Roberts, D. (1966) *Biochemistry 5*, 3546-3548.
Santi, D. V., & McHenry, C. S. (1972) *Proc. Natl. Acad. Sci. U.S.A. 69*, 1855-1857.
Scott, J. M. (1976) *Biochem. Soc. Trans.* 4, 845-850.
Segel, I. H. (1975a) *Enzyme Kinetics*, pp 57-58, Wiley, New York.
Segel, I. H. (1975b) *Enzyme Kinetics*, p 115, Wiley, New York.
Shin, Y. S., Buehring, K. U., & Stokstad, E. L. R. (1972) *J. Biol. Chem. 247*, 7266-7269.
Wahba, A. J., & Friedkin, M. (1962) *J. Biol. Chem. 237*, 3794-3801.

Multiple Species of Mammalian *S*-Adenosylmethionine Synthetase. Partial Purification and Characterization†

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ABSTRACT: Two species of *S*-adenosylmethionine (*S*-Ado-Met) synthetase (EC 2.5.1.6) exist in rat liver cytosol and a distinct species of the enzyme exists in kidney cytosol. *S*-Ado-Met synthetases α and β in rat liver cytosol have been partially purified about 200- and 80-fold, respectively. The apparent molecular weight estimated by gel filtration and the sedimentation coefficient are 210000 and 9 S for *S*-Ado-Met synthetase α and 160000 and 5.5 S for *S*-Ado-Met synthetase β. Both enzymes absolutely require Mg^{2+} and K^+ for the activity and are completely inhibited by *p*-(chloromercuri)-benzoate. Kinetic studies indicate that *S*-Ado-Met synthetases α and β exhibit negative cooperativity with low $S_{0.5}$ (ligand concentration required for half-maximal velocity) for L-methionine (17 μM) and ATP (0.5 mM) and positive cooperativity with much higher $S_{0.5}$ values ($S_{0.5}$ (L-methionine) = 0.5 mM, $S_{0.5}$ (ATP) = 2 mM), respectively. The cryoprotectants dimethyl sulfoxide and glycerol markedly lower the $S_{0.5}$ values of *S*-Ado-Met synthetase β without significant effect on V_{max}. A single species of *S*-Ado-Met synthetase has been purified about 1000-fold from rat kidney cytosol. The kidney enzyme, termed *S*-Ado-Met synthetase γ, has an apparent molecular weight of 190000 and a sedimentation coefficient of 7.5 S and is resistant to the inhibition by *p*-(chloromercuri)benzoate. *S*-Ado-Met synthetase γ exhibits slightly negative cooperativity with an apparent $S_{0.5}$ value for L-methionine of 6 μM and for ATP of 70 μM.

S-Adenosylmethionine synthetase [ATP:L-methionine *S*-adenosyltransferase, EC 2.5.1.6] catalyzes the formation of *S*-adenosylmethionine (*S*-Ado-Met)[1] which is the methyl donor for transmethylation reactions as well as the propylamine-group donor in the biosynthesis of polyamines (Lombardini & Talalay, 1970; Cantoni, 1975; Raina & Jänne, 1975). We have been studing the properties of rat *S*-Ado-Met synthetase and the regulation of *S*-Ado-Met biosynthesis under several conditions, including induction of DNA and RNA syntheses. *S*-Ado-Met synthetase from yeast has been purified to homogeneity and characterized in detail (Chiang & Cantoni, 1977). In mammals, however, only the hepatic enzyme has been partially purified (Cantoni & Durell, 1957; Pan & Tarver, 1967; Lombardini et al., 1970; Liau et al., 1977), and little formation is available on precise characteristics of the enzyme. Recently, evidence for the existence of two distinct species of *S*-Ado-Met synthetase has been reported in rat liver (Liau et al., 1977; Hoffman & Kunz, 1977; Okada et al., 1979), but the purification of each of the two enzymes in rat liver and their kinetic and molecular properties have not yet been established. One of the two enzyme species in rat liver is strikingly stimulated by Me_2SO at a low concentration (25 μM) of L-methionine and the other is only slightly activated (Hoffman & Kunz, 1977; Okada et al., 1979). The less Me_2SO-stimulated enzyme and the Me_2SO-stimulated enzyme were tentatively termed *S*-Ado-Met synthetases α and β, respectively (Okada et al., 1979). In contrast to the rat liver enzymes, the enzyme activities in the cytosol from rat brain, heart, and kidney are reported to be slightly inhibited by Me_2SO (Hoffman & Kunz, 1977). From kinetic evidence using the crude enzyme preparation of rat and human livers, Liau et al. (1979a,b) have reported the existence of three isozymes of *S*-Ado-Met synthetase which are termed low-K_m, intermediate-K_m, and high-K_m isozymes according to their K_m values for L-methionine.

As will be described in this paper, *S*-Ado-Met synthetase activity in crude extracts from various nonheptatic tissues examined similarly responded to tripolyphosphate, an intermediate of the enzyme reaction, as well as to Me_2SO. Therefore, we compared hepatic *S*-Ado-Met synthetases α and β with the enzyme from kidney having the highest specific activity of the nonhepatic tissues examined. We wish to describe some molecular and catalytic properties of *S*-Ado-Met synthetases α and β in rat liver and of the kidney enzyme, tentatively termed *S*-Ado-Met synthetase γ.

Materials and Methods

Chemicals. L-[*methyl*-^{3}H]Methionine (8.7 Ci/mmol) and [2-^{3}H]ATP (16 Ci/mmol) were obtained from Radiochemical Centre (Amersham, England). Potassium tripolyphosphate, spectroquality Me_2SO, and poly(ethylene glycol) 6000 were

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[1] Abbreviations used: *S*-Ado-Met, *S*-adenosylmethionine; Me_2SO, dimethyl sulfoxide; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)aminomethane.

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obtained from Nakarai Chemicals (Kyoto, Japan). ATP and 1-aminocyclopentanecarboxylic acid (cycloleucine) were from Sigma (St. Louis, MO). DEAE-Sephadex A-50, QAE-Sephadex A-25, Sephadex G-50, Sepahdex G-150 (superfine), and Blue Sepharose CL-6B were products of Pharmacia (Uppsala, Sweden). Hydroxylapatite was a product of Seikagaku-kogyo (Tokyo, Japan). Phosphocellulose, DEAE-cellulose, and cellulose phosphate paper were purchased from Whatman (Kent, England). All other reagents were of analytical grade.

Buffers. Buffers A (50 mM Tris-HCl, pH 7.8, 20% (v/v) glycerol, 0.2 mM DTT, 0.1 mM EDTA and 10 mM $MgCl_2$), B (50 mM potassium phosphate, pH 6.2, 20% (v/v) glycerol, 1 mM DTT, and 0.1 mM EDTA), and C (20 mM potassium phosphate, pH 6.2, 20% (v/v) glycerol, and 0.1 mM EDTA) were used. The pH of the buffers was adjusted at 20–25 °C. DTT was added immediately before use from a 0.1 M stock solution.

Enzyme Assay. The activity of *S*-Ado-Met synthetase was determined according to the method of Liau et al. (1977) with a slight modification. The standard reaction mixture in a total volume of 0.1 mL contained 0.1 M Tris-HCl (pH 9.0), 20 mM $MgCl_2$, 0.15 M KCl, 5 mM DTT, 10 mM ATP (Tris salt), 25 μM L-[*methyl*-^{3}H]methionine (0.25 μCi), and the enzyme. The incubation was carried out at 37 °C for 10 min. The reaction was terminated by the addition of 20 μL of 2 M $HClO_4$ and 5 mM L-methionine in an ice bath. The resulting precipitate was removed by centrifugation at 1000*g* for 10 min, and a 100-μL portion of the supernatant was spread onto a cellulose phosphate paper disk (2.5-cm diameter). The disk was dropped into a beaker containing 5 mM potassium phosphate buffer (pH 7.0) and extensively washed with the same buffer. The washed paper was transferred to a counting vial and immersed in 1 mL of 1.5 M NH_4OH. The radioactivity was measured in a Packard scintillation spectrometer. The scintillator solution contained 667 mL of toluene, 333 mL of Triton X-100, 4 g of 2,5-diphenyloxazole, and 0.1 g of 2,2′-*p*-phenylenebis(5-phenyloxazole). Radioactivity of blanks containing no enzyme was subtracted out. One unit of the enzyme was defined as the amount of enzyme that catalyzes the formation of 1 nmol of *S*-Ado-Met/min under the standard assay conditions. Under the assay conditions employed, the enzyme activity was linear with respect to the incubation time and the amount of protein. Reaction velocity (v) was expressed as total picomoles of *S*-Ado-Met formed per minute in the reaction mixture.

Stoichiometry of the Enzyme Reaction. The stoichiometric relationships between the consumption of ATP and the formation of *S*-Ado-Met were determined by using either 0.25 μCi of [^{3}H]ATP (2.5 nmol) plus 2.5 nmol of L-methionine or 0.25 μCi of [^{3}H] L-methionine (2.5 nmol) plus 2.5 nmol of ATP in the reaction mixture containing 0.03–0.05 unit of the enzyme. [^{3}H]ATP was separated from [^{3}H]AMP and [^{3}H]ADP by QAE-Sephadex A-25 (formate form) column (0.5 × 3 cm). AMP, ADP, and ATP are successively eluted from the column with 0.1, 0.2, and 0.4 M ammonium formate.

Preparation of Various Tissue Extracts. Wistar male rats (350-g body weight) were killed by decapitation, and the tissues examined were removed. The tissues were homogenized in 4 volumes of 20 mM Tris-HCl, pH 7.5, 15% (v/v) glycerol, 10 mM $MgCl_2$, 0.5 mM DTT, and 0.1 mM EDTA in a glass–Teflon homogenizer at 0 °C. The homogenates were centrifuged at 105000*g* for 1 h at 2 °C. The resulting supernatant solutions were dialyzed against 20 mM Tris-HCl, pH 7.5, 15% (v/v) glycerol, 10 mM $MgCl_2$, 0.5 mM DTT, and 0.1 mM EDTA for 5 h at 0 °C. The enzyme activity in the cytosols was determined in the standard reaction mixture.

Purification Procedures of the Enzyme. All operations were carried out at 0–4 °C. Wistar male rats weighing 150–250 g were used.

(*1*) *Purification of Rat Liver Enzymes.* The livers removed from decapitated rats were homogenized in 4 volumes of 0.25 M sucrose, and 3.3 mM $MgCl_2$ in a glass–Teflon homogenizer. The homogenate was centrifuged at 105000*g* for 1 h. To 100 mL of the supernatant solution (step 1) was added 31.3 g of solid ammonium sulfate, followed by stirring for 30 min. The precipitate recovered by centrifugation at 20000*g* for 30 min was dissolved in 10 mL of buffer A containing 75 mM KCl. Ammonium sulfate was removed from the solution by gel filtration on a Sephadex G-50 column (2.2 × 19 cm) previously equilibrated with the same buffer. The enzyme fraction (step 2, 17 mL) was applied to a DEAE-Sephadex column (1.3 × 16 cm) previously equilibrated with buffer A containing 75 mM KCl. The column was washed with 5 column volumes of the same buffer, and then the enzyme fraction was eluted with a linear KCl gradient (0.075–0.25 M) in 100 mL of buffer A. Fractions of 2.5 mL were collected. The active fractions (34 mL) eluted around 0.16 M KCl were pooled and precipitated with ammonium sulfate fractionation (0–50% saturation). The resulting precipitate was suspended in a minimal volume of buffer A containing 0.1 M KCl and well dissolved by dialysis against the same buffer. The enzyme fraction (step 3, 1.5 mL) was chromatographed on a column (1.75 × 145 cm) of Sephadex G-150 (void volume: fraction number 93) equilibrated with buffer B containing 0.5 M KCl. Fractions of 1.35 mL were collected, and fraction numbers 106–113 (*S*-Ado-Met synthetase α) and 120–128 (*S*-Ado-Met synthetase β) were pooled (step 4). The intermediate fractions were discarded to avoid possible cross-contamination. Immediately before the fractions were applied to a hydroxylapatite column, the pH of both fractions was carefully adjusted to 6.3 with a dropwise addition of 0.4 M acetic acid with mechanical stirring. Each enzyme solution was applied to a column (0.9 × 5.5 cm) of hydroxylapatite previously equilibrated with 10 mM potassium phosphate, pH 6.3, 0.2 mM DTT, and 20% (v/v) glycerol, and the column was washed with 8 mL of the same buffer. The enzyme was eluted with pH and concentration gradients of potassium phosphate buffer containing 20% (v/v) glycerol and 0.2 mM DTT between 20 mL of 10 mM potassium phosphate, pH 6.3, and 20 mL of 30 mM potassium phosphate, pH 8.8. Fractions of 1 mL were collected. Each *S*-Ado-Met synthetase, α and β, was eluted at pH 7.2–7.5 and concentrated with the aid of an immersible Millipore filter (Amicon Far East) (step 5).

(*2*) *Purification of Kidney Enzyme.* Kidneys were removed from decapitated rats and stored at −70 °C for less than 3 weeks, if necessary. The pooled kidneys (65 g) were homogenized in 4 volumes of 0.25 M sucrose and 3.3 mM $MgCl_2$ in a glass–Teflon homogenizer. The homogenate was centrifuged at 105000*g* for 1 h to obtain the supernatant solution (step 1, 290 mL). To the solution adjusted to 10 mM potassium phosphate by the addition of 1 M potassium phosphate, pH 7.0, was added 300 mL of calcium phosphate gel (15 mg dry weight/mL) with mechanical stirring. After being stirred for 5 min, the mixture was centrifuged at 2000*g* for 10 min. The packed gel was washed once with 600 mL of 10 mM potassium phosphate, pH 7.0, and 0.1 mM EDTA and then eluted twice with 300 mL of 0.15 M potassium phosphate, pH 7.0, and 0.1 mM EDTA. To the eluate (step 2, 615 mL) was added the poly(ethylene glycol) solution (50%, w/v) to

a final concentration of 6% (w/v). After being stirred for 30 min, the solution was centrifuged at 15000*g* for 15 min. The resulting supernatant solution was adjusted to pH 5.0 by the dropwise addition of 1 M acetic acid. The precipitate obtained by a 30-min centrifugation at 15000*g* was dissolved in a minimal volume of 20 mM potassium phosphate, pH 7.0, and 0.1 mM EDTA. The solution was dialyzed against 500 mL of buffer C for 6 h and then centrifuged at 25000*g* for 15 min to remove the precipitate. The clear supernatant solution (step 3, 21 mL) was applied to a phosphocellulose column (2 × 20 cm) previously equilibrated with buffer C to obtain the pass-through fraction (step 4, 34 mL). The enzyme fraction was applied to a DEAE-cellulose column (1.3 × 12 cm) previously equilibrated with buffer C. The column was washed with 4 column volumes of buffer C, and the enzyme fraction was eluted with a linear concentration gradient of potassium phosphate between buffer C (70 mL) and 0.35 M potassium phosphate, pH 7.0, 20% (v/v) glycerol, and 0.1 mM EDTA (70 mL). Fractions (3.5 mL) were collected, and the active fractions pooled were concentrated with ammonium sulfate precipitation (0–50% saturation). The precipitate obtained by a 30-min centrifugation at 105000*g* was dissolved in a minimal volume of buffer C and dialyzed against buffer C for 6 h. The dialyzed fraction was centrifuged at 15000*g* for 15 min. The enzyme solution (step 5, 3.6 mL) was applied to a Blue Sepharose column (1.2 × 13 cm) previously equilibrated with buffer C. The column was washed with 2 column volumes of buffer C, and then the enzyme was eluted with a linear KCl gradient (0–0.35 M) in 120 mL of buffer C. The fractions (3.8 mL) containing the bulk of the enzyme activity were combined. The precipitate obtained by the addition of ammonium sulfate (50% saturation) was dissolved in a minimal volume of buffer B containing 0.5 M KCl for 6 h. A 1-mL portion of the dialyzed fraction (step 6, 1.3 mL) was applied to a Sephadex G-150 column (0.95 × 130 cm) previously equilibrated with buffer B containing 0.5 M KCl. Fractions (0.92 mL) were collected, and active fractions were concentrated with the aid of an immersible Millipore filter. The solution was dialyzed against buffer C for 4 h to obtain step 7 enzyme.

Analytical Gel Filtration. Analytical gel chromatography was carried out at 4 °C on a column of Sephadex G-150 (0.95 × 130 cm) previously equilibrated with buffer B containing 0.5 M KCl. The molecular weights of *S*-Ado-Met synthetase α, β, and γ were estimated according to the method of Andrews (1964). Catalase (M_r 240 000), lactate dehydrogenase (M_r 140 000), and bovine serum albumin (M_r 67 000) were used as external marker proteins. Blue Dextran was employed to determine the void volume.

Glycerol Density Gradient Centrifugation. *S*-Ado-Met synthetases α and β from step 5 and γ from step 6 were dialyzed for 12 h against 100 mL of buffer B containing 5% (v/v) glycerol instead of 20%. A 0.1-mL portion of the enzyme solution was layered on a 4.9 mL of 10–30% (v/v) linear glycerol gradient in 50 mM potassium phosphate, pH 6.2, 1 mM DTT, 0.1 mM EDTA, and 0.5 M KCl and centrifuged in a Hitachi SW50 rotor at 40 000 rpm for 19 h. Bovine serum albumin ($s_{20,w}$ = 4.4 S) and catalase ($s_{20,w}$ = 11.3 S) were used as external marker proteins, and lactate dehydrogenase ($s_{20,w}$ = 7 S) was used as an external and internal marker protein. Sedimentation coefficients were calculated as described by the method of Martin & Ames (1961).

Protein Determination. Protein was determined by the method of Lowry et al. (1951) or of Bradford (1976) by using crystalline bovine serum albumin as a standard. The protein concentration in column fractions was estimated by measuring absorbance at 280 nm.

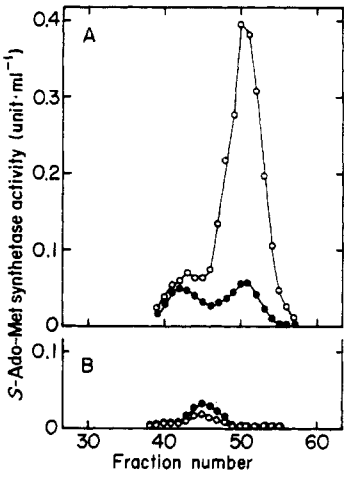

FIGURE 1: Gel-filtration profiles of *S*-Ado-Met synthetase in rat liver (A) and kidney (B) cytosols. The cytosols of rat liver and kidney were prepared as described under Materials and Methods, followed by dialysis against buffer A containing 0.1 M KCl for 4 h. A 0.42-mL portion of the liver cytosol (0.8 unit) or the kidney cytosol (0.2 unit) was chromatographed on a column (0.93 × 110 cm) of Sephadex G-150 previously equilibrated with buffer A containing 0.1 M KCl. Fractions of 0.92 mL were collected, and the 20-μL aliquots were assayed for the enzyme activity with (○) or without (●) 10% (v/v) Me_2SO in the standard reaction mixture, except for the use of 0.5 μCi of L-[^{3}H]methionine (25 μM). The recovery of the enzyme activity was 50–65%. The void volume (fraction number 33) of the column was determined with Blue Dextran.

Results

S-Ado-Met Synthetase Activity in Various Rat Tissues. When *S*-Ado-Met synthetase in the cytosols from various tissues of rat was assayed at 25 μM L-methionine, the specific activity of the enzyme (munits/mg of protein) was in the following order: liver (75.5); kidney (32.0); bone marrow (20.9); thymus (16.2); testis (14.2); small intestine (12.1); spleen (11.9); brain (10.7); lung (6.3); heart (4.8); skeletal muscle (2.5). The enzyme activity in the liver cytosol was stimulated 5- to 10-fold by 10% Me_2SO and about 3-fold by 0.15 mM tripolyphosphate whereas the enzyme activity in the cytosol of the nonhepatic tissues was inhibited by 10% Me_2SO (60–80% of control) and by 0.15 mM tripolyphosphate (65–80% of control). In contrast to the hepatic enzyme activity, the enzyme activity in the nonhepatic tissues was not considerably susceptible to the inhibition by *p*-(chloromercuri)benzoate (see Table III). These results suggest that the nonhepatic tissues examined predominantly contain the enzyme species distinguishable from hepatic *S*-Ado-Met synthetases α and β. When the kidney cytosol was applied to a Sephadex G-150 column, a single, symmetrical peak of *S*-Ado-Met synthetase activity was observed between the elution position of *S*-Ado-Met synthetase α, which was less activated by Me_2SO, and that of *S*-Ado-Met synthetase β, which was strikingly activated by Me_2SO (Figure 1). We therefore intended to purify the kidney enzyme, termed *S*-Ado-Met synthetase γ, as well as liver *S*-Ado-Met synthetases α and β.

The same results as stated above were obtained by using fresh crude extracts from rat tissues without dialysis. When partially purified *S*-Ado-Met synthetase α (step 4, Table I) was incubated in the fresh liver extract for 1 h at 0 °C, the activities in the mixture, which were determined in the presence and absence of 10% Me_2SO, were nearly equal to the additive values of the activities in the partially purified enzyme and

Table I: Summary of Purification of *S*-Ado-Met Synthetases α and β from Rat Liver[a]

step	protein (mg)	total activity (units)	specific activity (units/mg)	yield (%)	purification (*n*-fold)
(1) crude extract	2054	142	0.07	100	1
(2) ammonium sulfate fractionation	870	136	0.16	96	2.2
(3) DEAE-Sephadex column	84	100	1.19	70	17.2
(4) Sephadex G-150 column					
S-Ado-Met synthetase α	12.4	20	1.63	14	(46.5)
S-Ado-Met synthetase β	11.2	14	1.26	10	(36.0)
(5) hydroxylapatite column					
S-Ado-Met synthetase α	2.4	18	7.5	5	(214)
S-Ado-Met synthetase β	3.0	9	3.0	2	(86)

[a] The enzyme activity was determined as described under Materials and Methods. Values in parentheses were obtained by assuming that the activity of *S*-Ado-Met synthetase α was equal to that of *S*-Ado-Met synthetase β in step 1 under the standard assay conditions (see Figure 1).

Table II: Summary of Purification of *S*-Ado-Met Synthetase γ from Rat Kidney[a]

step	protein (mg)	total activity (units)	specific activity (units/mg)	yield (%)	purification (*n*-fold)
(1) crude extract	3349	143	0.04	100	1
(2) calcium phosphate gel	1021	130	0.12	90	3
(3) poly(ethylene glycol)	360	132	0.36	92	8
(4) phosphocellulose column	244	113	0.46	79	18
(5) DEAE-cellulose column	29.5	68.4	2.31	48	55
(6) Blue Sepharose column	1.3	35.6	27.4	25	658
(7) Sephadex G-150 column	0.2	7.8	39.0	5.4	975

[a] The enzyme activity was determined as described under Materials and Methods.

in the crude extract. This suggests that *S*-Ado-Met synthetase β is not derived from *S*-Ado-Met synthetase α by partial proteolysis during the preparation of the crude extract from liver. Similarly, the partially purified *S*-Ado-Met synthetase α was not converted to *S*-Ado-Met synthetase γ, which was assayed in the presence of 0.5 mM *p*-(chloromercuri)benzoate, in the fresh crude extract from kidney. It seems likely that liver *S*-Ado-Met synthetases α and β and kidney *S*-Ado-Met synthetase γ exist in vivo.

Enzyme Purification. The results of a typical purification of *S*-Ado-Met synthetases α and β are summarized in Table I. Both *S*-Ado-Met synthetases α and β bound to DEAE-Sephadex at pH 7.8 were eluted at about 0.16 M KCl. The two enzyme species were separated by Sephdex G-150 column chromatography (cf. Figure 1). The overall purification was about 200-fold for *S*-Ado-Met synthetase α and about 80-fold for *S*-Ado-Met synthetase β. The partially purified liver enzymes (steps 4 and 5) were stable in buffer A containing 0.1 M KCl at 0 °C for at least a month. In the absence of glycerol and DTT, *S*-Ado-Met synthetases α and β were spontaneously inactivated during the purification procedures and the storage at 0 °C.

As shown in Table II, *S*-Ado-Met synthetase γ was purified about 1000-fold with a yield of 5.4% from kidney cytosol. The kidney enzyme (step 7) was spontaneously inactivated within a week, even in the presence of glycerol and DTT. Therefore, the enzyme preparation of step 6 was used for the following experiments unless otherwise noted.

More than 90% of [^{3}H]ATP consumed was converted to [^{3}H]-*S*-Ado-Met in the reactions of the partially purified enzymes from rat liver (steps 4 and 5) and from kidney (steps 6 and 7), and nearly equal amounts of [^{3}H]-*S*-Ado-Met were obtained by using L-[^{3}H]methionine instead of [^{3}H]ATP. Since stoichiometric relationships between the consumption of ATP and the formation of *S*-Ado-Met in the reactions of the partially purified enzymes were observed, these enzyme preparations seem to be freed from interfering enzymes, such as ATPase.

Table III: Catalytic Properties of *S*-Ado-Met Synthetases from Rat Liver and Kidney

assay conditions[a]	relative activity of *S*-Ado-Met synthetase (% of control) α	β	γ
control system	100	100	100
$-Mg^{2+}$	1	1	1
$-K^+$	3	1	1
−DTT	35	30	97
−DTT, + pCl-HgBzO$^-$[b] (0.5 mM)	3	6	78
+Me_2SO (10% v/v)	130	1700	86
+glycerol (20% v/v)	250	610	18
+tripolyphosphate (0.15 mM)	70	493	75
+tripolyphosphate (1 mM)	23	171	32
+cycloleucine[c] (0.1 mM)	83	126	92
+cycloleucine[c] (1 mM)	36	72	48

[a] *S*-Ado-Met synthetase activity was determined as described under Materials and Methods. The enzyme activities in the control system for liver *S*-Ado-Met synthetases α and β (step 5) and kidney *S*-Ado-Met synthetase γ (step 6) were 4, 5, and 6.5 pmol/min, respectively. [b] *p*-(Chloromercuri)benzoate. [c] The reaction was carried out at pH 8.0 instead of pH 9.0.

General Properties. The pH optima for the activity of *S*-Ado-Met synthetases α, β, and γ were broad between pH 7.5 and 9.5. The enzyme activity at pH 7.5 and 9.5 was about 70–80% of the maximal activity. The liver enzymes and the kidney enzyme absolutely required Mg^{2+} and K^+ for the activity (Table III), as shown in the yeast enzyme (Mudd & Cantoni, 1958). Mg^{2+} and K^+ were not replaced by Mn^{2+} and Na^+, respectively. *S*-Ado-Met synthetases α and β required DTT for the activity and were completely inhibited by *p*-(chloromercuri)benzoate (Table III), indicating that SH groups are essential for the enzyme activity. In contrast, the kidney enzyme, *S*-Ado-Met synthetase γ, showed full activity

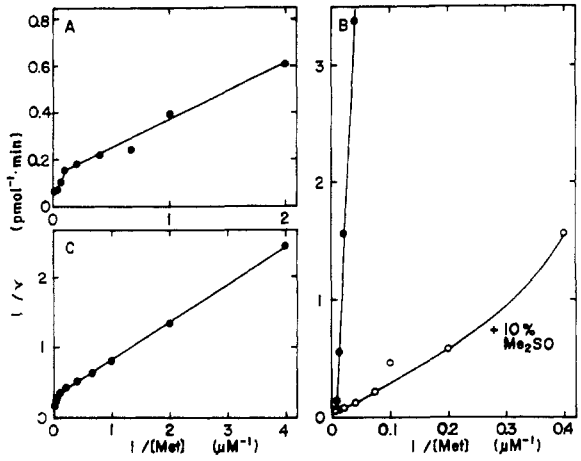

FIGURE 2: Double-reciprocal plots of the reaction velocity as a function of L-methionine concentration for *S*-Ado-Met synthetases α (A), β (B), and γ (C). The reaction velocity (v) was determined as described under Materials and Methods with (○) or without (●) 10% (v/v) Me_2SO, except that the reaction mixture contained the following concentrations of L-[^{3}H] methionine (40 Ci/mol): 0.5 μM–0.5 mM for *S*-Ado-Met synthetase α; 2.5 μM–2 mM for *S*-Ado-Met synthetase β; 0.25 μM–0.5 mM for *S*-Ado-Met synthetase γ. The apparent $S_{0.5}$ value was estimated as the ligand concentration giving half V_{max} determined graphically by using the double-reciprocal plots.

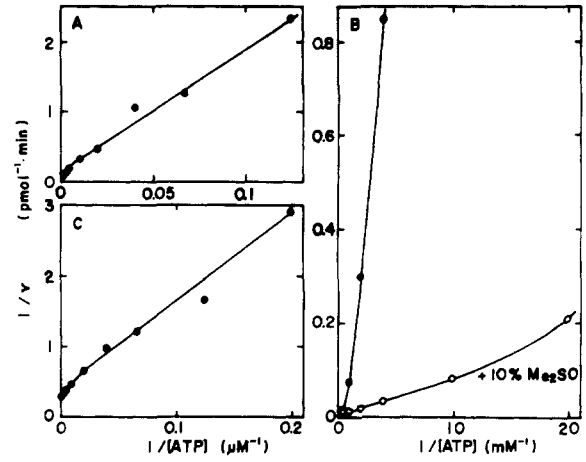

FIGURE 3: Double-reciprocal plots of the reaction velocity as a function of ATP concentration for *S*-Ado-Met synthetases α (A), β (B), and γ (C). The reaction velocity (v) was determined as described under Materials and Methods with (○) or without (●) 10% (v/v) Me_2SO, except that the reaction mixture contained 0.25 mM L-[^{3}H]methionine (2 μCi) and 5 μM–2.5 mM ATP for *S*-Ado-Met synthetases α and γ and 1.5 mM L-[^{3}H]methionine (2 μCi) and 20 μM–10 mM ATP for *S*-Ado-Met synthetase β. The apparent $S_{0.5}$ value was estimated as in the legend of Figure 2.

even in the absence of DTT and was slightly inhibited by *p*-(chloromercuri)benzoate.

Influence of Effectors. By the addition of 10% Me_2SO to the standard reaction mixture containing 25 μM L-methionine, *S*-Ado-Met synthetases α and β were activated by 1.3- and by 17-fold, respectively (Table III). The stimulation ratio with respect to *S*-Ado-Met synthetase β was decreased with an increasing concentration of L-methionine (see Figure 2B). As in the case of Me_2SO, another cryoprotectant glycerol (20%, v/v) similarly affected the activity of *S*-Ado-Met synthetases α and β. The optimal concentration for the stimulation of *S*-Ado-Met synthetase β was 10–15% for Me_2SO and about 20% for glycerol, and at their higher concentrations, the stimulation was gradually decreased. Even in the presence of either 10% Me_2SO or 20% glycerol, the enzyme reaction proceeded linearly within at least 15 min. The kidney enzyme was slightly inhibited by Me_2SO and was susceptible to the inhibition by glycerol (Table III).

Tripolyphosphate is a powerful inhibitor of *S*-Ado-Met synthetase (Greene, 1969; Chou & Talalay, 1972; Lombardini et al., 1973; Liau et al., 1977), which is competitive with ATP. Table III shows the contrasting resonses of *S*-Ado-Met synthetases α and β to tripolyphosphate. At 25 μM L-methionine, *S*-Ado-Met synthetase β was gradually activated up to 5-fold with an increasing concentration of tripolyphosphate (0.01–0.2 mM) and inhibited at a higher concentration than 0.2 mM with an apparent $I_{0.5}$[2] value of about 0.4 mM. In the presence of 2.5 mM L-methionine, the inhibitory effect on *S*-Ado-Met synthetase α as well as the stimulatory effect on *S*-Ado-Met synthetase β tends to diminish. Lombardini et al. (1973) previously demonstrated that the partially purified enzyme from rat liver, which is presumed to contain *S*-Ado-Met synthetases α and β, was activated 2.5-fold by about 0.2 mM tripolyphosphate and was inhibited by the higher concentrations. The dual effect of tripolyphosphate was dependent on the concentration of L-methionine.

When assayed at 25 μM L-methionine, *S*-Ado-Met synthetase β was stimulated 1.3-fold by 0.1 mM cycloleucine, a nonsubstrate analogue of L-methionine, and was inhibited by the higher concentrations with an apparent $I_{0.5}$ value of 1 mM (see Table III). The stimulatory effect tends to diminish in the presence of 2.5 mM L-methionine. The dual effect of L-methionine analogues including cycloleucine was also observed by using the partially purified enzyme from rat liver (Lombardini et al., 1973). *S*-Ado-Met synthetase α was invariably inhibited by cycloleucine with an apparent $I_{0.5}$ value of 0.6 mM. *S*-Ado-Met synthetase γ was susceptible to the inhibition by tripolyphosphate and cycloleucine as in the case of *S*-Ado-Met synthetase α (Table III).

Kinetic Properties. Plots of reciprocal reaction velocities of *S*-Ado-Met synthetase against reciprocal concentrations of one of the two substrates, L-methionine and ATP, are shown in Figures 2 and 3. As for *S*-Ado-Met synthetase α, the double-reciprocal plots exhibited slightly negative cooperativity, yielding an apparent $S_{0.5}$[3] value of 17 μM for L-methionine (Figure 2A) and 0.5 mM for ATP (Figure 3A). Hill coefficients obtained were 0.6 for L-methionine and 0.8 for ATP. Similar negative cooperativity with respect to substrates is also observed in the case of yeast *S*-Ado-Met synthetases I and II (Chiang & Cantoni, 1977). Kinetic patterns of *S*-Ado-Met synthetase β showed positive cooperativity, yielding an apparent $S_{0.5}$ value of 0.5 mM for L-methionine (Figure 2B) and 2 mM for ATP (Figure 3B). Hill coefficients obtained were 1.6 for L-methionine and 1.8 for ATP. Me_2SO markedly lowered the $S_{0.5}$ value of *S*-Ado-Met synthetase β for L-methionine to 50 μM and the $S_{0.5}$ value for ATP to 0.5 mM without significant effect on V_{max}. The Hill coefficient was decreased to 1.1 for L-methionine and to 1.2 for ATP by Me_2SO. In contrast, neither the V_{max}, the $S_{0.5}$ value, nor the Hill coefficient of *S*-Ado-Met synthetase α for substrates was significantly affected by Me_2SO (data not shown). Similar results were obtained with 20% glycerol instead of Me_2SO (data not shown). Kinetic patterns of *S*-Ado-Met synthetase γ were similar to those of *S*-Ado-Met synthetase α. The apparent $S_{0.5}$ values for L-methionine and ATP were 6 and 70 μM, respectively (Figures 2C and 3C). Hill coefficients

[2] The notation was defined as a ligand concentration required for 50% inhibition of maximal activity.

[3] The notation was defined as a ligand concentration required for half-maximal velocity.

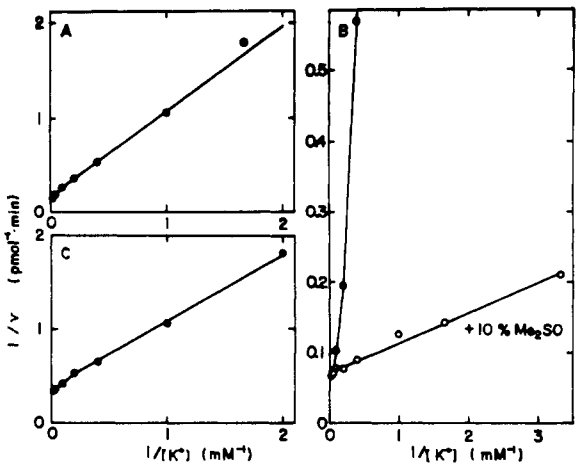

FIGURE 4: Double-reciprocal plots of the reaction velocity as a function of K^+ concentration for *S*-Ado-Met synthetases α (A), β (B), and γ (C). The reaction velocity (v) and $S_{0.5}$ values were determined as in the legend of Figure 3 except for the use of 10 mM ATP and 0.3–200 mM KCl.

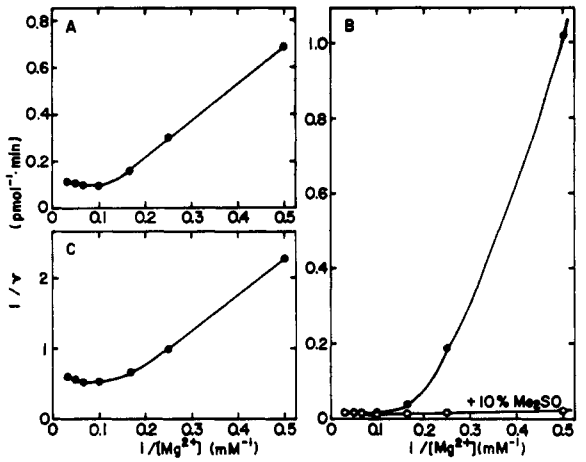

FIGURE 5: Double-reciprocal plots of the reaction velocity as a function of Mg^{2+} concentration for *S*-Ado-Met synthetases α (A), β (B), and γ (C). The reaction velocity and $S_{0.5}$ values were determined as in the legend of Figure 3 except for the use of 10 mM ATP and the following concentrations of $MgCl_2$: 2–30 mM for *S*-Ado-Met synthetases α and γ; 1–30 mM for *S*-Ado-Met synthetase β.

obtained were 0.4 for L-methionine and 0.7 for ATP.

Plots of reciprocal reaction velocities of *S*-Ado-Met synthetases α, β, and γ against reciprocal concentrations of K^+ exhibited abnormal kinetic patterns as in the case of the substrates (Figure 4). The $S_{0.5}$ value for K^+ was 5 mM for *S*-Ado-Met synthetase α, 8 mM for β, and 2 mM for γ. Hill coefficients of *S*-Ado-Met synthetases α, β, and γ were 0.7, 1.6, and 0.7, respectively. Me_2SO (10%) lowered the $S_{0.5}$ value for K^+ of *S*-Ado-Met synthetase β to 2 mM and the Hill coefficient to 1.0.

Slightly positive cooperativity with respect to Mg^{2+} and the slight inhibition at the higher concentration were observed in the case of *S*-Ado-Met synthetases α and γ (Figure 5A,C). An apparent $S_{0.5}$ value for Mg^{2+} was about 6 mM for *S*-Ado-Met synthetase α and about 5 mM for *S*-Ado-Met synthetase γ. This positive cooperativity, in contrast to the negative cooperativity with respect to L-methionine, ATP, and K^+, may reflect the requirement of excess Mg^{2+} over the ATP concentration for the optimal activity. Yeast *S*-Ado-Met synthetase exhibiting similar kinetic patterns with respect to Mg^{2+} requires an excess of 5 mM Mg^{2+} over the ATP concentration for the optimal activity at pH 9 (Greene, 1969). *S*-Ado-Met synthetase β exhibited a markedly positive cooperativity with respect to Mg^{2+} in the presence of 10 mM ATP (Figure 5B). Me_2SO lowered the apparent $S_{0.5}$ value of 7 mM to about 2 mM along with the disappearance of the markedly positive cooperativity. Nearly identical results were obtained by using constant Mg^{2+}/ATP ratios of 1.0 and 1.5. Therefore, the markedly positive cooperativity is not due to the inhibition by excess ATP over Mg^{2+} concentration at low concentrations of Mg^{2+} but is due to the enhancement of the positive cooperativity, which is characteristic of *S*-Ado-Met synthetase β (see Figures 2B, 3B, and 4B), by requirement of excess Mg^{2+} over the ATP concentration for the maximal activity.

Molecular Properties. On the basis of gel filtration on a Sephadex G-150 column, the apparent molecular weight was estimated to be 210000 for *S*-Ado-Met synthetase α and 160000 for *S*-Ado-Met synthetase β. The kidney enzyme has an apparent molecular weight of 190000. On the basis of glycerol gradient centrifugation, sedimentation coefficients of *S*-Ado-Met synthetases α and β were estimated to be 9 and 5.5 S, respectively. The kidney enzyme has a sedimentation coefficient of 7.5 S. Both enzymes of liver were eluted from a DEAE-Sephadex column by the similar concentration of KCl (~0.16 M), suggesting that their charge properties are probably identical.

Discussion

Our present results indicate that two distinct species of *S*-Ado-Met synthetases α and β in rat liver cytosol and a single species of the enzyme in kidney cytosol are partially purified and that these three species of the enzyme are markedly different from one another in molecular and catalytic properties. *S*-Ado-Met synthetases α (M_r 210000, $s_{20,w}$ = 9 S) and β (M_r 160000, $s_{20,w}$ = 5.5 S) exhibit negative cooperativity with low $S_{0.5}$ values for the substrates and K^+ and positive cooperativity with high $S_{0.5}$ values, respectively. *S*-Ado-Met synthetase γ from kidney (M_r 190000, $s_{20,w}$ = 7.5 S) shows kinetic patterns similar to those of *S*-Ado-Met synthetase α. In contrast to the liver enzymes, the kidney enzyme is resistant to the inhibition by *p*-(chloromercuri)benzoate. Two isofunctional forms of *S*-Ado-Met synthetase have been purified to homogeneity from bakers' yeast, whose properties are not markedly different from each other (Chiang & Cantoni, 1977). Our preliminary experiment indicates that the enzyme activity in the extract from bakers' yeast is not stimulated by Me_2SO (10% v/v). *S*-Ado-Met synthetase from mammalian livers has been shown to exhibit abnormal kinetics (Lombardini et al., 1973; Finkelstein et al., 1975; Liau et al., 1977) and unusual responses to tripolyphosphate and cycloleucine (Lombardini et al., 1973). Now, these phenomena are understood to be due to the presence of *S*-Ado-Met synthetase β in the enzyme preparation.

Chromatographic and kinetic studies by Liau et al. (1977) have shown that Novikoff ascites hepatoma cells contain a single form of *S*-Ado-Met synthetase and that normal liver contains two forms of the enzyme. Recently, they have reported the existence of three isozymes of the enzyme in adult rat and human livers, which are termed low-K_m (3–4 μM), intermediate-K_m (17–24 μM), and high-K_m (0.6–1 mM) isozymes according to their K_m values for L-methionine (Liau et al., 1979a,b). These isozymes were kinetically detected by using the enzyme preparation obtained from one-step purification on DEAE-cellulose chromatography. It seems likely that the high-K_m isozyme corresponds to *S*-Ado-Met synthetase β with an apparent $S_{0.5}$ value for L-methionine of 0.5 mM. The low-K_m and intermediate-K_m isozymes may be intrinsic kinetic forms of *S*-Ado-Met synthetase α but not two distinct molecular species, for *S*-Ado-Met synthetase γ and yeast

S-Ado-Met synthetases I and II, as well as *S*-Ado-Met synthetase α, show negative cooperativity with respect to the substrates, from which apparently two K_m values could be obtained. As estimated from Figure 2A, *S*-Ado-Met synthetase α has two intrinsic K_m values for L-methionine of about 2 and 20 μM.

Yoshida ascites hepatoma AH 130, Morris hepatoma 7316A (Okada et al., 1979), and Novikoff hepatoma (Liau et al., 1977, 1979a) contain a single form of *S*-Ado-Met synthetase which may be similar to kidney *S*-Ado-Met synthetase γ rather than hepatic *S*-Ado-Met synthetase α with respect to apparent molecular weight and sensitivities to SH-blocking reagents. As described previously (Okada et al., 1979), rat liver of precancerous states induced by thioacetamide contains half of *S*-Ado-Met synthetase β relative to α. Furthermore, Morris hepatoma 7794A (Okada et al., 1979) and hepatoma induced by 2-(acetylamino)fluorene (Tsukada & Okada, 1980) contain a decreased amount of *S*-Ado-Met synthetase β and kidney-type enzyme, probably *S*-Ado-Met synthetase γ, instead of liver *S*-Ado-Met synthetase α. Therefore, we suggest that *S*-Ado-Met synthetases α and β disappear during liver carcinogenesis along with the appearance of *S*-Ado-Met synthetase γ. It is worth noting that the kidney-type enzyme also exists in fetal liver as a predominant species (Okada et al., 1980).

We are interested in the physiological importance and regulation of *S*-Ado-Met synthetases α and β in relation to hepatic functions. Our preliminary observation indicates that both enzymes are localized almost all in liver cytosol and the activity is negligible in particulate fractions including nuclei. On the basis of chromatography of the liver cytosol (see Figure 1), the activity ratio of *S*-Ado-Met synthetase β to α can be estimated to be 1.0 at 25 μM L-methionine. When assayed at 25 μM L-methionine in the presence of 10% Me_2SO, the activity of *S*-Ado-Met synthetase β is mainly measured in the liver cytosol (see Figure 1). In the assay conditions with and without 10% Me_2SO, we have estimated the level of liver *S*-Ado-Met synthetases α and β under several physiological conditions including partial hepatectomy (Okada et al., 1979), nutritional changes, and hydrocrotisone injections. The level of the enzyme activity was not significantly changed under these conditions. Me_2SO and glycerol markedly stimulate *S*-Ado-Met synthetase β activity at the suboptimal concentrations of ligands by increasing the affinity of the enzyme for the ligands. These cryoprotectants have been reported to exhibit stimulatory effects on several enzyme systems, such as glutamine-dependent carbamoyl-phosphate synthetase (Ishida et al., 1977) and pyruvate kinase (Ruwart & Suelter, 1971). These two enzymes are regulated by allosteric activators in their reactions. The concentration of L-methionine in rat liver cells is estimated to be within a range of 50–120 μM from the data of Sturman et al. (1969) and Adibi et al. (1973), which is much lower than the apparent $S_{0.5}$ value of *S*-Ado-Met synthetase β and higher than the apparent $S_{0.5}$ value of *S*-Ado-Met synthetase α. Therefore, we cannot rule out the possibility that *S*-Ado-Met synthetase β is regulated by an unknown, physiological, allosteric activator increasing the affinity of the enzyme for L-methionine.

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References

Adibi, S. A., Modesto, T. A., Morse, E. L., & Amin, P. M. (1973) *Am. J. Physiol. 225*, 408–414.
Andrews, P. (1964) *Biochem. J. 91*, 222–233.
Bradford, M. M. (1976) *Anal. Biochem. 72*, 248–254.
Cantoni, G. L. (1975) *Annu. Rev. Biochem. 44*, 435–451.
Cantoni, G. L., & Durell, J. (1957) *J. Biol. Chem. 225*, 1033–1048.
Chiang, P. K., & Cantoni, G. L. (1977) *J. Biol. Chem. 252*, 4506–4513.
Chou, T.-C., & Talalay, P. (1972) *Biochemistry 11*, 1065–1073.
Finkelstein, J. D., Kyle, W. E., & Martin, J. J. (1975) *Biochem. Biophys. Res. Commun. 66*, 1491–1497.
Greene, R. C. (1969) *Biochemistry 8*, 2255–2265.
Hoffman, J. L., & Kunz, G. L. (1977) *Biochem. Biophys. Res. Commun. 77*, 1231–1236.
Ishida, H., Mori, M., & Tatibana, M. (1977) *Arch. Biochem. Biophys. 182*, 258–265.
Liau, M. C., Lin, G. W., & Hurlbert, R. B. (1977) *Cancer Res. 37*, 427–435.
Liau, M. C., Chang, C. F., Belanger, L., & Grenier, A. (1979a) *Cancer Res. 39*, 162–169.
Liau, M. C., Chang, C. F., & Becker, F. F. (1979b) *Cancer Res. 39*, 2113–2119.
Lombardini, J. B., & Talalay, P. (1970) *Adv. Enzyme Regul. 9*, 349–384.
Lombardini, J. B., Coulter, A. W., & Talalay, P. (1970) *Mol. Pharmacol. 6*, 481–499.
Lombardini, J. B., Chou, T.-C., & Talalay, P. (1973) *Biochem. J. 135*, 43–57.
Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265–275.
Martin, R. G., & Ames, B. N. (1961) *J. Biol. Chem. 236*, 1372–1379.
Mudd, S. H., & Cantoni, G. L. (1958) *J. Biol. Chem. 231*, 481–492.
Okada, G., Sawai, Y., Teraoka, H., & Tsukada, K. (1979) *FEBS Lett. 106*, 25–28.
Okada, G., Watanabe, Y., & Tsukada, K. (1980) *Cancer Res. 40*, 2895–2897.
Pan, F., & Tarver, H. (1967) *Arch. Biochem. Biophys. 119*, 429–434.
Raina, A., & Jänne, J. (1975) *Med. Biol. 53*, 121–147.
Ruwart, M. J., & Suelter, C. H. (1971) *J. Biol. Chem. 246*, 5990–5993.
Sturman, J. A., Cohen, P. A., & Gaull, G. E. (1969) *Biochem. Med. 3*, 244–251.
Tsukada, K., & Okada, G. (1980) *Biochem. Biophys. Res. Commun. 94*, 1078–1082.