 M formic acid in the reservoir. Fractions of 1.2 ml of effluent were collected. The dimensions of the column were 0.3 cm x 30 cm.

as Qp (*E. coli*) was designated as Qp, and the other was designated as Q*p (Figure 2). The ultraviolet absorption spectra of Qp and Q*p from rat liver tRNA are shown in Figure 3. The spectra were completely identical with those of Qp and Q (*E. coli*). The thin-layer chromatographic mobilities of the nucleosides Q and Q* isolated from rat liver, ascites hepatoma and kidney, and rabbit liver were compared with that of *E. coli* Q (Table 1). All these preparations of Q had the same mobility as *E. coli* Q, suggesting that the structure of the Q's isolated from these mammalian cells is the same as that of *E. coli* Q. To confirm the structural identity of Q (rat liver) with Q (*E. coli*), the mass spectra of the trimethylsilyl derivatives of the two compounds were recorded. As shown in Figure 4, Q_{TMS} (rat liver) gave the same molecular ion, m/e 913, and the same fragmentation pattern as that of Q_{TMS} (*E. coli*), indicating that the structure of Q (rat liver) is in fact the same as that of Q (*E. coli*).[13] Although not shown in this table, Q and Q* isolated from other sources such as hagfish, starfish, lingula and wheat germ had the same chromatographic mobility on two-dimensional chromatography, and the same ultraviolet absorption characteristics as those of Q and Q* from rat liver. At present, the exact structure of Q* is not known. Mass spectral analysis of the trimethylsilyl derivative of Q* (rat liver) indicated that Q* may contain a different side chain from Q, which is probably a derivative of cyclopentenediol. This conclusion is based on the fact that a fragment ion, m/e 656, corresponding to the heterocyclic nucleus of Q was also present in the spectrum of Q^*_{TMS}, but the molecular ion of Q^*_{TMS} differed from that of Q_{TMS}. Structural studies on Q* are now in progress.

Figure 5 showed the results of quantitative analyses of the contents of Q and Q* in tRNAs isolated from 14 different sources. It was found that Q was present in most organisms examined including both animals and one plant. Q and Q* were found in a variety of animals, such as Brachiopoda (*Lingula unguis*), Echinodermata (*Asterias amurensis*) and Chordata (*Eptatretus furgeri*) in addition to Vertebrata. The only tRNAs examined which did not contain Q or Q* were yeast and the extreme thermophilic bacterium, *Thermus thermophilus*. So far Q* has only been found in the animal kingdom. The contents of Q and Q* seem to differ in different organs, because, in rats, the contents of Q and Q* are higher in liver than in kidney, and because scarcely any Q or Q* was detected in the ovary or testis of starfish, whereas substantial amounts were present in the liver. White *et al.* previously reported that the Q content of *Drosophila* tRNA changed during the life cycle and that late larvae contained scarcely any Q (3). We also could not detect Q or Q* in silkworm larvae

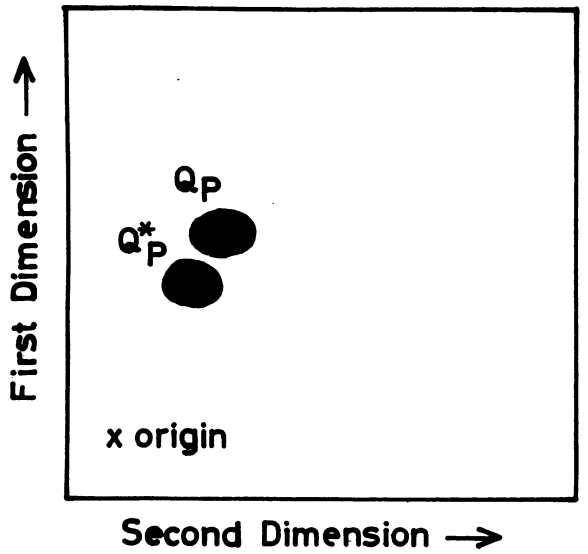


Figure 2. Schematic diagram showing the positions of the modified nucleotides, Qp and Q*p, on the two-dimensional thin-layer chromatogram.

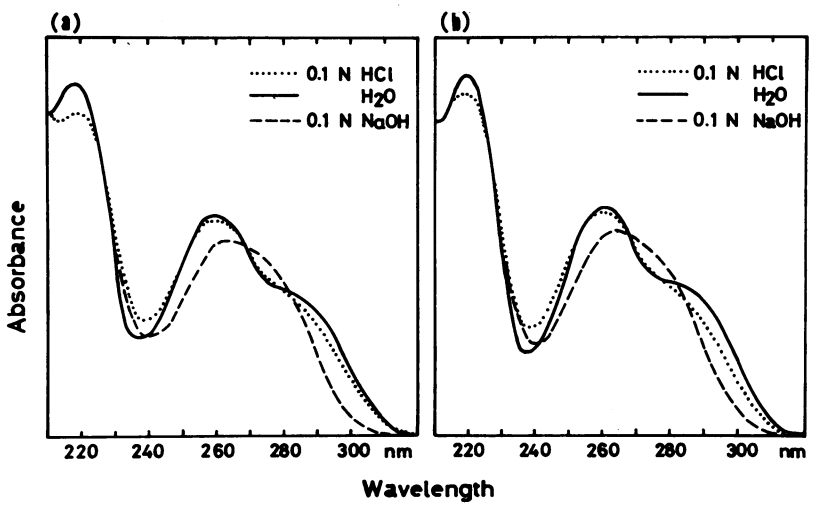


Figure 3. Ultraviolet absorption spectra of nucleosides Q (a) and Q* (b) isolated from rat liver.

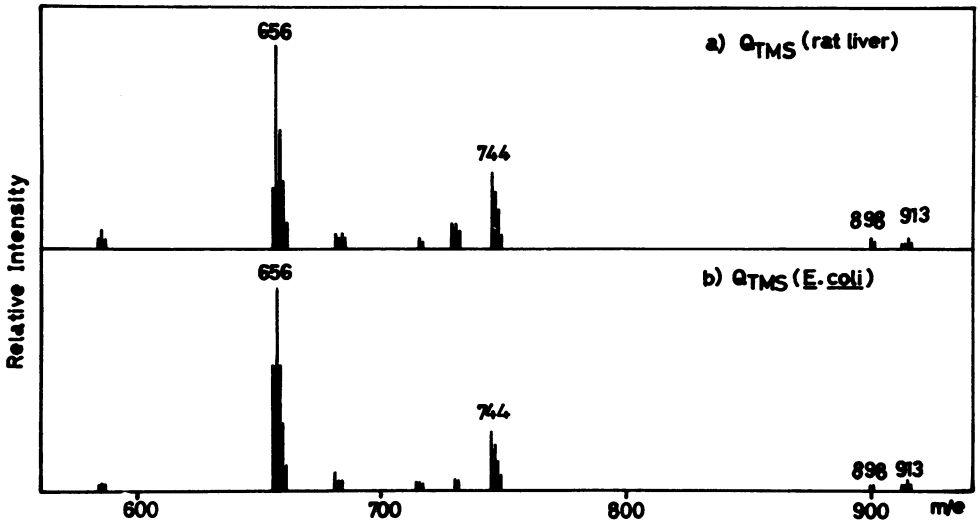


Figure 4. Mass spectra of Q_{TMS} (rat liver) and Q_{TMS} (*E. coli*) taken at 20 eV.

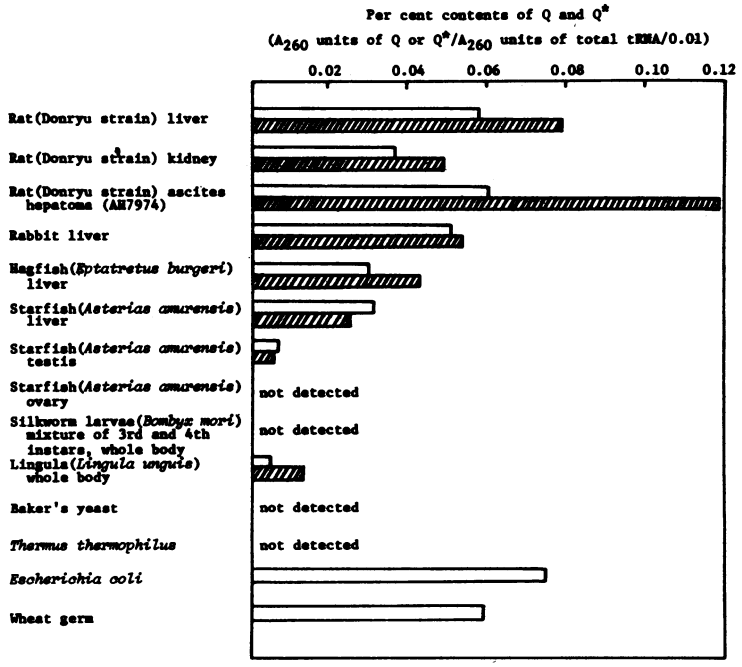


Figure 5. Contents of Q and Q* in unfractionated tRNA's from various organisms: ▭, content of Q; ▨, content of Q*.

Table 1. Comparison of thin-layer chromatographic mobilities of Q and Q* isolated from mammalian cells.

	Rf in Solvent System			
	A	B	C	D
Q (*E. coli*)	0.64	0.09	0.20	0.61
Q (Rat liver)	0.64	0.09	0.20	0.61
Q (Rat kidney)	0.65	0.09	0.21	0.60
Q (AH7974)	0.64	0.09	0.20	0.60
Q (Rabbit liver)	0.64	0.09	0.20	0.61
Q* (Rat liver)	0.56	0.05	0.10	0.55
Q* (Rat kidney)	0.55	0.05	0.10	0.54
Q* (AH7974)	0.56	0.05	0.10	0.55
Q* (Rabbit liver)	0.56	0.05	0.10	0.55

(*Bombyx mori*, 3rd and 4th instars) (Figure 5). Q and Q* were also found in tumor cells. Rat ascites hepatoma (AH7974) contained more Q* than normal rat liver (Figure 5), but the difference in the contents in normal rat liver and hepatoma seems less than that expected from indirect evidence (10,11,12).

In conclusion, the unique modified nucleosides, Q and Q* that are 7-deazaguanosine derivatives were found in tRNA's of a wide variety of organisms, including Vertebrata, Chordata, Brachiopoda, Echinodermata, and a plant, in addition to bacteria (*E. coli*). The presence of Q in tRNA's of such a wide range of organisms may reflect its importance in the process of cell growth or growth regulation, although at present its actual function is unknown.

ACKNOWLEDGEMENTS

We are indebted to Dr. H. J. Gross, Max-Planck-Institut für Biochemie, München for a gift of rabbit liver tRNA, to Dr. Y. Lapidot, The Hebrew University of Jerusalem, Jerusalem for a gift of wheat germ tRNA, and to Dr. A. Ninomiya, Product Development Laboratories, Sankyo Co. Ltd., Tokyo for providing a mass spectrometer.

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13 The mass spectrum of Q shown here differs from that reported previously (2). No peaks corresponding to a carboxylated form of Q were observed in the spectrum since it was taken at 20 eV instead of 70 eV.