

AN ADENOSINE 3':5'-MONOPHOSPHATE ADENOSINE-BINDING PROTEIN FROM MOUSE LIVER

Association with *S*-adenosylhomocysteinase activity

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1. Introduction

Cyclic AMP-binding proteins not associated with protein kinase activity have been described in various tissues [1–7]. Most of these proteins have been shown to bind adenosine [3,6,7].

We have purified to apparent homogeneity a cyclic AMP adenosine-binding protein from mouse liver [7]. The molecular properties of this protein and its interaction with adenosine, adenine and adenine nucleotides have been studied to some detail [7–11]. A fraction of adenosine bound to this protein is converted to adenine. Deamination of adenosine to inosine by the enzyme adenosine deaminase is decreased in the presence of the binding protein. On the basis of these results it was suggested that the protein may sequester adenosine in the cell [12].

It was reported [13] that an adenosine binding protein from human placenta was associated with *S*-adenosylhomocysteinase activity. In the light of this report we investigated whether the cyclic AMP adenosine-binding protein from mouse liver catalyzes the formation of *S*-adenosylhomocysteine from adenosine and homocysteine. This report brings the result of this investigation showing that the apparent homogenous preparation of the binding protein possesses both *S*-adenosylhomocysteine synthase and hydrolase activity. The enzyme activity and the adenosine/adenine-binding activity co-migrated upon

polyacrylamide gel electrophoresis under various conditions.

2. Experimental

2.1. Materials

S-Adenosyl-L-homocysteine, DL-homocysteine, adenosine, inosine, adenine, adenosine deaminase (type I from calf intestinal mucosa) were obtained from Sigma Chemical Co. Polyethyleneimine was from Serva, Heidelberg, cellulose powder, MN-300 from Macherey Nagel, FRG. [8-¹⁴C]Adenosine (59 mCi/mmol) was from the Radiochemical Centre, Amersham. The binding protein was purified to apparent homogeneity as in [11].

2.2. Polyacrylamide gel electrophoresis

Polyacrylamide gel electrophoresis of the native binding protein was performed in 75 × 5 mm tubes at 6.5%, 7.5% and 9% gel (30% crosslinking). The gel buffer was 10 mM Tris/glycine, pH 8.8, or 15 mM Tris/phosphate, pH 7.5. The electrophoresis was conducted at 4°C using 8 mA/gel. The gels were either stained or sliced and eluted as in [12].

2.3. Thin-layer chromatography

PEI-Cellulose thin-layer sheets on glass plates (20 × 20 cm) were made as in [14]. Three chromatographic systems were used:

System A. The PEI-cellulose plates were developed in 1.2 M LiCl;

Abbreviations: SAH, *S*-adenosylhomocysteine; PEI, polyethyleneimine

System B, *n*-Butanol/glacial acetic acid/water, 2:1:1 (v/v/v).

The R_F values for *S*-adenosylhomocysteine (SAH) in these systems were 0.64 and 0.43, respectively. The values for adenine and adenine derivatives are in [12,15].

System C. Adenine ($R_F = 0.61$), adenosine ($R_F = 0.52$), inosine ($R_F = 0.37$) and SAH ($R_F = 0.15$) were separated on PEI-cellulose plates using isobutanol/ethanol/water, 2:1:1 (v/v/v) as solvent.

2.4. Assay for *S*-adenosylhomocysteine synthase and hydrolase activity

The enzyme activity was usually assayed in the direction of synthesis. The incubation buffer was 150 mM potassium phosphate buffer (pH 7.3) containing 3 mM DL-homocysteine, 2 mM 2-mercaptoethanol, [$8\text{-}^{14}\text{C}$]adenosine (10 μM) and 0.2% bovine serum albumin. The assay was started by the addition of protein. The incubation was conducted at 37°C and was stopped by adding aliquots from the incubation mixture into equal volumes of 0.8 N perchloric acid. Neutralized samples [12] were subjected to chromatography in system C, the spots visualized by ultraviolet absorption, scraped off and counted by liquid scintillation [12].

3. Results

3.1. Formation of *S*-adenosylhomocysteine from adenosine and homocysteine

The binding protein was incubated in the presence of DL-homocysteine and [^{14}C]adenosine under the conditions in section 2.4. A radioactive substance was formed which was identified as SAH in 3 chromatographic systems (A–C) (data not shown). The amount of SAH formed was linear with respect to concentration of binding protein and time of incubation (data not shown). The double reciprocal plot for the formation of SAH versus the concentration of adenosine was nonlinear and consistent with a K_m value of 7×10^{-7} M and 2×10^{-5} M for adenosine (fig.1). The *S*-adenosylhomocysteine synthase activity was determined at 0.025–15 mM DL-homocysteine. Adenosine was 20 μM . The double reciprocal plot for the enzyme activity versus the concentration of DL-homocysteine was linear. An app. K_m of 0.15 mM was calculated for DL-homocysteine (data not shown).

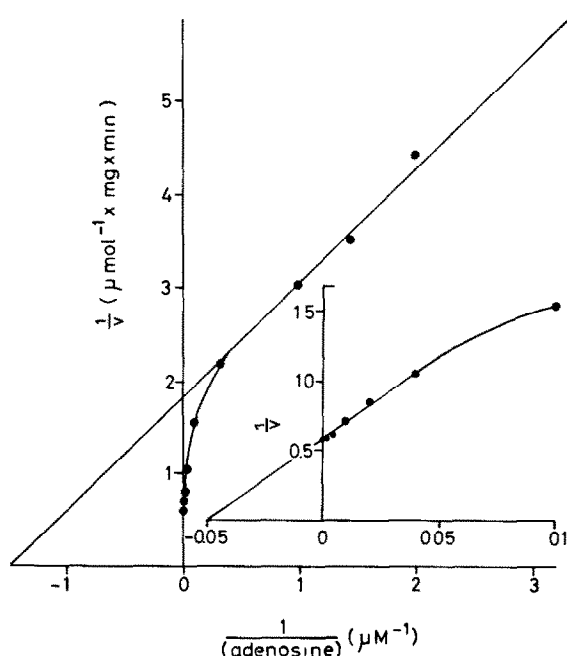


Fig.1. Double reciprocal plot for the *S*-adenosylhomocysteine synthase activity versus the concentration of adenosine. The enzyme activity was measured under the conditions in section 2.4, except that adenosine was varied between 0.3 μM and 2 mM. Inset shows the plot at high concentrations (10 μM to 2 mM) of adenosine.

3.2. Polyacrylamide gel electrophoresis

The adenosine/adenine-binding activity and the *S*-adenosylhomocysteine synthase activity co-migrated upon polyacrylamide gel electrophoresis under various conditions (see section 2.2). The results obtained at pH 8.8 are shown in fig.2. This observation indicates that these activities reside on the same protein molecule.

3.3. Hydrolysis of *S*-adenosylhomocysteine

Radioactive SAH was formed from [^{14}C]adenosine and homocysteine in the presence of the binding protein. Upon removal of free adenosine by the addition of adenosine deaminase, the concentration of SAH decreased as a function of time (fig.3). The radioactivity was recovered as inosine. No decrease in the concentration of SAH was observed if the binding protein was denatured by heating (100°C for 5 min) prior to the addition of adenosine deaminase. The

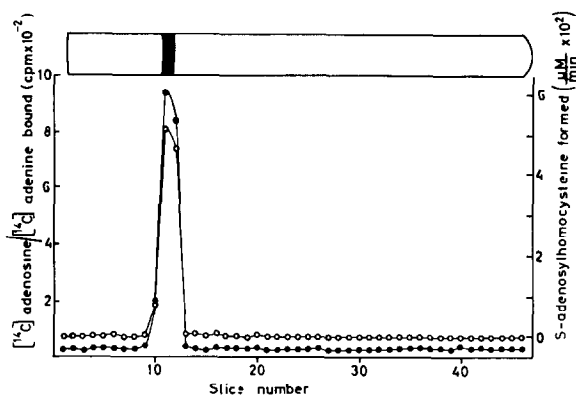


Fig. 2. Polyacrylamide gel electrophoresis of the native binding protein. Binding protein (40 μ g) was subjected to polyacrylamide gel electrophoresis at pH 8.8 and 9% gel. The gels were either stained or cut with a gel cutter. The gel slices were eluted and the binding of adenosine/adenine (\bullet — \bullet) measured as in [12]. The *S*-adenosylhomocysteine synthase activity (\circ — \circ) was determined at a 600-fold dilution of the eluate.

results are consistent with hydrolysis of SAH to adenosine and homocysteine upon removal of adenosine from the incubation mixture.

4. Discussion

The cyclic AMP adenosine-binding protein from mouse liver catalyzes both the synthesis of SAH from adenosine and homocysteine and the hydrolysis of SAH upon removal of adenosine by deamination (fig. 1,3). These observations show that the binding protein is associated with *S*-adenosylhomocysteinase activity. This enzyme was first discovered [16] in bovine liver and was shown to catalyze the thioether-bound formation between adenosine and homocysteine [16]. The equilibrium of the reaction lies far in the direction of condensation and removal of adenosine shifts the reaction towards hydrolysis [16]. The metabolic flow has been suggested to be in the direction of hydrolysis of SAH [17]. The enzyme has been named *S*-adenosylhomocysteinase according to this reaction.

SAH is a potent inhibitor of cellular methylation processes using *S*-adenosylmethionine as a methyl donor. The enzyme *S*-adenosylhomocysteinase has been suggested to play a role in the regulation of bio-

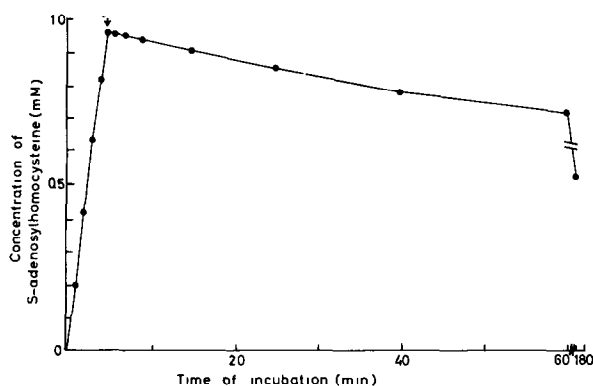


Fig. 3. Hydrolysis of *S*-adenosylhomocysteine upon deamination of adenosine. Binding protein (50 μ g/ml) was incubated in the presence of adenosine (1 mM) and homocysteine under the conditions in section 2.4. After 5 min incubation, the incubation mixture was made 15 units/ml in adenosine deaminase (arrow). The concentration of SAH (\bullet — \bullet) was plotted versus time of incubation.

logical methylation [18]. The cyclic AMP adenosine-binding protein from mouse liver has been shown to interact with cyclic AMP and other adenine nucleotides [7–11]. The identification of the binding protein as *S*-adenosylhomocysteinase thus suggests an association between the adenine nucleotide metabolism and biological methylation.

A fraction of adenosine bound to this protein is converted to adenine [12]. The conversion to adenine could not be detected under the condition of assay for *S*-adenosylhomocysteine synthase activity used in this paper as the protein was extensively diluted to meet with the requirement of linearity of enzyme activity versus time of incubation and concentration of protein.

Both the sequestration of adenosine without its conversion to SAH and hydrolysis of protein bound adenosine to adenine could be demonstrated at high (cellular) concentration of binding protein in the presence of homocysteine (unpublished results). PEI-cellulose chromatography (system C) allows the separation of adenosine, adenine, inosine and SAH, and is thus convenient for the simultaneous determination of the conversion of adenosine to adenine and the protection of adenosine against deamination to inosine under the condition of enzymic hydrolysis or formation of SAH.

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