Volume 4 Number 7 July 1977 Nucleic Acids Research

Chromatographic behavior of several mammalian tRNAs on acylated dihydroxyl-borate cellulose and Aminex A-28

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Received 29 March 1977

ABSTRACT

Studies of the chromatographic behavior of mammalian tRNAs, from several sources, on acylated DBAE-cellulose indicate that species of $tRNA^{Asn}$, $tRNA^{Asp}$ and $tRNA^{His}$ can be retained on this matrix, while species of $tRNA^{Tyr}$, $tRNA^{Asn}$ and $tRNA^{Asp}$ are not retained. Treatment of total rat liver tRNA with cyanogen bromide and subsequent chromatography on Aminex A-28 columns demonstrated that these tRNA species might contain Q (or Q*) nucleoside. However, comparable studies of the tRNA isolated from Walker 256 rat mammary tumor tissue demonstrated that this tumor tRNA almost totally lacks the hypermodified nucleosides Q and Q*. In addition, we have found that at least the major species of rat liver $tRNA^{Asn}$ contains the Q nucleoside.

These studies indicate that chromatography on the acylated DBAE-cellulose matrix, coupled with the analytical ion-exchange chromatography of cyanogen bromide treated and untreated amino-acyl-tRNA can be a valuable technique for the determination of alterations in the Q (or Q*) nucleoside content of the tRNAs isolated from normal and tumor tissues.

INTRODUCTION

It has recently been reported by McCutchan *et al.* (1) that the four families of isoaccepting species of tRNA which contain the hypermodified nucleoside Q can be selectively purified on acylated dihydroxyl-borate substituted cellulose columns (acylated DBAE-cellulose). In *E. coli* tRNA, the nucleoside Q, which contains the cis-diol group (2), occurs only in $tRNA^{Asn}$, $tRNA^{Asp}$, $tRNA^{His}$ and $tRNA^{Tyr}$ (3). It was postulated that (under alkaline conditions and at room temperature) a complex between the borate groups on the resin, and the cis-diol groups of the modified nucleoside Q, causes the retention of these Q containing tRNAs on the acylated DBAE-cellulose matrix (1).

Mammalian tRNA also has isoaccepting species which contain a hypermodified nucleoside identical in structure to the *E. coli* Q, as well as a derivative of Q, designated Q* (4,5). Alterations

in the tRNAs which contain Q (or Q*) have been demonstrated during Drosophila development (6). These same four isoaccepting families of mammalian tRNA (i.e. those which contain Q in E. coli and are altered during Drosophila development) have altered chromatographic elution in response to cell density and serum type in SV-40 transformed cells (7) and the $tRNA^{Asp}$ shows altered chromatographic mobilities when isolated from several different mammalian tumor tissues (8).

We now wish to report that those mammalian tRNAs which in E. coli tRNA contain Q significantly differ from the E. coli tRNAs in their behavior on acylated DBAE-cellulose. Chromatography of rat liver, rat mammary tumor Walker 256, human liver and human placenta tRNAs on acylated DBAE cellulose indicates that $tRNA^{His}$ from these tissues is mostly retained on the matrix, while the $tRNA^{Tyr}$ does not significantly interact with the matrix. In addition, the $tRNA^{Asn}$ from these four tissues contains species which are only partially retained, while $tRNA^{Asp}$ interacts only slightly with the matrix. In contrast, these tRNAs isolated from the rat mammary tumor Walker 256 show a significant decrease in the proportional amount of each species retained on the acylated DBAE-cellulose matrix. These results, coupled with the observed shifts in the ion exchange column chromatographic elution profiles upon treatment of rat liver tRNA with cyanogen bromide, and the observation that no shifts were obtained upon treatment of the Walker 256 tumor tRNA with cyanogen bromide, are consistent with the hypothesis that the rat liver tRNAs contain a modified nucleoside which is lacking in the Walker 256 tumor tRNAs. These results imply that the Walker 256 tumor tRNAs lack both Qp and Q^{*}_{p} and instead contain some other nucleotide in the wobble position. Our results also indicate that species of mammalian $tRNA^{Asn}$, $tRNA^{Asp}$ and $tRNA^{His}$ might contain the Q nucleoside while other species of $tRNA^{Asn}$ and $tRNA^{Asp}$ as well as $tRNA^{Tyr}$ might contain the Q* nucleoside. A direct analysis of a highly purified species of rat liver $tRNA^{Asn}$ demonstrated that the Q nucleoside was present in this tRNA.

MATERIALS AND METHODS

Preparation and Aminoacylation of tRNA. Total mammalian tRNA and a preparation of crude mammalian aminoacyl-tRNA synthetases were

isolated as described previously (9). Total E. coli tRNA was a product of Biogenics Research Corp., Chagrin Falls, Ohio, and crude E. coli aminoacyl-tRNA synthetase was prepared from E. coli MRE600 as described earlier (10). Rat liver and Walker 256 mammary carcinosarcoma tissue from Sprague-Dawley Albino rats (strain ARS) was obtained through the courtesy of Grand Island Biological Co., Grand Island, N.Y. Aminoacylation conditions were as reported (9,10) for mammalian and E. coli tRNA, respectively. All amino acid concentrations were 0.01 mM, and asparagine aminoacylation was performed in the presence of 0.05 mM non-radioactive aspartic acid.

Preparative aminoacylation reactions were performed by scaling up the volume of the aminoacylation incubation mixtures 10 fold. After incubation at 37°C for 30 minutes the aminoacyl tRNA was isolated by phenol extraction and ethanol precipitation as described earlier (11). Radioactive amino acids, which were obtained from Schwarz/Mann and New England Nuclear, were diluted to a concentration of 50 μCi/μmole. Radioactivity was determined in Omnifluor-Toluene or Aquasol using a refrigerated Isocap 300 liquid scintillation spectrometer as described earlier (9,11).

Cyanogen Bromide Treatment of tRNA. Crude tRNA was treated with 3 times sublimed cyanogen bromide as described by Saneyoshi and Nishimura (12).

Chromatographic Procedures. Acylated DBAE-cellulose (4 grams) was purchased from Collaborative Research, Waltham, Mass., equilibrated with 1 M NaCl, 0.1 M $MgCl_2$, 0.05 M morpholine (pH 8.7) (buffer I in Ref. #1), packed into a 1.0 cm x 15.0 cm glass column and further washed with buffer I, with the aid of a Milton Roy instrument mini-pump, at a flow rate of 3 ml per 3 min. per fraction at room temperature. The maximum column capacity was found to be 500 mg of crude E. coli tRNA and in the experiments shown later (see Table 1) 50 mg of each tRNA sample (at a concentration of 5 mg tRNA per ml of buffer I) was applied to a column which had been previously equilibrated with buffer I. The column was first washed with 100 ml buffer I to elute the non-absorbed tRNA and then washed with 0.2 M NaCl, 0.05 M sodium acetate pH 4.5 (buffer G in Ref. #1) to elute the Q containing tRNAs. The column was then stored at room temperature in buffer G and equilibrated with buffer I immediately before re-use. Total

recovery of applied tRNA was, in all experiments, greater than 95% and the results were reproducible for the tRNAs studied.

Aminex A-28 (Bio-Rad Laboratories, Richmond, California) was pre-treated, equilibrated, and packed into a 0.5 cm x 30 cm stainless steel tube with Swagelock fittings top and bottom as previously described (13). The column was built and run at room temperature and the flow rate was maintained at 1 ml per 1.2 min. per fraction using a Mil-Roy D pump, as described previously (10). Approximately 20,000 CPM each of ^{14}C- and ^{3}H-labeled tRNA were dissolved in 200 microliters of buffer B (0.01 M sodium acetate, pH 4.5, 0.01 M $MgCl_2$, and 0.001 M sodium thiosulfate) containing 0.1 M NaCl. This sample was then pumped onto the column and washed with 15 ml of buffer B containing 0.1 M NaCl, while 1 ml fractions were collected in half dram vials (Rochester Scientific Co.). The sample was eluted with a 73 ml concave gradient (42 ml of buffer B containing 0.1 NaCl as the low salt and 31 ml of buffer B containing 1.2 M NaCl as the high salt). Upon completion of the gradient, the column was washed with 30 ml of buffer B containing 2.0 M NaCl and 10% ethanol. 1.2 ml Aquasol was pipetted into each half dram vial and the radioactivity (^{14}C and ^{3}H) in each fraction was determined using the dual label channel in a Nuclear Chicago refrigerated Isocap 300 liquid scintillation counter.

Chromatography on RPC-5 was performed as previously described (13). The tRNA was eluted from the column (0.5 cm x 100 cm), by using a linear salt gradient (0.3 M NaCl to 1.2 M NaCl 200 ml each) containing 0.01 M Tris-HCl pH 7.6, 0.01 M $MgCl_2$, 0.001 M sodium thiosulfate at a flow rate of 3 ml per 3 min. per fraction.

The direct analysis for Qp (or Q_p^*) nucleotide was performed by chromatography of a KOH digested tRNA on Dowex 1X8 according to the procedure described by Kasai, et al. (4). Briefly, the tRNA to be analysed was dissolved in 0.3 M KOH, incubated at 37° overnight and subsequently adjusted to pH 8 with Dowex 50 (H^+ form), and centrifuged twice to clarify the neutralized digestion mixture. Dowex 1X8 resin (a gift from Dr. R. Singhal) was pre-treated with formic acid, washed with water and packed into a 0.5 x 50 cm glass column. The flow rate was maintained at 1.0 ml per minute per fraction with the aid of a Milton Roy minipump.

After equilibration of the column with water, the neutralized tRNA digestion mixture was applied and the column was washed with water until the nucleosides were eluted. The Qp (and Q_p^*) peak was eluted with a 100 ml linear gradient (water to 0.05 M formic acid) and the remaining nucleotides as well as alkali stable dinucleotides were eluted with 3.0 M formic acid.

Nucleoside Analysis of tRNA. In addition to the analysis of Qp and Q_p^* nucleotides using the above mentioned procedure we employed a 2-dimensional cellulose TLC to discriminate between Qp and Q_p^* (4). UV spectra were determined for the suspected Qp (and Q_p^*) UV detected spots eluted from the cellulose TLC plates and also for the ultraviolet (UV) containing peak from the Dowex 1X8 column over the range 320 nM to 210 nM using a Cary Model 116C recording spectrophotometer. Analysis of major and modified nucleosides contained in the tRNAs was performed using the tritium post-labeling technique described by Randerath, et al. (14).

RESULTS AND DISCUSSION

The chromatographic behavior of Q containing tRNAs on acylated DBAE cellulose at room temperature is due to the formation of a stable complex between the borate groups on the cellulose matrix and the cis-diol groups of the hypermodified Q nucleoside (1). In our preliminary experiments aimed at isolation of E. coli Q containing tRNAs, we essentially reproduced the data reported by McCutchan, et al. (1) using the commercial acylated DBAE cellulose. As can be seen from Table 1, all four Q containing species of E. coli tRNA were separated from the total tRNA by this column. We then attempted a similar isolation for the mammalian Q containing tRNAs. In the case of the human placenta, human liver and rat liver, the $tRNA^{His}$ had the greatest affinity for the acylated DBAE-cellulose matrix, while $tRNA^{Tyr}$ showed the lowest affinity for the matrix. Intermediate affinities were shown by the mammalian $tRNA^{Asn}$ and $tRNA^{Asp}$ since both isoaccepting families contained species of tRNA with different affinities for the acylated DBAE-cellulose matrix. Rechromatography of those tRNAs resolved by one passage through this matrix resulted in a slight increase in the purity for the later eluting species but did not yield significant alterations in their elu-

Table 1. Separation of tRNA on Acylated DBAE-Cellulose. 50 mg of each crude tRNA was chromatographed on acylated DBAE-cellulose as described in Materials & Methods. The total nanomoles of 4 isoaccepting tRNAs in each pool are given.

	Peak I*				Peak II**			
tRNA \ amino acid source	Asp	Asn	His	Tyr	Asp	Asn	His	Tyr
E. Coli	0.2	1.1	-	-	6.8	24.7	11.4	29.5
Human Liver	1.1	3.2	-	17.1	0.2	4.8	4.2	0.2
Human Placenta	2.3	1.5	0.4	10.5	0.6	5.0	3.0	0.3
Rat Liver	20.2	11.8	4.7	47.1	9.2	10.0	9.7	2.0
Walker 256 Mammary Tumor	12.9	16.7	5.1	14.9	0.4	4.9	3.0	0.9

*Peak I - Those tRNAs not retained on acylated DBAE-Cellulose, 43 mg recovered.

**Peak II - Those tRNAs retained on acylated DBAE-Cellulose, 6 mg recovered.

- Less than 0.10 nmoles

tion positions. This data is consistent with the hypothesis that species of mammalian $tRNA^{His}$, $tRNA^{Asn}$ and $tRNA^{Asp}$ contains the E. coli like Q nucleoside while species of mammalian $tRNA^{Tyr}$, $tRNA^{Asn}$ and $tRNA^{Asp}$ contain the Q* nucleoside. This latter possibility has been recently confirmed by the observations that both $tRNA^{Asp}$ and $tRNA^{Tyr}$ isolated from several mammalian tissues contain the Q* nucleoside (15,20). Since the Q* nucleoside has a sugar attached through an ether linkage to the number 4 carbon of the 5 membered cyclopenten-diol ring (5), the Q* containing tRNAs might not interact with the acylated DBAE-cellulose matrix. However, it is also possible that other species of mammalian $tRNA^{Asp}$ as well as $tRNA^{Tyr}$ and some species of $tRNA^{Asn}$ are not retained on the acylated DBAE-cellulose matrix because they lack Q nucleoside and instead contain some other nucleoside in the wobble position.

Figure 1 shows the typical elution profiles obtained by co-chromatography of cyanogen bromide treated and untreated rat liver $tRNA^{Tyr}$. It can be seen that the cyanogen bromide treatment causes the $tRNA^{Tyr}$ to be eluted from the Aminex A-28 column

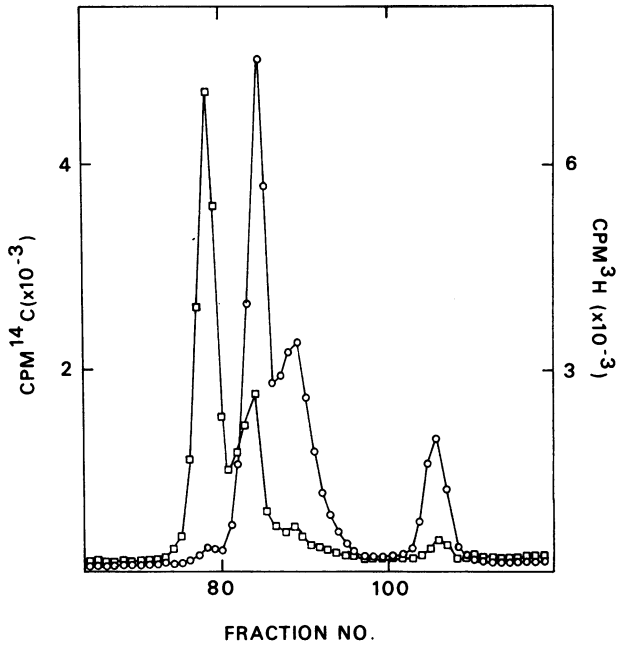

Figure 1. Cochromatography of ^{14}C-Tyr-tRNA^{Tyr} from rat liver treated with cyanogen bromide prior to aminoacylation (O-O) and ^{3}H-Tyr-tRNA^{Tyr} from rat liver aminoacylated without prior cyanogen bromide treatment (□-□). 30 A_{260} units of each aminoacyl-tRNA was applied to an Aminex A-28 column, eluted and the radioactivity determined as described in Materials and Methods.

at a higher salt concentration. Since essentially all the rat liver tRNA^{Tyr} is not retained on the acylated DBAE-cellulose column and its elution position is shifted to higher salt concentrations upon treatment with cyanogen bromide, it is quite probable that this tRNA contains Q*. Further studies of those isoaccepting species of tRNA^{Asn}, tRNA^{Asp} and tRNA^{His}, which were not retained on the acylated DBAE cellulose matrix, showed cyanogen bromide induced shifts in their elution profiles on Aminex A-28 similar to those found with tRNA^{Tyr} (data not shown). We cannot, at present, rule out the possibility that the cyanogen bromide treatment alters a nucleoside other than Q or Q*, thereby causing the later chromatographic elution. The effect of cyanogen bromide treatment on several modified nucleosides has been discussed by Katze (7) and it has also been reported that cyanogen bromide treatment of tRNAs containing X, whose structure

has been shown to be 3-(3-amino-3-carboxyl-n-propyl)uridine (16), causes their later elution from RPC-5 columns (17).

Those mammalian tRNAs which were retained on acylated DBAE-cellulose also have been found to be sensitive to cyanogen bromide treatment. When these peak II tRNAs are aminoacylated and co-chromatographed with unfractionated aminoacyl-tRNA on Aminex A-28, their elution position corresponds to that of the earlier eluting, cyanogen bromide sensitive species (data not shown). This evidence suggests that only the Q containing tRNAs are retained on the acylated DBAE-cellulose matrix, while Q* containing tRNAs are not retained. In addition, our evidence also indicates that both Q and Q* containing tRNAs experience a shift in their chromatographic elution profiles on Aminex A-28, after treatment with cyanogen bromide. However, it is possible that the Q* containing tRNAs are retained on acylated DBAE cellulose, due to interactions of the galactose or mannose present in Q* (5) with this matrix. At present we have no direct experimental evidence to discriminate between these two alternatives. However, based on studies reported below concerning a highly purified species of rat liver $tRNA^{Asn}$, we believe that the former possibility (i.e. only Q containing tRNAs are retained on the acylated DBAE-cellulose matrix and that Q* containing tRNAs are not retained on this matrix) is the most plausible. Further insight into this problem will be gained by the isolation and direct nucleotide sequence analysis for the several Q and Q* containing mammalian tRNAs. However, in principle, the use of acylated DBAE-cellulose coupled with the analysis of untreated and cyanogen bromide treated mammalian tRNAs on Aminex A-28 can provide evidence for the absence or presence of Q (or Q*) in tRNAs isolated from different tissues.

We therefore attempted such a determination for the tRNAs isolated from the rat mammary tumor Walker 256. As can be seen in Table 1, the tRNA isolated from the tumor tissue showed a greatly decreased affinity for the acylated DBAE-cellulose matrix. Considering our previous discussion, these results imply that the tumor tRNA either contains an increased amount of Q* in place of a comparable amount of Q nucleoside or lacks both Q* and Q. The latter conclusion seems to be the most reasonable

explanation when the following experimental evidence is considered.

When the total tRNA from both rat liver and Walker 256 tumor tissue were subjected to alkaline hydrolysis and subsequently analysed for their Qp (or Q^*_p) content by chromatography on Dowex 1X8, the results shown in figure 2a,b were obtained. It can be seen in figure 2a that the rat liver tRNA contains an alkaline hydrolysis product which elutes in the position corresponding to Qp (or Q^*_p), fractions 34-37, and as reported (4). Two-dimensional cellulose TLC, followed by elution and spectral analysis confirmed that this alkaline hydrolysis product contained a mixture of both Qp and Q^*_p nucleotide (data not shown but similar to that in ref. 4). The results of a similar series of experiments, shown in figure 2b, indicate that there is a marked decrease in the Qp (and Q^*_p) content of Walker 256 tumor tRNA. These results suggest that the Walker 256 tumor tRNA either contains a modified Qp (or Q^*_p) nucleotide which cannot be resolved on the Dowex 1X8 column or that the Walker 256 tumor tRNA lacks these hypermodified nucleotides and instead contains some other nucleotide in the wobble position. Since, based on these observations, both conclusions are plausible, we proceeded to compare the Aminex A-28 chromatographic elution profiles for rat liver, Walker 256 tumor tissue, cyanogen bromide treated rat liver and cyanogen bromide treated Walker 256 tumor tissue tRNA. The results of these experiments for $tRNA^{Tyr}$ are shown in figures 1,3 and similar results were also obtained for $tRNA^{Asp}$, $tRNA^{Asn}$ and $tRNA^{His}$ (data not shown). These studies indicate that the Walker 256 tumor tRNA contains increased amounts of species which lack the hypermodified nucleosides Q and Q*, as they are not as sensitive to cyanogen bromide treatment as are the tRNAs isolated from rat liver. In addition, these results also show that species of Q (or Q*) lacking tRNAs are present in rat liver as the minor species, while they are present in the Walker 256 tumor tRNA as the major species. Furthermore, while these Q (or Q*) lacking tRNAs from Walker 256 tumor are not affected by cyanogen bromide treatment, the Q (or Q*) containing species from both rat liver and Walker 256 tumor tissue do show altered chromatographic mobilities upon treatment with cyanogen bromide. Therefore,

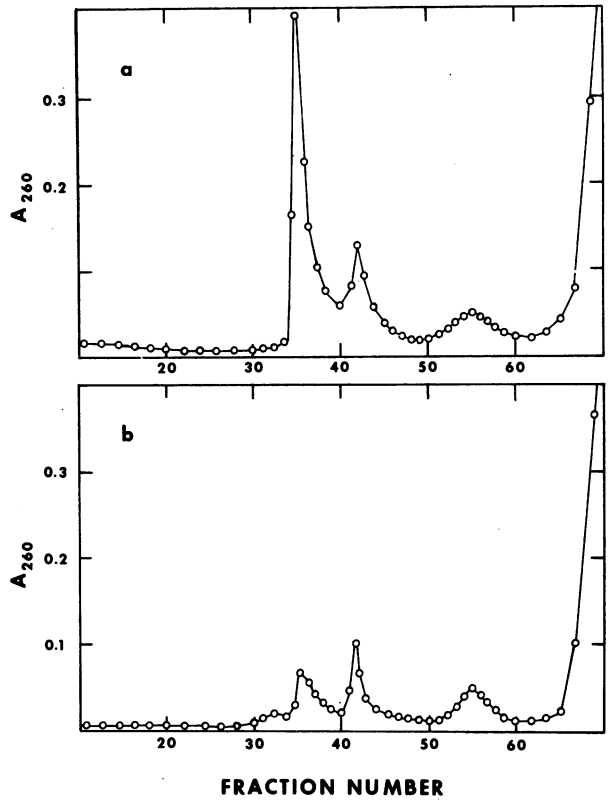

Figure 2. Chromatography of an alkaline digest of mammalian tRNA on Dowex 1X8.

a. Chromatography of an alkaline digest of 20 A_{260} units of total rat liver tRNA on Dowex 1X8 as described in Materials and Methods.

b. Chromatography of an alkaline digest of 20 A_{260} units of total Walker 256 tumor tRNA on Dowex 1X8 as described in Materials and Methods.

based on these three independent observations we conclude that the Walker 256 tumor tRNA does not contain increased amounts of Q* but rather contains some other nucleoside (modified or unmodified) in the wobble position. The exact nature of the nucleoside present, however, awaits the isolation and subsequent nucleotide sequence analysis for these tumor tRNAs.

With these future studies in mind, we therefore devised an isolation procedure aimed at purification of a Q containing

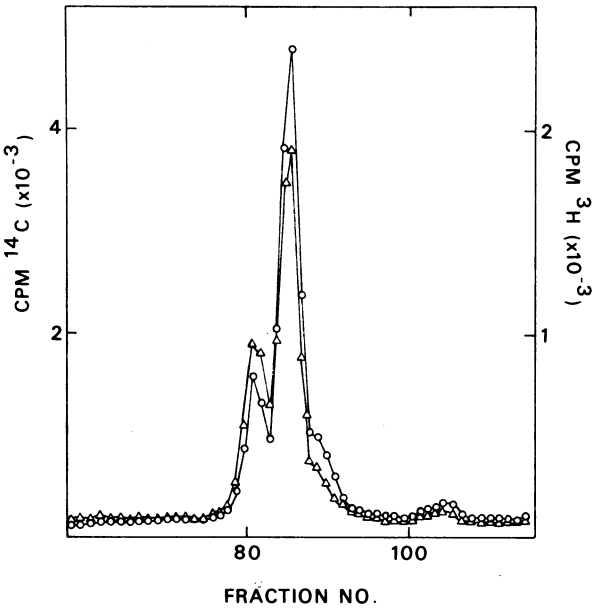

Figure 3. Cochromatography of ^{14}C-Tyr-tRNA^{Tyr} from Walker 256 tumor treated with cyanogen bromide prior to aminoacylation (0-0) and ^{3}H-Tyr-tRNA^{Tyr} from Walker 256 tumor aminoacylated without prior cyanogen bromide treatment (X-X). 30 A_{260} units of each aminoacyl-tRNA was applied to an Aminex A-28 column, eluted and the radioactivity determined as described in Materials and Methods.

species of mammalian tRNA as the first series of experiments leading to a determination of the exact nature of the observed alterations in these tRNAs when comparing rat liver and Walker 256 tumor tissue. The protocol for our isolation of a rat liver Q containing tRNA is as follows: Total crude mammalian tRNA was first chromatographed on BD-cellulose (9), followed by fractionation on acylated DBAE-cellulose, with the subsequent purification of the individual Q containing tRNAs to apparent homogeneity on RPC-5 and Aminex A-28 columns (10,13). A compilation of those tRNAs isolated to date using the above protocol, their apparent purity (based on amino acid acceptor activity) and their nucleoside compositions are shown in Table 2. Since the Q (and Q*)

Table 2. Nucleoside Composition of Mammalian tRNAs[a,b]

Nucleoside	$tRNA^{His}$ Human Liver	$tRNA^{Asn}$ Human Placenta	$tRNA^{Asn}$ Rat Liver
G	21.50	20.80	20.20
Ψ	1.43	2.70	2.88
A	13.21	11.70	11.40
U	10.30	9.60	9.40
m^2G	0.92	0.90	1.00
m^1G	0.97	0.78	0.97
m^5C	1.20	nd	nd
m^1A	1.20	1.06	0.80
C	21.00	20.96	21.06
hU	1.95	2.12	2.20
m^7G	0.90	0.94	0.90
m_2^2G	nd	0.77	0.95
X	nd	0.95	1.04
t^6A	nd	0.86	0.95
Qp	na	na	1.00[c]
rT	nd	nd	nd

[a] Molar ratios based on 75 nucleosides per tRNA as determined using the tritium post-labelling method (14). All experiments were performed in quadruplicate and the average values are given. The error was less than 5% for any quadruplicate data point.

[b] Each tRNA was isolated using the protocol discussed in Results and Discussion, and was eluted as Peak II on acetylated DBAE cellulose. The purity, based on amino acid acceptor activity, was 1.0, 1.1 and 1.2 nmoles per A_{260} unit for $tRNA^{His}_{H.L.}$, $tRNA^{Asn}_{H.P.}$ and $tRNA^{Asn}_{R.L.}$, respectively.

[c] The value for the Qp nucleotide content of rat liver $tRNA^{Asn}$ was determined by Dowex 1X8 chromatography of an alkaline digest as described in Materials and Methods, as it is not amenable to analysis using the tritium post-labeling technique (14). This value is an estimate based on terminal nucleoside present.

nd. Not detected

na. The Qp nucleotide content of human liver $tRNA^{His}$ and human placenta $tRNA^{Asn}$ was not analyzed.

nucleosides cannot be resolved using the tritium post labelling technique under the reported conditions (14 and our unpublished observations), analysis for Q (and Q*) was performed as described earlier (see Materials and Methods). Briefly, a neutralized alkaline hydrolysate of rat liver $tRNA^{Asn}$ was fractionated on the Dowex 1X8 column, the Qp (or Q^*_p) peak (fractions 34-37 of figure 2a) was subsequently resolved by 2-dimensional cellulose TLC and the UV absorption spectrum was determined on the eluted material. The results of this analysis demonstrated that the rat liver $tRNA^{Asn}$ contained the Qp, rather than the Q^*_p nucleotide, (data not shown). It can also be seen that,

although the mammalian tRNAs whose nucleoside compositions are listed in Table 2 are from different eucaryotic sources, they are similar (if not identical). This observation is in agreement with our earlier studies which indicated the similar nucleotide sequences for $tRNA^{Met}_{I}$ isolated from several eucaryotic tissues (18) and also the similar nucleotide sequences for $tRNA^{Phe}$ isolated from several eucaryotic tissues (19).

In conclusion, chromatography on acylated DBAE-cellulose can be a valuable technique for both the isolation of mammalian Q containing tRNAs, as well as for the determination of alterations in the Q nucleoside content for normal and tumor tRNAs. Nucleotide sequence analysis of the mammalian tRNAs which contain Qp (and Q^{*}_{p}) are needed to further understand the results reported here, as well as to determine the function of these hypermodified nucleotides and their observed alterations in normal and tumor tissue. Nucleotide sequence analysis of these tRNAs from human liver, human placenta and rat liver are presently underway in our laboratory.

While this manuscript was being written, a report by Okada, Shindo-Okada and Nishimura (20) was published which presented evidence that mammalian $tRNA^{Asp}$ and $tRNA^{Tyr}$ contain the Q^{*}_{p} nucleotide (mannose and galactose derivative, respectively). In this present communication our independent experiments confirm these results and extend them to show directly that mammalian $tRNA^{Asn}$ and indirectly that $tRNA^{His}$, as well as some species of $tRNA^{Asp}$ might contain the Qp nucleotide. In addition, the recent report of the chromatographic behavior of each calf liver tRNA isoacceptor on acylated DBAE-cellulose by Chinault, et al. (21) is in agreement with our observations for the mammalian tRNAs listed in Table 1.

ACKNOWLEDGMENTS

We thank Kevin Roesch, Debbie Pike and William Mook for their technical assistance at various stages during these experiments.

This work was supported by grants from the American Cancer Society (NP-230) and the National Institutes of Health (GM-21405). B. A. R. is a recipient of a Research Career Development Award (KO4-GM-00178).

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