Translation in vitro and regulation of mouse liver *S*-adenosylmethionine synthetase messenger RNA

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Total RNA was isolated from adult mouse liver tissues. The α- and β-form isozymes of *S*-adenosylmethionine synthetase existing in liver were synthesized in a reticulocyte lysate cell-free system under the direction of total RNA and were immunoprecipitated with antibody to the β-form. The newly synthesized and the in vivo labeled *S*-adenosylmethionine synthetase subunits were compared by SDS–polyacrylamide gel electrophoresis. Both the α- and β-forms consist of the same size M_r 48000 subunit. The level of the β-form mRNA activity in mouse liver was shown to increase following intraperitoneal transplantation of Ehrlich ascites tumor cells and the changes in the mRNA activity parallel those in the cellular level of *S*-adenosylmethionine synthetase β.

S-Adenosylmethionine synthetase isozymes *Mouse liver* *Cell-free translation* *Ehrlich ascites tumor cells* *mRNA* *Regulation*

1. INTRODUCTION

Isozymes α, β and γ of *S*-adenosylmethionine (AdoMet) synthetase (ATP : L-methionine *S*-adenosyltransferase, EC 2.5.1.6) have been shown in animal tissues; their properties differ from each other in their sensitivity to dimethylsulfoxide, app. M_r-value and the effects of some chemical reagents [1]. Isozymes α and β are present in normal liver [1–3]; isozyme γ is present in the kidney and most other tissues [1]. The β-form has been purified to homogeneity from rat livers, and shown to consist of two identical subunits of M_r 48000 [4]. The antibody against the β-form enzyme cross-reacted with the α-form enzyme, but not with the γ-form from kidney [4]. The activity of the α-form in rat liver after treatment with ethionine and adenine for 2 consecutive days [5,6] and that of the β-form in mouse liver on the 11th day after transplantation of Ehrlich ascites tumor cells, were increased several-fold compared to normal liver [7], and the observed increase in the levels of each activity was shown to be due to an increase in the cellular content of the enzyme [8].

To understand the mechanism underlying the increase in the synthesis of this enzyme in the liver, we examined the cellular level of the mRNA coding for these isozymes. In this communication, we report the synthesis of AdoMet synthetase from liver directed by liver mRNA in a cell-free rabbit reticulocyte system, and show that the cellular level of the synthetase mRNA activity increases with increased content of the enzyme.

2. MATERIALS AND METHODS

2.1. *Animals*

Ehrlich ascites tumor cells were maintained in male *dd* mice by weekly intraperitoneal transplantation in our laboratory. Tumor cells were prepared by inoculation of 0.1 ml ascites fluid as in [7].

2.2. *Preparation of AdoMet synthetase and antibody*

AdoMet synthetase β from rat livers was purified to homogeneity as in [7]. Antibody against the β-form of AdoMet synthetase was prepared as

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in [7]. These antisera, as well as serum from a non-immunized rabbit, were subjected to ammonium sulfate fractionation [9] followed by DEAE-cellulose chromatography [10] to obtain the respective antibodies or immunoglobulin G.

2.3. *Labeled amino acid incorporation studies*

L-[^{14}C(U)]Leucine (>300 mCi/mmol), 0.5 mCi in 0.5 ml 0.9% NaCl, was administered into the tail vein of normal mouse. After 3 h, the liver was homogenized with 4 vol. 0.25 M sucrose in 3.3 mM $MgCl_2$ and the soluble fraction was prepared after centrifugation at $105000 \times g$ for 60 min. The solution was applied onto a phenyl–Sepharose column to separate the α- and β-forms as in [8]. Each isozyme was dialyzed against 50 mM Tris–HCl (pH 7.8), 0.2 mM dithiothreitol, 0.1 mM EDTA, 10 mM $MgCl_2$ and 20% (v/v) glycerol (buffer A).

2.4. *Isolation of total RNA*

All manipulations were performed at 0–4°C unless otherwise specified. Mouse livers were homogenized in 9.5 vol. 10 mM sodium-acetate (pH 5.1) containing 10 mM EDTA (buffer B), 0.5 vol. 10% sodium dodecyl sulfate (SDS) and 10 vol. buffer B-saturated phenol [11]. After centrifugation, the supernatant was re-extracted 5 times with buffer B-saturated phenol. Total RNA was precipitated by the addition of 2 vol. ethanol at −20°C overnight. The resulting precipitate was collected by centrifugation, washed twice with ethanol and dried. The pellet was dissolved in 20 mM EDTA (pH 7.0) and this solution was made 3 M in sodium-acetate (pH 5.1) [12]. RNA was precipitated with ethanol as before, dissolved in distilled water and stored at −80°C. RNA was measured using an extinction coefficient of $E^{1\%}_{260\ nm} = 200$.

2.5. *Cell-free translation and immunoprecipitation*

Cell-free protein synthesis was carried out with a nuclease-treated rabbit reticulocyte lysate system [13]. The translation mixture (0.1 ml) contained 20 mM Tris–HCl (pH 7.6), 1.0 mM dithiothreitol, 90 mM potassium acetate, 1.2 mM magnesium acetate, 1 mM ATP, 0.4 mM GTP, 5.0 mM creatine phosphate, 20 μg creatine kinase/ml, 20 μg hemin, 20 μM of each amino acids (all except methionine), 100 μCi [^{35}S]methionine/ml (760–1190 Ci/mmol), 40 μg tRNA, various amounts of total RNA and 400 μl/ml of nuclease-treated rabbit reticulocyte lysate. After incubation at 25°C for 2 h, 0.9 ml 10 mM Tris–HCl (pH 7.8)/2 mM EDTA/0.1% SDS (buffer C) was added to the reaction mixture. AdoMet synthetase was immunoprecipitated with anti-β-form isozyme and 50 μl 10% *Staphylococcus aureus*, and shaken at 24°C for 60 min. After centrifugation, antigen–antibody–bacteria complexes were washed 4 times with buffer C, the final precipitate was suspended in 30 μl of buffer C and boiled for 5 min. The supernatant was subjected to electrophoresis on 12% SDS–polyacrylamide slab gels as in [14]. The radioactive bands on slab gels were located by fluorography. Fluorography was performed as in [15]. The developed film was scanned at 500 nm.

3. RESULTS AND DISCUSSION

Antibody against the β-form isozyme gave a similar quantitative response to the α-form isozyme [4,8]. Thus, anti-β-isozyme antibody was used to isolate both the α- and β-forms synthesized in vitro. However, the α- and β-forms of AdoMet synthetase in mouse liver were shown to separate completely by chromatography on a hydrophobic resin, phenyl–Sepharose [8].

Isolation and identification of the [^{14}C]leucine-labeled AdoMet synthetase isozymes synthesized in mouse liver were accomplished by means of immunoadsorption, SDS–polyacrylamide gel electrophoresis and fluorography. After labeled amino acid was administered into a mouse, the α- and β-forms of AdoMet synthetase in the soluble fraction from the liver were separated by a phenyl–Sepharose. Each labeled isozyme was immunoadsorbed with antibody and resolved by SDS–polyacrylamide gel electrophoresis. As shown in fig.1, M_r 48000 for the β-form (b) is in good agreement with that observed in [4]. A faint band corresponding to M_r 48000 was also detected in the α-form preparation (a). These results indicate that the α-form consists of same size subunit as the β-form. Since the α-form was estimated to be M_r ~200000 by gel filtration ([1]; unpublished), it consists of 4 subunits of M_r 48000.

After cell-free translation system, the total pro-

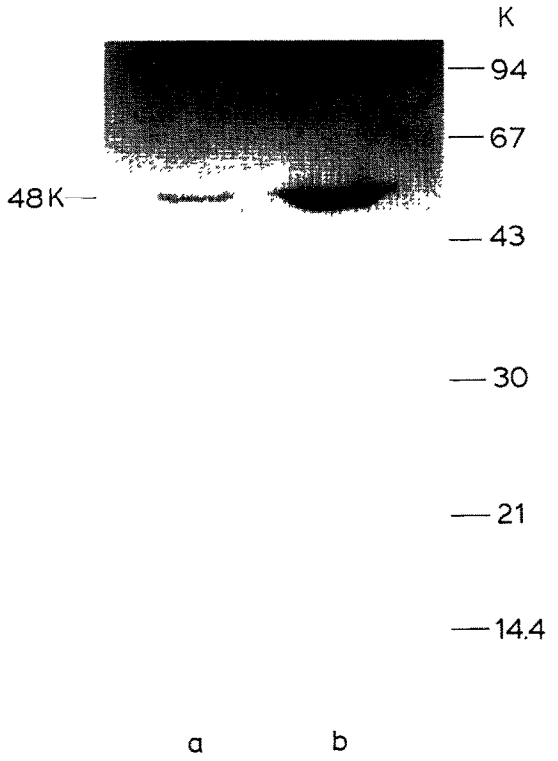

Fig.1. Fluorogram of ^{14}C-labeled AdoMet synthetase isozymes in vivo. The soluble fractions from a mouse liver after administration of labeled leucine were separated into the α- and β-forms by phenyl–Sepharose. Immunoprecipitation was performed using an excess of antibody to the β-form. After washing the precipitate, the final precipitate was subjected to electrophoresis on a 12% SDS–polyacrylamide slab gel. The gel was subjected to fluorography. The cathode is at the top. (a) The α-form isozyme fraction; (b) the β-form isozyme fraction. The marker polypeptides used were ($M_r \times 10^{-3}$): phosphorylase *b* (94); bovine serum albumin (67); AdoMet synthetase β (48); ovalbumin (43); carbonic anhydrase (30); trypsin inhibitor (21); α-lactalbumin (14.4).

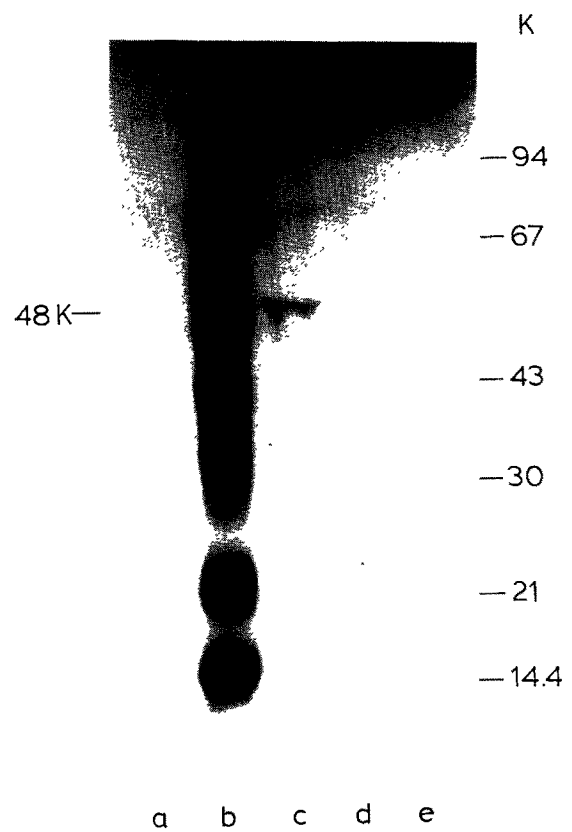

Fig.2. Fluorogram of SDS–polyacrylamide gel electrophoresis of the cell-free translation product. Cell-free translation was performed with 0.8 mg/ml of normal mouse liver cell RNA in the presence of [^{35}S]methionine: (a) total translation product formed without added RNA; (b) total translation product; (c) immunoprecipitate formed with antibody to AdoMet synthetase β; (d) immunoprecipitate formed with control immunoglobulin G; (e) immunoprecipitate formed with antibody to AdoMet synthetase β and 20 μg (assayed as in [16]) AdoMet synthetase β. The markers used were the same as those given in fig.1.

tein products as well as the products isolated by indirect immunoprecipitation with antibody to the β-form were analyzed by electrophoresis on a SDS–polyacrylamide slab gel. The fluorogram of this analysis is presented in fig.2. Practically no protein was synthesized in the absence of exogeneous RNA (a). The immunoprecipitate formed with antibody to the β-form enzyme contained only one band of M_r 48000 (c). In contrast, the immunoprecipitate formed with control immunoglobulin G did not contain this product (d). Furthermore, the addition of a large excess of unlabeled β-form isozyme to the translation product prior to immunoprecipitation with antibody to the β-form resulted in the disappearance of the radioactive band corresponding to M_r 48000 (e). These results indicate that the product of M_r 48000 represents AdoMet synthetase isozymes in liver.

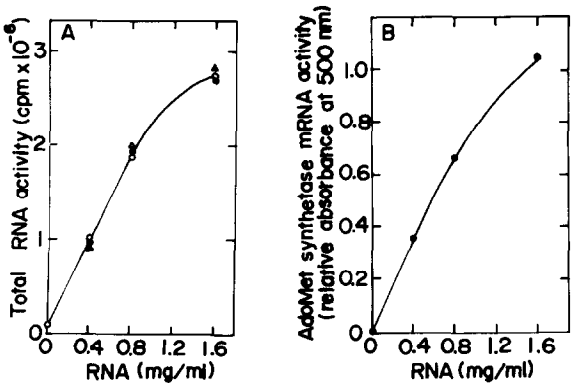

Fig.3. Effects of various amounts of total RNA on [^{35}S]methionine incorporation into total protein (A) and AdoMet synthetase (B). The indicated amounts of total RNA from mouse liver were used per 100 μl of reaction mixture to direct protein synthesis. Total mRNA activity is expressed as the amount of [^{35}S]methionine incorporated into trichloroacetic acid-insoluble material per each reaction mixture. (○) Normal mouse liver; (●, △) liver 4 and 9 days after transplantation of tumor cells into a mouse, respectively. The relative activity of AdoMet synthetase mRNA was assayed by scanning the fluorogram of electrophoresis of the immunoprecipitated samples and by measuring the density of the radioactive band corresponding to the product of M_r 48000.

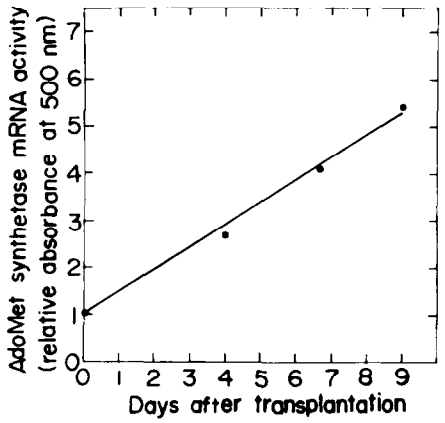

Fig.4. Levels of AdoMet synthetase mRNA activity from mouse liver after transplantation of tumor cells. The assay of mRNA activity was performed with 0.8 mg/ml of total RNA per 100 μl reaction mixture as in fig.3.

Reticulocyte lysate was incubated with mouse liver RNA and [^{35}S]methionine. The amount of AdoMet synthetase as well as of total proteins synthesized in the cell-free system was almost proportional to the amount of total RNA added up to 100 μg/0.1 ml reaction mixture (fig.3). Therefore, this assay system was employed for quantitative purposes. Total RNA from normal and from tumor cell-inoculated mouse livers directed the synthesis of total proteins to essentially the same extent (fig.3A).

The activity of the β-form markedly increased following intraperitoneal transplantation of Ehrlich ascites tumor cells, whereas the activity of the α-form did not change as strikingly [7], and this increase was accompanied by a change in the amount of the immuno-reactive protein [8]. To determine the levels of AdoMet synthetase mRNA, the activity of the synthetase and its mRNA in these preparations were assayed. Cellular levels of the synthetase mRNA increased with increasing activity of its enzyme (fig.4). These results show that the increased synthesis of AdoMet synthetase β in the normal mouse liver after transplantation of Ehrlich ascites tumor cells is due to an increased level of mRNA coding for the enzyme.

The α- and β-form isozymes are immunochemically identical, and composed of same M_r 48000 subunit, although in several points, the properties of both enzymes are different from each other [1–8]. Further investigation of this discrepancy about the catalytic properties of these enzymes and peptide maps formed by protease is now in progress.

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