Inactivation of liver *S*-adenosylhomocysteine hydrolase *in vitro* of rats treated with *erythro*-9-(2-hydroxynon-3-yl)adenine

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S-Adenosylhomocysteine hydrolase activity decreased *in vitro* time-dependently in liver homogenates obtained from rats treated *in vivo* with *erythro*-9-(2-hydroxynon-3-yl)adenine, a potent inhibitor of adenosine deaminase. The inhibitor in itself had no effect on the stability of the hydrolase. The inactivation of *S*-adenosylhomocysteine hydrolase was irreversible, proceeded fairly rapidly at a low temperature (0°C) and showed first-order reaction kinetics. Adenosine was found to accumulate in these tissue homogenates during storage. Several lines of evidence suggest that adenosine caused the observed suicide-like inactivation *post mortem*. Pre-incubation of purified *S*-adenosylhomocysteine hydrolase at 0°C with adenosine showed a half-maximal inactivation rate at 33 µM substrate concentration; the rate constant of inactivation was 0.01 min⁻¹. Inactivation during tissue preparation and storage complicates the assay of *S*-adenosylhomocysteine hydrolase activity in samples that contain an inhibitor of adenosine deaminase. These results also suggest that the decrease of *S*-adenosylhomocysteine hydrolase activity reported to occur in several disturbances of purine metabolism should be re-examined to exclude the possibility of inactivation of the enzyme *in vitro*.

The major adenosine-metabolizing enzymes in mammals are adenosine kinase and adenosine deaminase (Fox & Kelley, 1978). *S*-Adenosylhomocysteine hydrolase, catalysing the synthesis of Ado-Hcy from adenosine and homocysteine, also has high activity in several tissues including the liver (Kajander & Raina, 1981). However, hydrolysis of Ado-Hcy is the biological function of this enzyme (de la Haba & Cantoni, 1959; Cortese *et al.*, 1974). Because the equilibrium of the enzymic reaction strongly favours synthesis over hydrolysis, efficient removal of adenosine is critical for hydrolysis to proceed. Adenosine kinase and *S*-adenosylhomocysteine hydrolase from rat liver have a high affinity for adenosine, i.e. about 100 times that of the deaminase. K_m values of 0.5 µM, 0.6 µM and 46 µM for adenosine have been reported for adenosine kinase, *S*-adenosylhomocysteine hydrolase and adenosine deaminase respectively (Arch & Newsholme, 1978*a*; Kajander & Raina, 1981). Physiological concentrations of adenosine are low, in the micromolar range (see Arch & Newsholme, 1978*b*). Elevation of intracellular adenosine levels may lead to accumula-

tion of Ado-Hcy and to impairment of biological methylation reactions (Kredich & Martin, 1977; Pike *et al.*, 1978; Kredich & Hershfield, 1979).

S-Adenosylhomocysteine hydrolase seems to be the target for adenosine toxicity when adenosine deaminase activity is inhibited or is genetically deficient. Hershfield (1979) and others (Chiang *et al.*, 1981; Kajander & Raina, 1981) have shown that this enzyme is inactivated *in vitro* in a suicide-like process by several adenine nucleosides, including its natural substrates adenosine and Ado-Hcy. This kind of inactivation seems to accompany several clinical disorders of purine metabolism as a secondary enzyme deficiency. Virtual absence of the hydrolase activity has been found in erythrocytes of adenosine deaminase-deficient patients (Hershfield *et al.*, 1979). Decreased hydrolase activities have been found in erythrocytes of purine nucleoside phosphorylase- and hypoxanthine/guanine phosphoribosyltransferase-deficient patients (Kaminska & Fox, 1980; Hershfield, 1981). Inhibitors of adenosine deaminase, like 2'-deoxycytosine and *erythro*-9-(2-hydroxynon-3-yl)adenine, can produce metabolic alterations similar to those found in adenosine deaminase deficiency (Tedde *et al.*, 1980; Newby, 1981;

Abbreviations used: Ado-Hcy, *S*-adenosyl-L-homocysteine; Ado-Met, *S*-adenosyl-L-methionine.

Hershfield *et al.*, 1981). Inactivation of *S*-adenosylhomocysteine hydrolase in human lymphoblasts has been observed after such treatment *in vivo* (Hershfield *et al.*, 1981) and in cell cultures after addition of *erythro*-9-(2-hydroxynon-3-yl)adenine and 2'-deoxyadenosine to the growth medium (Hershfield & Kredich, 1980).

Inhibitors of adenosine deaminase have recently found value in clinical use because of their ability to increase the potency of cytotoxic and antiviral nucleoside analogues that are inactivated by deamination (Plunkett & Cohen, 1975; LePage *et al.*, 1976; Lapi & Cohen, 1977; North & Cohen, 1978; Plunkett *et al.*, 1979). Therefore, a closer characterization of the effect of *erythro*-9-(2-hydroxynon-3-yl)adenine on Ado-Hcy metabolism in an animal model seemed desirable. In these experiments we have observed an unexpected inactivation of *S*-adenosylhomocysteine hydrolase *in vitro* in rat liver preparations obtained from animals treated with the inhibitor *in vivo*. The inactivation was shown to occur rapidly *post mortem* in tissue extracts, probably due to accumulation of adenosine.

Experimental

Chemicals

The following chemicals were supplied by Sigma Chemical Co., St. Louis, MO, U.S.A.: Ado-Hcy, L-homocysteine thiolactone, Coomassie Brilliant Blue G, crystalline bovine serum albumin and 2'-deoxyadenosine. L-Homocysteine was prepared from L-homocysteine thiolactone by the method of Duerre & Miller (1966). [8-¹⁴C]Adenosine (sp. radioactivity 49 Ci/mol) and *S*-adenosyl-L-[Me-¹⁴C]-methionine (sp. radioactivity 55 Ci/mol) were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Radioactive Ado-Hcy was prepared enzymically by the procedure described previously (Eloranta *et al.*, 1976). *Erythro*-9-(2-hydroxynon-3-yl)adenine was supplied by Burroughs Wellcome Co., Research Triangle Park, NC, U.S.A. Adenosine was from Boehringer, Mannheim, Germany. All other chemicals used were Reagent-Grade materials from various commercial sources.

Animals and treatment

The animals used were female Wistar rats weighing 80–120 g. The animals were housed at 20°C and 40% humidity, maintained under regularly alternating 12 h periods of light and dark. They were allowed to eat (a standard diet made by Hankkija Oy, Turku, Finland) and drink *ad libitum*. Unless otherwise indicated, the food was removed 12 h before killing.

Erythro-9-(2-hydroxynon-3-yl)adenine was dissolved in 0.9% NaCl to a final concentration of

50 mM and 0.2–0.5 ml/100 g body wt. was injected intraperitoneally into rats.

Assay of *S*-adenosylhomocysteine hydrolase activity

S-Adenosylhomocysteine hydrolase (EC 3.3.1.1) activity was assayed in the direction of Ado-Hcy synthesis as described previously (Kajander *et al.*, 1976). For enzyme activity assays, a portion of the liver was homogenized with a Potter–Elvehjem homogenizer in 2–8 vol. of ice-cold 10 mM-potassium phosphate buffer, pH 7.4, containing 1 mM-dithiothreitol. Enzyme activities were usually assayed in the 100 000 *g*_{av} supernatant fractions of the homogenates. In experiments with the purified enzyme, bovine serum albumin (0.5 mg/ml) was added to the incubation mixtures. In some enzyme-inactivation experiments the hydrolase activity was measured immediately from fresh homogenates. The standard incubation mixture contained, in a final volume of 75 μ l, 3 mM-L-homocysteine, 0.5 mM-[8-¹⁴C]adenosine (sp. radioactivity 1.0 Ci/mol), 2 mM-dithiothreitol, 100 mM-potassium phosphate buffer (pH 7.4) and 20–60 μ g of crude enzyme protein. The accumulation of adenosine in *erythro*-9-(2-hydroxynon-3-yl)adenine-treated tissue extracts did not interfere with the assay procedure, since the concentration of this substrate was high in the incubation medium and the enzyme samples were greatly diluted.

The radioactive product was separated by paper electrophoresis. Enzyme activity is expressed as nmol of Ado-Hcy formed/min per mg of protein. The enzyme was purified to apparent homogeneity from rat livers by the published affinity-chromatographic method (Kajander & Raina, 1981).

Assay of Ado-Hcy and Ado-Met

The rats were decapitated under diethyl ether anaesthesia, and the livers were rapidly removed. Portions of livers containing 2–3 g of wet tissue were immediately homogenized with an Ultra-Turrax homogenizer (Janke and Kunkel KG, Staufen i. Breisgau, Germany) in 3 vol. of 10% (w/v) trichloroacetic acid containing 1–3 nCi of both *S*-[8-¹⁴C]adenosyl-L-homocysteine and *S*-adenosyl-L-[Me-¹⁴C]methionine/ml. After centrifugation at 10 000 *g* for 15 min at 4°C, the supernatant was extracted three times with an equal volume of diethyl ether and was then used for the analysis of Ado-Hcy and Ado-Met by the phosphocellulose-column-chromatographic method of Eloranta *et al.* (1976). Purity of Ado-Hcy and Ado-Met fractions was checked by high-pressure liquid chromatography on a Partisil-10-SCX-column (Whatman Inc., Clifton, NJ, U.S.A.). The system consisted of a Perkin–Elmer model 601 liquid chromatograph equipped with a Perkin–Elmer model LC-55 spectrophotometer (Perkin–Elmer Corp., Norwalk, CT, U.S.A.). The

column was run at room temperature with a stepwise gradient of ammonium formate buffer, pH 4.0. Ado-Hcy was eluted with 25 mM buffer and Ado-Met with 0.5 M buffer.

Assay of adenosine

Pieces of livers were homogenized in 2 vol. of 10% trichloroacetic acid within 10 s after tissue removal from anaesthetized rats. Adenosine concentration was measured in the diethyl ether-extracted supernatants by high-pressure liquid chromatography on a column (190 mm × 4.6 mm) packed with Spherisorb-S5-ODS-2 (Phase Separations, Deeside Industrial Estate, Queensferry, Clwyd, U.K.). Isocratic elution was performed with 10 mM-potassium acetate buffer, pH 5.0, containing 8% (v/v) methanol. At an elution rate of 1 ml/min, the retention times were 3.0, 5.0, 6.3, 8.9, 14.5 and 17.9 min for hypoxanthine, inosine, adenine, Ado-Hcy, adenosine and 2'-deoxyadenosine respectively. The specificity of the assay method was strengthened by demonstrating that the adenosine peak disappeared when neutralized extracts were incubated with purified adenosine deaminase from calf intestine (Boehringer).

Protein determination

Protein was measured by the method of Lowry *et al.* (1951) or with Coomassie Brilliant Blue as described by Spector (1978). Crystalline bovine serum albumin (Sigma) was used as a standard.

Results

Decrease of liver *S*-adenosylhomocysteine hydrolase activity in enzyme preparations obtained from rats treated with erythro-9-(2-hydroxynon-3-yl)-adenine *in vivo*

Table 1 shows the effect of a single dose of erythro-9-(2-hydroxynon-3-yl)adenine (25 µmol/100 g body wt.) on *S*-adenosylhomocysteine hydrolase activity found in the 100 000 g_{av} supernatant fraction of liver homogenate after storage for 15 h at 0°C. As seen in Table 1, the enzyme activity at

30 min after the treatment was only about 6% of the control value and then gradually increased to about 50% at 4 h after injection. A thorough dialysis (48 h) could not restore enzyme activity, indicating the irreversible nature of the process. Surprisingly, liver Ado-Hcy and Ado-Met concentrations remained nearly unchanged, as shown in Table 2. Because this finding was incompatible with the markedly lowered hydrolase activity, the influence of multiple injections with the adenosine deaminase inhibitor during a longer time period was studied. The enzyme activities of the liver homogenates were determined immediately after killing the animals and from the supernatant fractions as rapidly as possible (within 90 min after killing). As demonstrated in Table 3, no inhibition was observed if the enzyme activity was determined immediately after tissue homogenization. In the supernatant fraction assayed immediately after preparation, the enzyme activity was decreased by about 30%. Since *S*-adenosylhomocysteine

Table 1. *S*-Adenosylhomocysteine hydrolase activity in crude liver extracts of rats treated with erythro-9-(2-hydroxynon-3-yl)adenine *in vivo*

A single dose of erythro-9-(2-hydroxynon-3-yl)-adenine (25 µmol/100 g body wt.) was injected intraperitoneally in 0.5 ml of 0.9% NaCl at indicated times before killing. Control animals received 0.9% NaCl only. Hydrolase activity was determined from 100 000 g_{av} supernatant fraction after storage for 15 h at 0°C. Thereafter, a portion of the supernatant was dialysed against 100 mM-potassium phosphate buffer, pH 7.4. The values are means of four to five animals ± s.d.

Time of treatment (min)	Enzyme activity (nmol/min per mg of protein)	
	Undialysed	Dialysed
60 (control)	47.7 ± 1.7	49.1 ± 2.4
30	2.7 ± 0.3	3.9 ± 2.1
60	3.7 ± 0.4	5.3 ± 1.8
90	4.6 ± 0.4	5.4 ± 1.4
120	8.0 ± 2.6	9.1 ± 2.1
240	23.8 ± 2.3	24.5 ± 6.6

Table 2. Effect of erythro-9-(2-hydroxynon-3-yl)adenine on the concentration of *S*-adenosylhomocysteine and *S*-adenosylmethionine in rat liver

Erythro-9-(2-hydroxynon-3-yl)adenine was administered intraperitoneally in 0.5 ml of 0.9% NaCl at indicated times before killing. The values shown are means of duplicate assays of pooled samples of two livers.

Treatment (µmol/100 g body wt.)	Time period of treatment (h)	Concentration (nmol/g of liver fresh wt.)	
		<i>S</i> -Adenosylhomocysteine	<i>S</i> -Adenosylmethionine
0.9% NaCl	1	44	69
25	1	43	83
25	4 and 2	51	77
25	4	38	69

Table 3. *S*-Adenosylhomocysteine hydrolase activity in crude liver extracts of rats treated with multiple doses of erythro-9-(2-hydroxynon-3-yl)adenine *in vivo*

Erythro-9-(2-hydroxynon-3-yl)adenine ($10\mu\text{mol}/100\text{g}$ body wt.) was given intraperitoneally in 0.2ml of 0.9% NaCl 4h before killing or six times at 4h intervals (total dose $60\mu\text{mol}/100\text{g}$ body wt.), the last dose 4h before killing. Control animals received the vehicle only (4h before killing). The enzyme activity was determined from the homogenate immediately, and from the supernatant fraction about 90min . after killing the animals. The values are means of four animals. $*P < 0.001$, for the significance of the differences (based on Student's *t* test) compared with the activity in the supernatant fraction of the control animals.

Treatment	Enzyme activity (nmol/min per mg of protein)	
	Homogenate	Supernatant
0.9% NaCl	28.0 ± 1.3	43.6 ± 1.2
$10\mu\text{mol} \times 1$	27.5 ± 3.7	$34.4 \pm 1.9^*$
$10\mu\text{mol} \times 6$	27.6 ± 2.7	$27.0 \pm 2.7^*$

hydrolase of rat liver has a long half-life (considerably longer than 4h ; T. O. Eloranta & E. O. Kajander, unpublished work), irreversible inactivation of the enzyme taking place *in vivo* should have decreased the hydrolase activity of the homogenate, especially with multiple doses. These results suggested that inactivation of liver *S*-adenosylhomocysteine hydrolase of erythro-9-(2-hydroxynon-3-yl)adenine-treated animals took place during preparation and storage of the enzymes, either by the inhibitor itself or by compound(s) accumulating *post mortem* in homogenates and crude tissue extracts. The possibility that the inhibitor itself would be deleterious to the hydrolase was excluded by incubating purified *S*-adenosylhomocysteine hydrolase from rat liver with 0.25 and 2.5mM -erythro-9-(2-hydroxynon-3-yl)adenine in 50mM -Tris/HCl, pH 7.4 , at 0°C for 48h . No change in the hydrolase activity was observed with either concentration of the inhibitor (results not shown). A similar finding was previously reported for crude rat liver enzyme (Kajander *et al.*, 1976).

The time course of the inactivation process *in vitro* was followed after administration *in vivo* of the adenosine deaminase inhibitor. Fig. 1 shows that inactivation of *S*-adenosylhomocysteine hydrolase proceeded fairly rapidly after tissue homogenization even at low temperatures. The rate of inactivation depended on the time elapsed from erythro-9-(2-hydroxynon-3-yl)adenine injection to the killing of the animal, probably reflecting the concentration of the inhibitor left in tissues at the time of enzyme preparation.

Fig. 1. Decrease in liver *S*-adenosylhomocysteine hydrolase activity in enzyme preparations obtained from rats treated with erythro-9-(2-hydroxynon-3-yl)adenine *in vivo*

Erythro-9-(2-hydroxynon-3-yl)adenine ($25\mu\text{mol}/100\text{g}$ body wt.) was injected 30 (Δ) or 240min ($\square$) before killing. The enzyme preparations were stored at 0°C . The zero-point activity was determined from the homogenates immediately after killing the animals. Thereafter the enzyme activity was measured from the 100000g -supernatant fractions at the time points indicated. The zero-point activity of the untreated control ($\circ$) (mean specific activity of the homogenate $26.7\text{nmol}/\text{min}$ per mg of protein) was taken as 100% . Values are means of duplicate assays.

Characterization of disappearance of *S*-adenosylhomocysteine hydrolase activity *in vitro*

Hershfield (1979) has shown that the natural substrates of *S*-adenosylhomocysteine hydrolase (adenosine or Ado-Hcy) can protect the enzyme from being inactivated by other nucleosides like 2'-deoxyadenosine or arabinosyladenine. In the absence of homocysteine, adenosine or Ado-Hcy can also bring about inactivation of the enzyme even at low temperature (Kajander & Raina, 1981). In hypoxic conditions, adenine nucleotides are rapidly degraded at least partly to adenosine and further to inosine (Kaufman *et al.*, 1977; Arch & Newsholme, 1978b). Under such conditions accumulation of adenosine would be a logical consequence of inhibition of adenosine deaminase. In fact, it was observed in the present study that treatment of the rat with the adenosine deaminase inhibitor resulted in a rapid and profound accumulation *post mortem* of adenosine in liver homogenates. When erythro-9-(2-hydroxynon-3-yl)adenine was administered

intraperitoneally ($10\text{ }\mu\text{mol}/100\text{ g}$ body wt.) to the rats (four animals in each group) 45 min before killing. Adenosine concentration measured immediately after death was $91 \pm 19\text{ nmol/g}$ of fresh liver. The adenosine level rose to 774 ± 130 and $1470 \pm 100\text{ nmol/g}$ of tissue after 10 and 30 min storage of the homogenates at 0°C respectively. In the control homogenates, adenosine concentrations were 47 ± 14 , 96 ± 16 and $41 \pm 14\text{ nmol/g}$ at the same time points. Since the concentration of Ado-Hcy also rises *post mortem* (Eloranta, 1977), the effects of the natural substrates of *S*-adenosylhomocysteine hydrolase on the inactivation process were studied.

In the experiment described in Fig. 2, changes in the *S*-adenosylhomocysteine hydrolase activity were followed in the enzyme preparations incubated *in vitro* in the presence or absence of the adenosine deaminase inhibitor (final concentration 25 and $250\text{ }\mu\text{M}$). Homogenate and $100\,000\text{ g}$ supernatant fractions prepared from normal liver were incubated at 0°C with the inhibitor, and the remaining enzyme activity was assayed at various times. Incubation of the homogenate in the presence of the inhibitor (Fig. 2a) caused a rapid inactivation of the hydrolase, which followed the first-order reaction kinetics. The rate of inactivation was practically the same with

both concentrations of the inhibitor. In the supernatant fraction (Fig. 2b) the hydrolase remained stable for about 4 h, after which the activity disappeared with accelerating velocity. Addition of adenosine at 0.5 mM concentration to the supernatant fraction together with the deaminase inhibitor resulted in a rapid and irreversible inactivation of the hydrolase without any lag period (Fig. 2b). The kinetics of the process was analogous to that observed in the homogenates after administration *in vivo* or *in vitro* of erythro-9-(2-hydroxynon-3-yl)-adenine. Ado-Hcy at 0.5 mM concentration brought about only a slight inactivation (results not shown). Addition of L-homocysteine or ATP to the pre-incubation mixture totally prevented the decrease of the hydrolase activity (see the legend to Fig. 2). During incubation of the supernatant fraction in the presence of erythro-9-(2-hydroxynon-3-yl)adenine (but not in the absence of the inhibitor), adenosine was slowly accumulated; this was prevented by the addition of L-homocysteine or ATP to the incubation mixture (results not shown). In the presence of L-homocysteine adenosine was used for Ado-Hcy synthesis (Ado-Hcy was accumulated). The mechanism of how ATP prevented the increase in adenosine level is not known at present. The action

Fig. 2. Inactivation of *S*-adenosylhomocysteine hydrolase *in vitro* in rat liver homogenate (a) and supernatant fraction (b) in the presence of erythro-9-(2-hydroxynon-3-yl)adenine and/or adenosine

Fresh enzyme preparations were pre-incubated at 0°C in the presence of $25\text{ }\mu\text{M}$ - (Δ) or $250\text{ }\mu\text{M}$ - ($\square$) erythro-9-(2-hydroxynon-3-yl)adenine or $250\text{ }\mu\text{M}$ -inhibitor plus $500\text{ }\mu\text{M}$ -adenosine ($\blacksquare$). The initial enzyme activities of the untreated controls ($\circ$) were 27.2 (a) and 44.5 (b) nmol/min per mg of protein. The activities (results not shown) of samples of the supernatant fraction treated with $250\text{ }\mu\text{M}$ -inhibitor plus 1 or 10 mM -ATP, or with $250\text{ }\mu\text{M}$ -inhibitor plus 0.5 mM -homocysteine were indistinguishable from the control values. The values are means of duplicate assays.

of L-homocysteine, the other substrate besides adenosine in the synthetic reaction catalysed by this enzyme, together with the observed inactivation kinetics, suggested that the causative agent was adenosine.

Adenosine and 2'-deoxyadenosine as inactivators of purified S-adenosylhomocysteine hydrolase

The effect of the pre-incubation temperature on S-adenosylhomocysteine hydrolase activity in the presence of adenosine and 2'-deoxyadenosine was then studied. 2'-Deoxyadenosine was included because it has been reported to cause inactivation of this enzyme in adenosine deaminase-inhibited cells incubated at 37°C (Kredich & Hershfield, 1980). Kinetics of the inactivation of S-adenosylhomocysteine hydrolase in the presence of adenosine is presented in Fig. 3. At 0°C the inactivation obeyed typical first-order kinetics (Fig. 3a). From a reciprocal plot of rate constants versus adenosine concentration, a K_i value of 33 μM and maximal inactivation velocity of 0.01 min^{-1} were obtained (see Walsh, 1977).

At 37°C, the process was more complex. The progress curves presented in Fig. 3(b) show curvilinearity. It is evident that at this temperature the initial inactivation velocity was several times faster than at 0°C.

In contrast with the inactivation caused by adenosine, pre-incubation of the hydrolase at zero temperature with 2'-deoxyadenosine up to 1 mM concentration had no effect on enzyme activity. At 37°C, inactivation caused by 2'-deoxyadenosine

showed pseudo-first-order kinetics. The maximal inactivation velocity obtained from the double-reciprocal plot was 0.05 min^{-1} and the K_i value 40 μM (results not shown).

Discussion

In the present work it was shown that S-adenosylhomocysteine hydrolase activity is not inactivated *in vivo* in rat liver after intraperitoneal administration of high doses of erythro-9-(2-hydroxynon-3-yl)adenine. Irreversible inactivation of the hydrolase took place only *post mortem*. The extent of this process reflected the dose and the time elapsed between the administration of the drug and killing of the animals. It seemed that inactivation of S-adenosylhomocysteine hydrolase paralleled the extent of the deaminase inhibition and thus the concentration of erythro-9-(2-hydroxynon-3-yl)adenine present in tissues at the time of killing the animals.

The mechanism of inactivation *post mortem* of S-adenosylhomocysteine hydrolase is interesting, since the deaminase inhibitor itself had no effect on this enzyme. At 0°C the process followed first-order reaction kinetics and was irreversible. Several lines of evidence indicate that it was a suicide-like inactivation brought about by adenosine: (1) adenosine was found to accumulate in liver homogenates obtained from animals treated with the inhibitor *in vivo*; (2) addition of adenosine to the crude supernatant fraction produced an inactivation time course identical with that obtained from tissue

Fig. 3. Kinetics of inactivation of the purified S-adenosylhomocysteine hydrolase at 0°C or 37°C in the presence of adenosine

S-Adenosylhomocysteine hydrolase (sp. activity 5.9 $\mu\text{mol/min}$ per mg; 6.7 μg of protein in a final volume of 100 μl) was pre-incubated at 0°C (a) or 37°C (b) in the presence of adenosine at the concentrations shown in the inserts of the Figure. The pre-incubation buffer was 50 mM-Tris/HCl, pH 7.4, containing 5 mM-dithiothreitol and bovine serum albumin (0.5 mg/ml). During pre-incubation portions (10 μl) were transferred to the incubation mixture (75 μl) and assayed for hydrolase activity (incubation time 5 min at 37°C). Correction has been made for the dilution of the radioactive adenosine in the enzyme assay. The values are means of two determinations.

homogenates treated with the deaminase inhibitor. With the supernatant fraction treated with the deaminase inhibitor there was a lag period of 4 h before inactivation became apparent. Plausibly, enough inactivating substance was produced during this time to start the process. (3) Inactivation by adenosine was readily observed at 0°C, whereas 2'-deoxyadenosine, another possible inactivating substance, had no effect on the hydrolase activity at this temperature. (4) Addition of homocysteine or ATP, both capable of preventing the accumulation of adenosine in crude tissue supernatant fractions *in vitro*, protected the enzyme from inactivation. The present data indicate that inactivation of *S*-adenosylhomocysteine hydrolase *in vitro* may be a source of artificial results. Adenosine deaminase inhibitors are widely used in combination with adenosine and its analogues, also in experiments aimed to study the effects on the hydrolase activity. The deaminase inhibition may lead to suicide-like inactivation of the hydrolase activity even on storage at low temperatures. This can be eliminated by performing the assay of enzyme activity without any delay.

Kredich & Hershfield (1980) have reported that *S*-adenosylhomocysteine hydrolase activity was reduced to less than 5% of normal in erythrocytes of a human patient treated with 2'-deoxycoformycin, a tight-binding inhibitor of adenosine deaminase. The disappearance of enzyme activity was suggested to occur *in vivo* and result from suicide inactivation by accumulated 2'-deoxyadenosine. The possibility of *S*-adenosylhomocysteine hydrolase inactivation during tissue preparation and storage makes it difficult to draw definitive conclusions about the enzyme activity *in vivo* under such conditions. Our results also suggest that the decrease in *S*-adenosylhomocysteine hydrolase activity reported to occur in several disturbances of purine metabolism (see the introduction section) should be re-examined to exclude the possibility of inactivation of the enzyme *in vitro*.

Inactivation of *S*-adenosylhomocysteine hydrolase by adenosine, in the absence of homocysteine, has been reported to be a first-order reaction and to resemble suicide inactivation (Hershfield, 1979; Chiang *et al.*, 1981). In the case of rat liver enzyme, the nature of the process varied according to the temperature used. At 0°C, inactivation obeyed typical first-order kinetics and a high adenosine concentration was necessary for the maximal inactivation rate ($K_i = 33 \mu\text{M}$). At 37°C the inactivation occurred at much lower adenosine concentrations. The inactivation rate curves were curvilinear and did not show first-order kinetics. It seems possible that at the higher temperature more than one mechanism is involved. Previously, both types of inactivation-rate curves have been obtained at 37°C for bovine and mice liver enzymes, varying

according to the particular inactivating substance (Chiang *et al.*, 1981; Helland & Ueland, 1981). In the present case, both types were obtained with a single substance, adenosine, at different temperatures.

Erythro-9-(2-hydroxy-3-yl)adenine did not elevate liver Ado-Hcy or Ado-Met concentrations measured 1–4 h after the inhibitor administration. This supports the view (see Arch & Newsholme, 1978b) that adenosine deaminase is of little importance (in the liver) in adenosine metabolism under normal, very low, adenosine levels. The deaminase may be important in the elimination of adenosine accumulated under specific conditions, for example, as a result of increased degradation of adenine nucleotides during tissue hypoxia.

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