Int. J. Biochem. Vol. 14, pp. 207 to 213, 1982
Printed in Great Britain.

0020-711X/82/030207-07$03.00/0

S-ADENOSYLHOMOCYSTEINASE FROM MOUSE LIVER. INACTIVATION OF THE ENZYME IN THE PRESENCE OF METABOLITES

PER MAGNE UELAND
Department of Pharmacology, University of Bergen, School of Medicine, MFH-bygget, 5016 Haukeland Sykehus, Bergen, Norway

(*Received* 6 *August* 1981)

Abstract—1. *S*-Adenosylhomocysteinase (*S*-adenosylhomocysteine hydrolase, EC 3.3.1.1) was slowly inactivated in the presence of adenine and adenine nucleotides (Ueland & Sæbø, 1979b).

2. The enzyme was stabilized by 2-mercaptoethanol and dithiothreitol, and was slowly inactivated at 37°C in the absence of reducing agents and rapidly inactivated in the presence of 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB). The inactivation (both in the absence and presence of DTNB) was partly prevented by adenine, AMP and ADP.

3. A slow decrease in enzyme activity was observed in the presence of AMP, ADP and ATP, and this process was enhanced by sulfhydryl compounds like L-homocysteine, L-cysteine, 2-mercapthoethanol and dithiothreitol.

4. Inactivation of the enzyme by adenine was independent of sulfhydryl compounds, and was characterized by an initial phase showing first-order kinetics and saturability with respect to adenine.

5. Inorganic phosphate nearly abolished the inactivation of *S*-adenosylhomocysteinase induced by both adenine nucleotides and adenine.

6. The enzyme activity was recovered when adenine was removed by dilution or gel filtration. Attempts to reverse the effect of adenine nucleotides on the enzyme were not successful.

7. The effect of adenine nucleotides was a V_{max}-effect, and the inactivation was not associated with dissociation or polymerization of the enzyme or dissociation of enzyme-bound NAD.

INTRODUCTION

S-Adenosylhomocysteine (SAH) is a product formed from *S*-adenosylmethionine (SAM) upon transmethylation from *S*-adenosylmethionine to cellular acceptors (Cantoni & Scarano, 1954). Inhibition of SAM-dependent transmethylations by SAH was first demonstrated by Gibson *et al.* (1961). This observation has been confirmed and extended to show that SAH is a potent inhibitor of many transmethylation reactions using SAM as a methyl donor. On this basis it has been suggested that SAH is a regulator of biological methylation (Mann & Mudd, 1963; Hurwitz *et al.*, 1964; Zappia *et al.*, 1969; Deguchi & Barchas, 1971; Kerr, 1972; Coward *et al.*, 1973; Pugh *et al.*, 1977).

The inhibition of transmethylation reactions by SAH is probably relieved by hydrolysis of SAH to adenosine and L-homocysteine. A reversible hydrolysis of SAH is catalyzed by the enzyme *S*-adenosylhomocysteinase (*S*-adenosylhomocysteine hydrolase, EC 3.3.1.1.)(De La Haba & Cantoni, 1959). *S*-Adenosylhomocysteinase may be a central enzyme in the regulation of the tissue level of SAH, and may indirectly influence biological methylation reactions (Finkelstein & Harris, 1973; Walker & Duerre, 1975; Schatz *et al.*, 1977). Therefore, knowledge of the regulation of *S*-adenosylhomocysteinase may give insight into the control of biological methylation.

Adenine and adenine nucleotides competitively inhibit both the synthesis and hydrolysis of SAH catalyzed by *S*-adenosylhomocysteinase from mouse liver. Furthermore, a time-dependent decrease in enzyme activity was observed in the presence of these purines (Ueland & Sæbø, 1979b). The present paper reports on further data on the inactivation of *S*-adenosylhomocysteinase in the presence of adenine and adenine nucleotides. At least three processes could be distinguished.

(1) Low stability of the enzyme in the absence of reducing agents.

(2) Irreversible inactivation induced by adenine nucleotides.

(3) Reversible inactivation in the presence of adenine.

MATERIALS AND METHODS

Chemicals

Sources to most reagents used have been given previously (Ueland, 1978; Sæbø & Ueland, 1978). *S*-[8-^{14}C] adenosylhomocysteine was synthesized enzymatically and purified as described elsewhere (Ueland & Sæbø, 1979b). Bovine serum albumin (crystallized) was from British Drug House (BDH), England and was devoid of adenosine deaminase activity which is often present in albumin from other sources. 5,5'-Dithiobis-(2-nitrobenzoic acid) (DNTB), DL-homocysteine, L-homocysteine thiolactone, L-cysteine and DL-dithiothreitol were obtained from Sigma Chemical Co., St Louis, U.S.A. L-Homocysteine was prepared from L-homocysteine thiolacetone by treatment of L-homocysteine thiolactone with 5 M NaOH for 5 min at room temperature (Duerre & Miller, 1966) followed by neutralization to pH 7.0 with 1 M HEPES. The sulfhydryl compounds were dissolved or prepared immediately before use.

Purification of S-adenosylhomocysteinase from mouse liver

The enzyme was purified to apparent homogeneity according to a slight modification (Ueland, 1978) of a purification scheme published elsewhere (Ueland & Døskeland, 1977).

Purification of nucleotides

AMP, ADP and ATP were purified as described (Ueland, 1978).

Assay for S-adenosylhomocysteine synthase and hydrolase activity

This was performed essentially as described previously (Ueland & Sæbø, 1979b). The incubations were performed at 37°C, and the incubation buffer was 15 mM HEPES pH 7.0 containing 5 mM Mg-acetate, 150 mM KCl and 0.25% bovine serum albumin (buffer A) supplemented with 2 mM 2-mercaptoethanol when not otherwise indicated. The assay mixture used for the measurement of SAH synthesis contained 5 μM [8-^{14}C]adenosine and 3 mM DL-homocysteine. These reagents were replaced by 5 μM S-[8-^{14}C]adenosylhomocysteine and adenosine deaminase (100 U ml^{-1}) when the enzyme activity was determined in the hydrolytic direction.

Determination of enzyme-bound NAD

Samples of 40 ul containing enzyme 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. The enzyme, excluded from the gel, was eluted in 150 μl and denaturated by adding 60 μl of 0.8 N perchloric acid. The precipitated protein was removed by centrifugation. The solution was then neutralized to pH 6.0 by addition of alkali (0.36 M KOH/0.3 M $KHCO_3$). After 5 min at 0°C, perchloric acid was removed by centrifugation and the solution analyzed by HPLC. A Spectra Physics model SP 8000B chromatograph, a μ-Bondapak C18 reverse-phase column and a model SP 8300 u.v.-detector were used. The absorbance at 254 nm was recorded. Isocratic elution (2 ml min^{-1}) was carried out at ambient temperature (23°C) using 10 mM potassium acetate (pH 5.5) containing 4% methanol as solvent. NAD was eluted after 8 min clearly separated from adenine and guanine nucleotides, adenine, guanine, IMP, adenosine, SAH, SAM and oxypurines.

Determination of adenine and adenosine

Samples (60 μl) to be analyzed were deproteinized by treatment with perchloric acid and neutralized as described in the preceding paragraph. Adenine and adenosine were determined by HPLC. The same chromatograph, column and solvent were used as above except that the concentration of methanol in the elution buffer was increased to 8%. The flow rate was 2 ml min^{-1}. Adenine and adenosine eluted after 7.5 and 14.5 min, respectively. ATP was eluted in the flow-through, ADP after 1.9 min and AMP after 3.5 min. The detection limit for adenine and adenosine in this system was about 0.3 μM.

Separation of adenine nucleotides by TLC

AMP, ADP and ATP were separated by chromatography on PEI-cellulose plates. Before development of the chromatograms, salt was eluted from the application spots by methanol. The plates were developed in 1.2 M LiCl as described by Randerath & Randerath (1967).

Polyacrylamide gel electrophoresis

Polyacrylamide gel electrophoresis was carried out in a Bio-Rad vertical slab cell (Model 220). Slab thickness was 1.5 mm. The electrophoresis was run for 3 hr, at 50 mA, pH 8.8 (30 mM Tris-glycine buffer) and a gel concentration of 6%. The gel was stained in Coomassie Blue and destained in 7% acetic acid containing 30% methanol.

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 (Ueland *et al.*, 1978).

RESULTS

Stability of the enzyme in the presence of sulfhydryl compounds, DNTB, adenine and adenine nucleotides

The enzyme was slowly inactivated when incubated at 37°C in the absence of reducing agents and was stabilized by reducing agents like 2-mercaptoethanol and dithiothreitol. There was a rapid inactivation of the enzyme in the presence of DTNB. The inactivation, both in the absence and presence of DTNB was less pronounced when adenine, AMP or ADP was

Table 1. The stability of the enzyme in the presence of sulfhydryl compounds, adenine, adenine nucleotides and DTNB

Preincubation in the presence of	Addition	Enzyme activity (μmol min^{-1} mg^{-1})		
		Time of preincubation		
		0 min	10 min	20 min
No sulfhydryl compound		2.20	1.12	0.66
2 mM 2-mercaptoethanol		2.24	2.10	1.92
2 mM dithiothreitol		2.25	2.25	2.10
No sulfhydryl compound	2 mM AMP	2.10	1.62	1.30
—	2 mM ADP	2.18	1.48	1.20
—	2 mM ATP	2.21	1.02	0.55
—	50 μM adenine	2.16	1.36	1.12
2 mM DTNB	—	2.00	0.10	0.06
—	2 mM AMP	2.16	1.30	0.75
—	2 mM ADP	2.20	0.41	0.15
—	2 mM ATP	1.92	0.08	0.03
—	50 μM adenine	1.94	0.35	0.08

The enzyme (22 μg ml^{-1}) was preincubated for the times indicated in the presence of the compounds listed in the table. The preincubation was performed at 37°C in buffer A, and was terminated by transferring 5 μl to the assay mixture (300 μl). The enzyme activity was determined in the synthetic direction.

present (Table 1). These data suggest that sulfhydryl groups of the enzyme may be essential for maintenance of catalytic activity.

Adenine nucleotides

The enzyme was preincubated in the presence of increasing concentrations (0.1–5 mM) AMP, ADP or ATP in buffer A containing 2 mM 2-mercaptoethanol, diluted 60-fold, and assayed for SAH synthase and hydrolase activity. Temperature was 37°C. The preincubation was performed with and without DL-homocysteine (3 mM).

The hydrolytic and synthetic activity decreased in parallel as a function of time of preincubation. The rate of inactivation increased by increasing the concentration of AMP, ADP or ATP, and in the presence of DL-homocysteine. The process was not saturable with respect to these nucleotides within the concentration range tested. The effect of DL-homocysteine was more pronounced with ADP (about 2-fold increase in rate of inactivation) than with AMP or ATP (data not shown).

Kinetics of the inactivation in the presence of adenine

The progress curves for the decrease in enzyme activity during preincubation with various concentrations (0.5–50 μM) of adenine were determined. The curves were characterized by an initial phase showing pseudo first-order kinetics, after which a plateau was obtained (Fig. 1). The process was saturable with respect to adenine. The rate constant for the inactivation (k_{inact}) was determined from the linear part of the curve (Fig. 1) at various concentrations of adenine. Double-reciprocal plots for the rate constants vs the concentrations of adenine was linear (Fig. 1, inset) and consistent with a rate constant (k_{max}) of 0.45 min^{-1} at saturating concentration of adenine. The concentration of adenine where the rate of inactivation was half-maximal (K_I), was 1.56×10^{-6} M (inset, Fig. 1).

Sulfhydryl compounds

The adenine nucleotide dependent decrease in enzyme activity (Ueland & Sæbø, 1979a) was enhanced when 2 mM dithiothreitol was included in the preincubation mixture as compared to the effect observed with 2 mM 2-mercaptoethanol. The effect of 2-mercaptoethanol was dose dependent, and the rate of inactivation increased by increasing the concentration to 10 mM (data not shown).

In the presence of 2 mM 2-mercaptoethanol, which stabilized the enzyme (Table 1), the slow transition towards the inactive form effected by AMP, ADP or ATP was further increased when the preincubation mixture was supplemented with DL-homocysteine, L-homocysteine or L-cysteine (Table 2). L-Cysteine was more potent in this respect than L-homocysteine (Fig. 2).

The time-dependent inactivation of the enzyme by adenine was essentially independent of L-cysteine, L-homocysteine (Table 2) and reducing agents (data not shown).

Inorganic phosphate

Both in the absence and presence of L-cysteine or L-homocysteine, inorganic phosphate decreased the rate of inactivation of the enzyme effected by AMP, ADP, ATP and adenine (Table 2). Similar dose–response curves for phosphate were obtained with adenine and ADP. Half-maximal effect was observed at 2–5 mM of phosphate (Fig. 3). About the same results with adenine were obtained when magnesium was omitted from the preincubation mixture (data not shown) showing that the effect of phosphate was not

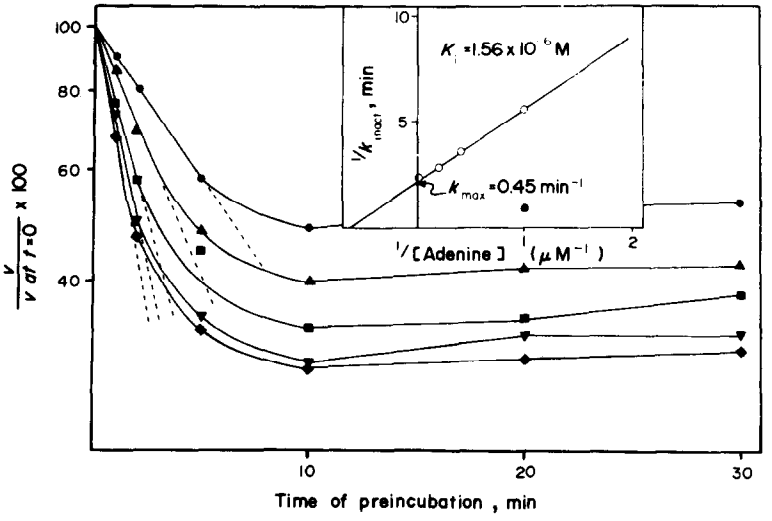

Fig. 1. Kinetics of the inactivation of the enzyme in the presence of adenine. The enzyme (22 μg ml^{-1}) was preincubated at 37°C in the presence of 0.5 (●), 1 (▲), 2.5 (■), 5 (▼) and 50 μM (◆) of adenine in buffer A containing 2 mM 2-mercaptoethanol. At times indicated on the figure, aliquots of 5 μl were transferred to the assay mixture (300 μl) and assayed for SAH synthase activity. From the linear part of the curves the rate constants (k_{inact}) of the inactivation was determined. Inset shows the double-reciprocal plot for k_{inact} vs the concentration of adenine.

Table 2. The activity remaining after preincubation of the enzyme in the presence of various metabolites

Addition	Enzyme activity (μmol min^{-1} mg^{-1})				
	Control	AMP	ADP	ATP	Adenine
	2.10	1.90	1.58	1.21	0.82
3 mM DL-Hcy		1.48	0.32	0.89	0.81
3 mM L-Hcy		1.70	0.72	0.92	—
3 mM L-Hcy, 30 mM P_i		2.01	1.79	2.01	—
0.15 mM L-cysteine		1.18	0.25	0.85	0.80
0.15 mM L-cysteine, 30 mM P_i		1.70	1.70	1.28	1.20
0.15 mM L-cysteine, 10 μM adenosine		1.31	0.51	1.50	—

The enzyme (22 μg ml^{-1}) was preincubated for 15 min at 37°C in the presence of 2 mM AMP, ADP, ATP or 50 μM adenine and the compounds listed in the left row of the table. The experimental design was as described in legend to Table 1.

related to the formation of a magnesium–phosphate complex (Rudolph & Fromm, 1969).

The competitive inhibition of the enzyme catalysis by AMP and ADP, but not adenine, was less pronounced in the presence of phosphate. In the absence of phosphate, the inhibitor constants for adenine, AMP and ADP as calculated from Dixon plots (Dixon, 1953), were 2.0×10^{-6}, 2.4×10^{-5} and 2.5×10^{-4} M, respectively (Ueland & Sæbø, 1979b). The corresponding values were 2.0×10^{-6}, 9.8×10^{-5} and 11.5×10^{-4} M when 30 mM inorganic phosphate was included in the incubation mixture. The same results were obtained in the absence and presence of magnesium (data not shown).

Miscellaneous

Adenosine (10 μM) decreased the rate of inactivation of the enzyme effected by AMP, ADP or ATP (Table 2).

When the enzyme was preincubated for 20 min with DL-homocysteine (3 mM), L-homocysteine (3 mM), L-cysteine (0.15 mM), inorganic phosphate (30 mM), adenosine (10 μM), methionine (3 mM) or NAD (0.1 mM) in the absence of adenine nucleotides, no change in enzyme activity was observed. Apart from adenosine, these compounds did not affect the synthetic activity when included in the assay mixture. Preincubation of the enzyme with 2 mM GDP or 2 mM GTP was also without effect (data not shown).

Reversibility

The progress curve for the synthesis of SAH in the presence of enzyme preincubated with adenine was characterized by an initial linear phase for 1–2 min after dilution of the enzyme in the incubation mixture. This phase was followed by an upward curvature after which the curve acquired about the same slope as the curve for the synthesis in the presence of enzyme not

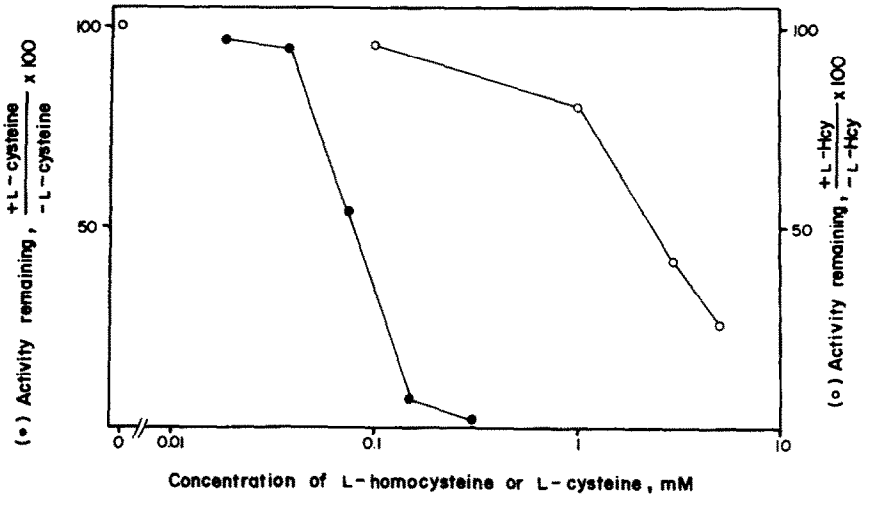

Fig. 2. Dose-effect curves for L-cysteine and L-homocysteine. The enzyme (22 μg ml^{-1}) was preincubated for 15 min at 37°C with 2 mM ADP and various concentrations of either L-cysteine or L-homocysteine (prepared from L-homocysteine thiolactone). The preincubation buffer and experimental design were as described in legend to Fig. 1. The enzyme activity was determined in the synthetic direction. The ratio between the activity remaining after preincubation in the presence of L-cysteine (●) or L-homocysteine (○) and the activity determined after preincubation without these compounds, is plotted vs the concentration of L-cysteine or L-homocysteine.

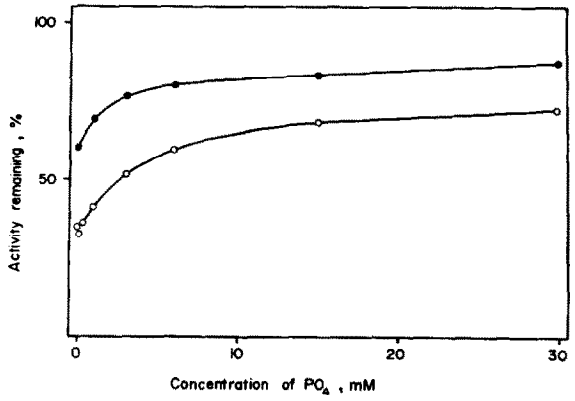

Fig. 3. Effect of various concentrations of inorganic phosphate on the activity remaining after preincubation of the enzyme in the presence of adenine or ADP. The enzyme (22 μg ml^{-1}) was preincubated at 37°C for 15 min with either 2 mM ADP (○) or 50 μM adenine (●) and various concentrations of inorganic phosphate (potassium salt) in buffer A containing 2 mM 2-mercaptoethanol and 3 mM DL-homocysteine. The experimental design was as described in legend to Fig. 1. The enzyme activity was measured in the synthetic direction.

preincubated with adenine (Fig. 4). This indicates reversal of the adenine effect upon dilution of the adenine–enzyme complex. In a similar experiment, adenosine or homocysteine was omitted from the incubation mixture into which the enzyme was diluted. The synthetic reaction was initiated after various periods of time (0–15 min) after dilution by addition of either adenosine or homocysteine. The initial velocity of the reaction gradually approached that observed for the enzyme not preincubated with adenine (data not shown), suggesting that the reversion is not dependent on the presence of adenosine and homocysteine.

The catalytic activity of the enzyme preincubated with adenine was also recovered when adenine was removed by gel filtration (data not shown).

In contrast, the inactivation induced by treatment of the enzyme with adenine nucleotides was not reversed by dilution or gel filtration.

Physio-chemical and kinetic properties of the enzyme preincubated with adenine and adenine nucleotides

The enzyme preincubated (for 5–20 min) in the presence of adenine (50 μM) and adenine nucleotides (2 mM) showed exactly the same mobility on polyacrylamide gel electrophoresis and contained the same amount of enzyme-bound NAD/NADH as the non-treated enzyme. The amount of NAD bound per subunit (mol wt = 46,500 (Ueland *et al.*, 1978) was 0.84 ± 0.04 mol mol^{-1} (n = 12)).

Double-reciprocal plots for the synthesis of SAH vs the concentration of adenosine showed that the effect of preincubation with adenine nucleotides was a V_{max} effect, and K_m for adenosine was not affected. The inhibitor constants for adenine, AMP and ADP were determined using enzyme treated with AMP, ADP or ATP, and the same values were obtained as those reported for the non-treated enzyme (Ueland & Sæbø, 1979b) (data not shown). In these experiments the concentration of free nucleotides was reduced by gel filtration and dilution prior to kinetic analysis.

Test for formation of adenosine and degradation of adenine nucleotides

No formation of adenosine from AMP, ADP or ATP (2 mM) could be detected after incubation for 30 min under the condition of the inactivation experiments. Furthermore, a slight conversion of [^{3}H]ATP to [^{3}H]ADP (5–7%) and from [^{3}H]ADP to [^{3}H]AMP (3–9%) was observed after 30 min of incubation under the same condition (data not shown).

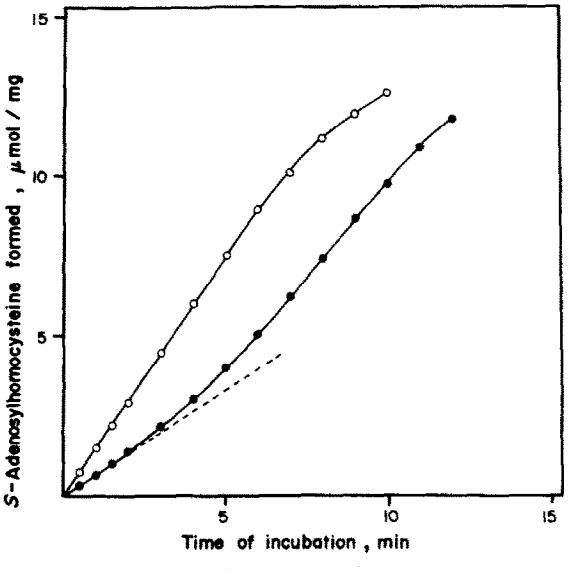

Fig. 4. Progress curve for the synthesis of SAH in the presence of enzyme preincubated with adenine. The enzyme (22 μg ml^{-1}) was preincubated for 0 or 20 min at 37°C in the presence of 50 μM adenine. The preincubation buffer was the same as that used in the experiment presented in Fig. 1. Aliquots of 5 μl were transferred to the incubation mixture (300 μl) containing 5 μM [^{14}C]adenosine and 3 mM DL-homocysteine. The amount of SAH formed in the presence of enzyme transferred to the incubation mixture without preincubation (○) and after preincubation (●) is plotted vs time of incubation.

DISCUSSION

Based on data presented in this paper, three processes leading to a slow inactivation of S-adenosylhomocysteinase can be distinguished.

(1) The catalytic activity of the enzyme progressively decreased in the absence of reducing agents and in the presence of the sulfhydryl reagent, 5,5'-dithiobis-(2-nitrobenzoic acid). This observation indicates that sulfhydryl groups of the enzyme may be essential for maintenance of catalytic activity, as suggested by Kajander & Raina (1981) for the rat liver enzyme. The inactivation was counteracted by adenine and adenine nucleotides (Table 1).

(2) An irreversible inactivation of S-adenosylhomocysteinase was induced by adenine nucleotides. This process was enhanced by several sulfhydryl compounds including cysteine, homocysteine, 2-mercaptoethanol and dithiothreitol, and was partly inhibited by inorganic phosphate.

(3) A reversible inactivation of *S*-adenosylhomocysteinase was observed in the presence of adenine. The inactivation was independent on sulfhydryl compounds, but was counteracted by inorganic phosphate (Table 1).

S-Adenosylhomocysteinase from various sources is irreversibly inactivated by an active site directed mechanism in the presence of adenosine analogues (Hershfield, 1979; Hershfield *et al.*, 1979). The inactivation is distinguished from the processes described in this work on the basis of its kinetic characteristics, essentially no effect of homocysteine (Hershfield *et al.*, 1979), enhancement (Hershfield, 1981) or no effect of added phosphate (Helland & Ueland, 1981).

The mechanism of action of adenine nucleotides on *S*-adenosylhomocysteinase has not been uncovered. There is no polymerization or dissociation of the enzyme exposed to adenine nucleotides as judged by polyacrylamide gel electrophoresis (Ueland & Sæbø, 1979a) (see Result) or sucrose gradient centrifugation (Ueland & Døskeland, 1977).

S-Adenosylhomocysteinase from beef liver contains tightly bound NAD (Palmer & Abeles, 1976). The enzyme from mouse liver treated with ATP has high binding capacity for adenine nucleotides (Ueland, 1978; Ueland & Døskeland, 1977). Adenine nucleotides have been shown to interact with the NAD site of glyceraldehyde dehydrogenase (Yang & Deal, 1969). In the light of these data we investigated whether AMP, ADP or ATP induce conformational changes in *S*-adenosylhomocysteinase leading to dissociation of tightly-bound NAD from the enzyme. Data supporting this possibility were not obtained.

ATP induce conformational changes in the enzyme as judged by altered reactivity of sulfhydryl groups in the enzyme (Ueland *et al.*, 1978). The observation is in accordance with the finding (Table 1) that AMP and ADP protected the enzyme against inactivation in the presence of 5,5'-dithiobis-(2-nitrobenzoic acid). Furthermore, the inactivation of the enzyme observed in the presence of adenine nucleotides was promoted by 2-mercaptoethanol and dithiothreitol suggesting the involvement of sulfhydryl group of the enzyme in the mechanism of action of adenine nucleotides.

The kinetics of inactivation of the enzyme with adenine point to a possible mode of action of this agent. The initial phase of inactivation obeyed first-order kinetics and was saturable with respect to adenine, suggesting that formation of the inactive enzyme proceeds from an adenine–enzyme complex. Adenine probably binds to the catalytic site. This suggestion is based on the following observation. Adenosine partly protected the enzyme from inactivation (Table 2). The concentration of adenine where the rate of inactivation was half-maximal (K_I for adenine) (Fig. 1, inset) equals the inhibitor constant for adenine calculated from initial velocity studies (Ueland & Sæbø, 1979a).

The progress curve for the synthesis of SAH in the presence of adenine acquired a slope after 5–10 min of incubation which was about half the initial velocity of the reaction (Ueland & Sæbø, 1979b). This indicates that the plateau observed after prolonged preincubation with adenine (Fig. 1) is not an artifact of the dilution prior to assay. The plateau may represent an equilibrium between the inactive adenine–enzyme complex and its (active) precursor.

The effect of phosphate on the enzyme catalysis suggests interaction of inorganic phosphate with the enzyme. The interaction may induce conformational changes in the enzyme, counteracting the inactivation effected by adenine and adenine nucleotides. Phosphate may reduce the binding of adenine nucleotides by an allosteric mechanism (Monod *et al.*, 1965) or by competitive inhibition of the binding of nucleotides to the enzyme. A competitive relationship between the binding of inorganic phosphate and nucleotides has been reported for AMP nucleosidase (Schramm & Fullin, 1978).

The cellular level of inorganic phosphate is about 1–15 mM (Furchgott & De Gubareff, 1956; Seraydarian *et al.*, 1961). The data of Fig. 3 shows that the effect of phosphate on the inactivation in the presence of ADP or adenine, was observed in this concentration range. This suggests that phosphate may be a regulator or *S*-adenosylhomocysteinase *in vivo*. Inorganic phosphate is a potential regulator of hexokinase (Lueck & Fromm, 1974), adenosine triphosphatase (Penefsky, 1977), phosphofructokinase (Banuelos *et al.*, 1977) and adenosine monophosphate nucleosidase (Schramm & Fullin, 1978) indicating that inorganic phosphate may be factor in metabolic control.

Phosphate-buffer is often a constituent of the assay mixture used for the measurement of *S*-adenosylhomocysteine synthase and hydrolase activity (Finkelstein & Harris, 1973; Walker & Duerre, 1975; Kajander *et al.*, 1976; Hershfield & Kredich, 1978; Richards *et al.*, 1978). Furthermore, L-homocysteine present in the assay mixture for the synthesis of *S*-adenosylhomocysteine is prepared from L-homocysteine thiolactone by exposure of the thiolactone to 5 M NaOH. Potassium phosphate has been recommended for the neutralization of NaOH (Duerre & Miller, 1966) and would thus be included in the assay mixture. Data presented in this paper show that inorganic phosphate affects the catalytic properties of *S*-adenosylhomocysteinase, at least *in vitro*. Thus, attention should be paid to the absence or presence of phosphate when comparing data on the catalytic properties of this enzyme from different laboratories.

In conclusion, metabolites other than the natural substrates of *S*-adenosylhomocyteinase may affect the activity of the enzyme, at least *in vitro*. The slow response of the enzyme to adenine and adenine nucleotides is modified by both naturally occurring and synthetic sulfhydryl compounds and by inorganic phosphate. These observations may form the basis for detailed studies into the mechanism of action of these compounds and their possible role as regulators of the enzyme *in vivo*.

Acknowledgements—The technical assistance of H. Bergesen is highly appreciated. This work was supported by grants from Nordisk Insulinfond, Langfeldts fond and the Norwegian Research Council for Science and the Humanities.

REFERENCES

BANUELOS M., GANCEDO C. & GANCEDO J. M. (1977) Activation by phosphate of yeast phosphofructokinase. *J. biol. Chem.* **252**, 6394–6398.

CANTONI G. L. & SCARANO E. (1954) The formation of S-adenosylhomocysteine in enzymatic transmethylation reactions. *J. Am. chem. Soc.* **76**, 4744.

COWARD J. K., SLISZ P. E. & WU F. Y.-H. (1973) Kinetic studies on catechol O-methyltransferase. Product inhibition and the nature of the catechol binding site. *Biochemistry* **12**, 2291–2297.

DEGUCHI T. & BARCHAS J. (1971) Inhibition of transmethylations of biogenic amines by S-adenosylhomocysteine. Enhancement of transmethylation by adenosylhomocysteinase. *J. biol. Chem.* **246**, 3175–3181.

DE LA HABA G. & CANTONI G. L. (1959) The enzymatic synthesis of S-adenosyl-L-homocysteine from adenosine and homocysteine. *J. biol. Chem.* **234**, 603–608.

DIXON M. (1953) The determination of enzyme inhibitor constants. *Biochem. J.* **55**, 170–171.

DUERRE J. A. & MILLER C. H. (1966) Preparation of L-homocysteine from L-homocysteine thiolactone. *Analyt. Biochem.* **17**, 310–315.

FINKELSTEIN J. D. & HARRIS B. (1973) Methionine metabolism in mammals: synthesis of S-adenosylhomocysteine in rat tissues. *Archs Biochem. Biophys.* **159**, 160–165.

FURCHGOTT R. F. & DE GUBAREFF T. (1956) The determination of inorganic phosphate and creatine phosphate in tissue extracts. *J. biol. Chem.* **223**, 377–388.

GIBSON K. D., WILSON J. D. & UDENFRIEND S. (1961) The enzymatic conversion of phospholipid ethanolamine to phospholipid choline in rat liver. *J. biol Chem.* **236**, 673–679.

HELLAND S. & UELAND P. M. (1981) Interaction of 9-β-D-arabinofuranosyladenine, 9-β-D-arabinofuranosyladenine 5′-monophosphate, and 9-β-D-arabinofuranosyladenine 5′-triphosphate with S-adenosylhomocysteinase. *Cancer Res.* **41**, 673–678.

HERSHFIELD M. S. (1979) Apparent suicide inactivation of human lymphoblast S-adenosylhomocysteine hydrolase by 2′-deoxyadenosine and adenine arabinoside. *J. biol. Chem.* **254**, 22–25.

HERSHFIELD M. S. (1981) Proposed explanation for S-adenosylhomocysteine hydrolase deficiency in purine nucleoside phosphorylase and hypoxanthine-guanine phosphoribosyltransferase-deficient patients. *J. clin. Invest.* **67**, 696–701.

HERSHFIELD M. S. & KREDICH N. M. (1978) S-Adenosylhomocysteine hydrolase is an adenosine-binding protein: a target for adenosine toxicity. *Science* **202**, 757–760.

HERSHFIELD M. S., KREDICH N. M., SMALL W. C. & FREDERICKSEN M. L. (1979) Suicide-like inactivation of human S-adenosylhomocysteine hydrolase by analogs of adenosine. In *Transmethylation* (Edited by USDIN E., BORCHARDT R. T. & CREVELING C. R.), pp. 173–186. Elsevier/North-Holland, New York.

HURWITZ J., GOLD M. & ANDERS M. (1964) The enzymatic methylation of ribonucleic acid and deoxyribonucleic acid—IV. The properties of the soluble ribonucleic acid-methylating enxymes. *J. biol Chem.* **239**, 3474–3482.

KAJANDER E. O. & RAINA A. M. (1981) Affinity-chromatographic purification of S-adenosyl-L-homocysteine hydrolase. Some properties of the enzyme from rat liver. *Biochem. J.* **193**, 503–512.

KAJANDER O., ELORANTA T. & RAINA A. (1976) A sensitive isotopic assay method for S-adenosylhomocysteine hydrolase. Some properties of the enzyme from rat liver. *Biochim biophys. Acta* **438**, 522–531.

KERR S. J. (1972) Competing methyltransferase systems. *J. biol. Chem.* **247**, 4248–4252.

LUECK J. D. & FROMM H. J. (1974) Kinetics, mechanism, and regulation of rat skeletal muscle hexokinase. *J. biol. Chem.* **249**, 1341–1347.

MANN J. D. & MUDD S. H. (1963) Alkaloid and plant metabolism—IV. The tyramine methylpherase of barley roots. *J. biol. Chem.* **238**, 381–385.

MONOD J., WYMAN J. & CHANGEUX J.-P. (1965) On the nature of the allosteric transitions: a plausible model. *J. molec. Biol.* **12**, 88–118.

PALMER J. L. & ABELES R. H. (1976) Mechanism for enzymatic thioether formation. Mechanism of action of S-adenosylhomocysteinase. *J. biol. Chem.* **251**, 5817–5819.

PENEFSKY H. S. (1977) Reversible binding of P_i by beef heart mitochondrial adenosine triphosphatase. *J. biol. Chem.* **252**, 2891–2899.

PUGH C. S. G., BORCHARDT R. T. & STONE H. O. (1977) Inhibition of Newcastle disease viron messenger RNA (guanine-7)-methyltransferase by analogues of S-adenosylhomocysteine. *Biochemistry* **16**, 3928–3932.

RANDERATH K. & RANDERATH E. (1967) Thin-layer separation methods for nucleic acid derivatives. *Meth. Enzym.* **12A**, 323–347.

RICHARDS H. H., CHIANG P. K. & CANTONI G. L. (1978) Adenosylhomocysteine hydrolase. Crystallization of the purified enzyme and its properties. *J. biol. Chem.* **253**, 4476–4480.

RUDOLPH F. B. & FROMM H. J. (1969) Initial rate studies of adenylosuccinate synthetase with product and competitive inhibitors. *J. biol. Chem.* **244**, 3832–3839.

SCHATZ R. A., VUNNAM C. R. & SELLINGER O. Z. (1977) S-Adenosyl-L-homocysteine in brain. Regional concentrations, catabolism, and the effects of methionine sulfoximine. *Neurochem. Res.* **2**, 27–38.

SCHRAMM V. L. & FULLIN F. A. (1978) Kinetics of adenosine monophosphate nucleosidase inactivation by phosphate and protection by substrate and allosteric activator. *J. biol. Chem.* **253**, 2161–2167.

SERAYDARIAN K., MOMMAERTS W. F. H. M., WALLNER A. & GUILLORY R. J. (1961) An estimation of the true inorganic phosphate content of frog sartorius muscle. *J. biol. Chem.* **236**, 2071–2075.

SÆBØ J. & UELAND P. M. (1978) An adenosine 3′,5′-monophosphate adenosine-binding protein from mouse liver. Association with S-adenosylhomocysteinase activity. *FEBS Lett*, **96**, 125–128.

UELAND P. M. (1978) An adenosine 3′,5′-monophosphate/adenosine binding protein from mouse liver. A study of its interaction with synthetic and naturally occurring adenosine derivatives. *Eur. J. Biochem.* **86**, 27–34.

UELAND P. M. & DØSKELAND S. O. (1977) An adenosine 3′,5′-monophosphate-adenosine binding protein from mouse liver. Purification and partial characterization. *J. biol. Chem.* **252**, 677–686.

UELAND P. M. & SÆBØ J. (1979a) Cyclic AMP-adenosine binding protein/S-adenosylhomocysteinase from mouse liver. A fraction of adenosine bound is converted to adenine. *Biochim. biophys. Acta* **585**, 512–526.

UELAND P. M. & SÆBØ J. (1979b) S-Adenosylhomocysteinase from mouse liver. Affect of adenine and adenine nucleotides on the enzyme catalysis. *Biochemistry* **18**, 4130–4135.

UELAND P. M., SKOTLAND T., DØSKELAND S. O. & FLATMARK T. (1978) An adenosine 3′,5′-monophosphate-adenosine binding protein from mouse liver: some physico-chemical properties. *Biochim. Biophys. Acta* **533**, 57–65.

WALKER R. D. & DUERRE J. A. (1975) S-Adenosylhomocysteine metabolism in various species. *Can. J. Biochem.* **53**, 312–319.

YANG S. T. & DEAL W. C. (1969) Metabolic control and structure of glycolytic enzymes—VI. Competitive inhibition of yeast glyceraldehyde 3-phosphate dehydrogenase by cyclic adenosine monophosphate, adenosine triphosphate, and other adenine-containing compounds. *Biochemistry* **8**, 2806–2813.

ZAPPIA V., ZYDEK-CWICK C. R. & SCHLENK F. (1969) The specificity of S-adenosylmethionine derivatives in methyl transfer reactions. *J. biol. Chem.* **244**, 4499–4509.