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Substrate specificity, plasma membrane localization, and lipid modification of the aldehyde dehydrogenase ALDH3B1

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ABSTRACT

The accumulation of reactive aldehydes is implicated in the development of several disorders. Aldehyde dehydrogenases (ALDHs) detoxify aldehydes by oxidizing them to the corresponding carboxylic acids. Among the 19 human ALDHs, ALDH3A2 is the only known ALDH that catalyzes the oxidation of long-chain fatty aldehydes including C16 aldehydes (hexadecanal and *trans*-2-hexadecenal) generated through sphingolipid metabolism. In the present study, we have identified that ALDH3B1 is also active in vitro toward C16 aldehydes and demonstrated that overexpression of ALDH3B1 restores the sphingolipid metabolism in the *ALDH3A2*-deficient cells. In addition, we have determined that ALDH3B1 is localized in the plasma membrane through its C-terminal dual lipidation (palmitoylation and prenylation) and shown that the prenylation is required particularly for the activity toward hexadecanal. Since knockdown of *ALDH3B1* does not cause further impairment of the sphingolipid metabolism in the *ALDH3A2*-deficient cells, the likely physiological function of ALDH3B1 is to oxidize lipid-derived aldehydes generated in the plasma membrane and not to be involved in the sphingolipid metabolism in the endoplasmic reticulum.

1. Introduction

A wide variety of aldehydes is generated in the organism from endogenous and exogenous substrates such as lipids, amino acids, and neurotransmitters, as well as foods, drugs, and pollutants [1]. Being highly reactive molecules, the accumulation of aldehydes often has toxic effects on cells. Aldehyde dehydrogenases (ALDHs) are a group of enzymes that catalyze the oxidation of aldehydes to less reactive carboxylic acids, thus alleviating the aldehyde-induced cytotoxicity. There are 19 ALDHs known in humans with different substrate specificity, tissue distribution, and intracellular localization [1]. Mutations in these *ALDH* genes and consequent aldehyde accumulation have been implicated in the pathogenesis of several inherited diseases, including Sjögren–Larsson syndrome (*ALDH3A2*) [2], type II hyperprolinemia (*ALDH4A1*) [3], pyridoxine-dependent seizures (*ALDH7A1*) [4], and γ-hydroxybutyric aciduria (*ALDH5A1*) [5].

The mammalian ALDH3 subfamily is comprised of ALDH3A1, ALDH3A2, ALDH3B1, and ALDH3B2, which share sequence similarity. ALDH3A1 is a highly expressed corneal soluble protein and protects the cornea and underlying lens against UV-induced oxidative stress (as lipid peroxidation) by eliminating the toxic aldehydes including 4-hydroxy-2-nonenal [6]. ALDH3A2 is expressed ubiquitously and exhibits activity toward aliphatic aldehydes of C6 to C24 [7], such as those derived from dietary phytol [8], leukotriene B_4 [9], and sphingosine 1-phoshosphate (S1P) [10]. Two splicing isoforms of ALDH3A2 are known: a major isoform localized in the endoplasmic reticulum (ER) and a minor isoform in the peroxisomes [11]. ALDH3B1 is expressed in the kidney, liver, and brain and has been shown active in vitro toward C6 to C9 aldehydes [12]; however, its activity toward ≥ C10 aldehydes is currently unknown. In addition, although ALDH3B1 is suggested to be localized in the plasma membrane and cytosol [13,14], the precise intracellular localization still remains to be elucidated. Unlike other ALDH3 family members, ALDH3B2 has so far not been well characterized.

We recently reported the involvement of ALDH3A2 in the metabolism of sphingolipid-derived C16 aldehydes to glycerophospholipids [10]. Sphingolipids and glycerophospholipids are major lipid components of the eukaryotic plasma membrane. The hydrophobic backbone of sphingolipids is a ceramide (Cer) composed of a long-chain base (LCB) and an amide-linked fatty acid [15]. In mammals, the major LCB

Abbreviations: ABE, acyl–biotinyl exchange; ALDH, aldehyde dehydrogenase; Cer, ceramide; DHS, dihydrosphingosine; ER, endoplasmic reticulum; GlcCer, glucosylceramide; IPC, inositol phosphorylceramide; LCB, long-chain base; MIPC, mannosylinositol phosphorylceramide; $M(IP)_2C$, mannosyldiinositol phosphorylceramide; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PlsC, plasmanylcholine; PlsE, plasmenylethanolamine; PS, phosphatidylserine; S1P, sphingosine 1-phoshosphate; SM, sphingomyelin

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is sphingosine with a *trans* double bond between the 4th and 5th carbons, accompanied by the saturated analog dihydrosphingosine (DHS). These LCBs are phosphorylated by sphingosine kinases to the LCB 1-phosphates (S1P and DHS 1-phosphate) and further degraded by S1P lyase to phosphoethanolamine and the corresponding C16 aldehydes (*trans*-2-hexadecenal and hexadecanal, respectively) [15]. The generated C16 aldehydes are subsequently oxidized by ALDH3A2 and are eventually metabolized to glycerophospholipids [10]. Interestingly, during our investigation, we noticed that the *ALDH3A2*-mutant CHO-K1 cells (FAA-K1A) were still able to metabolize LCBs to glycerophospholipids, albeit to a lesser extent compared to the wild-type CHO-K1 cells. We therefore suspected that another ALDH might be responsible for the residual activity [10].

So far, ALDH3A2 is the only known ALDH that exhibits activity toward C16 aldehydes. In the present study, we have identified ALDH3B1 as another ALDH3 family member capable of oxidizing C16 aldehydes and biochemically characterized its substrate specificity, intracellular localization, and lipid modification.

2. Material and methods

2.1. Cell culture and transfection

HEK 293 T and HeLa cells were grown in Dulbecco's modified Eagle's medium (Sigma, St. Louis, MO) and CHO-K1 cells in Ham's F-12 medium (Sigma), with each medium supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin. HEK 293 T cells were grown in dishes coated with 0.3% collagen. The *ALDH3A2*-null FAA-K1A cells were described previously [10,16]. Transfections were performed using Lipofectamine Plus™ Reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions.

2.2. Yeast strains and media

The yeast *Saccharomyces cerevisiae* strain BY4741 (*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*) [17] and its Δ*hfd1*::*KanMX4* derivative (strain 6550) [18] were obtained from Open Biosystems (Huntsville, AL). Cells were grown in either YPD medium (1% yeast extract, 2% bactopeptone, and 2% D-glucose) or synthetic complete medium lacking uracil (0.67% yeast nitrogen base, 2% D-glucose, 0.5% casamino acids, 20 mg/l adenine, and 20 mg/l tryptophan).

2.3. Plasmids

The pCE-puro 3xFLAG-1 [19], pCE-puro His_6-Myc-1 [19], and pEGFP-C1 (Clontech, TAKARA Bio, Palo Alto, CA) plasmids are mammalian expression vectors designed to produce proteins tagged with an N-terminal 3xFLAG, an N-terminal tandemly oriented His_6 and Myc epitope (His_6-Myc), and an N-terminal enhanced GFP protein, respectively. The pAKNF316 (*URA3* marker) plasmid is a yeast expression vector designed to produce an N-terminal 3xFLAG-tagged protein [10]. The plasmids encoding *3xFLAG*-tagged human *ALDH3A2* cDNA (pCE-puro 3xFLAG-ALDH3A2 for expression in mammalian cells and pNK5 for expression in yeast) were described previously [10].

The human *ALDH3A1*, *ALDH3B1*, and *ALDH3B2* cDNAs were amplified by PCR using appropriate templates (for *ALDH3A1*, human EST clone ID 3610317 (Thermo Fisher Scientific, Waltham, MA); for *ALDH3B1*, human lung cDNA (Clontech, TAKARA Bio); and for *ALDH3B2*, human placenta cDNA (Clontech, TAKARA Bio)) and primers (for *ALDH3A1*, 5′-TCTAGAATGAGCAAGATCAGCGAGGCCGTGA-3′ (*Xba*I site underlined) and 5′-TCAGTGCTGGGTCATCTTGGCCGGG-3′; for *ALDH3B1*, 5′-GGATCCATGGACCCCCTTGGGGACACGCTG-3′ (*Bam*HI site underlined) and 5′-TCAGAGCAGTGTGCAGCTGCAGCC-3′; and for *ALDH3B2*, 5′-GGATCCATGAAGGATGAACCACGGTCCACG-3′ (*Bam*HI site underlined) and 5′-GGATCCTCACAGGAGGGTGCAGCTCTGGGAG-3′ (*Bam*HI site underlined)). The amplified fragments were first cloned into the pGEM-T Easy Vector (Promega, Madison, WI). The cDNA fragment was then prepared from each resulting plasmid by digesting with appropriate restriction enzymes and cloned into the pAKNF316, pCE-puro 3xFLAG-1, or pCE-puro His_6-Myc-1 vector. The pNK10 (*3xFLAG-ALDH3A1*), pNK12 (*3xFLAG-ALDH3B1*), and pNK14 (*3xFLAG-ALDH3B2*) plasmids were derived from the pAKNF316 plasmid. The pCE-puro 3xFLAG-ALDH3A1, pCE-puro 3xFLAG-ALDH3B1, pCE-puro His_6-Myc-ALDH3B1, and pCE-puro 3xFLAG-ALDH3B2 plasmids were constructed using either pCE-puro 3xFLAG-1 or pCE-puro His_6-Myc-1 vector.

The plasmids encoding *ALDH3B1* mutants (*C463S*, *C465S*, and *C463/465S*) were constructed using QuikChange Site-Directed Mutagenesis Kit (Stratagene, Agilent Technologies, La Jolla, CA) with primers (for *C463S*, 5′-CCATGGAGGCCCAAGGCTCCAGCTGCACACTGCTC-3′ and 5′-GAGCAGTGTGCAGCTGGAGCCTTGGGCCTCCATGG-3′; for *C465S*, 5′-GGAGGCCCAAGGCTGCAGCTCCACACTGCTCTG-3′ and 5′-CAGAGCAGTGTGGAGCTGCAGCCTTGGGCCTCC-3′; and for *C463/465S*, 5′-GGAGGCCCAAGGCTCCAGCTCCACACTGCTCTG-3′ and 5′-CAGAGCAGTGTGGAGCTGGAGCCTTGGGCCTCC-3′). The wild-type and *ALDH3B1* mutant cDNAs were cloned into the following vectors: the pCE-puro 3xFLAG-1 vector to produce the pCE-puro 3xFLAG-ALDH3B1-C463S, pCE-puro 3xFLAG-ALDH3B1-C465S, and pCE-puro 3xFLAG-ALDH3B1-C463/465S plasmids; the pEGFP-C1 vector to generate the pEGFP-ALDH3B1, pEGFP-ALDH3B1-C463S, pEGFP-ALDH3B1-C465S, and pEGFP-ALDH3B1-C463/465S plasmids; and the pAKNF316 vector to create the pTK71 (*3xFLAG-ALDH3B1-C463/465S*) plasmid.

2.4. [^{3}H]DHS labeling assay

The assay was performed using [4,5-^{3}H]DHS (50 Ci/mmol; American Radiolabeled Chemicals, St Louis, MO) as described previously [10].

2.5. Production of stable transformants of FAA-K1A cells

To obtain FAA-K1A derivatives stably expressing the human ALDH3B1 protein, the pCE-puro His_6-Myc-ALDH3B1 plasmid was transfected into FAA-K1A cells. The transfected cells were subjected to selection in 10 μg/ml puromycin for 10 days. The FAA-ALDH3B1-A and FAA-ALDH3B1-B cells expressing the highest level of His_6-Myc-ALDH3B1 were used for further analyses.

2.6. Immunoblotting

Immunoblotting was performed as described previously [20] using anti-FLAG (M2; 1 μg/ml; Stratagene), anti-Myc (9E10; 1 μg/ml; Enzo Life Sciences, Farmingdale, NY), anti-calnexin (H-10; 0.2 μg/ml; Santa Cruz Biotechnology, Santa Cruz, CA), anti-GAPDH (1 μg/ml; Ambion, Austin, TX), and anti-Pgk1 (0.25 μg/ml; Molecular Probes, Invitrogen, Eugene, OR) as the primary antibodies and HRP-conjugated anti-rabbit and anti-mouse IgG $F(ab')_2$ fragment (each 1:7500 dilution; GE Healthcare Life Sciences, Buckinghamshire, UK) as the secondary antibodies. Labeling was detected using a Pierce Western Blotting Substrate (Thermo Fisher Scientific).

2.7. Cell fractionation

Cells treated with or without the geranylgeranyltransferase I inhibitor GGTI-2133 (Sigma) were suspended in buffer A (50 mM HEPES–NaOH (pH 7.4), 150 mM NaCl, 10% glycerol, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, and 1× Complete™ protease inhibitor mixture (EDTA-free; Roche Diagnostics, Indianapolis, IN)) and lysed by sonication. Unlysed cells and aggregated proteins were removed by centrifugation at 20,400 ×*g* for 5 min. The supernatant (total lysate) was centrifuged at 100,000 ×*g* for 30 min at 4 °C, and the resulting pellet (membrane fraction) and supernatant (soluble fraction) were subjected to immunoblotting.