Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 368 (2008) 30–36
BBRC
www.elsevier.com/locate/ybbrc

S-Adenosylhomocysteine hydrolase (AdoHcyase) deficiency: Enzymatic capabilities of human AdoHcyase are highly effected by changes to codon 89 and its surrounding residues

R. Belužić [a], M. Ćuk [b], T. Pavkov [c], I. Barić [b], O. Vugrek [a,*]

[a] *Institute Ruđer Bošković, Division of Molecular Medicine, Bijenička 54, 10000 Zagreb, Croatia*
[b] *University Hospital Center, School of Medicine, Department of Pediatrics, Zagreb, Croatia*
[c] *Karl-Franzens-University, Institute of Chemistry, Structural Biology, Graz, Austria*

Received 18 December 2007
Available online 22 January 2008

Abstract

Recently, *S*-adenosylhomocysteine hydrolase deficiency was confirmed for the first time in an adult. Two missense mutations in codons 89 (A>V) and 143 (Y>C) in the AdoHcyase gene were identified [N.R.M. Buist, B. Glenn, O. Vugrek, C. Wagner, S. Stabler, R.H. Allen, I. Pogribny, A. Schulze, S.H. Zeisel, I. Barić, S.H. Mudd, *S*-Adenosylhomocysteine hydrolase deficiency in a 26-year-old man, J. Inh. Metab. Dis. 29 (2006) 538–545]. Accordingly, we have proven the Y143C mutation to be highly inactivating [R. Belužić, M. Ćuk, T. Pavkov, K. Fumić, I. Barić, S.H. Mudd, I. Jurak, O. Vugrek, A single mutation at tyrosine 143 of human *S*-adenosylhomocysteine hydrolase renders the enzyme thermosensitive and effects the oxidation state of bound co-factor NAD, Biochem. J. 400 (2006) 245–253]. Now we report that the A89V exchange leads to a $\geqslant$70% loss of enzymatic activity, respectively. Circular dichroism analysis of recombinant p.A89V protein shows a significantly reduced unfolding temperature by 5.5 °C compared to wild-type. Gel filtration of mutant protein is almost identical to wild-type indicating assembly of subunits into the tetrameric complex. However, electrophoretic mobility of p.A89V is notably faster as shown by native polyacrylamide gel electrophoresis implicating changes to the overall charge of the mutant complex.

'Bioinformatics' analysis indicates that Val^{89} collides with Thr^{84} causing sterical incompatibility. Performing site-directed mutagenesis changing Thr^{84} to 'smaller' Ser^{84} but preserving similar physico-chemical properties restores most of the catalytic capabilities of the mutant p.A89V enzyme. On the other hand, substitution of Thr^{84} with Lys^{84} or Gln^{84}, thereby introducing residues with higher volume in proximity to Ala^{89} results in inactivation of wild-type protein. In view of our mutational analysis, we consider changes in charge and the sterical incompatibility in mutant p.A89V protein as main reason for enzyme malfunction with AdoHcyase deficiency as consequence.

Keywords: Human mutation; Thermosensitivity; Circular dichroism; Functional genomics; Site-directed mutagenesis

Recently, we have shown that missense mutations in the human AdoHcyase gene might be linked to AdoHcyase deficiency in an adult [1]. AdoHcyase represents a key enzyme in the mammalian methionine metabolism, and disfunction causes serious health problems. Main clinical and biochemical consequences are severe myopathy, developmental delay, elevated serum creatine kinase concentrations, increased *S*-adenosylmethionine (AdoMet) and *S*-adenosylhomocysteine (AdoHcy), and hypermethioninaemia [1,3,4]. AdoHcy is formed as a product of AdoMet through transmethylation reactions [5]. AdoMet is the major methyl donor for delivery of methyl groups to DNA, RNA, proteins and cellular metabolites in eukaryotes, and AdoHcy hydrolysis is the only source of homocysteine in mammals [6]. AdoHcy is a strong competitive inhibitor of many AdoMet-dependent methyltransferases [7]. Thus, AdoMet/AdoHcy turnover is believed to play a

* Corresponding author. Fax: +38514680119.
E-mail address: ovugrek@irb.hr (O. Vugrek).

0006-291X/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2008.01.042

critical in the regulation of biological methylation, and aberrant methylation processes might be the reason for the characteristic clinical consequences in AdoHcyase deficient patients.

AdoHcy hydrolysis, catalyzed by *S*-adenosylhomocysteine hydrolase (EC 3.3.1.1), is a reversible reaction with an equilibrium favoring AdoHcy formation, but proceeds under physiological conditions in the hydrolytic direction due to the rapid removal of reaction products adenosine (Ado) and homocysteine [8].

Human AdoHcyase is a tetramer consisting of chemically identical and functionally equivalent subunits [9], each with one molecule NAD tightly bound [10]. The proposed mechanism for reversible hydrolysis of AdoHcy involves coenzyme NAD in a cyclic redox reaction, producing several intermediates to form the final products, Ado or AdoHcy [10,11].

The 3-dimensional structure of human AdoHcyase has been resolved [12] and mutational studies have allowed detailed insights into the processes important for catalytic activity [13].

In this report, we elucidate the role of identified mutation in codon Ala^{89} for enzymatic activity and holoenzyme formation of AdoHcyase. We used circular dichroism to determine structural aspects such as protein unfolding and stability during thermal induction. Also, we measured the oxidation state of bound co-factor NAD and determined the exact enzymatic capabilities of recombinant p.A89V protein. Additionally, we performed a series of site-directed mutations targeting Thr^{84}, which is in proximity to residue 89 to evaluate the consequences of the alanine to valine exchange in mutant protein. We show, that the A89V mutation must be considered as highly negative modification of human AdoHcyase resulting in an intramolecular collision with neighboring residue Thr^{84}, leading to enzyme inactivation, and that it represents one basis for severe AdoHcyase deficiency in human.

Materials and methods

Cloning and overexpression of recombinant wild-type and mutant AdoHcyase for expression in Escherichia coli. The expression vector harboring the wild-type AdoHcyase gene (p32AHHwt) was constructed as described previously [2] and used as template for site-directed mutagenesis with specific oligonucleotides (listed in Table 1) and the GeneTailor™ system (Invitrogen, Carlsbad, CA, U.S.A). The exchanges were confirmed by dideoxy sequencing using the BigDye® chemistry (Applied-Biosystems, Foster City, CA, USA).

A detailed protocol for expression and purification of recombinant AdoHcyases in *E. coli* BL21 (DE3) RIL is given in Belužić et al. [2].

Additional expression plasmids pQE30AHHwt and pQE30A89V were constructed by using vector pQE30 (Qiagen, Hilden, Germany) as host. Briefly, AdoHcyase coding sequences were removed from vectors p32AHHwt and p.A89V by restriction with endonucleases KpnI and HindIII and inserted into KpnI/HindIII restricted pQE30. Positive clones were transformed into *E. coli* M15 (Qiagen, Hilden, Germany) and recombinant protein was generated and purified according to Belužić et al. [2].

Enzymatic assays. *S*-Adenosylhomocysteine hydrolase activity in purified enzyme preparations was assayed according to Takata et al. [14] using 5 μg recombinant protein.

The synthetic activity of the purified AdoHcyase was determined by the rate of disappearance of adenosine according to Belužić et al. [2].

Quantitation of enzyme bound NAD^+ and NADH was performed using a fluorescence technique described by Hohman et al. [15] using 200 μg of recombinant protein.

Polyacrylamide gel electrophoresis (PAGE) and gel filtration chromatography. SDS–PAGE and determination of protein concentrations followed standard laboratory procedures. Additionally, purity and electrophoretic behavior of the recombinant AdoHcyase protein was analyzed using native polyacrylamide gel electrophoresis (native PAGE) as described previously 2. The molecular weights of recombinant mutant and wild-type forms of AdoHcyase were analyzed by gel filtration chromatography according to Belužić et al. [2].

Circular dichroism analysis (CD). CD measurements were performed on Jasco J-715 spectropolarimeter (Jasco Europe S.R.L, Cremella) using a 0.02 cm water-jacket cylindrical cell, thermostated by an external computer-controlled water bath as described previously [2]. The protein concentrations used for measurements were 0.73 mg/ml for the wild-type and 0.53 mg/ml for the p.A89V protein.

Bioinformatics. We used software applications nnPredict (Donald Kneller, University of California) for secondary structure prediction. Deepview/Swiss-PdbViewer [16] was used for in silico mutation and 3D-structural analysis of the crystal structure of the human AdoHcyase (1A7A) [12]. PeptideMass (http://www.expasy.ch/tools) was used for molecular weight prediction of recombinant AdoHcyase protein. Physicochemical characteristics of amino acid were retrieved from http://prowl.rockefeller.edu/.

Results

Purification of recombinant AdoHcyases

Cloning, expression and purification of recombinant AdoHcyases was achieved as described previously [2]. We constructed vectors that expressed recombinant protein with single mutations in the AdoHcyase gene at amino acid positions 84 (p.WT_A84; p.WT_S84; p.WT_K84; p.WT_Q84) and 89 (p.A89V), and double mutations at

Table 1
Oligonucleotides used for site-directed mutagenesis (purchased from Invitrogen, Carlsbad, CA, USA)

Construct	Oligonucleotide (5′–3′) forward	Oligonucleotide (5′–3′) reverse	Codon change
p.A89V	ATGCGGTGGCTGCAATTGCCAA	TTGGCAATTGCAGCCACCGCAT	GCG→GTG
p.WT_A84	GTTGCAACATCTTCTCCGCCCA	GCGGAGAAGATGTTGCAACTGGA	ACC→GCC
p.WT_S84	GTTGCAACATCTTCTCCTCCCA	GAGGAGAAGATGTTGCAACTGGA	ACC→TCC
p.A89V_A84	GTTGCAACATCTTCTCCGCCCA	GCGGAGAAGATGTTGCAACTGGA	ACC→GCC
p.A89V_S84	GTTGCAACATCTTCTCCTCCCA	GAGGAGAAGATGTTGCAACTGGA	ACC→TCC
p.WT_K84	GTTGCAACATCTTCTCCAAACAGGA	TTGGAGAAGATGTTGCAACTGGA	ACC→AAA
p.WT_Q84	GTTGCAACATCTTCTCCCAACAGGA	TGGGAGAAGATGTTGCAACTGGA	ACC→CAA

position 84 and 89 (p.A89V_A84; p.A89V_S84). The mutation in codon 89 has been detected in an adult AdoHcyase deficient patient, whereas the codon 84 mutations were deliberately introduced into the AdoHcyase cDNA. The same purification steps used to purify the wild-type enzyme were used in the purification of the mutant enzymes.

Approximately 5–10 mg of homogeneous AdoHcyase was obtained from a 1-L culture. pET32 based expression yielded in less formation of insoluble protein than pQE30 expression probably as a result of the presence of a thioredoxin tag.

pET32 based recombinant AdoHcyases have an increased molecular weight (63.9 kDa) due to the additional 152 amino acid residues contributed by the plasmid expression vector tag region. Cleavage with thrombin reduced the size of the recombinant proteins by 130 residues (~14 kDa), leaving only 22 additional amino acids fused to the AdoHcyase protein that are identical for all expressed variants. SDS–PAGE analysis of cleaved or uncleaved pET32 derived protein showed their mobility is in agreement with the predicted molecular weight (Fig. 1). The pQE30 expressed AdoHcyases consist of a fusion protein with only 18 additional residues when compared to human placental AdoHcyase (not shown).

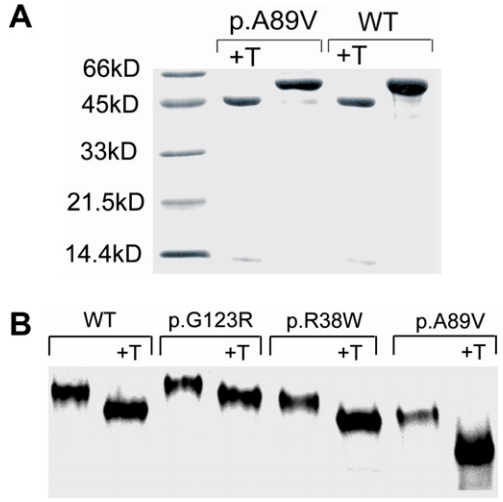

Fig. 1. (A) SDS–PAGE of 4 μg of freshly prepared Ni–NTA affinity-purified thrombin-cleaved or uncleaved recombinant p.A89V AdoHcyase; wild-type (WT) protein is derived from a 12 month frozen stock. (B) Native PAGE of 5 μg of Ni–NTA affinity-purified thrombin-cleaved or uncleaved recombinant AdoHcyases; WT; p.R38W, isoform SAHH-2; p.G123R, isoform SAHH-3; p.A89V; +T, thrombin-cleaved protein; (B) Recombinant proteins were resolved on 7.5% native PAGE or 12% SDS–PAGE, respectively.

Characterisation of p.A89V AdoHcyase

The overall catalytic activity was determined in the directions of AdoHcy synthesis or hydrolysis. Table 2 summarizes the catalytic properties of wild-type and mutant enzymes. The p.A89V enzyme showed a dramatic decrease of the overall catalytic activity in both directions ($\geqslant$70% compared to wild-type). Also, we found that the enzymatic activity of p.A89V protein has a very short half-life of approximately 1–2 weeks at 4 °C and even storage at −20 °C does not preserve its activity for more than 1 month (not shown). On the other hand wild-type protein is extremely robust and retains activity for at least one year including several freeze/thaw cycles. Comparison of enzymatic activity of recombinant protein generated by expression systems based on pQE30 or pET32 vectors did not show any differences. This indicates that the tag-region of pET32 (152 additional residues) does not interfere by any means with AdoHcyase activity (not shown).

Further, native-PAGE analysis shows an electrophoretic mobility for the p.A89V protein significantly different to the mobility of other human AdoHcyase isoforms (wild-type; isoforms SAHH-2, and SAHH-3; [17]). Thus, the p.A89V protein migrates notably faster than the wild-type protein or the other human isoforms (Fig. 1B). The same behavior is observed for thrombin-digested AdoHcyase or pQE30 derived proteins, indicating that the fusion part of the recombinant protein does not interfere with holoenzyme formation (Fig. 1B).

Gel filtration chromatography showed elution of recombinant wild-type enzyme as a single symmetrical peak at the position corresponding to Mr of 260,000 (Fig. 2), indicating that the recombinant enzyme is a tetramer as is the native enzyme found in human tissues. The p.A89V protein elutes also with a single symmetrical peak, but with a slightly different retention time (5.4 s) compared to wild-type. At a flowrate of 0.5 ml/ml this represents a difference in the retention volume of only 45 μl.

Quantitation of the NAD^+ and NADH content in the recombinant p.A89V enzyme showed approximately 70.5% of the coenzyme bound as NAD^+, i.e. similar to the values determined for wild-type protein (81%). The coenzyme content of recombinant p.A89V AdoHcyase

Table 2
Kinetic parameters of wild-type and mutant enzymes assessed

	Km (μM) hydrolysis	Enzymatic activity (μmol $min^{-1}mg^{-1}$) hydrolysis	Enzymatic activity (μmol $min^{-1}mg^{-1}$) synthesis
WT	15.09	0.748 ± 0.013 (100%)	1.230 ± 0.043 (100%)
p.A89V	14.6	0.170 ± 0.022 (22.7%)	0.400 ± 0.061 (32.5%)
p.WT_S84	14.4	0.74 ± 0.02 (98.9%)	N/A
p.A89V_S84	17.0	0.59 ± 0.038 (78%)	N/A

The data represent 5 technical replicates shown as mean values ± S.D. Values in parentheses are percentage of wild-type.

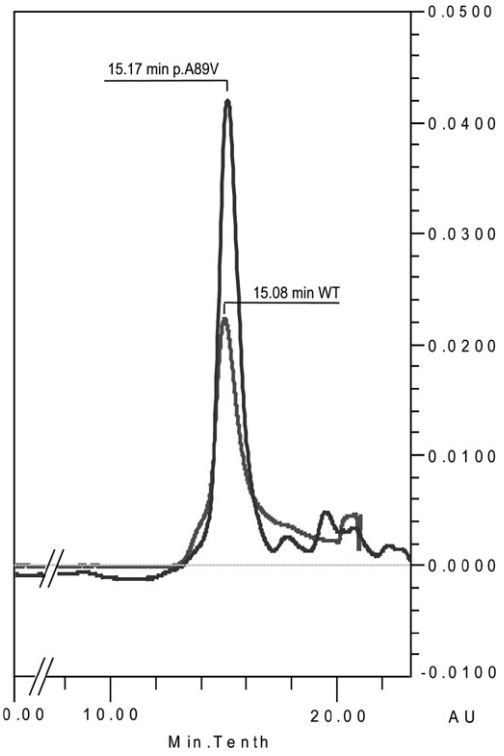

Fig. 2. Gel filtration chromatography. A range of 50–100 μg of Ni–NTA affinity purified uncleaved wild-type and p.A89V, suspended in 50 μl of 50 mM K_2HPO_4, pH 7.2, 300 mM NaCl, 1 mM EDTA (buffer A), was applied to a BIO-SIL SEC 250-5 column (Biorad, Munich), equilibrated with buffer A. Elution was performed with equilibration buffer at a flow rate of 0.5 ml/min. The protein was monitored at 280 nm.

was determined to be 3.9 molecules NAD per tetramer, in agreement with previously published data [18].

The far-UV CD spectra of wild-type and p.A89V protein in water at room temperature were almost identical (not shown). A significant difference was observed in the thermal stability of the recombinant protein. The p.A89V protein undergoes an unfolding process between 42.2 °C and 49.8 °C with a T_m of 46.0 ± 0.5 °C, while unfolding of wild-type occurs from 46.0 °C to 57.2 °C with a T_m of 51.5 ± 0.6 °C (not shown).

Heat inactivation experiments for wild-type and p.A89V protein show that a 15 min incubation in buffer A at 50 °C results in a 45% loss of enzymatic activity for p.A89V. Enzymatic activity of wild-type protein is not effected at 50 °C, but is reduced to 50% at an incubation temperature of 55 °C. Mutant protein is fully inactivated above 55 °C, whereas wild-type retains 12% activity even after incubation at 57 °C.

Analysis with the software package DeepView did not show changes to the H-bond network in p.A89V protein (not shown). Also, secondary structure prediction does not indicate changes in the region containing residue Val^{89}. However, physico-chemical properties of alanine and valine differ in their residue volume (alanine: 88.6; valine: 140) and their surface area (alanine: 115; valine: 155), respectively. Both values are higher for valine.

Site-directed mutagenesis

Fig. 3 gives a ribbon diagram overview of the series of mutations introduced in region containing Thr^{84} and Ala^{89} in human AdoHcyase and emphasizes possible implications of the alanine to valine exchange, focusing on the volumes of side-chains in proximity to residue Ala^{89} or Val^{89}, respectively. Thus, in wild-type protein a small gap between residues Ala^{89}, Thr^{84} and Ala^{101} is shown to exist, that is closed in the p.A89V protein indicating sterical interference of the side-chain of Thr^{84} with Val^{89} in the mutant AdoHcyase.

Results of the site-directed mutagenesis study are summarized in Fig. 4 showing a graphical representation of enzymatic activities in the hydrolytic direction of recombinant mutant and wild-type enzymes (WT, p.A89V, p.WT_A84, p.WT_S84, p.A89V_A84 and p.A89V_S84). Both wild-type and p.A89V recombinant proteins with the Thr^{84} to Ala^{84} substitution are mostly inactivated and show only residual activity (⩽11%). In addition, p.A89V_A84 exhibits high instability and a half-life of only 1–2 days.

Enzymatic activity could not be detected for wild-type variants p.WT_K84 and p.WT_Q84. On the other hand, WT protein with the Thr^{84} to Ser^{84} mutation has an almost identical enzymatic activity as its unchanged WT counterpart (⩾91%), whereas the Ser^{84} exchange in p.A89V leads to recovery of enzymatic activity to ⩾70% compared to wild-type.

Discussion

The main task of our work was to evaluate whether the A89V exchange represents a harmless modification to the AdoHcyase such as observed for the AdoHcyase isoforms SAHH-2 or SAHH-3 or a negative modification involved in AdoHcyase deficiency. Thus, we found that the A89V substitution brings about a significant reduction in catalytic activity of recombinant mutant AdoHcyase without effecting assembly into the tetrameric holoenzyme. Since both alanine and valine are 'kindred' hydrophobic residues, we searched for reasons to explain the dramatic decrease in enzymatic activity.

First we performed a bioinformatical analysis on the 3-D model of AdoHcyase and the region containing the A89V mutation to seek clues to the reason for enzyme inactivation. The AdoHcyase subunit is composed of three domains, of which the catalytic and the NAD-binding domain are divided by a cleft [12]. Upon substrate binding the enzyme undergoes conformational changes and the cleft between both domains is closed, similar to a nutcracker mechanism.

The A89V mutation is located in the catalytic domain in alpha-helix 3 that is facing the cleft (residues 1–183 and 357–390; Fig. 3). We hypothesized, that differences in the amino acid properties regarding surface area and residue volume between alanine and valine cause disorder in

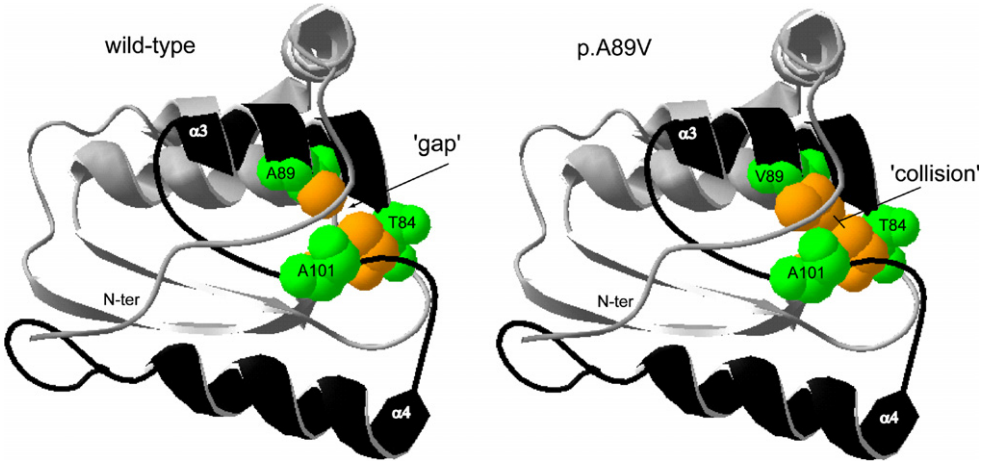

Fig. 3. Ribbon diagrams of a portion of the catalytic domain human AdoHcyase. The enlargements show the region containing residues Ser^{2} to Asn^{126} of human AdoHcyase and the amino acid exchanges introduced by site-directed mutagenesis. Investigated amino acids are represented with side-chains in 3-D rendering.

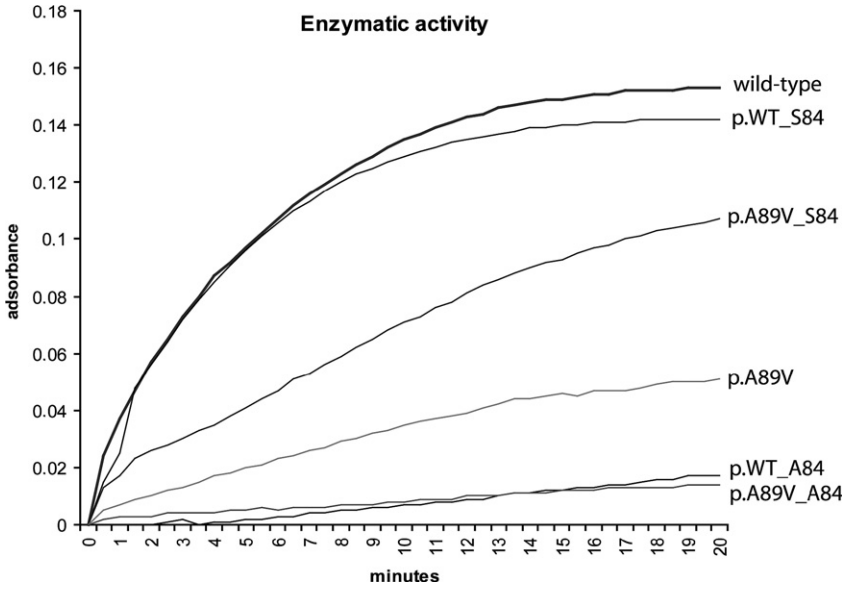

Fig. 4. Graphical representation of enzymatic activities in the hydrolytic direction of recombinant wild-type and mutant enzymes p.A89V, p.WT_A84, p.WT_S84, p.A89V_A84, and p.A89V_S84. Hydrolysis of AdoHcy generates Ado, which is converted to inosine by Ado Deaminase. Disappearance of Ado is measured at a wavelength of 265 nm and plotted against elapsed time.

alpha-helix 3 or its surrounding peptide chains, inflicted by collision of Val^{89} and Thr^{84}, and are possibly translated into structural changes in the catalytic domain or overall holoenzyme structure. The next step was to proof that collision of Val^{89} with Thr^{84} is the main reason for enzyme malfunction. Thus we planned site-directed mutagenesis of the residues in question. The goal was to replace Thr^{84} in mutant p.A89V protein with residues showing similar physico-chemical properties such as polarity and charge, but decreased volume to avoid collision with Val^{89}. We chose to replace Thr^{84} ($v = 116.1$) with Ala^{84} ($v = 88.6$) or with Ser^{84} ($v = 89$). By using Ala^{84} we were also able to see whether polarity of residues in the surrounding region of amino acid 89 is of importance for enzyme function. The feasibility of planned mutagenesis was evaluated first by introducing Ala^{84} or Ser^{84} into wild-type AdoHcyase to find any effect on enzymatic activity. To our surprise, the Ala^{84} exchange in wild-type protein (p.WT_A84) almost completely inactivated the enzyme, indicating importance of both polarity and residue size in the region. On the other hand, the Ser^{84} exchange had no significant impact on the enzymatic capabilities of recombinant AdoHcyase. Both exchanges were then introduced into p.A89V protein to produce double mutants, containing the A89V exchange and either Ala^{84} or Ser^{84} substitutions, i.e. proteins p.A89V_A84 and p.A89V_S84, respectively. As expected, the p.A89V protein with the Ser^{84} modification restored its enzymatic activity from formerly 20–30% to more than 70% if compared to wild-type, whereas the Ala^{84} exchange was highly deactivating as observed in

p.WT_A84. Also, we deliberately inactivated wild-type protein to foster our collision assumption. Therefore, we introduced Lys^{84} ($v = 168.6$) or Gln^{84} ($v = 143.8$) that have a higher residue volume than Thr^{84} ($v = 116.1$) to provoke an intra-molecular collision between residues 84 and 89.

Mutant protein was further investigated using circular dichroism analysis monitoring the unfolding behavior during thermal induction. We show that p.A89V enzyme has a reduced unfolding temperature with a significant lower Tm of 5.5 °C compared to wild-type. Although *in silico* analysis did not indicate changes to neither hydrogen-bond network nor secondary structure of p.A89V protein, we found evidence that some significant changes must occur in the mutant protein. We conclude this from the native PAGE analysis, which showed a clearly altered electrophoretic mobility profile for p.A89V protein. As native PAGE allows determination of several characteristics of proteins such as differences in charge or molecular weight, the different mobility must result from effects of the A89V mutation on protein charge, because the gel filtration profile of recombinant p.A89V is almost identical to wild-type protein. Interestingly, the calculated charge of mutant protein does not differ from wild-type values and thus a concise explanation for the differences in mobility is rather elusive.

Also, it is unclear how the collision actually causes AdoHcyase malfunction, although it might impair slow open–closed conformational changes that occur during the catalytic reaction and allow release of Hcy and access of a water molecule to the catalytic domain. Opening the cleft and releasing Ado from the active site completes the catalytic cycle. Because alpha-helix 3 and Ala^{89} locate close to the inner face of the cleft, conformational changes may interfere with product access and/or release, which on the other hand results in reduced enzymatic activity. Thus, crystallization and X-ray diffraction studies might provide new insights. Evaluation of another feature such as the frequency of vibration of the catalytic domain of substrate-free mutant AdoHcyase represents a challenging experiment [19]. Deviation from the 40 MHz frequency measured for wild-type enzyme might hint for the underlying mechanisms of enzyme inactivation in mutant protein.

In conclusion, we find that mainly the structural abnormalities in the mutant p.A89V protein represent one basis for severe human AdoHcyase deficiency and its pathological effects. This is in concurrence with our NAD^+/NADH measurements, which indicate that the redox cycle itself is not effected. Also, our site-directed mutagenesis study shows that AdoHcyase alpha-helix 3 plays an important role for enzyme function that was not anticipated previously. Towards this end, we have evidence based on a new clinical case of AdoHcyase deficiency (O. Vugrek and S.H. Mudd, unpublished data) that a mutation in alpha-helix 3 at position 86 is fatal for enzyme activity. Thus, changes to the examined region of AdoHcyase in general, and in particular to codons 84, 86, and 89 are highly negative to AdoHcyase function, an information eventually valuable in prenatal diagnostics.

Acknowledgments

This work was supported by Grants 0098086, 098-0000000-2463 (O.V.), and 0108016 (M.C., I.B.) of the Ministry of Science, Education and Sports from the Republic of Croatia and by the Austrian Science Fund (FWF) project P17885 (TP).

References

[1] N.R.M. Buist, B. Glenn, O. Vugrek, C. Wagner, S. Stabler, R.H. Allen, I. Pogribny, A. Schulze, S.H. Zeisel, I. Barić, S.H. Mudd, *S*-Adenosylhomocysteine hydrolase deficiency in a 26-year-old man, J. Inh. Metab. Dis. 29 (2006) 538–545.

[2] R. Belužić, M. Ćuk, T. Pavkov, K. Fumić, I. Barić, S.H. Mudd, I. Jurak, O. Vugrek, A single mutation at tyrosine 143 of human *S*-adenosylhomocysteine hydrolase renders the enzyme thermosensitive and effects the oxidation state of bound co-factor NAD, Biochem. J. 400 (2006) 245–253.

[3] I. Barić, K. Fumić, B. Glenn, M. Ćuk, A. Schulze, J.D. Finkelstein, S. Jill James, V. Mejaški-Bošnjak, L. Pažanin, I.P. Pogribny, M. Radoš, V. Sarnavka, M. Šćukanec-Špoljar, R.H. Allen, S. Stabler, L. Uzelac, O. Vugrek, C. Wagner, S. Zeisel, S.H. Mudd, *S*-adenosylhomocysteine hydrolase deficiency in a human: a genetic disorder of methionine metabolism, Proc. Natl. Acad. Sci. USA 101 (2004) 4234–4239.

[4] I. Barić, M. Ćuk, K. Fumić, O. Vugrek, R.H. Allen, B. Glenn, M. Maradin, L. Pažanin, I. Pogribny, M. Radoš, V. Sarnavka, A. Schulze, S. Stabler, C. Wagner, S. Zeisel, S.H. Mudd, *S*-Adenosylhomocysteine hydrolase deficiency: a second patient, the younger brother of the index patient, and outcomes during therapy, J. Inh. Metab. Dis. 28 (2005) 885–902.

[5] G.L. Cantoni, Biological methylation: selected aspects, Annu. Rev. Biochem. 44 (1975) 435–451.

[6] M. Fontecave, M. Atta, E. Mulliez, *S*-Adenosyl methionine: nothing goes to waste, Trends Biochem. Sci. 29 (2004) 243–249.

[7] S.T. Prigge, P.K. Chiang, *S*-Adenosylhomocysteine hydrolase, in: R. Carmel, D.W. Jacobsen (Eds.), Homocysteine in Health and Disease, Cambridge University Press, USA, 2001, pp. 79–90.

[8] G. Dela Haba, G.L. Cantoni, The enzymatic synthesis of *S*-adenosyl-L-homocysteine from adenosine and homocysteine, J. Biol. Chem. 234 (1959) 603–608.

[9] D.E. Coulter-Karis, M.S. Hershfield, Sequence of full length cDNA for human *S*-adenosylhomocysteine hydrolase, Ann. Hum. Genet. 53 (1989) 169–175.

[10] J.L. Palmer, R.H. Abeles, Mechanism for enzymatic thioether formation: mechanism of action of *S*-adenosylhomocysteinase, J. Biol. Chem. 251 (1976) 5817–5819.

[11] J.L. Palmer, R.H. Abeles, The mechanism of action of *S*-adenosylhomocysteinase, J. Biol. Chem. 254 (1979) 1217–1226.

[12] M.A. Turner, C.S. Yuan, R.T. Borchardt, M.S. Hershfield, G.D. Smith, P.L. Howell, Structure determination of selenomethionyl *S*-adenosylhomocysteine hydrolase using data at a single wavelength, Nat. Struct. Biol. 5 (1998) 369–376.

[13] T. Yamada, Y. Takata, J. Komoto, Y. Huang, T. Gomi, H. Ogawa, M. Fujioka, F. Takusagawa, Catalytic mechanism of *S*-adenosylhomocysteine hydrolase: roles of His 54, Asp130, Glu155, Lys185, and Aspl89, Int. J. Biochem. Cell Biol. 37 (2005) 2417–2435.

[14] Y. Takata, T. Yamada, Y. Huang, J. Komoto, T. Gomi, H. Ogawa, M. Fujioka, F. Takusagawa, Catalytic mechanism of *S*-adenosylhomocysteine hydrolase: site-directed mutagenesis of Asp-130, Lys-185, Asp-189, and Asn-190, J. Biol. Chem. 277 (2002) 22670–22676.

[15] R.J. Hohman, M.C. Guitton, M. Veron, Inactivation of *S*-adenosyl-L-homocysteine hydrolase by cAMP results from dissociation of enzyme-bound NAD^+, Proc. Natl. Acad. Sci. USA 82 (1985) 4578–4581.

[16] N. Guex, M.C. Peitsch, SWISS-MODEL and the Swiss-PdbViewer: an environment for comparative protein modeling, Electrophoresis 18 (1997) 2714–2723.
[17] K. Fumić, R. Belužić, M. Čuk, T. Pavkov, D. Kloor, I. Barić, I. Mijić, O. Vugrek, Functional analysis of human *S*-adenosylhomocysteine hydrolase isoforms SAHH-2 and SAHH-3, Eur. J. Hum. Gen. 15 (2007) 347–351.
[18] B. Matuszewska, R.T. Borchardt, The role of nicotinamide adenine dinucleotide in the inhibition of bovine liver *S*-adenosylhomocysteine hydrolase by neplanocin A, J. Biol. Chem. 262 (1987) 265–268.
[19] D. Yin, X. Yang, Y. Hu, K. Kuczera, R.L. Schowen, R.T. Borchardt, T.C. Squier, Substrate binding stabilizes *S*-adenosylhomocysteine hydrolase in a closed conformation, Biochemistry 39 (2000) 9811–9818.