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Isolation of mammalian $tRNA^{Asp}$ and $tRNA^{Tyr}$ by lectin-Sepharose affinity column chromatography

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ABSTRACT

$tRNA^{Asp}$ from rabbit liver, rat liver and rat ascites hepatoma was readily isolated by concanavalin A-Sepharose (Con A-Sepharose) affinity column chromatography. $tRNA^{Tyr}$ from these sources was extensively purified by *Ricinus communis* lectin-Sepharose column chromatography. These results, together with the chromatographic behaviour of four tRNAs ($tRNA^{Tyr}$, $tRNA^{His}$, $tRNA^{Asn}$ and $tRNA^{Asp}$) on acetylated DBAE-cellulose column chromatography suggested that $tRNA^{Asp}$ contains a Q^* nucleoside species having a mannose moiety while $tRNA^{Tyr}$ contains Q^* nucleoside with galactose. The sugars attached in 4-position of cyclopentene diol in the Q molecule are therefore not present at random in the four tRNAs, but present only in each specific tRNA. This is the first case which shows that plant agglutinin interacts with nucleic Acid as well as polysaccharide and glycoproteins.

INTRODUCTION

The modified nucleoside Q (also named queuosine or Quo) was previously found in the first position of the anticodon of *Escherichia coli* $tRNA^{Tyr}$, $tRNA^{His}$, $tRNA^{Asn}$ and $tRNA^{Asp}$ (1,2). In addition to Q nucleoside, Q^*, a derivative of Q was found in mammalian tRNA (3). The structure of Q^* nucleoside was recently determined to be a derivative of Q having mannose or galactose units linked to position 4 of its cyclopentene diol moiety (4). It is the first modified nucleoside isolated from tRNA to carry sugar units in the side chain. Thus the possibility is open to purify sugar-containing tRNAs by affinity chromatography using lectin-Sepharose columns which have an affinity for either mannose or galactose. This technique was previously used for isolation of polysaccharides and glycoproteins or cell surface membranes having a specific sugar moiety (5). In fact we are able to purify $tRNA^{Asp}$ from mammalian cells by simply passing unfractionated tRNA through concanavalin A-Sepharose which has affinity for mannose. No other tRNAs were retained in the column, and $tRNA^{Asp}$ thus isolated was found to be more than 95 % pure. When a *Ricinus communis* lectin-Sepharose column that has

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affinity for galactose was used instead of concanavalin A-Sepharose, only $tRNA^{Tyr}$ was retarded. These results indicated that modification of the Q nucleoside with sugars is specific for certain tRNA species, namely mannose for $tRNA^{Asp}$ and galactose for $tRNA^{Tyr}$.

MATERIALS AND METHODS

tRNA and aminoacyl-tRNA synthetase. Rat liver tRNA and rat ascites hepatoma AH7974 tRNA were prepared according the procedure described previously (6). Rabbit liver tRNA was a gift from Dr. H. Gross, Max-Planck-Institut für Biochemie, München. Crude rat liver aminoacyl-tRNA synthetase was also prepared as described previously (7). Amino acid acceptor activity of the three mammalian tRNAs for tyrosine, histidine, asparagine and aspartic acid was performed using rat liver aminoacyl-tRNA synthetase. The assay condition was also described in the same literature (7).

Lectin-Sepharose. Concanavalin A-Sepharose (Con A-Sepharose) was obtained from Pharmacia Fine Chemicals, Uppsala. *R. communis* lectin-Sepharose was prepared by the procedure described by Adair and Kornfeld (8), and kindly donated from Drs. Y. Kobayashi and T. Osawa of Tokyo University.

Finger-print analysis of RNase T_1 digest of rabbit liver tRNA by post-labeling. The 5'-end of oligonucleotides were labeled with ^{32}P by the polynucleotide kinase reaction according the modified procedure described by Sugisaki (9). In this procedure, *E. coli* alkaline phosphomonoesterase which was used for removal of terminal 3'-phosphate was temporally inactivated by heat treatment, and the γ-^{32}P-ATP which remained in the reaction mixture was not decomposed by myosine ATPase or yeast hexokinase as originally described (10,11). Oligonucleotides labeled with ^{32}P were separated by two-dimensional PEI-cellulose chromatography as described by Mirzabekov and Griffin (12). RNase T_1 and *E. coli* alkaline phosphomono-esterase used for the experiment were obtained from Sankyo Co. Ltd., Tokyo, and Boehringer Mannheim Gmbh, Mannheim, West Germany, respectively. T4-phage induced polynucleotide kinase was a gift from Dr. M. Takanami of Kyoto University. γ-^{32}P-ATP was prepared as described by Glynn and Chappell (13). PEI-cellulose plates were obtained from Nacherey-Nagel Co., Postfack, West Germany.

Fractionation of rabbit liver tRNA by acetylated DBAE-cellulose. The procedure described by McCutchan *et al.* (14) was adopted. After unfraction-

ated rabbit liver tRNA was loaded on a column (0.8 x 10 cm), the column was washed with Buffer I [1 M NaCl-0.1 M $MgCl_2$-0.05 M morpholine (pH 8.7)] at 4°C. Then tRNAs that presumably contain Q nucleoside were eluted with Buffer G (0.2 M NaCl-0.05 M sodium acetate buffer, pH 5.0), at a flow rate of 2 ml/hr. One ml of effluent was collected in each fraction. Acetylated DBAE-cellulose was a gift from Dr. D. Söll, Yale University.

RESULTS

Figure 1 shows fractionation of rabbit liver, rat liver and rat ascites hepatoma tRNAs by concanavalin A-Sepharose column chromatography at 4°C. In each case, almost all of the tRNA was passed through the column by washing with 0.05 M sodium acetate buffer (pH 6.0) containing 0.9 % NaCl, 1 mM $MnCl_2$, 5 mM $MgCl_2$ and 1 mM $CaCl_2$. When the elution buffer was changed to

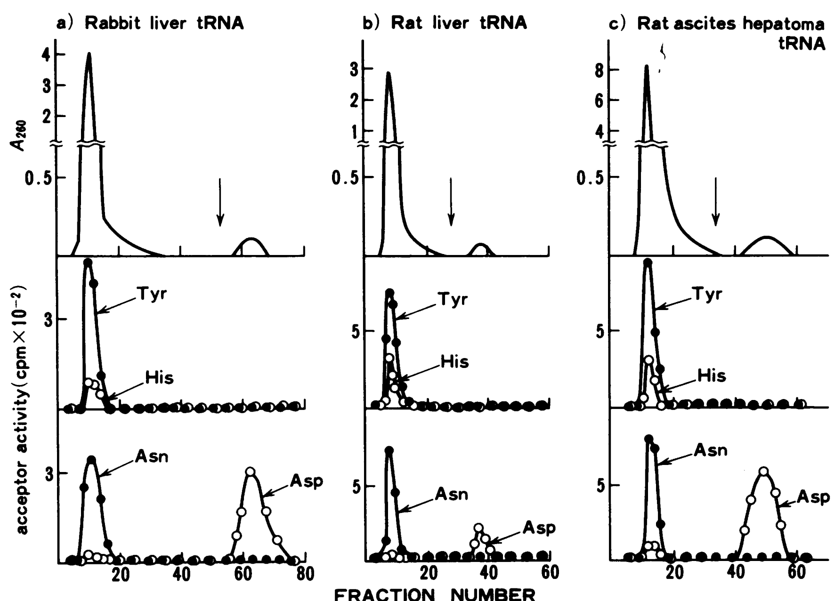

Figure 1. Fractionation of rabbit liver, rat liver and rat ascites hepatoma tRNAs by concanavalin A-Sepharose affinity column chromatography. Unfractionated tRNA (12 A_{260} units of rabbit liver tRNA, 11 A_{260} units of rat liver tRNA, 25 A_{260} units of rat ascites hepatoma tRNA) dissolved in 0.1 ml of 0.05 M sodium acetate buffer (pH 6.0)-0.9 % NaCl-1 mM $MnCl_2$-1 mM $MgCl_2$-1 mM $CaCl_2$ was loaded on a column (0.6 x 12 cm) that was previously equilibrated with the same buffer. The column was washed with the buffer until UV absorbance in eluate was diminished. tRNA absorbed in the column was then eluted with the above buffer containing 50 mM methyl-α-D-glucopyranoside. Flow rate was 3 ml/hr; 0.6 ml of effluent was collected in each fraction. Chromatography was carried out at 4°C. Arrow indicated in the figure shows the point at which the elution buffer was changed.

the above buffer containing 0.05 M methyl-α-D-glucopyranoside which is known to dissociate interaction of concanavalin A with sugars, small amount of ultraviolet absorbing material (approximately 2 to 3 % of the originally applied tRNA) was eluted. This small fraction of tRNA accepted only aspartic acid (0.96 nmole of ^{14}C-aspatic acid accepted per 1 A_{260} unit of tRNA). The other three tRNAs, i.e. $tRNA^{Tyr}$, $tRNA^{His}$ and $tRNA^{Asn}$, that are possible candidates to contain Q* were not absorbed on the column, and were eluted without retention. The purity of $tRNA^{Asp}$ thus isolated was found to be more than 95 % as judged by finger-printing of an RNase T_1 digest of rabbit liver $tRNA^{Asp}$ labeled with ^{32}P at the 5'-end, as shown in Figure 2. The purity of $tRNA^{Asp}$ was also checked by two-dimensional polyacrylamide gel electrophoresis according to the procedure described by Ikemura and Dahlberg (15). Although not shown in the figure, $tRNA^{Asp}$ gave a single major spot and a very faint spot indicating that it is almost homogeneous.

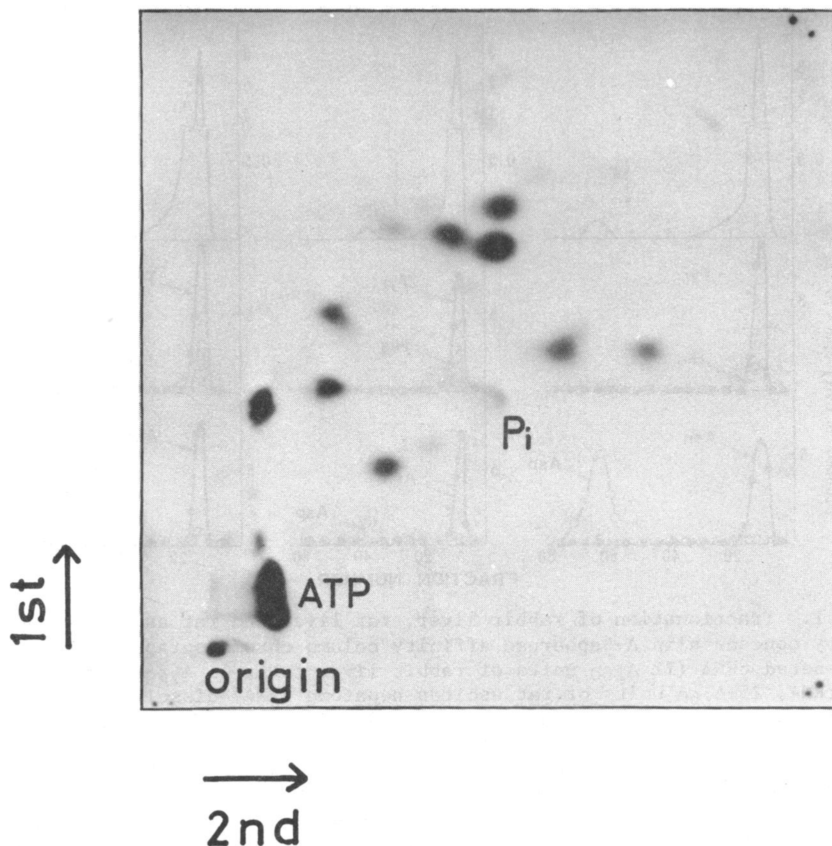

Figure 2. Radioautogram of ^{32}P-labeled oligonucleotides derived from RNase T_1 digest of rabbit liver $tRNA^{Asp}$.

When fractionation of tRNA by concanavalin A-Sepharose was performed at 20°C instead of 4°C, $tRNA^{Asp}$ was less tightly bound to the column, and eluted even without using methyl-α-D-glucopyranoside, as shown in Figure 3. However, $tRNA^{Asp}$ was eluted much later, and completely separated from other tRNAs. Therefore, almost the same extent of purification can be achieved by operating the chromatography at 20°C.

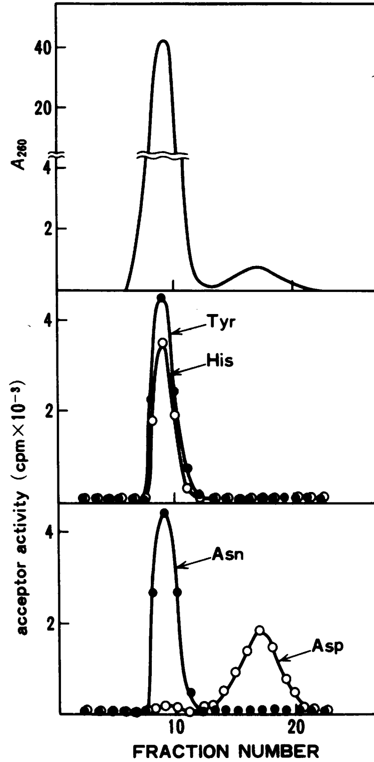

Figure 3. Fractionation of rabbit liver tRNA by concanavalin A-Sepharose affinity column chromatography at 20°C. The conditions are the same as described in Figure 1 except the fractionation was carried out at 20°C. 55 A_{260} units of rabbit liver tRNA was loaded on the column.

Nucleoside Q* found in rabbit liver tRNA is a mixture of two components, i.e. Q with mannose (Quo-Man) and Q with galactose (Quo-Gal). Since concanavalin A has affinity for either a α-D-mannosyl and α-D-glucosyl group or sterically related residues (16), it is likely that $tRNA^{Asp}$ purified by this affinity chromatography contains Quo-Man. Weak binding of $tRNA^{Asp}$ to concanavalin A at 20°C may be due to less favorable structure of Quo-Man to interact with concanavalin A, because the mannosyl residue in Q* is

attached to cyclopentenediol in β form, and concanavalin A is known to interact preferably with α forms of mannosyl and glucosyl residues (16). In order to examine whether Quo-Gal is present in other tRNA species, other lectin-Sepharose affinity chromatography, i.e. *R. communis* lectin-Sepharose that has a specificity for galactosyl group (5) was performed. As shown in Figure 4, $tRNA^{Tyr}$ was eluted later than other tRNAs from a column of *R. communis* lectin-Sepharose in all three cases of rabbit liver, rat liver and rat ascites hepatoma tRNAs. In fact, second the half portion of the $tRNA^{Tyr}$ fraction was purified more than 10 fold by this procedure. It should be noted that $tRNA^{Asp}$ as well as $tRNA^{His}$ and $tRNA^{Asn}$ were not retained in the column, indicating that isolation of $tRNA^{Asp}$ by concanavalin A-Sepharose is due to specific interaction between concanavalin A and $tRNA^{Asp}$, but is not due to Sepharose itself.

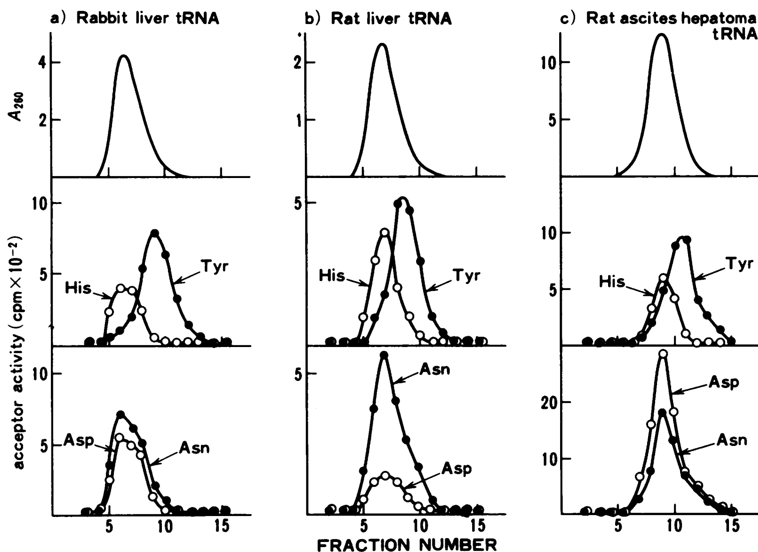

Figure 4. Fractionation of rabbit liver, rat liver and rat ascites hepatoma tRNAs by *R. communis* lectin affinity column chromatography. The fractionation was carried out as described in Figure 1, using a column (0.6 x 12 cm) of *R. communis* lectin-Sepharose. Flow rate was 1 ml/hr. tRNA loaded on the column: rabbit liver tRNA, 8 A_{260} units; rat liver tRNA, 5 A_{260} units; rat ascites hepatoma tRNA, 25 A_{260} units.

DISCUSSION

It was demonstrated that mammalian $tRNA^{Asp}$ is easily isolated by applying total unfractionated tRNA to a column of concanavalin A-Sepharose. This procedure opens the way to study the sequence or other properties of

specific tRNAs in mammalian cells, since it is simple to quantitatively isolate pure species of $tRNA^{Asp}$. Chemical characterization of sugars, in $tRNA^{Asp}$ and $tRNA^{Tyr}$ has not been done at the present time. However, it is reasonable to assume that $tRNA^{Asp}$ contains Q^* with mannose and $tRNA^{Tyr}$ contains Q^* with galactose, according to the specificity of each lectin-Sepharose used for purification of these tRNAs. It should be noted that only four tRNAs, i.e. $tRNA^{Tyr}$, $tRNA^{His}$, $tRNA^{Asn}$ and $tRNA^{Asp}$ contain Q nucleoside both in *E. coli* and *Drosophila* (1,17). Among them, $tRNA^{His}$ and $tRNA^{Asn}$ which were not purified by the affinity chromatography were instead purified by acetylated DBAE-cellulose chromatography as shown in Figure 5. The principle of acetylated DBAE-cellulose involves interaction with the cis-diol group of the Q molecule (14). The reason why $tRNA^{Asp}$ and $tRNA^{Tyr}$ were not retained by accetylated DBAE-cellulose is reasonably explained by

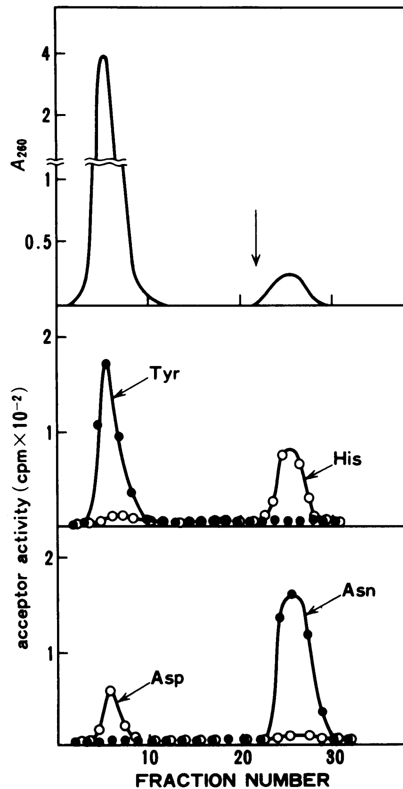

Figure 5. Fractionation of rabbit liver tRNA by acetylated DBAE-cellulose column chromatography. The condition for fractionation were as described in the Materials and Methods section. Arrow indicated in the figure shows the point at which the elution buffer was changed. 10 A_{260} units of rabbit liver tRNA was loaded on the column.

assuming that these tRNAs contain Q^* instead of Q. Q^* in which the hydroxy group at the 4 position is blocked by sugars should no longer interact with acetylated DBAE-cellulose. In fact, it was shown that rabbit liver $tRNA^{Asp}$ contains Q^* but not Q (F. Harada, M. Yamada, S. Nishimura and H. J. Gross, unpublished results). Additional evidence to support the idea of the presence of each sugar in specific tRNAs is that enzymatic conversion of Q to Q^* was achieved with a rat ascites hepatoma cell-free system only using *E. coli* $tRNA^{Asp}$ but not other *E. coli* Q-containing tRNAs (18). Thus it is very likely that Q^* with mannose (Quo-Man) and Q^* with galactose (Quo-Gal) are specifically present in $tRNA^{Asp}$ and $tRNA^{Tyr}$, respectively.

It should be noted that more than 90 % of $tRNA^{Asp}$ was eluted from concanavalin A-Sepharose after the column was washed with the buffer containing methyl-α-D-glucopyranoside. This suggested that modification of Quo-Man is almost complete in all three mammalian tRNAs tested so far. However, it should be mentioned that in the case of rat ascites hepatoma tRNA, more $tRNA^{Asp}$ was eluted without retaining in the column, indicating that content of $tRNA^{Asp}$ having either G or Q is higher than that from normal rat liver (Figure 1). It was previously shown that tRNA from rat ascites hepatoma AH7974 was a more active acceptor than normal rat liver tRNA for guanylation when Ehrich ascites tumor cells were used as an enzyme source (Y. Ito, F. Harada, S. Nishimura and I. Watanabe, unpublished results). The guanylation reaction in which Q base or guanine in the first position of anticodon is replaced by guanine (19) may be restricted by modification of Q to Q^*.

It is interesting to mention that mannose and galactose are components which specify the properties of glycoprotein receptors in membranes (5). The presence of the same sugars in specific tRNAs may reflect an important role of these tRNAs in membrane function. This fascinating idea and other possible functions of sugar-containing tRNAs is to be tested by further investigation. In anycase, action of a plant hemagglutinin, on mammalian cells must also be considered from the point of its interaction with specific tRNA.

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