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S-Adenosylhomocysteinase from Mouse Liver. Effect of Adenine and Adenine Nucleotides on the Enzyme Catalysis†

Per Magne Ueland* and John Saebø‡

ABSTRACT: The kinetics of the synthesis and hydrolysis of *S*-adenosylhomocysteine catalyzed by the enzyme *S*-adenosylhomocysteinase from mouse liver was determined in the presence of adenine and adenine nucleotides. At a concentration of 1 μM adenosine, the time course of the synthesis of *S*-adenosylhomocysteine in the presence of adenine acquired a smaller slope after about 5 min of incubation. The synthesis in the presence of AMP was linear for about the same time as that in the absence of inhibitor whereas the curve obtained with ADP and ATP gradually leveled off until a plateau was reached after about 10 min of incubation. The hydrolysis of *S*-adenosylhomocysteine in the presence of AMP, ADP, and ATP was linear with respect to time for about the same time as that in the absence of these nucleotides. The time course in the presence of adenine acquired a smaller slope after about 5 min of incubation. The enzyme was preincubated in the presence of adenine (25 μM), AMP (2 mM), ADP (2 mM), and ATP (2 mM), diluted to minimize the effect of purines during incubation, and assayed for *S*-adenosylhomocysteine synthase activity. After a lag phase lasting for 2–5 min, the activity decreased in a time-dependent manner in the presence of ADP and ATP, and about 50% of the initial activity remained after 30 min of preincubation. Only a slight decrease was observed with AMP. Adenine effected a rapid initial fall in synthase activity after which no further decrease was observed. Kinetic analysis of initial velocity data indicates linear competitive inhibition of *S*-adenosylhomocysteine hydrolase activity in the presence of adenine, AMP, and ADP, and the inhibitor constants (K_i) increased in the order mentioned. The inhibitor constants for these purines seem to be the same during synthesis and hydrolysis of *S*-adenosylhomocysteine. Cyclic AMP and ATP were not or only slightly inhibitory. The hydrolysis of *S*-adenosylhomocysteine (50 μM) was allowed to proceed to equilibrium. Adenine, AMP, and ADP increased the time needed for equilibrium to be obtained.

A protein not associated with cyclic AMP dependent protein kinase was identified in mouse liver because of its ability to bind cyclic AMP (Døskeland & Ueland, 1975). Further binding kinetic studies revealed the following binding properties of this protein. The protein was isolated in a form possessing low binding capacity for cyclic AMP (Ueland & Døskeland, 1977). The cyclic AMP binding was increased by preincubating the protein in the presence of ATP. Thus, the ATP-treated protein had two distinct binding sites, called the adenosine binding site(s) and the cyclic AMP binding site, respectively (Ueland & Døskeland, 1977). The adenosine binding site(s) showed rather high specificity toward this nucleoside whereas the structural requirements for the binding of ligands to the cyclic AMP site was low. The following adenine derivatives were shown to interact with this site with decreasing affinity in the order mentioned: cyclic AMP, adenosine, AMP, ADP, and ATP (Ueland, 1978).

The binding protein which interacts with various adenine analogues was eventually identified as *S*-adenosylhomocysteinase (EC 3.3.1.1) (Saebø & Ueland, 1978; Hershfield & Kredich, 1978). This enzyme catalyzes the reversible thioether bond formation between adenosine and L-homocysteine according to the equation

adenosine + L-homocysteine ⇌ *S*-adenosylhomocysteine

(De la Haba & Cantoni, 1959). This enzyme has been implicated in the regulation of the cellular level of *S*-adenosylhomocysteine (SAH[1]) which is a product formed from *S*-adenosylmethionine (SAM) upon transmethylation from SAM to cellular acceptors (Cantoni & Scarano, 1954; De la Haba & Cantoni, 1959). SAH is a potent inhibitor of most transmethylases using SAM as a methyl donor (Hurwitz et al., 1964; Zappia et al., 1969; Deguchi & Barchas, 1971; Kerr, 1972; Coward et al., 1974; Pugh et al., 1977). The tissue level of SAH equals that of SAM (Salvatore et al., 1971; Hoffman, 1975). On this basis SAH has been suggested to be a regulator of biological methylation (Hurwitz et al., 1964; Zappia et al., 1969; Deguchi & Barchas, 1971; Salvatore et al., 1971; Kerr, 1972; Coward et al., 1974; Hoffman, 1975; Pugh et al., 1977).

In this paper the effect of adenine and adenine nucleotides on the metabolism of SAH in the presence of *S*-adenosylhomocysteinase from mouse liver was investigated for the following reasons. (a) The binding of adenine and adenine nucleotides to the enzyme (Ueland & Døskeland, 1977; Ueland, 1978) suggests that the metabolism of SAH may be regulated by the concentration of these purines. To our knowledge, a detailed study on the effect of the adenine and adenine nucleotides on *S*-adenosylhomocysteinase has not been carried out before. (b) By measurement of the synthesis and hydrolysis of SAH in the presence of adenine and adenine nucleotides, data on the binding properties of the catalytic site(s) may be obtained which may suggest the relation between the adenosine binding site(s), the cyclic AMP site, and the catalytic site(s).

† From the Department of Pharmacology, University of Bergen, School of Medicine, MFH-bygget, N-5016 Haukeland sykehus, Bergen, Norway. *Received March 16, 1979.* This work was supported by grants from the Norwegian Research Council for Science and the Humanities and by the Nordisk insulinfond.

‡ Deceased.

[1] Abbreviations used: SAH, *S*-adenosylhomocysteine; SAM, *S*-adenosylmethionine; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; Hcy, homocysteine; Ado, adenosine.

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Materials and Methods

Purification of S-Adenosylhomocysteinase from Mouse Liver. The enzyme was purified to apparent homogeneity according to a purification scheme published previously (Ueland & Døskeland, 1977; Ueland, 1978). The enzyme was not treated with ATP or other purines prior to use.

Purification of AMP, ADP, and ATP. This was performed as described previously (Ueland, 1978).

Thin-Layer Chromatography. Chromatography was performed on poly(ethylenimine)-impregnated cellulose thin-layer sheets (0.25 mm) on glass plates (20 × 20 cm). Three solvent systems were used (Saebø & Ueland, 1978): glacial acetic acid–water–1-butanol (1:1:2 v/v/v) (system A); 1.2 M LiCl (system B); 2-methyl-1-propanol–water–ethanol (2:1:1 v/v/v) (system C).

Preparation of [^{14}C]-S-Adenosylhomocysteine. [8-^{14}C]-Adenosine (59 mCi/mmol; 0.5 mM) and DL-homocysteine (3 mM) were incubated for 10 min at 37 °C in the presence of purified *S*-adenosylhomocysteinase (100 μg/mL) from mouse liver. The incubation buffer was 5 mM Hepes buffer, pH 7.0, containing 2 mM 2-mercaptoethanol. The reaction was terminated by heating (100 °C) for 5 min, and the reaction products were separated by thin-layer chromatography in system C. *S*-Adenosylhomocysteine was eluted from PEI–cellulose powder into distilled water, lyophilized, and redissolved to a final concentration of 0.7 mM. More than 99% of the radioactive material comigrated with *S*-adenosylhomocysteine in systems A–C.

Assay for S-Adenosylhomocysteine Synthase Activity. [8-^{14}C]Adenosine and DL-homocysteine (3 mM) were incubated at 37 °C in the presence of enzyme under the conditions given in the legends to the figures. The incubation buffer was 15 mM Hepes, pH 7.0, containing 5 mM magnesium acetate, 150 mM KCl, 2 mM 2-mercaptoethanol, and 0.25% bovine serum albumin.

Bovine serum albumin was included in the assay mixture and in the buffer in which the enzyme was diluted to increase stability of the enzyme. At high dilutions (i.e., concentration of enzyme less than 1 μg/mL), the enzyme was unstable, especially at low ionic strength.

The incubations were stopped by adding samples of 25 μL into 25 μL of 0.8 N perchloric acid (0 °C). After 5 min at 0 °C, the acid was neutralized to pH 6.0 (Ueland & Saebø, 1979). Samples of 25 μL were subjected to thin-layer chromatography in system C. The chromatographic spots were visualized by absorption in UV light, scrapped off, and counted by liquid scintillation.

Assay for S-Adenosylhomocysteine Hydrolase Activity. [^{14}C]-*S*-Adenosylhomocysteine was incubated at 37 °C in the presence of enzyme under the conditions given in the legends to the figures. The incubation buffer was the same as that described above except that it was supplemented with adenosine deaminase (50 units/mL). The reaction products (SAH and inosine) were separated, and the radioactivity was determined as described above.

Determination of Protein. Protein was determined by measuring the absorbance of 280 nm, using a specific extinction coefficient ($E_{280nm}^{1\%}$) of 13.0 for the protein (Ueland et al., 1978).

Results

Kinetic Parameters of the Enzyme Catalysis. The double-reciprocal plot of the concentration of adenosine vs. the *S*-adenosylhomocysteine synthase activity was linear and consistent with a K_m of 0.2 μM for adenosine and a V_{max} of 1.9 μmol/(min mg) in the synthetic direction under the conditions used. The *S*-adenosylhomocysteine hydrolase activity was characterized by a K_m of 0.75 μM for SAH and a V_{max} of 0.54 μmol/(min mg) under the same conditions.

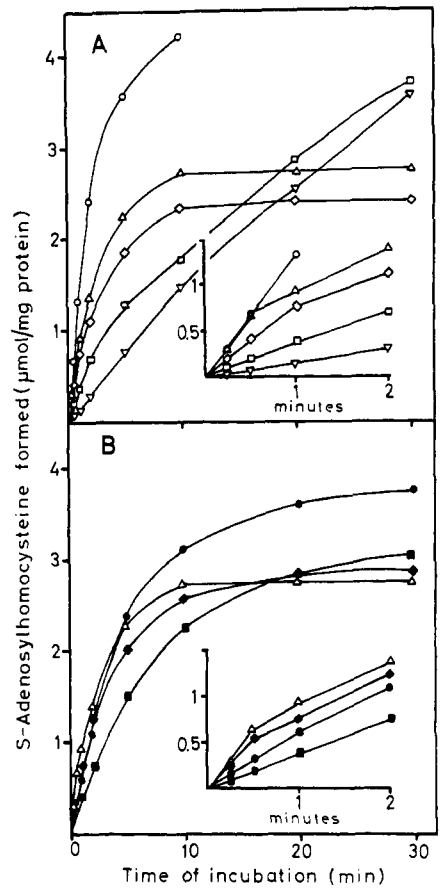

FIGURE 1: Time course of the synthesis of *S*-adenosylhomocysteine. (A) [^{14}C]Adenosine (1 μM) was incubated in the presence of homocysteine and enzyme (0.23 μg/mL) under the conditions described under Materials and Methods. The incubation mixture was supplemented with 30 μM adenine (□), 1 mM AMP (▽), 1 mM ADP (◇), 2.5 mM ATP (△), or no addition (○). The amount of SAH formed is plotted vs. time of incubation. The inset shows the same data at a short time of incubation. (B) The formation of SAH was determined in the presence of 2.5 mM ATP as described above except that the incubation buffer contained no Mg and KCl (●), 5 mM Mg (◆), 5 mM Mg and 150 mM KCl (▲), or 5 mM Mg, 150 mM KCl, and 5 μM SAH (■). The data are plotted as described in (A).

Time Course of the Synthesis of S-Adenosylhomocysteine in the Presence of Adenine and Adenine Nucleotides. The amount of SAH formed was linear with respect to time until about 50% of adenosine (1 μM) was incorporated into SAH under the conditions of Figure 1A. The initial velocity was inhibited in the presence of adenine (30 μM), AMP (1 mM), and ADP (1 mM). The velocity of the synthetic reaction was reduced to about half of its initial rate after about 5 min of incubation in the presence of adenine. The time course observed with AMP was linear for about the same time as that in the absence of the nucleotide whereas the curve obtained in the presence of ADP and ATP gradually leveled off until a plateau was reached after about 10 min of incubation (Figure 1A). Cyclic AMP (0.1 mM) was without effect (data not shown).

The effect of SAH, KCl, and Mg^{2+} on the time course of the synthesis in the presence of ATP was investigated. SAH decreased the initial velocity, but the progressive decline in the synthetic activity was less pronounced. The leveling off of the formation of SAH was promoted in the presence of KCl

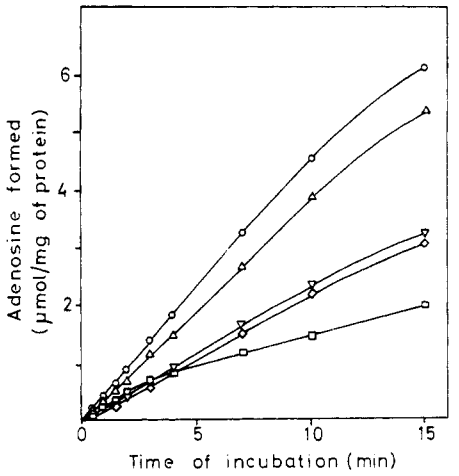

FIGURE 2: Time course of the hydrolysis of *S*-adenosylhomocysteine. [^{14}C]Adenosylhomocysteine (5 μM) was incubated in the presence of enzyme (0.57 μg/mL) under the conditions described under Materials and Methods. The incubation mixture contained 10 μM adenine (□), 0.2 mM AMP (▽), 2 mM ADP (◇), 2 mM ATP (△), or no addition (○). The amount of adenosine formed is plotted against time of incubation.

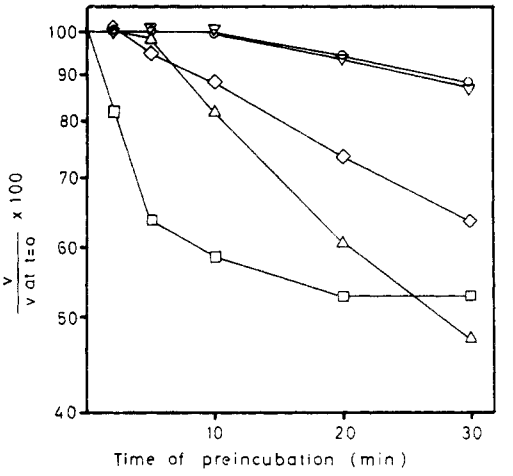

FIGURE 3: Effect of preincubation of the enzyme in the presence of adenine and adenine nucleotides on SAH synthase activity. *S*-Adenosylhomocysteinase (25 μg/mL) was preincubated for various periods of time in the presence of 25 μM adenine (□), 2 mM AMP (▽), 2 mM ADP (◇), 2 mM ATP (△), or no addition (○). The preincubation buffer was 15 mM Hepes, pH 7.0, containing 0.25% bovine serum albumin, 150 mM KCl, 5 mM magnesium acetate, and 2 mM 2-mercaptoethanol, and the temperature was 37 °C. At times indicated on the figure, aliquots of 5 μL were transferred to the assay mixture (300 μL) (see Materials and Methods) containing 5 μM [^{14}C]adenosine. The logarithm of the initial velocity is plotted against time of preincubation.

and Mg (Figure 1B). These ions were also shown to stimulate the initial velocity of the reaction both in the absence (data not shown) and in the presence of ATP (Figure 1B).

The time course of the synthesis of SAH in the presence of ATP was determined at various concentrations of adenosine (1.5×10^{-7} to 10^{-4} M). The decline in the enzyme activity with respect to time was more pronounced, and the plateau was reached at a shorter time of incubation at low concentrations of the nucleoside (data not shown).

The effect of SAH described above (Figure 1B) could not be explained by an increase in the concentration of adenosine through hydrolysis of SAH. No hydrolysis of radioactive SAH (5 μM) could be detected under the conditions used (i.e., in the presence of 3 mM homocysteine and 1 μM adenosine and in the absence of adenosine deaminase; data not shown).

Time Course of the Hydrolysis of S-Adenosylhomocysteine in the Presence of Adenine and Adenine Nucleotides. The initial velocity of the hydrolysis of SAH (5 μM) was inhibited in the presence of adenine, AMP, and ADP and only slightly by ATP (Figure 2). The time curve acquired about half its initial slope after about 5 min of incubation in the presence of adenine. The curve obtained in the presence of AMP, ADP, and ATP was linear for the same time as that in the absence of these nucleotides (Figure 2). Principally, the same results were obtained over a wide concentration range (0.5–50 μM) of SAH.

Effect of Preincubation in the Presence of Adenine, AMP, ADP, and ATP on the Synthesis of SAH. The enzyme was preincubated in the presence of adenine (25 μM), AMP (2 mM), ADP (2 mM), and ATP (2 mM), diluted 60-fold to minimize the effect on the enzyme of purines carried over from the preincubation mixture, and then assayed for SAH synthase activity. The logarithm of enzyme activity was plotted vs. time of preincubation (Figure 3). After an initial lag phase lasting for 2–5 min, there was a slow decrease in enzyme activity in the presence of ADP and ATP. About half of the initial activity remained after 30 min of preincubation. Only a slight decrease was observed in the presence of AMP after prolonged incubation. In contrast, the curve obtained in the presence of adenine showed a rapid initial fall after which a plateau was obtained (Figure 3).

Competitive Inhibition of the Synthesis and Hydrolysis of SAH by Adenine and Adenine Nucleotides. The initial velocity of the synthesis and hydrolysis of SAH was determined at various concentrations of substrates in the presence of adenine, AMP, or ADP or no addition. The enzyme was diluted to the extent that less than 25% of the substrates was consumed within 2 min of incubation. In the synthetic direction, the amount of SAH formed was determined between 15 s and 4 min of incubation, and there was apparent linearity of the amount of SAH formed vs. time of incubation for at least 1 min under the conditions of the experiment (see inset of Figure 1).

The double-reciprocal plots for the synthesis (Figure 4) and hydrolysis (Figure 5) were linear both in the absence and in the presence of inhibitor. The plots obtained suggest that the inhibition is of competitive type.

The initial velocity of the hydrolysis of SAH (1, 3, and 10 μM) was determined in the presence of the increasing concentrations of adenine (1–20 μM), AMP (10 μM–0.2 mM), and ADP (0.1–2 mM). Data were plotted as $1/v$ vs. the concentration of inhibitor (Dixon, 1953) as shown in Figure 6. The graphs obtained are consistent with linear competitive inhibition in the presence of adenine, AMP, and ADP.

The inhibitor constants (K_i) of adenine, AMP, and ADP could be calculated both in the synthetic and in the hydrolytic direction from the data of Figures 4 and 5 by using the equation (Dixon, 1953)

$$K_i = \frac{K_m[I]v}{V_{max}[S] - ([S] + K_m)v}$$

where v = the initial velocity, [I] = concentration of competing molecule, and [S] = concentration of substrate. The K_i values could also be determined from the Dixon plots (Dixon, 1953) (Figure 6). The results listed in Table I show that the enzyme–inhibitor complex is characterized by dissociation constants which are nearly the same during synthesis and hydrolysis. The affinity is highest for adenine, followed by AMP and ADP in the order mentioned.

Inhibition of the Synthesis and Hydrolysis of SAH by Adenine Nucleotides at the Cellular Level of Adenosine and SAH. The initial velocity of the synthesis and hydrolysis of

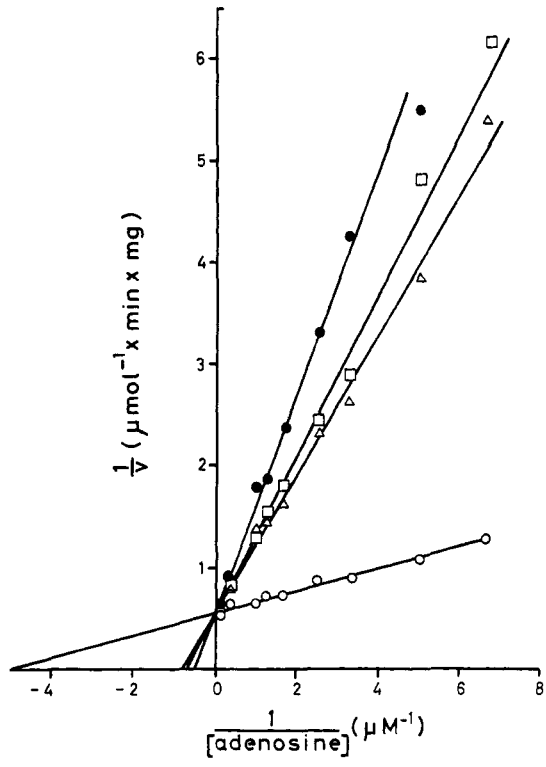

FIGURE 4: Double-reciprocal plot for the synthesis of SAH vs. the concentration of adenosine in the presence of inhibitors. The initial velocity of the synthesis of SAH was determined at various concentrations of adenosine in the presence of 10 μM adenine (△), 0.2 mM AMP (●), 1 mM ADP (□), or no addition (○). Details are given in the text.

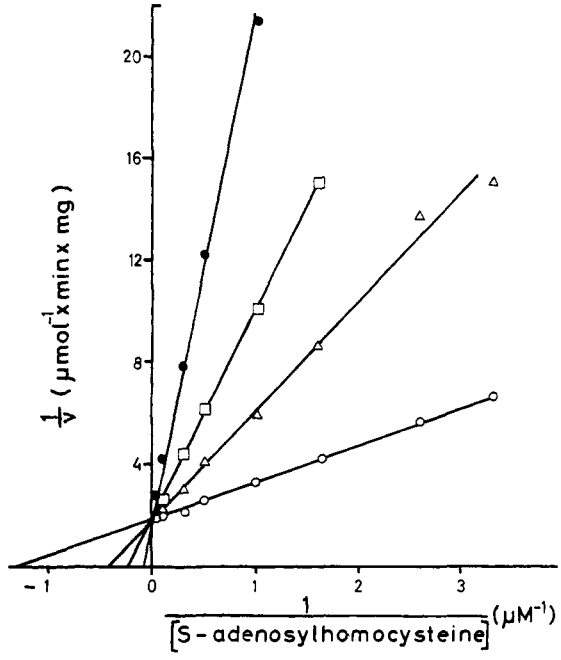

FIGURE 5: Double-reciprocal plot for the hydrolysis of SAH vs. the concentration of SAH in the presence of inhibitors. The initial velocity of the hydrolysis of SAH was determined at various concentrations of SAH in the presence of 3 μM adenine (△), 0.3 mM AMP (●), and 1 mM ADP (□) or no addition (○). Details are given in the text.

SAH is determined at the cellular level of adenosine (1 μM) (Nordström et al., 1977; Newman & McIlwain, 1977; Fox & Kelley, 1978) and SAH (50 μM) (Salvatore et al., 1971; Hoffman, 1975) and in the presence of AMP, ADP, or ATP. At the cellular level of AMP [about 0.3 mM (Lund et al., 1975)] or ADP [1 mM (Lund et al., 1975)], the synthesis was inhibited by 50 and 70%, respectively, whereas the hydrolysis was inhibited by 25% in the presence of AMP and by 10% in the presence of ADP. The synthesis and hydrolysis were only slightly inhibited by ATP (1–6 mM) (data not shown). These data indicate that *S*-adenosylhomocysteinase may be competitively inhibited by adenine nucleotides in vivo.

Table I: Inhibitor Constants (K_i) for Adenine and Adenine Nucleotides[a]

inhibitor	inhibitor constant, K_i (M)		
	synthesis of SAH	hydrolysis of SAH	
adenine	1.9×10^{-6}	1.5×10^{-6}	2.0×10^{-6}
AMP	2.3×10^{-5}	2.3×10^{-4}	2.4×10^{-5}
ADP	1.7×10^{-4}	2.1×10^{-4}	2.5×10^{-4}

[a] The inhibitor constants (K_i) in the synthetic and hydrolytic direction listed in the second and third columns in the table are calculated from the data of Figures 4 and 5 as described in the text, whereas the values given in the column to the right are determined from the Dixon plots (Figure 6).

Effect of Adenine and Adenine Nucleotides on the Hydrolysis of SAH Allowed to Proceed to Equilibrium. The hydrolysis of SAH, at the cellular level of this compound (50 μM) (Salvatore et al., 1971; Hoffman, 1975), was allowed to proceed to equilibrium in the presence of adenine, AMP, ADP, and ATP (Figure 6). The equilibrium was determined from the hydrolysis of SAH for two reasons. First, the metabolic flow has been suggested to be in the hydrolytic direction (Cortese et al., 1974). Second, the formation of 50 μM SAH would require about 50 μM adenosine, which exceeds the cellular level of this nucleoside.

Adenine, AMP, and ADP increase the time needed to reach equilibrium with decreasing efficiency in the order mentioned (Figure 7). The equilibrium constant (K_{eq}), defined by the equation (De la Haba & Cantoni, 1959)

$$K_{eq} = \frac{[\text{Hcy}][\text{Ado}]}{[\text{SAH}]}$$

is calculated to be 0.8×10^{-6} M from the data in Figure 7.

Discussion

An apparent K_m value of 2×10^{-7} M was observed for adenosine under the conditions used in this study, i.e., in the presence of mercaptoethanol, Mg, KCl, and bovine serum albumin. This value differs somewhat from the results obtained when different incubation conditions were used (Saebø & Ueland, 1978). K_m values for adenosine of *S*-adenosylhomocysteinase reported by others vary from 0.7 μM to 1.5 mM (Walker & Duerre, 1975; Kajander et al., 1976; Hershfield & Kredich, 1978; Richards et al., 1978; Saebø & Ueland, 1978). This may be explained by interspecies variation, the presence of isozyme forms, or by different incubation conditions used. In favor of the latter possibility is the fact that K_m values for adenosine of the enzyme from rat liver have been reported to be 0.7 μM (Kajander et al., 1976) and 1.5 mM (Walker & Duerre, 1975). This inconsistency, together with the seemingly unfavorable kinetic conditions existing when the K_m value of an enzyme is 1000 times the concentration of its substrate (Atkinson, 1977), leads us to suggest that particular attention should be paid to the incubation conditions used when performing kinetic studies on this enzyme.

The synthesis of *S*-adenosylhomocysteine decreases progressively in the presence of ADP and ATP, and nearly no further synthesis is observed after 10 min of incubation under the conditions of the experiment (Figure 1A). There was only

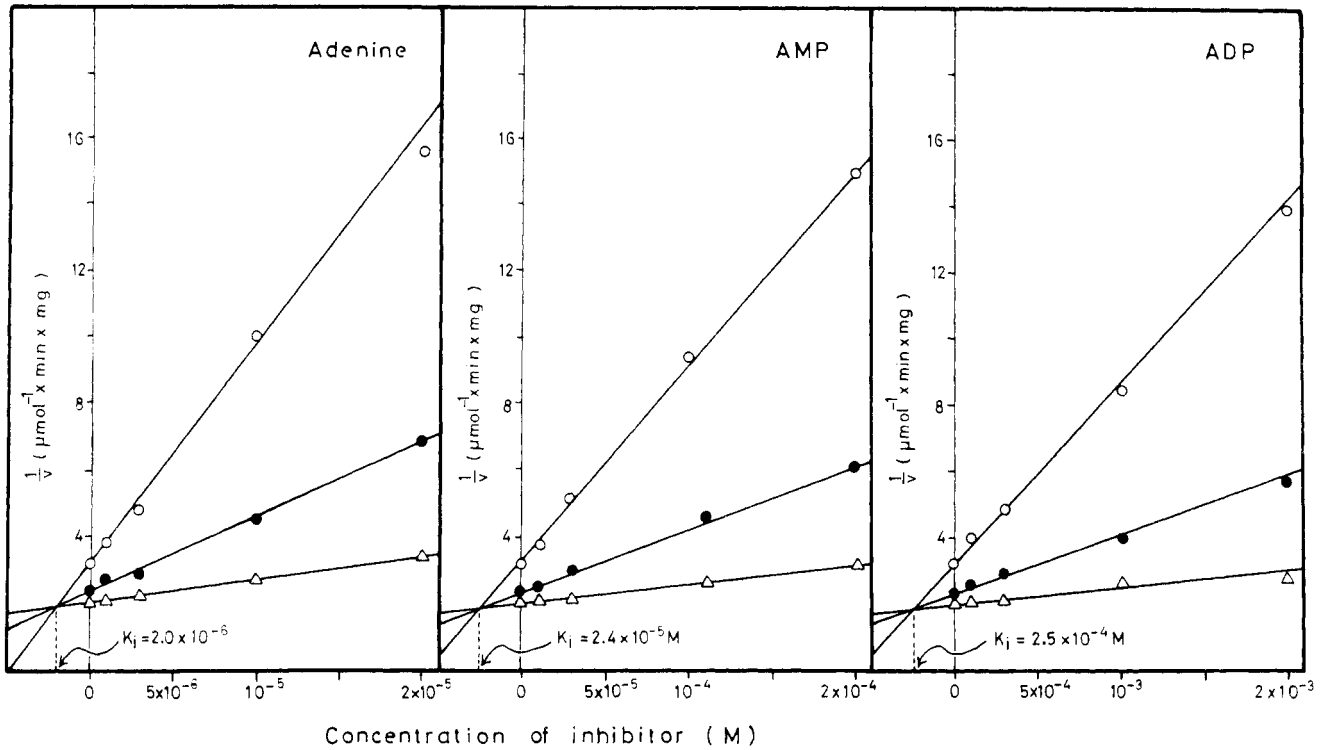

FIGURE 6: Inhibition of the hydrolysis of SAH by various concentrations of adenine, AMP, and ADP. The initial velocity (v) of the hydrolysis of 1 (○), 3 (●), and 10 μM (△) SAH was determined in the presence of adenine, AMP, and ADP at concentrations indicated on the figure. The data are plotted according to Dixon (1953).

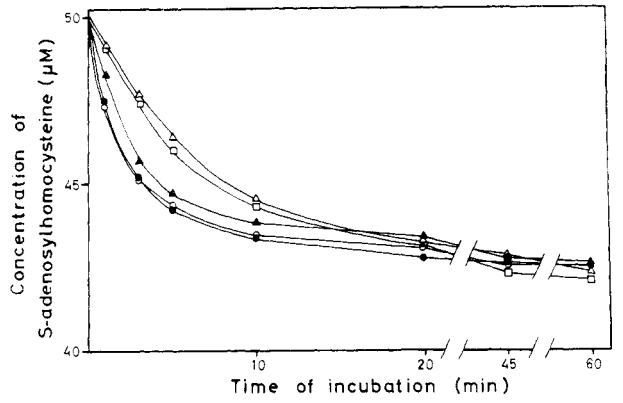

FIGURE 7: Effect of adenine and adenine nucleotides on the hydrolysis of SAH allowed to proceed to equilibrium. SAH (50 μM) was incubated in the presence of enzyme (12 μg/mL) and 0.2 mM adenine (△), 2 mM AMP (□), 2 mM ADP (▲), 2 mM ATP (●), or no addition (○). The incubation conditions are as described under Materials and Methods except that adenosine deaminase was omitted from the incubation mixture. The concentration of SAH is plotted against time of incubation.

a slight decrease in the synthetic activity after preincubation for 10 min in the presence of ADP and ATP and extensive dilution of the preincubation mixture prior to assay (Figure 3). These data suggest that the low synthetic activity (Figure 1) is dependent on the presence of the adenine nucleotide. This interpretation should perhaps be related to the observation that the enzyme preincubated in the presence of ATP has a high binding capacity for adenine nucleotides (Ueland, 1978).

S-Adenosylhomocysteinase from human lymphoblast is inactivated by 2-deoxyadenosine and adenine arabinoside (Hershfield, 1979). The inactivation shows first-order kinetics, is saturable, and is irreversible, and the inactivators bind tightly to the enzyme. These data suggest "suicide" inactivation which is explained by tight binding of a substrate analogue to the active site (Walsh, 1977). The effect of adenine and adenine nucleotides reported here does not conform to the concept of suicide inactivation. The decrease in synthetic activity does not display first-order kinetics (Figure 3) and seems partly dependent on the presence of effector. Furthermore, bound ATP is dissociated from the enzyme upon removal of the free fraction (Ueland & Døskeland, 1978a).

The following observations suggest that the effect of adenine and adenine nucleotides is not caused by a partial denaturation of the enzyme. The time-dependent decline in the synthesis of SAH is enhanced in the presence of Mg^{2+} and KCl which both stimulate the initial velocity of the reaction (Figure 1B). In the presence of these ions, ATP decreases the reactivity of slow-reaction sulfhydryl groups in this protein (Ueland et al., 1978) whereas denaturation of the protein would probably unmask sulfhydryl groups. Proteolysis of this protein could not be detected after treatment with ATP (Ueland & Døskeland, 1978b). Finally, the hydrolysis of SAH is linear with respect to time in the presence of ADP and ATP for about as long as that in the absence of these nucleotides (Figure 2).

The effect of adenine and adenine nucleotides could be interpreted in terms of hysteresis as defined by Frieden (1970). These purines may effect a slow transition of the enzyme toward a form(s) possessing altered catalytic properties. Both the synthesis (Figures 1 and 3) and hydrolysis (Figure 2) of SAH acquire a reaction rate after about 5 min of incubation in the presence of adenine which is about half of the initial velocity. This may be interpreted as a transition of the enzyme toward a form with low synthetic and hydrolytic activity.

There are some similarities between the activation of the cyclic AMP binding capacity of this protein and the effects of adenine and adenine nucleotides discussed above. The activation of the cyclic AMP binding site by ATP and the effect of this nucleotide on the synthesis of SAH (Figure 1) are promoted by KCl and Mg but not by NaCl (Ueland & Døskeland, 1978a). The leveling off of the synthetic reaction was observed in the presence of purines (ATP and ADP),

which increase the binding capacity for adenine nucleotides of this protein, but not in the presence of metabolites (adenine and AMP) inhibiting this process (Figure 1A) (Ueland, 1978; Ueland & Døskeland, 1978a,b). Thus, the activation of the cyclic AMP binding site and the hysteretic effect of ATP and ADP seem to be related phenomena.

Reasoning along the lines suggested above, it was somewhat surprising that cyclic AMP (0.1 mM) did not affect the enzyme activity either in the synthetic or in the hydrolytic direction. This nucleotide has been shown to activate its own site (homologous activation) (Ueland & Døskeland, 1978b). Whether this could be explained by inhibition of the cyclic AMP effect by adenosine (Ueland & Døskeland, 1978b), SAH, or homocysteine remains to be established. However, this observation adds to the data (Sugden & Corbin, 1976) suggesting that *S*-adenosylhomocysteinase is not a major mediator of cyclic AMP effects in the cell.

Data on the competitive inhibition of the synthesis and hydrolysis of SAH by other purines (Figures 4–6 and Table I) show that the site catalyzing the hydrolysis of SAH has nearly the same affinity toward adenine and adenine nucleotides as the site catalyzing the synthesis of SAH. This indicates that the synthesis and hydrolysis take place at a common site, which is in accordance with a reaction mechanism suggested for this enzyme (Palmer & Abeles, 1976). The catalytic site shows similar binding properties with the adenosine binding site(s) previously identified from binding studies (Ueland & Døskeland, 1977; Ueland, 1978), indicating that the enzyme catalysis takes place at the adenosine binding site(s) and not at the cyclic AMP site.

The progressive decline in the synthetic activity in the presence of ATP was observed at 1 μM of adenosine which is in the concentration range reported for the tissue level of this nucleoside (Nordström et al., 1977; Newman & McIlwain, 1977; Fox & Kelley, 1978). Thus, from a functional point of view, one may speculate whether an increase in the cellular concentration of adenosine results in a slow increase of the level of SAH at high concentrations of ADP and ATP, relative to the increase at low concentrations of these nucleotides. In general, this may indicate that the metabolism of adenosine and SAH may be regulated by the energy charge (Atkinson, 1977) of the adenylate pool through the hysteretic effect(s) of adenine nucleotides on the enzyme *S*-adenosylhomocysteinase. In addition, adenine and adenine nucleotides may affect the metabolism of adenosine and SAH through competitive inhibition of the binding of substrates to this enzyme (Figures 4 and 5).

There are several indications that the hypothesis outlined above is an oversimplification. The hysteretic effect of ATP decreased in the presence of SAH (Figure 1B). Furthermore, the hysteretic effect of adenine on both the synthesis and hydrolysis suggests that this purine may be an important regulator in this system. Finally, to measure the time course of the enzyme catalysis, the enzyme concentrations used in this study are several orders of magnitude lower than the cellular level of this enzyme which has been calculated to be about 10 μM in mouse liver (Ueland & Saebø, 1979). Thus, the enzyme concentration is of the same order of magnitude as the concentrations of its substrates which may have regulatory implications (Sols & Marco, 1970).

Acknowledgments

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