CHANGES IN THE ACTIVITIES OF *S*-ADENOSYLMETHIONINE SYNTHETASE ISOZYMES FROM RAT LIVER ON ETHIONINE ADMINISTRATION

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1. Introduction

We have indicated that three isozymes of *S*-adenosylmethionine (AdoMet) synthetase (ATP: L-methionine *S*-adenosyltransferase, EC 2.5.1.6) are present in rat tissues. The α- and β-forms of the enzyme are characteristic of normal liver, and the γ-form is present in kidney, most other tissues and also in fetal liver [1,2]. These isozymes differ from one another in M_r, sensitivity to dimethylsulfoxide (Me_2SO), and their response to sulfhydryl reagents [1,2]. Similar isozymes of AdoMet synthetase have been demonstrated in adult rat and human livers [3–5]. Administration of ethionine, a liver carcinogen, to rats is known to result in the accumulation of undermethylated transfer RNA (tRNA) as well as adenosylethionine (AdoEt) in livers [6–9]. When ethionine was administered to rats daily for 2 days, activity of the α-form is induced temporarily, decreasing to control level on day 7 after treatment, whereas activity of the β-form is unaffected [10].

This communication reports that AdoMet synthetase in liver cytosol will catalyse the formation of AdoEt as well as AdoMet, and that when ethionine plus adenine were given daily to rats for 9 days, the activity of the α-form of AdoMet synthetase gradually decreased after temporarily increasing, and activity of the β-form decreased with the period of prolonged treatment. The activities of tRNA methyltransferases increased following administration of ethionine plus adenine, whereas the activity of glycine methyltransferase (AdoMet: glycine methyltransferase, EC 2.1.1.20) decreased in the livers of female rats, as did the activity of the β-form.

2. Materials and methods

All materials used in these experiments except labelled ethionine were as in [1]. L-[*ethyl*-^{3}H]Ethionine (50 mCi/mmol) was obtained from New England Nuclear. Wistar female rats (150–200 g) received daily i.p. injections of D,L-ethionine (250 mg/kg body wt) plus adenine (120 mg/kg body wt). The animals were killed by decapitation on the morning following the last injection. Livers were removed rapidly and placed on ice. After weighing, the tissues were homogenized in 4 vol. 0.25 M sucrose/3.3 mM $CaCl_2$ with a glass–Teflon homogenizer. The homogenates were centrifuged at 1000 × *g* for 10 min and the supernatant fluids centrifuged at 105 000 × *g* for 60 min to obtain the cytosol fractions. The cytosol fractions were dialyzed against buffer A (50 mM Tris–HCl (pH 7.8)/0.2 mM dithiothreitol/0.1 mM EDTA/10 mM $MgCl_2$/20% (v/v) glycerol) containing 0.075 M KCl for 3–5 h.

AdoMet synthetase activity was determined as in [1,2]. 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) or 50 μM L[*ethyl*-^{3}H]ethionine (0.5 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 or AdoEt in the incubation mixture without Me_2SO.

tRNA methyltransferase activity was determined by a method slightly modified from that in [5]. 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 *E. coli* tRNA and the various liver cytosol solu-

tions (30 μg protein), listed in fig.3, as tRNA methyltransferases. After incubation at 37°C for 40 min, the tubes were chilled and 1 mg carrier RNA was added, followed by 5 ml 5% trichloroacetic acid. After extensive washing with trichloroacetic acid, acetone, ethanol and ether, the samples were counted in a liquid scintillation spectrometer. In the assay for methylation of glycine, the reaction mixture (0.1 ml) contained 0.05 M Tris–HCl (pH 8.6), 0.1 M KCl, 0.2 mM [*methyl*-^{3}H]AdoMet (0.1 μCi), 1 mM dithiothreitol, 0.02 M glycine and the various cytosol solutions (40 μg protein), listed in fig.3, as glycine methyltransferase. After incubation at 37°C for 20 min, the reaction was stopped by addition of 0.25 ml of 10% trichloroacetic acid. The reaction mixture was then made up to 1 ml with distilled water and was centrifuged at 10 000 × *g* for 10 min. The supernatant was neutralized with 0.2 ml 1 M Tris, and was then applied to a Dowex 50-X8 column (H^+ forms, 100–200 mesh) (0.6 × 3 cm) and followed by 3 ml H_2O. An aliquot containing [*methyl*-^{3}H]methylglycine was determined with a water-miscible counting fluor.

Gel filtration chromatography of cytosol fractions was performed as in [11], and protein was determined by Lowry's method [12].

3. Results and discussion

When the cytosol from rat liver was applied to Sephadex G-150, AdoMet synthetase activity was separated into two peaks as shown in fig.1 [1,2]. The first peak corresponding to the α-form, with low K_m for ligands and an M_r of 220 000, was less sensitive to Me_2SO while the second peak corresponding to β-form, with a high K_m and an M_r of 160 000, was markedly stimulated by Me_2SO [1,2]. Both isozymes absolutely required Mg^{2+} and K^+ for activity and were inhibited by *p*-chloromercuribenzoate. The activity of the first peak increased ~4-fold after 2 days' treatment with ethionine plus adenine, whereas the activity of the β-form is not affected (fig.1) [10]. The activity of the β-form is very low in rats after 7 days' treatment as shown in fig.1. When [*ethyl*-^{3}H]ethionine was used as a substrate for AdoMet, the activity of the α-form is almost 2-fold higher than that of the β-form, and the stimulation ratio of the synthetase by Me_2SO is increased compared to that when AdoMet was used as a substrate, and the isozyme patterns of the synthetase activity, when ethionine plus adenine had been given daily to rats, are strictly similar to those of the activity using [*methyl*-^{3}H]methionine as a substrate as shown in fig.1. From these results it is clear that the synthetase catalyzes the formation of AdoMet as well as AdoEt, although the rate of AdoMet formation is much higher than that of AdoEt.

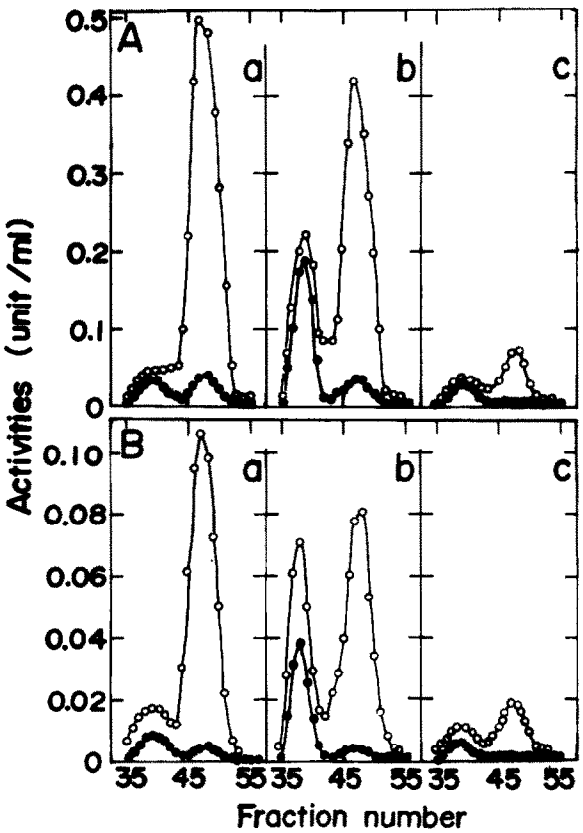

Fig.1. Sephadex G-150 column chromatography of AdoMet synthetase from rat livers after treatment with ethionine plus adenine. The cytosol fraction (0.45 ml) from pooled livers of 2–4 rats was applied to a column (0.95 × 110 cm) of Sephadex G-150 equilibrated with buffer A containing 0.1 M KCl. Fractions 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 60–70% in each case. The apparent relative molecular masses were estimated according to Andrews [11]. Catalase (M_r 240 000), lactate dehydrogenase (M_r 140 000) and bovine serum albumin (M_r 67 000) were used as external standard proteins. Void volume (fraction 32) was determined by employing blue dextran. L-[*methyl*-^{3}H]Methionine (A) or L-[*ethyl*-^{3}H]ethionine (B) was used as substrate in the standard assay mixture. (a) Normal rat livers; (b,c) livers 2 and 7 days after administration of ethionine plus adenine to the rats, respectively.

Fig.2 illustrates the change in the activity of AdoMet synthetase isozymes from rat liver when rats had received injections of ethionine plus adenine. The activity of the α-form was strikingly enhanced after 2 days' treatment, then it decreased towards day 9 of treatment, whereas the activity of the β-form decreased gradually, and was very low in rats after 9 days' treatment. However the activity of the α-form was >50% of that of control level. Thus, the behavior of the activities of these isozymes in rat liver is very different, when the rats were injected daily with ethionine plus adenine.

The specific activities of liver tRNA methyltransferases and glycine methyltransferase of rats after the treatment were measured at various times. As shown in fig.3, following administration of ethionine plus adenine, tRNA methyltransferase activity increased almost 2-fold on day 2, following which it gradually increased, while the activity of glycine methyltransferase decreased with prolonged treatment just like the activity of the β-form of AdoMet synthetase.

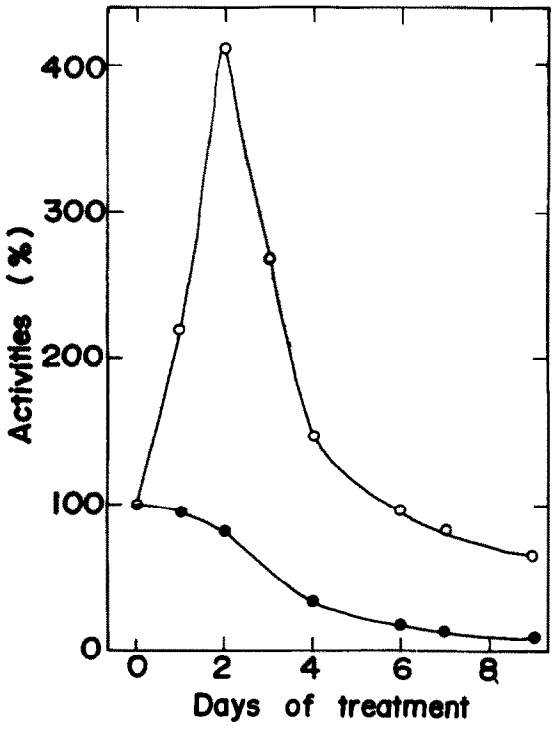

Fig.2. Changes in the activities of AdoMet synthetase isozymes from livers of ethionine-plus-adenine treated rats. Analysis of AdoMet synthetase activity was carried out as in fig.1A for each time point. The values of the activities of the α-form and the β-form from control rat liver are taken as 100%. Each point represents the average of the results obtained from 2 independent analyses. (○) α-Form of AdoMet synthetase; (●) β-form of AdoMet synthetase.

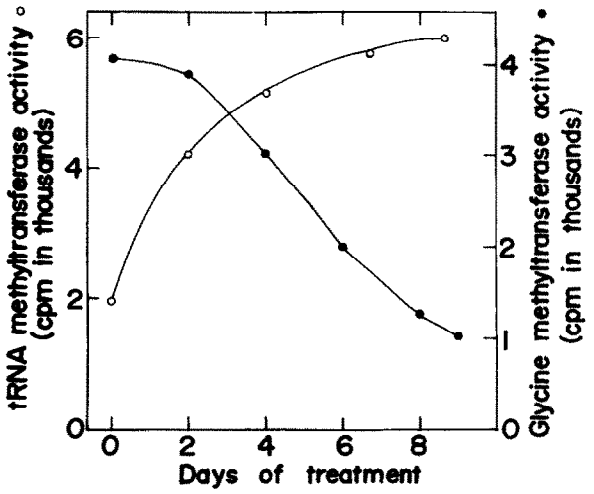

Fig.3. The activities of tRNA methyltransferases (○——○) and glycine methyltransferase (●——●) from livers of ethionine plus adenine treated rats. Each point shows the average of the results obtained from 3–4 rats. The values of tRNA methyltransferase activity are cpm *methyl*-^{3}H incorporated per 20 μg *E. coli* tRNA (MRE 600, Boehringer Mannheim) per 40 min, and the values of glycine methyltransferase activity are cpm *methyl*-^{3}H incorporated to glycine per 20 min.

A hepatocarcinogen, ethionine [14], induces a rapid decrease in hepatic ATP concentration, accumulation of AdoEt [15] and inhibition of RNA synthesis [15]. This inhibition of RNA synthesis is reversed in vivo by adenine administration [16]. AdoEt has been shown to inhibit the methylation of tRNA [17]. Hepatic tRNA isolated from rats given ethionine plus adenine are hypomethylated [6–10].

Increased activities of tRNA methyltransferases of malignant cells have been demonstrated [18–20]. Administration of ethionine to female rats results in an increase in tRNA methyltransferase activity in the liver [21,22]. Significant levels of glycine methyltransferase activity were found mainly in liver [23], but the minimal deviation Morris hepatoma had very low activity, while the enzyme activity could be detected in Novikoff hepatoma or Ehrlich ascites cells [23].

In hepatoma induced by *N*-fluorenylacetamide, the γ-form of the enzyme appears concomitantly with the disappearance of the α- and β-forms as carcinogenesis progressed, and when the activity of the α-form had disappeared, a slight activity of the β-form still remained [13]. The activity of glycine methyltransferase decreased in this hepatoma and the decreased

activity of the glycine methyltransferase indicated the change corresponding to that of the β-form of AdoMet synthetase as carcinogenesis progresses (unpublished).

It is possible that these two isozymes in liver are involved in the supply of AdoMet for different biological functions in different subcellular compartments. Part of the low K_m isozyme may be functionally associated with tRNA methyltransferase and part of the high K_m isozyme functionally associated with glycine methyltransferase [5]. The existence of AdoMet synthetase-tRNA methyltransferase complex was supported by the demonstration of cochromatography of these enzymes on Sepharose 6B, co-precipitation at pH 5, and a higher efficiency of utilization of AdoMet synthesized by AdoMet synthetase by tRNA methyltransferases in the enzyme complex than of that provided exogeneously [5]. However, from these experiments, the β-form of AdoMet synthetase which is characteristic of liver, may be functionally concerned with the enzymes that catalyze methylation, which occurs primarily on the hydroxyl or amino groups of low M_r compounds, e.g., glycine in the liver.

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