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Enzymatic synthesis of Q* nucleoside containing mannose in the anticodon of tRNA: isolation of a novel mannosyltransferase from a cell-free extract of rat liver

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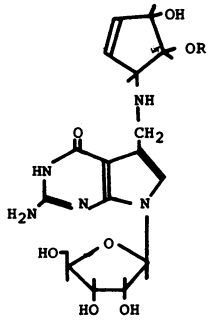
ABSTRACT

The Q* nucleosides isolated from rabbit liver tRNA are known to have sugars (mannose or galactose) linked to their cyclopentene diol moiety. A Q* nucleoside containing mannose (manQ) was synthesized by a cell-free system from rat liver, using purified *E. coli* $tRNA^{Asp}$ as an acceptor and GDP-mannose as a donor molecule. The novel mannosyltransferase catalyzing this reaction was purified from a particulate-free soluble enzyme fraction and found to be strictly specific for $tRNA^{Asp}$. These results, together with the anomeric configuration of mannose in Q* nucleoside, indicate that no lipid intermediate is involved in the biosynthesis of Q* nucleoside.

INTRODUCTION

E. coli $tRNA^{Tyr}$, $tRNA^{His}$, $tRNA^{Asn}$ and $tRNA^{Asp}$ are known to have the hypermodified nucleoside Q in the first position of the anticodon (1,2). In addition to Q, Q* was identified as a derivative of Q nucleoside having mannose or galactose units linked to position 4 of its cyclopentene diol moiety (Figure 1, reference 3). Lectin-Sepharose column chromatography



Q, R=H
manQ, R=β-D-mannosyl

Figure 1. Structure of Q and manQ.

showed that modification of the Q nucleoside with sugars was specific for certain tRNA species, namely with mannose for $tRNA^{Asp}$ and with galactose for $tRNA^{Tyr}$ (4). This paper reports the isolation of a novel mannosyltransferase, specific for $tRNA^{Asp}$. This enzyme catalyzes transfer of D-mannose from GDP-α-mannose to Q in *E. coli* $tRNA^{Asp}$. During the transfer reaction, inversion of the anomeric configuration at the C-1 carbon of the sugar seems to occur to yield the β-D-mannosyl Q nucleoside. The mannosyltransferase described in this paper has different characteristics from other mannosyltransferases so far isolated (5); namely it can be isolated easily from a particulate-free soluble enzyme fraction and no lipid intermediate seems to be involved in the mannose transfer reaction.

MATERIALS AND METHODS

Isolation of mannosyltransferase from rat liver cells. Adult male rats of the Donryu strain were killed by decapitation and their livers were quickly excised; 50 g of liver was homogenized in a Teflon homogenizer; the homogenate was centrifuged at 105,000 x g for 90 min; the resulting supernatant was dialyzed against 0.01 M Tris-HCl (pH 7.5)-0.01 M $MgCl_2$-0.001 M EDTA-0.006 M β-mercaptoethanol-10 % glycerol [Buffer A] for 5 hours, and then applied to a DEAE-cellulose (DE-52) column (4 x 15 cm) equilibrated with buffer A; the column was washed with 150 ml of buffer A, and then eluted with a linear gradient obtained by placing 500 ml of buffer A in the mixing chamber and 500 ml of buffer A containing 0.4 M NaCl in the reservoir, and fractions of 5 ml were collected.

Assay of mannosyltransferase. The standard reaction mixture contained 0.3 ml of fractionated rat liver enzyme, 30 μmoles of Tris-HCl (pH 7.5), 15 μmoles of $MgCl_2$, 1.7 A_{260} units of purified *E. coli* tRNA as specified and 2 nmole of GDP-[U-^{14}C]mannose (specific activity 179 mCi/mmole) in a final volume of 3 ml. The reaction mixture was incubated at 37°C and samples (0.4 ml) were taken at various times. The tRNA was precipitated by addition of one volume of 20 % (w/v) trichloroacetic acid, and collected on a glass fiber disc, and its radioactivity was counted in a liquid scintillation counter.

Analysis of ^{14}C-labeled reaction product. Reaction mixture containing 2.5 A_{260} units of purified *E. coli* $tRNA^{Asp}$ in a total volume of 5 ml was incubated at 37°C for 1 hour. Then the reaction mixture was

shaken with 2 volumes of phenol saturated with water and the aqueous phase containing $tRNA^{Asp}$ was applied to a column (0.3 x 1 cm) of DEAE-cellulose (DE-52). The column was washed with 5 ml of 0.02 M Tris-HCl (pH 7.5)-0.01 M $MgCl_2$-0.2 M NaCl, and then $tRNA^{Asp}$ was eluted with 1 ml of 0.02 M Tris-HCl (pH 7.5)-0.01 M $MgCl_2$-1 M NaCl and precipitated with 2.5 volumes of ethanol. After centrifugation, the precipitated $tRNA^{Asp}$ was dissolved in 0.05 ml of 0.05 M potassium acetate buffer (pH 4.7) and completely hydrolyzed with 5.0 units of RNase T_2. The hydrolyzate was subjected to two-dimensional thin-layer chromatography as described previously (7).

Materials. *E. coli* $tRNA^{Tyr}$, $tRNA^{His}$, $tRNA^{Asn}$ and $tRNA^{Asp}$ were isolated from cells of *E. coli* B by combinations of several column chromatographies as described previously (7). GDP-[U-^{14}C]mannose (specific activity 179 mCi/mmole) was obtained from the Radiochemical Centre, Amersham, England. RNase T_2 was a product of Sankyo Co. Ltd., Tokyo.

RESULTS

Figure 2 showed the elution profile of the mannosyltransferase on DEAE-cellulose column chromatography. The enzyme was eluted in later fractions as two peaks. The main fraction (fraction No 26 - No 30) was used as the enzyme source in the following experiment. Incorporation of radioactivity from GDP-[U-^{14}C]mannose into *E. coli* tRNAs was assayed. As

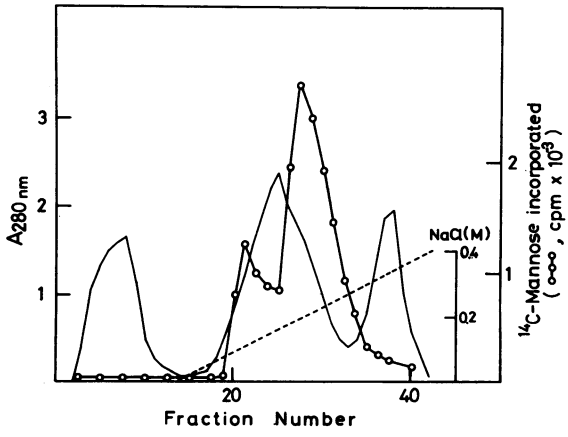


Figure 2. Isolation of a mannosyltransferase from a crude extract of rat liver by DEAE-cellulose column chromatography. Assay conditions were as described in the Materials and Methods, except that 0.5 A_{260} unit of *E. coli* $tRNA^{Asp}$ and 0.1 ml of eluate were used in a final volume of 0.4 ml.

shown in Figure 3 incorporation was observed only when aspartic acid specific *E. coli* tRNA was used as acceptor. Three other *E. coli* tRNAs that contain Q nucleoside, i.e. $tRNA^{Tyr}$, $tRNA^{His}$ and $tRNA^{Asn}$, were unable to act as acceptors. Incorporation of radioactivity was proportional to the time of incubation for up to 1 hour. On incubation for 2 hours, about 1/30 of the *E. coli* $tRNA^{Asp}$ added to the reaction mixture accepted radioactive mannose. When a crude extract was used as an enzyme source, instead of the enzyme purified by DEAE-cellulose column chromatography, no mannosyltransferase activity was detected, even when *E. coli* $tRNA^{Asp}$ was used as an acceptor. This was probably due to degradation of the GDP-[U-^{14}C]mannose.

Table 1 shows results on the requirements of the reaction. Magnesium ion was absolutely necessary for the incorporation of the radioactivity, and addition of EDTA completely abolished the incorporation. Addition of 0.1 M NaCl stimulated the activity more than 2 fold. The pH optimum of the reaction was found to be 7.5.

In order to characterize the ^{14}C-labeled product, the ^{14}C-labeled $tRNA^{Asp}$ was hydrolyzed with RNase T_2 and the digest was subjected to two-dimensional thin-layer chromatography as described in the Materials and Methods. As shown in Figure 4, the radioactive spot coincided with the position of the Q* nucleotide, indicating that the product synthesized was in fact Q* nucleotide.

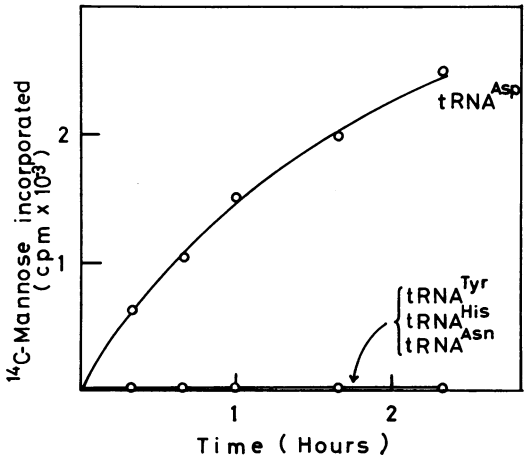


Figure 3. Acceptor activities of the four *E. coli* tRNAs for mannosylation. Assay conditions were as described in the Materials and Methods.

TABLE 1. Effects of pH, monovalent cation and magnesium ion on incorporation of radioactivity from GDP-[^{14}C]mannose into *E. coli* $tRNA^{Asp}$.

Conditions					Incorporation of radioactivity (pmole/A_{260} unit of $tRNA^{Asp}$)
Experiment 1					
pH 7.0	Mg^{2+}	5 mM			7.65
pH 7.5	Mg^{2+}	5 mM			8.39
pH 8.0	Mg^{2+}	5 mM			7.28
pH 8.8	Mg^{2+}	5 mM			4.36
pH 7.5	Mg^{2+}	1 mM			3.74
pH 7.5	Mg^{2+}	1 mM	EDTA	1 mM	0.02
Experiment 2					
pH 7.5	Mg^{2+}	5 mM			4.42
pH 7.5	Mg^{2+}	5 mM	NaCl	100 mM	10.70
pH 7.5	Mg^{2+}	5 mM	NaCl	150 mM	8.78
pH 7.5	Mg^{2+}	5 mM	NaCl	200 mM	4.98

Assay conditions were as described for Figure 2.

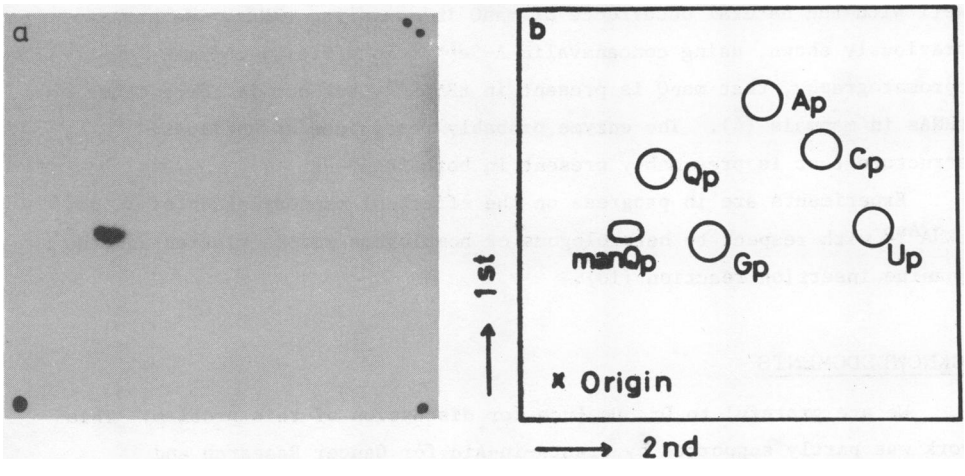


Figure 4. Autoradiogram of a two-dimensional thin-layer chromatogram of the RNase T_2 digest (a) autoradiogram of the chromatogram. (b) composite tracing of (a) in relation to the locations of the four normal nucleotides and Q nucleotide.

DISCUSSION

In the biosynthesis of glycoprotein, it is known that several lipid intermediates are involved in the transfer of carbohydrate from sugar nucleotides to glycoproteins in mammalian systems (8). In some instances the lipid moiety has been identified as dolichol (8), and there in also evidence that retinol is involved as a lipid intermediate (9). In contrast to the synthesis of these glycoproteins, no lipid intermediate seems to be involved in the biosynthesis of manQ: namely, the mannosyltransferase reported here can be isolated from 105,000 x g supernatant that is free from a particulate fraction, and it can be purified further by DEAE-cellulose column chromatography. Additional evidence to support the conclusion that there is no lipid intermediate is that the mannosyl residue in manQ is attached to cyclopentene diol in the β form (3). The mannose moiety is probably incorporated into cyclopentene diol in the same configuration in the cell-free system reported here, since an inversion of the anomeric configuration of D-mannose is known to occur in the transfer of the sugar from GDP-D-α-mannose (8). Therefore, GDP-mannose is probably the direct donor molecule, transferring mannose to the Q residue in tRNA.

Only *E. coli* $tRNA^{Asp}$ was utilized as an acceptor for the biosynthesis of manQ in this heterologous system; the other three *E. coli* tRNAs, i.e. $tRNA^{Tyr}$, $tRNA^{His}$ and $tRNA^{Asn}$, that also contain Q were ineffective as acceptors. This strict specificity of mannosyltransferase coincides very well with the natural occurrence of manQ in mammalian tRNAs. We have previously shown, using concanavalin A-Sepharose affinity column chromatography, that manQ is present in $tRNA^{Asp}$, but not in three other tRNAs in mammals (4). The enzyme probably recognizes an anticodon structure that is presumably present in both $tRNA^{Asp}$s.

Experiments are in progress on the effect of mannosylation of *E. coli* $tRNA^{Asp}$ with respect to heterologous or homologous aminoacylation and the guanine insertion reaction (10).

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