Volume 4 Number 12 December 1977 Nucleic Acids Research

Detection of nucleoside Q precursor in methyl-deficient E.coli tRNA

Norihiro Okada, Tomoko Yasuda and Susumu Nishimura

Biology Division, National Cancer Center Research Institute, Tsukiji 5-1, Chuo-ku, Tokyo, Japan

Received 28 July 1977

ABSTRACT

^{32}P-Labeled tRNA^{Asn} was isolated from methyl-deficient *E. coli* tRNA. Nucleotide sequence analysis showed that tRNA^{Asn} contains three derivatives of the Q nucleoside, possibly Q precursors, in addition to guanosine in the first position of the anticodon. One of the Q precursors was isolated on a large scale. Its UV spectra were identical with those of normal Q, indicating that the 7-deazaguanosine structure having a side chain at position C-7 is complete in the Q precursor. No radioactivity was incorporated into Q or Q precursors from either [methyl-^{14}C]methionine, [1-^{14}C]methionine or [U-^{14}C]methionine, showing that methionine was not directly involved in the formation of Q.

INTRODUCTION

The modified nucleoside Q is found in the first position of the anticodon of *E. coli* tRNA^{Tyr}, tRNA^{His}, tRNA^{Asn} and tRNA^{Asp} (1). Its structure was determined to be 7-(4,5-*cis*-dihydroxy-1-cyclopenten-3-ylaminomethyl)-7-deazaguanosine, namely 7-deazaguanosine having a cyclopentenediol attached to the deazapurine C-7 position through a $-CH_2-NH_2-$ linkage (2). It is of interest to study the biosynthesis of Q, since it is the only nucleoside in which the purine skeleton itself is modified, and it is widely distributed in a variety of organisms, such as microorganisms, insects, animals and plants (3).

Katze previously reported that *E. coli* methyl-deficient tRNA lacked the Q nucleoside (4). This conclusion was reached indirectly by comparing the chromatographic profiles of the four Q-containing tRNAs on RPC-5 column chromatography before and after modification of unfractionated tRNA with cyanogen bromide.

A guanine insertion reaction in rabbit reticulocytes, previously described by Farkas (5), was found to be a reaction for exchange of guanine with Q base or guanine located in the first position of the

anticodons of $tRNA^{Tyr}$, $tRNA^{His}$, $tRNA^{Asn}$ and $tRNA^{Asp}$ (6). It was found that a guanine insertion enzyme, isolated from *E. coli*, catalyzed the exchange of guanine for guanine, but not for the Q base, unlike the rabbit reticulocyte enzyme (7). When the *E. coli* enzyme was used to test for the guanine insertion reaction, methyl-deficient *E. coli* tRNA was found to be an active acceptor, while normal *E. coli* tRNA was not utilized, suggesting that the formation of Q is incomplete in methyl-deficient tRNA. These results led us to make further studies on the effect of methionine on the biosynthesis of Q.

^{32}P-Labeled methyl-deficient $tRNA^{Asn}$ was isolated by two-dimensional polyacrylamide gel electrophoresis, and its nucleotide composition and sequence were analyzed. Surprisingly, it was found that methyl-deficient $tRNA^{Asn}$ contained three derivatives of the Q nucleoside in addition to guanosine in the first position of the anticodon. One of the Q precursors ($preQ_{1p}$) was isolated on a large scale from unlabeled methyl-deficient *E. coli* tRNA. UV absorption spectra showed that formation of 7-deazaguanosine and attachment of a side chain at the purine C-7 position is already complete in the Q precursor molecule.

MATERIALS AND METHODS

Preparation of ^{32}P-labeled methyl-deficient $tRNA^{Asn}$ from E. coli. A methionine-requiring *E. coli* mutant 58-161 (rel^-, met^-) was grown in 20 ml of medium A ($Na_2HPO_4 \cdot 12H_2O$, 17.6 g; KH_2PO_4, 3 g; NH_4Cl, 1 g; NaCl, 0.5 g; $MgSO_4$, 0.12 g; glucose, 2 g; and methionine, 0.05 g/l). When the culture reached an absorbance of 0.5 at 540 nm, the cells were harvested by centrifugation, washed twice with medium B (medium A in which phosphate buffer is replaced by 0.1 M Tris-HCl (pH 7.5), containing either 2.5 or 25 µg/ml of methionine). The cells were resuspended in 20 ml of medium B and incubated for 15 min at 37°. Then, 20 mCi of carrier free ^{32}P was added and the cells were grown at 37° for 3 hr. The cells were harvested, washed once with cold medium A and suspended in 1 ml of 0.2 M sodium acetate buffer (pH 6.0)-0.01 M $MgCl_2$. The cell suspension was shaken for one hour at 20° with 1 ml of phenol saturated with water, and then centrifuged. The aqueous phase was loaded on a small column of DEAE-cellulose, which was then washed with 0.02 M Tris-HCl (pH 7.5)-0.01 M $MgCl_2$-0.2 M NaCl. ^{32}P-Labeled tRNA was then eluted from the column with 0.02 M Tris-HCl (pH 7.5)-0.01 M $MgCl_2$-1 M NaCl, and precipitated by adding 2.5 volumes of ethanol.

The ^{32}P-labeled unfractionated tRNA thus obtained (specific activity, 5 x 10^7 cpm/A_{260}) was fractionated by two-dimensional polyacrylamide gel electrophoresis as described by Ikemura and Dahlberg (8). This procedure permitted isolation of $tRNA^{Asn}$ as a pure species.

Separation of oligonucleotides and analysis of nucleotide compositions. ^{32}P-Labeled oligonucleotides in the RNase T_1 digest were separated by two-dimensional PEI-cellulose chromatography as reported by Mirzabekov and Griffin (9). The nucleotide composion of each oligonucleotide was determined by two-dimensional thin-layer chromatography of its RNase T_2 digest (10). Assignment of the nucleotide sequence was done by comparing the results with those obtained previously on the primary structure of *E. coli* $tRNA^{Asn}$ (11).

Isolation of $preQ_1$ on a large scale from unfractionated methyl-deficient tRNA. *E. coli* mutant 58-161 was grown in medium A containing 2.5 µg/ml of methionine in a 600 l tank. The cells were collected in the late logarithmic stage of growth, and tRNA was isolated as described by Zubay, except that 0.05 M sodium acetate (unbuffered) was used instead of Tris buffer for suspension of cells (12). Approximately 100,000 A_{260} units of unfractionated tRNA were obtained. 12,000 A_{260} units of the tRNA were incubated with 500 units of RNase T_2 in 0.05 M potassium acetate buffer (pH 4.7) at 37° for 18 hr. The hydrolyzate was adjusted to pH 7.5 with tris(hydroxymethyl)aminomethane, and fractionated by Dowex 1 column chromatography (x 2; column size, 1 x 50 cm) as described previously (3). The peak of material that contained Qp and preQp together with m^7Gp and mnm^5s^2Up, which was eluted before Cp, was collected and lyophilized, to obtain 40 A_{260} units of nucleotide residue. This material was fractionated by two-dimensional thin-layer chromatography (20 x 20 cm) as described earlier (10). The spot corresponding to $preQ_{1p}$ was cut out, eluted with water, and applied to a column of Dowex 1 (x 2; column size, 0.2 x 5 cm). The column was washed with water, and $preQ_{1p}$ was eluted from the column with 0.02 M formic acid.

Labeling of a methionine-requiring E. coli mutant with ^{14}C-labeled methionine, and isolation of ^{14}C-labeled tRNA and modified nucleosides from it. *E. coli* mutant 58-161 (rel^-, met^-) was grown in 10 ml of medium A. When the absorbance of the culture reached 0.4 at 540 nm, the cells were harvested by centrifugation, and washed twice with medium A from which

methionine was omitted. The cells were resuspended in 10 ml of the same medium and shaken for 15 min at 37°. Then, 50 μCi of L-[1-^{14}C]methionine (specific activity 60 Ci/mole), L-[methyl-^{14}C]methionine (specific activity 55 Ci/mole) or L-[U-^{14}C]methionine (specific activity 260 Ci/mole) was added. The final concentration of methionine was adjusted to 20 μg/ml by adding unlabeled methionine. The cells were grown with vigorous shaking at 37° for 4 hr. Then they were washed with the standard buffer containing 0.01 M Tris HCl (pH 7.5)-0.01 M $MgCl_2$-0.06 M KCl and 0.006 M 2-mercaptoethanol, suspended in 10 ml of the same buffer and shaken with 10 ml of phenol. The aqueous layer was mixed with 10 A_{260} units of cold *E. coli* tRNA and the total tRNA was precipitated by adding 2.5 volume of ethanol. ^{14}C-Labeled tRNA thus obtained was passed through DEAE-cellulose, mixed with 250 A_{260} units of unlabeled *E. coli* tRNA, and hydrolyzed with RNase T_2, and the digest was fractionated by Dowex 1 column chromatography as described previously (3). On this column, the four constituent nucleotides were separated in the order of Cp, Ap, Gp and Up. Qp emerged before Cp; m^2Ap was eluted between Cp and Ap, and acp^{3}Up was eluted immediately after Ap. The fractions containing Qp, m^2Ap and acp^{3}Up, respectively, were collected, evaporated to dryness, and purified further by two-dimensional thin-layer chromatography (10). Spots corresponding to these nucleotides were eluted and their radioactivities and UV absorbances were analyzed.

Materials. L-[1-^{14}C]Methionine (specific activity, 60 Ci/mole) and L-[methyl-^{14}C]methionine (specific activity, 55 Ci/mole) were obtained from the Radiochemical Centre, Amersham, England, and L-[U-^{14}C]methionine (specific activity, 260 Ci/mole) was from New England Nuclear Corp. PEI-cellulose sheets were obtained from Macherey-Nagel Co., Postfach, West Germany. RNase T_1 and T_2 were products of Sankyo Co., Ltd., Tokyo. RNase A (5 x recrystallized) was obtained from Sigma Chemical Company.

RESULTS

Figure 1 shows the autoradiogram obtained after two-dimensional polyacrylamide gel electrophoresis of ^{32}P-labeled *E. coli* methyl-deficient tRNA isolated from cells grown in medium containing 2.5 μg/ml of methionine. The single spot located in the upper left of the autoradiogram was identified as $tRNA^{Asn}$. The spot corresponding to $tRNA^{Asn}$ was separated into two parts, i.e. $tRNA^{Asn}$[A] and $tRNA^{Asn}$[B]. Each was hydrolyzed with RNase T_1 and the digest were fractionated by two-dimensional PEI-cellulose chromato-

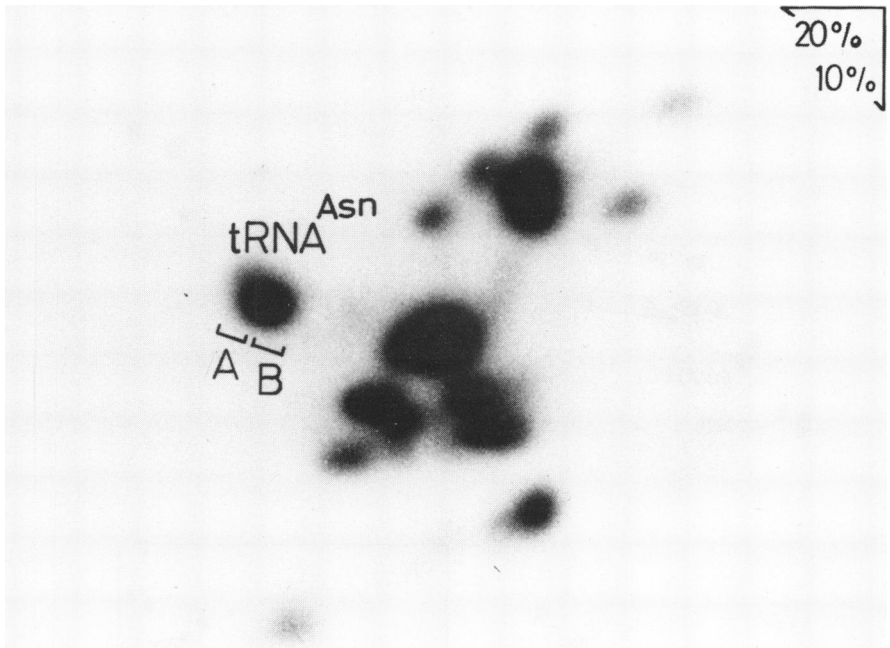

Figure 1. Autoradiogram after two-dimensional polyacrylamide gel electrophoresis of ^{32}P-labeled methyl-deficient tRNA.

graphy. As shown in Figure 2, $tRNA^{Asn}$[A] and $tRNA^{Asn}$[B] gave somewhat different fingerprint patterns. The chromatogram of $tRNA^{Asn}$[A] showed dense spots, designated as 1a and 1b, while that of $tRNA^{Asn}$[B] showed spots 2a and 2b. Analysis of the nucleotide compositions of these oligonucleotides made it possible to assign the sequences shown in Figure 2(d). The materials in spots 1a and 1b are both oligonucleotides derived from the anticodon. Their different chromatographic mobilities are due to the replacement of tA in 1a by atA [tris(hydroxymethyl)aminomethane-containing tA (13)] in 1b. Spots 2a and 2b also contained tA and atA, respectively, indicating that they were also derived from the anticodon region.

The oligonucleotides in spots 1a, 1b, 2a and 2b were further hydrolyzed by RNase A, and the digests were fractionated by two-dimensional PEI-cellulose chromatography. It was found that the materials in spots 1a and 1b both gave the dinucleotides A-Cp and Q-Up, which were both found to be missing from spots 2a and 2b, indicating that the materials in spots 2a and

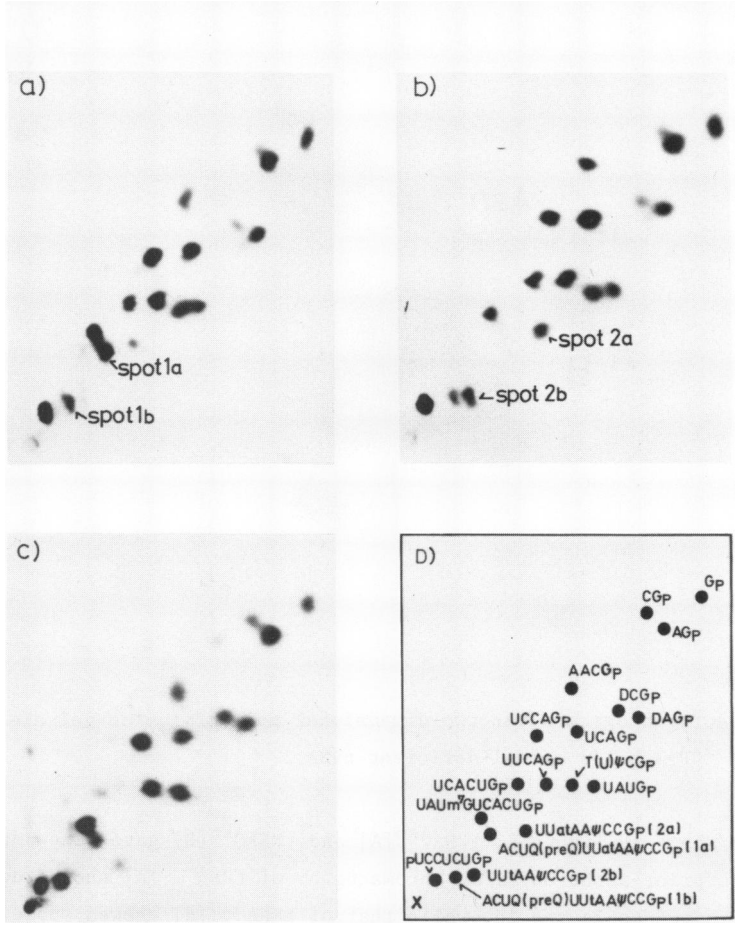

Figure 2. Two-dimensional PEI-cellulose chromatogram of the complete RNase T_1 digestion products of $tRNA^{Asn}$. (a) autoradiogram of methyl-deficient $tRNA^{Asn}$[A]; (b) autoradiogram of methyl-deficient $tRNA^{Asn}$[B]; (c) autoradiogram of $tRNA^{Asn}$ isolated from cells grown with a sub-optimal concentration of methionine; (d) composite tracing of oligonucleotides of RNase T_1 digestion products of $tRNA^{Asn}$. The solvent systems used were: first dimension, 1.4 and 1.8 M lithium formate (pH 3.8)-7 M urea; second dimension, 0.6 M lithium chloride-0.02 M Tris-HCl buffer (pH 8.0)-7 M urea.

2b are shorter oligonucleotides cleaved at the first position of the anticodon, due to replacement of Q by G in $tRNA^{Asn}$[B] (Figure 3). For confirmation of the presence of Q, the Q-Up fractions from the materials in spots 1a and 1b were combined and hydrolyzed completely with RNase T_2, and the digest was analyzed by two-dimensional thin-layer cellulose chromatography

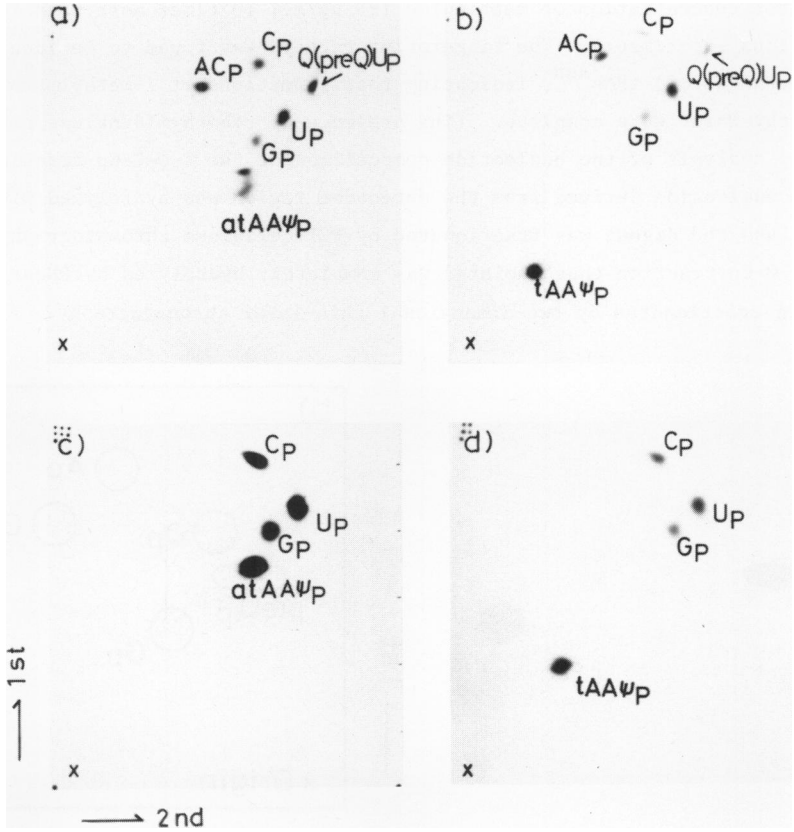

Figure 3. Two-dimensional PEI-cellulose chromatogram of the complete RNase A digestion products of oligonucleotides derived from $tRNA^{Asn}$. (a) autoradiogram of RNase A digests of spot 1a; (b) autoradiogram of RNase A digests of spot 1b; (c) autoradiogram of RNase A digests of spot 2a; (d) autoradiogram of RNase A digests of spot 2b. The solvent systems used were; first dimension, 1.3 M and 1.8 M lithium formate (pH 3.8)-7 M urea; second dimension, 0.5 M lithium chloride-0.02 M Tris-HCl buffer (pH 8.0)-7 M urea.

as described previously (10). Surprisingly, it was found that a derivative of Q, presumably a precursor (designated as $preQ_1$), was present in place of normal Q in the Q-Up fraction (Figure 4). This $preQ_1$ nucleoside appears to have a positive charge, like Q, since $preQ_1$-Up behaved identically with Q-Up on two-dimensional PEI-cellulose chromatography.

Figure 2 (c) shows a chromatogram of the RNase T_1 digest of methyl-deficient $tRNA^{Asn}$ prepared from *E. coli* cells grown in the presence of a sub-optimal concentration of methionine (25 µg/ml, 10 times more than in the previous experiment). The fingerprint pattern was found to be identical with that of normal $tRNA^{Asn}$, indicating that formations of 7-methylguanosine and ribothymidine were complete. (The presence of ribothymidine was confirmed by analysis of the nucleotide composition of the T-ψ-C-Gp fraction). The oligonucleotide derived from the anticodon region was hydrolyzed with RNase A, and the digest was fractionated by PEI-cellulose chromatography. Then the Q-Up fraction thus isolated was completely hydrolyzed by RNase T_2 and again fractionated by two-dimensional thin-layer chromatography. As

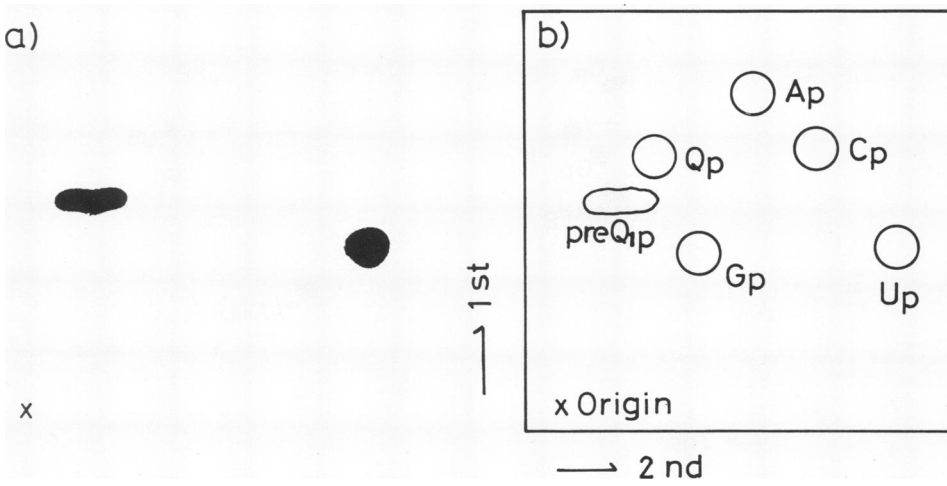

Figure 4. Two-dimensional thin-layer cellulose chromatogram of the RNase T_2 digestion products of Q(preQ)Up separated by PEI-cellulose chromatography as shown in Figure 3. (a) autoradiogram of the RNAase T_2 digest; (b) composite tracing of (a) in relation to the locations of the four normal nucleotides. The solvent systems used were: first dimension, isobutyric acid-0.5 M NH_4OH (5:3, v/v); second dimension, 2-propanol-concentrated HCl-water (70:15:15, v/v/v).

shown in Figure 5, it was found that two other derivatives of Q, designated as $preQ_2$ and $preQ_3$, were present in addition to $preQ_1$ and Q.

In order to measure the UV spectra of $preQ_1$, a large amount of $preQ_{1p}$ was isolated from unlabeled methyl-deficient *E. coli* tRNA by Dowex 1 column chromatography (see Materials and Methods section). The $PreQ_{1p}$ thus isolated gave identical UV spectra to those of normal Q nucleotide, as shown in Figure 6. This strongly suggests that formation of the 7-deazaguanosine nucleus and attachment of a side chain at the C-7 position are already complete in the $preQ_1$ molecule.

Goodman *et al.* previously reported that the label from [methyl-^{14}C]-methionine was not incorporated into Q nucleoside in *E. coli* $tRNA^{Tyr}$ (14). Katze *et al.* also recently showed that incorporation of [2-^{14}C]methionine or [methyl-^{14}C]methionine into growing cells of *E. coli* did not result in labeling of nucleoside Q (15). It is, however, possible that no normal Q nucleosides are synthesized under their growth conditions (<2.5 µg L-methionine/ml). In order to clarify this issue, a methionine-requiring *E. coli* mutant was grown in the presence of either [methyl-^{14}C]-, [1-^{14}C]- or [U-^{14}C]methionine at a concentration of 20 µg/ml; [1-^{14}C]- and [U-^{14}C]-

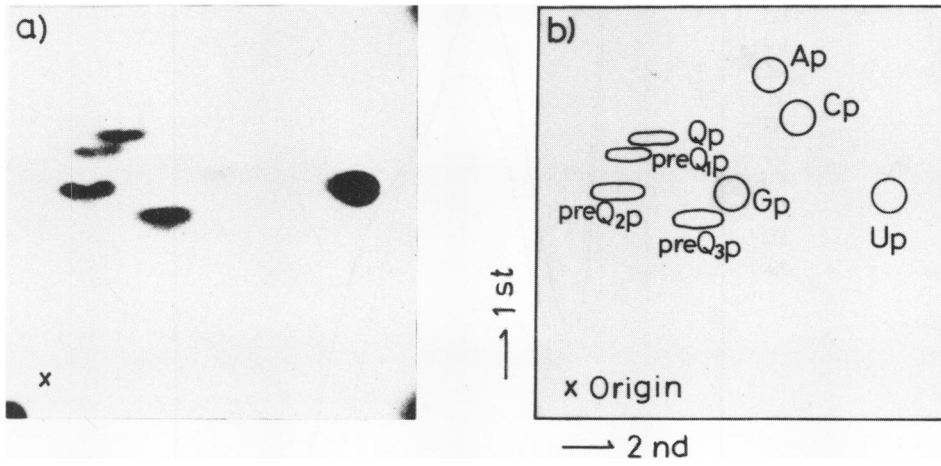

Figure 5. Two-dimensional thin-layer cellulose chromatogram of the RNase T_2 digestion products of Q(preQ)Up derived from $tRNA^{Asn}$ grown with a sub-optimal concentration of methionine. (a) autoradiogram of the RNase T_2 digest; (b) composite tracing of (a) in relation to the locations of the four normal nucleotides. The solvent systems used were as described for Figure 4.

methionine were used to check whether the 3-amino-3-carboxypropyl group of methionine is utilized in biosynthesis of Q nucleoside, as in the case of 3-(3-amino-3-carboxypropyl)uridine (acp^3U) in tRNA (16). ^{14}C-Labeled tRNAs were isolated from each suspension, and the radioactivities incorporated into Q, m^2A and acp^3U were measured. As shown in Table I, no radioactivity was incorporated into Q or precursor Q nucleosides, but m^2A and acp^3U were labeled appreciably, labeling depending on the type of ^{14}C-methionine. Thus it is concluded that no carbon unit of methionine is directly utilized for biosynthesis of the Q nucleoside.

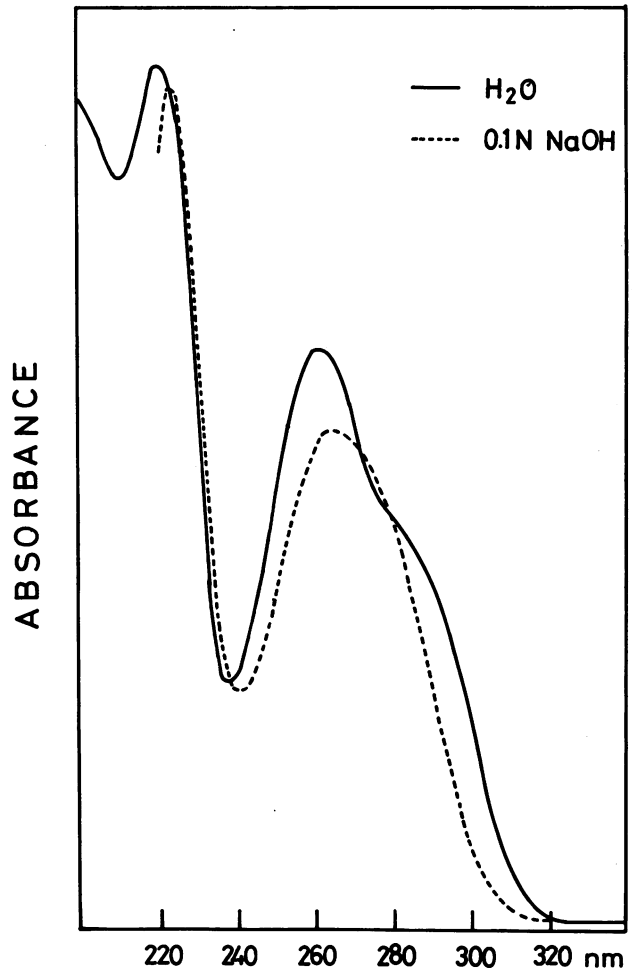

Figure 6. Ultraviolet absorption spectra of $preQ_1p$ (———) pH 6; (-----) pH 12.

Table I. No incorporation of radioactivity into Q and Q precursors from ^{14}C-methionine.

	Total radioactivity recovered (cpm)		
	[methyl-^{14}C]-methionine	[1-^{14}C]-methionine	[U-^{14}C]-methionine
Qp	ND	ND	ND
preQ$_1$p	ND	ND	ND
preQ$_2$p	ND	ND	ND
preQ$_3$p	ND	ND	ND
acp^{3}Up	ND	1.7×10^4	1.3×10^4
m^{2}Ap	9.6×10^3	ND	6.5×10^3

ND, not detected

DISCUSSION

Nucleotide sequence analysis of ^{32}P-labeled *E. coli* methyl-deficient tRNA^{Asn} clearly demonstrates that this tRNA^{Asn} lacks the Q nucleoside, as previously suggested by Katze (4). In addition, the most notable finding in this work was the detection of three derivatives of Q, as well as guanosine, in the first position of the anticodon where Q nucleoside is present in normal tRNA. These derivatives are presumably precursors of Q, and we named them preQ$_1$, preQ$_2$ and preQ$_3$, respectively. PreQ$_1$ accumulates when *E. coli* cells are grown in severely methionine-deficient conditions. On the other hand, preQ$_2$ and preQ$_3$ accumulate in cells grown in the presence of a sub-optimal concentration of methionine; under these conditions, normal Q is also synthesized. Therefore, it is likely that the biosynthetic route for Q in tRNA is G→preQ$_1$→(preQ$_2$, preQ$_3$)→Q.

The UV spectra of preQ$_1$ are identical with those of Q. Therefore, formation of a 7-deazaguanosine moiety with attachment of a side chain at the C-7 position should be complete in the preQ$_1$ molecule. However, further work is required on the exact structure of preQ$_1$.

We have previously shown that a guanine insertion reaction in the rabbit reticulocyte system that was discovered by Farkas (5) is in fact an exchange reaction between guanine and either Q base or guanine in the first anticodon position of tRNA^{Tyr}, tRNA^{His}, tRNA^{Asn} and tRNA^{Asp} (6). Contrary to the rabbit reticulocyte enzyme system, the corresponding enzyme isolated from *E. coli* only catalyzed exchange of guanine with guanine in the same location (7). We presume that this guanine insertion enzyme is involved in the biosynthesis of Q. Unfractionated *E. coli* methyl-deficient tRNA was found to be an acceptor of guanine for the *E. coli* guanine insertion enzyme

(7). It was found that the species accepting guanine in this preparation is tRNA having guanosine, but not preQ, in the anticodon, as shown by the following experiment. ^{32}P-Labeled methyl-deficient $tRNA^{Asn}$ was incubated with the guanine insertion enzyme and cold guanine. After incubation, the amounts of $preQ_1p$, $preQ_2p$ and $preQ_3p$ in the tRNA were analyzed. Results showed that the amounts of the Q precursors had not diminished during the incubation (7). Our hypothesis is that the base of $preQ_1$ or its precursor is synthesized without participation of tRNA, and is then inserted into tRNA, by the guanine insertion enzyme to replace guanine base. Then the $preQ_1$ incorporated into tRNA is converted to Q, *via* $preQ_2$ and $preQ_3$. The guanine insertion enzyme may not catalyze the reverse reaction (i.e. conversion of Q base or preQ to guanine).

It was previously shown that during biosynthesis of Q, the carbon atom at position 8 in the precursor molecule guanine was expelled in a fashion similar to that in the biosynthesis of the nucleoside antibiotic toyocamycin (17). Elstner and Suhadolnik proposed that GTP is an intermediate in the synthesis of toyocamycin (18). It is probable that the Q precursor is also synthesized from GTP. No radioactivity was incorporated into Q nucleoside from either [methyl-^{14}C]-, [1-^{14}C]- or [U-^{14}C]methionine, indicating that no carbon from methionine is directly utilized for the synthesis of Q. Methionine may be involved in the enzymatic reaction by which $preQ_2$ and $preQ_3$ are converted to Q. Kitchingman and Fournier reported that leucine starvation in the growing *E. coli* mutant (rel^-, leu^-) resulted in formation of modification-deficient $tRNA^{Leu}$ in which pseudouridine and dihydrouridine are replaced by uridine (19). The effect of methionine on the biosynthesis of Q may be similar to that reported by Kitchingman and Fournier. Formation of normal Q nucleoside was incomplete in $tRNA^{Asn}$ isolated from *E. coli* cells grown with a sub-optimal concentration of methionine, whereas formation of methylated nucleosides, such as ribothymidine and 7-methylguanosine, in the tRNA was complete. Thus it seems that biosynthesis of the final Q structure requires a higher concentration of methionine than that necessary for the synthesis of methylated nucleotides. This result also implies that inhibition of the biosynthesis of Q by methionine starvation is not due to the absence of other methylated nucleosides in the tRNA. We have recently isolated an *E. coli* mutant which accumulates a large amount of $preQ_1$ together with a small amount of Q in tRNA but shows normal methylation (20). This mutant should be useful for further investigations on the biosynthesis of the Q nucleoside.

ACKNOWLEDGMENTS

We are indebted to Dr. F. Harada of this laboratory for analysis of oligonucleotides by PEI-cellulose chromatography. We thank Dr. Katze for showing us his manuscript before its publication. This work was partly supported by Grants-in-Aid for Cancer Research and Scientific Research from the Ministry of Education, Science and Culture, Japan.

The abbreviations used are: Q, 7-(4,5-*cis*-dihydroxy-1-cyclopenten-3-ylaminomethyl)-7-deazaguanosine; acp^3U, 3-(3-amino-3-carboxypropyl)uridine; tA, N-[9-(β-D-ribofuranosyl)purin-6-ylcarbamoyl]threonine; atA, N-[N-[(9-β-D-ribofuranosylpurin-6-yl)carbamoyl]threonyl]-2-amido-2-hydroxymethylpropane-1,3-diol; m^2A, 2-methyladenosine; T, ribothymidine.

REFERENCES

1. Harada, F. and Nishimura, S. (1972) *Biochemistry 11,* 301-308.
2. Kasai, H., Ohashi, Z., Harada, F., Nishimura, S., Oppenheimer, N. J., Crain, P. F., Liehr, J. C., von Minden, D. L. and McCloskey, J. A. (1975) *Biochemistry 14,* 4198-4208.
3. Kasai, H., Kuchino, Y., Nihei, K. and Nishimura, S. (1975) *Nucl. Acids Res. 2,* 1931-1939.
4. Katze, J. R. and Mosteller, R. D. (1976) *J. Bacteriol. 125,* 205-210.
5. Farkas, W. R. and Singh, R. (1973) *J. Biol. Chem. 248,* 7780-7785.
6. Okada, N., Harada, F. and Nishimura, S. (1976) *Nucl. Acids Res. 3,* 2593-2603.
7. Okada, N. and Nishimura, S., manuscript in preparation.
8. Ikemura, T. and Dahlberg, J. E. (1973) *J. Biol. Chem. 248,* 5024-5032.
9. Mirzabekov, A. D. and Griffin, B. E. (1972) *J. Mol. Biol. 72,* 633-643.
10. Nishimura, S. (1972) *Progr. Nucl. Acid Res. Mol. Biol. 12,* 49-85.
11. Ohashi, K., Harada, F., Ohashi, Z., Nishimura, S., Stewart, T. S., Vögeli, G., McCutchan, T. and Söll, D. (1976) *Nucl. Acids Res. 3,* 3369-3376.
12. Zubay, G. (1962) *J. Mol. Biol. 4,* 347-356.
13. Kasai, H., Murao, K., Nishimura, S., Liehr, G., Crain, P. F. and McCloskey, J. A. (1976) *Eur. J. Biochem. 69,* 435-444.
14. Goodman, H. M., Abelson, J. N., Landy, A., Zadrazil, S. and Smith, J. D. (1970) *Eur. J. Biochem. 13,* 461-483.
15. Katze, J. R., Simonian, M. H. and Mosteller, R. D., *J. Bacteriol.,* submitted.
16. Nishimura, S., Taya, Y., Kuchino, Y. and Ohashi, Z. (1974) *Nucl. Acids Res. 57,* 702-708.
17. Kuchino, Y., Kasai, H., Nihei, K. and Nishimura, S. (1976) *Nucl. Acids Res. 3,* 393-398.
18. Elstner, E. F. and Suhadolnik, R. J. (1971) *J. Biol. Chem. 246,* 6973-6981.
19. Kitchingman, G. R. and Fournier, M. J. (1976) *Biochem. Biophys. Res. Commun. 73,* 314-322.
20. Noguchi, S. and Nishimura, S., unpublished results.