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Aldehyde dehydrogenase 7A1 (ALDH7A1) attenuates reactive aldehyde and oxidative stress induced cytotoxicity

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ABSTRACT

Mammalian aldehyde dehydrogenase 7A1 (ALDH7A1) is homologous to plant ALDH7B1 which protects against various forms of stress such as increased salinity, dehydration and treatment with oxidants or pesticides. Deleterious mutations in human ALDH7A1 are responsible for pyridoxine-dependent and folinic acid-responsive seizures. In previous studies, we have shown that human ALDH7A1 protects against hyperosmotic stress presumably through the generation of betaine, an important cellular osmolyte, formed from betaine aldehyde. Hyperosmotic stress is coupled to an increase in oxidative stress and lipid peroxidation (LPO). In this study, cell viability assays revealed that stable expression of mitochondrial ALDH7A1 in Chinese hamster ovary (CHO) cells provides significant protection against treatment with the LPO-derived aldehydes hexanal and 4-hydroxy-2-nonenal (4HNE) implicating a protective function for the enzyme during oxidative stress. A significant increase in cell survival was also observed in CHO cells expressing either mitochondrial or cytosolic ALDH7A1 treated with increasing concentrations of hydrogen peroxide (H_2O_2) or 4HNE, providing further evidence for anti-oxidant activity. *In vitro* enzyme activity assays indicate that human ALDH7A1 is sensitive to oxidation and that efficiency can be at least partially restored by incubating recombinant protein with the thiol reducing agent β-mercaptoethanol (BME). We also show that after reactivation with BME, recombinant ALDH7A1 is capable of metabolizing the reactive aldehyde 4HNE. In conclusion, ALDH7A1 mechanistically appears to provide cells protection through multiple pathways including the removal of toxic LPO-derived aldehydes in addition to osmolyte generation.

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1. Introduction

Aldehyde dehydrogenases facilitate the $NAD(P)^+$ dependent oxidation of aldehydes to their corresponding carboxylic acids and NAD(P)H. These enzymes participate in many physiologically important biosynthetic pathways but also play a crucial role in removing toxic aldehydes produced during oxidative stress and the metabolism of xenobiotics. Aldehyde dehydrogenase 7A1 (ALDH7A1), originally named antiquitin, was first identified in plants as an enzyme that was upregulated during various insults such as increased salinity, dehydration and treatment with oxidants or pesticides [1–4]. Deleterious mutations in human ALDH7A1 cause pyridoxine-dependent and folinic acid-responsive seizures [5,6]. In mammals, ALDH7A1 plays a role in lysine catabolism where it metabolizes α-aminoadipic semialdehyde (AASA) to α-aminoadipic acid [7]. Lysine is an essential amino acid and its metabolism is important for the maintenance of cellular nitrogen pools, synthesis of glutamate and formation of ketone bodies [8]. Recently, the enzyme was also found to play a significant role in protecting mammalian cells from hyperosmotic stress, presumably through the generation of the osmolyte betaine from betaine aldehyde [9]. The same study also identified that ALDH7A1 is enzymatically active against a number of lipid peroxidation-derived aldehydes which are formed under oxidative conditions. Hyperosmotic stress is coupled to the generation of reactive oxygen species (ROS) and elevated oxidative stress within the cell [10]. The tight correlation between osmotic and oxidative stress suggests that the cytoprotective functions of ALDH7A1 may be two fold: (1) the generation of osmolytes to counteract osmotic stress and (2) the removal of reactive aldehydes generated as a result of increased oxidative stress.

ROS and lipoxidation products generated during oxidative stress can contribute significantly to cytotoxicity. ROS including hydroxyl

Abbreviations: AASA, α-aminoadipic semialdehyde; 4HNE, 4-hydroxy-2-nonenal; ALDH7A1, aldehyde dehydrogenase 7A1; BME, β-mercaptoethanol; CHO, Chinese hamster ovary; DTT, dithiothreitol; H_2O_2, hydrogen peroxide; LPO, lipid peroxidation; MDA, malondialdehyde; ROS, reactive oxygen species.

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radicals, superoxide and hydrogen peroxide (H_2O_2) are readily formed during oxidative stress and can trigger the oxidative degradation of biological membranes, known as lipid peroxidation (LPO). LPO occurs when free radicals remove electrons from lipids found within cellular membranes, preferentially attacking polyunsaturated fatty acids (PUFAs) [11]. This process produces fatty acid radicals which in turn react with molecular oxygen to form lipid peroxyl radicals. The lipid peroxyl radicals then react with neighboring fatty acids to generate lipid peroxides and additional fatty acid radicals that initiate additional rounds of oxidation. Antioxidants, such as vitamin E, or antioxidant enzymes, including catalase, superoxide dismutase and peroxidases, can terminate redox cycling by directly neutralizing radicals [12]. Aldehydes are produced in large quantities during LPO as a result of lipid chain cleavage and are considered secondary toxic metabolites [13,14]. LPO produces over 200 different aldehydes, many of which are highly reactive and extremely toxic even at low concentrations. Of those produced, propanal, hexanal, MDA and 4HNE are the most abundant [13,14]. Aldehydes can covalently bind to protein and DNA resulting in protein inactivation and DNA damage. Moreover, aldehydes are associated with the pathophysiology of several diseases including Parkinson's disease, Alzheimer's disease, cataract formation, atherosclerosis and alcoholic liver disease, to name a few [15–19]. As such, understanding the enzymes and pathways associated with aldehyde removal has direct relevance to disease prevention and treatment.

The present studies were performed to determine the cytoprotective actions of ALDH7A1 during oxidative stress. More specifically, we examine whether ALDH7A1 can protect mammalian cells from oxidants, such as hydrogen peroxide, and toxic LPO-derived aldehydes.

2. Materials and methods

2.1. Materials

Lipofectamine Plus reagent and hygromycin B were purchased from Invitrogen (Carlsbad, CA). The affinity resin, 5′-AMP-Sepharose 4B, used for fast protein liquid chromotography (FPLC) was obtained from GE Healthcare (Piscataway, NJ). PVDF membranes were purchased from Millipore (Bedford, MA). Complete Protease Inhibitor Cocktail tablets were obtained from Roche (Indianapolis, IN). Horseradish peroxidase-conjugated goat anti-rabbit secondary antibodies were obtained from Jackson ImmunoResearch Laboratories (West Grove, PA). Reagents for chemiluminescence were purchased from NEN Life Science Products (Boston, MA). Aldehyde substrates, NAD^+, sodium pyrophosphate, sodium phosphate, potassium phosphate and Amberlyst® 15 ion exchange resin were purchased from Sigma–Aldrich (St. Louis, MO). Allysine ethylene acetal was purchased from Chiralix (Nijmegen, The Netherlands). 4HNE was purchased from Cayman Chemical Company (Ann Arbor, MI). Protein assay reagent was obtained from BioRad (Hercules, CA). All chemicals were of analytical grade. Unless otherwise specified, all tissue-culture media, supplements, assay reagents and buffers were purchased from Invitrogen (Carlsbad, CA) or Fisher Scientific (Hampton, NH).

2.2. Cell culture

Chinese hamster ovary (CHO) cells were grown in F-12 nutrient medium (Ham's) supplemented with 7% (vol/vol) heat-inactivated fetal bovine serum (HI-FBS), 20 mM HEPES (pH 7.4), penicillin (100 U/mL) and streptomycin (100 mg/mL) at 37 °C in a humidified atmosphere containing 5% CO_2.

2.3. Construction and expression of recombinant plasmids

Mammalian and baculovirus constructs were created using human ALDH7A1 cDNA sequences and either ΔpCEP4Δ or pBlueBac4.5 expression vectors, respectively, as previously described [9]. Both mitochondrial (ΔpCEP4Δ-ALDH7A1v1) and cytosolic (ΔpCEP4Δ-ALDH7A1v2) ALDH7A1 coding sequences were cloned into the ΔpCEP4Δ expression vector. Baculovirus expression constructs were plaque-purified and amplified in Sf9 (*Spodoptera frugiperda*) insect cells by the University of Colorado Cancer Center Protein Production/MoAB/Tissue Culture Shared Core as described previously [20,21].

2.4. Generation of ALDH7A1-transfected cell lines

Stable cell lines were created by transfecting cells with either vector (ΔpCEP4Δ) or mitochondrial ALDH7A1 (ΔpCEP4Δ-ALDH7A1v1) using Lipofectamine Plus reagent as previously described [22]. Stable cell populations were then selected for in media containing hygromycin B (0.4 mg/mL). ALDH7A1 expression was screened by Western blot. Two stable cell lines, CHO-ALDH7A1v1 #14 and #35, were generated and the higher expressing cell line (CHO-ALDH7A1v1 #35) was used in subsequent experiments to determine cytoprotection and LC50 values towards various aldehydes (benzaldehyde, hexanal, octanal, 4HNE, malondialdehyde (MDA) and AASA). Pooled populations of transiently transfected cells were used in SRB assays to compare protective effects of mitochondrial (ALDH7A1v1) and cytosolic (ALDH7A1v2) variants against H_2O_2 and 4HNE. CHO cells were transiently transfected in 100 mm dishes at 80% confluence using Lipofectamine Plus reagent and ΔpCEP4Δ, ΔpCEP4Δ-ALDH7A1v1 or ΔpCEP4Δ-ALDH7A1v2 to create CHO-Vector, CHO-ALDH7A1v1 and CHO-ALDH7A1v2 cells, respectively. Western blotting was then used to determine ALDH7A1 expression levels. These cells were then used to seed multi-well plates for subsequent treatments.

2.5. Western immunoblotting

Cell lysates were prepared using radio-immunoprecipitation assay (RIPA) buffer (25 mM Tris–HCl (pH 7.6), 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS) plus Complete Protease Inhibitor Cocktail. Cells were rocked at 4 °C with RIPA for 15 min then sonicated four times for 10 s. Crude lysates were centrifuged at 15,000 rpm for 10 min to pellet debris and supernatants transferred to a fresh microfuge tube. Lysates (20 μg per well) and recombinant ALDH7A1 (30 ng per well) were separated by SDS–PAGE (12%). Analysis was performed as described previously using polyclonal anti-human ALDH7A1 antibody (1:5000 dilution) and horseradish peroxidase (HRP)-conjugated secondary antibody at 1:5000 [9]. Protein concentrations were determined using BioRad Protein Assay reagent according to the manufacturer's instructions. Densitometry was performed on the resulting Western data using BioRad's Quantity One (version 4.6.5) image analysis software. The results represent the means ± standard deviation from three separate experiments.

2.6. Aldehyde treatments

Unless otherwise stated, aldehyde substrates were obtained from Sigma–Aldrich. AASA was generated from allysine ethylene acetal as described previously [5]. The concentration of AASA synthesized was determined by derivatization to 2-aminobenzaldehyde followed by absorbance measurements at 465 nm [23].

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