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COMPARISON OF SOME PHYSICOCHEMICAL AND KINETIC PROPERTIES OF *S*-ADENOSYLHOMOCYSTEINE HYDROLASE FROM BOVINE LIVER, BOVINE ADRENAL CORTEX AND MOUSE LIVER

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S-Adenosyl-L-homocysteine hydrolase (EC 3.3.1.1) was purified to apparent homogeneity from bovine liver, bovine adrenal cortex and mouse liver. All enzymes were tetramers, composed of two types of subunit present in the proportion 1:1, as judged by SDS-polyacrylamide gel electrophoresis. The partition coefficient was exactly the same for these enzymes on high-performance gel permeation chromatography, and they co-sedimented in density gradients, suggesting the same molecular size and form of *S*-adenosylhomocysteine hydrolase from these sources. The bovine enzymes differed from the mouse liver enzyme with respect to isoelectric point (pI = 5.35, versus pI = 5.7), affinity for DEAE-cellulose, and migration of subunits on SDS-polyacrylamide gel electrophoresis with SDS from some commercial sources. The enzymes were not substrates for cAMP-dependent protein kinase. The apparent K_m values for adenosine (0.2 μM) and *S*-adenosylhomocysteine (0.75 μM) were the same for all three enzymes. The ratio between V_{max} for the synthesis and hydrolysis of *S*-adenosylhomocysteine was about 4 for the mouse liver enzyme, and about 6 for the bovine enzymes. It is concluded that only subtle kinetic and physicochemical differences exist between *S*-adenosylhomocysteine hydrolase from these bovine and mouse tissues. This suggests that differences in experimental procedures rather than species- and organ-differences of *S*-adenosylhomocysteine hydrolase are responsible for the variability in kinetic and physicochemical parameters reported for the mammalian hydrolase.

Introduction

S-Adenosylhomocysteine hydrolase (EC 3.3.1.1.) catalyzes the reversible hydrolysis of the endogenous transmethylase inhibitor, *S*-adenosylhomocysteine, to adenosine and L-homocysteine [1]. This enzyme was first described in rat liver by De La Haba and Cantoni in 1959 [2], and has recently been purified to apparent homogeneity from various mammalian tissues [3–9]. Evidence has been provided by Hershfield [6] that *S*-adenosylhomocysteine hydrolase from human placenta is identical to the so-called cyclic AMP-adenosine binding protein [10], and this observation has been confirmed for the enzyme from human lymphoblasts [11], mouse liver [12] and bovine adrenal cortex and liver (data to be presented in the present report).

Disagreement exists as to whether these proteins are tetramers [3–7,9,13] or pentamers [8,14]. The M_r has been reported as from 180000 to

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Abbreviations: SDS, sodium dodecyl sulfate; STS, sodium tetradecyl sulfate; Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid.

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240000 and subunit M_r from 45000 to 60000 [3–9,13–15]. Furthermore, data on the kinetic properties of homogeneous *S*-adenosylhomocysteine hydrolase from mammalian tissues are rather inconsistent, the K_m values for adenosine ranging from 0.2 to 420 μM and the K_m values for *S*-adenosylhomocysteine from 0.75 to 63 μM [5–9,12,13,16].

The different kinetic and physicochemical properties reported for *S*-adenosylhomocysteine hydrolase isolated from various mammalian sources may be due to species- or tissue-differences. In fact, the differential metabolic response of various tissues to the synthetic substrate 3-deazaadenosine has tentatively been ascribed to different properties of *S*-adenosylhomocysteine hydrolase [17]. Alternatively, the discrepancy may be related to varations in experimental design or procedures from one laboratory to another. As a first attempt to evaluate these two possibilities, some physical and kinetic properties of *S*-adenosylhomocysteine hydrolase from bovine liver, bovine adrenal cortex and mouse liver have been determined and compared.

Experimental

Chemicals

Adenosine, *S*-adenosyl-L-homocysteine, DL-homocysteine, adenosine deaminase (type I from calf intestinal mucosa) and crystalline serum albumin were from Sigma Chemical Co. St. Louis. MO, U.S.A. Acrylamide and bisacrylamide were from Eastman Kodac Co., Rochester, NY, U.S.A. Sodium dodecyl sulfate was obtained from the following sources: Bio-Rad Lab., Richmond, CA, U.S.A. (Electrophoresis purity reagent, 22251), BDH Chemicals Ltd., Poole, Dorset, U.K. (Red Label, specially pure, 660863109), Pierce Chem. Co., Rockford, IL, U.S.A. (Sequenal grade, No. 28312), Serva (Krist. Reinst, 20760), Merck, Darmstadt, F.R.G. (Art, 2969, Lot No. 4170185), Sigma (No. L-5750, stated to contain 60% dodecyl sulfate, 32% tetradecyl sulfate and 8% hexadecyl sulfate; Lot 49-0438), Koch-Light, Colnbrook, Bucks, U.K. (Pract. grade, Lot 74169). Sodium tetradecyl sulfate (STS) was from Eastman Kodak (Lot. A912). [8-^{14}C]Adenosine (0.59 m Ci/m mol), cyclic [8-^{3}H]AMP (27 Ci/mmol) and [α-^{32}P]ATP (5 Ci/mmol) were from the Radiochemical Centre, Amersham, U.K. *S*-[8-^{14}C]Adenosyl-L-homocysteine (0.59 m Ci/mmol) was synthesized enzymatically from [^{14}C]adenosine and DL-homocysteine [16].

High-performance gel permeation liquid chromatography

Samples of 20 μl were subjected to analysis on a G 3000SW protein column (0.75 × 60 cm, Toyo Soda Manufacturing Co. Tokyo, Japan) eluted with 15 mM Hepes-NaOH, pH 7.0, containing 150 mM KCl/0.3 mM EGTA/0.1 mM EDTA/10 mM 2-mercaptoethanol, at ambient temperature and a flow rate of 1 ml · min^{-1}. The solvent delivery system was a ConstaMetric III high-pressure pump, from Laboratory Data Control. The absorbance of the effluent was recorded at 280 nm, using an ultraviolet-detector, model 1203 from Laboratory Data Control.

The void volume of the column was determined with Blue Dextran (Pharmacia Chemical Co., Uppsala, Sweden). The column was calibrated with the following standard proteins, and their Stokes radii were taken from the references in the publications of Ackers [18], Siegel and Monty [19] and Laurent and Killander [20]: horse heart cytochrome *c* (1.7 nm), skeletal muscle myoglobin (1.9 nm), bovine pancrease chymotrypsinogen (2.1 nm), soybean trypsin inhibitor (2.3 nm), ovalbumin (2.8 nm), bovine serum albumin (3.55 nm), human transferrin (3.6 nm), bovine liver catalase (5.2 nm), bovine γ-globulin (5.2 nm), and equine spleen ferritin (7 nm).

Polyacrylamide gel electrophoresis

Discontinuous SDS-polyacrylamide gel electrophoresis was performed according to the method of Laemmli [21], using cylindrical running gels (7 mm i.d.) of 10% w/v polyacrylamide (2.6% cross-linking with *N*,*N*′-methylene bisacrylamide). Treatment of samples and composition of electrophoresis buffers and stacking gel were exactly as described by Laemmli [21]. The gels were run (1 mA/gel) at room temperature in a Pharmacia GE-2/4LS apparatus with buffer recirculation. Continuous SDS-polyacrylamide gel electrophoresis was carried out according to Weber and Osborne [22], and electrophoresis of the native enzyme as described earlier [4]. The gels were fixed

in 1.2 M acetic acid/10 M methanol, stained in the same solution containing 0.04% (w/v) Coomassie brilliant blue R (Gurr, Bucks, U.K.), destained in acetic acid/methanol, and stored in 1.2 M acetic acid. When sodium tetradecyl sulfate was used instead of SDS, precipitates formed when the gels were immersed in tubes with acetic acid/methanol. The precipitates dissolved, however, when the tubes were placed in a shaking waterbath at 70°C for 3 h. The gels were scanned at 633 nm using a Zeine soft laser densitometer (Biomedical Instruments, Chicago, IL, U.S.A.).

Isoelectric focusing

This was carried out either in a vertical glass column as described [4], cylindrical polyacrylamide gels according to the method of Catsimpoolas [23], polyacrylamide slabs according to the manufacturer (Separation News, 4-5, 1979, Pharmacia Fine Chemicals, Uppsala, Sweden), or a horizontal layer of Sephadex G-75 (Ultradex) using an LKB 2117 Multiphor. In the last case the bed volume was 70 ml and contained 5 g Ultradex and 1.5 ml of Ampholine (LKB) pH 5–8 and 3 ml of Ampholine pH 4–6. The focusing was performed at 7 W constant power for 16 h at 4°C.

The pH was measured [4] directly in the fractions elulted from the glass column, and in the distilled-water eluates from Ultradex fractions or from slices (1 mm thickness) of cylindrical polyacrylamide gels. For the measurement of pH in the Ultradex-bed, a LKB 2117-111 Multiphor surface electrode was used. The enzyme band was localized by inspection of the plate under ultra-violet light. The isoelectric point of proteins focused in slab gels was determined by reference to standards (Broad p*I* calibration kit, Pharmacia).

Purification of cAMP-dependent protein kinase and preparation of its catalytic subunit

cAMP-dependent protein kinases, types I and II, were purified from rabbit skeletal muscle and bovine heart, respectively [24]. The free catalytic subunit from bovine heart protein kinase was prepared as described [25].

Purification of S-adenosylhomocysteine hydrolase

Tissue was homogenized (1/4, w/v) for 1 min at 0°C in a Silverson mixer-emulsifier in 20 mM Tris-HCl buffer (pH 7.5)/0.25 M sucrose/5 mM EDTA/10 mM 2-mercaptoethanol, containing 14% w/v poly(ethylene glycol) (mean M_r 6000). The homogenate was centrifuged for 15 min at 12000 $\times g_{av}$. To the supernatant thus obtained a further 170 g/l (to the mouse liver homogenate) or 190 g/l (to the bovine tissue homogenates) of poly(ethylene glycol) were added. The precipitate was collected by centrifugation, and further purified by a modification of a previously described [4] procedure: DEAE-cellulose chromatography, ammonium sulfate fractionation, gel chromatography (Ultrogel AcA-34), isoelectric focusing (in a granulated horizontal gel), hydroxyapatite chromatography (on a 2.6 × 2 cm column equilibrated with 3 mM potassium phosphate, pH 6.8, and eluted with a 0.5 litre linear gradient of 3–30 mM potassium phosphate, pH 6.8), and density gradient (10–25% w/v glycerol) centrifugation. The enzyme from bovine adrenal cortex was subjected to a second isoelectric focusing in a vertical column with sucrose as the stabilizing medium after the hydroxyapatite chromatography, and then to a second hydroxyapatite chromatography before the density gradient centrifugation.

Assay for S-adenosylhomocysteine synthase and hydrolase activity

This was performed by a radiochemical method described elsewhere [16]. The incubation buffer was 15 mM Hepes-NaOH, 150 mM KCl, 5 mM magnesium acetate, 2.5 mg/ml of bovine serum albumin and (when not otherwise indicated) 5 mM 2-mercaptoethanol. The temperature was 37°C.

Other assays

Adenosine deaminase [26], protein kinase [27] and cAMP-adenosine-binding [28] activities were determined according to published procedures.

Determination of protein

This was performed by the method of Klungsøyr [29] using bovine serum albumin as standard.

Results

Notes on the purification of S-adenosylhomocysteine hydrolase

Some differences were noted in the behaviour

of the mouse enzyme and bovine enzymes during purification. Thus, the mouse enzyme was eluted from DEAE-cellulose with 15 mM Tris-HCl, pH 7.5, and 4 mM EDTA, whereas inclusion of 30 mM NaCl was required for the elution of the bovine enzymes. The bovine enzymes focused at pH 5.35–5.50, and the mouse liver enzyme at pH 5.65–5.75 in granulated bed. A slightly higher concentration (about 1.5% w/v) of poly(ethylene glycol) was required for the precipitation of the bovine enzymes in crude extract. Whether this was related to intrinsic differences between the enzymes or differential interaction of the enzymes with factors present in the extracts could not be decided because none of the purified enzymes precipitated at any concentration of poly(ethylene glycol). All enzymes behaved similarly on Ultrogel chromatography or density gradient centrifugation (9.0 S).

The binding activity for adenosine and cAMP [4] co-purified with the *S*-adenosylhomocysteine hydrolase activity during all purification steps.

All enzyme preparations were contaminated with adenosine deaminase up to the Ultrogel-step, which separated the low-M_r deaminase of liver from the *S*-adenosylhomocysteine hydrolase. In the case of bovine adrenal cortex, however, about 20% of the deaminase chromatographed like *S*-adenosylhomocysteine hydrolase. Upon rechromatography of the *S*-adenosylhomocysteine hydrolase peak, part of the deaminase appeared in a low-M_r form and part chromatographed near *S*-adenosylhomocysteine hydrolase. Upon gradient centrifugation the adenosine deaminase appeared as a 3 S form and an 11 S form, the latter overlapping with a 10 S protein and *S*-adenosylhomocysteine hydrolase. The *S*-adenosylhomocysteine hydrolase was completely separated from the 10 S protein and adenosine deaminase by repeated isoelectric focusing and hydroxyapatite chromatography. Mixing of the 3 S form of adenosine deaminase with the 10 S protein converted part of the deaminase to the 11 S form. No such shift occurred when the deaminase was mixed with *S*-adenosylhomocysteine hydrolase. The 10 S protein could thus be tentatively identified as an adenosine deaminase-binding protein [30–33]. The 10 S protein had a subunit M_r of about 90000 as judged by SDS-polyacrylamide gel electrophoresis according to Weber and Osborn [22], co-chromatographed exactly with *S*-adenosylhomocysteine hydrolase on Ultrogel, and had a p*I* of 5.40. These are physical properties which resemble those of adenosine deaminase-binding proteins [30–33]. It did not bind adenosine or cAMP.

The preparations of *S*-adenosylhomocysteine hydrolase were nearly homogeneous as judged by native or SDS-polyacrylamide gel electrophoresis and by high-performance gel permeation chromatography. They were stable for at least 1 year when stored as concentrated solution (2–20 mg/ml) at −80°C or in liquid nitrogen. They were progressively inactivated at −18°C, especially in the absence of reducing agents (such as 2-mercaptoethanol).

High-performance gel permeation liquid chromatography

This was carried out with a mobile phase supplemented with 150 mM KCl, since preliminary experiments showed that *S*-adenosylhomocysteine hydrolase was partly retained by the stationary phase at low ionic strength. Single sharp peaks,

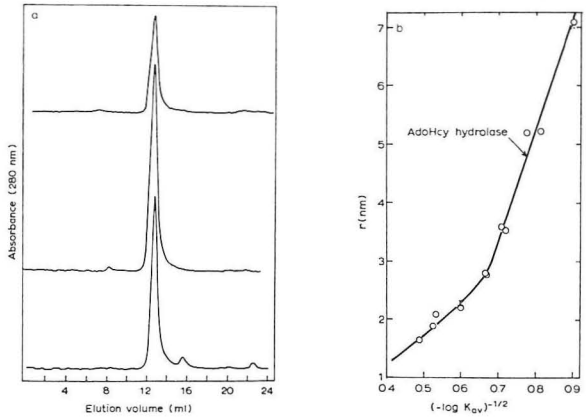

Fig. 1. High-performance gel permeation liquid chromatography. a. The elution profile of *S*-adenosylhomocysteine hydrolase for bovine adrenal cortex (upper), bovine liver (middle) and mouse liver (lower) on high-performance gel permeation chromatography. From 20 to 80 μg of enzyme protein were injected. b. Calibration curve for determination of Stokes radius from the elution volume of standard proteins (ferritin, catalase, γ-globulin, bovine serum albumin, transferrin, ovalbumin, soybean trypsin inhibitor, chymotrypsinogen, myoglobin and cytochrome) on the size-exclusion column. The data are plotted according to Laurent and Killander [20]. See Experimental section for details. AdoHcy, *S*-adenosylhomocysteine.

eluting at exactly the same retention time (12.43–12.46 min) were obtained for each enzyme (Fig. 1a). The plot of Stokes radii of standard proteins versus K_{av} (see Ref. 20), though not linear, indicated a Stokes radius of 4.8 nm for the enzymes (Fig. 1b). A non-linear relation between Stokes radius and K_{av} has been observed for agarose gels by Le Maire et al. [34], and has been ascribed to more than one set of pore sizes in the gel.

SDS-polyacrylamide gel electrophoresis

In the continuous system of Weber and Osborn [22] used as described [4], all enzyme subunits migrated as M_r 45000 proteins regardless of the source of SDS used.

With the discontinuous system of Laemmli [21] sharper bands were observed, and when the protein load was low (1–5 μg) and the distance of migration long (5–10 cm), two closely spaced bands staining with equal intensity were observed (Fig. 2). This doublet was observed for all enzyme preparations whether SDS from Bio-Rad or Sigma, or STS was used. The difference in migration distance of the two bands corresponded to a difference in M_r of about 1000. The reliability of discontinuous SDS-electrophoresis for M_r determination is doubtful, however. Different shapes of the M_r calibration curves were observed depending on whether commercial SDS of high (Bio-Rad or BDH) or low (Sigma) purity or STS was used (Fig. 3). SDS from Pierce and Serva produced standard curves similar to those with SDS from Bio-Rad or BDH, whereas SDS from Merck or Koch-Light gave standard curves similar to that with SDS from Sigma (data not shown).

Whereas the doublet for each species of *S*-adenosylhomocysteine hydrolase was observed with detergent of different composition and purity

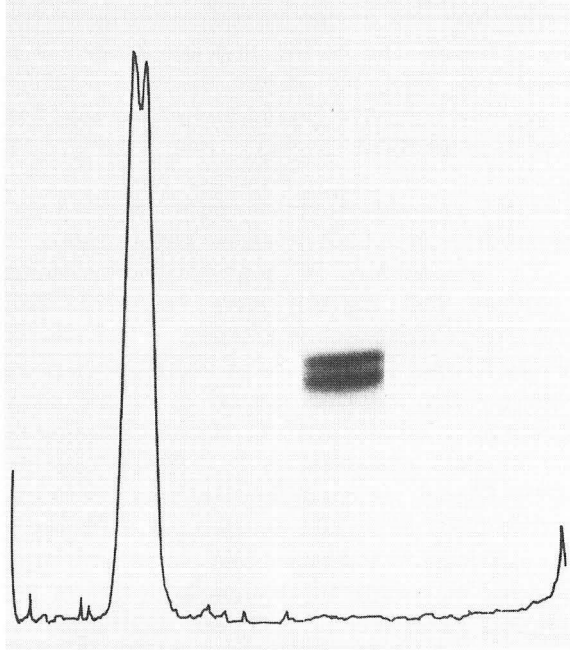

Fig. 2. Resolution of two peaks on SDS-polyacrylamide gel electrophoresis of homogeneous *S*-adenosylhomocysteine hydrolase. Mouse liver *S*-adenosylhomocysteine hydrolase (3 μg) was subjected to discontinuous SDS (from Bio-Rad) -polyacrylamide gel electrophoresis. The migration distance of the enzyme subunits was about 10 cm, which was required to obtain sufficiently high resolution to separate the subunits. The separation gel (stained for protein with Coomassie blue) was scanned at 633 nm (top of the gel to the right and bottom to the left.). The inset shows a photo of the part of the gel containing the two bands.

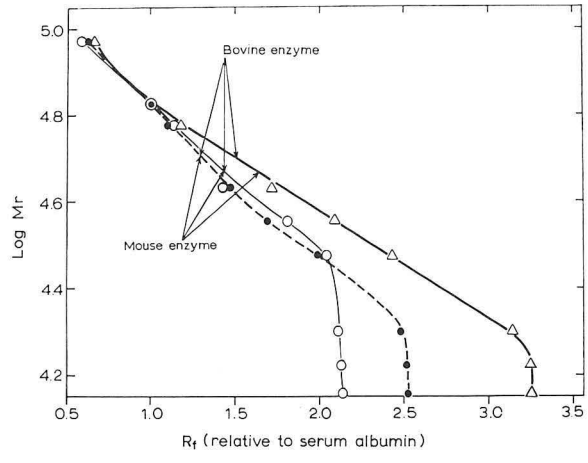

Fig. 3. Calibration curves for M_r determination of protein chains on discontinuous polyacrylamide gel electrophoresis with detergent from different sources. The log M_r values of the following marker proteins, Phosphorylase *b* (subunit M_r 94000), albumin (67000), catalase (60000), ovalbumin (43000), carbonic anhydrase (30000) and trypsin inhibitor (20100), were plotted against their migration in the gels relative to the migration of albumin. The use of albumin rather than Bromphenol blue for the calculation of R_f was due to the variation of Bromphenol blue migration with the type of detergent used. All marker proteins (except catalase which was from Sigma) were from the low M_r Calibration kit from Pharmacia. The R_f of mouse and bovine *S*-adenosylhomocysteine hydrolase is indicated by arrows. ○, SDS (Bio-Rad); ●, STS; △, SDS (Sigma).

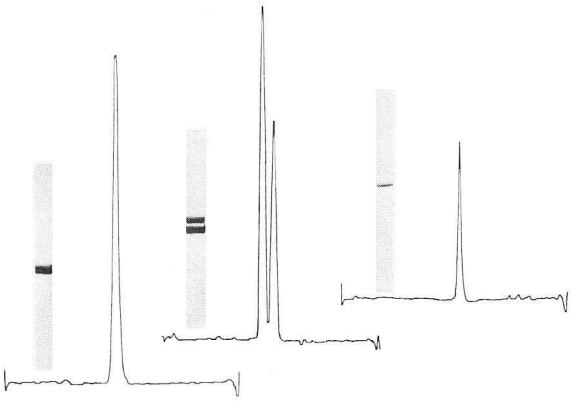

Fig. 4. Separation of bovine and mouse *S*-adenosylhomocysteine hydrolase on discontinuous polyacrylamide gel electrophoresis. The electrophoresis was with impure SDS (from Sigma). The gels were scanned as described in the legend to Fig. 2. The migration distance of the enzyme subunits was about 4 cm. The insets show photos of each gel. Mouse liver enzyme (30 μg) (gel to the left), a mixture of enzyme from mouse liver (30 μg) and bovine adrenal cortex (15 μg) (gel in the middle) and bovine liver enzyme (4 μg) (gel to the right) were subjected to gel electrophoresis.

(see above), separation of the bovine enzymes and the mouse liver enzyme was obtained with SDS from Merck, Koch-Light or Sigma (Fig. 4), but not with STS or SDS of high purity. Thus, it is likely that separation of bovine and mouse enzyme subunits is due to differential binding of some species of detergent contaminating certain SDS preparations. Using the calibration curve of Fig. 3, the estimated subunit M_r of the mouse and bovine enzyme would be 46000 and 50000, respectively. Whereas the doublet probably would escape detection [4] under the conditions used for continuous SDS-polyacrylamide gel electrophoresis, protein chains of M_r 46000 and 50000 would easily be separated. The separation between the protein chains of enzyme from bovine and mouse tissue with impure SDS was thus only apparent in the discontinuous system.

Isoelectric focusing

As indicated in the Experimental section the bovine enzyme has a p*I* of 5.35–5.50 and the mouse enzyme a p*I* of 5.65–5.75 on focusing in granulated gel during purification. The same p*I* was found when tissue extract (dialyzed overnight against 2 mM Hepes-NaOH (pH 7.0)/1 mM EDTA/10 mM 2-mercaptoethanol) was focused. The bovine enzymes focused at pH 5.25 to pH 5.55 (mean p*I* 5.35) and the mouse liver enzyme between pH 5.60 and pH 5.80 (mean p*I* 5.70) in a series of experiments carried out in a vertical glass column [4]. The estimated p*I* value was not affected by the concentration (1–6% w/v) or pH range (pH 4–6, pH 5–8 or a mixture of pH 3–5 and pH 5–8) of the ampholytes, the orientation of the pH gradient or the stabilizing medium (sucrose or glycerol) used. Biphasic peaks were often observed, but refocusing of each peak gave rise to a similar biphasic pattern, suggesting that the peaks did not represent stable distinct forms of the enzymes. On focusing in polyacrylamide gels, the mouse and bovine enzymes were clearly separated when applied together, whereas the enzymes from bovine liver and bovine adrenal cortex could not be separated. The bovine enzymes focused at a lower pH than the mouse enzymes whether Ampholine (LKB) or Pharmalyte (Pharmacia) was used as ampholytes.

Test of S-adenosylhomocysteine hydrolase as a possible substrate for cAMP-dependent protein kinase

The preparations of *S*-adenosylhomocysteine hydrolase (1.4 mg/ml) were incubated with cAMP-dependent protein kinase type I or type II in the absence or presence of cAMP, as well as with the free catalytic subunit of bovine heart kinase for 2–60 min at 30°C. No incorporation of the terminal phosphate of [γ-^{32}P]ATP was detected. Under the conditions used an incorporation of 0.007 phosphate moieties per *S*-adenosylhomocysteine hydrolase subunit would have been detected. In parallel incubation up to 80% of the terminal phosphate of ATP was transferred to histone (type II from Sigma, present at 0.6 mg/ml). The phosphorylation of histone was not impaired in the presence of *S*-adenosylhomocysteine hydrolase. To test the possibility that the enzymes might be isolated in a fully phosphorylated form and thus not accept additional phosphate, they were preincubated for 2–120 min at 4 or 30°C with a phosphohistone phosphatase (purified about 120-fold from bovine renal cortex) in the presence of 5 mM Mg^{2+} or Mn^{2+}. This treatment did not im-

prove the ability of the enzymes to serve as phosphate acceptors.

Kinetic parameters for the synthesis and hydrolysis of S-adenosylhomocysteine

The apparent K_m values for adenosine and *S*-adenosylhomocysteine were 0.2 μM (range 0.15–0.30 μM) and 0.75 μM (range 0.52–1.2 μM), respectively, and were exactly the same for the enzymes from bovine adrenal cortex, bovine liver (Fig. 5) and mouse liver (Fig. 5 and Ref. 16).

It has previously been shown that reducing agents are essential for maintenance of catalytic activity of *S*-adenosylhomocysteine hydrolase [8,35]. The progress curves for both the synthesis and hydrolysis of *S*-adenosylhomocysteine levelled off at a shorter time of incubation in the absence of 2-mercaptoethanol than in its presence, and this was particularly pronounced at low concentrations of adenosine or *S*-adenosylhomocysteine (data not shown). However, the apparent K_m values for adenosine and *S*-adenosylhomocysteine (based on the determination of initial velocity) were not affected by the concentration (0, 2 or 10 mM) of 2-mercaptoethanol (data not shown).

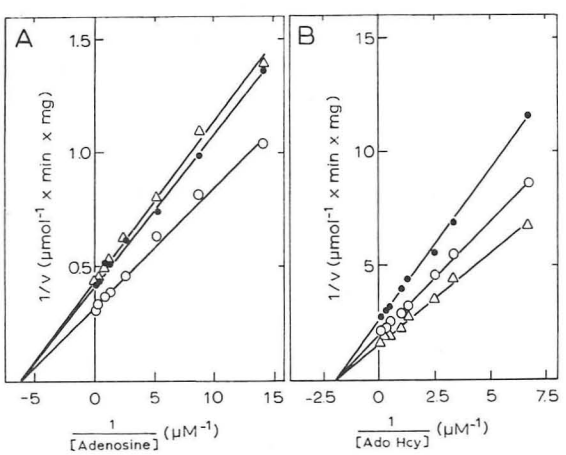

Fig. 5. Double-reciprocal plots for the synthesis and hydrolysis of *S*-adenosylhomocysteine. A, the initial velocities of the synthesis of *S*-adenosylhomocysteine in the presence of *S*-adenosylhomocysteine hydrolase from bovine adrenal cortex (●), bovine liver (○) and mouse liver (△) were determined at various concentrations of adenosine (0.075–10 μM). The concentration of DL-homocysteine was 3 mM. B, the initial velocities of the hydrolysis of *S*-adenosylhomocysteine catalyzed by the enzyme from bovine adrenal cortex (●), bovine liver (○) and mouse liver (△) were determined at various concentrations (0.15–10 μM) of *S*-adenosylhomocysteine. AdoHcy, *S*-adenosylhomocysteine.

The catalytic activity at saturating concentrations of substrates in the synthetic direction was higher than the rate of hydrolysis of *S*-adenosylhomocysteine (in the presence of excess adenosine deaminase). The ratio obtained when dividing the value for V_{max} for the synthesis of *S*-adenosylhomocysteine by V_{max} for the hydrolytic reaction, was 4.16 ± 0.63 ($n = 8$) for the mouse liver enzyme and 6.00 ± 0.51 ($n = 7$) and 5.91 ± 0.35 ($n = 7$) for the enzymes from bovine adrenal cortex and bovine liver, respectively. Thus, this ratio is significantly ($P < 0.0001$) lower for the mouse liver enzyme than for the bovine enzymes.

The specific activities of the enzyme purified from bovine adrenal cortex, bovine liver and mouse liver were of the same order of magnitude (0.3–0.9 μmol/min per mg) when determined in the hydrolytic direction.

Discussion

Homogeneous enzyme from bovine adrenal cortex, bovine liver, and mouse liver were all found to be associated with cAMP-adenosine-binding activity. Taken together with previously published data from the enzyme from other sources [6,11,12,36], this indicates that *S*-adenosylhomocysteine hydrolase and thr so-called cAMP-adenosine-binding proteins [4,10,14,28] or adenine analogue-binding proteins [37] represent the same entity.

Both the bovine and mouse enzyme preparations contained two types of subunit, present in the proportion 1:1 and separable on SDS-polyacrylamide gel electrophoresis when long gels and low protein loads were used (Fig. 2). The two types of subunit could be separated by polyacrylamide gel electrophoresis either in the presence of tetradecyl sulfate or SDS of varying degree of purity. The existence of two types of subunit in *S*-adenosylhomocysteine hydrolase has not been noted previously, and indicates that each enzyme molecule has two types of subunit present in the same proportion, or that *S*-adenosylhomocysteine hydrolase isolated from three different sources exists in two equally abundant forms which have different types of subunits.

All enzyme preparations sedimented as 9.0 S on density gradient centrifugation and eluted like proteins with Stokes radius of 4.8 nm on high-performance gel permeation chromatography (Fig. 1). This indicates an M_r 180000–190000 for *S*-adenosylhomocysteine hydrolase from bovine liver, bovine adrenal cortex and mouse liver, assuming they have the partial specific volume (0.74 cm^3/g) previously calculated for the mouse liver enzyme [15]. In conjunction with the data from continuous SDS-polyacrylamide gel electrophoresis (apparent subunit M_r 45000) and discontinuous polyacrylamide gel electrophoresis in the presence of pure SDS (M_r 46000) or sodium tetradecyl sulfate (M_r 50000; see Fig. 3), it is concluded that the three enzyme preparations investigated are tetramers. A similar conclusion has been reached by others for the enzyme from human placenta [6] calf and bovine liver [5,13], rat brain [7] and rat liver [9], whereas a pentameric composition has been suggested for the rat liver enzyme by Kajander and Raina [8] and for the cAMP-adenosine-binding protein from rabbit erythrocytes by Yuh and Tao [14]. If our finding of different subunits present in the proportion 1:1 (Fig. 2) reflects the fact that each enzyme molecule has two types of subunit, this is definitely in favour of the existence of a tetrameric rather than a pentameric composition of the enzyme.

The data of Richards et al. [5] on the calf liver enzyme are at variance with our studies and the study by Palmer and Abeles [13] on bovine liver enzyme with regard to M_r (237000) and subunit M_r (60000). They also report a p*I* of 5.8–6.0, which is about 0.5 pH unit higher than we have found in the present study. Whether this discrepancy reflects changes of the enzyme during ontogeny and growth, or is due to other factors, remains to be investigated.

Whereas no difference in physicochemical properties was noted between enzyme from bovine liver and bovine adrenal cortex, the bovine enzymes differed from the mouse liver enzyme in p*I* (5.35 versus 5.7), in having a higher affinity for DEAE-cellulose, and in migration on discontinuous polyacrylamide gel electrophoresis in the presence of SDS from certain commercial sources (Fig. 4). The basis for this is unknown, but several authors have reported separation of proteins by discontinuous SDS-polyacrylamide gel electrophoresis that is not related to M_r, but that is dependent on the composition of the commercial SDS preparations used [38–40]. Zoller et al. [41] were able to separate the nonphosphorylated and autophosphorylated forms of the regulatory subunit of cAMP-dependent protein kinase II using discontinuous SDS-polyacrylamide gel electrophoresis. The difference in migration between mouse and bovine hydrolase subunits on SDS-polyacrylamide gel electrophoresis (Figs. 3 and 4) could not be ascribed to varying degrees of cAMP-dependent phosphorylation, since we found none of the enzymes to be substrates for cAMP-dependent protein kinase.

Several explanations can be offered for the inconsistent data on the kinetic properties of *S*-adenosylhomocysteine hydrolase [5–8,11–13,16]. We have found that the enzyme must be extensively diluted (to about 0–5 μg/ml) to allow a reliable measurement of enzyme activity when the concentration of substrate (adenosine or *S*-adenosylhomocysteine) is below 1 μM, and that such highly diluted enzyme is rapidly inactivated, even at 0°C, in the absence of stabilizing factors such as serum albumin, reducing agents, physiological ionic strength, glycerol [8,16,35], as well as adenosine and *S*-adenosylhomocysteine (unpublished observations). The requirement for a very extensive purification of *S*-adenosylhomocysteine hydrolase from bovine adrenal cortex to remove contaminating adenosine deaminase (see Results section) indicates that even highly purified enzyme preparations may contain traces of adenosine deaminase activity, which will affect the determination of K_m for adenosine [42]. Alternatively, the enzyme may be modified during purification by partial denaturation, or by proteolysis. We found that the *S*-adenosylhomocysteine hydrolase activity of dialyzed tissue extracts focused in a pH gradient like purified enzyme (see Results section), that the activity in crude extracts chromatographed like purified enzyme on conventional (Sephadex G-150) or high-performance gel permeation chromatography (unpublished observations) and sedimented like purified enzyme on density gradient centrifugation [4,28].

In conclusion, the present study has demonstrated that *S*-adenosylhomocysteine hydrolase from bovine adrenal cortex and liver differ from

mouse liver enzyme in the ratio of V_{max} in synthetic and hydrolytic directions, in isoelectric point, and in migration on discontinuous polyacrylamide gel electrophoresis when certain commercial preparations of SDS are used. It has earlier been shown that the enzyme from bovine adrenal cortex has a higher affinity for DEAE-cellulose and binds [^{3}H]adenosine with an apparently higher affinity than the mouse liver enzyme [28]. The most striking feature is, however, the similarity between the enzymes from such relatively disparate species as mouse and ox, with respect to physicochemical (similar sedimentation coefficient and Stokes radius) as well as kinetic (same apparent K_m for adenosine and *S*-adenosylhomocysteine) properties.

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