Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical Pharmacology 66 (2003) 2117–2123
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

Adenosine binding sites at S-adenosylhomocysteine hydrolase are controlled by the NAD^+/NADH ratio of the enzyme

Doris Kloor[a,*], Angelika Lüdtke[a], Stanka Stoeva[b], Hartmut Osswald[a]

[a]*Department of Pharmacology and Toxicology, Faculty of Medicine, University of Tübingen, Wilhelmstrasse 56, D-72074 Tübingen, Germany*
[b]*Department of Physical Biochemistry, University of Tübingen, Hoppe-Seyler-Strasse 4, D-72076 Tübingen, Germany*

Received 17 June 2003; accepted 24 July 2003

Abstract

S-Adenosylhomocysteine hydrolase (AdoHcy hydrolase) catalyzes the reversible hydrolysis of S-adenosylhomocysteine (AdoHcy) to adenosine (Ado) and homocysteine. On the basis of the kinetics of Ado binding to AdoHcy hydrolase we have shown that AdoHcy hydrolase binds Ado with different affinities [Kidney Blood Press. Res. 19 (1996) 100]. Since AdoHcy hydrolase in its totally reduced form binds Ado with high affinity we determined in the present study the Ado binding characteristics of purified AdoHcy hydrolase from bovine kidney (native form) and of reconstituted forms with defined NAD^+/NADH ratios. AdoHcy hydrolase in its native form and at a ratio of 50% NAD^+ and 50% NADH exhibits two binding sites for Ado with a K_{D1} of 9.2 ± 0.6 nmol/L and a K_{D2} of 1.4 ± 0.1 μmol/L, respectively. Binding of Ado to AdoHcy hydrolase in its NADH form and in its NAD^+ form exhibits only one binding site with high affinity 48.3 ± 2.7 nmol/L for the NADH form and with a low affinity of 4.9 ± 0.3 μmol/L for the NAD^+ form. To identify these two Ado binding sites, AdoHcy hydrolase was covalently modified with [2-^{3}H]-8-azido-Ado. After irradiation of the native AdoHcy hydrolase two different photolabeled peptides were isolated and identified as Asp^{307}-Val^{325} and Tyr^{379}-Thr^{410}. When the reconstituted AdoHcy hydrolase in its NADH and in its NAD^+ form was irradiated with [2-^{3}H]-8-azido-Ado only one peptide was identified as Asn^{312}-Lys^{318} from the NADH form and as Asp^{391}-Ala^{396} from the NAD^+ form. Based on the crystallographic data, the labeled peptide Asp^{391}-Ala^{396} (low affinity binding site), appears to belong to the catalytic domain of AdoHcy hydrolase, whereas the labeled peptide, identified as Asn^{312}-Lys^{318} (high affinity binding site), is located in the NAD domain. In conclusion, our data show that AdoHcy hydrolase has two different Ado binding sites which are dependent upon the enzyme-bound NAD^+/NADH ratios.

Keywords: S-Adenosylhomocysteine hydrolase; Apo-enzyme; Adenosine binding sites; NAD^+/NADH ratio; Azido-adenosine; Photolabeled peptides

1. Introduction

The product of all methylation reactions, using AdoMet as methyl donor, is AdoHcy a potent inhibitor of methyltransferases [1–3]. In eukaryotic cells, AdoHcy is metabolized solely by AdoHcy hydrolase (EC 3.3.1.1) producing Ado, which has been suggested to play an important role in renal [4,5], cardiovascular [6] and neuronal functions [7], and homocysteine (Hcy) which has been implicated in causing vascular disease [8]. Thus, AdoHcy hydrolase is an essential enzyme playing an important role in regulating processes like transmethylation [9], transsulfuration [10] and purine metabolism [6]. Deletion of the AdoHcy hydrolase gene is associated with embryo lethality in mice [11].

The mammalian AdoHcy hydrolase is a homotetrameric enzyme which contains tightly bound NAD^+ as a cofactor, with a ratio of four mol NAD^+ per mol enzyme. The mechanism of action of AdoHcy hydrolase, in which a cycle of reciprocal oxidation–reduction of substrate and NAD^+ is involved has been studied by Palmer and Abeles [12]. Binding of Ado to AdoHcy hydrolase resulted in a 3′-keto-Ado by the enzyme-bound NAD^+ which is reduced to NADH. At this stage of the catalytic cycle, the enzyme is turned into a ‘‘closed’’ form [13,14] which is enzymatically inactive. The inactive AdoHcy hydrolase retains its ability to bind Ado with high affinity [15,16]. In earlier studies, it was shown that AdoHcyase from different species exhibits one or two Ado-binding sites with high

* Corresponding author. Tel.: +49-7071-2974941; fax: +49-7071-294942.
E-mail address: doris.kloor@uni-tuebingen.de (D. Kloor).
Abbreviations: Ado, adenosine; AdoHcy, S-adenosylhomocysteine; AdoMet, S-adenosylmethionine; NAD^+, NADH.

0006-2952/$ – see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/S0006-2952(03)00581-1

and low affinity and with a capacity of 2 mol Ado/mol enzyme [17–19]. Therefore, AdoHcy hydrolase could serve as a Ado-binding protein in the cytosol of the kidney [16]. Furthermore, Ado-binding to AdoHcy hydrolase leads to inhibition of the enzyme. Therefore, intracellular Ado is likely to be involved in the physiological regulation of AdoHcy hydrolase activity. Since the ratio of NAD^+/NADH at the enzyme modifies the Ado-binding site at the AdoHcy hydrolase, we analyzed in the present study the Ado-binding characteristics of AdoHcy hydrolase with defined ratios of NAD^+/NADH. Using [2-^{3}H]-8-azido-Ado we identified the amino acids that are part of the Ado-binding sites at the substrate–enzyme complex.

2. Materials

The following materials were purchased from the sources indicated: [2,8,5′-^{3}H]-Ado (2.3 TBq/mmol), [adenine-2,8-^{3}H]-NAD (1.4 TBq/mmol) NEN; [2-^{3}H]-8-azido-Ado (568 GBq/mmol) Bio-Trend; Ado, NAD^+, NADH Boehringer; Trypsin, Endoproteinase Asp-N, sequencing grade, Roche. Reagents for automated sequence analysis were obtained from the supplier of the instrument. All buffer chemicals were of the highest available quality exclusively from Merck.

3. Methods

3.1. Purification of AdoHcy hydrolase

The purification of AdoHcy hydrolase from bovine kidney was carried out as described previously [17]. The purified enzyme was frozen at −20° until use. The protein concentration was determined by the method of Bradford [20] using bovine serum albumin as a standard.

3.2. Preparation of apo-AdoHcy hydrolase

Apo-AdoHcy hydrolase was produced by treatment of freshly purified AdoHcy hydrolase with 1.5 mol/L KCl, 80 mmol/L ATP and 80 mmol/L $MgCl_2$ to remove the NAD^+ as described previously [16]. The apo-enzyme was reconstituted with the following NAD^+/NADH ratios: 1/0, 0.9/0.1, 0.5/0.5, 0.1/0.9, 0/1.

3.3. Determination of enzyme-NAD^+ content

Apo-AdoHcy hydrolase (120 μg) was reconstituted with ^{3}H-NAD^+ (2 μCi/mmol) and NADH (1 mmol/L) in 20 mmol/L Tris, 40 mmol/L Hepes, pH 7.4 with the following ratios: 0.9 ^{3}H-NAD^+/0.1 NADH, 0.5 ^{3}H-NAD^+/0.5 NADH, 0.1 ^{3}H-NAD^+/0.9 NADH. After 90 min incubation at room temperature, the reaction mixture was filtered through GF/B filter, presoaked with 0.3% polyethylenimine. Radioactivity adsorbed on the filters was determined by liquid scintillation counting.

3.4. Assay of AdoHcy hydrolase activity

The activity of AdoHcy hydrolase in its native and reconstituted form was assayed in the hydrolytic direction as described earlier [17]. This assay measures the rate of uric acid formation at 292 nm.

3.5. Binding assay procedure

Saturation binding experiments were performed in a final volume of 300 μL 20 mmol/L Tris, 40 mmol/L Hepes, pH 7.4 with ^{3}H-Ado (0.01–60 μmol/L) and 10 μg/mL AdoHcy hydrolase with the NAD^+/NADH ratios mentioned above. The reaction mixture was incubated overnight at 4°. Untreated (native) AdoHcy hydrolase served as control. After incubation samples were filtered as described above.

3.6. Isolation of photolabeled peptides

Native AdoHcy hydrolase (1 mg) and AdoHcy hydrolase in its totally active (NAD^+) and in its totally reduced (NADH) form (400 μg) were incubated overnight at 4° with [2-^{3}H]-8-azido-Ado (30 μCi/μmol) in a protein-to-probe molar ratio of 1:20. In separate experiments, we tested whether [2-^{3}H]-8-azido-Ado and Ado exhibit the same binding properties (data not shown). After irradiation with a UV lamp at 254 nm for 5 min, the reaction mixture was dialyzed either against 50 mmol/L sodium-phosphate buffer, pH 8.0 for Asp-N proteinase digestion or against 100 mmol/L Tris–HCl, pH 8.6 for trypsin digestion.

The Asp-N or trypsin digests were separated by reverse phase HPLC on a Grom-Sil ODS column (250 mm × 4 mm; 5 μm, Herrenberg). The solvent system consisted of eluent A (0.1% trifluoroacetic acid (TFA) in water) and eluent B (80% acetonitrile in eluent A). The elution was carried out using a linear concentration gradient from 10 to 50% B (by volume) in 80 min at a flow rate of 0.8 mL/min. The UV absorbance of the eluate was monitored at 214 nm. Fractions were manually collected and radioactivity in each fraction was determined by liquid scintillation counting.

3.7. Identification of labeled peptides

The peptide peaks containing the highest radioactivity were sequenced with on-line analysis of the phenylthiohydantoin (PTH) derivatives [21] by automated Edman degradation using an Applied Biosystem 473A pulsed-liquid Protein Sequencer (Weiterstadt). The peptides were dissolved in 0.1% (v/v) TFA and spotted onto polybrene-coated filters. The PTH amino acids produced by the sequential Edman degradation of the analyzed peptides were identified using the retention times of a PTH standard run as a reference.

Mass spectroscopic analysis of the HPLC fractions containing the labeled peptide fragments was done by MALDI-MS (Kratos, MALDI II equipment, Shimadzu). Peptides (10–50 pmol) were dissolved in 0.1% (v/v) TFA and applied onto a target. Analysis was carried out in α-cyano-4-hydroxycinnamic acid or 2,5-dihydroxybenzoic acid [22,23]. Solution of human substance P (1347.7 Da) and bovine insulin (5733.6 Da) were used to calibrate the mass scale. The mass values assigned to the amino-acid residues are the average masses.

4. Calculation

Data were analyzed using two fitting procedure, LIGAND [24] and nonlinear regression analysis. The run test was used to determine the goodness of fit data to a given curve. The F test was applied to compare the curve fitting for a one versus two or more receptor site model. $P < 0.05$ was considered significant.

5. Results

5.1. Determination of enzyme-bound NAD^+

In order to determine the ratios of NAD^+/NADH incorporation into the apo-AdoHcy hydrolase, we first conducted experiments with ^{3}H-NAD^+ to confirm the NAD^+/NADH ratios of the different prepared AdoHcy hydrolase forms used in this study. As shown in Table 1, the reconstituted apo-enzyme shows to the full extent the ^{3}H-NAD ratio set in the incubation medium.

Table 1
Incorporation of ^{3}H-NAD into apo-AdoHcy hydrolase

^{3}H-NAD/NADH ratio	^{3}H-NAD incorporated (%)
0.9/0.1	90.05
0.5/0.5	52
0.1/0.9	11.4

5.2. Enzyme activity and Ado-binding of the different AdoHcy hydrolase forms

Figure 1 shows enzyme activity of AdoHcy hydrolase and ^{3}H-Ado-binding related to the NAD^+/NADH ratios of the enzyme. NAD^+-AdoHcy hydrolase exhibits the highest enzyme activity of 0.12 ± 0.02 U/mg and the lowest ^{3}H-Ado binding of 16.1 ± 1.1 pmol/mg. The enzyme activity was reduced to 0.04 U/mg with increase of NADH (0.1 NAD^+/0.9 NADH), while ^{3}H-Ado was bound with a 7-fold higher capacity (112.3 ± 3.2 pmol/mg). Correspondingly, AdoHcy hydrolase in its totally reduced (NADH) form is enzymatically inactive and has a ^{3}H-Ado binding capacity of 111.9 ± 2.1 pmol/mg.

Saturation experiments were performed by increasing concentrations of [^{3}H]-Ado up to 60 μM. Nonspecific binding increased linearly with increasing [^{3}H]-Ado concentrations and represented approximately 5–15% of total binding. Nonlinear regression (Fig. 2A) and Scatchard analysis (Fig. 2B) of the binding isotherms of native AdoHcy hydrolase and of the AdoHcy hydrolase form with 50% NAD^+ and 50% NADH indicated two binding sites for [^{3}H]-Ado with a high and a low affinity. On the other hand, the nonlinear regression (Fig. 2C) and Scatchard analysis (Fig. 2D) of the binding isotherms of the

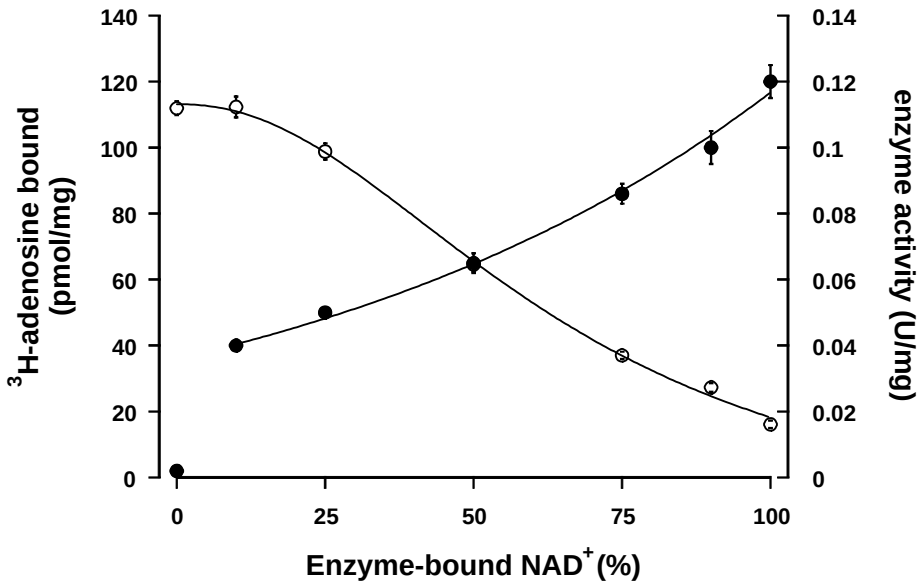

Fig. 1. ^{3}H-Ado binding (○) and enzyme activity (●) of AdoHcy hydrolase dependent upon the NAD^+/NADH ratio. Binding was performed with 10 nM ^{3}H-Ado and AdoHcy hydrolase 10 μg/mL. Enzyme activity was performed in the direction of hydrolysis with 50 μM AdoHcy and AdoHcy hydrolase 20 μg/mL. Data were obtained from three independent experiments.

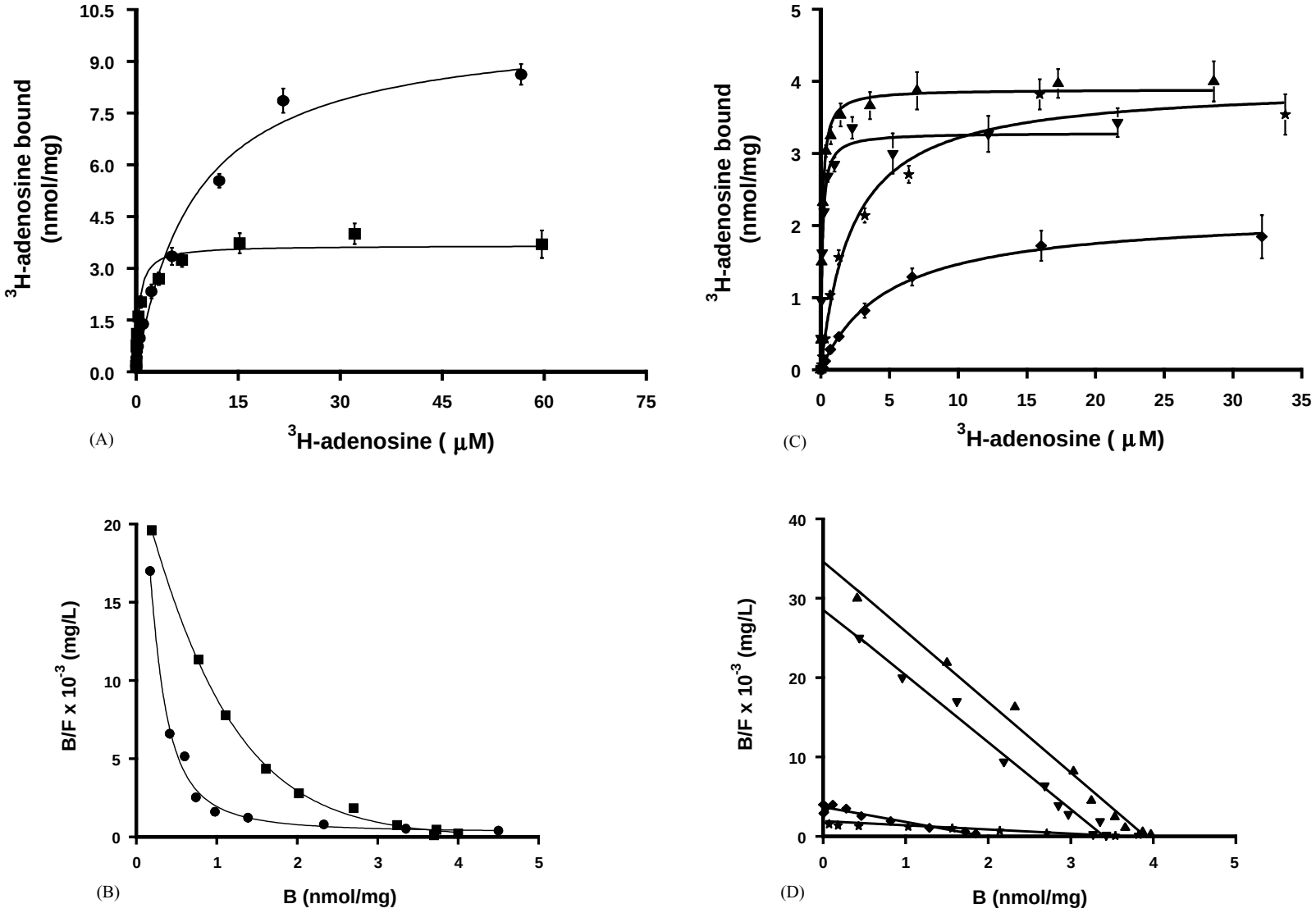

Fig. 2. Specific binding of [^{3}H]-Ado to AdoHcy hydrolase. Native AdoHcy hydrolase (●) and AdoHcy hydrolase containing 50% NAD^+ and 50% NADH (■) (A) and AdoHcy hydrolase containing 100% NADH (▼), 90% NADH and 10% NAD^+ (▲), 100% NAD^+ (✦), 90% NAD^+ and 10% NADH (◆) (C), respectively, were incubated with increasing concentrations of ^{3}H-Ado. Transformation of the data according to Scatchard are shown in (B) and (D). Data were consisting of duplicate determinations and were obtained from four independent experiments.

Table 2
Kinetic data obtained in saturation binding experiments with native AdoHcy hydrolase and AdoHcy hydrolase with defined NAD^+/NADH ratios

AdoHcy hydrolase	K_{D1} (nmol/L)	K_{D2} (μmol/L)	B_{max1} (nmol/mg)	B_{max2} (nmol/mg)
Native	9.2 ± 0.6	1.4 ± 0.1	0.62 ± 0.03	8.6 ± 1.1
NAD^+/NADH ratios				
1/0	–	4.9 ± 0.3	–	2.3 ± 0.1
0.9/0.1	–	3.7 ± 0.1	–	3.1 ± 0.2
0.5/0.5	6.5 ± 0.3	0.6 ± 0.03	0.65 ± 0.01	3.5 ± 0.3
0.1/0.9	98.5 ± 7.7	–	–	3.9 ± 0.8
0/1	48.3 ± 2.7	–	–	3.5 ± 1.0

Table 3
Amino-acid sequence analysis of the photolabeled peptides

NAD^+/NADH	[2-^{3}H]-8-Azido-adenosine	Labeled peptide
1.0/0.0		DEAVA Asp^{391}-Ala^{396}
Native	DVKWLN**NENAVEK**VNIKPQV Asp^{307}-Val^{325}	VGVHFLPKKL**DEAVA**EAHLG Tyr^{379}-Thr^{410}
0.0/1.0	NENAVEK Asn^{312}-Lys^{318}	

AdoHcy hydrolase forms which contain either 100 or 90% NAD^+ and the forms which contain 100 or 90% NADH exhibits only one [^{3}H]-Ado binding site. The equilibrium dissociation constants (K_d) and the maximal number of binding sites (B_{max}) labeled by [^{3}H]-Ado are shown in Table 2. Comparing the data of the modified AdoHcy hydrolase with the data of the native AdoHcy hydrolase, four aspects attract attention: (i) two Ado-binding sites, with a low and a high affinity exhibits only the AdoHcy hydrolase form with equal concentrations of NAD^+ and NADH, (ii) the high affinity binding site is lowered 5–10 times at the AdoHcy hydrolase forms which contain mainly NADH, (iii) the enzymatically most active AdoHcy hydrolase form exhibits no Ado-binding site with high affinity, (iv) the Ado-binding capacity of these reconstituted AdoHcy hydrolase forms is reduced. These enzymes bind approximately 1 mol Ado/mol enzyme.

5.3. Photolabeled peptides

As shown in Table 3 two different photolabeled peptides could be isolated from the native AdoHcy hydrolase: Tyr^{379} -Thr^{410} (low affinity binding site) and Asp^{307}-Val^{325} (high affinity binding site). When AdoHcy hydrolase with defined NAD^+/NADH ratios was photolabeled, only one peptide was identified as Asp^{391}-Ala^{396} from the NAD^+ form, whereas another peptide, Asn^{312}-Lys^{318}, of the enzyme in its NADH form incorporated the azido-probe.

6. Discussion

To elucidate further the mechanism of the reversible hydrolysis of AdoHcy to Ado and Hcy several crystal structures of AdoHcy hydrolase, including site-directed mutagenesis have been determined [13,14,25–27]. Each structure represents a different conformation of AdoHcy hydrolase, either an open [25] or closed [13,14] conformation structure of the enzyme. Furthermore, Ado or other AdoHcy hydrolase inhibitors (e.g. D-eritadenine) interacted with the same amino-acid residues which are considered to represent the active site of the enzyme [14,27]. Since in these studies no attempts were made to determine the influence the different NAD^+/NADH ratios on Ado-binding to the enzyme we carried out Ado-binding and photoaffinity labeling experiments to study the effect of defined NAD^+/NADH ratios of Ado-binding.

In a previous study we have shown that Ado induces the reduction of the tightly bound NAD^+ to NADH, resulting in an enzymatically inactive enzyme [17]. The enzymatically inactive AdoHcy hydrolase, reconstituted with NADH retains its ability to bind Ado with a K_D of 35 nM [16]. As shown in Fig. 1, the enzyme activity of the reconstituted AdoHcy hydrolase with defined NAD^+/NADH ratios decreases reciprocally with the increase of NADH content. Since Ado is removed in the reaction mixture the actual ratio of NAD^+/NADH in the enzyme is unlikely to be changed during the cycle of AdoHcy hydrolysis which generates Ado. At a ratio of 90% NADH and 10% NAD^+ the enzyme activity is still 30% when compared to the oxidized form suggesting that the enzyme retains sufficient activity for hydrolysis of AdoHcy at even 10% NAD^+. The enzyme activity of this AdoHcy hydrolase form might be sufficient to remove intracellular AdoHcy which has a tissue content in the kidney of 0.7 nmol/g wet weight, corresponding to approximately 0.9 μM. In addition, AdoHcy hydrolase in its NADH form might serve as an intracellular Ado-binding protein [28].

Saturation binding experiments with defined NAD^+/NADH ratios clearly demonstrated that the affinity of Ado and the Ado-binding sites at AdoHcy hydrolase are dependent upon the enzyme-bound NAD^+/NADH ratio.

Two binding sites are detected, both, in the native AdoHcy hydrolase and in the 50% NAD^+ and 50% NADH reconstituted AdoHcy hydrolase form, indicating that freshly isolated AdoHcy hydrolase from bovine kidney contains NAD in its oxidized and in its reduced form, which is in accordance with observations made by Porter and Boyd [15] and Kloor *et al.* [29]. The corresponding peptides of the native AdoHcy hydrolase that are labeled with [2-^{3}H]-8-azido-Ado are Asp-307-Val-325 and Tyr-379-Thr-410. It was not possible to identify the amino acid associated with the radioactivity since the ^{3}H-label dissociated from the modified amino acid during sequencing. A peptide of the same region was identified by Yuan and Borchardt in recombinant human AdoHcy hydrolase using also photoaffinity labeling [30]. This binding domain was proposed to participate to the adenine-ring-binding pocket. However, both azido-Ado labeled peptides from bovine kidney do not correspond to the assumed pockets of crystallized recombinant AdoHcy hydrolase published by other investigators [13,14,25–27]. These discrepancies may be due to the different procedures of enzyme purification and differences in species from which the enzyme was isolated. This interpretation is supported by the observation that the most striking difference between our data and those of the literature refers to the enzymatic activity of AdoHcy hydrolase. Takata *et al.* [27] reported for the recombinant wild type AdoHcy hydrolase which was used for crystallization an enzymatic activity of 5 nmol/min/mg whereas our AdoHcy hydrolase freshly isolated from bovine kidney exhibits an activity of 250 nmol/min/mg [17], representing a 50-fold difference.

AdoHcy hydrolase in its reduced form exhibits only one Ado-binding site with high affinity. The high affinity binding site disappears when AdoHcy hydrolase contains only NAD^+. This enzyme form binds Ado with low affinity, comparable with the K_M value for Ado in direction of synthesis suggesting the interaction of Ado with the active site of the enzyme [31]. The two different single Ado-binding sites of the NAD^+ and NADH forms of the reconstituted enzyme reported in the present study correspond to the respective two Ado-binding sites of native AdoHcy hydrolase. Compared to the native AdoHcy hydrolase which binds 2 mol Ado/mol enzyme, all reconstituted AdoHcy hydrolase forms bind Ado with a maximal capacity of 1 mol Ado/mol enzyme (Fig. 2). We cannot exclude, that during the preparation of the apo-enzyme and subsequent reconstitution with NAD^+/NADH changes of the enzyme conformation may occur resulting in a loss of Ado-binding capacity compared to the native enzyme.

Our studies show a mechanism of intracellular Ado action to reduce the enzyme-bound NAD^+ to NADH. Under pathophysiological conditions, such as hypoxia or tissue injury tissue levels of Ado and AdoHcy increase [28]. It has been reported, that high Ado concentrations can induce apoptosis [32,33]. Since AdoHcy hydrolase activity determines the methylation potential (AdoMet/AdoHcy) high AdoHcy tissue levels results in an inhibition of AdoMet-dependent transmethylation. Therefore, this intracellular Ado action might be part of the mechanism of Ado-induced apoptosis.

In summary, our results show a high affinity Ado-binding site of AdoHcy hydrolase in its NADH form. The existence of two separate binding sites at the enzyme suggested by previous studies were confirmed with azido-Ado experiments. The putative function of the high affinity binding site may serve allosteric purposes. Further experimental work is necessary to match our findings with the crystallographic data of AdoHcy hydrolase.

Acknowledgments

The work carried out was supported by a grant from the Federal Ministry of Education, Science, Research and Technology (BMBF-Fö 01 EC 9405).

References

[1] Cantoni GL, Chiang PK. The role of S-adenosylhomocysteine and S-adenosylhomocysteine hydrolase in the control of biological methylation. In: Cavallini D, Gaull GE, Zappia V., editors. Natural sulfur compounds: novel biochemical and structural aspects. New York and London: Plenum Press; 1980. p. 67–80.
[2] Barber JR, Clarke S. Inhibition of protein carboxyl methylation by S-adenosyl-L-homocysteine in intact erythrocytes. Physiological consequences. J Biol Chem 1984;259:7115–22.
[3] Schatz RA, Wilens TE, Sellinger OZ. Decreased transmethylation of biogenic amines after *in vivo* elevation of brain S-adenosyl-L-homocysteine. J Neurochem 1981;36:1739–48.
[4] Osswald H. The role of adenosine in the regulation of glomerular filtration rate and renin secretion. TIPS 1984;5:94–7.
[5] Osswald H, Mühlbauer B, Schenk F. Adenosine mediates tubuloglomerular feedback response: an element of metabolic control of kidney function. Kidney Int 1991;39:128–31.
[6] Kroll K, Decking UK, Dreikorn K, Schrader J. Rapid turnover of the AMP-adenosine metabolic cycle in the guinea pig heart. Circ Res 1993;73:846–56.
[7] Fredholm BB, Hedqvist P. Modulation of neurotransmission by purine nucleotides and nucleosides. Biochem Pharmacol 1980;29:1635–43.
[8] Hankey GJ, Eickelboom JW. Homocysteine and vascular disease. Lancet 1999;354:407–13.
[9] Shatkin AJ. Capping of eukaryotic mRNAs. Cell 1976;9:645–53.
[10] Finkelstein JD, Martin JJ. Methionine metabolism in mammals. Distribution of homocysteine between competing pathways. J Biol Chem 1984;259:9508–13.
[11] Miller MW, Duhl DM, Winkes BM, Arredondo-Vega F, Saxon PJ, Wolff GL, Epstein CJ, Hershfield MS, Barsh GS. The mouse lethal nonagouthi (a(x)) mutation deletes the S-adenosylhomocysteine hydrolase (Ahcy) gene. EMBO J 1994;13:1806–16.
[12] Palmer JL, Abeles RH. Mechanism of action of S-adenosylhomocysteinase. J Biol Chem 1979;254:1217–26.
[13] Turner MA, Yuan CS, Borchardt RT, Hershfield MS, Smith GD, Howell PL. Structure determination of selenomethionyl S-adenosylhomocysteine hydrolase using data at a single wavelength. Nature Struc Biol 1998;5:369–76.
[14] Huang Y, Komoto J, Takata Y, Powell DR, Gomi T, Ogawa H, Fujioka M, Takusagawa F. Inhibition of S-adenosylhomocysteine hydrolase by "acyclic sugar" adenosine analogue D-eritadenine: crystal structure of

S-adenosylhomocysteine hydrolase complexed with D-eritadenine. J Biol Chem 2002;277:7477–82.
[15] Porter DJT, Boyd FL. Reduced S-adenosylhomocysteine hydrolase. Kinetics and thermodynamics for binding of 3′-ketoadenosine, adenosine, and adenine. J Biol Chem 1992;267:3205–13.
[16] Kloor D, Kozo Y, Delabar U, Osswald H. Simple and sensitive binding assay for measurement of adenosine using reduced S-adenosylhomocysteine hydrolase. Clin Chem 2000;46:537–42.
[17] Kloor D, Fuchs S, Kurz J, Faust B, Osswald H. S-Adenosylhomocysteine hydrolase from bovine kidney: enzymatic and binding properties. Kidney Blood Press Res 1996;19:100–8.
[18] Olsson RA. Ligand binding to the adenosine analogue binding protein in rabbit erythrocyte. Biochemistry 1978;17:367–75.
[19] Hershfield MS, Kredich NM. S-Adenosylhomocysteine hydrolase is an adenosine binding protein: a target for adenosine toxicity. Science 1978;202:757–60.
[20] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Anal Biochem 1976;72:248–54.
[21] Hunkapiller MW, Hood LE. Protein sequence analysis: automated microsequencing. Science 1983;219:650–9.
[22] Hillenkamp F, Karas M. Mass spectrometry of peptides and proteins by matrix-assisted ultraviolet laser desorption/ionization. Methods Enzymol 1990;193:280–95.
[23] Gevaert K, Demol H, Puype M, Broekaert D, De Boek S, Houthaeve T, Vandekerckhove J. Peptides adsorbed on reverse-phase femtomole sequencing by post-source decay matrix assisted laser desorption ionization-reflectron time of flight mass spectrometry (MALDI-RE-TOF-MS). Electrophoresis 1997;18:2950–60.
[24] Munson PJ, Rodbard D. LIGAND: a versatile computerized approach for the characterization of ligand binding systems. Anal Biochem 1980;107:220–39.
[25] Hu Y, Komoto J, Huang Y, Gomi T, Ogawa H, Takata Y, Fujioka M, Takusagawa F. Crystal structure of S-adenosylhomocysteine hydrolase from rat liver. Biochemistry 1999;38:8323–33.
[26] Komoto J, Huang Y, Gomi T, Ogawa H, Takata Y, Fujioka M, Takusagawa F. Effects of site-directed mutagenesis on structure and function of recombinant rat liver S-adenosylhomocysteine hydrolase. J Biol Chem 2000;275:32147–56.
[27] Takata Y, Yamada T, Huang Y, Komoto J, Gomi T, Ogawa H, Fujioka M, Takusagawa F. Catalytic mechanism of S-adenosylhomocysteine hydrolase. J Biol Chem 2002;277:22670–6.
[28] Kloor D, Delabar U, Mühlbauer B, Luippold G, Osswald H. Tissue levels of S-adenosylhomocysteine in the rat kidney: effects of ischemia and homocysteine. Biochem Pharmacol 2002;63:809–15.
[29] Kloor D, Danielyan L, Osswald H. Characterization of the cAMP binding site of purified S-adenosylhomocysteine hydrolase from bovine kidney. Biochem Pharmacol 2002;64:1201–6.
[30] Yuan CS, Borchardt RT. Photoaffinity labeling of human placental S-adenosyl-homocysteine hydrolase with 2-[^{3}H]-8-azido-adenosine. J Biol Chem 1995;270:16140–6.
[31] Kloor D, Fuchs S, Petroktistis F, Delabar U, Mühlbauer B, Quast U, Osswald H. Effects of ions on adenosine binding and enzyme activity of purified S-adenosylhomocysteine hydrolase from bovine kidney. Biochem Pharmacol 1998;56:1493–6.
[32] Rounds S, Yee LW, Dawicki DD, Harrington E, Parks N, Cutaia MV. Mechanism of extracellular ATP- and adenosine-induced apoptosis of cultured pulmonary artery endothelial cells. Am J Physiol 1998;275:L378–88.
[33] Cerutti S, Giammarioli AM, Camurri A, Falzano L, Rufini S, Frank C, Fiorentini C, Malorni W, Abbracchio MP. Adenosine- and 2-chloroadenosine-induced cytopathic effects on myoblastic cells and myotubes: involvement of different intracellular mechanisms. Neuromuscul Disord 2000;10:436–46.