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Binding of Adenosine to Intracellular *S*-Adenosylhomocysteine Hydrolase in Isolated Rat Hepatocytes*

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Per Magne Ueland and Svein Helland

From the Department of Pharmacology, University of Bergen, School of Medicine, MFH-bygget, 5016 Haukeland sykehus, Bergen, Norway

Binding of adenosine to *S*-adenosyl-L-homocysteine (AdoHcy) hydrolase (EC 3.3.1.1.) and partial conversion of bound adenosine to a substance liberating adenine has been demonstrated under conditions of enzymatic synthesis and hydrolysis of AdoHcy (Ueland, P. M., and Helland, S. (1980) *J. Biol. Chem.* 255, 7722-7727). Gel filtration of cytosol from isolated rat hepatocytes treated with [^{14}C]adenosine on a high performance liquid chromatography protein column showed that labeled adenine/adenosine eluted as a peak which cochromatographed exactly with AdoHcy hydrolase. Formation of this peak was inhibited by exposure of the cells to compounds (ara-A, 3-deazaadenosine, and homocysteine) interacting with the catalytic site of the enzyme. Furthermore, the adenine/adenosine-protein complex and AdoHcy hydrolase focused at exactly the same pH (pI = 5.76) in a granulated bed. On this basis it was concluded that labeled adenosine formed a stable complex with AdoHcy hydrolase.

A substantial portion (about 50%) of endogenous adenosine in rat hepatocytes seemed to be associated with AdoHcy hydrolase, and this portion equaled the amount of cellular adenosine which was not readily mobilized by high level of extracellular adenosine deaminase. Exposure of the hepatocytes to compounds which block the formation of the adenosine-AdoHcy hydrolase complex (ara-A, 3-deazaadenosine, and homocysteine) for 1 to 2.5 h only slightly reduced the amount of adenosine associated with the enzyme, indicating a slow turnover of the complex under the conditions of the experiment.

It was concluded that adenosine is sequestered in rat hepatocytes through the interaction with AdoHcy hydrolase. The physiological implication of this process may be related to the metabolism and biological effects of adenosine as well as the regulation of AdoHcy hydrolase activity.

Adenosine seems to be a regulator of various biological processes, including vasodilatation of the heart and other organs, platelet aggregation, lipolysis in adipose tissue, neurotransmission, cyclic AMP metabolism, and immunofunctions. Knowledge of the regulatory functions of adenosine has stimulated investigation of cellular components interacting with this purine (1, 2). Soluble adenosine binding proteins was first demonstrated by Yuh and Tao in rabbit erythrocytes (3), and has later been purified from various mammalian tissues (4–9). The physiological role of these binding proteins for adenosine remained unknown until Hershfield (10) and Hershfield and Kredich (11) identified adenosine binding proteins from human placenta and lymphoblasts as *S*-adenosyl-L-homocysteine hydrolase (EC 3.3.1.1.), the enzyme responsible for the metabolic degradation of the endogenous transmethylase inhibitor, AdoHcy[1] (12). This observation has later been confirmed by others (13–15).

Adenosine forms a stable complex with purified AdoHcy hydrolase (10, 16) and with this enzyme in crude tissue extract (17). A fraction of adenosine bound to AdoHcy hydrolase is converted to adenine or a substance liberating adenine (15, 17–19). Both tight binding of adenosine to AdoHcy hydrolase and formation of adenine can be demonstrated under conditions of enzyme catalysis (20). This observation is in favor of the hypothesis (17) that AdoHcy hydrolase functions as an adenosine binding protein in the intact cells. The present paper presents evidence for the existence of a stable adenosine-AdoHcy hydrolase complex in intact rat hepatocytes.

EXPERIMENTAL PROCEDURES

Materials—Adenosine, inosine, adenine, AdoHcy, DL-homocysteine, ara-A, Hepes, adenosine deaminase (type I from calf intestinal mucosa), collagenase (type I) were purchased from Sigma Chemical Co. 2′-Deoxycoformycin was a gift from the Developmental Therapeutics Program, Chemotherapy, National Cancer Institute, Bethesda, MD. [8-^{14}C]Adenosine (0.59 Ci/mmol) and [2,5′,8-^{3}H]adenosine (42 Ci/mmol) was obtained from the Radiochemical Centre, Amersham, ODS Hypersil 3 μm microparticle medium for reverse phase chromatography was from Shandon Southern Products Ltd. The ODS-Hypersil columns (0.5 × 10 cm, 9000–11000 theoretical plates) were packed by Dr. Schanche at our institute, using a Shandon column packer. The protein column for HPLC (I-250) was from Waters Associates, Milford, MA.

Purification of AdoHcy Hydrolase—The enzyme was purified to apparent homogeneity from mouse liver according to a published procedure (5).

Preparation and Incubation of Isolated Rat Hepatocytes—Hepatocytes were prepared by a slight modification (21) of the collagenase perfusion technique of Berry and Friend (22). Cell viability (21) was higher than 95% at the start of the experiments. The cells were incubated in an isotonic solution containing salts, bovine serum albumin, antibiotics, and glucose (21). The temperature was 37 °C.

Thin Layer Chromatography—Samples to be analyzed were deproteinized by addition of perchloric acid to a final concentration of 0.5 N. High speed supernatant of this extract was neutralized to pH 6 (18), cooled (0 °C), precipitated potassium perchlorate removed by centrifugation, and the supernatant analyzed by TLC on polyethyl-

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[1] The abbreviations used are: AdoHcy, *S*-adenosylhomocysteine; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; ara-A, 9-β-D-arabinofuranosyladenine; c^{3}Ado, 3-deazaadenosine; Hcy, L-homocysteine; TLC, thin layer chromatography; HPLC, high performance liquid chromatography.

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eneimine-impregnated cellulose plates, developed in glacial acetic acid/water/l-butanol (1:1:2) as described by Fain and Shepherd (23).

High Performance Liquid Chromatography—Samples of 25 μl were analyzed on an ODS-Hypersil column eluted isocratically with 7% methanol in 10 mM potassium phosphate buffer, pH 6.0, at a flow rate of 1 ml/min. The solvent delivery system was a SP 8000B or a SP 8700 from Spectra-Physics. The elution pattern was monitored by UV absorption at 254 nm, using a SP 8300 UV detector. Adenine (retention time of 4.34 min), adenosine (11.82 min) and 3-deazaadenosine (7.34 min) were separated in this system.

Determination of [^{14}C]Adenosine Binding to Intracellular AdoHcy Hydrolase—Rat hepatocytes (4–7.5 × 10^6 cells/ml) were incubated with [^{14}C]adenosine (20 μM, 59 mCi/mmol) and 1 μM of 2′-deoxycoformycin. The incubations were terminated by centrifugation of samples (600–1000 μl) in pre-cooled 1.5-ml polyethylene tubes for 15 s at 9000 × *g* in a Beckman Microfuge, type B. The cell pellets were homogenized in 150 μl of ice-cold buffer. The homogenization buffer was 15 mM Hepes-NaOH, pH 7.0, containing 10 mM 2-mercaptoethanol, 0.5% Triton X-100, adenosine (1.0–10 mM), and when not otherwise indicated, 10 mM AMP. The homogenate was centrifuged at 100,000 × *g* for 10 min, using a Beckman Air-fuge. Aliquots of 40 μl from the supernatant were analyzed on a HPLC protein column (I-250, Waters) eluted at a flow rate of 0.8 ml/min with 15 mM Hepes, pH 7.5, containing 10 mM 2-mercaptoethanol and 150 mM KCl. Inclusion of salt in the mobile phase was required for the quantitative elution of proteins from the column. The temperature was 23 °C. Fractions of 240 μl were collected, a portion of these fractions (100 μl) was added to scintillation vials and mixed with 5 ml of Scint-Hei 4 (Ingeniør Heidenreich, Oslo), and the radioactivity determined by liquid scintillation counting. The remaining portion was deproteinized and analyzed by TLC as described above.

Isoelectric Focusing of ^{3}H-labeled Adenine/Adenosine-Protein Complex—Rat hepatocytes (5.2 × 10^6 cells/ml) were incubated in the presence of 20 μM [^{3}H]adenosine (10.5 Ci/mmol) for 30 min. The cells were extracted in the presence of excess unlabeled adenosine, and the high speed supernatant prepared and chromatographed on I-250 protein column as described in the paragraph on the determination of [^{14}C]adenosine binding to the enzyme, except that the concentration of KCl was 50 mM. The fractions corresponding to the adenine/adenosine peak, which co-eluted with AdoHcy hydrolase (see "Results"), were collected. The effluent corresponding to this peak from eight separate injections were pooled and subjected to isoelectric focusing in a horizontal layer of Sephadex G-75 (Ultradex) using a LKB 2117 Multiphore. The bed volume was 70 ml and contained 5 g of Ultradex and 3 ml of Ampholine, pH 5–7, and 10 mM 2-mercaptoethanol. The sample was applied to the gel after the pH gradient was established and the focusing was continued for further 12 h at 2 °C and 6 watt. For measurement of pH in the Ultradex-bed, a LKB 2117-111 Multiphore surface electrode was used. The bed was fractionated using a fractionating grid, and the protein eluted from gel with 2 ml of distilled water containing 10 mM 2-mercaptoethanol.

In the fractions from the isoelectric focusing, the following parameters were determined. (*a*) The total radioactivity was determined by adding 50 μl from each fraction to scintillation vials, and mixed with 5 ml of Scint Hei 4 (Ingeniør, Heidenreich, Oslo). (*b*) Radioactive adenine and adenosine was determined by thin layer chromatography. (*c*) The AdoHcy hydrolase activity was determined in the fractions by a radiochemical method (13). The enzyme activity was determined in the synthetic direction, and the assay mixture contained 100 μM [8-^{14}C]adenosine, 3 mM DL-homocysteine, 0.2% bovine serum albumin, 80 mM KCl, 1 μM 2′-deoxycoformycin, 2 mM 2-mercaptoethanol in 80 mM potassium phosphate buffer, pH 7.0.

Determination of the Amount of Endogenous Adenosine Associated with Intracellular AdoHcy Hydrolase—This was carried out by a slight modification of the procedure described in the preceding paragraph. Cell pellet (from 1 ml of cell suspension) was homogenized in 150 μl of 80 mM potassium phosphate buffer, pH 7.0, containing 10 mM 2-mercaptoethanol, 0.5% Triton X-100, and 3 mM 3-deazaadenosine and 3 mM DL-homocysteine (to block possible binding of adenosine after cellular homogenization). High speed supernatant of the homogenate was analyzed (40 μl) on the HPLC protein column as described above, and the concentration of KCl in the mobile phase was 50 mM. The effluent was either fractionated (200 μl per fraction) or the effluent corresponding to the retention time (10.24 min) of AdoHcy hydrolase was pooled (1.6 ml). The fractions were immediately cooled (0 °C) and then deproteinized by addition of ice-cold perchloric acid (final concentration of 0.2 N), which was neutralized after 30 min with 0.36 N KOH/0.30 N $HKCO_3$, to pH 6.0. The solution obtained was lyophilized, resuspended in 150 μl of distilled water, and analyzed by HPLC. 3-deazaadenosine and UV-absorbing material present in 2-mercaptoethanol (Merck) were used as internal standards.

Uptake of [^{14}C]Adenosine—Rat hepatocytes were incubated with [^{14}C]adenosine (20 μM) for increasing periods of time. The cells were separated from the medium by centrifugation through oil, and the radioactivity associated with cells determined as described previously (24).

Determination of Cellular Content of Adenosine—Samples of 1 ml of the cell suspension were centrifuged for 15 s at 9000 × *g* in pre-cooled tubes. The cells were homogenized in 150 μl of ice-cold perchloric acid (final concentration of 0.4 N) the precipitated protein removed by centrifugation, and the acid neutralized to pH 6.0 by the addition of 0.36 N KOH/0.30 N $KHCO_3$. High speed supernatant of this solution was analyzed by HPLC on an ODS Hypersil column. Identification of adenosine in the chromatogram was based on comparison of its retention time with that of adenosine standard, spiking of the adenosine peak by addition of exogenous adenosine to the tissue extract, and the absence of the peak in neutralized perchloric acid extract treated with adenosine deaminase.

RESULTS

Distribution of Radioactive Metabolites in Hepatocytes Incubated with [^{14}C]Adenosine—The chromatographic pattern of radioactive material eluting from a HPLC protein column loaded with cytosol from hepatocytes treated with [^{14}C]adenosine can be divided into four parts (Fig. 1). A distinct peak (*I*) eluted between fraction 36 and 39. This peak co-chromatographed exactly with AdoHcy hydrolase, and contained mainly adenine (about 50%) and adenosine (about 20%). Peak I was partly overlapped by a less defined part of the chromatographic profile (*region II*) containing mainly nucleotides. After region II, two sharp peaks eluted from the column (*peaks III* and *IV*) (Fig. 1.) Peak III contained mainly adenine nucleotides, and peak IV, which included the total volume of the column, contained adenosine (which was formed during processing of the cellular extract) and small amounts of oxypurines.

Unlabeled adenosine was included in the buffer in which

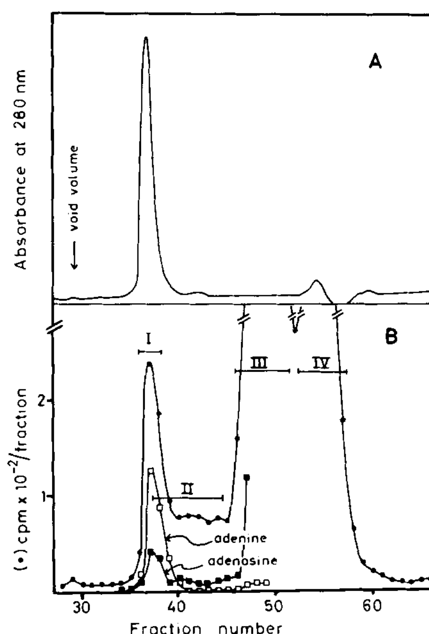

FIG. 1. **HPLC gel filtration elution profile of purified AdoHcy hydrolase (*A*) and cytosol from rat hepatocytes incubated with [^{14}C]adenosine (*B*).** *A*, a sample (40 μl) containing 48 μg of enzyme was injected into the column. *B*, hepatocytes (7 × 10^6 cells/ml) were incubated with [^{14}C]adenosine for 30 min. See "Experimental Procedures" for details.

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the cell pellet was homogenized, to prevent binding of radioactive adenosine to AdoHcy hydrolase after cellular disrupture. No decrease in the amount of radioactive adenine/adenosine associated with AdoHcy hydrolase was observed when the concentration of adenosine in the extraction buffer was increased from 1 to 3 mM and further to 10 mM (data not shown). Furthermore, addition of purified AdoHcy hydrolase (1 mg/ml) to the cellular extract (supplemented with 10 mM unlabeled adenosine) gave no increase in peak I (data not shown). These data suggest that there was no binding of radioactive adenosine to AdoHcy hydrolase during or after homogenization of the hepatocytes.

Inclusion of AMP in the homogenization buffer did not affect peak I, but selectively suppressed region II, which in the absence of AMP nearly obscured peak I (data not shown).

Effect of ara-A, 3-Deazaadenosine, and Homocysteine—ara-A is an active site directed inactivator of AdoHcy hydrolase (25, 26), and 3-deazaadenosine is even a better substrate for the enzyme than adenosine itself (12). Homocysteine condenses with adenosine to form AdoHcy in the presence of AdoHcy hydrolase (12), and prevents tight binding of adenosine to the enzyme (25). Thus, these compounds may inhibit the formation of the adenosine-AdoHcy hydrolase complex in the intact cell.

A marked decrease in the amount of radioactive adenine/adenosine associated with AdoHcy hydrolase (peak I) was observed with hepatocytes preincubated in the presence of ara-A (50 μM) or 3-deazaadenosine (50 μM) before the addition of [^{14}C]adenosine. Homocysteine (3 mM) was less effective (Fig. 2). ara-A and 3-deazaadenosine did not exert any effect on other parts of the elution profile, and preincubation with these compounds did not inhibit the uptake of radioactive adenosine by the hepatocytes under the conditions of the experiment. Preincubation with homocysteine partly prevented the formation of peak I (Fig. 2*D*), but also reduced peak III and peak IV and inhibited the uptake of radioactive adenosine by about 50% (data not shown). Treatment of hepatocytes with ara-A or 3-deazaadenosine or homocysteine (for 45 min) after the cells were exposed to [^{14}C]adenosine gave a moderate reduction in peak I relative to control (Fig. 2).

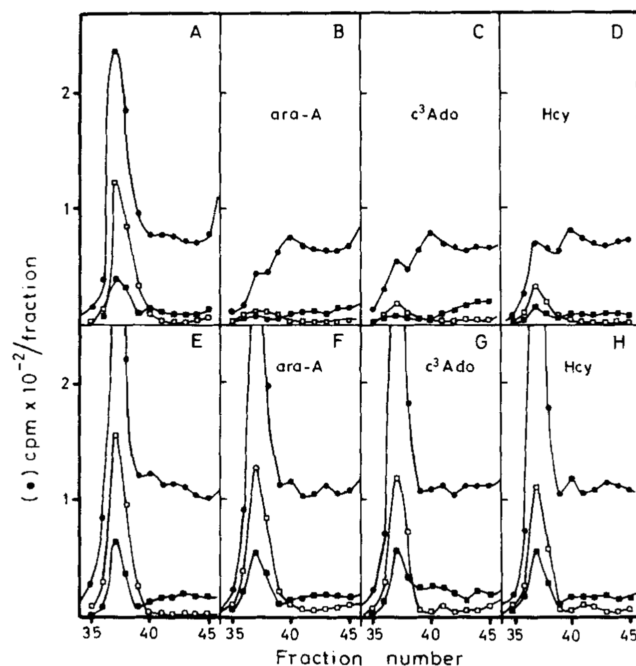

Fig. 2. Effect of various compounds on the amount of radioactive adenine/adenosine associated with AdoHcy hydrolase. Rat hepatocytes (7 × 10^6 cells/ml) were preincubated for 30 min with 50 μM ara-A (*B*) or 50 μM c^{3}Ado (*C*) or 3 mM Hcy (*D*) or no addition (control, *A*), and then exposed for 30 min to [^{14}C]adenosine (initial concentration of 20 μM). *E, F, G,* and *H* show the results from similar experiments with cells incubated with [^{14}C]adenosine (20 μM) for 30 min, and then for further 45 min with 50 μM ara-A (*F*) or 50 μM c^{3}Ado (*G*) or 3 mM Hcy (*H*) or no addotion (*E*). All incubations were carried out in the presence of 1 μM 2′-deoxycoformycin. Cytosol from the hepatocytes was subjected to gel filtration on a HPLC protein column. The part of the elution profiles for adenine (□), adenosine (■), and the total radioactivity (●) corresponding to the retention time of AdoHcy hydrolase (see Fig. 1) is shown on the figure.

Isoelectric Focusing—A gel filtration experiment was performed as described in the legend to Fig. 1, except that the hepatocytes were incubated with [^{3}H]adenosine instead of [^{14}C]adenosine. The effluent corresponding to peak I was subjected to isoelectric focusing. AdoHcy hydrolase and the adenine/adenosine-protein complex focused both at pH 5.76 (Fig. 3). The ratio of the amount of adenine to adenosine was 2:1 (data not shown), and the recovery of the radioactive complex applied to the isoelectric focusing bed was about 80%.

Adenosine Content in Rat Hepatocytes—Adenosine bound to purified AdoHcy hydrolase liberates adenine when the complex is denatured by certain procedures (18). Preliminary experiments were therefore conducted to investigate whether the adenosine content in hepatocytes is underestimated because of partial conversion of adenosine to adenine. Rat hepatocytes were incubated with [^{14}C]adenosine (20 μM) for 30 min, and at this time point adenosine deaminase was added to remove extracellular adenosine. Cells exposed for 5, 10, 30, and 60 min to adenosine deaminase were pelleted and extracted in ice-cold perchloric acid. The extract was neutralized and analyzed by TLC. The amount of radioactive adenine and adenosine remained nearly constant for at least 60 min of incubation with adenosine deaminase, and the ratio between the amounts of adenine and adenosine was 1.32 ± 0.21 (mean ± S.D., n = 11) for one cell preparation, but varied (in the range 1.0–1.8) from one cell preparation to another (data not shown). The amount of ^{14}C-labeled adenine was suppressed by about 70% when the cells were treated with either ara-A or 3-deazaadenosine, as described in legend to Fig. 2. This sug-

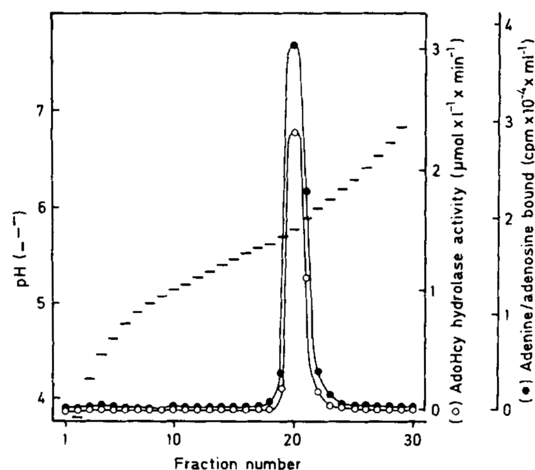

Fig. 3. Isoelectric focusing of the radioactive adenine/adenosine-protein complex formed in rat hepatocytes. Rat hepatocytes were incubated with [^{3}H]adenosine, the cells extracted in the presence of high concentration of unlabeled adenosine, and the high speed supernatant was fractionated on a HPLC gel filtration column. The radioactive adenine/adenosine-protein complex co-eluting with AdoHcy hydrolase (Fig. 1), was subjected to flat bed electrofocusing in a granulated gel. Details are given in the text. AdoHcy hydrolase activity, assayed in the synthetic direction (○), and the amount of [^{3}H]adenine and [^{3}H]adenosine (●), were determined in the isoelectric fractions.

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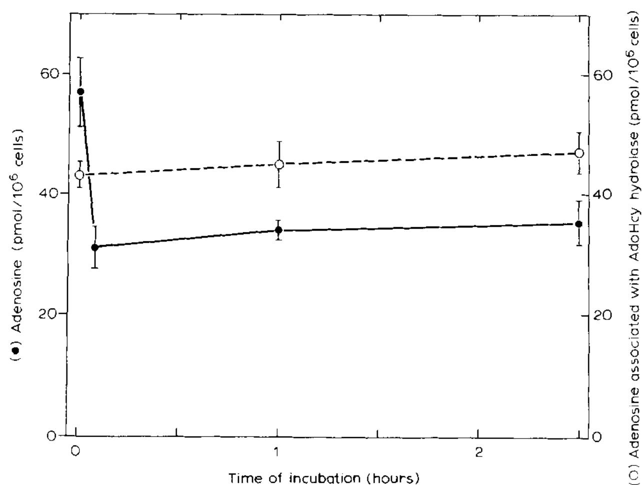

FIG. 4. **Adenosine content and adenosine associated with AdoHcy hydrolase in rat hepatocytes exposed to extracellular adenosine deaminase.** Suspension of rat hepatocytes (5.4×10^6 cells/ml) was supplemented with adenosine deaminase (16 units/ml), and the cellular content of adenosine (●) and adenosine associated with AdoHcy hydrolase (○) were determined at various time points after the addition of the enzyme. The first values (at time 0 min of incubation) represent the amount of adenosine immediately before the addition of adenosine deaminase. Each value is mean of four determinations ± S.E.

gests that most [^{14}C]adenine is derived from [^{14}C]adenosine tightly bound to AdoHcy hydrolase. However, the total amount of adenine in these cells determined by HPLC, was about 10-fold higher than the amount of adenosine (data not shown), suggesting additional sources of adenine in hepatocytes and/or formation of adenine from unlabeled purines in the extract after cellular homogenization.

The cellular content of adenosine in rat hepatocytes[2] decreased rapidly to 50–70% when the extracellular medium was supplemented with high level of adenosine deaminase. The result of a typical experiment is shown in Fig. 4. The residual amount of adenosine was not mobilized by prolonged (up to 2.5 h) exposure of the cells to adenosine deaminase (Fig. 4).

Association of Endogenous Adenosine with AdoHcy Hydrolase—Determination of the amount of endogenous adenosine associated with AdoHcy hydrolase involved the extraction of the cells in a buffer containing 3-deazaadenosine and homocysteine. Under these conditions no binding of adenosine to AdoHcy hydrolase in the extract seemed to take place during or after homogenization. This statement is based on the observation that there was no increase in adenosine associated with AdoHcy hydrolase when the extract was supplemented with purified AdoHcy hydrolase (1 mg/ml).

When high speed supernatant of extract from hepatocytes was fractionated on a HPLC protein column, a similar result was obtained as that depicted in Fig. 1 for hepatocytes treated with [^{14}C]adenosine, *i.e.* adenosine eluted as a peak which cochromatographed exactly with AdoHcy hydrolase. Under the conditions of these experiments (deproteinization of the fractions with 0.2 N perchloric acid at 0 °C), the amount of adenine was less than 20% of the amount of adenosine.

The amount of adenosine associated with AdoHcy hydrolase was 37 ± 15 pmol/10^6 cells (mean ± S.D., n = 16). The value is of the same order of magnitude as the fraction of adenosine in hepatocytes which is not mobilized by extracellular adenosine deaminase (Fig. 4). Incubation of hepatocytes for 1 or 2.5 h in the presence of adenosine deaminase (Fig. 4) or ara-A or 3-deazaadenosine or homocysteine only slightly reduced the amount of endogenous adenosine associated with AdoHcy hydrolase. The amount of adenosine associated with AdoHcy hydrolase in hepatocytes exposed to 50 μM ara-A was 40 ± 5 (1 min) and 33 ± 3 pmol/10^6 cells (2.5 h). The data obtained with 50 μM c^{3}Ado were 35 ± 2 (1 min) and 30 ± 4 pmol/10^6 cells (2.5 h), and with 3 mM DL-homocysteine 36 ± 3 (1 min) and 36 ± 4 pmol/10^6 cells (2.5 h). The corresponding values for control cells were 32 ± 3 (1 min) and 34 ± 2 pmol of adenosine/10^6 cells (2.5 h). Each value represents mean of four determinations ± S.E.

[2] The amount of adenosine in the rat hepatocytes varied in the range from 25 to 70 pmol/10^6 cells from one cell preparation to another.

DISCUSSION

Radioactive adenosine added to a suspension of rat hepatocytes forms a stable complex with intracellular AdoHcy hydrolase. This statement is based on the following observations. ^{14}C-labeled adenine/adenosine co-chromatographed exactly with AdoHcy hydrolase on a HPLC protein column (Fig. 1). A fraction of the radioactive material in this peak was identified as adenine (Figs. 1 and 2), which is in accordance with the observation (17–19) that adenosine bound to AdoHcy hydrolase in cell-free systems is partly converted to adenine or a substance liberating adenine. Furthermore, the formation of the complex between adenosine and the macromolecule(s) eluting as AdoHcy hydrolase, was blocked by compounds (ara-A (25), 3-deazaadenosine (12), and homocysteine (25)) which are expected to inhibit tight binding of adenosine to this enzyme. Among these compounds, 3-deazaadenosine seems to be a rather specific substrate for AdoHcy hydrolase and is neither a substrate nor an inhibitor of adenosine deaminase or adenosine kinase (12). Finally, the adenine/adenosine-protein complex formed in the intact hepatocytes and AdoHcy hydrolase from these cells focused both at pH 5.76 (Fig. 3), which is the isoelectric point reported for AdoHcy hydrolase from rat (27) and mouse liver (5).

ara-A, 3-deazaadenosine, and homocysteine inhibit the formation of the adenosine-AdoHcy hydrolase complex in rat hepatocytes (Fig. 2), but the rate of decrease in the amount of this complex in the presence of these compounds was low (Fig. 2, see "Results"). Furthermore, lowering the cellular content of adenosine by suspending the cells in a medium containing adenosine deaminase insignificantly decreased the amount of adenosine associated with AdoHcy hydrolase (Fig. 3). These data suggest that the adenosine-AdoHcy hydrolase complex has long half-life in rat hepatocytes, at least under the conditions of the experiments. The fraction of adenosine which resists mobilization by treatment of hepatocytes with adenosine deaminase (28, 29) is of the same order of magnitude as the amount of adenosine associated with AdoHcy hydrolase (Fig. 3). Thus AdoHcy hydrolase may sequester adenosine in intact hepatocytes, as previously shown for adenosine in crude extract from various tissue (17). The physiological implications of the possibility that a substantial fraction of adenosine is tightly bound to intracellular protein(s) have been discussed in some detail by others (29–33).

Compartmentalization of adenosine in the dog and rat heart has recently been studied by Olsson *et al.* (33). In analogy with the data obtained with rat hepatocytes, they found that intracellular adenosine was not mobilized by treatment of dispersed cardiocytes with adenosine deaminase. An intracellular pool of adenosine was also demonstrated in the dog heart *in situ*. The sequestration of adenosine was explained by the existence of a long-lived adenosine-AdoHcy hydrolase complex (33).

The mechanism of action of AdoHcy hydrolase has been studied in detail by Palmer and Abeles (34). They found that

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the enzyme contains tightly bound NAD^+ which participates in the catalytic process. Adenosine entering the catalytic cycle (in the presence of homocysteine) as well as adenosine interacting with the enzyme in the absence of homocysteine, is oxidized to 3′-ketoadenosine with a concomitant reduction of NAD^+ to NADH (34). Thus, the possibility exists that adenosine bound to intracellular AdoHcy hydrolase is chemically modified.

The determination of the amount of adenosine complexed with AdoHcy hydrolase, as well as the total amount of adenosine in hepatocytes may be obscured by the liberation of adenine from adenosine (or a derivative thereof) bound to AdoHcy hydrolase (17, 18). The fraction of adenosine converted to adenine is critically dependent on the time of incubation, the presence of reducing agents and adenine derivatives, the procedure used for denaturation of the enzyme (18, 19), and probably other factors. Attempts to avoid this problem when measuring total adenosine content in hepatocytes were not successful. When the samples were deproteinized by procedures involving homogenization of the cells in ethanol or ethanol containing acetic acid, denaturation of AdoHcy hydrolase and liberation of adenosine was obtained, but enzymes degrading or forming adenosine in the extract were not completely inactivated.[3] The fraction of adenosine converted to adenine cannot be corrected for by simultaneous measurement of adenine in the extract, because most adenine in intact cells is probably formed by the action of other enzyme(s) (35) than AdoHcy hydrolase. Thus, liberation of adenine from adenosine bound to AdoHcy hydrolase may be a source of erratic results when determining the adenosine content in tissues rich in this enzyme. Furthermore, the conditions used to denature the isolated adenosine-AdoHcy hydrolase complex eluting from the gel filtration column provoke only a moderate conversion of adenosine to adenine, relative to the conversion taking place under the conditions required to denature interfering enzymes and AdoHcy hydrolase in crude extract from hepatocytes. This may offer an explanation to the observation (Fig. 4) that the amount of adenosine bound to the enzyme slightly exceeds the total amount of adenosine in the hepatocytes.

The observation that only a small fraction of the endogenous adenosine bound to AdoHcy hydrolase eluting from the HPLC protein column is converted to adenine may be explained by the exposure of the enzyme to mercaptoethanol (19) during the migration through the column and/or the denaturation procedure (dilute acid at 0 °C). The possibility also exists that a fraction of adenosine bound to AdoHcy hydrolase is dissociated at a sufficiently high rate to be completely liberated from the enzyme during gel filtration. In other words, the gel filtration technique may be limited to the determination of complexes characterized by long half-life. This possibility is supported by data for the plant enzyme provided by Jakubowski and Guranowski (36) demonstrating that adenosine binds to two sites, distinguishable on the basis of half-lives of the complexes. Similar data have been obtained with the mouse liver enzyme (19).

Results obtained with isolated mammalian AdoHcy hydrolase (25, 37) suggest that the enzyme which forms a stable complex with adenosine is converted into an inactive form. Based on the data in the literature and in the present paper, the following calculations can be made. AdoHcy hydrolase activity in isolated rat liver cells is about 10 nmol/min/10^6 cells when determined in the synthetic direction.[4] The specific activity of homogenous AdoHcy hydrolase is 2–3 μmol/mg/min when assayed under similar conditions (27, 38, 39), and the molecular weight of the enzyme is 185,000 to 240,000 (27, 34, 37, 38, 40). Thus, the cellular concentration of enzymatically active AdoHcy hydrolase is about 16 pmol/10^6 cells in rat hepatocytes. The cellular content of AdoHcy hydrolase which exists as a stable complex with adenosine is about 10 pmol/10^6 cells. This value is based on the assumption that adenosine is tightly bound to the catalytic site of the enzyme, and each enzyme molecule has four catalytic sites (34). These data suggest that a substantial fraction of intracellular AdoHcy hydrolase (or catalytic sites residing on this enzyme) forms a stable complex with adenosine, and this enzyme may not participate in the metabolic handling of AdoHcy. The possible interconversion between these two forms of AdoHcy hydrolase is probably related to the half-life of the adenosine-Ado-Hcy hydrolase complex. Further studies on this process may reveal more fully the role of AdoHcy hydrolase in adenosine metabolism as well as the effect of tight binding of adenosine to the enzyme on the metabolism of AdoHcy.

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[3] S. Helland and P. M. Ueland, unpublished results.
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