

Effects of DL-ethionine on mouse liver tRNA base composition

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ABSTRACT

Treatment of mice with DL-ethionine and adenine causes a reduction of all methylated bases of liver tRNA. This effect is dose-dependent and specific for the methylated bases. Individual methylated components are affected to different extents, m₂G being most sensitive to inhibition.

INTRODUCTION

Administration of DL-ethionine, the ethyl analog of methionine, to rodents induces hepatocellular carcinomas¹⁻⁵ but the exact mechanism underlying the carcinogenic action of this compound is far from being understood. Ethionine inhibits the synthesis of protein⁶ and RNA⁷ and, in addition, leads to the ethylation⁸⁻¹⁷ of proteins and nucleic acids. Hancock⁴, Hancock and Forrester⁵, Moore and Smith¹⁸, and Wainfan *et al*¹⁹ have demonstrated enhanced activity of liver tRNA methyltransferases following the administration of ethionine. Other carcinogens such as dimethylnitrosamine²⁰, dimethylaminoazobenzene⁵, and N-nitrosomethylurea²¹ have also been found to induce increased activity of these enzymes.

As recently reported by Rajalakshmi, tRNA exhibiting significant *in vitro* methyl acceptor activity in a homologous assay system can be isolated from livers of rats treated with a mixture of ethionine and adenine²². This work has been confirmed and extended by Wainfan *et al*¹⁹ and Kerr²³, who also characterized the undermethylated tRNA by its ability to accept methyl groups *in vitro*.

We have reported earlier on the isolation of mammalian tRNA specifically lacking 5-methylcytidine (m⁵C⁻ tRNA)²⁴.

Since we are interested in comparing the properties of m^5C^- tRNA with those of generally undermethylated tRNA, we have isolated tRNA from the livers of mice that had been treated with DL-ethionine plus adenine and determined its base composition by direct measurement, using a tritium derivative method developed in our laboratory^{25,26}. Base analysis led to the observation that the individual methylated bases are affected to very different extents by the drug treatment.

MATERIALS AND METHODS

Female BALB/Crg1 mice (about 25g), bred and maintained at the mouse colony of the Department of Cell Biology of Baylor College of Medicine, were used. DL-Ethionine and adenine were purchased from Sigma Chemical Co. DL-Ethionine was dissolved in 0.9% NaCl solution. Adenine was dissolved in a small volume of 0.1 N HCl, diluted with 0.9% NaCl and the pH was adjusted to 3. Mice (5 groups, 5 animals per group) were given DL-ethionine and adenine at a ratio of 25 : 12²² by intraperitoneal injections, once daily for 3 days, and were killed by cervical dislocation 24 hr after the last injection. Control animals received 0.9% NaCl.

Livers were removed immediately and kept frozen at $-80^{\circ}C$ until extraction. Crude 4S RNA was prepared by phenol extraction at pH 4.5 and adsorption to DEAE-cellulose^{27,28}. tRNA was further purified by gel electrophoresis²⁹. The base composition of various tRNA samples was analyzed by a tritium derivative method^{25,26}. Separations of nucleoside trialcohols were carried out on cellulose sheets (EM Laboratories, No. 5502) in solvents A and B of ref. 26.

RESULTS

In Table 1, base composition data are given for liver tRNA of mice that had been treated with various doses of ethionine plus adenine, as well as for control tRNA. The results demonstrate a dose-dependent decrease of all methylated components of tRNA following drug administration, while the non-methyla-

Base Composition (mole %, mean \pm S.D.) ^a					
Dose (mg/kg/day) Ethionine/Adenine 0/0		50/24	100/48	150/72	250/120
Nucleoside					
Uridine	14.01 \pm 0.10	13.88 \pm 0.08	14.11 \pm 0.12	14.42 \pm 0.15	14.35 \pm 0.24
Adenosine	17.12 \pm 0.12	16.73 \pm 0.11	17.05 \pm 0.07	17.17 \pm 0.24	17.37 \pm 0.10
Cytidine	25.98 \pm 0.20	26.56 \pm 0.53	26.45 \pm 0.20	26.74 \pm 0.27	26.54 \pm 0.14
Guanosine	27.00 \pm 0.25	27.94 \pm 0.11*	27.81 \pm 0.08*	27.95 \pm 0.22*	27.62 \pm 0.08*
m ⁵ U	0.57 \pm 0.01	0.45 \pm 0.01*	0.42 \pm 0.01*	0.40 \pm 0.00*	0.42 \pm 0.01*
hU	2.96 \pm 0.02	2.96 \pm 0.01	3.00 \pm 0.01	2.86 \pm 0.11	2.95 \pm 0.06
Ψ	3.88 \pm 0.02	3.89 \pm 0.06	3.99 \pm 0.05	3.97 \pm 0.08	4.00 \pm 0.04
X	0.64 \pm 0.07	0.70 \pm 0.04	0.71 \pm 0.05	0.65 \pm 0.06	0.62 \pm 0.08
m ¹ A	1.26 \pm 0.02	1.14 \pm 0.02*	1.15 \pm 0.03*	1.03 \pm 0.03*	1.09 \pm 0.01*
Inosine	0.29 \pm 0.01	0.28 \pm 0.01	0.32 \pm 0.01	0.31 \pm 0.03	0.29 \pm 0.02
m ³ C	0.39 \pm 0.01	0.38 \pm 0.01	0.29 \pm 0.03*	0.25 \pm 0.02*	0.26 \pm 0.01*
m ⁵ C	1.88 \pm 0.04	1.52 \pm 0.05*	1.37 \pm 0.02*	1.23 \pm 0.04*	1.30 \pm 0.02*
ac ⁴ C	0.24 \pm 0.02	0.27 \pm 0.01	0.27 \pm 0.02	0.25 \pm 0.02	0.24 \pm 0.02
m ² ₂ G	0.62 \pm 0.01	0.45 \pm 0.01*	0.40 \pm 0.00*	0.37 \pm 0.02*	0.40 \pm 0.01*
m ² ₁ G	1.31 \pm 0.02	1.12 \pm 0.01*	1.03 \pm 0.02*	0.94 \pm 0.03*	0.99 \pm 0.02*
m ¹ G	0.80 \pm 0.00	0.78 \pm 0.01	0.75 \pm 0.01*	0.67 \pm 0.01*	0.70 \pm 0.01*
m ⁷ G	1.06 \pm 0.04	0.97 \pm 0.03	0.86 \pm 0.05*	0.79 \pm 0.04*	0.84 \pm 0.06*
Total	100.01	100.02	99.98	100.00	99.98
Unknown 1 ^b	-	0.11 \pm 0.01	0.16 \pm 0.00	0.17 \pm 0.01	0.15 \pm 0.01
Unknown 2 ^b	-	0.04 \pm 0.01	0.05 \pm 0.02	0.06 \pm 0.01	0.05 \pm 0.01
Unknown 3 ^b	-	0.04 \pm 0.02	0.07 \pm 0.02	0.06 \pm 0.02	0.05 \pm 0.02
Modified N ^c	15.90 ^c 0.14	14.91 \pm 0.10*	14.56 \pm 0.10*	13.72 \pm 0.11*	14.10 \pm 0.17*
Methylated N ^c	7.89 \pm 0.09	6.81 \pm 0.10*	6.27 \pm 0.06*	5.68 \pm 0.04*	6.00 \pm 0.08*
Modified N - Methylated N ^c	8.01 \pm 0.08	8.10 \pm 0.06	8.29 \pm 0.08	8.04 \pm 0.10	8.10 \pm 0.13

Table 1

The effects of DL-ethionine plus adenine administration on the base composition of bulk tRNA isolated from mouse liver.

Footnotes for Table 1:

*Probability < 0.001 (student's t-test).

^aEach labeled tRNA digest, derived from pooled liver samples of each group of animals, was subjected to 5 chromatographic analyses.

^bThe count rates of the unidentified spots were not included in the total count rates for the calculation of the base compositions. The mole% of unknown spots is expressed as fraction of the total count rates.

^cModified N, Modified nucleosides.
Methylated N, m³C, m⁵C, m⁵U, m¹A, m²₂G, m²₁G, m¹G, m⁷G.
Modified N - Methylated N, Ψ, hU, X, I, ac⁴C. X is 3-(3-amino-3-carboxypropyl)uridine⁴⁴.

ted modified nucleosides do not exhibit any dose-dependent changes. Except for m^3C , m^1G , and m^7G at the lowest dose used, the decrease in the methylated nucleosides is statistically highly significant (t-test, $P < 0.001$). The slight increase in the amounts of the major nucleosides appears to be a direct result of decreased methylation, since U_{total} (= U + all modified derivatives of U), A_{total} , C_{total} , and G_{total} did not show any dose-dependent changes. As shown in Table 1, the sums of both the modified and methylated nucleosides exhibit dose-dependent decreases, while the sum of the non-methylated, modified nucleosides ($U + hU + X + I + ac^4C$) is not affected. The dose-dependence of this inhibitory effect on methylation is illustrated in Figure 1.

The individual methylated bases were found to be influenced to different extents by the drug treatment, with m^2G being most strongly affected (Figure 2). At the highest dose used, a slight increase in the amount of all methylated bases was observed as compared to the values obtained from mice

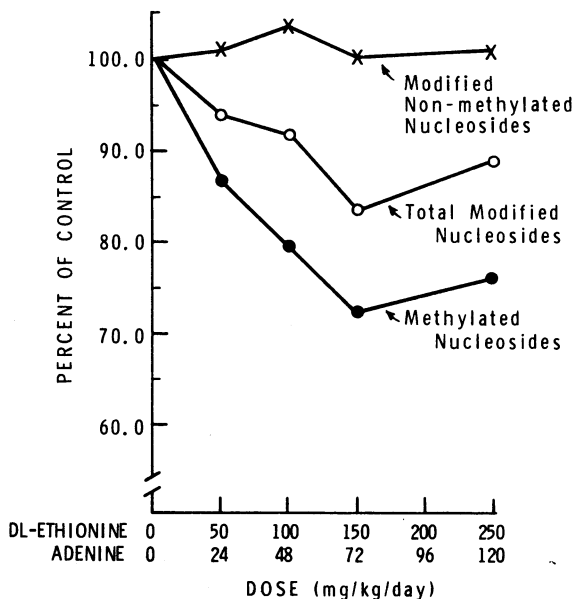


Figure 1. The selective effect of DL-ethionine plus adenine administration on the modification of mouse liver tRNA. The decrease in the percent of total modified nucleosides is a result of the decreased amount of methylated bases.

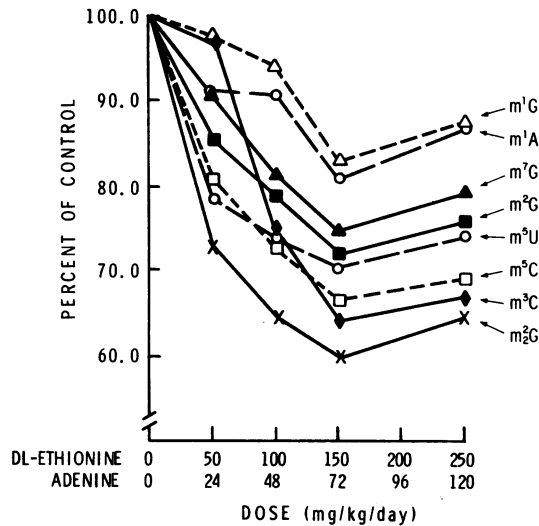


Figure 2. Effects of DL-ethionine on individual methylated bases in mouse liver tRNA.

that had received 150 mg/kg of ethionine plus 72 mg/kg of adenine.

Three unidentified spots were detected on fluorograms obtained from drug-treated mice (Table 1; Fig. 3, spots marked U1, U2, and U3). The chromatographic mobilities of these compounds relative to the mobility of adenosine trialcohol (R_A) were 1.25 and 1.94 (Unknown 1); 1.58 and 2.00 (Unknown 2); 0.70 and 0.64 (Unknown 3), respectively, for the 1st and 2nd dimensions of the map.

DISCUSSION

Since it was not possible until recently to prepare undermethylated tRNA from mammalian tissues, heterologous tRNA preparations, i.e. from *E. coli* or yeast, had to be used to assay the activity of mammalian tRNA methyltransferases. In view of numerous reports on increased tRNA methyltransferase activities in neoplasms³⁰ as well as following the administration of carcinogens, the preparation of homologous undermethylated mammalian tRNAs appears highly desirable since this may enable one to characterize and assay mammalian tRNA methyltransferases in a more precise way than has been hereto-

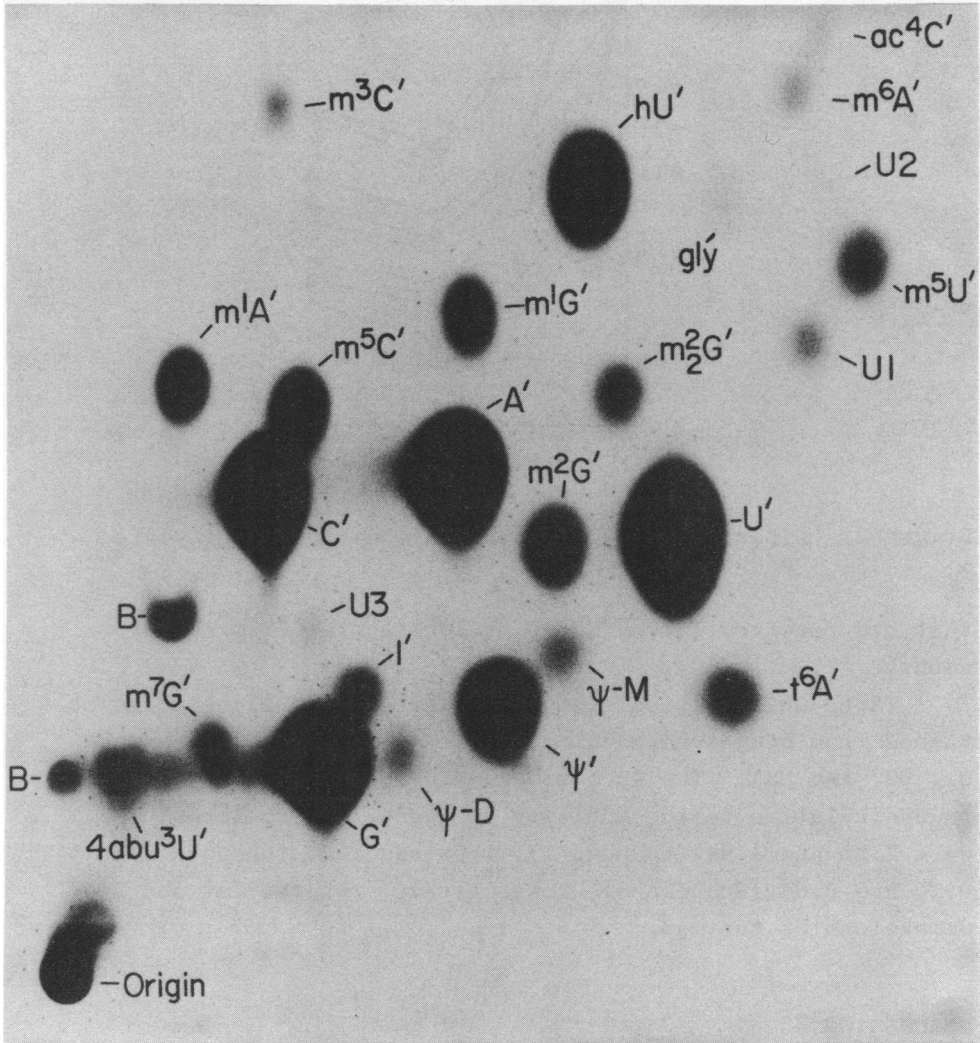


Figure 3. Fluorogram of cellulose thin-layer map obtained by digestion to nucleosides and tritium labeling²⁵ of liver tRNA from mice treated with 150 mg/kg of DL-ethionine and 72 mg/kg of adenine once daily for three days. 4abu³U, 3-(3-amino-carboxypropyl) uridine; ψ-D and ψ-M, traces of labeled products derived from pseudouridine; spots marked B, background label (not from RNA).

fore possible.

We have recently isolated a specifically 5-methylcytidine deficient tRNA from the livers of mice that had been treated

with the antileukemic agent, 5-azacytidine²⁴. To compare the properties of m^5C^- tRNA with those of randomly undermethylated mouse liver tRNA, we have adopted a procedure similar to the one recently reported by Rajalakshmi²² for the production of undermethylated rat liver tRNA.

Such undermethylated liver tRNA, obtained from rats given ethionine plus adenine, has been characterized thus far by in vitro methyl acceptor assays^{19,22,23,31}, using crude enzyme preparations. In such assays, individual methylations can be inhibited or stimulated differentially by various compounds^{32,33}. Thus, in vitro methyl acceptor activity of undermethylated tRNA may not correlate with the actual degree of undermethylation, and such measurements do not provide information about possible drug effects on other modified constituents.

The tritium derivative method for base analysis enabled us not only to measure directly the degree of undermethylation but also to detect a selective inhibitory effect of ethionine on mouse liver tRNA methylation. A dose-dependent decrease of the individual methylated bases but not of other modified constituents was also demonstrated by applying this method.

Adenine was administered together with ethionine since this compound is known to alleviate a major acute toxic effect of ethionine, i.e. the depletion of the ATP pool³⁴. Wainfan et al have reported that adenine alone does not inhibit mammalian tRNA methylation¹⁹.

A significant finding of the present study is that different methylated bases are affected to different extents by ethionine administration. Of all the methylated bases analyzed, m^2G appears to be most sensitive to the drug treatment, while the methylation of N1 of adenine and guanine is least affected (Figure 2). For unknown reasons, the amount of m^3C is only slightly reduced at the lowest dose used. It has been suggested that the inhibitory effect of ethionine on methylation is mediated by S-adenosyl ethionine¹⁸. As to the mechanism underlying the differential degree of inhibition, the individual tRNA methyltransferases may possibly exhibit different K_i 's for S-adenosyl ethionine, as has been reported

for S-adenosyl homocysteine³⁵.

As demonstrated in the present study, the inhibition of methylation of tRNA after ethionine treatment has no effect on the other base-modifications, i.e. the amounts of hU, Ψ , X, I, and ac⁴C remain unchanged, suggesting that the biosynthesis of the latter compounds is independent of prior methylation. The previously reported isolation of mammalian tRNA specifically lacking m⁵C²⁴ demonstrates also that the inhibition of the synthesis of this individual methylated base has no influence on the formation of the other methylated or modified bases assayed. These observations are in agreement with results of Davis and Nierlich³⁶, which suggest a random sequence of methylations in *E. coli* tRNA.

At the highest dose used (250 mg/kg of ethionine), the percent decrease in the methylated bases is slightly less than that observed at 150 mg/kg of ethionine (Table 1, Figure 2). This effect may be due to several factors, such as decreased synthesis of tRNA⁷ or altered tRNA methyltransferase activity. Hancock⁴ reported increased tRNA methyltransferase activity following administration of ethionine to animals, and also demonstrated that the enzyme activity increases more rapidly in response to relatively high levels of dietary ethionine³⁷. Liver tRNA obtained from rats treated for 5 days with ethionine was also reported to be a poorer methyl acceptor than liver tRNA obtained from rats given ethionine for 3 days only, while the tRNA methyltransferase activity was higher in the former preparation¹⁹. Thus, the slightly higher degree of methylation observed for the highest dose used may in part result from the enhanced enzyme activity, which in turn led to an increased frequency of methylation.

A comparison of our results with data reported by Wildenauer and Gross³⁸ and Jank and Gross³¹ suggests that chronic feeding (for 4 weeks or 3 months) of L-ethionine to rats may lead to a different pattern of undermethylation than treatment with DL-ethionine and adenine for a few days. To decide whether, in addition to differences in dosing schedule, these differences are due to species variation it will be necessary to measure directly the base composition of

mouse liver tRNA following chronic ethionine feeding.

The 3 additional radioactive products observed on the fluorograms, which have thus far not been identified, may represent ethylated base derivatives as described by others^{17,38,39}.

Results from a number of laboratories have indicated an enhanced activity of tRNA methyltransferases in tumors³⁰, while the actual degree of methylation of tumor tRNA may be similar to or even lower than that of tRNA from normal control tissue⁴⁰⁻⁴³. It is interesting to note that among the early effects following the administration of the carcinogen, DL-ethionine, are decreased tRNA methylation and increased methyltransferase activity. The possible relationship of these early effects to the carcinogenic action of ethionine deserves further exploration.

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ABBREVIATIONS

Abbreviations used are as recommended by the IUPAC-IUB Commission on Biochemical Nomenclature, except X = 3-(3-amino-3-carboxypropyl) uridine⁴⁴.

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