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NUCLEOSIDE Q IN tRNA OF YELLOW LUPIN (*LUPINUS LUTEUS*) SEEDS *

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The nucleoside Q, 7-(4,5-cis-dihydroxy-1-cyclopenten-3-yl-trans-aminomethyl)-7-deazaguanosine, was found in lupin seed tRNA.

The nucleoside occurs in only one of the two histidine tRNA species, as proved by their chromatographic properties (RPC-5) following treatment with cyanogen bromide or periodate, and dihydroxyborylphenyl-succinamyl-aminoethyl-cellulose chromatography.

Hypermodified nucleoside 7-(4,5-cis-dihydroxy-1-cyclopenten-3-yl-trans-aminomethyl)-7-deazaguanosine (nucleoside Q1) and its two known glycosyl derivatives (nucleosides Q*) with mannose or galactose units linked to position 4 of cyclopentene diol moiety of Q, occur in some isoacceptor tRNAs, recognizing XA11 codons, *i.e.* tRNA^{Tyr}, tRNA^{His}, tRNA^{Asp} and tRNA^{Asn}. The content of these nucleosides varies in populations of tRNAs of different origin, and according to Kasai *et al.* (1975) ranges from zero (*e.g.* in yeast and *Thermus thermophilus* tRNA) to 0.18% (in the rat, Doryn strain, ascites hepatoma tRNA).

Unfractionated tRNA of wheat germ, the only higher plant in which nucleoside Q was found, contains about 0.06% of Q. This prompted us to attempt detection and a possible determination of the nucleoside Q-family in tRNA of yellow lupin seeds studied in this laboratory. It was shown that tRNA of this dicotyledon plant contains a very small amount of Q, probably present only in one of the two isoacceptor histidine tRNAs.

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1 Nucleoside Q is also called queuosine and the Q base queuine.
MATERIALS AND METHODS

General. Adogen 464, DEAE-cellulose DE52, acetylated DEAE-cellulose were purchased from Serva (Heidelberg, F.R.G.). Plaskon CTFE powder was from Allied Chemical Co. (Morristown, N.J., U.S.A.), concanavaline A-Sepharose from Pharmacia (Uppsala, Sweden), Dowex AG-1-X8 and AG 50W-X4 were from Bio-Rad Labor. (Richmond, Calif., U.S.A.), TLC-cellulose plates from Merck (Darmstadt, F.R.G.), tRNA from E.coli and sRNA from baker's yeast were from Calbiochem (San Diego, Calif., U.S.A.).

Radioactive amino acids: [3H]tyrosine (41 Ci/mmol), [14C]asparagine acid (216 mCi/mmol), [3H]aspartic acid (300 mCi/mmol), [14C]asparagine (84 mCi/mmol), [3H]histidine (47 Ci/mmol) were from the Radiochemical Centre (Amersham, England), and [14C]tyrosine (33 mCi/mmol), [14C]histidine (210 mCi/mmol), [14C]valine (175 mCi/mmol), [14C]phenylalanine (317 mCi/mmol) from the Institute for Research, Production and Application of Radioisotopes (Prague, Czechoslovakia).

All other chemicals were reagent grade products from POCh (Gliwice, Poland). Lupinus luteus seeds var. Express were obtained from Seed Companies in Poland.

Isolation of tRNA and preparation of aminoacyl-tRNA-synthetases. Crude tRNA from lupin seeds was isolated as previously described (Legocki et al., 1967; Augustyniak et al., 1974). Wheat germ tRNA was prepared by Zubay's procedure (Zubay, 1962). Preparations of crude aminoacyl-tRNA synthetases from L.luteus seeds and from wheat germ were isolated as described by Jakubowski & Pawelkiewicz (1973).

Aminoacylation of tRNA. The reaction mixture contained in the total volume of 75 μl: Hapes, 7.5 μmol, pH 8.0; MgCl₂, 0.75 μmol; ATP, 0.075 μmol; 2-mercaptoethanol, 0.6 μmol; EDTA, 0.012 μmol; BSA, 5 μg; 14C-or 3H-labelled amino acid (2-5 nmol); 0.5-2 A₂₆₀ units of tRNA, and the enzyme (1-5 μg of purified synthetases, or 0.1 mg of crude synthetase preparations). Histidine tRNA was also aminoacylated using the heterologous enzyme from wheat germ in the following reaction mixture (75 μl): Hapes, 7.5 μmol, pH 7.5; MgCl₂, 0.75 μmol; ATP, 0.225 μmol; 2-mercaptoethanol, 0.4 μmol; KCl, 0.75 μmol; EDTA, 0.0125 μmol; [14C]histidine, 2 nmol; 0.05-2 A₂₆₀ units of tRNA, and about 0.2 mg of the crude enzyme preparation. The mixture was incubated for 15 min at 25°C, in the presence of 19 unlabelled amino acids (all amino acids were at the concentration of 10 μm). The amount of aminoacyl-tRNA synthesized was estimated by the filter paper disc technique of Mans & Novelli (1961).

Preparative aminoacylation for reversed-phase chromatography. This was performed by scaling up 10-20-fold the volume of the aminoacylation incubation mixtures. After incubation, radioactive aminoacyl-tRNA was recovered on a small DEAE-cellulose column and precipitated by ethanol.
Periodate modification of aminoacyl-tRNA. Aminoacyl-tRNA was modified by treatment with NaIO₄ essentially as reported by Farkas & Chernoff (1976). The ³H- or ¹⁴C-labelled aminoacyl-tRNAs were dissolved in 0.1 M-sodium acetate, pH 5. Then 0.1 vol. of 0.1 M-NaIO₄ was added and the mixture left for 30 min at 20°C in the dark. The IO₄⁻ reaction was stopped by the addition of ethylene glycol and the labelled aminoacyl-tRNA was recovered on a DEAE-cellulose column (0.6 × 4 cm). Low-molecular-weight impurities were removed with 10 mm-sodium acetate buffer, pH 4.5, containing 0.3 M-NaCl, 10 mm-MgCl₂ and 1 mm-EDTA. Aminoacyl-tRNA was eluted with 0.8 M-NaCl dissolved in the same buffer, and precipitated with cold ethanol or diluted with an equal volume of RPC-5 buffer in the case of immediate RPC-5 chromatography.

Cyanogen bromide treatment of tRNA. Crude tRNA was treated with BrCN as described by Katze (1975). tRNA, 20 mg, was dissolved in 9 ml of 0.1 M-NaHCO₃-NaCO₃ buffer, pH 8.9. To this solution 2.5 mg of BrCN was added (in 1 ml of the same buffer). After 10 min the tRNA was precipitated with ethanol, collected by centrifugation, dissolved in 1 M-potassium acetate buffer, pH 4.5, and reprecipitated with ethanol.

Reversed-phase chromatography. The RPC-5 chromatography system of Pearson et al. (1971) was used. The 1 × 50 cm column was developed at room temperature with a linear salt gradient containing 10 mm-sodium acetate buffer, pH 4.8, 10 mm-MgCl₂ and 1 mm-EDTA, at a flow rate of 3 ml per 2 min per fraction. Concentration of the gradient solution was determined by conductivity measurement. Radioactivity of the fractions was determined by the addition of 10 vol. of Tritosol scintillator solution, and counted in scintillation counter.

Chromatography of Asp-tRNA on concanavalin A-Sepharose. This was carried out as described by Wosnick & White (1978). For analytical purposes, 10 A₂₆₀ units of [¹⁴C]Asp-tRNA was applied onto a column (0.8 × 18 cm) of Con A-Sepharose that had been equilibrated with buffer A (0.05 M-sodium acetate, pH 6, 0.15 M-NaCl, 1 mm-MnCl₂, 1 mm-CaCl₂ and 5 mm-MgCl₂). The unbound tRNA was eluted with the same buffer at a flow rate of 3 ml per hour. The bound tRNA was recovered by elution with buffer A containing 50 mm-α-methylmannoside.

Acylated DBAE-cellulose chromatography. Unfractionated tRNA was chromatographed at room temperature on acylated DBAE-cellulose essentially as reported by McCutchan et al. (1975). Crude tRNA, 50 A₂₆₀ units, was applied onto a 0.8 × 10 cm column in the buffer containing 1 M-NaCl, 0.1 M-MgCl₂ and 0.05 M-morpholine, pH 8.7, then washed with the same buffer to elute the non-adsorbed tRNA; next the Q-containing tRNAs were eluted with 0.2 M-NH₄Cl [instead of 0.2 M-NaCl and 0.05 M-acetate buffer, used by McCutchan et al. (1975)]. The flow rate was 4 ml per 20 min. The peak fractions were pooled as indicated by absorbance at 254 nm; the first peak fraction was concentrated directly by ethanol
precipitation, after adjusting the pH to 4.5. To the second combined fraction, carrier yeast RNA was added (2 A_260 units/ml) and RNA was then precipitated with ethanol. Specific aminoacylation activities of both preparations were determined. The activity of the second fraction was calculated on the basis of its absorbance measured before the addition of carrier RNA.

Isolation of Qp from alkaline hydrolysates of tRNA. The method described by Kasai et al. (1975) was used. Unfractionated tRNA (0.02 - 1 g) was hydrolysed in 0.3 m-KOH at 37°C for 18 h. Then pH of the hydrolysate was adjusted to 8 by adding Dowex 50 (H^+ form) and the hydrolysate was applied onto a column of Dowex 1 (formate form, 0.3 × 50 cm or 1.4 × 50 cm). Elution was performed with a linear gradient of 0 to 0.05 m-formic acid. The fractions corresponding to Qp were pooled and lyophilized.

Identification of Qp by two-dimensional thin-layer chromatography. The Qp fraction separated on Dowex 1 column was evaporated to dryness, dissolved in a small volume of water, mixed with 0.2 A_260 unit each of Gp, Ap, Up and Cp as markers, applied to a thin-layer cellulose plate and subjected to two-dimensional separation in solvents A and B of Kasai et al. (1975). Control experiments were carried out with E.coli tRNA. In both tests, fluorescent spots were located at the same position on chromatographic plates.

RESULTS AND DISCUSSION

RPC-5 chromatography of tRNA modified with cyanogen bromide and periodate. tRNAs containing Q-base (but not Q^* base) treated with cyanogen bromide prior to aminoacylation, underwent modification that altered their chromatographic properties. A similar effect was brought about by treatment of the aminoacylated tRNAs with periodate.

The modified aminoacyl-tRNAs were eluted at much higher NaCl concentration than the unmodified molecules when chromatographed on RPC-5 column.

RPC-5 chromatography of tRNA from yellow lupin seeds yielded two major peaks of tyrosyl-tRNA. The treatment of the lupin Tyr-tRNA with periodate did not change the elution pattern of these isoaccepting tRNA species although two additional minor peaks appeared at higher salt concentrations (Fig. 1A), in agreement with the previous observation of Kędzierski et al. (1980).

On the other hand, the treatment of lupin tRNAs with cyanogen bromide caused retardation of Tyr-tRNA_{2tyr} on the RPC-5 column (Fig. 1B). This indicates that some other nucleoside present in tRNA_{2tyr}, was changed by BrCN.

It is known that cyanogen bromide reacts not only with Q-nucleotides but also with other nucleosides of tRNA, e.g. with thiolated uridine, or
3-N-(3-amino-3-carboxypropyl)uridine. The former nucleotides react also with periodate.

Thus, the above results argue against the presence of nucleoside Q in tyrosine tRNA. Also the cochromatography of Asp-tRNA treated and untreated with periodate or cyanogen bromide indicates the absence of nucleoside Q in this tRNA species. Neither reagent affected the elution profile of Asp-tRNA. Similar conclusions were drawn from an analogous analysis of tRNA\textsuperscript{Asp} (data not shown).

On the other hand, a large shift characteristic for the Q-nucleoside-containing tRNA species was found in the elution pattern of one of the histidine isoaccepting tRNAs after periodate or cyanogen bromide treatment (Fig. 2A,B). To verify this suggestion, lupin tRNA was fractionated on acetylated DBAE-cellulose column.
Fig. 2. RPC-5 cochromatography of His-tRNA from lupin seeds. A, [14C]His-tRNA modified with periodate (- - -), and [3H]His-tRNA unmodified (——); B, [3H]His-tRNA treated with BrCN prior to aminoacylation (- - -), and [14C]His-tRNA unmodified (——).

The introduction of absorbents with organic derivatives of boric acid, to the chromatography of Q-containing tRNAs facilitates considerably the isolation and analysis of these tRNA species. The commercially available DBAE-cellulose forms stable complexes with the Q-nucleoside-containing tRNAs at high salt concentration and alkaline pH, at room temperature. These complexes dissociate at moderate salt concentration in slightly acidic medium.

It was found that 97 - 99% of tRNA from yellow lupin seeds was not retained on the column under the binding conditions, whereas in control experiments with the E. coli tRNA this value did not exceed 93%. Moreover, the specific acceptor activities of lupin tRNA in fraction I (unbound) and II (bound on the column) were similar for tRNAAsp and tRNAAsn. On the contrary, the values of specific acceptor activities of histidine tRNA of fraction I and II were about 10 and 40 pmol of histidine per A260 unit, respectively. The last value seems to be under-
estimated because fraction II from DBAE column contained also some quantities of unspecifically adsorbed tRNA and u.v.-absorbing impurities which enhance absorbance of the fraction.

Higher concentration of histidine tRNA species in fraction II is a further indirect proof for the presence of nucleoside Q in lupin tRNAHis.

*Column chromatography on concanavalin A-Sepharose. It has been shown that mammalian tRNA^Tyr and tRNA^Asp contain Q derivatives with galactosyl-Q and mannosyl-Q moieties, respectively (Kasai et al., 1976). The Q* nucleosides are detectable neither by the cyanogen bromide or periodate method, nor by chromatography on DBAE column. However, tRNA^Asp with mannosyl-Q moiety can be separated by the use of the affinity chromatography on Con A-Sepharose which has a specific affinity for mannose (and glucose), but not for galactose (Okada et al., 1977).

The application of this method for the analysis of [14C]Asp-tRNA from lupin seeds showed the absence of mannosyl-Q in tRNA^Asp, because all the radioactivity introduced to the column passed along unretained.

*Direct detection of nucleoside Q in alkaline digest of lupin tRNA. The group of Nishimura (Kuchino et al., 1976) elaborated a simple method for the detection and isolation of the Q-family nucleosides. The method is based on the enzymatic or alkaline hydrolysis of tRNA, followed by fractionation of the released mononucleotides on an anionic resin, and subsequent separation of the Q and Q* nucleotides by thin-layer chromatography (Kasai et al., 1975).

Figure 3 shows the elution pattern of the alkaline digest of lupin tRNA on Dowex-1 column. It can be seen that a very small absorption peak (fractions No. 26 - 28) precedes the elution of cytidylate (fractions No. 35 - 48). This peak has been consistently observed on chromatograms of the E.coli and wheat germ hydrolysates, but was never found on chromatograms of yeast tRNA hydrolysates.

![Fig. 3. Dowex-1 column chromatography of an alkaline digest of lupin seed tRNA. Fractions No. 26 - 28 contain Q-nucleotides. For details see Materials and Methods.](image-url)
To isolate a larger amount of the nucleoside represented by this extremely small peak, 15 000 $A_{260}$ units of crude lupin tRNA was hydrolysed and fractionated. The pooled fractions (No. 26 - 28) were lyophilized and subjected to two-dimensional cellulose TLC. The results are presented in Fig. 4. The fluorescent spot No. 1 shows the mobility corresponding to that reported for Qp (Kuchino et al., 1976). Its amount was, however, too low to examine it more closely (Spot No. 2 may represent $t^6$Ap).

![Fig. 4. Two-dimensional thin-layer chromatography of the modified nucleotides separated on Dowex-1 column (fractions No. 26 - 28). The positions of these nucleotides (1, 2) are related to the location of nucleotides added as markers. The fluorescent spot No. 1 shows the mobility of Qp.](image)

This direct evidence of the presence of Q in lupin tRNA is consistent with the results obtained from cyanogen bromide and periodate tRNA treatment, as well as acetylated DBAE-cellulose column experiments. In conclusion, the above studies have shown that yellow lupin seed tRNA contains minute amounts of nucleoside Q, probably exclusively in one of the two isotopic species, tRNA$\text{His}^\text{2}$.

The presence of queuine only in tRNA$\text{His}^\text{2}$ and its absence in the other tRNA species may suggest a comparatively greater affinity of this tRNA to tRNA transglycosylase (an enzyme involved in the incorporation of queuine into polynucleotide chain).

Comparison of the chromatograms from Dowex-1 fractionation allows to conclude that lupin tRNA contains at least 3 and 5 times less of the hypermodified nucleoside than wheat germ and E.coli tRNAs, respectively.

On the other hand, since the content of histidine-tRNA$\text{2}$ in lupin seed tRNA amounts to about 0.8% (unpublished data), the real content of queuine in lupin His-tRNA does not exceed 0.01%.

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REFERENCES


NUKLEOZYD Q W tRNA NASION ŁUBINU ŻÓLTEGO

Streszczenie

W pracy wykazano, że tRNA izolowany z nasion łubinu żółtego zawiera tzw. nukleozyd Q, 7-(4,5-cis-dihydroksyl-1-cyklopenten-3-ylo-aminometylo)-7-dezazaganozynę. Nukleozyd ten znaleziono tylko w jednym z dwóch obecnych w łubinie izoakceptorów histydynowego tRNA. Udokumentowano to metodę chromatograficzną (RPC-5), stwierdzając przesunięcie szczytu elucji His-tRNAHs po traktowaniu zaminocylumowego tRNA bromocyanem lub nadjadanem. Wynik potwierdzono metodą izolacji tRNA zawierających Q, na DBAE-celulozie.

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