Volume 330, number 3, 307–311 FEBS 12922 September 1993

Immunohistochemical analysis of rat *S*-adenosylmethionine synthetase isozymes in developmental liver

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Received 8 June 1993; revised version received 19 July 1993

Mammalian *S*-adenosylmethionine (AdoMet) synthetase exists as two isozymes, liver-type and kidney(non-hepatic)-type enzymes. The developmental expression of these two isozyme proteins has been investigated in rat liver using immunohistochemical techniques. The liver-type AdoMet synthetase is expressed only in adult liver, but not in fetal liver. On the other hand, the kidney-type AdoMet synthetase is predominantly expressed in fetal liver and faintly detected in adult liver. It was also found that both isozymes were localized to the hepatocytes of rat liver. These results clearly show that AdoMet synthetase isozymes are developmentally regulated within hepatocytes. In addition, in rat kidney we have shown that the kidney-type AdoMet synthetase is predominantly localized to the distal tubule

S-Adenosylmethionine synthetase; Isozyme; Immunohistochemical analysis

1. INTRODUCTION

S-Adenosylmethionine synthetase (ATP:L-methionine *S*-adenosyltransferase (EC 2.5.1.6)), first reported by Cantoni, is the enzyme that catalyzes the formation of *S*-adenosylmethionine (AdoMet) from methionine and ATP [1]. AdoMet is an important methyl donor in most transmethylation reactions and is also the propylamino donor in the biosynthesis of polyamines. Mammalian AdoMet synthetase exists as three different isoforms, designated α (or I), β (or III), and γ (or II) [2,3]. The α and β forms are confined to the liver, whereas the γ form is widely distributed in extrahepatic tissues [2,4–6]. The α and β forms purified from rat liver have been shown to be composed of four and two identical subunits, respectively, of molecular mass 48 kDa on polyacrylamide gel electrophoresis. Although the α and β forms differ in their properties, they are thought to be products of the same gene and, therefore, represent different forms of the same enzyme (liver-type isozyme). On the other hand, the γ form (non-hepatic- or kidney-type isozyme) is abundantly expressed in the kidney at levels higher than in all tissues examined [4,7]. The γ form purified from human lymphocytes [5] and bovine brain [8] is composed of two different types of subunits. The bovine brain enzymes has two polypeptide bands of 48 and 38 kDa proteins [8]. However, the function of each subunit remains to be established.

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We have previously reported, by determining the enzyme activities, that the kidney-type AdoMet synthetase predominantly exists in fetal rat liver and is progressively replaced by the liver-type AdoMet synthetase during development [2]. However, whether the expression of these two AdoMet synthetase isozymes occurs within the same cells has not been resolved. Immunohistochemical techniques with antibodies specific for each of the two AdoMet synthetase isozymes allowed us to distinguish the two isozymes in liver sections.

Here we have investigated the expression of the two AdoMet synthetase isozymes in rat liver during development and in rat kidney using immunohistochemical techniques.

2. MATERIALS AND METHODS

Hybond-C nitrocellulose filters and protein A-TSK gel were obtained from Amersham Corp. Alkaline phosphatase-conjugated goat anti-rabbit IgG was from Vector Laboratories. 5-Bromo-4-chloro-3-indolyl phosphate (BCIP) and nitro blue tetrazolium chloride (NBT) were from Promega. The labeled streptavidin-biotin kit was from Dakopatts (Copenhagen, Denmark). Keyhole limpet hemocyanine was from Calbiochem. All other reagents were of analytical grade.

2.1. *Preparation of antibodies*

Two antibodies directed against AdoMet synthetase isozymes were used for the immunodetection of the two isozymes in rat liver and kidney extracts. A hexadecapeptide with a sequence of NGQLNGFHEAFIEEGT (amino acid residues 2–17) near the NH_2 terminus segment of rat kidney-type AdoMet synthetase [4] was selected as an immunogen based on its low homology with the other isozyme (liver-type AdoMet synthetase) [9]. The peptide was synthesized using an Applied Biosystems model 430A peptide synthesizer and the product was confirmed by analytical HPLC and automated amino acid sequencing (Applied Biosystems Model 470A gas phase

sequencer) and coupled to keyhole limpet hemocyanine with glutaraldehyde [10]. The antibody was raised in rabbit and purified from the antiserum by the protein A-TSK gel kit (Amersham). An anti-rat liver-type AdoMet synthetase antibody was prepared as described [9].

2.2. *Western blot analysis*

Tissues were removed from male Wistar rats that were killed by decapitation. Tissues were homogenized with a glass-Teflon homogenizer in 10 vols of 0.25 M sucrose containing 3.3 mM $MgCl_2$ and centrifuged at $8,000 \times g$ for 5 min at 4°C. The supernatants were separated by SDS-PAGE according to Laemmli [11] using 10% polyacrylamide gels. After electrophoresis, the fractionated proteins were electrophoretically blotted onto nitrocellulose filters overnight at 4°C using constant current (100 mA) in 0.01% SDS, 25 mM Tris, 190 mM glycine, pH 8.3. The filters were blocked for 1 h at room temperature with 3% nonfat dry milk in TBS (150 mM NaCl, 50 mM Tris-HCl, pH 7.4). The filters were incubated for 1 h at room temperature with a primary antibody in TBST (TBS with 0.05% Tween 20) containing 3% nonfat dry milk. The blots were washed with TBST (3 times 10 min) to remove unbound antibodies and incubated for 1 h at room temperature with a secondary antibody (goat anti-rabbit IgG coupled to alkaline phosphatase) in TBST containing 3% nonfat dry milk. After washing with TBST (3 times 10 min), the immunoreactive bands were visualized using 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium chloride. Protein concentration was determined by the method of Bradford [12].

2.3. *Immunohistochemical analysis*

Selected portions of rat fetal (19-days gestation rats) and adult livers and rat kidney were fixed in 4% paraformaldehyde/0.1 M phosphate buffer, pH 7.4, and embedded in paraffin. Paraffin-embedded sections of 3 μm thickness were used. After deparaffinization and rehydration of the tissue sections, the endogenous peroxidase activity was blocked by incubating the samples in methanol that contained 3% hydrogen peroxide for 30 min. The sections were incubated with normal goat serum for 20 min at room temperature to minimize nonspecific staining. The sections were then covered with the primary antibody overnight at 4°C. The slides were then incubated with biotinylated antibody against rabbit IgG for 20 min at room temperature. Then, the samples were incubated in peroxidase-conjugated streptavidin/0.05 M Tris-HCl, pH 7.6, for 20 min at room temperature. Color was developed with diaminobenzidine and counterstained with hematoxylin.

3. RESULTS AND DISCUSSION

3.1. *Characterization of antibodies to the rat liver-type and kidney-type AdoMet synthetases by Western blot analysis*

The specificity of antibodies used in this study was examined by Western blot analysis of rat kidney and liver extracts (Fig. 1). The anti-rat liver-type AdoMet synthetase antibody reacted only with a single band (48 kDa) in rat liver extracts (Fig. 1A, lane 2). In contrast, no immunoreactive band was observed in extracts from rat kidney (Fig. 1A, lane 1). The apparent molecular weight of the band was similar to that reported previously [13], indicating that the antibody to the rat liver-type AdoMet synthetase significantly recognizes the liver-type enzyme in rat liver. On the other hand, the anti-peptide antibody to kidney-type AdoMet synthetase generated against the 16 N-terminal amino acids of kidney-type AdoMet synthetase reacted only with a single band (48 kDa) in rat kidney extracts (Fig. 1B, lane 1), but not with extracts from rat liver (Fig. 1B, lane 2). These results clearly show that the immunoreactivity found with antibodies to the liver-type and kidney-type AdoMet synthetases in rat liver and kidney extracts is specific for the kidney-type and liver-type isozyme, respectively.

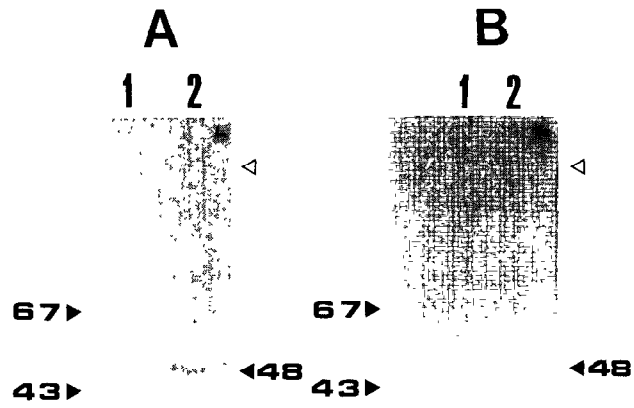

Fig. 1. Immunoblots of liver-type and kidney-type *S*-adenosylmethionine synthetase isozymes. Rat liver and kidney extracts (50 μg/lane) were subjected to SDS-PAGE followed by immunoblotting. (A) anti-rat kidney-type AdoMet synthetase antibody; (B) anti-rat liver-type AdoMet synthetase antibody; lane 1, liver extracts, lane 2, kidney extracts. Molecular mass markers used are bovine serum albumin (67 kDa) and ovalbumin (43 kDa). The protein bands are shown in kDa. The open triangles show the starting points

3.2. *Immunohistochemical analysis*

The expression of AdoMet synthetase isozymes was examined in rat fetal (19-days gestation rats) and adult livers using immunohistochemical techniques. In the fetal liver, only the kidney-type AdoMet synthetase was detected (Fig. 2A,a). In contrast, no reaction product was found when the anti-rat liver-type AdoMet synthetase antibody was used (Fig. 2A,b). On the other hand, the adult liver tissues were predominantly immunostained with anti-rat liver-type AdoMet synthetase antibody. In addition, the adult liver samples were also faintly stained with anti-rat kidney-type AdoMet synthetase antibodies (Fig. 2B,a and b). Nonimmune rabbit serum showed no staining (data not shown). These findings are correlated well with the results of the reverse transcription-polymerase chain reaction

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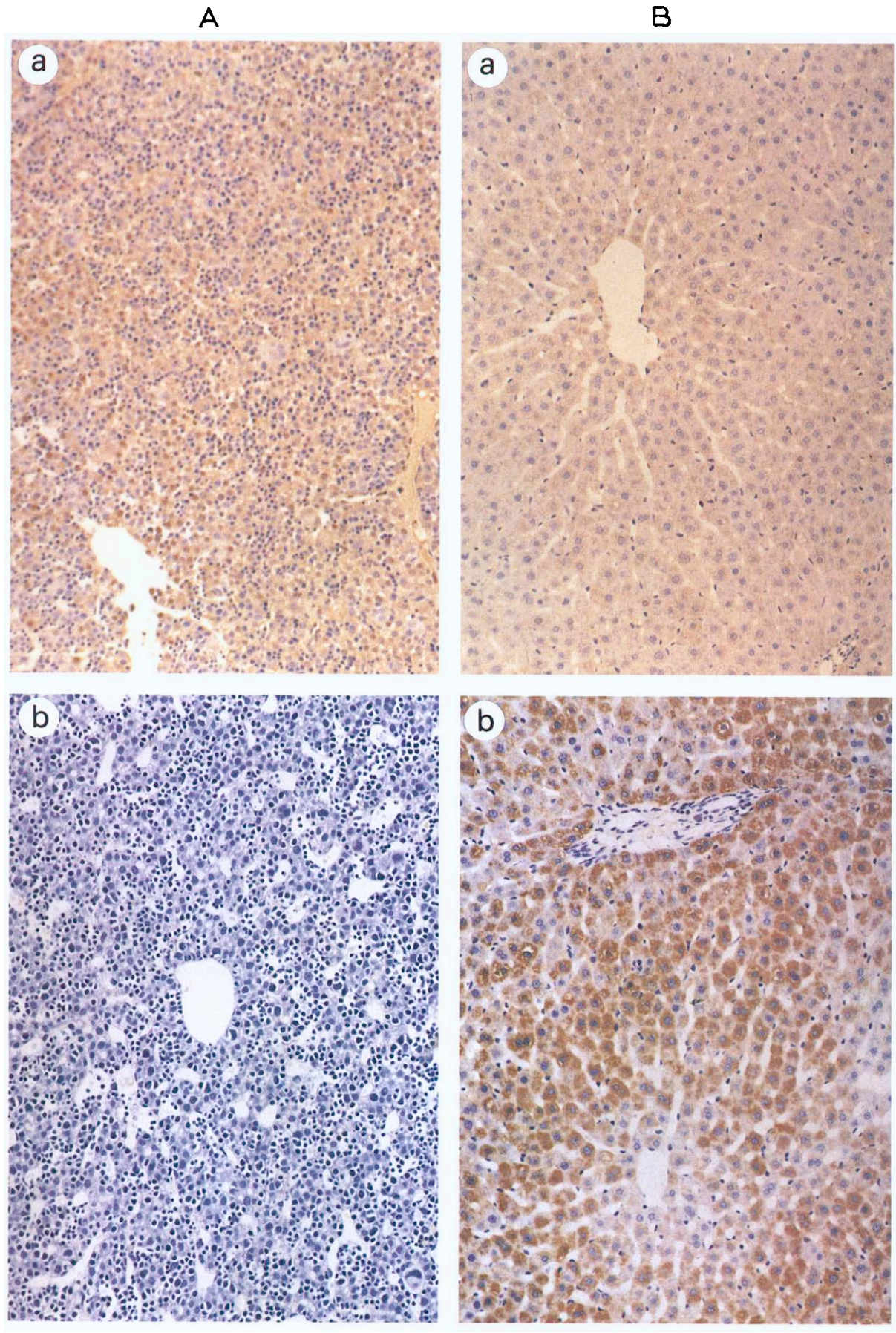
Fig. 2. Immunohistochemical analysis of *S*-adenosylmethionine synthetase isozymes in rat fetal and adult livers. Sections of rat fetal (A) and adult (B) livers were stained with antibodies against rat kidney-type (a) and liver-type (b) AdoMet synthetases. Magnification, ×45.


A
B
a
a
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b

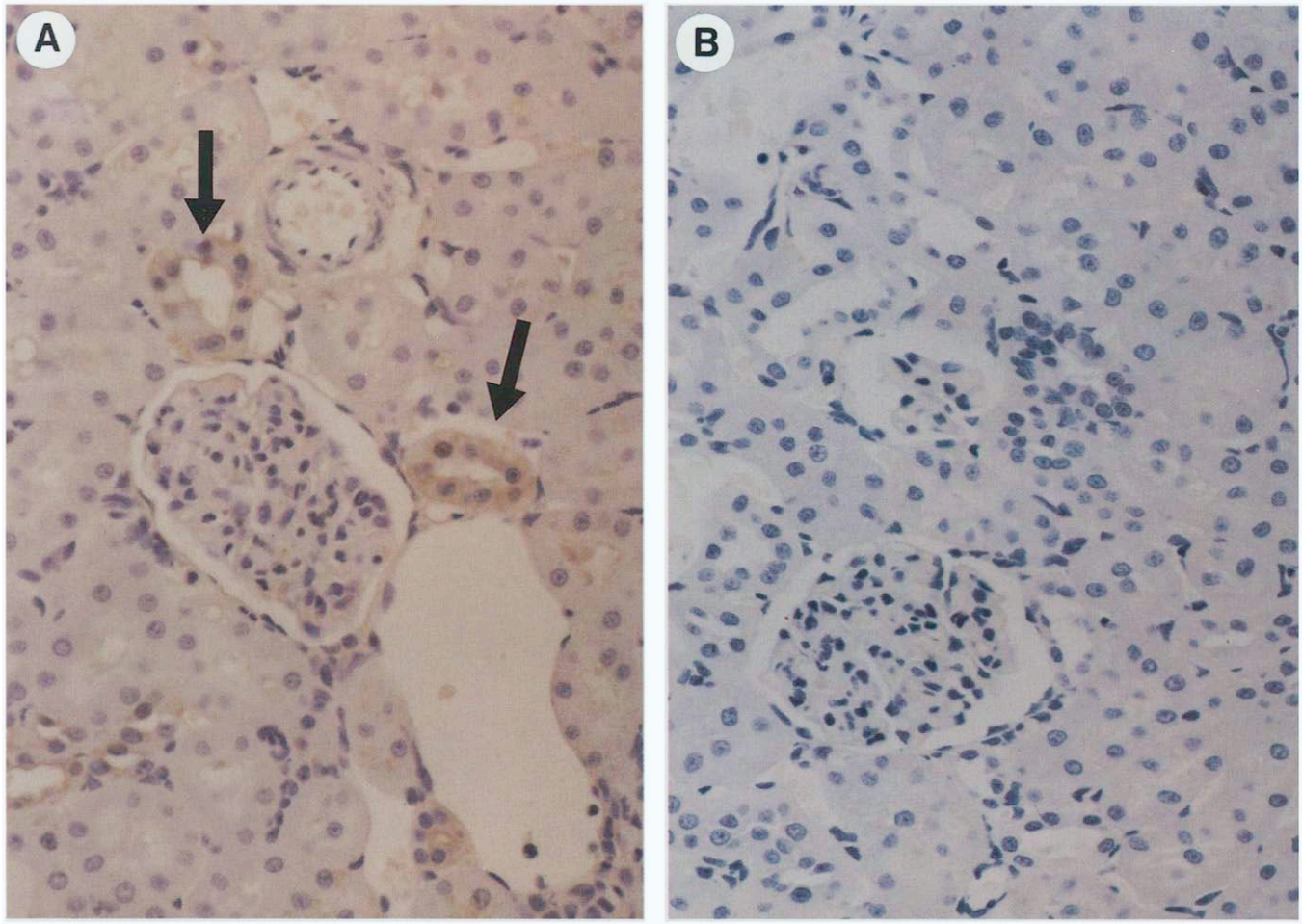

Fig. 3. Immunohistochemical localization of kidney-type *S*-adenosylmethionine synthetase in rat kidney. A section of rat kidney was stained with antibodies against rat kidney-type *S*-adenosylmethionine synthetase (A) and rat liver-type *S*-adenosylmethionine synthetase (B), and visualized with the peroxidase reaction. Arrowheads show the distal tubule. Magnification, × 75.

(RT-PCR) analysis in human livers [15]. In addition, we have found that the AdoMet synthetases are localized in the cytoplasm of hepatocytes.

Furthermore, we have attempted to examine the localization of the kidney-type AdoMet synthetase in rat kidney (Fig. 3). It was obvious that the immunoperoxidase activity of the kidney-type AdoMet synthetase was restricted to the region of the distal tubule (Fig. 3A). Other types of kidney cells did not display a significant level of kidney-type enzyme immunostaining. We can, however, not exclude the possibility that a small amount of the kidney-type *S*-adenosylmethionine synthetase might exist in other portions of the kidney. No immunoreactivity was detected in the kidney with anti-rat liver-type antibodies (Fig. 3B).

We have prepared two antibodies that show specific reactivity to two AdoMet synthetase isozymes, respectively. In this study we have used these antibodies to immunolocalize AdoMet synthetase isozymes in the liver and kidney of rats. This study provides the first definitive evidence that the expression of AdoMet synthetase isozymes are developmentally regulated and that the changes occur within the same cell. In addition, in the kidney we have shown that the kidney-type AdoMet synthetase immunoreactivity is restricted to the distal tubule. Further work is needed to characterize the role of the kidney-type AdoMet synthetase in the distal tubule.

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