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

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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

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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