[CANCER RESEARCH 41, 673-678, February 1981]
0008-5472/81/0041-0000$2.00

Interaction of 9-β-D-Arabinofuranosyladenine, 9-β-D-Arabinofuranosyladenine 5′-Monophosphate, and 9-β-D-Arabinofuranosyladenine 5′-Triphosphate with S-Adenosylhomocysteinase[1]

Svein Helland[2] and Per Magne Ueland

Department of Pharmacology, University of Bergen, School of Medicine, MFH-bygget, and the Department of Dermatology, N-5016 Haukeland Sykehus, Bergen, Norway

ABSTRACT

9-β-D-Arabinofuranosyladenine (ara-A), 9-β-D-arabinofuranosyladenine 5′-monophosphate, and 9-β-D-arabinofuranosyladenine 5′-triphosphate competitively inhibit both the synthesis and hydrolysis of *S*-adenosylhomocysteine catalyzed by *S*-adenosylhomocysteinase [*S*-adenosylhomocysteine hydrolase (EC 3.3.1.1.)] from mouse liver, and the inhibitor constants were 5.0×10^{-6}, 1.1×10^{-4}, and 1.0×10^{-3} M, respectively. A time-dependent inactivation of the enzyme was observed when the enzyme was preincubation with ara-A, 9-β-D-arabinofuranosyladenine 5′-monophosphate, or 9-β-D-arabinofuranosyladenine 5′-triphosphate. ara-A was the most potent inactivator. The inactivation with ara-A was less pronounced in the presence of adenosine, *S*-adenosylhomocysteine, adenine, adenosine 5′-monophosphate, or adenosine 5′-diphosphate, showed first-order kinetics, saturability, and irreversibility. The rate of inactivation was half-maximal at 5×10^{-6} M ara-A, and the rate constant of inactivation was 0.43 min^{-1} at saturating concentrations of ara-A. ara-A was tightly but not covalently bound to the enzyme. ara-A bound to the enzyme was not available for deamination to 9-β-D-arabinofuranosylhypoxanthine catalyzed by the enzyme adenosine deaminase.

INTRODUCTION

The antibiotic ara-A[3] is an oncostatic (7, 26, 28) and antiviral agent (36). This nucleoside is either phosphorylated to the corresponding nucleotides or is deaminated to ara-Hx (4). Deamination of ara-A is catalyzed by the enzyme adenosine deaminase [adenosine aminohydrolase (EC 3.5.4.4)] (8, 48) which is widely distributed in mammalian tissues (2). The biological effect of ara-A is probably limited by its conversion to ara-Hx (3, 4, 7). Potent inhibitors of adenosine deaminase have recently become available (13, 32, 34, 46), and these inhibitors increase the biological activity of ara-A in several cell systems (1, 6, 28) and *in vivo* (21, 23, 27, 33).

The mechanism of action of ara-A is a matter of some controversy. ara-A inhibits tRNA methylases (44). It has been stated that the cytotoxic effect of ara-A probably results from its conversion to ara-ATP. This nucleotide is an inhibitor of ribonucleotide reductase (25) and some DNA polymerases (14, 15, 22, 48), and its incorporation into nascent DNA may inhibit the replication of DNA (20).

Inhibition of *S*-adenosylhomocysteinase [AdoHcy hydrolase (EC 3.3.1.1.)] by ara-A was first demonstrated by Trewyn and Kerr (37). This finding has recently been extended by Hershfield (17) showing that ara-A, among other adenosine derivatives, irreversibly inactivates the enzyme from human lymphoblasts. *S*-Adenosylhomocysteinase from mouse liver interacts with various synthetic and naturally occurring adenosine derivatives (38). Adenine and adenine nucleotides are potential regulators of the enzyme activity (42), and some effects of these purines on the enzyme are less pronounced in the presence of P_i.[4] These data point to the possibility that the response of the enzyme to ara-A may be modulated by metabolites like adenine, adenine nucleotides, and P_i.

S-Adenosylhomocysteinase catalyzes the reversible hydrolysis of AdoHcy (11) which is both a product from (5) and a potent inhibitor of (9, 10, 18, 19, 24, 29, 49) various biological transmethylation reactions using *S*-adenosylmethionine as methyl donor. Cellular accumulation of AdoHcy would be expected to occur upon inhibition of the enzyme. Thus, inactivation of the enzyme by analogs of adenosine may influence biological methylation, which may contribute to the effects of these agents.

The interaction of ara-A and its metabolic products, ara-AMP and ara-ATP, with *S*-adenosylhomocysteinase from mouse liver is the subject of the present work. This study was undertaken for the following reasons: (*a*) to obtain data on the nature of the interaction of ara-A with the enzyme; (*b*) to investigate whether the inhibition by ara-A is relieved by its conversion to nucleotides and modulated by the presence of naturally occurring metabolites known to interact with the enzyme; and (*c*) to investigate whether binding of ara-A to *S*-adenosylhomocysteinase may influence inactivation (deamination) of the drug.

MATERIALS AND METHODS

Chemicals. Adenosine, inosine, AdoHcy, DL-homocysteine, adenosine deaminase (type I from calf intestinal mucosa), AMP, ADP, ATP, and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid were obtained from Sigma Chemical Co., St. Louis, Mo. ara-A, ara-AMP, and ara-ATP were kindly supplied by the Developmental Therapeutics Program, Chemotherapy, National Cancer Institute, Bethesda, Md. [8-^{14}C]Adenosine (59 mCi/mmol) and [2, 8-^{3}H]ara-A (22 Ci/mmol) were purchased from the Radiochemical Centre, Amersham, England. [8-^{14}C]AdoHcy (59 mCi/mmol) was synthesized enzymatically from [8-^{14}C]adenosine and purified as described elsewhere (42). Polyethyleneimine-impregnated cellulose thin-layer sheets (0.25 mm) on glass plates (20×20 cm) were prepared

[1] Supported by grants from Nordisk insulinfond, Langfeldts fond, and the Norwegian Research Council for Science and the Humanities.

[2] To whom requests for reprints should be addressed.

[3] The abbreviations used are: ara-A, 9-β-D-arabinofuranosyladenine; ara-Hx, 9-β-D-arabinofuranosylhypoxanthine; ara-ATP, 9-β-D-arabinofuranosyladenine 5′-triphosphate; AdoHcy, *S*-adenosyl-L-homocysteine; ara-AMP, 9-β-D-arabinofuranosyladenine 5′-monophosphate.

Received June 23, 1980; accepted October 21, 1980.

[4] P. M. Ueland, manuscript in preparation.

as described by Randerath and Randerath (30) and were developed in distilled water before use.

Purification of S-Adenosylhomocysteinase from Mouse Liver. The enzyme was purified to apparent homogeneity according to a slight modification (38) of a purification scheme published elsewhere (39).

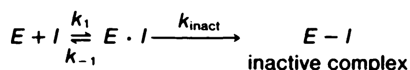
Measurement of the Conversion of ara-A to ara-Hx. Samples from the incubation mixture containing [^{3}H]ara-A were mixed with perchloric acid, neutralized, and then subjected to thin-layer chromatography on polyethyleneimine-cellulose plates. The chromatograms were developed in isobutyl alcohol: water:ethanol (2:1:1). ara-Hx showed the same mobility as inosine (31). The radioactivity in the separate spots was determined by liquid scintillation counting.

Assay for AdoHcy Synthase and Hydrolase Activity. This was performed according to a radiochemical method described elsewhere (42). When the enzyme activity was determined in the synthetic direction, the incubation mixture contained enzyme, [^{14}C]adenosine, and DL-homocysteine (3 mM). Measurement of hydrolysis of AdoHcy was performed by incubating enzyme in the presence of [8-^{14}C]AdoHcy and an adenosine trap (*i.e.*, adenosine deaminase, 50 units/ml). The incubation buffer was 15 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH 7.0, containing 5 mM magnesium acetate, 150 mM KCl, 0.25% bovine serum albumin, and 2 mM 2-mercaptoethanol. The temperature was 37°.

Polyacrylamide Gel Electrophoresis. This was performed at pH 8.8 (15 mM Tris:glycine buffer), 7.5% gel in 0.7- x 8.5-cm tubes. The enzyme (preincubated with [^{3}H]ara-A) was applied to the gel in 20% sucrose. The electrophoresis proceeded at 4° for about 3 hr at 3 mamp/gel. The gels were either stained in Coomassie blue and destained in 7% acetic acid containing 30% methanol or cut into slices (1.7 mm) with a gel cutter. The slices were placed in scintillation vials containing 1 ml 2% sodium dodecyl sulfate and eluted for 18 hr at room temperature. Then, 7 ml scintillation fluid were added.

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 (43).

Definition of Some Kinetic Parameters and their Theoretical Basis. Suicide inactivation of an enzyme (*E*) by an active site directed agent (*I*) is described by the equation (45):



$$E + I \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E \cdot I \xrightarrow{k_{inact}} \underset{\text{inactive complex}}{E - I}$$

The formation of the inactive complex ($E - I$) from $E \cdot I$ is a first-order process. The observed rate of inactivation at a given concentration of *I* corresponds to the rate constant k_{inact}. The double-reciprocal plot of k_{inact} *versus* the concentration of *I* is linear. From the vertical intercept of this plot, the limiting rate constant k_2 could be determined. The parameter k_2 corresponds to the rate of inactivation at saturating concentrations of *I*. The horizontal intercept gives K_I which equals the concentration of *I* where the rate of inactivation is half-maximal and is a measure of the affinity of *I* for the enzyme (45).

RESULTS

Competitive Inhibition of Enzyme Catalysis by ara-A, ara-AMP, and ara-ATP. Synthesis of AdoHcy was determined at various concentrations of adenosine (0.5, 1.5, and 5 μM) and increasing concentrations of ara-A, ara-AMP, or ara-ATP. The data obtained were plotted according to Dixon (12). The plots were consistent with linear competitive inhibition of the synthetic reaction by ara-A, ara-AMP, and ara-ATP, and the inhibitor constants were 5.0×10^{-6}, 1.1×10^{-4}, and 1.0×10^{-3} M, respectively (Chart 1). Dixon plots for the hydrolysis of AdoHcy (1 and 3 μM) in the presence of increasing concentration of ara-AMP and ara-ATP were linear, and the same inhibitor constants for ara-AMP and ara-ATP were obtained as those determined from Chart 1 (data not shown). These data show that the inhibition of enzyme catalysis observed with ara-AMP and ara-ATP was not caused by contamination with ara-A, as the nucleoside would be expected to be degraded by adenosine deaminase (8, 48) present in the assay mixture used for the measurement of hydrolysis of AdoHcy. The same results were obtained when 30 mM P_i was included in the incubation mixture (data not shown).

Inactivation of the Enzyme in the Presence of ara-A. The rate of inactivation of the enzyme in the presence of ara-A obeyed first-order kinetics, and the process was saturable with respect to ara-A (Chart 2). The double-reciprocal plot for the rate constant of inactivation *versus* the concentration of ara-A was linear, and the concentration of ara-A where the rate of inactivation was half-maximal (K_I for ara-A) was 5.0×10^{-6} M. The rate constant of inactivation at saturating concentrations of ara-A (k_2) was 0.43 min^{-1} (Chart 2). About the same results were obtained when dithiothreitol (2 mM) was replaced by 2 mM 2-mercaptoethanol and when reducing agent was not present during preincubation (data not shown). In the absence of reducing agent, there was interference by low stability of the enzyme.[4] The enzyme could not be reactivated by removal of free ara-A by gel filtration and extensive dilution (data not shown).

Inactivation of the Enzyme in the Presence of ara-AMP and ara-ATP. There was a time-dependent decrease in enzyme activity when the enzyme was preincubated with ara-AMP (Chart 3; Table 1) or ara-ATP (Table 1). The semilogarithmic plot for the inactivation of the enzyme in the presence of ara-AMP was characterized by an initial phase after which the curves leveled off. The process was not saturable with respect to ara-AMP within the concentration range tested (Chart 3).

Effect of Various Metabolites on the Inactivation. The

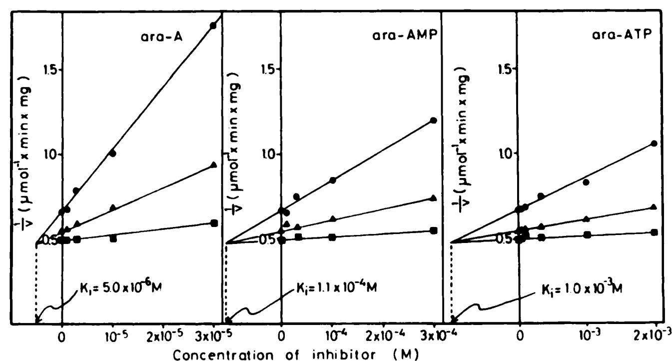
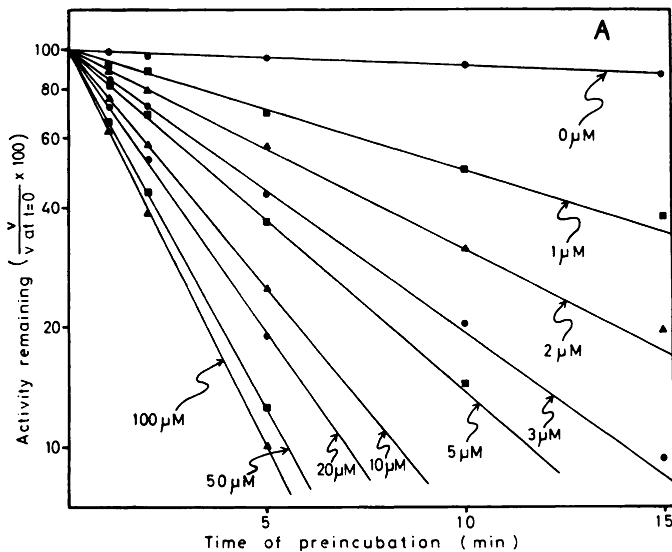


Chart 1. Inhibition of the synthesis of AdoHcy by ara-A, ara-AMP, and ara-ATP. The initial velocity of the synthesis of AdoHcy was determined in the presence of 0.5 μM (●), 1.5 μM (▲), and 5 μM (■) adenosine and increasing concentration of ara-A, ara-AMP, or ara-ATP. The data were plotted according to Dixon (12).



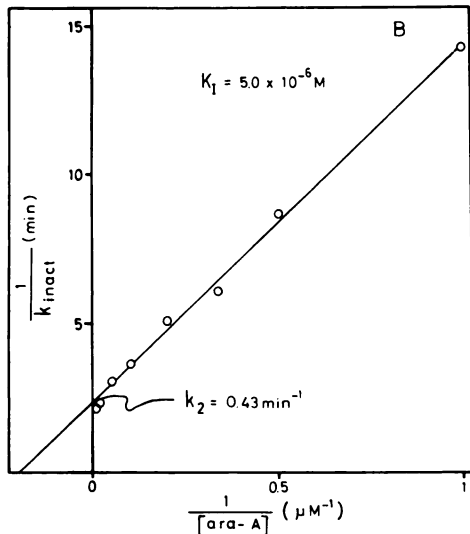


Chart 2. Kinetics of inactivation of the enzyme in the presence of ara-A. The enzyme (22 μg/ml) was preincubated at 37° for increasing periods of time in the presence of ara-A at concentrations indicated in *A*. The preincubation buffer was 15 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH 7.0, containing 150 mM KCl, 5 mM magnesium acetate, 0.25% bovine serum albumin, and 2 mM dithiothreitol. Aliquots of 5 μl were transferred to the incubation mixture (300 μl) and assayed for AdoHcy synthase activity. *A*, progress curves for the inactivation of the enzyme; *B*, double-reciprocal plot for the rate constant of inactivation (k_{inact}) *versus* the concentration of ara-A.

inactivation of the enzyme in the presence of ara-A, ara-AMP, or ara-ATP was not affected by or slightly less pronounced in the presence of homocysteine or cysteine. The rate of inactivation observed with ara-ATP but not with ara-A and ara-AMP was decreased when P_i (30 mM) was present during preincubation (Table 1).

Adenosine, AdoHcy, adenine, AMP, and ADP protected the enzyme from inactivation by ara-A. The substrates, adenosine and AdoHcy, were most effective in this respect (Table 1).

Binding of [^{3}H]ara-A to the Enzyme. Enzyme incubated with [^{3}H]ara-A was subjected to Sephadex G-25 chromatography. Radioactivity was excluded from the gel showing that a fraction of ara-A was bound to the enzyme (Chart 4). The amount of radioactive material bound to the enzyme increased as a function of time of incubation. The maximal amount of radioactivity associated with the enzyme corresponded to the binding of 3.1 mol of ara-A per mol of enzyme (data not shown). An aliquot of the fraction excluded from the gel was subjected to polyacrylamide gel electrophoresis. The radioactivity comigrated exactly

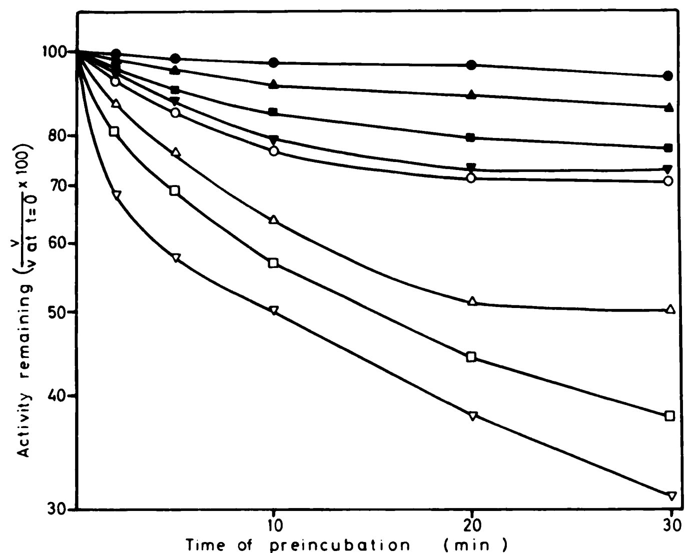


Chart 3. Progress curves for the inactivation of the enzyme in the presence of ara-AMP. The enzyme (22 μg/ml) was preincubated for increasing periods of time in the presence of 0 μM (●), 1 μM (▲), 3 μM (■), 5 μM (▼), 20 μM (○), 0.5 mM (△), 1 mM (□), and 4 mM (▽) ara-AMP. The preincubation buffer and the experimental design was as described in the legend to Chart 2.

Table 1

Effect of various metabolites on the inactivation of S-adenosylhomocysteinase by ara-A, ara-AMP, and ara-ATP

The enzyme (22 μg/ml) was preincubated in the presence of ara-A, ara-AMP, or ara-ATP and various metabolites at concentrations indicated in the table. The preincubation buffer was 15 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH 7.0, containing 5 mM magnesium acetate, 150 mM KCl, 0.25% bovine serum albumin, and 2 mM 2-mercaptoethanol. At times indicated, aliquots of 5 μl were transferred to the incubation mixture (300 μl) and assayed for AdoHcy synthase activity.

		Enzyme activity (μmol/mg/min)		
Inactivator	Metabolite	0 min preincubation	5 min preincubation	10 min preincubation
Control		2.08	2.04	2.00
ara-A (1 μM)		2.02	1.25	0.43
	3 mM L-homocysteine	2.08	1.48	0.64
	3 mM L-cysteine	2.10	1.28	0.45
	30 mM inorganic phosphate	2.01	1.30	0.43
ara-A (10 μM)		2.10	0.51	0.19
	10 μM adenosine	2.04	1.91	1.75
	10 μM AdoHcy	2.09	1.77	1.56
	50 μM adenine	2.01	1.22	0.91
	2 mM AMP	2.12	1.67	1.44
	2 mM ADP	2.06	1.06	0.66
	2 mM ATP	2.04	0.42	0.18
ara-AMP (2 mM)		2.08	1.64	1.39
	3 mM L-homocysteine	2.06	1.70	1.40
	3 mM L-cysteine	2.02	1.65	1.42
	30 mM P_i	2.10	1.70	1.41
	50 μM adenosine	2.09	2.02	1.88
ara-ATP (2 mM)		2.00	0.94	0.80
	3 mM L-homocysteine	2.06	0.98	0.88
	3 mM L-cysteine	2.01	0.99	0.84
	30 mM P_i	2.09	1.55	1.30

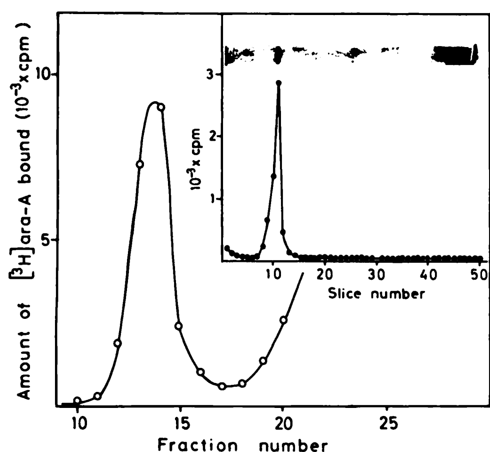


Chart 4. Binding of [^{3}H]ara-A to *S*-adenosylhomocysteinase. The enzyme (180 μg/ml) was incubated for 30 min at 37° with 20 μM [^{3}H]ara-A. The incubation buffer was 15 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH 7.0, containing 150 mM KCl, 5 mM magnesium acetate, 0.25% bovine serum albumin, and 2 mM dithiothreitol. A sample of 20 μl was applied to a Sephadex G-25 column (0.45 x 6 cm) equilibrated with 15 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH 7.0, and eluted with the same buffer. Fractions of 134 μl were collected, aliquots of 25 μl were added to scintillation vials, and the radioactivity was determined. The elution profile is shown on the chart. Aliquots of 20 μl from the top fraction (Fraction 14) were subjected to polyacrylamide gel electrophoresis. *Inset*, association of radioactivity with the enzyme.

with the protein band (Chart 4, *inset*) which shows that [^{3}H]ara-A was bound to *S*-adenosylhomocysteinase.

Enzyme was inactivated by incubation (for 40 min) with high concentration (700 μM) of ara-A and then subjected to Sephadex G-25 chromatography. The inactive enzyme which was excluded from the gel was preincubated in the absence and presence of enzyme not exposed to ara-A, and the mixture was diluted and assayed for AdoHcy synthase activity (Chart 5). There was no reactivation of the inactive enzyme and there was no effect of the inactive enzyme (to which ara-A was bound) on the enzyme not exposed to ara-A (Chart 5). In contrast, when the enzyme inactivated by ara-A was denatured by heating prior to preincubation with the nontreated enzyme, the activity of the latter decreased as a function of time (Chart 5). These data indicate tight but not covalent binding of ara-A to the enzyme. Furthermore, heat treatment of the enzyme induced release of inactivator from the enzyme.

Sequestration of ara-A. ara-A is converted to the biologically inactive metabolite, ara-Hx, by the enzyme adenosine deaminase (7). The interaction of ara-A with *S*-adenosylhomocysteinase protected ara-A against deamination. This phenomenon, termed sequestration of ara-A, was demonstrated by preincubation of ara-A with the enzyme for various periods of time before the addition of excess adenosine deaminase. Under these conditions, the progress curve for the formation of ara-Hx was characterized by an initial burst phase after which a plateau was obtained. The fraction of ara-A not available for deamination increased as a function of time before addition of adenosine deaminase (Chart 6).

DISCUSSION

The competitive inhibition of enzyme catalysis by ara-A and the corresponding nucleotides, ara-AMP and ara-ATP (Chart 1), indicates that these compounds interact with the catalytic site of the enzyme. The affinity towards this site decreases in the order ara-A, ara-AMP, and ara-ATP, as judged by the inhibitor constants of these agents. In addition, ara-A, ara-AMP, and ara-ATP induce a time-dependent inactivation of the enzyme (Charts 2 and 3; Table 1). The nucleotides are less potent in this respect than their congener, ara-A. These data suggest that the inhibition and inactivation of *S*-adenosylhom-

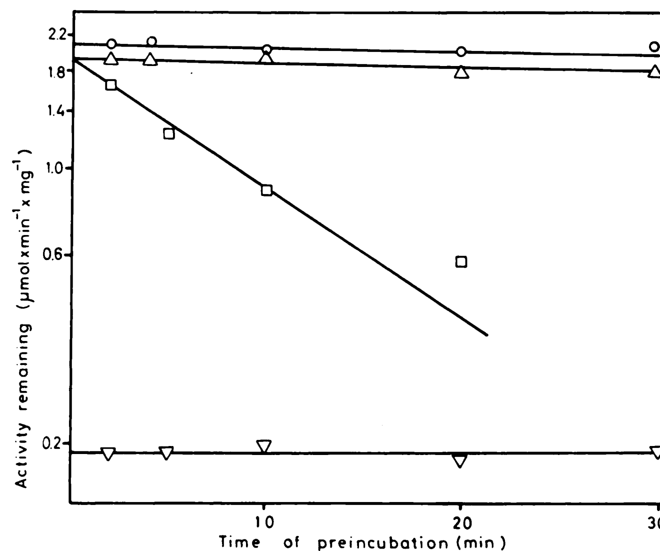


Chart 5. Test for dissociation of inactivator from the native enzyme and from the enzyme denatured by heating. Enzyme (1.4 mg/ml) was preincubated for 40 min at 37° in the presence of 700 μM ara-A. An aliquot of 50 μl was subjected to gel filtration on a Sephadex G-25 column (0.45 x 6 cm). The fraction excluded from the gel (200 μl) was divided into 2 parts. One portion was heated for 7 min at 100° (denatured enzyme) and the other portion was kept at 0° (inactivated enzyme). Nontreated enzyme (22 μg/ml) was preincubated for increasing periods of time under the conditions depicted in Chart 2 in the presence of denatured enzyme (diluted 4-fold) (□) or inactivated enzyme (diluted 4-fold) (○) or with no addition (△). The inactivated enzyme was also preincubated under the same condition in the absence of nontreated enzyme (▽). At times indicated on the chart, aliquots of 5 μl were transferred to the incubation mixture (300 μl) and assayed for AdoHcy synthase activity.

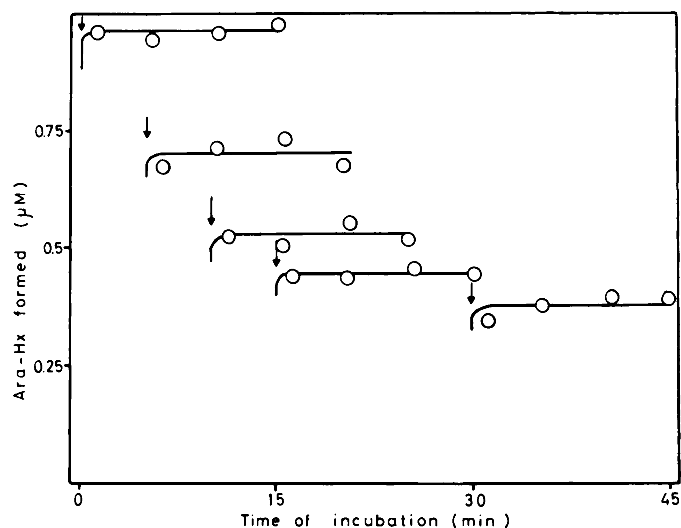


Chart 6. Protection of ara-A against deamination. [^{3}H]ara-A (1 μM) was incubated with enzyme (100 μg/ml) at 37°. The incubation buffer was 15 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH 7.0, containing 150 mM KCl, 5 mM magnesium acetate, 0.25% bovine serum albumin, and 2 mM 2-mercaptoethanol. At times indicated on the chart (*arrows*), the incubation mixture was supplemented with excess adenosine deaminase (100 units/ml). The amount of ara-Hx formed is plotted *versus* time of incubation.

ocysteinase by ara-A may be partly relieved by its conversion to nucleotides.

The inactivation observed with ara-A obeys first-order kinetics, shows saturability and irreversibility, and is associated with tight binding of inactivator. The enzyme is protected against inactivation by its substrates (Table 1). These data suggest suicide-like inactivation (44) of the enzyme by ara-A, as previously demonstated for *S*-adenosylhomocysteinase from human lymphoblasts (17). This implies that the formation of the inactive ara-A-enzyme complex proceeds from a reversible binding of ara-A to the active site of the enzyme. This interpretation is supported by the observation that the inhibition constant for ara-A determined from initial velocity studies (Chart 1) equals the concentration of ara-A where the rate of inactivation is half-maximal (K_I for ara-A) (Chart 2).

The inactivation of the enzyme in the presence of ara-AMP shows rather complex kinetics (Chart 3), and the mechanism of inactivation is not readily apparent from the kinetic data. Thiol compounds and P_i do not influence the inactivation with ara-AMP whereas these agents have pronounced effects on the response of the enzyme to adenine nucleotides.[4] This may indicate that the mechanism of action of ara-AMP differs from that of its natural counterpart.

The interaction of adenine, AMP, and ADP in addition to the substrates, adenosine and AdoHcy, with the catalytic site may be the molecular basis for the protection of the enzyme against inactivation by ara-A (Table 1). This statement is supported by the observation that the efficiency to protect the enzyme from inactivation (Table 1) parallels the affinity of adenosine, AdoHcy, adenine, and adenine nucleotides to the catalytic site as judged by K_m values or inhibitor constants for these compounds (42).

The protection of *S*-adenosylhomocysteinase against inactivation by adenosine and naturally occurring adenosine derivatives (Table 1) suggests that inactivation of the enzyme in the intact cell[5] may be influenced by its metabolic status. Adenosine deaminase inhibitors increase the cellular level of adenosine but also affect the metabolism of adenine nucleotides in the cell (16). The effect of erythro-9-(2-hydroxy-3-nonyl)adenine on the adenine nucleotide pool size seems to be dependent on the concentration of this agent (35). Thus, it is possible that adenosine deaminase inhibitors have a dual action on the inactivation of *S*-adenosylhomocysteinase in the cell. These agents may promote the inactivation of the enzyme by inhibiting the inactivation of ara-A but may also affect the inactivation by changing the cellular level of adenosine and adenine nucleotides.

Adenosine is protected against deamination through its interaction with *S*-adenosylhomocysteinase. This phenomenon, termed sequestration of adenosine, could be demonstrated in crude extracts from mouse liver and other tissues (41). The observation that the sequestration of adenosine occurs both during synthesis and hydrolysis of AdoHcy (40) suggests that the sequestration process may operate *in vivo*. Data presented in Chart 6 show that ara-A bound to *S*-adenosylhomocysteinase is not available for deamination to ara-Hx. The possibility of irreversible inactivation of *S*-adenolsylhomocysteinase in the intact cell suggests that the effects of ara-A may be influenced by the intracellular turnover of the enzyme (17).

[5] S. Helland and P. M. Ueland, manuscript in preparation.

The inhibition of *S*-adenosylhomocysteinase by ara-A raises the possibility that the effectiveness of this agent stems from blocking degradation of AdoHcy (37). Studies on the inactivation of the enzyme in intact cells and *in vivo* and the effects of ara-A on the metabolism of AdoHcy and on cellular methylation reactions are subjects for further investigation to solve this question.

ACKNOWLEDGMENTS

The authors are grateful to H. Bergesen for skillful technical assistance and to S. Dybvik for correcting the manuscript. The authors also wish to thank the Developmental Therapeutics Program, Chemotherapy, National Cancer Institute, Bethesda, Md., which provided some of the drugs used in this study.

REFERENCES

1. Borondy, P. E., Chang, T., Maschewske, E., and Galzko, A. J. Inhibition of adenosine deaminase by co-vidarabine and its effect on the metabolic disposition of adenine arabinoside (vidarabine). Ann. N. Y. Acad. Sci., *284:* 9–20, 1977.
2. Brady, T. G., and O'Donovan, C. I. A study of the tissue distribution of adenosine deaminase in six mammal species. Comp. Biochem. Physiol., *14:* 101–120, 1965.
3. Brink, J. J., and LePage, G. A. Metabolic effects of 9-D-arabinosylpurines in ascites tumor cells. Cancer Res., *24:* 312–318, 1964.
4. Brink, J. J., and LePage, G. A. Metabolism and distribution of 9-β-D-arabinofuranosyladenine in mouse tissues. Cancer Res., *24:* 1042–1049, 1964.
5. Cantoni, G. L., and Scarano, E. The formation of *S*-adenosylhomocysteine in enzymatic transmethylation reactions. J. Am. Chem. Soc., *76:* 4744, 1954.
6. Cass, C. E., and Au-Yeung, T. H. Enhancement of 9-β-D-arabinofuranosyladenine cytotoxicity to mouse leukemia L1210 *in vitro* by 2'-deoxycoformycin. Cancer Res., *36:* 1486–1491, 1976.
7. Cohen, S. S. Introduction to the biochemistry of D-arabinosyl nucleosides. Progr. Nucleic Acid Res. Mol. Biol., *5:* 1–88, 1966.
8. Cory, J. G., and Suhadolnik, R. J. Structural requirements of nucleosides for binding by adenosine deaminase. Biochemistry, *4:* 1729–1732, 1965.
9. Coward, J. K., Slisz, E. P., and Wu, F. Y-H. Kinetic studies on catechol *O*-methyltransferase. Product inhibition and the nature of the catechol binding site. Biochemistry, *12:* 2291–2297, 1973.
10. Deguchi, T., and Barchas, J. Inhibition of transmethylations of biogenic amines by *S*-adenosylhomocysteine. Enhancement of transmethylation by adenosylhomocysteinase. J. Biol. Chem., *246:* 3175–3181, 1971.
11. de la Haba, G., and Cantoni, G. L. The enzymatic synthesis of *S*-adenosyl-L-homocysteine from adenosine and homocysteine. J. Biol Chem., *234:* 603–608, 1959.
12. Dixon, M. The determination of enzyme inhibitor constants. Biochem. J., *55:* 170–171, 1953.
13. Evans, B., and Wolfender, R. J. A potential transition state analog for adenosine deaminase. J. Am. Chem. Soc., *92:* 4751–4752, 1970.
14. Furth, J. J., and Cohen, S. S. Inhibition of mammalian DNA polymerase by the 5'-triphosphate of 9-β-D-arabinofuranosyladenine. Cancer Res., *27:* 1528–1533, 1967.
15. Furth, J. J., and Cohen, S. S. Inhibition of mammalian DNA polymerase by the 5'-triphosphate of 1-β-D-arabinofuranosylcytosine and the 5'-triphosphate of 9-β-D-arabinofuranosyladenine. Cancer Res., *28:* 2061–2067, 1968.
16. Henderson, J. F., Brox, L., Zombor, G., Hunting, D., and Lomax, C. A. Specificity of adenosine deaminase inhibitors. Biochem. Pharmacol., *26:* 1967–1972, 1977.
17. Hershfield, M. S. Apparent suicide inactivation of human lymphoblast *S*-adenosylhomocysteine hydrolase by 2'-deoxyadenosine and adenine arabinoside. A basis for direct toxic effects of analogs of adenosine. J. Biol. Chem., *254:* 22-25, 1979.
18. Hurwitz, J., Gold, M., and Anders, M. The enzymatic methylation of ribonucleic acid and deoxyribonucleic acid. J. Biol. Chem., *239:* 3474–3482, 1964.
19. Kerr, S. J. Competing methyltransferase systems. J. Biol. Chem., *247:* 4248–4252, 1972.
20. Lee, M. Y. W. T., Byrnes, J. J., Downey, K. M., and So, A. G. Mechanism of inhibition of deoxyribonucleic acid synthesis by 1-β-D-arabinofuranosyladenosine triphosphate and its potentiation by 6-mercaptopurine ribonucleoside 5'-monophosphate. Biochemistry, *19:* 215–219, 1980.
21. Lee, S. H., Caron, N., and Kimball, A. P. Therapeutic effects of 9-β-D-arabinofuranosyladenine and 2'-deoxycoformycin combinations on intracerebral leukemia. Cancer Res., *37:* 1953–1955, 1977.
22. LePage, G. A. Resistance to 9-β-D-arabinofuranosyladenine in murine tumor cells. Cancer Res., *38:* 2314–2316, 1978.

23. LePage, G. A., Worth, L. S., and Kimball, A. P. Enhancement of the antitumor activity of arabinofuranosyladenine by 2'-deoxycoformycin. Cancer Res., *36:* 1481-1485, 1976.
24. Mann, J. D., and Mudd, S. H. Alkaloids and plant metabolism. IV. The tyramine methylpherase of barley roots. J. Biol. Chem., *238:* 381-385, 1963.
25. Moore, E. C., and Cohen, S. S. Effects of arabinonucleotides on ribonucleotide reduction by an enzyme system from rat tumor. J. Biol. Chem., *242:* 2116-2118, 1967.
26. Müller, W. E. G., Maidhof, A., Zahn, R. K., and Shannon, W. M. Effect of 9-β-D-arabinofuranosyladenine on DNA synthesis *in vivo*. Cancer Res., *37:* 2282-2290, 1977.
27. Plunkett, W., Alexander, L., Chubb, S., and Loo, T. L. Comparison of the activity of 2'-deoxycoformycin and erythro-9-(2-hydroxy-3-nonyl)adenine *in vivo*. Biochem. Pharmacol., *28:* 201-206, 1979.
28. Plunkett, W., and Cohen, S. S. Two approaches that increase the activity of analogs of adenine nucleosides in animal cells. Cancer Res., *35:* 1547-1554, 1975.
29. Pugh, C. S. G., Borchardt, R. T., and Stone, H. O. Inhibition of Newcastle disease virion messenger RNA (guanine-7-)methyltransferase by analogues of *S*-adenosylhomocysteine. Biochemistry, *16:* 3928-3932, 1977.
30. Randerath, K., and Randerath, E. Thin-layer separation methods for nucleic acid derivatives. Methods Enzymol., *12A:* 323-347, 1967.
31. Saebø, J., and Ueland, P. M. An adenosine 3':5'-monophosphate adenosine-binding protein from mouse liver. Association with *S*-adenosylhomocysteinase activity. FEBS Lett., *96:* 125-128, 1978.
32. Sawa, T., Fukagawa, Y., Homma, I., Takeuchi, T., and Umezawa, H. Mode of inhibition of coformycine on adenosine deaminase. J. Antibiot. (Tokyo) (Ser. A), *20:* 227-231, 1967.
33. Schabel, F. M., Jr., Trader, M. W., and Laster, W. R., Jr. Increased therapeutic activity of 9-β-D-arabinosyladenine (ara-A) against leukemia p388 and L1210 by an adenosine deaminase inhibitor. Proc. Am. Assoc. Cancer Res., *17:* 46, 1976.
34. Schaeffer, H. J., and Schwender, C. F. Enzyme inhibitors. 26. Bridging hydrophobic and hydrophilic regions on adenosine deaminase with some 9-(2-hydroxy-3-alkyl)adenines. J. Med. Chem., *17:* 6-8, 1974.
35. Skupp, S., Vugrek, G., and Ayvazian, J. H. Effect of erythro-9-(2-hydroxy-3-nonyl)adenine on purine and pyrimidine metabolism in the human peripheral lymphocyte during the early phases of phytohemagglutinin-mediated blastogenesis. Biochem. Pharmacol., *28:* 3323-3331, 1979.
36. Tilles, J. G. Antiviral agents. Annu. Rev. Pharmacol., *14:* 469-489, 1974.
37. Trewyn, R. W., and Kerr, S. J. Regulation of tRNA methyltransferase activity in onco-developmental systems. *In:* W. H. Fishman and S. Sell (eds.), Onco-Developmental Gene Expression, pp. 101-106. New York: Academic Press, Inc., 1976.
38. Ueland, P. M. 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, 1978.
39. Ueland, P. M., and Døskeland, S. O. An adenosine 3':5'-monophosphate-adenosine binding protein from mouse liver. Purification and partial characterization. J. Biol. Chem., *252:* 677-686, 1977.
40. Ueland, P. M., and Helland, S. *S*-Adenosylhomocysteinase from mouse liver. Catalytic properties at cellular enzyme level. J. Biol. Chem., *255:* 7722-7727, 1980.
41. Ueland, P. M., and Saebø. J. Sequestration of adenosine in crude extract from mouse liver and other tissues. Biochim. Biophys. Acta, *587:* 341-352, 1979.
42. Ueland, P. M., and Saebø, J. *S*-Adenosylhomocysteinase from mouse liver. Effect of adenine and adenine nucleotides on the enzyme catalysis. Biochemistry, *18:* 4130-4135, 1979.
43. Ueland, P. M., Skotland, T., Døskeland, S. O., and Flatmark, T. An adenosine 3':5'-monophosphate-adenosine binding protein from mouse liver: some physicochemical properties. Biochim. Biophys. Acta, *533:* 57-65, 1978.
44. Wainfan, E., and Landsberg, B. Inhibition of transfer ribonucleic acid methylating enzymes by cytotoxic analogues of adenosine. Biochem. Pharmacol., *22:* 493-500, 1973.
45. Walsh, C. Recent developments in suicide substrates and other active site-directed inactivating agents of specific target enzymes. *In:* E. Quagliariello (ed.), Horizons in Biochemistry and Biophysics, Vol. 3, pp. 36-81. Reading, Mass.: Addison-Wesley Publishing Co., Inc., 1977.
46. Woo, P. W. K., Dion, H. W., Lange, S. M., Dahl, L. F., and Durham, L. J. A novel adenosine and ara-A deaminase inhibitor, (R)-3-(-2-deoxy-β-D-erythro-pentofuranosyl)-3,6,7,8-tetrahydroimidazo[4,5-*d*][1,3]diazepin-8-ol. J. Heterocycl. Chem., *11:* 641-643, 1974.
47. York, J. L., and LePage, G. A. A proposed mechanism for the action of 9-β-D-arabinofuranosyladenine as an inhibitor of the growth of some ascites cells. Can. J. Biochem., *44:* 19-26, 1966.
48. York, J. L., and LePage, G. A. A kinetic study of the deamination of some adenosine analogues. Can. J. Biochem., *44:* 331-337, 1966.
49. Zappia, V., Zydek-Cwick, C. R., and Schlenk, F. The specificity of *S*-adenosylmethionine derivatives in methyl transfer reactions. J. Biol. Chem., *244:* 4499-4509, 1969.