[CANCER RESEARCH 42, 2861–2866, July 1982]
0008-5472/82/0042-0000$02.00

Reactivation of *S*-Adenosylhomocysteine Hydrolase Activity in Cells Exposed to 9-β-D-Arabinofuranosyladenine[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) inactivates isolated *S*-adenosyl-L-homocysteine (AdoHcy) hydrolase (EC 3.3.1.1) as well as AdoHcy hydrolase in intact cells. Whereas the inactivation in cell-free systems is an irreversible process, the AdoHcy hydrolase activity in rat hepatocytes exposed to ara-A gradually recovered upon prolonged incubation of the cells in a medium devoid of ara-A. This process, tentatively termed reactivation of the enzyme, was nearly totally dependent on a high level of adenosine deaminase in the extracellular medium, which induced a decrease in intracellular content of adenosine as well as ara-A. Reactivation of intracellular enzyme was inhibited by adenosine deaminase inhibitors [2′-deoxycoformycin and *erythro*-9-(2-hydroxy-3-nonyl)adenine] and the synthetic substrate for AdoHcy hydrolase, 3-deazaadenosine. An inhibitor of protein synthesis (cycloheximide) was without effect. Homocysteine, which protected the intracellular AdoHcy hydrolase against inactivation by ara-A, induced no reactivation of the enzyme. The half-life of the intracellular ara-A-AdoHcy hydrolase complex was about 90 min and was not affected by adenosine deaminase, 3-deazaadenosine, or homocysteine added to the cell suspension. However, the rate of elimination of the complex in the hepatocytes exceeded the rate of reactivation of AdoHcy hydrolase. Thus, the elimination process accounted for the reactivation, but no correlation between these two processes was observed. Reactivation of intracellular AdoHcy hydrolase caused a pronounced fall in cellular content of AdoHcy. The possibility that reduced cellular level of AdoHcy induced the reactivation of AdoHcy hydrolase seemed unlikely. This statement was based on the observation that reactivation was observed also under conditions of high concentrations of AdoHcy (obtained by the addition of homocysteine to the cell suspension). Reactivation of AdoHcy hydrolase with a concomitant decrease in cellular level of AdoHcy could also be demonstrated with mouse plasmacytoma (MPC-11) cells and mouse fibroblasts (L-929) exposed to ara-A, but the reactivation process was far less pronounced than with hepatocytes.

INTRODUCTION

The antibiotic, ara-A,[3] an antiviral and antitumor agent (4), irreversibly inactivates isolated AdoHcy hydrolase (10), the enzyme responsible for the metabolic degradation of the endogenous transmethylase inhibitor, AdoHcy (3). The kinetics of the inactivation process suggest that ara-A is an active site-directed agent (5, 7, 10). ara-A also inactivates AdoHcy hydrolase in intact cells (9, 11), leading to a massive buildup of AdoHcy (9, 25) and inhibition of cellular methylation.[4] These data are in favor of the hypothesis (19) that some effects of ara-A stem from blocking the degradation of AdoHcy.

Whereas the inactivation of isolated AdoHcy hydrolase by ara-A is an irreversible process, the enzyme activity in intact cells gradually recovers. This process is greatly enhanced when the extracellular medium contains high levels of adenosine deaminase. Data are presented suggesting that the inactive ara-A-enzyme complex is reactivated in intact cells.

MATERIALS AND METHODS

Chemicals. AdoHcy, DL-homocysteine, adenosine, inosine, HEPES, collagenase (type I), albumin (Fraction V), ara-A, and cycloheximide were from Sigma Chemical Co., St. Louis, Mo. *erythro*-9-(2-hydroxy-3-nonyl)adenine and dCF were gifts from Burroughs Wellcome Co., Research Triangle Park, N. C., and from the Developmental Therapeutics Program, Chemotherapy, National Cancer Institute, Bethesda, Md., respectively. [8-^{14}C]Adenosine (590 mCi/mmol) and [2,8-^{3}H]ara-A (22 Ci/mmol) were obtained from the Radiochemical Centre, Amersham, England. [8-^{14}C]AdoHcy (590 mCi/mmol) was synthesized enzymatically from [8-^{14}C]adenosine and purified as described elsewhere (23). AdoHcy hydrolase was purified to apparent homogeneity from mouse liver according to a published procedure (21).

Assay for AdoHcy Synthase and Hydrolase Activities. This was performed by a radiochemical method described elsewhere (23). The assay mixture used for measurement of the synthetic reaction contained 50 or 200 μM [8-^{14}C]adenosine and 3 mM DL-homocysteine. The hydrolysis of AdoHcy was determined in the presence of 50 μM [8-^{14}C]AdoHcy and adenosine deaminase (50 units/ml). The assay buffer was 80 mM potassium phosphate, pH 7.0, containing 80 mM KCl, 0.2% bovine serum albumin, and 10 mM 2-mercaptoethanol. The temperature was 37°.

Preparation of Isolated Rat Hepatocytes. Hepatocytes were prepared by a slight modification (17) of the collagenase perfusion method described by Berry and Friend (2). Cell viability (17) was higher than 95% at the start of the experiments.

Cell Lines and Culture Conditions. Mouse plasmacytoma (MPC-11) cells and mouse fibroblasts (L-929) were grown in suspension culture under conditions described previously (9).

Incubation Conditions. The isolated rat hepatocytes and the cultured cells were incubated in the same medium, consisting of HEPES buffer, pH 7.4, salts, bovine serum albumin, glucose, and antibiotics, as described in detail in a previous publication (9).

Extraction of AdoHcy Hydrolase from Intact Cells. Cells were incubated with ara-A or other effectors and were separated from the medium by centrifugation of aliquots of 200 μl through oil (9). The cell

[1] Supported by grants from the Norwegian Society for Fighting Cancer. A preliminary report on this work was presented at the Conference on Transmethylation, Lake of the Ozarks, Mo., October 26 to 29, 1981.

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

[3] The abbreviations used are: ara-A, 9-β-D-arabinofuranosyl-adenine; AdoHcy, *S*-adenosyl-L-homocysteine; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; dCF, 2′-deoxycoformycin; HPLC, high-performance liquid chromatography.

Received January 4, 1982; accepted April 8, 1982.

[4] J-S. Schanche and T. Schanche, unpublished results.

pellets were immediately homogenized in (200 μl) ice-cold 80 mM potassium phosphate buffer, pH 7.0, containing 20% glycerol, 2 mM dithiothreitol, 3 mM DL-homocysteine, and 0.5% Triton X-100. The suitability of this buffer for the extraction of AdoHcy hydrolase from intact cells has been documented (9).

Determination of Cellular Content of AdoHcy. Samples (1 ml) from the cell suspension were centrifuged, the cell pellets were extracted with 5% sulfosalicylic acid, and the high-speed supernatant of the extract was analyzed by HPLC on a Partisil 10 SCX column, essentially as described (9). The ammonium phosphate buffer was replaced by 50 mM ammonium formate, pH 4.0, because this volatile buffer caused no precipitation of salt at the piston seals and other junctions of the solvent delivery system.

AdoHcy was also determined by reverse-phase liquid chromatography on a 3-μm octadecyl silane Hypersil column (0.5 x 10 cm) packed by Dr. Solheim, using a Shandon column packer. The cellular extract was neutralized (to pH 7.5) with 2 N Tris, and the mobile phase was 7.7% methanol in 9 mM potassium phosphate buffer, pH 6.0, and the flow rate was 1 ml/min.

Determination of Cellular Content of Adenosine and ara-A. This was performed by reverse-phase liquid chromatography, using the same column and mobile phase as described above, except that in some instances the concentration of methanol was reduced to 5 to 6%.

Determination of the Half-Life of the Intracellular ara-A-Enzyme Complex. Rat hepatocytes (4.4×10^6 cells/ml) were incubated for 30 min with 0.5 μM [^{3}H]ara-A (22 Ci/mmol) and then for 5 min with 150 μM unlabeled ara-A (to block the remaining sites of AdoHcy hydrolase). Cells so treated were washed in an ara-A-free medium and resuspended in a medium containing adenosine deaminase, homocysteine (3 mM), 3-deazaadenosine (50 μM), or no addition. At various time points, samples (250 μl) from the cell suspension were centrifuged through oil (9), and the cell pellets were homogenized in 75 μl ice-cold buffer (15 mM HEPES, pH 7.0, supplemented with 0.5% Triton X-100 and 10 mM 2-mercaptoethanol) containing 10 mM adenosine, to prevent binding of ara-A after cellular disrupture. The high-speed supernatant from the cellular extract (40 μl) was subjected to HPLC on a protein column (I-250; Waters Associates, Inc., Milford, Mass.), as described previously (9).

RESULTS

ara-A-induced Accumulation of AdoHcy in the Absence and Presence of dCF. The adenosine deaminase inhibitor (6) dCF did not enhance the rate and extent of inactivation of intracellular AdoHcy hydrolase in rat hepatocytes (9). The accumulation of AdoHcy in hepatocytes exposed to ara-A plus dCF increased progressively for at least 3 hr. In the absence of dCF, the AdoHcy accumulation induced by ara-A was less pronounced. The cellular content of AdoHcy reached a plateau after about 1 hr and then declined (Chart 1*A*).

Reactivation of AdoHcy Hydrolase in Rat Hepatocytes. When hepatocytes were exposed to ara-A without dCF, the inactivation of intracellular AdoHcy hydrolase was followed by a slight progressive increase in enzyme activity. This process, tentatively termed reactivation of AdoHcy hydrolase, was enhanced greatly by supplementing the extracellular medium with a high level of adenosine deaminase and was associated with a pronounced fall in the cellular content of AdoHcy (Chart 1*B*). The velocity of the reactivation process increased in a dose-dependent manner as a function of extracellular adenosine deaminase activity (Chart 2*A*). The same results were obtained when the enzyme activity was determined in the synthetic and hydrolytic direction (data not shown).

To investigate whether the increase in AdoHcy hydrolase activity was an artifact related to consumption of inhibitor(s) of AdoHcy hydrolase, the following experiment was conducted. The recovery of purified AdoHcy hydrolase added to the extraction buffer in which the hepatocytes were homogenized was determined at various time points after the addition of adenosine deaminase to the cell suspension. The amount of exogenous AdoHcy hydrolase recovered was high (about 70%) and independent of the duration of cellular treatment with adenosine deaminase (Chart 2*A*).

Reactivation of AdoHcy hydrolase in rat hepatocytes could be demonstrated under conditions of high levels of cellular AdoHcy by adding homocysteine (3 mmol/liter) to the cell suspension at the time of addition of adenosine deaminase (*i.e.*, after 30 min of cellular exposure to ara-A). The experimental design was otherwise as described in the legend to Chart 1*B*. Under these conditions, the rate of reactivation was reduced by about 20% (Chart 2*B*), and the AdoHcy content of

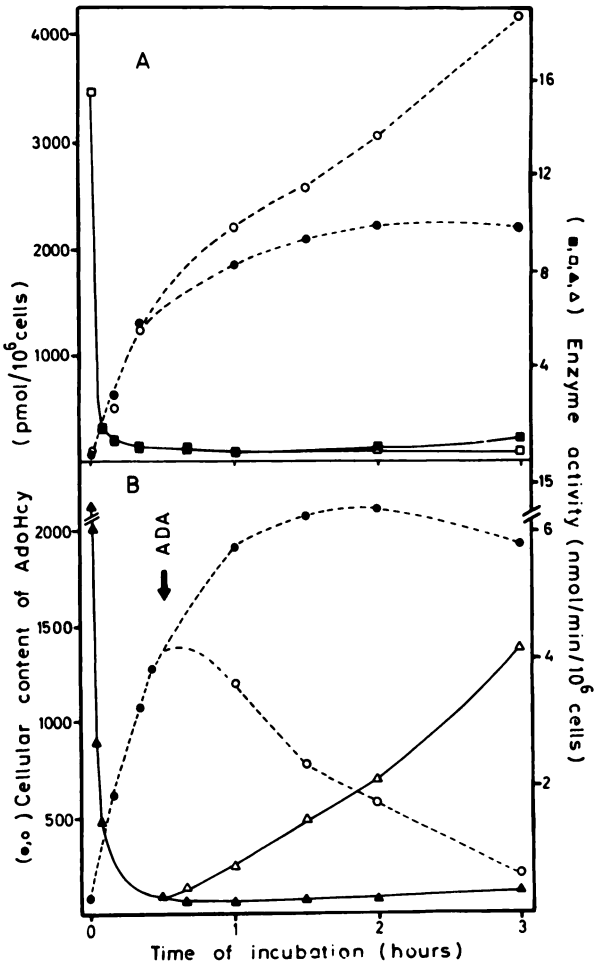

Chart 1. AdoHcy hydrolase activity and accumulation of AdoHcy in rat hepatocytes exposed to ara-A. In *A*, rat hepatocytes (4.3×10^6 cells/ml) were incubated with 100 μM ara-A in the absence (●, ■) and presence (○, □) of 1 μM dCF. The cells exposed to dCF were preincubated with this compound for 30 min before the addition of ara-A. The cellular content of AdoHcy (●, ○) and AdoHcy hydrolase activity (assayed in the synthetic direction) (■, □) are plotted *versus* time of incubation with ara-A. In *B*, hepatocytes (4.5×10^6 cells/ml) were incubated in the presence of 100 μM ara-A. After 30 min of incubation, a portion of the cell suspension was made 16 units/ml in adenosine deaminase (*ADA*) (*arrow*). AdoHcy hydrolase activity (▲, △) and cellular content of AdoHcy (●, ○) in the absence (●, ▲) and presence (○, △) of extracellular adenosine deaminase are plotted *versus* time of incubation.

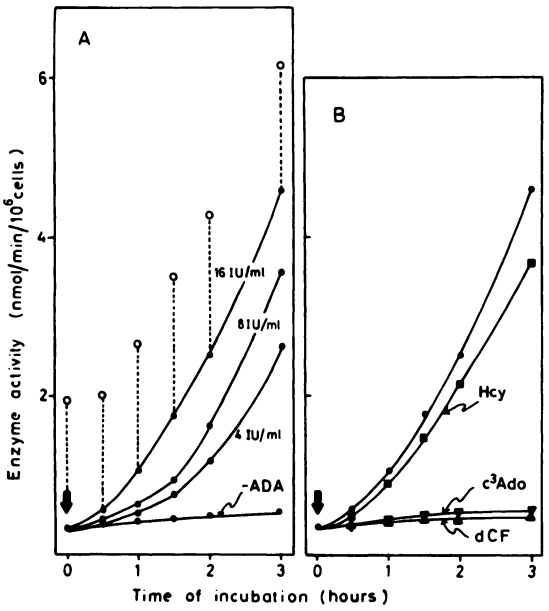

Chart 2. Factors affecting the reactivation of intracellular AdoHcy hydrolase. In *A*, rat hepatocytes (4.3 × 10^6 cells/ml) were preincubated with 100 μM ara-A for 30 min, and then adenosine deaminase (*ADA*) was added to the cell suspension (*arrow*). The concentration of extracellular adenosine deaminase varied from 0 to 16 units/ml (●). In a parallel experiment, the ara-A-treated cells were made 16 units/ml in adenosine deaminase, and the cell pellets were homogenized in an extraction buffer supplemented with purified AdoHcy hydrolase (3 μg/ml) (○). - - - -, recovery of exogenous AdoHcy hydrolase. In *B*, the increase in AdoHcy hydrolase activity induced by extracellular adenosine deaminase (16 units/ml) was determined in the presence of 3 mM DL-homocysteine (*Hcy*) (■), 50 μM 3-deazaadenosine (*c³Ado*) (▼), 1 μM dCF (▲), or no addition (●). These compounds were added to the cell suspension together with adenosine deaminase. The experimental design was otherwise as described in *A*.

hepatocytes increased from 80 to 1800 pmol/10^6 cells (30 min after the addition of ara-A to the cell suspension), 4800 pmol/10^6 cells (90 min), and 2100 pmol/10^6 cells (3 hr). These data suggest that reactivation of the enzyme is not induced by low cellular level of AdoHcy but points to the increase in AdoHcy hydrolase activity as the primary event.

Repetitive additions of homocysteine (3 mmol/liter) at intervals of 1 hr to rat hepatocytes induced a continuous high level of AdoHcy (about 3900 pmol/10^6 cells) in these cells. When ara-A (100 μM) was added to hepatocytes so treated, AdoHcy hydrolase activity was only moderately reduced (to about 50%) after 4 hr of incubation. Thus, the inactivation of AdoHcy hydrolase by ara-A was in this way essentially inhibited. Treatment of hepatocytes with ara-A (100 μM) for 30 min reduced the AdoHcy hydrolase activity to 2 to 3% (9). Reactivation of the enzyme in these cells was not obtained by repetitive additions (3 mmol/liter at intervals of 1 hr) of homocysteine, although a continuous massive accumulation of AdoHcy (up to 13,000 pmol/10^6 cells) was observed (data not shown). Washing the hepatocytes in an ara-A-free medium did not effect a marked increase in the rate of reactivation of AdoHcy hydrolase (data not shown).

Treatment of rat hepatocytes not exposed to ara-A with either homocysteine or adenosine deaminase did not alter the AdoHcy hydrolase activity (data not shown).

Effect of Adenosine Deaminase Inhibitors, Inosine, 3-Deazaadenosine, and Cycloheximide. The reactivation of inactivated AdoHcy hydrolase induced by extracellular adenosine deaminase was inhibited by the inclusion of dCF (1 μM) (Chart 2*B*) or *erythro*-9-(2-hydroxy-3-nonyl)adenine (10 μM) in the cell suspension. The reactivation process was also arrested when dCF was added to hepatocytes (exposed to ara-A) 1 hr after the reactivation was initiated by addition of adenosine deaminase (Chart 3). These data suggest that the effect of extracellular adenosine deaminase is related to the catalytic activity of the enzyme and is not mediated by contaminants in the enzyme preparation. Furthermore, a continuous high level of extracellular adenosine deaminase is required for maintenance of the reactivation process. The effect of adenosine deaminase is not related to the production of inosine, because inosine (100 μM) induced no reactivation of inactivated AdoHcy hydrolase in rat hepatocytes (data not shown).

Incubation of hepatocytes with 3-deazaadenosine (50 μM) for up to 3 hr did not affect the AdoHcy hydrolase activity of these cells. 3-Deazaadenosine (50 μM) nearly completely inhibited the reactivation of AdoHcy hydrolase when added to the cell suspension together with adenosine deaminase (Chart 2*A*). Cycloheximide (100 μg/ml), an inhibitor of protein synthesis (24), had no effect on the reactivation process (data not shown).

Test for Reactivation with Broken-Cell Preparation. Hepatocytes (5 × 10^6 cells/ml; 2 ml) were incubated for 30 min with 100 μM ara-A. The cells were then pelleted and resus-

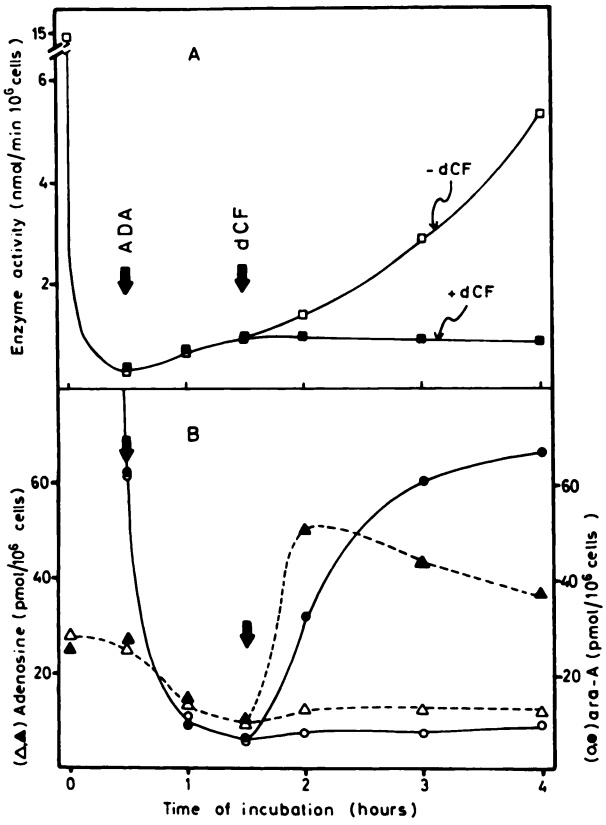

Chart 3. The relation between reactivation of AdoHcy hydrolase and cellular content of adenosine and ara-A. Rat hepatocytes (4.4 × 10^6 cells/ml) were incubated with 100 μM of ara-A. After 30 min of incubation, adenosine deaminase (*ADA*) (16 units/ml) was added to the cell suspension (*arrow*), and after 90 min, one portion of the cell suspension was made 1 μM in dCF (*arrow*). *A*, AdoHcy hydrolase activity of hepatocytes treated with ara-A and adenosine deaminase (□) and the enzyme activity of cells exposed to ara-A, adenosine deaminase, and then dCF (■). *B*, corresponding values for the amounts of ara-A and adenosine associated with the cells. ●, ▲, ara-A or adenosine content in cells supplemented with dCF at 90 min; ○, △, amounts in cells not exposed to dCF.

pended (0.5 ml) in a hypotonic medium (30 mM potassium phosphate, pH 7.4, containing 2 mM dithiothreitol). AdoHcy hydrolase in crude homogenate from hepatocytes (not exposed to ara-A) was stable in this medium and lost only about 20% of the activity after 3 hr of incubation at 37°. The cells treated with ara-A were broken by freezing and thawing, after which the broken-cell preparation was made 4 units/ml in adenosine deaminase and incubated for increasing periods of time (0, 0.5, 1, 2, and 3 hr) at 37°. Aliquots (50 μl) were assayed for AdoHcy synthase activity (in the presence of 50 μM dCF). No reactivation of AdoHcy hydrolase could be demonstrated (data not shown).

Cellular Content of Adenosine and ara-A. Treatment of hepatocytes with adenosine deaminase reduced the amount of adenosine associated with the cells from about 30 pmol/10^6 cells (the amount varied in the range of 20 to 80 pmol/10^6 cells from one cell preparation to another) to about 12 pmol/10^6 cells within 5 min. No further reduction in cellular adenosine content was observed (data not shown). The amount of intracellular ara-A may be difficult to determine in cells suspended in a medium containing high concentrations of ara-A because of interference from ara-A in the adherent extracellular water. This problem is avoided by measurement of ara-A associated with cells treated with adenosine deaminase, *i.e.*, under conditions favoring reactivation of AdoHcy hydrolase. Addition of dCF to hepatocytes during the reactivation process induced by extracellular adenosine deaminase increased the cellular content of both ara-A and adenosine 3- to 4-fold (Chart 3).

The amount of adenosine in the extracellular medium was less than 0.5 μM for hepatocytes not exposed to adenosine deaminase and was reduced to below the detection limit (0.1 μM) when the hepatocytes were treated with adenosine deaminase. Thus, adenosine in adherent extracellular medium does not account for adenosine associated with the hepatocytes.

Inactivation of Reactivated AdoHcy Hydrolase in Rat Hepatocytes. Rat hepatocytes exposed to ara-A (100 μM) for 30 min were treated with adenosine deaminase for 3 hr to reactivate AdoHcy hydrolase. At this time point, the cell suspension was made 1 μM in dCF and incubated for a further 10 min (to inhibit adenosine deaminase). The rate of inactivation of reactivated AdoHcy hydrolase in these cells (rate constant of 0.71 min^{-1}) by 100 μM ara-A was the same as the rate of inactivation of the enzyme in nontreated cells (9) (data not shown).

Half-Life of the Intracellular ara-A-AdoHcy Hydrolase Complex. The amount of the ara-A-AdoHcy hydrolase complex in hepatocytes and the half-life of the complex were determined by subjecting extract from hepatocytes to gel filtration on a HPLC protein column (Chart 4). Measurement of the AdoHcy synthase activity in the fractions (of extract from cells not treated with ara-A) showed that the enzyme activity coeluted with the ara-A-binding activity (data not shown). The half-life of the intracellular complex was about 90 min (Chart 4) and was not affected by extracellular adenosine deaminase (16 units/ml), the addition of 3-deazaadenosine (50 μM), or repetitive additions of homocysteine (3 mmol/liter each hr) to the cell suspension. 3-Deazaadenosine and its metabolite, S-3-deazaadenosylhomocysteine, as well as a high level of AdoHcy induced by homocysteine, interact with the catalytic site of AdoHcy hydrolase (3) and thereby may block the binding of ara-A to the enzyme. Thus, lack of effect of homocysteine and 3-deazaadenosine suggests no rebinding of ara-A to reacti-

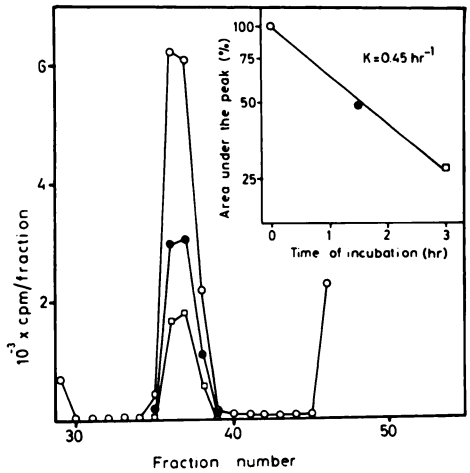

Chart 4. Half-life of the intracellular ara-A-AdoHcy hydrolase complex. The half-life of the ara-A-enzyme complex was determined by subjecting cytosol from hepatocytes (4.5 × 10^6 cells/ml) exposed to [^{3}H]ara-A to chromatography on a HPLC protein column as described in "Materials and Methods." Samples from the cells suspension were analyzed at 0 min (○), 90 min (●), and 3 hr (□) after the cells were resuspended in a ara-A-free medium. The radioactive peak eluting between *Fractions 35* and *39* coeluted exactly with purified AdoHcy hydrolase (retention time, 10.94 min) (9). *Inset*, area under the peaks plotted *versus* time of incubation.

vated AdoHcy hydrolase under the conditions of the experiment (*i.e.*, in the absence of extracellular ara-A). Only trace amounts of the ara-A-enzyme complex were detected in the extracellular medium, suggesting that the decrease in cellular content of the complex is not a result of cellular leakage. This suggestion is in accordance with the finding that AdoHcy hydrolase activity of rat hepatocytes (not exposed to ara-A) decreased only slightly (less than 20%) after prolonged (4 hr) incubation.

Reactivation of AdoHcy Hydrolase in Mouse Fibroblasts (L-929) and Mouse Plasmacytoma (MPC-11) Cells. AdoHcy hydrolase in L-929 cells and MPC-11 cells was inactivated by ara-A (100 μM). After 30 min, the cell suspensions were supplemented with adenosine deaminase (16 units/ml). AdoHcy hydrolase activity increased by 30 to 200% in these cultured cells after 3.5 hr of incubation with adenosine deaminase. The slight reactivation of the enzyme activity in these cultured cells was associated with a slight reduction in cellular content of AdoHcy, relative to the AdoHcy content in cells not exposed to adenosine deaminase (Chart 5). Thus, reactivation of AdoHcy hydrolase could be demonstrated with both L-929 cells and MPC-11 cells, but this process was far less pronounced than in rat hepatocytes under the conditions of the experiments.

DISCUSSION

The possibility existed that the increase in AdoHcy hydrolase activity in cells induced by adenosine deaminase in the extracellular medium is an artifact related to inhibition or inactivation of the enzyme during or after homogenization. This possibility is excluded on the basis of the following observations. The buffer used for the extraction of AdoHcy hydrolase ensures essentially no inactivation of the enzyme after cellular lysis (9). The recovery of purified AdoHcy hydrolase added to the extraction buffer was high and independent of the time of cellular exposure to adenosine deaminase (Chart 2*A*). Furthermore, the same results were obtained when AdoHcy hydrolase activity was determined in the hydrolytic and synthetic direction.

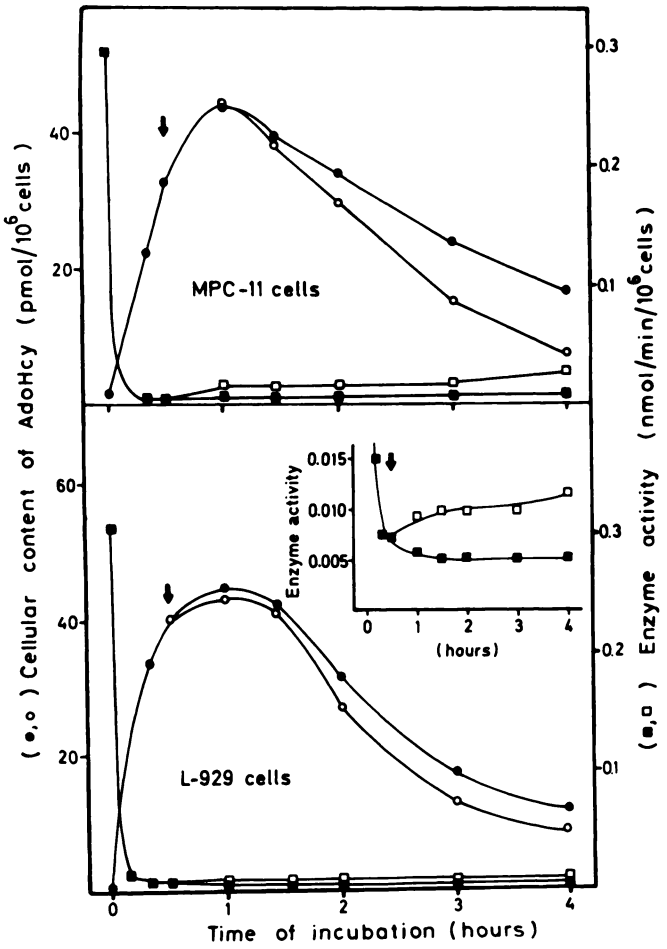

Chart 5. Reactivation of AdoHcy hydrolase and cellular content of AdoHcy in MPC-11 cells and L-929 cells. MPC-11 cells (6.1×10^6 cells/ml) and L-929 cells (5.9×10^6 cells/ml) were incubated with 100 μM ara-A. After 30 min of incubation, a portion of the cell suspensions was made 16 units/ml in adenosine deaminase (*arrow*). AdoHcy hydrolase activity (assayed in the synthetic direction) (▪, □) and cellular content of AdoHcy (●, ○) in the absence (▪, ●) and presence (□, ○) of adenosine deaminase are plotted *versus* time of incubation. *Inset*, replot of the data for the enzyme activity in L929 cells. The same *symbols* are used as above.

The synthesis of AdoHcy, which is assayed in the presence of dCF, is insensitive to adenosine deaminase in the assay mixture. On the other hand, hydrolysis of AdoHcy in the presence of excess adenosine deaminase is not influenced by the presence of substrates of this enzyme. Finally, the increase in AdoHcy hydrolase activity is associated with a decrease in cellular content of AdoHcy (Chart 1*B*), indicating that the reactivation process is an intracellular event.

At least 3 possibilities exist as to the nature of the reappearance of AdoHcy hydrolase in cells exposed to ara-A: (*a*) intracellular synthesis of AdoHcy hydrolase; (*b*) unmasking of AdoHcy hydrolase activity; and (*c*) reactivation of the inactive ara-A-enzyme complex.

An inhibitor of protein synthesis, cycloheximide, did not block the increase in enzyme activity induced by extracellular adenosine deaminase. This observation argues against synthesis of enzyme. The possibility that increase in AdoHcy hydrolase activity was related to unmasking of AdoHcy hydrolase was considered in the light of the fact that the enzyme may form an inactive complex with adenosine, AdoHcy (13, 22), and possibly other metabolites. The possibility existed that lowering the cellular content of adenosine leads to formation of active enzyme from an inactive adenosine-enzyme complex. If this was the case, one would expect to observe an increase in AdoHcy hydrolase activity in hepatocytes not exposed to ara-A upon treatment with adenosine deaminase. No unmasking of AdoHcy hydrolase was observed with these cells. Thus, based on the observations listed above, it is concluded that ara-A-AdoHcy hydrolase complex is reactivated in the intact rat hepatocytes.

The concept of reactivation of inactivated AdoHcy hydrolase is supported by the observation that the ara-A-enzyme complex shows a half-life in hepatocytes (rate constant of 0.4 hr^{-1}) which may account for the increase in enzyme activity [about 20% of the enzyme activity is reactivated after 3.5 hr (Charts 2 and 3)]. No effect of adenosine deaminase, 3-deazaadenosine, or homocysteine on the half-life of the complex suggests that the degradation or dissociation of the complex does not determine the rate of reactivation under the conditions of the experiment.

A high level of extracellular adenosine deaminase decreases the cellular content both of adenosine and ara-A in cells exposed to ara-A (Chart 3). Adenosine deaminase may enhance the reactivation process by trapping ara-A continously formed from 9-β-D-arabinofuranosyladenine 5′-monophosphate. However, data shown in "Results," suggest that no rebinding of ara-A to AdoHcy hydrolase takes place in cells suspended in a medium devoid of ara-A. Furthermore, procedures which protect intracellular AdoHcy hydrolase against inactivation by ara-A (*i.e.*, repetitive additions of homocysteine to the cell suspension) do not increase the enzyme activity in ara-A-treated cells. These data, taken together, indicate that trapping of ara-A is not the mechanism of action of extracellular adenosine deaminase.

A role of low cellular content of adenosine in the reactivation process is strongly supported by the observation that the adenosine analog, 3-deazaadenosine, nearly completely inhibits the reactivation of AdoHcy hydrolase. 3-Deazaadenosine is a better substrate for AdoHcy hydrolase than adenosine itself (3) and is neither a substrate nor an inhibitor of other enzymes metabolizing adenosine, *i.e.*, adenosine deaminase (15) or adenosine kinase (16).

The possible role of adenosine in the reactivation process should perhaps be related to the finding that this nucleoside forms a stable complex with AdoHcy hydrolase (13), also under conditions of enzyme catalysis (22) and in intact rat hepatocytes (20). In addition, adenosine is a potent inhibitor of the hydrolysis of AdoHcy catalyzed by AdoHcy hydrolase (3). Reactivation of AdoHcy hydrolase is possibly enhanced under conditions favoring hydrolysis of AdoHcy. This suggestion reconciles the finding that 3-deazaadenosine and to a less degree homocysteine (Chart 2) are inhibitors of the reactivation process. Both 3-deazaadenosine and homocysteine inhibit the hydrolytic reaction (3).

ara-A (8, 12) and 2′-deoxyadenosine (1) inactivate AdoHcy hydrolase *in vitro* by a mechanism which involves irreversible reduction of enzyme-bound NAD^+. Furthermore, enzyme-bound NAD^+ is also reduced in the presence of adenosine (18), but adenosine is a far less potent inactivator of the enzyme than ara-A and 2′-deoxyadenosine (5). Thus, one may speculate whether AdoHcy hydrolase inactivated by ara-A is

reactivated through the action of mechanism(s) protecting the cells against naturally occurring nucleosides (such as 2′-deoxyadenosine and adenosine) exerting a continuous impact on AdoHcy hydrolase.

The possibility of a cellular process reactivating AdoHcy hydrolase in human lymphoblasts exposed to 2′-deoxyadenosine has been suggested in the light of the finding that the enzyme activity recovered at a higher rate than was expected from the turnover of proteins in these cells (14). Furthermore, AdoHcy hydrolase of erythrocytes in patients treated with ara-A was inactivated (11), and the enzyme reappeared faster than could have been expected on the basis of production of new erythrocytes after the end of ara-A treatment.[5]

Reactivation of AdoHcy hydrolase could be demonstrated with cultured cells (Chart 5). Furthermore, AdoHcy hydrolase in most tissues of mice injected with ara-A was almost completely recovered 8 to 10 hr after a single administration of the compound.[6] These findings, together with those cited above, suggest that reactivation of inactivated AdoHcy hydrolase occurs in several cell types and animal tissues.

In the absence of dCF, the amount of AdoHcy decreases slightly in rat hepatocytes (Chart 1*B*) and markedly in the cultured cells (Chart 5) after prolonged incubation with ara-A, even under conditions of no enhancement of the reactivation mechanism by extracellular adenosine deaminase. Lowering of AdoHcy content in these cells may be caused by metabolism of AdoHcy catalyzed by the fraction of AdoHcy hydrolase which is not inactivated and by export of AdoHcy into the extracellular medium (9).

dCF inhibits the reactivation of intracellular AdoHcy hydrolase (Chart 2*B*) and may in this way enhance the effect of ara-A on cellular metabolism of AdoHcy. Furthermore, dCF increases the cellular level of adenosine (Chart 3*B*) which may inhibit residual AdoHcy hydrolase activity and thereby lead to accumulation of AdoHcy. These suggestions are in accordance with the finding that dCF greatly increases the accumulation of AdoHcy in various tissues of mice treated with ara-A.[6] Adenosine deaminase inhibitors, including dCF, have been found to potentiate the biological effects of ara-A in intact cells and in whole animals (4, 6). Thus, the present work adds to published data (4, 6), suggesting the usefulness of a combination therapy with ara-A plus dCF.

ACKNOWLEDGMENTS

The authors are grateful to Dr. I. F. Pryme and Dr. A. K. Abraham at the Biochemical Institute, University of Bergen, for their generous gift of the cultured cells used in this study. The expert technical assistance of H. Bergesen is highly appreciated.

REFERENCES

1. Abeles, R. H., Tashjian, A. H., and Fish, S. The mechanism of inactivation of *S*-adenosylhomocysteinase by 2′-deoxyadenosine. Biochem. Biophys. Res. Commun., *95:* 612–617, 1980.
2. Berry, M. N., and Friend, D. S. High-yield preparation of isolated rat liver parenchymal cells. A biochemical and fine structural study. J. Cell. Biol., *43:* 506–520, 1969.
3. Cantoni, G. L., and Chiang, P. K. The role of *S*-adenosylhomocysteine and *S*-adenosylhomocysteine hydrolase in the control of biological methylations. *In:* D. Cavallini, G. E. Gaull, and V. Zappia (eds.), Natural Sulfur Compounds. Novel Biochemical and Structural Aspects, pp. 67–80. New York: Plenum Publishing Corp., 1980.
4. Cass, C. E. 9-Beta-D-arabinofuranosyladenine (ara-A). *In:* F. E. Hahn (ed.), Antibiotics, Vol. 5, Part 2, pp. 85–109. Berlin: Springer Verlag, 1979.
5. Chiang, P. K., Guranowski, A., and Segall, J. E. Irreversible inhibition of *S*-adenosylhomocysteine hydrolase by nucleoside analogs. Arch. Biochem. Biophys., *207:* 175–184, 1981.
6. Glazer, R. I. Adenosine deaminase inhibitors: their role in chemotherapy and immunosuppression. Cancer Chemother. Pharmacol., *4:* 227–235, 1980.
7. Helland, S., and Ueland, P. M. 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, 1981.
8. Helland, S., and Ueland, P. M. The relation between the functions of 9-β-D-arabinofuranosyladenine as inactivator and substrate of *S*-adenosylhomocysteine hydrolase. J. Pharmacol. Exp. Ther., *218:* 758–763, 1981.
9. Helland, S., and Ueland, P. M. Inactivation of *S*-adenosylhomocysteine hydrolase by 9-β-D-arabinofuranosyladenine in intact cells. Cancer Res., *42:* 1130–1136, 1982.
10. 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.
11. Hershfield, M. S. Active site directed inactivation of *S*-adenosylhomocysteine hydrolase: a possible basis for antiviral effects of adenine arabinoside. Clin. Res., *27:* 346, 1979.
12. Hershfield, M. S. Inactivation of *S*-adenosylhomocysteine hydrolase: mechanism and occurrence *in vivo* in disorders of purine nucleoside catabolism. *In:* R. T. Borchardt, C. R. Creveling, and E. Usdin (eds.), The Biochemistry of *S*-Adenosylmethionine and Related Compounds. London: Macmillan Publishers, in press, 1982.
13. Hershfield, M. S., and Kredich, N. M. *S*-Adenosylhomocysteine hydrolase is an adenosine-binding protein: a target for adenosine toxicity. Science (Wash. D. C.), *202:* 757–760, 1978.
14. Hershfield, M. S., and Kredich, N. M. Resistance of an adenosine kinase-deficient human lymphoblastoid cell line to effects of deoxyadenosine on growth, *S*-adenosylhomocysteine hydrolase inactivation, dATP accumulation. Proc. Natl. Acad. Sci. U. S. A., *77:* 4292–4296, 1980.
15. Ikehara, M., and Fukui, T. Studies of nucleosides and nucleotides. LVIII. Deamination of adenosine analogs with calf intestine adenosine deaminase. Biochim. Biophys. Acta, *338:* 512–519, 1974.
16. Miller, R. L., Adamezyk, D. L., Miller, W. H., Koszalka, G. W., Rideout, J. L., Beacham, L. M., Chao, E. Y., Haggerty, J. J., Krenitsky, T. A., and Elion, G. B. Adenosine kinase from rabbit liver. II. Substrate and inhibitory specificity. J. Biol. Chem., *254:* 2346–2352, 1979.
17. Mørland, J., and Bessesen, A. Inhibition of protein synthesis by ethanol in isolated rat liver parenchymal cells. Biochim. Biophys. Acta, *474:* 321–320, 1977.
18. Palmer, J. L., and Abeles, R. H. The mechanism of action of *S*-adenosylhomocysteinase. J. Biol. Chem., *254:* 1217–1226, 1979.
19. 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.
20. Ueland, P. M. Intracellular sequestration of adenosine in rat hepatocytes. Acta Pharmacol. Toxicol., *49* (*Suppl.* 1): 80, 1981.
21. 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.
22. Ueland, P. M., and Helland, S. *S*-Adenosylhomocysteinase from mouse liver. Catalytic properties at cellular enzyme level. J. Biol. Chem., *255:* 7722–7727, 1980.
23. Ueland, P. M., and Sæbø, J. *S*-Adenosylhomocysteinase from mouse liver. Effect of adenine and adenine nucleotides on the same enzyme catalysis. Biochemistry, *18:* 4130–4135, 1979.
24. Vazquez, D. Inhibitors of protein synthesis. FEBS Lett., *40:* S63–S84, 1974.
25. Zimmerman, T. P., Wolberg, G., Duncan, G. S., and Elion, G. B. Adenosine analogues as substrates and inhibitors of *S*-adenosylhomocysteine hydrolase in intact lymphocytes. Biochemistry, *19:* 2252–2259, 1980.

[5] M. S. Hershfield, personal communication.
[6] S. Helland and P. M. Ueland, unpublished results.