Structure determination of a nucleoside Q precursor isolated from E. coli tRNA: 7-(aminomethyl)-7-deazaguanosine

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ABSTRACT

A precursor of modified nucleoside Q isolated from E. coli methyl-deficient tRNA was determined to be 7-(aminomethyl)-7-deazaguanosine. The structure was deduced by means of its chromatographic and electrophoretic mobilities, and UV and mass spectra, in addition to comparison with the synthesized authentic compound. The same molecule is also found in tRNA of an E. coli mutant selected for deficient synthesis of modified nucleosides.

INTRODUCTION

The structure of the modified nucleoside Q is unique with regard to components of tRNA because of replacement of N-7 by carbon, and because of the unusual side chain attached at that position. It is therefore of interest to elucidate the biosynthetic pathways through which these complex modifications are introduced. In this regard we have earlier shown that several precursors of the Q nucleoside accumulate in methyl-deficient E. coli tRNA. One of these compounds (hereafter designated preQ) has now been isolated in large quantity. Its UV spectra were found to be identical with those of Q nucleoside, indicating that formation of the 7-deazaguanine moiety has been completed in the preQ molecule. In the present communication we report the structure of preQ as 7-(aminomethyl)-7-deazaguanosine (compound I), deduced mainly by mass spectrometry and by comparison with the chemically synthesized...
The same precursor is also found in an E. coli mutant which was selected for its deficiency in synthesis of Q nucleoside.

**MATERIALS AND METHODS**

Selection of an E. coli Mutant which Accumulates Q Precursor (preQ₃) in tRNA. An E. coli K-12 strain (PA3092) was treated with N-methyl-N'-(nitro-nitrosoguanidine (NMNG); the ts mutants for growth were isolated as previously described and kindly provided by Dr. Y. Hirota of the National Institute of Genetics, Nishina, Japan. Cells were grown at 30° in L-broth supplemented with 50 μg/ml of thymine. Two hundred μCi of ³²P was added to 5 ml of the medium after the temperature was shifted to 42°, and the cells were grown for 1 hr. Transfer RNA isolated from each cell culture was hydrolyzed by RNase T₂, and the digests were analyzed by two-dimensional thin-layer chromatography using 20 x 20 cm plates for detection of modified nucleosides. It was found that tRNA isolated from one of the mutants (JE10651) showed almost complete replacement of nucleoside Q by a derivative of Q (preQ₃), which behaves identically with preQ₃ isolated from methyl-deficient tRNA. This Q precursor also accumulated in tRNA when the cells were grown at 30°. It should be noted that the growth rate of the E. coli mutant was approximately half that of the parent strain PA3092, which suggests that the presence of mature Q in tRNA is essential for normal cell growth. However, additional genetic studies are necessary to confirm this conclusion, because multiple mutations may have been caused in the cells by NMNG.

Isolation of preQ₃ from the E. coli Mutant. E. coli mutant JE10651 was grown in 500 l of L-broth in a 1000 l tank at 37°. 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-HCl buffer for cell suspension. Approximately 80,000 A₂₆₀ units of unfractionated tRNA were obtained, of which 10,000 A₂₆₀ units were hydrolyzed by incubation with 500 units of RNase T₂ in 0.05 M potassium acetate buffer (pH 4.7) at 37° for 18 hrs. The hydrolysate was fractionated by Dowex 1 column chromatography as described earlier. The fraction which contains the 3'-nucleotide of Q precursor (preQ₃ mp), and which elutes before the Cp fraction, was collected and lyophilized. The residue was dissolved in a small volume of water, applied to Whatman No. 3MM paper (40 x 40 cm), and subjected to two-dimensional paper chromatography. The spot corresponding to preQ₃ mp was cut out, eluted with water, and applied to a column of Dowex 1 (x2 0.2 x 5 cm). The column was washed with water, and preQ₃ mp was eluted from the column with...
0.02 M formic acid. PreQ,mp thus isolated (9.1 A260 units) was treated with
0.4 unit of E. coli alkaline phosphatase in 0.15 ml of 0.02 M triethylammonium
bicarbonate buffer (pH 7.5) for 16 hrs. The nucleoside preQ,m thus obtained
was finally purified by paper chromatography using Whatman No. 40 paper with a
solvent system of isobutyric acid - 0.5 M ammonium hydroxide (5:3, v/v).

Isolation of preQ, from Unfractionated Methyl-Deficient tRNA. E. coli
mutant 58-161 (rel', met') was grown under methyl-deficient conditions as pre-
viously described2. Transfer RNA was isolated and preQ1 was isolated from it
by the same procedure described in the preceeding section.

Mass Spectrometry. Mass spectral data were obtained from preQ1, preQ,m,
and the synthesized authentic compound after conversion to their volatile tri-
methylsilyl derivatives7. A typical derivatization reaction involved treat-
ment of 0.1 - 0.2 A260 units with N2O-bis(trimethylsilyltrifluoroacetamide) *
1 % trimethylchlorosilane (Regisil RC-2, Regis Chemical Co., Morton Grove,
Ill.) and dry pyridine (9:1), followed by heating in a sealed capillary tube
for 1 hr at 75°. Low-resolution mass spectra were obtained on an LKB 9000S
gas chromatograph-mass spectrometer (70 eV, ion source and separator tempera-
tures 270°) interfaced to a DEC PDP 11/40 computer8; samples were introduced
through the gas chromatograph (6' x 6' 1 % SE-30, temperature-programmed at
5°/min from 185°). High resolution mass spectra were photographically recor-
ded using a Varian MAT 731 instrument (70eV, ion source temperature 220°, reso-
lution 18,000). Photoplates were obtained from Ionomet Co., Waban, Mass.

Chemical Synthesis of 7-(Aminomethyl)-7-deazaguanosine. 7-(Aminomethyl)-
7-deazaguanosine (I) was chemically synthesized by the route shown in Fig. 1.
Compound III, synthesized from II, was treated with NaN3 and then acetylated
to give IV. Ammonolysis of IV yielded V, which was then converted to VI by
catalytic hydrogenation. Compound VI was hydrolyzed with hydrochloric acid to
furnish I. These procedures will be published in detail elsewhere.

RESULTS AND DISCUSSION

Comparison of Properties of preQ, with Authentic 7-(Aminomethyl)-7-
deazaguanosine. In a previous study it was found that the UV spectra of preQ1
were identical with those of normal Q nucleoside, indicating that the 7-
deazaguanine base is already formed in the preQ1 molecule2. Elution behavior
of preQ, upon Dowex 1 column chromatography indicates that one positive charge
is present, as in the case of normal Q, which suggests that preQ1 bears a pri-
mary or secondary amino function2. In addition, initial mass spectrometry
experiments on preQ1 as its trimethylsilyl derivative indicated a molecular
weight of 743 (addition of six trimethylsilyl residues), corresponding to a value of 311 for the free nucleoside. These data suggested that the structure of preQ₁ is 7-(aminomethyl)-7-deazaguanosine (I). In order to unambiguously characterize the structure of preQ₁, the authentic compound (I) was chemically synthesized according to the synthetic route illustrated in Fig. 1, in order to compare its properties with those of preQ₁.

Table 1 shows a comparison of thin-layer mobilities of preQ₁ with those of authentic 7-(aminomethyl)-7-deazaguanosine, using four different solvent systems. Both compounds behaved identically in all solvent systems tested, strongly supporting the structure of preQ₁ as I.

UV spectra and electrophoretic mobility (at pH 3.5) of synthetic 7-(aminomethyl)-7-deazaguanosine were also found to be identical with those of preQ₁ (data not shown).

Isolation of preQ₁ from an E. coli Mutant. As described in the Materials
Table 1. Comparison of thin-layer chromatographic mobilities of \(\text{preQ}_1\) and synthetic 7-(aminomethyl)-7-deazaguanosine

<table>
<thead>
<tr>
<th>Nucleoside</th>
<th>(R_f) in Solvent System</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>preQ₁</td>
<td>0.73</td>
</tr>
<tr>
<td>Authentic 7-(aminomethyl)-7-deaza-</td>
<td>0.73</td>
</tr>
<tr>
<td>guanosine</td>
<td>Adenosine</td>
</tr>
<tr>
<td>Uridine</td>
<td>0.64</td>
</tr>
<tr>
<td>Guanosine</td>
<td>0.62</td>
</tr>
<tr>
<td>Cytidine</td>
<td>0.75</td>
</tr>
</tbody>
</table>

Solvent systems used are:
- Solvent A; isobutyric acid - 0.5 \(\text{NH}_4\)OH (5:3)
- Solvent B; isopropanol - conc. \(\text{HCl}\) - \(\text{H}_2\text{O}\) (70:15:15)
- Solvent C; n-butanol - acetic acid - \(\text{H}_2\text{O}\) (4:1:2)
- Solvent D; n-propanol - conc. \(\text{NH}_4\)OH - \(\text{H}_2\text{O}\) (55:10:35)

and Methods section, \(^{32}\text{P}\)-labeled tRNA was prepared from cells of \(\text{E. coli}\) mutant JE10651. Fig. 2 shows the radioautogram from the thin-layer chromatogram of \(^{32}\text{P}\)-labeled tRNA; a spot corresponding to \(\text{preQ}_1\)-mp is clearly seen while normal Q is missing. It should be noted that other modified nucleosides such as dihydrouridine (D), pseudouridine (\(\text{\textsuperscript{4}\text{U}}\)), 4-thiouridine (s\(\text{\textsuperscript{4}\text{U}}\)), ribothymidine (T), N\(^6\)-methyladenosine (m\(^6\)A), 2-methyladenosine (m\(^2\)A), 2-methylthio-N\(^6\)-isopentenyladenosine (ms\(^2\)i\(\text{\textsuperscript{6}\text{A}}\)), and 2'-O-methylguanosine (Gm) are present as in wild-type \(\text{E. coli}\), indicating that the mutation specifically blocks formation of mature Q nucleoside. Since various methylated nucleosides are synthesized in the mutant, it is evident that accumulation of the Q precursor is not due to block of production of methionine in the cells.

In order to confirm whether the Q precursor accumulating in the mutant is the same compound as \(\text{preQ}_1\) present in methyl-deficient tRNA, it was isolated from the mutant on a large scale, as described in Materials and Methods, for further characterization. Its UV spectra were found to be identical with those of authentic 7-(aminomethyl)-7-deazaguanosine. For final characterization, mass spectrometric analysis was employed as described in the following section.

**Mass Spectrometry of \(\text{preQ}_1\).** Unambiguous structural characterization of \(\text{preQ}_1\) from methyl-deficient tRNA, and of \(\text{preQ}_1\)-m from tRNA of the mutant
Figure 2. Autoradiogram from thin-layer chromatograms of an RNase T2 digest of 32p-labeled tRNA from the E. coli mutant JE10651 (left) and parent strain K-12, PA3092 (right). JE10651, was made by means of low- and high-resolution mass spectrometry. Both precursor compounds as well as synthetic 7-(aminomethyl)-7-deazaguanosine were examined as their trimethylsilyl derivatives.

Mass spectrometry of derivatized preQ₁ showed a molecular weight of 743 (Figure 3). Repetitive scanning techniques and the resulting mass chromatograms based on m/e 743 were used to insure acquisition of a mass spectrum free of significant peaks from other components, particularly in and above the molecular ion region. Measurements of exact mass (Figure 3) and use of deuterium-labeled silyl groups [Si(CH₃)₅]₃ showed incorporation of 3 silyl groups in the base, 3 in the sugar, and a molecular composition of C₃₀H₆₅N₅O₁₆Si₅ (calc. 743.3601). The elemental composition of native preQ₁ is therefore C₁₂H₁₇N₅O₅ (mol. wt. 311), corresponding to structure I.

The unusual intensity ratio M > M - CH₃ (743 > 728), although exhibited by other nucleosides as well, is characteristic of guanosine derivatives, including the Q nucleoside. Ions of mass 742 and 394 are attributed to loss of hydrogen from the side chain amino function with subsequent stabilization by O, as similarly proposed in the case of Q. Other ions in the upper mass region are due to small amounts of the pentasilyl derivative (M = 671, M - CH₃ = 656 in part; see below). Peaks at m/e 395, 323 (base + H from the lower derivative), 424 (base + CH₂O) and 510 (base + C₅H₁₂OSi) represent the typical ion series composed of the intact base moiety and certain portions of the sugar. Peaks of m/e 307, 421 and (in part) 656 are characteristic of the Q base and are derived from cleavage of the side chain C₅N bond. The structure of the m/e 656 ion is similar to that derived from Q but its mechanism of formation may differ. The common series of sugar ions of masses 349 (intact
Figure 3. Mass spectrum of the trimethylsilyl derivative of PreQ isolated from E. coli methyl-deficient tRNA.

sugar fragment), 259, 217 and 103 denote an unmodified ribose moiety, while m/e 73 and 147 are common silyl ions and bear no structural information in the present case.

The mass spectrum shown in Figure 3 was found to correspond to that from synthetic 7-(aminomethyl)-7-deazaguanosine, including molecular mass (fd. for synthetic compound, 743.3623), as well as to the spectrum of preQ,m d., 743.3580). The structures of modified nucleosides preQ and preQ,m are thus firmly established as I.

SUMMARY

The present work shows the major precursor of nucleoside Q, isolated from both methyl-deficient tRNA and tRNA isolated from a mutant unable to fully synthesize Q nucleoside, is 7-(aminomethyl)-7-deazaguanosine. We have previously shown that two additional Q precursors, designated preQ, and preQ, accumulate in methyl-deficient tRNA. These compounds appear to be intermediates between mature Q and preQ. It can be reasonably concluded that the cyclopentenediol moiety in Q is attached to 7-(aminomethyl)-7-deazaguanosine in tRNA through a multi-step process. The characterization of preQ and consequent isolation of mutants of E. coli which accumulate additional precursors should be beneficial to further studies of the biosynthesis of Q nucleoside.

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