DIFFERENTIAL EFFECTS OF DIMETHYLSULFOXIDE ON *S*-ADENOSYLMETHIONINE SYNTHETASE FROM RAT LIVER AND HEPATOMA

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1. Introduction

The presence of two distinct species of *S*-adenosylmethionine (AdoMet) synthetase (ATP:L-methionine *S*-adenosyltransferase, EC 2.5.1.6) has been demonstrated in yeast [1] and rat liver [2–4]. One species of rat liver enzyme is strikingly stimulated by dimethylsulfoxide (Me_2SO) at low concentration (25 μM) of methionine, and the other is stimulated only 1.5-fold [2]. Novikoff rat ascites hepatoma cells have been reported [3,4] to contain a single form of the enzyme corresponding to the less-Me_2SO sensitive enzyme in elution position on gel filtration. Recently, we have partially purified two forms of AdoMet synthetase from rat liver and a single form from kidney, representing non-hepatic rat tissues [5]. The liver enzymes, termed α with low K_m for ligands (correspond to less-Me_2SO sensitive enzyme) and β with high K_m for ligands (correspond to Me_2SO-stimulated enzyme), have app. mol. wt 2.1×10^5 and 1.6×10^5, respectively. The kidney enzyme with an app. mol. wt 1.9×10^5 is partially inhibited by Me_2SO and exhibits low sensitivity to SH-blocking reagents.

Here we have investigated the effect of Me_2SO on AdoMet synthetase activity from rat liver of precancerous states induced by thioacetamide, regenerating liver, Morris hepatomas 7316A and 7794A, and Yoshida ascites hepatoma AH 130. Gel-filtration chromatography of the cytosol fractions indicates that the decrease in Me_2SO-stimulation ratio of the enzyme activity in thioacetamide-treated rat liver and hepatoma is due to the reduction of liver β enzyme.

2. Materials and methods

L-[*methyl*-^{3}H]Methionine (8.7 Ci/mmol) was obtained from Radiochemical Centre, England. Male Wistar rats (150–200 g) were injected intraperitoneally with thioacetamide in 0.15 M NaCl (50 mg/kg body wt) at intervals of 24 h. After daily doses of thioacetamide for 9 days, livers were homogenized in 4 vol. 0.25 M sucrose/3.3 mM $MgCl_2$ with a glass–Teflon homogenizer. The homogenate was centrifuged at $1000 \times g$ for 10 min and the supernatant fluid was centrifuged at $105\,000 \times g$ for 1 h to obtain the cytosol extract. Nuclei were isolated from the $1000 \times g$ precipitate as in [6] with 2.2 M sucrose/3.3 mM $MgCl_2$. Buffalo strain rats, bearing Morris hepatoma 7316A (survival days, 20–25) and 7794A (survival days, 40–180), were a kind gift from Dr Shigeaki Sato. Yoshida rat ascites hepatoma cells (AH 130) were a kind gift from Dr Masami Muramatsu. Cells of Morris hepatomas (about 20 days after subcutaneous transplantation) and Yoshida ascites hepatoma were sonicated in an equal vol. of 25 mM Tris–HCl (pH 7.5)/0.25 M sucrose/3.3 mM $MgCl_2$ on ice for 15 s ($\times$ 4) at 20 W, and centrifuged at $105\,000 \times g$ for 1 h. The cytosol extracts from rat liver and hepatoma were dialyzed against Buffer A (50 mM Tris–HCl, (pH 7.5)/0.2 mM dithiothreitol/0.1 mM EDTA/10 mM $MgCl_2$/20% (v/v) glycerol) containing 0.1 M KCl for 4–6 h.

AdoMet synthetase activity was determined as in [3] with a slight modification. The standard reaction mixture (0.1 ml) contained 0.1 M Tris–HCl (pH 9.0),

Table 1
Biochemical changes after treatment of thioacetamide on rat liver

Treatment	RNA polymerase I (nmol/mg DNA)	Nuclear RNase H		DNA synthesis (cpm/mg DNA)
		Mn^{2+} (nmol/mg DNA)	Mg^{2+}	
Control	0.43	3.30	1.62	510
Thioacetamide (9 days)	2.62	10.10	6.88	8980

RNA polymerase I was measured by its insensitivity to high levels of α-amanitin with liver nuclei as in [16]. Assays of RNase H were described [17]. DNA synthesis was measured by the incorporation of [^{3}H]thymidine injected into the tail vein 1 h before killing. Specific activities of nuclear DNA were measured as in [18]. Each value is the mean of the results obtained from 4–5 rats

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 fraction. The incubation 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. Protein was determined by the method [7] and DNA by the method [8].

3. Results and discussion

A hepatocarcinogen, thioacetamide, increases the nucleolar size [9] and stimulates ribosomal RNA synthesis in liver [10–12], both of which are characteristic of many tumors. Moreover, it has been shown to produce liver tumors after prolonged treatment [13,14]. The activities of Mg^{2+}-dependent RNase H [15] as well as RNA polymerase I [11,12] increase several fold within 24 h after a single injection of thioacetamide. Alterations in biochemical events after administration of thioacetamide for 9 days are shown in table 1. A nearly 6-fold increase in the activity of RNA polymerase I in nuclei was observed. Increased activities in nuclear Mg^{2+}- and Mn^{2+}-dependent RNase H (c.f., [15]) and in incorporation of [^{3}H]thymidine also occurred.

The stimulation ratio by Me_2SO of AdoMet synthetase activity from rat liver of precancerous states induced by repeated daily injections of thioacetamide for 9 days decreases to ~70% of that of control livers (table 2). AdoMet synthetase activities, their sensitiv-

Table 2
Effect of Me_2SO on AdoMet synthetase activity from rat liver and hepatomas

Source of enzyme	AdoMet synthetase activity (units/mg protein)		Stimulation ratio (+/−)
	− Me_2SO	+ Me_2SO	
Normal liver	0.089	1.069	12.7
Regenerating liver	0.110	1.360	12.4
Thioacetamide-treated liver	0.059	0.549	9.2
Hepatoma AH 130	0.136	0.102	0.8
Hepatoma 7316A	0.074	0.049	0.7
Hepatoma 7794A	0.120	0.210	1.8

The enzyme activity in the cytosol fraction from each source was assayed in the standard reaction mixture with and without 10% (v/v) Me_2SO. Each value from the liver is the mean of the results obtained from 4–5 rats

ity to Me_2SO, in regenerating rat liver 24 h after operation were not essentially changed compared with those from normal livers. The enzyme activities from hepatoma AH 130 and hepatoma 7316A were slightly inhibited by Me_2SO, whereas the activity from hepatoma 7794A was significantly activated by Me_2SO (table 2).

The species of AdoMet synthetase in these cytosol fractions were analyzed by Sephadex G-150 column chromatography. As shown in fig.1A, two peaks of AdoMet synthetase activity were observed in normal livers, and their apparent molecular weights were estimated to be $2.1–2.3 \times 10^5$ for the first peak (α) and $1.4–1.6 \times 10^5$ for the second peak (β) which was

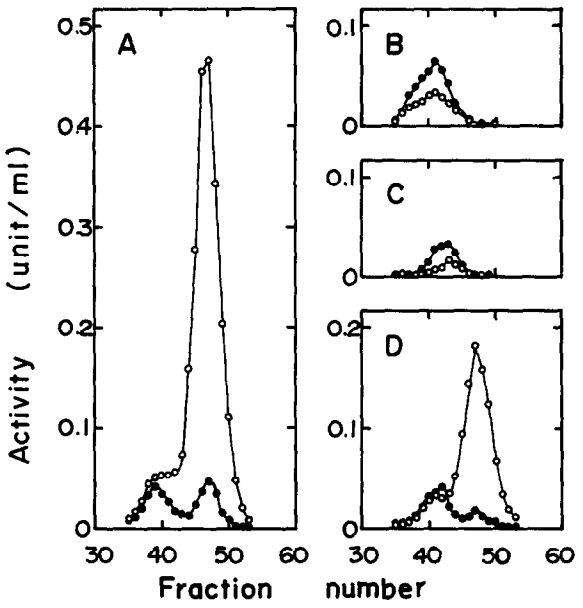

Fig.1. Sephadex G-150 column chromatography of AdoMet synthetase from rat liver and hepatomas. The cytosol fraction (0.45 ml) from normal rat liver (A, 0.43 unit), Yoshida ascites hepatoma AH 130 (B, 1 unit), Morris hepatoma 7316A (C, 0.3 unit) and Morris hepatoma 7794A (D, 0.6 unit) was chromatographed on a column (0.95 × 110 cm) of Sephadex G-150 (superfine, Pharmacia) 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 50–70% in each case. The apparent molecular weights were estimated as in [19]. Catalase (mol. wt 2.4×10^5), lactate dehydrogenase (mol. wt 1.4×10^5) and bovine serum albumin (mol. wt 6.7×10^4) were used as external standard proteins. Void volume (fraction no. 32) was determined by employing blue dextran.

strikingly stimulated by Me_2SO. Both α and β enzymes absolutely required Mg^{2+} and K^+ for activity and were completely inhibited by *p*-chloromercuribenzoate (c.f. [5]). Compared with normal livers, with α and β enzyme activities nearly equal in the standard assay conditions without Me_2SO, the thioacetamide-treated livers contained approximately half of the β enzyme activity relative to α enzyme activity (not shown). The enzymes from hepatoma AH 130 (fig.1B) and hepatoma 7316A (fig.1C) eluted as a single peak between the positions of the α and β enzymes. The enzyme activity was slightly inhibited by Me_2SO and reduced to 70–50% by the addition of *p*-chloromercuribenzoate (0.5 mM), suggesting that the enzymes from these hepatomas are similar to the kidney enzyme [5] rather than liver α enzyme. In contrast to hepatomas AH 130 and 7316A, the enzyme from hepatoma 7794A has been shown to have two peaks of activity (fig.1D). The first, main peak, corresponding in elution position to the enzyme from hepatomas AH 130 and 7316A, was slightly inhibited by Me_2SO and *p*-chloromercuribenzoate, whereas the second, small peak was remarkably stimulated by Me_2SO, which corresponds to liver β enzyme.

The results suggest that liver β enzyme disappears during liver carcinogenesis and that in three hepatoma strains examined but not in the livers of precancerous states induced by thioacetamide the kidney-type enzyme appears along with the disappearance of liver α and β enzymes. Preliminary experiments show that the liver from rat fetus contains almost all kidney-type enzyme. Further investigation of the relationship between kidney enzyme and the hepatoma-specific enzyme is now in progress.

From these experiments, it is noteworthy that we can obtain insight into the stage of liver carcinogenesis by assaying AdoMet synthetase with and without Me_2SO, although the biological action of Me_2SO remains to be clarified.

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