Composition, Associated Tissue Methyltransferase Activity, and Catabolic End Products of Transfer RNA from Carcinogen-induced Hepatoma and Normal Monkey Livers


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SUMMARY

This investigation was designed to explore transfer RNA (tRNA) methyltransferase activity, urinary excretion levels of RNA degradation products, and tRNA base composition in normal monkeys and in those with hepatocellular carcinomas induced by N-nitrosodiethylamine. After the development of the tumor, 24-hr urine specimens were collected, and the monkeys were sacrificed, and the livers removed or hepatoma homogenates were found to possess increased tRNA methyltransferase activity. The tRNA methyltransferase activity and capacity and the base composition of the tRNA pools was analyzed by high-resolution liquid chromatography, and similar base compositions were found for the hepatoma-bearing monkeys when compared to those with normal liver. The isolated RNA pools were analyzed by high-resolution liquid chromatography, and similar base compositions were found for the hepatoma-bearing and normal monkeys. With the use of methyl-deficient Escherichia coli tRNA as the methyl receptor and the analytical procedure for tRNA analysis, the methylating ability of the tRNA methyltransferases in hepatoma and normal liver extracts was determined. The hepatoma methyltransferase homogenates were found to produce increased levels of 7-methylguanine, N\(^2\),N\(^2\)-dimethylguanosine, and \(\beta\)-aminoisobutyric acid for the hepatoma-bearing monkeys when compared to those with normal liver. The isolated RNA pools were analyzed by high-resolution liquid chromatography, and similar base compositions were found for the hepatoma-bearing and normal monkeys. With the use of methyl-deficient Escherichia coli tRNA as the methyl receptor and the analytical procedure for tRNA analysis, the methylating ability of the tRNA methyltransferases in hepatoma and normal liver extracts was determined. The hepatoma methyltransferase homogenates were found to produce increased levels of 7-methylguanine, N\(^2\),N\(^2\)-dimethylguanosine, and thymine, while the normal liver extracts gave higher levels of N\(^2\)-methylguanine. These differences were not apparent in the base composition of the tRNA pools. The increased urinary excretion and higher methyltransferase activity of the hepatoma-bearing monkeys without an apparent increase in the methylated base content of their RNA suggest increased tRNA turnover. However, subtle changes in the methylated base content of individual isoaccepting tRNA’s would be missed by analyzing the tRNA pools. The variations in the individual tRNA methyltransferase activities of the hepatoma and normal liver homogenates indicate a difference in the methylation of their RNA’s.

INTRODUCTION

tRNA (2, 3, 12, 24, 25, 29, 32) and variations in its cellular interactions and structure associated with neoplastic change have been of intense interest and study. In particular, the methylation of the intact tRNA macromolecule by methyltransferases of malignant cells has been investigated extensively, primarily by in vitro techniques using heterogeneous tRNA's (4, 8, 13, 14, 30, 33). Also, the urinary excretion levels of methylated degradation products and other minor base constituents derived predominantly from tRNA have been determined (10, 17-20, 22, 23, 34-37). It has been suggested that aberrant tRNA methylation and altered tRNA structure may be factors in the oncogenic process (3, 30). In 1971, Borek (3) reported that high tRNA methyltransferase activity had been found in more than 30 different malignant tumors when compared to their corresponding normal tissue of origin. Since then, these findings have been extended and verified in a variety of different tumor types involving both animal and human neoplasms (8, 13, 14, 33, 38). However, 20 benign tumors examined by Sheid et al. (28) had normal methyltransferase activity.

Initial investigations by Berquist and Matthews (2) and by Viale et al. (32) indicated that the tRNA’s isolated from tumor cells contain increased amounts of specific methylated nucleic acid derivatives when compared to their normal tissue counterpart. Recently, further analytical techniques have become available (17, 21, 26, 27); a report by Randerath et al. (25) indicated few, if any differences in the methylated tRNA base content between certain normal and related tumor tissues. More extensive comparative studies, however, are desirable in order to gain a better perspective regarding the relationship between alterations in tRNA-methylated base content and malignant change.

Increased urinary excretion of methylated and minor base nucleic acid derivatives that originate predominantly from tRNA as degradation products has been reported for a number of different malignant diseases (9, 10, 16, 19, 20, 22, 23, 34, 35, 37). Some of these compounds promise to be potential biological markers for diagnosis and of possible value in monitoring the effectiveness of therapy (31, 36). This investigation was designed to define more clearly the
possible relationship between increased tumor cell tRNA methyltransferase activity, the increased urinary excretion of minor base tRNA-catabolic end products, and the content of these bases in tRNA pools isolated from the same tumor cells. Previous preliminary study had shown that monkey hepatoma (hepatocellular carcinoma) cells, induced by N-nitosodimethylaniline, had increased tRNA methyltransferase activity (33) when compared to the cells of normal monkey liver. These original observations are being extended to determine the predominant individual methylated and minor nucleic acid base compounds and their levels found in urine from monkeys with hepatomas and, for comparative purposes, to isolate, purify, and degrade tRNA's from these hepatomas to determine their base composition. For control purposes, similar determinations were carried out using normal monkeys and tRNA isolated from their livers.

MATERIALS AND METHODS

Apparatus

A Mark II UV analyzer (Oak Ridge National Laboratories, Oak Ridge, Tenn.) equipped with a 150- x 0.455-cm (i.d.) column containing Aminex 27 anion-exchange resin (Bio-Rad Laboratories, Richmond, Calif.), a 2-channel gradient generating system (7), and a programmable temperature control system were used for separation and analysis of the major and minor bases present in tRNA's and for the analysis of the urinary excretion levels of the minor nucleosides and 7-MeGua. The eluate from the chromatographic column was monitored at 254 and 280 nm by a duo Monitor (Laboratory Data Control, River Beach, Fla.), and a Servo Riter II (Texas Instruments, Houston, Texas) dual-pen recorder was used to record the chromatograms.

A Packard Tri-Carb liquid scintillation spectrometer (Packard Instrument Company, Inc., Downers Grove, Ill.) was used to measure tRNA methyltransferase activities and to determine the extent of methylation of tRNA by the methyltransferases.

A sand bath with a variable temperature control (±2°C) was used for hydrolysis of the tRNA samples.

A Gilford 2400-S spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, Ottawa) was used to determine tRNA levels and protein content of tissue homogenates.

Samples and Sample Preparation

Animals and Induction of Cancer. N-Nitrosodimethylaniline was administered to normal healthy rhesus (Macaca mulatta) monkeys (male and female) as previously reported (1, 33). Periodic liver biopsies were done to determine the status of the organ, and monkeys with clinical and histopathological evidence of hepatocellular carcinoma were selected for study. The animals were killed by decapitation, the livers were removed, and tumor tissue was dissected free of surrounding liver. Portions of tumor were also taken for confirmatory histological examination. Normal control monkey livers were prepared at the same time. All tissues were stored at —170°C for later study. Just prior to decapsulation, 24-hr urine specimens from each hepatoma-bearing and normal control monkey were collected without preservative in containers chilled by surrounding ice. Aliquot samples were frozen and maintained at —170°C until analysis.

tRNA Methylase Activity. The tRNA methyltransferase activity of each liver tissue (4 hepatoma and 3 normal controls) was determined by the method reported earlier by Waalkes et al. (33). After thawing, the tissues were homogenized in 3 volumes of a solution containing 0.3 M sucrose, 1 mM dithiothreitol, 0.01 M MgCl₂, and 0.01 M Tris (pH 8.0), with a Teflon-glass motor-driven homogenizer. Tissue extracts were centrifuged at 105,000 × g for 1 hr at 4°C, and the supernatant fraction was filtered through glass wool. The filtrate was divided into aliquot samples and stored at —170°C for later enzyme assays.

Protein content of the tissue extracts were determined by the method of Lowry et al. (18).

For the measurement of the tRNA methyltransferase-specific activities (rates) of tissue homogenates, the reaction mixture contained 6.8 nmol S-adenosyl-L-[³⁵S]methionine (New England Nuclear, Boston, Mass.; 57.7 mCi/mmol), 25 μmol Tris-HCl (pH 9.0), 70 μmol ammonium acetate, 0.3 μmol dithiothreitol, variable amounts of tissue homogenates, and 60 μg undermethylated Escherichia coli tRNA prepared from a relaxed E. coli mutant K₁₂W₆ by the method of Fleissner and Borek (11). For determination of tRNA capacities (maximal extent of methylation), the reaction mixtures were the same except that only 1 μg of tRNA was used. For the latter assays, tRNA was shown to be limiting and the methyltransferases were in excess. Control samples without tRNA were run for each assay. Reactions were carried out at 37°C for 1 hr and terminated by immersion in ice water. Carrier tRNA, 0.05 ml of a 10-mg/ml solution, was added to each tube followed by 1.0 ml 95% ethanol. After 1 hr at —30°C, the tubes were centrifuged at 7000 rpm for 20 min at 4°C, and the ethanol was decanted off. To extract the tRNA from the precipitate, 1 ml of 1 M NaCl solution was added and incubated at 40°C for 45 min. After centrifugation at 12,000 rpm for 20 min, the supernatant was removed and mixed with 3 ml of 10% TCA. After 20 min in ice, the contents of each tube were filtered through type HA Millipore filters (pore size, 0.45 μm) and the precipitate was washed with 30 ml ice-cold 5% TCA. The filters were dried and put in vials containing 10 ml toluene phosphor, and radioactivity was determined in a Packard liquid scintillation counter.

Urinary Excretion Levels of Nucleic Acid Components. The urinary excretion levels of the nucleic acid components, pseudouridine m₂G, m₁, 7-MeGua, β-AlA, and β-alanine, were determined for the normal and hepatoma-bearing monkeys. The determination of the urinary excretion level of the minor nucleic acid derivatives was accomplished by a method previously reported, using high-resolution liquid chromatography for separation and quantitation.
(20, 34). The urine sample was first filtered through a 0.45-
\( \mu \)m Millipore filter and then placed directly on a 125- x
0.455-cm (i.d.) Aminex 27 anion-exchange column. The sample was eluted with a 0.015 M ammonium acetate buffer, pH 4.7, and the eluate was monitored at 254 and 280 nm. The urinary excretion levels of \( \beta \)-Ala and \( \beta \)-alanine were determined by a modification (C. W. Gehrke, and K. K. Kuo, unpublished observations) of the method previously reported (35). The analysis of m1 by the liquid chromatographic method was not possible due to an interfering urinary component. The excretion levels for m1 were determined by the gas-liquid chromatographic method reported by Chang et al. (6).

**Transfer RNA Isolation and Analysis.** tRNA was isolated from the hepatoma and normal monkey liver tissues by the method described by Gallo and Pestka (12) with an additional final purification by chromatography on DEAE-cellulose as described by Yang and Novelli (39). After thawing, the tissue was homogenized with a solution containing 0.01 M Tris, 0.15 M NaCl, 1 mM EDTA, 1 mM MgCl\(_2\), and 1% bentonite (1 g tissue per ml solution). Following centrifugation, the supernatant was extracted with phenol (freshly distilled), saturated with the homogenizing solution, and then with chloroform. The aqueous phase was mixed with 2.2 volumes 95% ethanol and 0.1 volume 20% potassium acetate and allowed to stand overnight at −30°. After centrifugation at 10,000 rpm, the ethanol was decanted and the tRNA precipitate was dissolved in the homogenizing solution. After 2 chloroform extractions and precipitation with ethanol-potassium acetate, the tRNA precipitate was dissolved in a solution of 1 M NaCl, 1 mM EDTA, and 10 mM MgCl\(_2\). The extract was centrifuged at 10,000 rpm, and the supernatant was removed. The residue was extracted again and centrifuged, and the supernatants were combined and precipitated with ethanol:potassium acetate. The tRNA precipitate was dissolved in deionized water and centrifuged, and the supernatant was subjected to DNase at 37° for 15 min. The enzyme was removed by extraction with phenol, and the aqueous layer was mixed with ethanol:potassium acetate to precipitate the tRNA. The sample was centrifuged at 10,000 rpm, and the precipitate was dissolved in deionized water and centrifuged at 40,000 rpm for 1 hr. The supernatant was removed, shell frozen, and lyophilized. The tRNA was dissolved in deionized water and placed on a 12.5- x 1.0-cm (i.d.) DEAE column. The column was washed with 50 ml of 1.2 M NaCl, 1 mM MgCl\(_2\), and the tRNA was eluted with 50 ml of 0.7 M NaCl at 1 ml/min. The fractions containing the tRNA were combined and precipitated with ethanol. The precipitate was dissolved in water, shell-frozen, and lyophilized. The tRNA was stored at −170° for later use.

The analytical procedure used to determine the methylated base content of the normal and hepatoma monkey liver tRNA pools was reported earlier (17). The tRNA pools were first hydrolyzed to the free bases with 400 \( \mu \)l of trifluoroacetic acid:formic acid (1:1, v/v) heated in a closed vial at 200° for 1.5 hr (15). The hydrolyzing agents were removed by simple evaporation, and the sample was dissolved in 1.0 ml 0.1 N NaOH. The sample was placed on the Mark II UV Analyzer, and the major and minor bases separated using a 2-chamber gradient system (7). The 1st chamber contained 0.015 M ammonium acetate, pH 9.2, and the 2nd contained 1.2 M ammonium acetate, pH 9.2. The flow rate was approximately 35 ml/hr, and the eluate was monitored at 254 and 280 nm. The temperature was at ambient for the first 1.75 hr and then programmed at 0.5°/min to 60° for the remainder of the analysis. To measure quantitatively the MeB content of the individual tRNA's, duplicate samples containing 200 to 300 \( \mu \)g tRNA were hydrolyzed and analyzed. The level of each MeB was calculated by measuring the peak height at 254 or 280 nm and comparing it to the peak height of a known amount of that MeB on a standard chromatogram. A similar procedure was used to determine the major base levels of each tRNA, except that 70 to 80 \( \mu \)g of tRNA were hydrolyzed.

Since pseudouridine was found to be degraded during the hydrolysis of the tRNA to the free base level, it was necessary to analyze the tRNA's at the nucleoside level to determine the pseudouridine content. The tRNA samples were enzymatically hydrolyzed to the nucleoside level in 0.1 ml 0.2 M sodium acetate and 0.02 M magnesium acetate (pH 8.8) containing 80 \( \mu \)g snake venom phosphodiesterase and 1 unit alkaline phosphatase (27). The samples were incubated at 37° for 4 hr, and the enzymes were removed by precipitation with ethanol. The resulting nucleosides were placed on the analyzer and separated with a 0.02 M ammonium acetate buffer, pH 3.2, and the pseudouridine level was determined from its extinction coefficient at 254 nm.

**Assessment and Comparison of Degree of Methylation.** To evaluate further and compare the possible differences in the individual activities and methylating ability of the tRNA methyltransferases present in normal liver and hepatoma homogenates, undermethylated E. coli tRNA, as substrate, was reacted in vitro with S-adenosyl-L-[\( ^{14} \)C]methionine in the presence of the individual tissue extracts. The amounts of the methyltransferase reaction described earlier were increased 10-fold each, the total protein content for each tissue homogenate was adjusted to exactly 20 mg, and the methyl-deficient E. coli tRNA content was 200 \( \mu \)g. The isolation and purification of the E. coli tRNA were as previously indicated. The final tRNA precipitate, however, was not collected on a Millipore filter after the 10% precipitation but was washed first with 5% TCA, centrifuged, and then precipitated with ethanol. The tRNA was hydrolyzed and subsequently analyzed by the high-resolution liquid chromatographic method discussed earlier. The eluate was collected in 5-ml fractions, dried, redissolved in 0.5 ml 0.1 N acetic acid, and counted in 10 to 15 ml Aquasol liquid scintillation fluid in a Packard Tri-Carb spectrometer. The identification of the various MeB was verified by the addition of 2.5 \( \mu \)g of each individual base to the hydrolyzed tRNA sample prior to liquid chromatographic analysis. For determination of the amount of the \( ^{14} \)C methyl group present in the isolated tRNA as methylated bases, an aliquot was taken and counted prior to hydrolysis; the prehydrolysis count was then compared to the summation of the MeB counts obtained after analysis.

For further study of the individual tRNA methyltransferase activities and methylating abilities, E. coli \( \beta \) tRNA and normal monkey liver and hepatoma tRNA were used as the methyl receptors. The methyltransferase reaction condi-
RESULTS

tRNA Methyltransferase Activity and Capacity. In Chart 1A, the results obtained when undermethylated E. coli tRNA was used as the methyl acceptor for varying amounts of tissue homogenates from hepatoma and normal monkey livers are shown. In these experiments, the 60 μg tRNA per sample were in excess, and the enzyme was limiting. The tRNA methyltransferase activities of the 4 hepatoma homogenates were 1.5 to 2.0 times higher than those of the 3 normal livers. Chart 1B depicts the curves obtained when the tRNA was limiting (1 μg/assay) with the same level of tissue extracts as previously used. At saturation, the 4 hepatoma homogenates produced a greater level of methyl group incorporation when compared to those of the normal livers.

Urinary Excretion Levels. The average urinary excretion levels of pseudouridine, m1G, m22G, and 7-MeGua expressed in mg/kg/24-hr urine volume are presented in Table 1. Two 24-hr collections were obtained and analyzed for hepatoma-bearing Monkeys 1, 2, and 3 and for normal Monkey 3. Also in Table 1, the average excretion levels of β-AIBA and β-alanine in μmoles/kg/24 hr volume for the hepatoma and normal monkeys are presented, along with the β-AIBA:β-alanine ratio. The standard error for each of the compounds was calculated and is given in Table 1. The excretion levels of the tRNA degradation products for the hepatoma-bearing monkeys were all higher than the levels of the corresponding compound from the normal monkeys. The degree of elevation varied among the hepatoma monkeys and for individual compounds. The average level of excretion for pseudouridine, m22G, and β-AIBA for the hepatoma-bearing monkeys was more than twice the average level for the normal monkeys.

Isolation and Analysis of tRNA. Using the procedure outlined in “Materials and Methods” for tRNA isolation, the livers of 3 normal and 3 hepatoma-bearing monkeys were homogenized and the tRNA was purified. A typical chromatogram obtained from the final DEAE column purification step is shown in Chart 2. Each isolated tRNA sample gave a single chromatographic peak indicating a homogeneous mixture of the various individual tRNA’s. Charts 3 and 4 present chromatograms for the analysis of MeB in hepatoma and normal monkey liver tRNA pools, respectively. The average mole % composition and the standard error for the normal and hepatoma tRNA’s are given in Table 2. The pseudouridine content of the tRNA’s was determined at the nucleoside level; chart 5 presents a typical chromatogram for pseudouridine analysis. The average μmole pseudouridine per μmole uracil % and the standard error for the normal and hepatoma tRNA pools are also given in Table 2.

Individual tRNA Methyltransferase Capacity. The methyltransferase capacity of the hepatoma and normal monkey

<table>
<thead>
<tr>
<th>Compound</th>
<th>Normal a</th>
<th>S.E.</th>
<th>Hepatoma a</th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudouridine</td>
<td>1.49</td>
<td>0.12</td>
<td>3.06</td>
<td>0.18</td>
</tr>
<tr>
<td>m22G</td>
<td>0.11</td>
<td>0.01</td>
<td>0.21</td>
<td>0.01</td>
</tr>
<tr>
<td>m1G</td>
<td>0.08</td>
<td>0.01</td>
<td>0.11</td>
<td>0.01</td>
</tr>
<tr>
<td>7-MeGua</td>
<td>0.16</td>
<td>0.02</td>
<td>0.27</td>
<td>0.01</td>
</tr>
<tr>
<td>β-AIBA</td>
<td>0.544</td>
<td>0.064</td>
<td>1.43</td>
<td>0.17</td>
</tr>
<tr>
<td>β-Alanine</td>
<td>10.45</td>
<td>0.69</td>
<td>11.36</td>
<td>0.58</td>
</tr>
<tr>
<td>β-AIBA:β-Alanine</td>
<td>0.052</td>
<td>0.006</td>
<td>0.132</td>
<td>0.018</td>
</tr>
</tbody>
</table>

a Average normal monkey excretion levels. Single 24-hr collections for two monkeys and two 24 hr collections for 1. All samples analyzed in duplicate.

b Average hepatoma monkey excretion levels. Two 24-hr collections for 3 monkeys. All samples analyzed in duplicate.
The results in Chart 6 show that by in vitro interaction the methyltransferases of the hepatoma and normal monkey livers possess different methylating abilities for specific bases in the same tRNA. The hepatoma methyltransferases, when compared to the average of the normal enzymes, gave increased levels of thymine, 2-MeGua, 1-MeGua, and 7-MeGua; decreased N²-methylguanine content; and similar levels for 5-MeCyt, 1-methylhypoxanthine, and 1-methyladenine:N⁶-methyladenine.

The average methyltransferase activity with undermethylated E. coli tRNA as substrate, the mole % of the isolated tRNA's and the urinary excretion levels are compared in Table 3 for the hepatoma-bearing and normal monkeys. Increased hepatoma methyltransferase activity for thymine, 2-MeGua, and 7-MeGua correlated well with the increased excretion of these compounds by the hepatoma-bearing monkeys; however, the hepatoma tRNA content for these
Comparison of methyltransferase activity, tRNA content, and urinary excretion level.

<table>
<thead>
<tr>
<th>Methyltransferase activity</th>
<th>tRNA content</th>
<th>Urinary excretion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>1.0</td>
<td>1.38</td>
</tr>
<tr>
<td>Hepatoma</td>
<td>3.2</td>
<td>5.67</td>
</tr>
<tr>
<td>2-MeGua</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>17.3</td>
<td>0.51</td>
</tr>
<tr>
<td>Hepatoma</td>
<td>22.7</td>
<td>0.48</td>
</tr>
<tr>
<td>1-Methylhypoxanthine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>0.8</td>
<td>0.40</td>
</tr>
<tr>
<td>Hepatoma</td>
<td>0.7</td>
<td>0.44</td>
</tr>
<tr>
<td>7-MeGua</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>12.1</td>
<td>0.57</td>
</tr>
<tr>
<td>Hepatoma</td>
<td>19.9</td>
<td>0.41</td>
</tr>
<tr>
<td>Pseudouridine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>1.16</td>
<td></td>
</tr>
<tr>
<td>Hepatoma</td>
<td>0.74</td>
<td></td>
</tr>
</tbody>
</table>

* Average of values obtained from 3 hepatoma-bearing and 3 normal monkeys.

Compounds was not increased; in fact pseudouridine tRNA content was reduced while the excretion levels were increased.

When E. coli β tRNA was the methyl receptor for the methyltransferases, the profiles for the MeB were similar to those obtained for undermethylated E. coli tRNA. The hepatoma methyltransferases for 5-MeCyt showed increased activity with E. coli β tRNA as substrate, whereas incorporation of 7-MeGua in E. coli β tRNA was greatly reduced for normal and hepatoma methyltransferases. Also, a methyltransferase for 2-methyladenine was detected with E. coli β tRNA.

Table 3

Comparison of methyltransferase activity, tRNA content, and urinary excretion level.

Chart 5. High-pressure liquid chromatography analysis of pseudouridine in hepatoma monkey tRNA. Column, 125 x 0.465 cm i.d.; Aminex A-27 anion-exchange resin. Eluent, 0.02 M ammonium acetate (pH 3.2). Temperature, as given for Chart 3. Flow rate, 20 ml/hr (1.4 ml/sq cm/hr). UV output 0.04 absorbance until full scale. Peak identification: 1, N^-methylcytosine, N^-methyladenine, 7^-methylguanine; 2, cytosine, N^-methylcytosine; 3, pseudouridine; 4, uracil, 5, adenine; 6, N^-methylguanine; 7, m22G; 8, guanine, N^-methylguanine, N^-methyladenine.

Chart 6. Distribution of methylated bases in undermethylated E. coli tRNA after methyltransferase reaction. Experimental conditions for methyltransferase reaction, tRNA purification and hydrolysis, liquid chromatographic separation and collection, and liquid scintillation counting given in text. —, average of normals; —, 1 S.D. from normal average; O, normals; —, Hepatoma 1; O, Hepatoma 2; Δ, Hepatoma 3; Δ, Hepatoma 4. Total counts: 10, 10,000; 20, 20,000. 1-MeAd, 1-methyladenine; 6-MeAd, N^-methyladenine; Thy, thymine; 1-MeHypo, 1-methylhypoxanthine.

Chart 7. Distribution of methylated bases in Normal 2 and Hepatoma 3 tRNA using their respective tRNA methyltransferases, calculated as percentage of total MeB. Bars: H-H, Hepatoma 3 tRNA and methyltransferases; N-H, Normal 2 tRNA and Hepatoma 3 methyltransferases; H-N, Hepatoma 3 tRNA and Normal 2 methyltransferases; N-N, Normal 2 tRNA and methyltransferases.

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prior saturation of the methylation sites or a difference in the methyl receptor sites between the monkey or E. coli tRNA’s. As shown in Chart 7, only minor variations were found by using the normal monkey methyltransferases with either the hepatoma or normal tRNA as substrate. Similar MeB incorporation levels were observed when the hepatoma methyltransferase-normal tRNA substrate were interacted except for an increased 2-methylguanine content. The hepatoma methyltransferase-hepatoma tRNA substrate gave increased thymine incorporation and reduced levels of 5-MeCyt and 1-MeGua.

**DISCUSSION**

The increased tRNA methyltransferase activity and capacity of the monkey hepatoma tissue homogenates confirm the results of an earlier report (33). The increased urinary excretion levels of specific tRNA degradation products for patients, including those with a number of different types of malignant disease, have been described before (16, 20, 33–37). The results presented here show a similarly increased excretion of pseudouridine m7G, 7-MeGua, and β-AIBA by monkeys with N-nitrosodiethylamine-induced hepatomas compared to normal control monkeys. The analysis of the isolated tRNA from hepatoma tissue and normal livers from these same monkeys showed similar, or slightly decreased, levels of tRNA-methylated bases. Randerath et al. (25) have reported similar or decreased levels of MeB’s in rat liver hepatoma tRNA which agrees with the data presented for the monkey liver tRNA’s. The values reported for thymine in the tRNA pools varied greatly, which could be caused by DNA contamination (5). Although the tRNA isolation procedure contained a DNase step and final tRNA purification by DEAE-cellulose chromatography, the thymine tRNA values are considered questionable and were not used in the evaluation of the results.

As presented in “Results” when undermethylated E. coli tRNA was used as the methyl receptor, the hepatoma liver homogenates were able to synthesize greater amounts of thymine, 2-MeGua, 1-MeGua, and 7-MeGua than the normal liver extracts with the same tRNA substrates. Similar ratios of the MeB, using the hepatoma and normal homogenates, were obtained when E. coli β tRNA was the substrate, except for 5-MeCyt, which was increased by the hepatoma methyltransferases. The ratio of 7-MeGua incorporation for the 2 E. coli tRNA’s as substrate was similar; however, a much lower level of synthesis of 7-MeGua was obtained with E. coli β tRNA. The increased methyltransferase activity for 2-MeGua, 1-MeGua, and 5-MeCyt does not show increased tRNA content in the hepatoma-bearing monkeys although an increased excretion level for M22G was noted. When the hepatoma methyltransferases were reacted with their own isolated tRNA, reduced levels of 5-MeCyt and 1-MeGua were found, correlating with the hepatoma tRNA composition data and suggesting different methyl receptor sites for the E. coli and monkey liver tRNA’s.

Comparison of the urinary excretion levels, tRNA base composition, and individual tRNA methyltransferase activity for the hepatoma-bearing and normal monkeys indicates both increased tRNA turnover and aberrant tRNA methylation in the hepatoma-bearing monkeys. The increased total tRNA methyltransferase activity and capacity and the greater urinary excretion levels for the catabolic tRNA end products without a corresponding increase in the tRNA composition suggest a more rapid tRNA turnover. This is further substantiated by increased pseudouridine excretion by the hepatoma-bearing monkeys, although pseudouridine content is actually decreased in the hepatoma-bearing monkeys. Also, Borek et al. (2) have recently found very high turnover of tRNA in rats with tumors of the bladder. The variations in the individual tRNA methyltransferase activities of the hepatoma and normal liver homogenates point to differences in the methylation of the tRNA’s. Since there are at least 60 tRNA species present in the mammalian cells (12) and since the tRNA base composition was determined on the total tRNA pool, it is possible that aberrant methylation occurs on only a few of the species, and these changes are not detected when the total tRNA pool is analyzed. Further fractionation of the tRNA pool into individual tRNA’s will be necessary to determine whether alterations occur on only a few of the tRNA’s that are present.

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