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S-Adenosylhomocysteinase from Mouse Liver

CATALYTIC PROPERTIES AT CELLULAR ENZYME LEVEL*

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The cellular level of *S*-adenosylhomocysteinase (*S*-adenosylhomocysteine hydrolase, EC 3.3.1.1) in mouse liver is about 10 μM (Ueland, P.M., and Sæbø, J. (1979) *Biochim. Biophys. Acta* 587, 341-352) which far exceeds the concentration of enzyme used for the measurement of enzyme kinetics. The synthesis and hydrolysis of *S*-adenosylhomocysteine was determined at cellular concentrations of adenosine (2 μM) and *S*-adenosylhomocysteine (50 μM) in the presence of high level of enzyme (up to 10 μM). Under these conditions a fraction of adenosine, isolated as adenine, was not available for the synthesis of *S*-adenosylhomocysteine and for deamination catalyzed by adenosine deaminase. This fraction increased as a function of time of incubation both during synthesis and hydrolysis of *S*-adenosylhomocysteine (in the absence of adenosine deaminase). A substantial amount of adenine was liberated from the enzyme as judged by Sephadex G-25 chromatography.

Hydrolysis of *S*-adenosylhomocysteine was determined at 1 and 10 μM enzyme in the presence of excess adenosine deaminase. Homocysteine was included in the incubation mixture to retard the rate of hydrolysis to allow the determination of the rate of the reaction. The progress curve for the hydrolysis was characterized by an initial rapid phase followed by slow hydrolysis of *S*-adenosylhomocysteine. The second slow phase was more pronounced at high concentration of enzyme, low level of substrate, and in the presence of homocysteine. The concentration of *S*-adenosylhomocysteine of half-maximal catalytic activity increased by increasing the concentration of enzyme and was 20 μM and 120 μM at 1 μM and 10 μM enzyme, respectively.

S-Adenosylhomocysteinase (*S*-adenosylhomocysteine hydrolase, EC 3.3.1.1) catalyzes the reversible hydrolysis of *S*-adenosylhomocysteine to adenosine and L-homocysteine (1). AdoHcy[1] is a product formed from *S*-adenosylmethionine upon transmethylation from AdoMet to cellular acceptors (2). AdoHcy is a potent inhibitor of most transmethylases using AdoMet as a methyl donor (3–11) and has been suggested to be a regulator of biological methylation (6, 12). *S*-Adenosylhomocysteinase probably participates in the regulation of tissue level of AdoHcy (13–16). Knowledge of the catalytic and regulatory properties of *S*-adenosylhomocysteinase may, therefore, give insight into the control of cellular methylation reactions.

Adenosine bound to *S*-adenosylhomocysteinase from mouse liver is not available for deamination catalyzed by the enzyme adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4) (17). The interaction of adenosine with *S*-adenosylhomocysteinase was associated with the formation of adenine or a compound liberating adenine (17). The concentration of *S*-adenosylhomocysteinase in mouse liver (10 μM) (18) is of the same order of magnitude as the tissue level of adenosine (19–21). Therefore, the possibility exists that a fraction of this nucleoside may be sequestered through its interaction with the enzyme *in vivo*. This possibility was reinforced by the observation that a fraction of adenosine added to crude tissue extract is not available for deamination catalyzed by both the endogenous and exogenous adenosine deaminase. This phenomenon was observed at high concentration of tissue extract from mouse liver and several other mammalian tissues (18).

In general, studies on the catalytic properties of enzymes are usually performed under conditions where the enzyme is highly diluted and substrate is present in excess. These *in vitro* conditions usually depart from the conditions *in vivo* where the concentration of enzyme is higher than used in enzymatic studies (22). This may represent a serious objection to metabolic interpretation to experiments *in vitro* (22). The sequestration of adenosine was demonstrated at enzyme levels which far exceed those used for studies on enzyme kinetics (17, 18). In the light of these observations, the metabolism of *S*-adenosylhomocysteine and adenosine in the presence of cellular concentration of *S*-adenosylhomocysteinase was investigated.

MATERIALS AND METHODS

Chemicals—Adenosine, adenine, inosine, *S*-adenosyl-L-homocysteine, DL-homocysteine, adenosine deaminase (type I from calf intestinal mucosa) and Hepes were obtained from Sigma Chemical Co. Polyethyleneimine 400 was purchased from Serva, Heidelberg, and cellulose powder (MN 300) was from Macherey-Nagel and Co., F.R.G. Polyethyleneimine-impregnated cellulose thin layer sheets (0.25 mm) on glass plates (20 × 20 cm) were prepared as described by Randerath and Randerath (23) and were developed in distilled water before use. [2-^{3}H]Adenosine (24 Ci/mmol) and [8-^{14}C]adenosine (59 mCi/mmol) were from the Radiochemical Centre, Amersham, and the purity was checked by thin layer chromatography (16). *S*-[2-^{3}H]Adenosylhomocysteine and *S*-[8-^{14}C]adenosylhomocysteine were synthesized enzymatically and purified as described previously (16).

Purification of S-Adenosylhomocysteinase from Mouse Liver—The enzyme was purified to apparent homogeneity using a slight modification (24) of a purification scheme published elsewhere (25).

Assay for Synthesis and Hydrolysis of S-Adenosylhomocysteine—Synthesis of AdoHcy was determined by incubating adenosine and

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[1] The abbreviations used are: AdoHcy, *S*-adenosylhomocysteine; AdoMet, *S*-adenosylmethionine; Hepes, 4(2-hydroxyethyl)-1-piperazineethanesulfonic acid; $S_{0.5}$, concentration of substrate of half-maximal catalytic activity.

DL-homocysteine (3 mM) at 37°C in the presence of enzyme. Hydrolysis of AdoHcy was measured under the same conditions except that adenosine and homocysteine were omitted from the incubation mixture (homocysteine was included in the incubation mixture when indicated), and the incubation mixture was supplemented with adenosine deaminase (100 units/ml). The incubation buffer was 15 mM Hepes, pH 7.0, containing 0.25% bovine serum albumin, 150 mM KCl, 5 mM Mg^{2+}-acetate, and 2 mM 2-mercaptoethanol. The reaction was terminated by adding aliquots (6 to 25 μl) from the incubation mixture into 0.8 N perchloric acid (30 μl) which after 5 min at 0°C was neutralized by addition of alkali (16). Adenosine, adenine, inosine, and AdoHcy were separated on polyethyleneimine cellulose plates, and radioactivity was determined as described previously (16).

Analytical Gel Chromatography—Samples of 60 μl were applied to an Ultrogel AcA 34 column (0.6 × 20 cm) equilibrated with the incubation buffer (see preceding paragraph) which in some experiments was supplemented with 50 μM AdoHcy. Elution was carried out with the same buffer at 25°C and a flow rate of 10 ml/h. Fractions (140 μl) were collected and assayed for *S*-adenosylhomocysteine hydrolase activity.

Determination of Protein—Protein was determined by measuring the absorbance at 280 nM, using a specific extinction coefficient ($E^{1\%}_{280\ nm}$) of 13.0 for the enzyme (26).

RESULTS

Sequestration of Adenosine During Synthesis of S-Adenosylhomocysteine—The synthesis of AdoHcy was determined at 2 μM [^{14}C]adenosine and increasing concentrations (0.1 to 10 μM) of enzyme (Fig. 1). A fraction of adenosine was not incorporated into AdoHcy. This fraction, which was mainly isolated as adenine, increased as a function of the concentration of enzyme and time of incubation. Less than 3% of the radioactivity was identified as adenosine under the condition of the experiment (data not shown).

After 45 min of incubation, excess adenosine deaminase (100 units/ml) was added to the incubation mixture (*arrow*) to remove adenosine. The synthetic reaction was immediately reversed as shown by a rapid decrease in the concentration of AdoHcy. The radioactivity was recovered as inosine. It is noted that the rate of reversion increased by increasing the concentration of enzyme from 0.1 to 1 μM, whereas a further increase in the enzyme level resulted in a decrease in the rate of hydrolysis.

When the incubate was supplemented with adenosine deaminase, the amount of adenine was slightly reduced (Fig. 1). [^{3}H]Adenine (not formed from adenosine) was not metabolized by the preparation of adenosine deaminase (data not shown). These data are in favor of the interpretation (17, 27) that adenine formed from adenosine and not liberated from the enzyme or a substance liberating adenine may be reconverted back to adenosine.

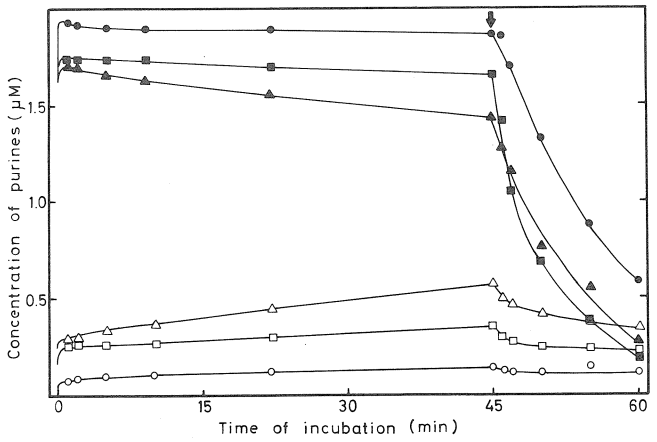


FIG. 1. **Synthesis of *S*-adenosylhomocysteine and the reversion of the synthetic reaction at increasing concentrations of enzyme.** [^{14}C]Adenosine (2 μM) and DL-homocysteine (3 mM) were incubated in the presence of various concentrations (0.1 to 10 μM) of enzyme. After 45 min of incubation, the incubation mixture was supplemented with adenosine deaminase (100 units/ml) (*arrow*). The amount of adenine (△, □, ○) and AdoHcy (●, ■, ▲) is plotted *versus* time of incubation. Results obtained at 0.1 μM (○, ●), 1 μM (□, ■), and 10 μM (△, ▲) enzyme are shown on the figure.

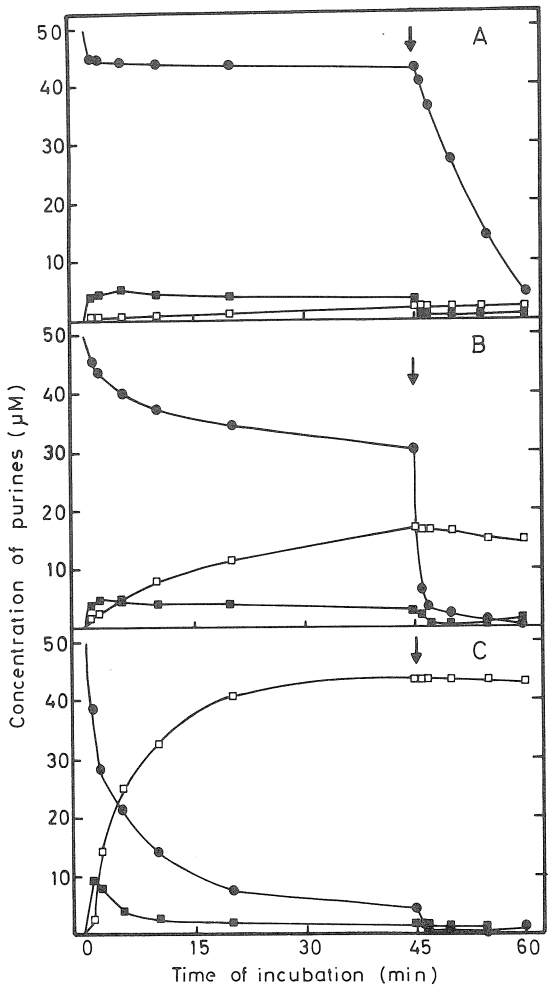


FIG. 2. **Metabolism of AdoHcy at increasing concentrations of enzyme.** [^{14}C]AdoHcy (50 μM) was incubated in the presence of 0.1 μM (*A*), 1 μM (*B*), and 10 μM (*C*) enzyme. After 45 min of incubation, the incubation mixture was supplemented with adenosine deaminase (100 units/ml) (*arrow*). The amount of AdoHcy (●), adenosine (■), and adenine (□) is plotted *versus* time of incubation.

Sequestration of Adenosine During Hydrolysis of S-Adenosylhomocysteine—The metabolism of AdoHcy (50 μM) was determined at increasing concentrations of enzyme (0.1 to 10 μM) in the absence of adenosine deaminase (Fig. 2). At low enzyme level (0.1 μM) AdoHcy was rapidly hydrolyzed to adenosine, and then the concentration of AdoHcy and adenosine was nearly constant, consistent with an equilibrium constant of 0.8×10^{-6} M (16). By increasing the concentration of enzyme, a slow decrease in the amount of AdoHcy was observed after the initial fall. This phenomenon was accompanied by a progressive formation of adenine or a substance liberating adenine (Fig. 2, *B* and *C*). At 10 μM enzyme, about 80% of AdoHcy initially present was recovered as adenine after 45 min of incubation (Fig. 2*C*).

When adenosine deaminase was added to the incubation mixture, AdoHcy was rapidly hydrolyzed whereas the amount of adenine decreased slightly (Fig. 2, *B* and *C*). The radioactivity was recovered as inosine.

Separation of Bound and Free Metabolites by Gel Filtration—Samples from the incubate (containing 50 μM AdoHcy and 10 μM enzyme) were subjected to Sephadex G-25 chro-

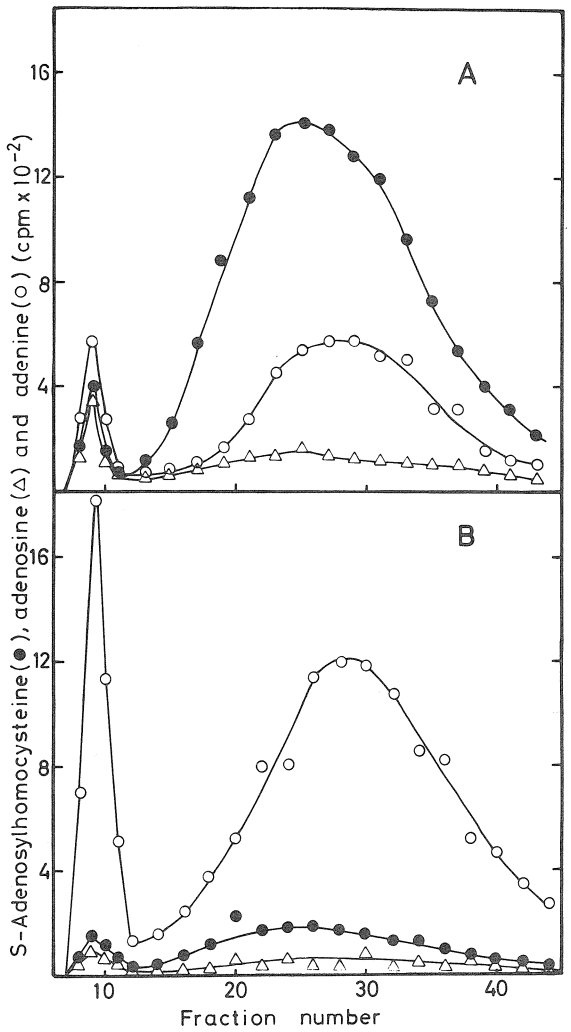


FIG. 3. **Separation of bound and free metabolites by Sephadex G-25 chromatographhy.** [^{14}C]AdoHcy (50 μM) was incubated in the presence of 10 μM enzyme. After 2 min (*A*) and 45 min (*B*) of incubation, samples (40 μl) from the incubation mixture were applied to a Sephadex G-25 column (0.45 × 6 cm) equilibrated with 15 mM Hepes, pH 7.0, and eluted with the same buffer. Fractions of 75 μl were collected and mixed with 30 μl of 0.8 N perchloric acid, neutralized (27), and samples either counted directly (25 μl) or analyzed by thin layer chromatography (60 μl) (16). The elution profiles for AdoHcy (●), adenine (○), and adenosine (△) are shown.

matography after 2 min (Fig. 3*A*) and 45 min (Fig. 3*B*) of incubation. The elution profiles for AdoHcy, adenosine, and adenine are shown on the figure. The protein-bound fraction (which was excluded from the gel) increased from 2 min to 45 min of incubation and the major metabolite was adenine. A portion of AdoHcy and adenosine appeared in the void volume (Fig. 3). This suggests the existence of complexes between these metabolites and enzyme characterized by sufficiently long half-life not to be completely dissociated upon removal of free metabolites during chromatography. However, the rather broad elution profiles corresponding to the free fraction indicate that metabolites were partly dissociated from the enzyme.

The major portion of adenine appeared in the free fraction (Fig. 3). The same elution profile for nonbound adenine was obtained when the fractions were exposed to perchloric acid (Fig. 3) or chromatographed directly (data not shown). This observation adds to the data (27) suggesting that formation of free adenine at least is not a result of acid hydrolysis of adenosine or a product thereof.

The Fraction of Adenosine Not Available for Deamination is Not Incorporated into S-Adenosylhomocysteine—The fraction of adenosine protected against deamination is progressively increased by preincubation of adenosine in the presence of the enzyme before the addition of adenosine deaminase (17). Adenosine was preincubated in the presence of the enzyme. After various periods of time, adenosine deaminase, homocysteine, or both were added to the incubation mixture, and the time course of the formation of inosine, AdoHcy, or

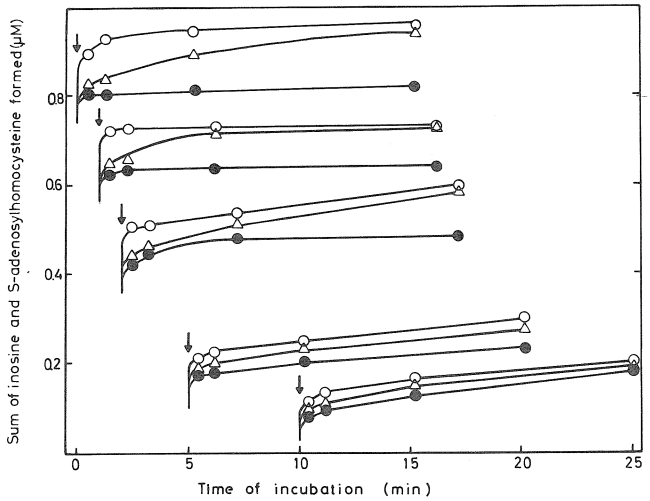


FIG. 4. **Nonavailability of adenosine for deamination and incorporation into AdoHcy.** Adenosine (1 μM) was preincubated in the presence of enzyme (150 μg/ml). After various periods of time, the incubation mixture was supplemented (*arrow*) with either adenosine deaminase (100 units/ml), DL-homocysteine (3 mM), or both. The amount of inosine formed (○) (after the addition of adenosine deaminase), AdoHcy formed (●) (after the addition of homocysteine), and the sum of inosine and AdoHcy formed (△) (after the addition of adenosine deaminase and homocysteine) is plotted against time of incubation.

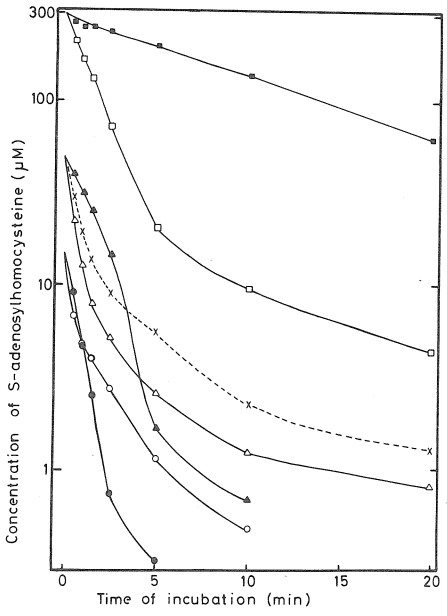


FIG. 5. **Progress curves for the hydrolysis of *S*-adenosylhomocysteine at high enzyme level.** The time course for the hydrolysis of 15 μM (○, ●), 50 μM (△, ▲), and 300 μM (□, ■) AdoHcy was determined at 1 μM enzyme (■, ▲, ●) and 10 μM enzyme (□, △, ○). DL-Homocysteine (0.5 mM) and adenosine deaminase (100 units/ml) were included in the assay mixture. ×---×, indicates hydrolysis of 50 μM in the presence of 1 mM DL-homocysteine and 10 μM enzyme. Note log scale.

the sum of inosine and AdoHcy was determined (Fig. 4). The fraction of adenosine not available for deamination increased in parallel with the fraction of adenosine not being incorporated into AdoHcy.

Time Course for the Hydrolysis of S-Adenosylhomocysteine at High Enzyme Level—Hydrolysis of AdoHcy was allowed to proceed to completion by trapping adenosine formed by including excess adenosine deaminase in the incubation mixture. At 10 μM enzyme, the progress curve for the hydrolysis of AdoHcy (50 μM) was biphasic. About 95% of AdoHcy present was hydrolyzed in less than 15 s whereas the remaining 5% showed a half-life of 5 to 10 min (data not shown). The initial rapid phase could hardly be recorded with the radiochemical method used. This problem was circumvented by including homocysteine in the assay mixture. Homocysteine inhibited the hydrolytic reaction. At low enzyme concentration at least, the inhibition by homocysteine was characterized by decreased V_{max} whereas K_m was not affected (data not shown). In the presence of DL-homocysteine (0.5 mM) the time course of the hydrolysis of AdoHcy (15 to 300 μM) in the presence of 1 and 10 μM enzyme could be determined (Fig. 5). The second slow phase of hydrolysis was more pronounced at low concentration of AdoHcy, at high enzyme level, and at high concentration of homocysteine (Fig. 5). By preincubation of AdoHcy (50 μM) in the presence of enzyme (10 μM) before the initiation of hydrolysis by addition of adenosine deaminase, the second slow phase became more pronounced, whereas the initial phase of hydrolysis was unaffected. After 5 min of incubation, the concentration of AdoHcy was 2.6 μM (Fig. 5), 3.1 μM, and 3.9 μM when the preincubation was 0 min, 2 min, and 5 min, respectively (data not shown).

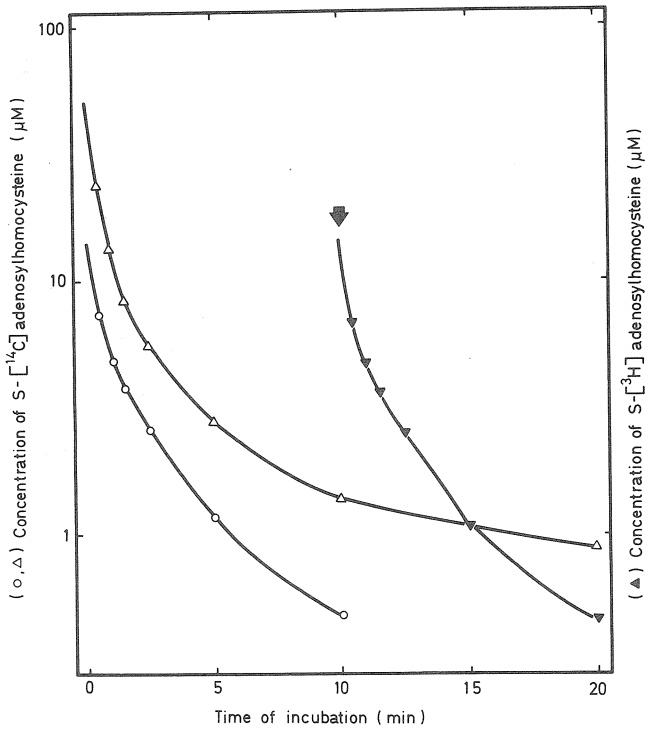


FIG. 6. **Progress curve for the hydrolysis of 15 μM S-[^{3}H]-adenosylhomocysteine in the presence of enzyme preincubated with 50 μM S-[^{14}C]adenosylhomocysteine for 10 min.** The time course of the hydrolysis of 15 μM (○) and 50 μM (△) S-[^{14}C]-adenosylhomocysteine was determined at 10 μM enzyme. DL-Homocysteine (0.5 mM) and adenosine deaminase (100 units/ml) were included in the incubation mixture. After 10 min of incubation, the incubation mixture containing S-[^{14}C]adenosylhomocysteine at an initial concentration of 50 μM was made 15 μM in S-[^{3}H]adenosylhomocysteine (*arrow*). The time course of the hydrolysis of S-[^{3}H]-adenosylhomocysteine (▼) is shown on the figure. Note log scale.

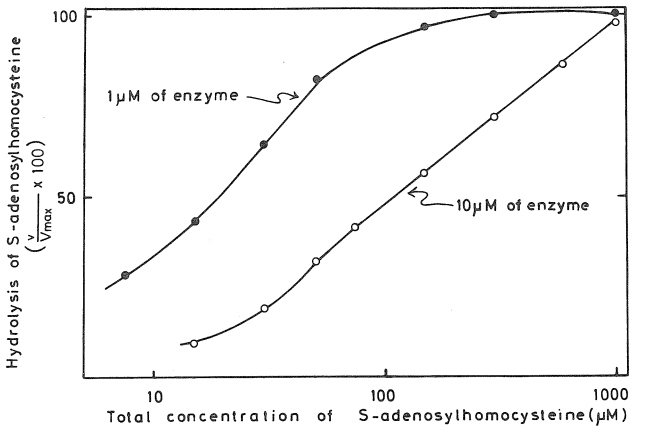


FIG. 7. **Substrate saturation curves for the hydrolysis of AdoHcy at 1 μM and 10 μM enzyme.** The initial velocity (v) of the hydrolysis of AdoHcy was determined at various concentrations (7.5 μM to 1 mM) of AdoHcy and 1 μM and 10 μM enzyme. DL-Homocysteine (3 mM) and adenosine deaminase (100 units/ml) were included in the assay mixture.

The time course of the hydrolysis of 15 μM and 50 μM [^{14}C]-AdoHcy was determined in the presence of 10 μM enzyme and 0.5 mM DL-homocysteine and under the same condition as used in the experiment presented in Fig. 5. The incubation mixture containing 50 μM [^{14}C]AdoHcy was made 15 μM in [^{3}H]AdoHcy after 10 min of incubation. The progress curve for the hydrolysis of 15 μM [^{3}H]AdoHcy was exactly the same as the curve obtained for the hydrolysis of 15 μM [^{14}C]AdoHcy, except that it was displaced 10 min to the right (Fig. 6). This indicates preservation of the catalytic properties of the enzyme during 10 min of hydrolysis of 50 μM AdoHcy.

Substrate Saturation at High Enzyme Level—The initial velocity (v) of hydrolysis of AdoHcy (7.5 to 1000 μM) was determined at 1 μM and 10 μM enzyme. Homocysteine (3 mM) was included in the incubation mixture for reasons given above. The maximal catalytic activity (V_{max}) at 10 μM of enzyme was 10 times V_{max} at 1 μM of enzyme (data not shown). The concentration of AdoHcy of half-maximal catalytic activity ($S_{0.5}$) was about 20 μM at 1 μM enzyme and 120 μM when the concentration of enzyme was 10 μM (Fig. 7).

Test for Polymerization of the Enyzme—The possibility existed that the catalytic properties of the enzyme at high enzyme level were related to enzyme polymerization under these conditions. This possibility was investigated by subjecting the enzyme to chromatography on an Ultrogel AcA 34 column equilibrated with the buffer used for the measurement of the enzyme activity. The enzyme was applied to the gel at concentrations of 0.25 μM and 12 μM, and the experiment was performed in the absence and presence of 50 μM AdoHcy. The enzyme activity eluted as a single symmetrical peak. The same elution volume for the enzyme was obtained at low and high enzyme concentration and in the absence and presence of AdoHcy. About 85% of the enzyme activity was recovered from the column (data not shown). These data suggest that the kinetics of the enzyme catalysis at high enzyme level is not a result of enzyme polymerization.

DISCUSSION

General—The cellular level of *S*-adenosylhomocysteinase is 10 μM in mouse liver (18) and seems to be of the same order of magnitude as the tissue level reported for adenosine (19) and AdoHcy (12, 28, 29). The average apparent concentration of protein in the cell has been estimated to 2×10^{-6} M which is higher than the enzyme concentration used for measurement of enzyme kinetics (22). The same relationship between other enzymes and their substrates as that existing for *S*-

adenosylhomocysteinase has been reported, *i.e.* the amount of a metabolite often equals the number of catalytic sites handling this metabolite (22, 30). There has been growing interest in the physiological implications of this relationship between enzymes and their substrates (30, 31). The present report deals with the metabolism of AdoHcy and adenosine at cellular concentration of *S*-adenosylhomocysteinase and its substrates. Under these conditions the four phenomena of potential physiological importance that could be demonstrated are 1) nonavailability of adenosine for deamination and incorporation into AdoHcy (sequestration of adenosine); 2) formation of adenine; 3) slow hydrolysis of AdoHcy; 4) $S_{0.5}$ for AdoHcy increased by increasing the enzyme level.

Sequestration of Adenosine—Sequestration of adenosine could be demonstrated both during synthesis (Fig. 1) and hydrolysis (Fig. 2) of *S*-adenosylhomocysteine. The fraction of adenosine not available for deamination increased as a function of the concentration of enzyme (Figs. 1 and 2), and the sequestration process could not be detected at concentrations of enzyme used for the measurement of enzyme kinetics. Furthermore, the fraction of adenosine not available for deamination was not incorporated into AdoHcy (Fig. 4). Thus, adenosine sequestered is not mobilized (or only slightly) by the presence of homocysteine.

Sequestration of adenosine has been observed in crude extract from various tissues (18) suggesting that it may be a general phenomenon. These studies were performed in the absence of homocysteine. Therefore, the possibility was not excluded that the sequestration process was an inherent step in the catalytic mechanism being demonstrated only when the catalytic process was arrested at a particular step because of lack of the other substrate, *i.e.* homocysteine. The observation that the sequestration of adenosine could be demonstrated during enzyme catalysis (Figs. 1 and 2) adds to the data suggesting (18) that the interaction of adenosine with *S*-adenosylhomocysteinase participates in the regulation of the tissue level of this nucleoside (19).

S-Adenosylhomocysteine hydrolase from human placenta is irreversibly inactivated in the presence of high concentrations of adenosine (32). The kinetics of inactivation suggests a suicide inactivation which implies that the inactivation proceeds from an adenosine·enzyme complex (32, 33). The inactivation of the enzyme and the sequestration process are probably related phenomena which are demonstrated at low enzyme concentration and high enzyme concentration, respectively.

Formation of Adenine—The fraction of adenosine not available for deamination was mainly isolated as adenine (Figs. 1 and 2). This shows that conversion of adenosine to adenine occurs during enzyme catalysis. A substantial amount of adenine was liberated from the enzyme under conditions of enzymic hydrolysis of AdoHcy (Fig. 3) pointing to the possibility that interaction of adenosine and AdoHcy with *S*-adenosylhomocysteinase may be a cellular source to adenine. Adenosine does not seem to be metabolized to adenine in isolated rat liver cells (34). Thus, it remains to be established whether adenosine and AdoHcy are converted to adenine through the interaction with *S*-adenosylhomocysteinase in the intact cell.

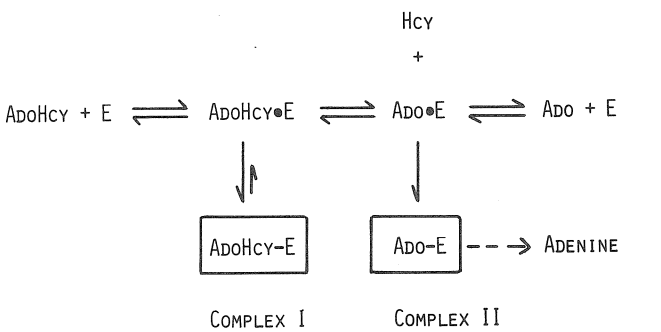
Data have been presented previously suggesting that the formation of adenine and the sequestration of adenosine are related phenomena (17). The formation of adenine or a substance liberating adenine may be an intermediate step in the tight binding of adenosine to the enzyme. Adenine or its precursor which is bound to the enzyme may be reconverted back to adenosine under certain conditions (17). Palmer and Abeles have recently suggested that adenosine is oxidized to 3-ketoadenosine in the presence of *S*-adenosylhomocysteinase (35). This compound is spontaneously converted to adenine (35). This may offer an explanation to the formation of adenine.

Biphasic Hydrolysis of AdoHcy—The data of Fig. 1 shows that the rate of hydrolysis of AdoHcy decreased as the enzyme concentration approached its cellular level. This finding should be related to the slow phase of hydrolysis of AdoHcy at high enzyme level (Fig. 5). Biphasic hydrolysis of AdoHcy (Fig. 5) could not be explained by conversion of the enzyme during incubation into a form characterized by altered catalytic properties, as demonstrated by the experiment depicted in Fig. 6.

The second slow phase of hydrolysis of AdoHcy predominated when the concentration of AdoHcy approached the concentration of enzyme (Fig. 5) suggesting that the slow hydrolysis is a saturable phenomenon. This phase may represent the half-life of an AdoHcy-enzyme complex. The existence of such a long lived complex is in fact suggested by the gel filtration experiment presented in Fig. 3. The amount of this complex may increase by increasing the time of interaction between the enzyme and AdoHcy. This suggestion is based on the observation that the second phase of hydrolysis was more pronounced when AdoHcy was preincubated in the presence of enzyme before initiation of hydrolysis and when the rate of hydrolysis was retarded by inclusion of homocysteine in the incubation mixture (Fig. 5). Furthermore, the concentration of free AdoHcy may fall far below the K_m for AdoHcy when a large fraction of the substrate is bound to the enzyme. This may also contribute to the kinetics of hydrolysis of AdoHcy at high enzyme level.

In physiological terms, high enzyme level ensures an efficient handling of AdoHcy but also counteracts cellular depletion of this metabolite. Furthermore, AdoHcy bound to the enzyme may not be available as an inhibitor of AdoMet-dependent transmethylation reactions.

Substrate Saturation at Cellular Enyzme Level—The K_m value for AdoHcy is 0.75 μM when determined in the presence of excess substrate relative to the amount of enzyme (16). Under these *in vitro* conditions the enzyme is saturated with respect to AdoHcy at concentrations reported for AdoHcy in tissues (28). Based on similar observations it has been suggested that the enzyme is saturated with AdoHcy *in vivo* (36, 37). However, the $S_{0.5}$ for AdoHcy increased by increasing the enzyme level (Fig. 7). In the presence of 10 μM enzyme, half-maximal catalytic activity was observed at 120 μM AdoHcy (Fig. 7) which is in the upper range reported for the tissue level of this metabolite (28).



ADOHCY = S-ADENOSYLHOMOCYSTEINE
ADO = ADENOSINE
HCY = L-HOMOCYSTEINE
E = ENZYME

SCHEME I

Hypothesis—The data of this paper is summarized in and discussed in relation to a tentative model (Scheme 1). The description of the catalytic process is based on the reaction mechanism of *S*-adenosylhomocysteinase suggested by Palmer and Abeles (35). The existence of two stable complexes is postulated. Complex I (AdoHcy-*E*) is a long lived nonproductive complex between AdoHcy (which may be chemically modified) and the enzyme. This complex may be formed from a transient intermediate (AdoHcy·*E*). The amount of Complex I would be expected to increase as a function of enzyme concentration, time of incubation before initiation of hydrolysis, and by the presence of homocysteine. The second slow phase of hydrolysis (Fig. 5) corresponds to the half-life of this complex. Complex II (Ado-*E*) refers to a complex between adenosine or a derivative thereof and the enzyme. The formation of Complex II from the short lived intermediate, Ado·*E*, is essentially irreversible. Complex II corresponds to the fraction of adenosine sequestered and to the inactive enzyme observed upon preincubation of the enzyme in the presence of high concentrations of adenosine (32). This complex is a precursor to adenine liberated from the enzyme (Fig. 3).

There are several objections to the scheme outlined above. Adenine, which seems to be a regulator of the enzyme (16), is formed at high enzyme level and may affect the kinetics of the enzyme catalysis under these conditions. The presence of factors affecting the catalytic properties of the enzyme in the enzyme preparation has not been excluded. Data in favor of the presence of such factors in crude extract from mouse liver was not obtained by the measurement of the recovery of enzyme activity after addition of homogenous enzyme to crude extract (18). Failure of detection of an inactive polymer by the gel filtration experiment (see under "Results") may be explained by no reactivation of the enzyme upon dilution in the assay mixture. However, enzyme polymerization has never been observed by subjecting concentrated enzyme solutions (10 to 50 mg/ml) to sucrose gradient centrifugation or polyacrylamide gel electrophoresis.[2]

Conclusion—The possible physiological implications of the metabolism of adenosine and AdoHcy at high enzyme level are rather apparent whereas the uncovering of the mechanisms underlying these phenomena will require further studies.

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