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Research paper

Mutations in S-adenosylhomocysteine hydrolase (AHCY) affect its nucleocytoplasmic distribution and capability to interact with S-adenosylhomocysteine hydrolase-like 1 protein

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ABSTRACT

S-adenosylhomocysteine hydrolase (AHCY) is thought to be located at the sites of ongoing AdoMet-dependent methylation, presumably in the cell nucleus. Endogenous AHCY is located both in cytoplasm and the nucleus. Little is known regarding mechanisms that drive its subcellular distribution, and even less is known on how mutations causing AHCY deficiency affect its intracellular dynamics.

Using fluorescence microscopy and GFP-tagged AHCY constructs we show significant differences in the intensity ratio between nuclei and cytoplasm for mutant proteins when compared with wild type AHCY.

Interestingly, nuclear export of AHCY is not affected by leptomycin B. Systematic deletions showed that AHCY has two regions, located at both sides of the protein, that contribute to its nuclear localization, implying the interaction with various proteins.

In order to evaluate protein interactions in vivo we engaged in bimolecular fluorescence complementation (BiFC) based studies. We investigated previously assumed interaction with AHCY-like-1 protein (AHCYL1), a paralog of AHCY. Indeed, significant interaction between both proteins exists. Additionally, silencing AHCYL1 leads to moderate inhibition of nuclear export of endogenous AHCY.

Introduction

S-Adenosylhomocysteine hydrolase (AHCY, EC 3.3.1.1) is a key cellular enzyme which catalyzes AdoHcy hydrolysis (Palmer and Abeles, 1976), preventing its accumulation and subsequent inhibition of methyl transferases (MTs). The importance of normal AHCY function is supported by the fact that the chromosomal deletion including the AHCY gene leads to embryonic mortality in mice (Miller et al., 1994). AHCY deficiency (OMIM #613752) in humans involves serious biochemical aberrations, retarded psychomotor development, myopathy and myelination disorders (Baric et al., 2004; Barić et al., 2005), and is potentially lethal in early stages of life (Baric et al., 2004; Barić et al., 2005; Vugrek et al., 2009). The newest findings link AHCY deficiency to hepatocellular carcinoma (Stender et al., 2015). The variety of possible outcomes of the disease, which is also indicated by functional studies of AHCY mutant proteins (Beluzić et al., 2006, 2008; Honzík et al., 2012; Vugrek et al., 2009) and differences in efficiency of therapeutic approaches to mitigate the effects of the disease on development (Strauss et al., 2015) indicate that AHCY might engage in multiple cellular processes, thereby being part of a larger network of proteins with distinct protein–protein interactions to suit particular metabolic requirements of the cell or organism.

One such process is transmethylation, which is crucial for a wide variety of different cellular functions (Tehlivets et al., 2013). The major methyl donor in all biological methylations is S-adenosylmethionine (AdoMet). The preference of methyltransferases for AdoMet over other potential methyl donors is thermodynamically favorable (Schubert et al., 2003). Thus, contin-

Abbreviations: Aa, amino acid; ADK, adenosine kinase; AdOx, adenosine-2′,3′-dialdehyde; AHCY, S-adenosylhomocysteinase; AHCYL1 (IRBIT), adenosylhomocysteinase-like 1 (inositol 1,4,5-trisphosphate receptor-binding protein); AdoMet, S-adenosylmethionine; AdoHcy, S-adenosylhomocysteine; BiFC, bimolecular fluorescence complementation; CMT, mRNA cap methyltransferase; CRM1, Exportin 1; FRAP, fluorescence recovery after photobleaching; Imp, importins; LMB, leptomycin B; MTs, methyltransferases; N/C, nuclear/cytoplasmic ratio; NLS, nuclear import signal; NES, nuclear export signal; wt, wild type.

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uous supply of AdoMet is needed for the methylation capacity of the cell. Apart from methylated substrate, transmethylation reactions produce S-adenosylhomocysteine (AdoHcy), which is a strong competitive inhibitor of many MTs (Lu and Mato, 2012).

Accordingly, it is proposed that the efficiency of transmethylation might profit from a close proximity between methyltransferases and AHCY due to its particular function of rapid removal of AdoHcy (Radomski et al., 1999). Transmethylation reactions occur likely in all cellular compartments (Kloor et al., 2007; Radomski et al., 1999). Since DNA, RNA and histone methylation takes place in the nucleus, significant amounts of AdoHcy are generated there. Nuclear pores are large enough to allow diffusion of AdoHcy molecule. However to overcome the limit of AdoHcy diffusion through nuclear pores, AHCY hydrolase must be in physical proximity or contact with the MTs on the sites of active nuclear AdoMet-dependent methylations to prevent feedback inhibition of MTs by AdoHcy (Radomski et al., 1999).

Human AHCY (Uniprot ID P23526) is enzymatically active only as a homotetramer (Turner et al., 1998). Each 47.7 kDa monomer has three domains: substrate binding (catalytic) domain (aa 1–181 and 355–385), cofactor binding domain (aa 197–351) and C-terminal domain (aa 386–432). Two large domains (SBD and CBD) are linked by a hinge region (Turner et al., 1998; Yang et al., 2003).

Homotetrameric structure of AHCY is "dimer of dimers" (Turner et al., 1998; Yang et al., 2003). Each dimer has a NAD-binding domain interface involving interaction between helices α17 of one monomer (aa 388–397) of one monomer and αC (aa 246–254) of the other, and between α18 (aa 411–416) of one monomer and residues of adenine side of the NAD binding site in the other monomer (Turner et al., 1998). Both K 426 and Y 430 of one monomer are involved interactions with the NAD bound predominantly to the other monomer (Turner et al., 1998). Site-specific mutagenesis experiments confirmed the importance of K 426 to AHCY activity and to maintaining of tetrameric structure (Ault-Riché et al., 1994).

The basis of for AHCY tetramer formation is the intersubunit interaction between helices αA1 (aa 184–187) and αA2 (aa 190–207) of each monomer (Turner et al., 1998).

Residues proven to be important for AHCY tetramer formation as well as the truncation boundaries are highlighted in Fig. 2A.

Each subunit has a molecular weight of 47.7 kDa and contains a single molecule of NAD^+ and a single binding site for adenosine. In general, proteins with a molecular mass >40 kDa require a nuclear localization signal (NLS) to gain entry into the nucleus (Cautain et al., 2015). Many NLSs are recognized by members of the importin (Imp) superfamily of proteins, NLS recognition commonly being through the Impα adaptor protein within the Impα/β1 heterodimer, or Impβ1 or homologues thereof directly (Cautain et al., 2015). Nuclear protein export is an analogous process, whereby NESs are recognized by the exportin family of Impβ homologues, CRM1 (exportin 1) being among the best described (Cautain et al., 2015). In *Arabidopsis*, nuclear targeting of AHCY depends on its interaction with adenosine kinase and mRNA cap MT, and involves an insertion region of 40–45 amino acids, which is specific to plants, parasitic protozoans and many bacteria (Lee et al., 2012a,b). The human enzyme lacks such a binding region. In *Xenopus laevis* cells AHCY is predominantly located in the nucleus (excluding nucleoli) of oocytes, embryonic cells at the end of gastrulation and transcriptionally active kidney cells (Radomski et al., 1999), where its enzymatic activity is required for pre-mRNA cap methylation and elongation (Radomski et al., 1999, 2002). Nuclear localization of AHCY was also detected in rat kidney cells under hypoxic conditions (Kloor et al., 2007). In chicken embryos AHCY is predominantly nuclear in most of the cells except in migratory neural crest cells where it is predominantly cytoplasmic (Vermillion et al., 2014). All these lines of evidence suggest that intracellular localization of AHCY depends on the cell type and context.

AHCY like 1 (AHCYL1, IRBIT; Uniprot ID O43865) is a paralog of AHCY (Devogelaere et al., 2008). It consists of IRBIT domain (aa 39–194) and AHCY domain (aa 105–530) that is 51% identical and 71% similar to AHCY enzyme.

Among its diverse functions are regulation of intracellular Ca^{2+} levels through binding to inositol 1,4,5-trisphosphate receptors (Ando et al., 2006) and inhibition of ribonucleotide reductase (Arnaoutov and Dasso, 2014). It was hypothesized (Devogelaere et al., 2008) that it could bind to, due to the high sequence homology, and inhibit AHCY.

Here, we determined the nucleo-cytoplasmic mobility of several AHCY variants (p.Y143C, p.R49C, p.Y328D) found in patients suffering from AHCY deficiency (Baric et al., 2004; Strauss et al., 2015; Vugrek et al., 2009), and identified AHCY domains that could be involved in its nucleoplasmic translocation. To identify the structural requirements for nuclear accumulation of AHCY, we investigated the localization of deletion mutants expressed in human cells by fluorescence microscopy. We used bimolecular fluorescence complementation (BiFC) (Hu et al., 2002) to further evaluate adverse effects of AHCY mutations on trafficking and protein–protein interactions.

Results

Enzymatic analysis of various recombinant AHCY variants

Enzymatic capabilities and macromolecular organization of AHCY variants, in particular for N-terminal variants p.Y7F, and truncated proteins p.M1_L14del, and p.M1_P30del, and the C-terminal deletion variant p.C421_Y432del were evaluated according to Beluzić et al. (2006).

Interestingly, p.M1_L14del mutant showed a macromolecular organization similar to wildtype AHCY, e.g. tetrameric (Fig. 2D), but it completely lacked enzymatic activity. Variants p.M1_P30del, and p.C421_Y432del were unable to form the AHCY tetrameric complex, and did not exhibit any enzymatic activity. Missense variant p.Y7F showed almost identical enzymatic capabilities when compared with AHCY wildtype. Also, tetrameric organization appeared to be similar to wildtype (Fig. 2D).

Additionally, we evaluated the effect of the GFP-tag on enzymatic capabilities of AHCY. Although recombinant GFP-tagged AHCY has an increased molecular mass (76.2 kDa) due to the additional 238 amino acid residues contributed by GFP when compared with the human serum protein subunit, the enzymatic capabilities are not effected (Table 1a). As AHCY is functional only as tetramer, we conclude that GFP is a suitable tag to investigate AHCY function by cell biological techniques. Previously we showed that other tagged variants of AHCY are enzymatically active, and that their behavior during gel filtration resembles wild type AHCY (Beluzić et al., 2006; Vugrek et al., 2009).

Export of the nuclear fraction of AHCY is CRM1-independent

The localization of endogenous AHCY was detected by immunofluorescence in human U2OS (Fig. 1A) and HeLa cells

Table 1a
Kinetic parameters of untagged (WT) and GFP-tagged AHCY in the directions of hydrolysis. Km and enzyme activity values were obtained by directly fitting the data into the Michaelis–Menten equation using a Lineweaver–Burke linearization. The data represent five technical replicates shown as mean values ± SD.

	Km (μM) hydrolysis	Enzymatic activity (μmol min^{-1} mg^{-1}) hydrolysis
WT	15.12	0.741 (100%) ± 0.014
GFP-tagged	16.01	0.689 (93%) ± 0.012