# 10. INTERACTION OF ADENOSINE WITH ADENOSINE-BINDING PROTEIN, S-ADENOSYLHOMOCYSTEINE HYDROLASE

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Soluble adenosine-binding proteins were first described by Yuh and Tao in rabbit erythrocytes [1], and similar proteins were later isolated from various eukaryotes [2–8]. The physiological role of these binding proteins for adenosine (Ado) remained obscure until Hershfield [9] and Hershfield and Kredich [10] demonstrated that such proteins from human placenta, spleen, and lymphoblasts were identical to S-adenosylhomocysteine (AdoHcy) hydrolase (EC: 3.3.1.1), the enzyme responsible for the metabolic degradation of the endogenous transmethylase inhibitor, AdoHcy [11]. This finding has been confirmed by others [12–14]. This chapter is a brief review of the properties of AdoHcy hydrolase, with emphasis on the interaction of the enzyme with Ado.

## PROPERTIES AND OCCURRENCE OF AdoHcy HYDROLASE

AdoHcy hydrolase catalyzes the reversible hydrolysis of AdoHcy to Ado and L-homocysteine (Hcy) (Fig. 10.1). At high concentrations of substrates (>1 $\mu M$), the AdoHcy hydrolase reaction favors synthesis of AdoHcy [15]. This can be more precisely expressed by the equilibrium constant ($K_{eq}$) of the reaction, defined by the following equation [15, 16]:

$$K_{eq} = \frac{[\text{Ado}] \times [\text{Hcy}]}{[\text{AdoHcy}]} = 10^{-6}\ M$$

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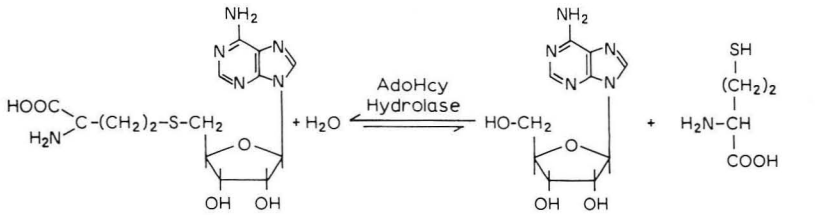


**Figure 10.1.** The reaction catalyzed by AdoHCy hydrolase.

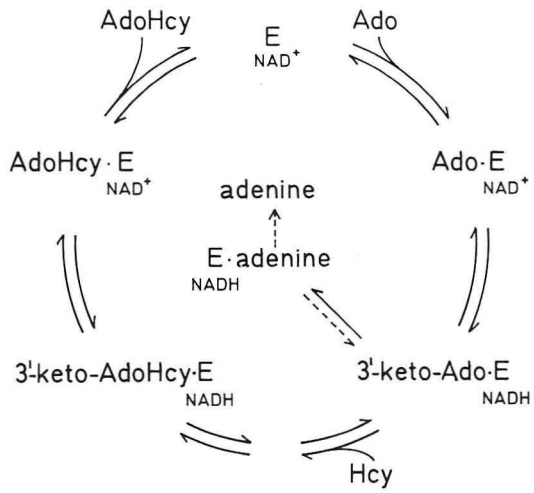


**Figure 10.2.** The catalytic cycle of AdoHcy hydrolase.

Ado and, to a lesser degree, Hcy are inhibitors of hydrolysis of AdoHcy, and the reaction can be directed toward hydrolysis by removal of Ado or Hcy [15].

Knowledge of the catalytic mechanism of AdoHcy hydrolase has been provided by the work of Palmer and Abeles [17]. They found that the calf liver enzyme contains tightly bound $NAD^+$, which participates in the catalytic cycle. Synthesis and hydrolysis of AdoHcy involve oxidation of Ado and AdoHcy, respectively, to their 3′-keto derivatives, with a concomitant reduction of $NAD^+$ to NADH (Fig. 10.2; for details, see [17]).

Data on physiochemical and kinetic properties of AdoHcy hydrolase purified to apparent homogeneity from various sources are summarized in Table 10.1. Because somewhat inconsistent data on this item exist (Table 10.1), a study was undertaken in my laboratory to compare the properties of AdoHcy hydrolase purified to homogeneity from three different sources, i.e.,

**Table 10.1.** Physiochemical and kinetic properties of AdoHcy hydrolase purified to homogeneity from various sources

| Source | $M_r$ | Subunits | | pI | $K_m$ | | | Reference |
|---|---|---|---|---|---|---|---|---|
| | | No. | $M_r$ | | Ado ($\mu M$) | AdoHcy ($\mu M$) | Hcy ($mM$) | |
| Rabbit erythrocytes | 240,000 | 5 | 48,000 | | | | | [1] |
| Beef liver | 192,000 | 4 | 48,000 | | 45 | 10.5 | | [18] |
| Mouse liver | 185,000 | 4 | 45,000–46,500 | 5.7 | 0.2 | 0.75 | 0.15 | [4, 12, 19] |
| Yellow lupin seeds | 110,000 | 2 | 55,000 | 4.9 | 2.3 | 12.0 | 4.6 | [20] |
| Calf liver | 237,000 | 4 | 50,000–60,000 | 5.8 | 420 | 63 | | [21] |
| Human placenta | 190,000 | 4 | | | 0.5 | | | [9] |
| Rat brain | 180,000 | 4 | 48,000 | 5.6 | | 36.6 | | [22] |
| Rat liver | 220,000 | 5 | 47,000 | 6.6 | 0.6 | 0.90 | 0.06 | [23] |
| Rat liver | 188,000 | 4 | 47,000 | 5.7 | 1.05 | 15.2 | 0.155 | [24] |
| Rat liver | | | 47,000–57,500 | | 0.94 | 12.3 | 0.164 | [25] |
| Bovine adrenal cortex | 185,000 | 4 | 45,000–46,000 | 5.35 | 0.2 | 0.75 | | [15] |
| Bovine liver | 185,000 | 4 | 45,000–46,000 | 5.35 | 0.2 | 0.75 | | [15] |

mouse liver, bovine liver, and bovine adrenal cortex [14]. Similar kinetic behavior was observed for these enzymes ($K_m$ for Ado and AdoHcy in the micromolar range), and all enzymes were quadrimers with $M_r$ of about 190,000 [14] (Table 10.1).

AdoHcy hydrolase is widely distributed in vertebrates, yeast, and plants, but bacteria are devoid of AdoHcy hydrolase activity [25]. In vertebrates, highest activity is found in liver and pancreas, but kidney is also rich in this enzyme. Smaller amounts are found in spleen, brain, skeletal muscle, heart, and testes, but some species variations have been observed [25–28]. This has been most clearly demonstrated in mammalian heart, where the enzyme activity is about 20-fold higher in guinea pig than in dog [29].

AdoHcy hydrolase from liver [26, 30], brain [21, 31], heart [29], and probably other tissues is a soluble protein localized to the cytosol fraction of the tissue homogenate.

## ADENINE ANALOGUE-BINDING PROTEINS

In 1971, Chambaut and co-workers demonstrated in rat liver the presence of a cyclic AMP-binding protein devoid of protein kinase activity [32]. Similar proteins were later described in various cells and tissues (Table 10.2). Some of these proteins bind Ado with high affinity [1, 3, 4, 6, 7] and also interact with adenine nucleotides other than cyclic AMP [3, 36]. Sugden and Corbin [3] suggested the name adenine analogue-binding proteins (AABP) for such proteins from rat and bovine tissues. The finding that Ado-binding proteins are associated with AdoHcy hydrolase activity [9, 10, 12, 14] raises the question of whether AdoHcy hydrolase may account for all soluble Ado-binding factors.

**Table 10.2.** Properties of adenine analogue binding proteins from various sources

| Source | Molecular size | Affinity ($K_d$) for | | Reference |
|---|---|---|---|---|
| | | Ado ($\mu M$) | cAMP ($\mu M$) | |
| Rat liver | | | | [33] |
| Rabbit erythrocytes | 240,000, 9S | 0.1 | 0.3 | [1] |
| Mouse liver | 185,000, 9S | 0.2 | 0.15 | [4, 19] |
| *Drosophila melanogaster* | 200,000 | | 0.1 | [34] |
| Rat liver | | 0.24 | | [2] |
| Rat liver | 180,500, 10.1 S<br>370,500, 14.2 S | | | [3] |
| Bovine liver | 185,000, 9.8 S | | | [3] |
| Rabbit erythrocytes | | 0.0053 | 0.009 | [7] |
| Human erythrocytes | 230,000 | | | [6] |
| Jerusalem artichoke rhizome tissues | 240,000 | | 0.23 | [35] |
| *Trypanosoma gambiense* | 150,000 | | 0.6 | [36] |
| Rat heart | 176,000 | | | [38] |

Binding proteins for Ado have been described in dog, cow [8], and rat heart [37], and these proteins are not associated with AdoHcy synthase or hydrolase activity. A cyclic AMP-Ado-binding protein from rat heart has been separated from AdoHcy hydrolase by affinity chromatography [37]. However, these reports do not definitely prove the existence *in vivo* of Ado-binding proteins not related to AdoHcy hydrolase. The enzyme is inactivated in the presence of various naturally occurring purines, including 2′-deoxyadenosine, Ado, inosine [38], and adenine nucleotides [39].

## INTERACTION OF ADENOSINE ANALOGUES WITH AdoHcy HYDROLASE

Numerous analogues of Ado interact with AdoHcy hydrolase (Table 10.3). Some Ado analogues serve as substrate for the enzyme and may thereby function as a metabolic precursor for the corresponding AdoHcy analogue. 3-deazaadenosine is an even better substrate for AdoHcy hydrolase than Ado itself, and this compound has been widely used as a pharmacological tool for studying methyltransfer reactions [11, and references therein]. Other analogues of Ado are reversible inhibitors of the enzyme. These compounds may induce a buildup of cellular AdoHcy derived from S-adenosyl-L-methionine (AdoMet). Carbocyclic Ado seems to be particularly effective [13]. Irreversible inactivation of AdoHcy hydrolase was first demonstrated with 9-β-D-arabinofuranosyladenine and 2′-deoxyadenosine [40], and these compounds inactivate the enzyme *in vitro* by a mechanism that involves irreversible reduction of enzyme-bound $NAD^+$ [38, 41, 42]. However, the intracellular enzyme exposed to 9-β-D-arabinofuranosyladenine [43] is slowly reactivated [44]. The existence of such a mechanism in the intact cell might be expected in the light

**Table 10.3.** Adenosine analogues as substrates, inhibitors, and inactivators of AdoHcy hydrolase

| Adenosine analogue | Substrate | Inhibitor | Inactivator | Reference |
|---|---|---|---|---|
| Adenosine | + + + | + + + | (+) | [13, 23, 46, 47] |
| 3-deazaadenosine | + + + | + + + | (+) | [11, 13, 46, 47] |
| Nebularin | + + | (+) | + + | [13, 46, 47] |
| Formycin | + | + | (+) | [13, 46, 47] |
| $N^6$-methyladenosine | + | + | (+) | [13, 46–48] |
| 8-azaadenosine | + | + | (+) | [13, 46, 47] |
| 2-chloroadenosine[a] | | | + + + | [13, 46] |
| Adeninearabinoside | (+) | + + | + + + | [13, 39, 41, 43, 44] |
| Carbocyclic adenosine | (+) | + + + | + + + | [13, 47] |
| 2′-deoxyadenosine | | | + | [13, 41, 42] |
| Adenosine dialdehyde | | | + + + | [49, 50] |

[a]2-Chloroadenosine seem to be a potent inactivator of the isolated enzyme [13] but induces only a moderate increase in AdoHcy content in intact cells [46].

of the fact that naturally occurring purines, including Ado, are inactivators of AdoHcy hydrolase [38].

Ado analogues that are reported to function as substrates, inhibitors, or inactivators of isolated AdoHcy hydrolase and the enzyme in intact cells are listed in Table 10.3.

### INTERACTION OF ADENOSINE WITH AdoHcy HYDROLASE *IN VITRO*

Ado forms a stable complex with AdoHcy hydrolase [4, 9, 10, 13, 50]. Compared with the site interacting with cyclic AMP, which accepts various adenine derivatives, the Ado-binding site is rather restrictive [4, 7, 36]. The ado-binding site may be identical to the catalytic site of the enzyme [16]. The Ado-enzyme complex formed after prolonged incubation shows a long half-life [50, 51], and the bound Ado is not displaced by excess unlabeled Ado [9, 50] and is not available for deamination catalyzed by adenosine deaminase [51–53].

A fraction of Ado bound to AdoHcy hydrolase is converted to adenine or a substance liberating adenine [52], and a significant amount of adenine is dissociated from the enzyme [52]. Tightly bound adenine (derived from Ado) is reconverted back to Ado under certain conditions [51], but synthesis of Ado from added adenine could not be demonstrated [52]. Adenine formation may be explained by liberation of the purine base from 3′-ketoadenosine [13, 53], a proposed intermediate in the catalytic cycle [17] (Fig. 10.2).

High concentrations of Ado in the absence of Hcy induce an irreversible inactivation of isolated AdoHcy hydrolase [22, 38]. The kinetics of the inactivation process suggest a suicide or $K_{cat}$ mechanism of action of Ado, which implies that the inactivation proceeds from a reversible Ado-enzyme complex [38]. A mechanism of action of Ado has been proposed [13] that involves irreversible reduction of enzyme-bound $NAD^+$ and liberation of adenine from 3′-ketoadenosine (Fig. 10.2), i.e., a mechanism of action similar to that of such inactivators as 2′-deoxyadenosine [41] and 9-β-D-arabinofuranosyladenine [42].

Tight binding of Ado to AdoHcy hydrolase, formation of adenine, and inactivation of the enzyme by Ado are probably closely related processes [53].

AdoHcy hydrolase from plant is a dimer (Table 10.1) and binds two molecules of Ado per molecule of enzyme [54]. The binding of the first Ado molecule is fast, whereas the binding of the second molecule is a slow process. This suggests negative cooperativity among binding sites. The Ado-enzyme complex reacts slowly to form adenine, ribose, and active enzyme. Thus, in contrast to mammalian AdoHcy hydrolase, Ado does not inactivate the plant enzyme. The half-life of the newly formed complex is short, whereas the old complex dissociates slowly [54].

Ado added to concentrated crude extracts from various tissues, including liver, kidney, adrenal cortex, brain, heart, skeletal muscle, and uterus, is not completely metabolized. A fraction of Ado added to the extracts is not available for deamination catalyzed by adenosine deaminase [28]. This phenome-

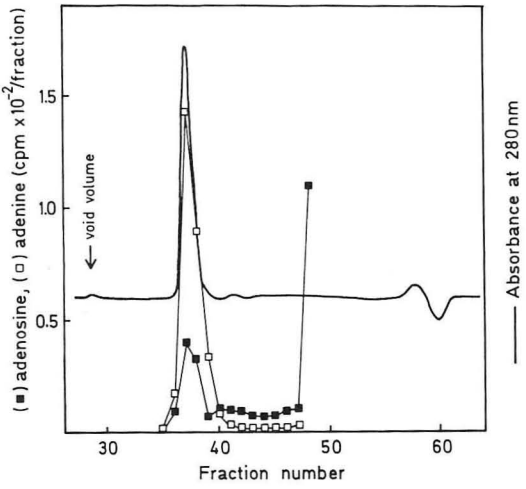


**Figure 10.3.** HPLC gel-filtration of purified AdoHcy hydrolase (−) and cytosol from rat hepatocytes incubated with [$^{14}$C]adenosine (■,□). Adenine (□), adenosine (■).

non, termed sequestration of Ado, is explained by formation of a stable Ado-AdoHcy hydrolase complex. The ability of various tissues to sequester Ado parallels the tissue level of AdoHcy hydrolase [28].

The possibility existed that tight binding of Ado to AdoHcy hydrolase and formation of adenine are *in vitro* phenomena related to the absence of the other substrates of the enzyme, namely, Hcy or AdoHcy. Therefore, synthesis and hydrolysis of AdoHcy were determined at assumedly physiological concentrations of enzyme (about 10 $\mu M$ [28]) and its substrates. Under these conditions, both sequestration of Ado and formation of adenine could be demonstrated. Furthermore, sequestered Ado is not available for synthesis of AdoHcy [53].

### BINDING OF ADENOSINE TO INTRACELLULAR AdoHcy HYDROLASE

In the light of the data given in the preceding paragraph, a study was carried out in my laboratory to investigate whether AdoHcy hydrolase functions as an Ado-binding protein in intact cells. Isolated rat hepatocytes were chosen as the experimental system because these cells are rich in AdoHcy hydrolase [25]. Hepatocytes incubated with [$^{14}$C]Ado were extracted in a buffer containing high concentrations of unlabeled Ado (10 m$M$) to prevent binding of radioactive Ado to the enzyme after cellular lysis. Extract from these cells was analyzed on a high-performance liquid chromatography (HPLC) protein column, and radioactive adenine and Ado eluted as a peak that co-chromatographed exactly with AdoHcy hydrolase (Fig. 10.3). Formation of this peak was inhibited by exposure of the cells to compounds (9-β-D-arabinofuranosyladenine, 3-deazaadenosine, or homocysteine) interacting with the catalytic

site of AdoHcy hydrolase. On this basis, it was concluded that labeled Ado forms a stable complex with intracellular AdoHcy hydrolase [55].

It has been reported that a fraction of endogenous Ado in rat hepatocytes is not mobilized by treating the cells with extracellular adenosine deaminase [44, 56]. This amount of Ado equals the amount of endogenous Ado associated with AdoHcy hydrolase, which was determined by a modification of the HPLC gel-filtration method, involving extraction of hepatocytes in the presence of 3-deazaadenosine and Hcy (to prevent binding of Ado to the enzyme after cellular lysis).

The amount of Ado associated with AdoHcy hydrolase in rat hepatocytes is $37 \pm 15$ pmol/$10^6$ cells (mean ± SD). This suggests that about 10 pmol of enzyme per $10^6$ cells form a stable complex with Ado (assuming that the enzyme molecule has four catalytic sites [17]). Based on data on the catalytic activity of AdoHcy hydrolase in hepatocytes [43, 44], it could be calculated that about 50% of AdoHcy hydrolase in these cells may exist as a stable complex with Ado. Thus, AdoHcy hydrolase functions as an Ado-binding protein in rat hepatocytes.

## POSSIBLE ROLE OF AdoHcy HYDROLASE IN ADENOSINE METABOLISM

AdoHcy hydrolase catalyzes the reversible hydrolysis of AdoHcy to Ado and Hcy. Although the reaction favors synthesis of AdoHcy, the metabolic flow is probably in the hydrolytic direction, because both Ado and Hcy are continuously trapped in the intact cell [11, 15]. Ado is either deaminated or phosphorylated [11] (Fig. 10.4). The cellular level of Hcy is low [57], suggesting no synthesis of AdoHcy under physiological conditions.

Apart from the work of Schütz et al. [58] investigating the site of production of Ado in the heart, no attention has been given to the possible role of the AdoHcy hydrolase reaction as a source of Ado *in vivo*. This question obviously deserves further attention.

Conversion of Ado to adenine is a side reaction of AdoHcy hydrolase, but this reaction is ineffective [52, 53]. Only small amounts of adenine are formed from Ado in isolated rat liver [56]. Thus, it seems that formation of adenine from Ado catalyzed by AdoHcy hydrolase is not a quantitatively important source of cellular adenine, relative to other metabolic pathways.

Addition of exogenous Ado leads to accumulation of AdoHcy, which is greatly enhanced by Hcy. This has been demonstrated with intact cells [59], perfused liver [57, 60], and heart [29] and in whole animals [61]. Thus, administration of these compounds is a convenient procedure for obtaining an elevated cellular level of AdoHcy. The mechanisms underlying this metabolic effect of Ado and Hcy involve inhibiting AdoHcy hydrolase or directing the AdoHcy hydrolase reaction toward synthesis of AdoHcy. Data provided by Duerre and Briske-Anderson [57], who perfused isolated rat liver with labeled Ado, suggest that reversal of the AdoHcy hydrolase reaction takes place even in the absence of exogenous Hcy. Because the cellular level of Hcy is far below

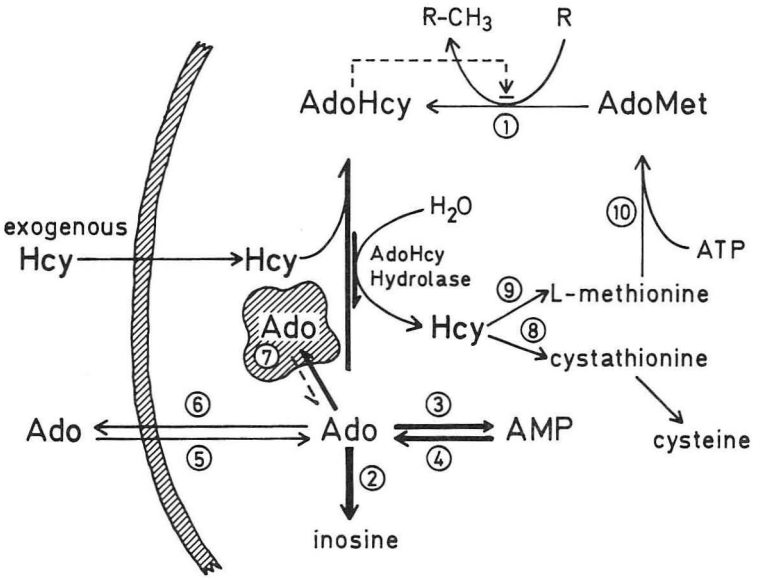


**Figure 10.4.** Role of AdoHcy hydrolase in intermediary metabolism. (1) AdoMet-dependent methyltransferases; (2) adenosine aminohydrolase (adenosine deaminase); (3) adenosine kinase; (4) adenosine 5′-monophosphate nucleotidase; (5) adenosine uptake; (6) adenosine release; (7) adenosine tightly bound to AdoHcy hydrolase; (8) cystathionine-β-synthase; (9) 5′-methyltetrahydrofolate: L-homocysteine methyltransferase and betaine: L-homocysteine S-methyltransferase; (10) ATP: L-methionine S-adenosyltransferase.

the apparent $K_m$ value for Hcy, the existence of a cellular pool of Hcy has been postulated to explain this observation [57]. However, under this condition (i.e., in the absence of exogenous Hcy), the bulk of AdoHcy may be derived from various methyltransfer reactions. When the liver is perfused with both Ado and Hcy, a significant amount of AdoHcy is formed via synthesis [57].

It seems reasonable to suggest that intracellular AdoHcy hydrolase functions as an Ado-binding protein in various tissues and cells in addition to rat hepatocytes. Green [62] has recently reported that Ado is taken up by murine neuroblastoma cells deficient in adenosine kinase by a concentrative and saturable process, and binding of Ado to intracellular protein such as AdoHcy hydrolase is suggested as an explanation for this finding. Furthermore, injection of mice with Ado decreases the AdoHcy hydrolase activity in brain extracts. This effect could not be reversed by dialysis and may be related to the formation of a stable Ado-AdoHcy hydrolase complex [61].

The fraction of Ado bound to AdoHcy hydrolase is probably not available for other enzymes participating in the metabolic handling of Ado [28] and for synthesis of AdoHcy [53]. Biological effects of Ado that are not mediated by AdoHcy hydrolase are probably induced by the free fraction of the nucleoside.

In my opinion, it is unlikely that the biological implications of the existence of a stable Ado-AdoHcy hydrolase complex are limited to a functional compartmentation of Ado. The size of a cellular pool of Ado existing as an Ado-AdoHcy hydrolase complex seems to be small relative to the metabolic capacity of other pathways leading to Ado formation (Fig. 10.4). It is conceivable that binding of Ado to AdoHcy hydrolase is important for the regulation of AdoHcy hydrolase activity *in vivo*. Studies should be carried out to investigate whether a long-lived complex between Ado and AdoHcy hydrolase plays a hitherto unrecognized role in the regulation of the metabolism of Ado or AdoHcy.

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## DISCUSSION

DR. H. MCILWAIN: In relation to further effects consequential to S-adenosylhomocysteine formation, we have found recently (Newman, McIlwain, and Reason) that addition of homocysteine to incubating neocortical tissues diminishes their cyclic AMP content. Preincubation of such tissues with both adenosine and homocysteine, however, permits unusually large responses to the later addition of other neurohumoral agents, including norepinephrine and histamine. We judge that, in the first case, adenosine is being sequestered; in the second, a reserve of AdoHcy has been formed and is supplying adenosine for activation of adenylate cyclase.

DR. UELAND: I think that the answer to your question is as follows: Addition of homocysteine (Hcy) alone will decrease adenosine content. The mechanism involves the condensation of adenosine with Hcy, through the action of AdoHcy hydrolase. When adenosine is added together with Hcy, this would lead to a large accumulation of AdoHcy. Dr. Zimmerman and co-workers have reported that high levels of AdoHcy inhibit cyclic AMP phosphodiesterase. We have recently obtained similar data [*FEBS Lett* 135:165, 1982].

DR. J. SCHRADER: Do you have recent estimates of the amount of free adenosine versus SAH-bound adenosine?

DR. UELAND: I would like to refer to our poster presented at this meeting. Briefly, the amount of adenosine associated with AdoHcy hydrolase (about 40 pmol/$10^6$ cells) was of the same order of magnitude as the amount of cellular adenosine not mobilized by extracellular adenosine deaminase. There is a problem here because adenosine bound to AdoHcy hydrolase liberates adenine when the complex is denatured. The conversion to adenosine depends on the denaturation procedure used, i.e., a large amount of adenine is formed in the presence of high concentrations of acid and small amounts when mild denaturation procedures were used. However, under the latter conditions, interfering enzymes, such as adenosine deaminase, are not inactivated. Despite these difficulties, it seems that the major fraction of adenosine in hepatocytes exposed to extracellular adenosine deaminase is associated with AdoHcy hydrolase.

DR. M.S. HERSHFIELD: Is the conversion of enzyme-bound adenosine to adenine reversible? Will $^{14}C$ adenosine exchange into enzyme-bound adenosine?

DR. UELAND: With isolated enzyme, we have demonstrated that $^{14}C$ adenine tightly bound to the enzyme, or more likely its precursor, is reconverted to $^{14}C$ adenosine. However, $^{14}C$ adenine added to the enzyme is not converted to $^{14}C$ adenosine.

DR. H. OSSWALD: I would like to make a comment on the intracellular binding of adenosine. According to your data shown in Table 10.3, you have a maximal sequestration of 25 pmol adenosine per mg protein of kidney tissue, which is 25 nmol per gm protein or 5 nmol per gm wet weight. This is very close to the tissue content we find in rat kidneys [Osswald et al., *Pfluegers Arch* 371:45, 1977]. In addition, we find an adenosine-binding protein in the dog kidney with a $K_d$ of 11 n$M$ at 0°C for adenosine [Sacher R, Osswald H: *Naunyn Schmidedebergs Arch Pharmacol* (Suppl.) 311:K49, 1980] that corresponds to your values. Therefore, it seems likely that more than 95% of the tissue adenosine is bound to the adenosine-binding proteins.

DR. C.I. THOMPSON: Is it possible that the stable adenosine-SAH hydrolase complex

maintains significant hydrolytic activity? If not, how does one account for the apparent steady-state activity of the hydrolytic pathway under control conditions?

DR. UELAND: Yes, I feel it might be possible. There are data suggesting that the enzyme has four catalytic sites. Besides, there is no definite evidence that the sites sequestering adenosine are identical to the catalytic site.

DR. V. CHAGOYA DE SANCHEZ: Do you know the role of NAD in the stability of the complex? Is it possible that the redox state of $NAD^+$, NADH, is implicated in the liberation of adenosine?

DR. UELAND: The formation of the complex is probably associated with reduction of tightly bound $NAD^+$. I feel that further studies into the fate of NADH and adenosine bound to the enzyme should be carried out by workers well trained in chemistry. I am aware of no data on the possible relation between the redox state of $NAD^+$ and the sequestration of adenosine.

DR. R. BÜNGER: In considering a possible physiological role of the stable SAH-adenosine complex, it would seem of critical importance to know the association constants of the complex as well as the $t_{1/2}$ of the system. Is it known whether the adenosine that is sequestered as SAH-adenosine complex can dissociate quickly enough (say, within 30 to 60 sec) in sufficient amounts to contribute substantially to metabolic vasoregulation in, for example, heart or liver? Perhaps Dr. Schrader would also comment on this question.

DR. UELAND: The adenosine-AdoHcy hydrolase complex in isolated rat hepatocytes seems to have a $t_{1/2}$ of several hours. The turnover of this complex is an important question related to the possible biological implication of this phenomenon, and the question has recently been addressed by Belloni et al. and in our laboratory. The long turnover of the intracellular complex may be related to the experimental conditions used. It may be possible that complexes with different $t_{1/2}$ exist, as was recently shown for adenosine binding to purified AdoHcy hydrolase from plant. The HPLC gel-filtration technique used by us may be limited to the detection of complexes characterized by long $t_{1/2}$.

DR. SCHRADER: The reported *in vitro* rate of dissociation of the adenosine-SAH complex is rather slow ($t_{1/2}$ = 2.5 hr). It therefore seems rather unlikely that adenosine released from this complex can contribute to metabolic control of flow on a short-term basis.