Vol. 95, No. 3, 1980 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
August 14, 1980 Pages 1160-1167

ETHIONINE-INDUCED CHANGES IN THE ACTIVITIES OF S-ADENOSYLMETHIONINE SYNTHETASE ISOZYMES FROM RAT LIVER

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Received June 30,1980

Summary; Isozyme patterns of S-adenosylmethionine synthetase have been measured with and without dimethylsulfoxide in rat liver induced by ethionine. The activity of the α-form is increased following administration of ethionine plus adenine for 2 consecutive days, and gradually decreased to control level on the 7th day after treatment, whereas the activity of β-form is relatively unaffected. Methyl-deficient transfer RNA and enhanced levels of transfer RNA-methylating enzymes were found in the livers of female rats after the treatment for 2 days, following which they gradually returned to control level.

The presence of three isozymes of S-adenosylmethionine (AdoMet) synthetase has been characterized in rat tissues (1-4). These enzymes can be distinguished by their molecular weights, sensitivities to dimethylsulfoxide (Me_2SO) and their responses to sulfhydryl reagents (1-4). The α- and β-forms of the enzyme are present in normal liver. The other, termed γ-form, is in kidney and most other tissues. During development from the fetal period to maturity, in rat fetal liver, a larger proportion of the γ-form enzyme is shown to be present, but the content of β-form, activated by Me_2SO, is very low (5). The β-form of the enzyme rises sharply to around 15th day after birth together with the α-form, which increases in amount to the tenth day (5). In rat hepatoma, the γ-form of the enzyme appears concomitant with the disappearance of the α- and β-forms as carcinogenesis progresses (6).

Abbreviations: AdoMet, S-adenosylmethionine; Me_2SO, dimethylsulfoxide; tRNA, transfer RNA; AdoEt, S-adenosylethionine.

0006-291X/80/151160-08$01.00/0

This communication reports that only the activity of α-form of AdoMet synthetase increases in rat liver after administration of ethionine plus adenine into a rat, accompanied by the accumulation of methyl-deficient transfer RNA (tRNA).

MATERIALS AND METHODS

L-[methyl-^{3}H] Methionine (8.7 Ci/mmol) and S-adenosyl-L-[methyl-^{3}H]methionine (25-50 Ci/mmol) were obtained from Radiochemical Centre, England. Spectroquality Me_2SO, L-ethionine and adenine were obtained from Nakarai Chemicals (Kyoto, Japan). tRNA (E. coli MRE 600) was purchased from Boehringer Mannheim. Sephadex G-150 (superfine) was a product of Pharmacia (Uppsala, Sweden). All other reagents were of analytical grade.

Female rats used were an albino Wistar strain. The rats were maintained on a diet of commercial laboratory rat chow and given food and water ad libitum. Experimental animals received daily i.p. injections of L-ethionine (200 mg/kg body weight) plus adenine (120 mg/kg body weight) at 6 p.m. for 2 consecutive days. No more injections were continued unless specified. The rats were killed by decapitation on each morning after the injection. Control rats did not receive any injections.

Animals (150-200 g) were sacrificed by decapitation and the livers were removed rapidly and placed on ice. After being weighed, the tissues were homogenized in 2 vol. of 0.25 M sucrose containing 3.3 mM $MgCl_2$ with a glass-Teflon homogenizer. The homogenates were centrifuged at 1,000 x g for 10 min and the supernatant fluids were centrifuged at 105,000 x g for 60 min to obtain the cytosol extracts. The cytosol extracts were dialyzed against Buffer A (50 mM Tris-HCl, pH 7.8, 0.2 mM dithiothreitol, 0.1 mM EDTA, 10 mM $MgCl_2$ and 20% (v/v) glycerol) containing 0.075 M KCl for 3-5 hr, in order to remove endogeneous methionine and S-adenosyl-compounds.

tRNA preparation was isolated from rat livers by the method described by Laking et al (7).

AdoMet synthetase activity was determined as described previously (1, 4).

tRNA methyltransferase activity was assayed by the slightly modified method described by Liau et al (3).

Gel filtration chromatography of cytosol fraction was performed as described previously (8).

Protein was determined by the method of Lowry et al (9) with bovine serum albumin taken as the standard, and RNA was quantitated by its absorbance at 260 nm; 50 μg/ml of RNA was taken to have an absorbance of 1.0.

RESULTS

The effect of Me_2SO on the AdoMet synthetase activity from rat liver after administration of ethionine plus adenine for 2 consecutive days was investigated. The stimulation ratio by Me_2SO of this enzyme activity from rat liver decreased after 2 days' treatment

Table I

Effect of Me_2SO on AdoMet synthetase activity from rat liver after administration of ethionine plus adenine

Days after rats received ethionine plus adenine	AdoMet synthetase activity units/mg protein		Stimulation ratio
	$-Me_2SO$	$+Me_2SO$	(+/-)
0	0.085	0.870	10.2
1	0.120	0.930	7.7
2	0.270	1.310	4.8
4	0.098	0.890	9.0
7	0.085	0.860	10.1

The enzyme activity in the cytosol fraction from each source was assayed in the standard mixture with and without 10% (v/v) Me_2SO. The standard reaction mixture (0.1 ml) contained 0.1 M Tris-HCl (pH 9.0), 20 mM $MgCl_2$, 0.15 M KCl, 5 mM dithiothreitol, 10 mM ATP, 25 μM L-[methyl-^{3}H]methionine (0.25 μCi) and enzyme solution. The reaction was carried out at 37°C for 10 min. One unit of enzyme activity was defined as being equivalent to the formation of 1 nmol/min of AdoMet in the incubation mixture without Me_2SO. Each value from the liver is the mean of the results obtained from 2-3 rats.

approximately to 50% of that of control liver, after which the ratio increased to control value on 7th day after administrations of ethionine plus adenine (Table I). Fig. 1 illustrated the gel filtration profiles of AdoMet synthetase from rat liver when rats had received injections of ethionine plus adenine. Two species of enzyme activities were observed in control liver (Fig. 1A). The first peak corresponding to the α-form with a molecular weight of 220,000 daltons was less sensitive to Me_2SO and second peak corresponding to β-form with a molecular weight of 160,000 daltons was markedly stimulated by Me_2SO (4, Fig. 1). The activity of the first peak increased approximately 4 fold after 2 days' administrations of ethionine plus adenine (Fig. 1C). Under these conditions, no significant change in the activity of the second peak activated by

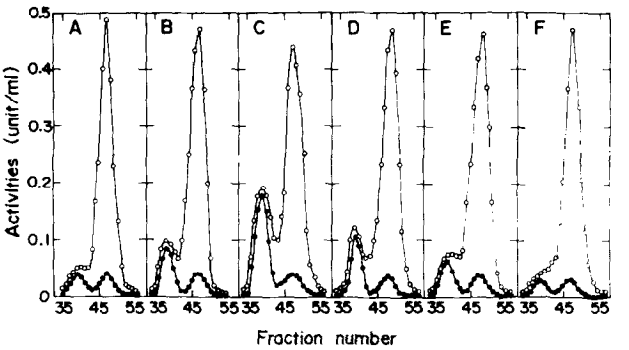

Fig. 1. Sephadex G-150 column chromatography of AdoMet synthetase from rat livers after treatment with ethionine plus adenine. The cytosol fraction (0.45 ml) from pooled livers of 2 to 4 rats was applied on a column (0.95 x 110 cm) of Sephadex G-150 equilibrated with Buffer A containing 0.1 M KCl. Fractions of 0.92 ml were collected and 20 µl aliquots were taken to determine the enzyme activity with (○) and without (●) 10% (v/v) Me_2SO. The recovery of the enzyme activity was 60 to 70% in each case. The apparent molecular weights were estimated according to Andrews (8). Catalase (mol. wt. 240,000), lactate dehydrogenase (mol. wt. 140,000) and bovine serum albumin (mol. wt. 67,000) were used as external standard proteins. Void volume (fraction number, 32) was determined by employing blue dextran. A, normal rat livers; B, C, E and F represent 1, 2, 4 and 7 days after administering ethionine plus adenine into rats, respectively, as described under "MATERIALS AND METHODS"; D, the livers from rats injected daily ethionine plus adenine for 3 days.

Me_2SO was detected. The level of α-enzyme activity began to decline and reached almost control level on 7th day after treatment. The maximal activity of α-enzyme was observed after 2 days' administration, even if the rats were injected ethionine plus adenine for 3 consecutive days (Fig. 1D).

tRNA preparations were isolated from the livers of rats after various time intervals during which they had received administration of ethionine plus adenine. Each of these tRNA preparations was assayed for its ability to accept methyl groups *in vitro* catalyzed by the tRNA-methylating enzymes in soluble fractions from livers of control rats. Fig. 2 shows the results of these experiments. Following the administration, a markedly enhanced ability of tRNA to accept methyl groups in the reaction catalyzed by enzymes was observed after 2 days' treatment. The methyl-accepting capacity of

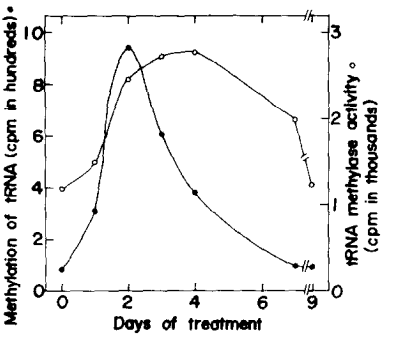

Fig. 2. Methylation of tRNA and tRNA-methylase activities from livers of ethionine plus adenine treated rats. tRNA was isolated from the livers of rats killed after the number of days indicated during which they had received ethionine plus adenine as described under "MATERIALS AND METHODS". In the assay for methylation of tRNA, the reaction mixture (0.1 ml) contained 0.05 M Tris-HCl (pH 7.8), 0.25 M KCl, 5 μM L-[methyl-^{3}H]AdoMet (0.25 μCi), 2.5 mM dithiothreitol, 0.5 mM $MgCl_2$, 20 μg of liver tRNA and the cytosol extract from normal rat liver as tRNA-methylating enzymes (30 μg of protein). The assay conditions for tRNA methylase activities were the same, except that E. coli tRNA (20 μg) replaced rat liver tRNA as substrate, and that the liver cytosol extracts (30 μg of protein) from the various sources listed above as tRNA methylases. After incubation at 37°C for 40 min, the tubes were chilled and 1 mg carrier RNA was added, followed by 5 ml of 5% trichloroacetic acid. After extensive washing with trichloroacetic acid, acetone, ethanol and ether, the samples were counted in a liquid scintillation spectrometer. Each point shows the average of the results obtained from 3-4 rats. The values of tRNA methylation are cpm methyl-^{3}H incorporated per 20 μg liver tRNA per 40 min, and the values of tRNA methylase activity are cpm methyl-^{3}H incorporated per 20 μg E. coli tRNA per 40 min.

the tRNA declined and reached almost the control level on the 7th day after onset of the injection as shown in Fig. 2. The specific activities of liver tRNA-methylating enzymes of rats after the treatment were measured for various time intervals in vitro with heterologous tRNA from E. coli as substrate. As shown in Fig. 2, following administrations for 2 consecutive days, enzyme activities had increased almost two-fold, later the level of the activities decreased almost to the control level by the 9th day after onset of treatment.

DISCUSSION

Ethionine, a liver carcinogen (10), when administered into rats, causes a rapid fall in the liver ATP concentration, as a result of

an imbalance between the accumulation of S-adenosylethionine (AdoEt) and de novo synthesis of adenine nucleotides (11, 12), followed by a striking inhibition of RNA (13) and protein synthesis (12, 14). The inhibition of RNA synthesis is reversed in vivo by adenine, even when protein synthesis is markedly inhibited (15). Ethionine inhibits tRNA methylation in vivo in bacteria as well as rats, and incompletely methylated tRNA can be isolated from these sources (16-19). AdoEt is shown to inhibit the methylation of tRNA by AdoMet (20). Wainfan et al (19) have reported that methyl-deficient tRNA was found in the livers of female rats that had received injection of 250 mg DL-ethionine plus 120 mg adenine per kg body weight, per day for 2 days after which the relative methyl deficiency of liver tRNA decreased. Our present results on the methyl-deficient tRNA are consistent with theirs, but their results that the specific activity of liver tRNA methylases was depressed below control for 2 days are contrary to ours. Most of the sites available for methylation by homologous enzymes were found in guanine moieties of the tRNA (19).

The methylation of the intact tRNA by tRNA methylases of malignant cells has been investigated extensively, primarily by in vitro techniques using heterologous tRNA (21-23). Administration of ethionine to female rats results in an increase in tRNA methylase activity in the liver (24, 25), which suggests that the inhibition reaction with AdoEt and the increased methylase activity may proceed by two different pathways (20). The data from our experiments and others suggest that AdoEt formed in vivo from ethionine competitively inhibits the methylation of tRNA in vivo by AdoEt, resulting in the production of methyl-deficient tRNA.

The activity of enzyme-α characteristic of adult liver rises after birth, and comes to the same level as enzyme-β by 30 to 40

days after birth (5). It is not clear from information available at present why methyl-deficient tRNA accumulated in the liver during the early period of 2 days' treatment, when tRNA methylase and α-form of AdoMet synthetase activities are increased, and also why only α-form among AdoMet synthetase isozymes is induced by ethionine treatment.

ACKNOWLEDGEMENTS

This investigation was supported in part by a Grant-in Aid for Cancer Research from the Ministry of Education, Science and Culture, Japan. The authors express their gratitude to Drs. Hirobumi Teraoka, and Masao Kajiyoshi for many helpful discussions.

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