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Research report

Corneal aldehyde dehydrogenases: Multiple functions and novel nuclear localization

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ABSTRACT

Aldehyde dehydrogenases (ALDHs) represent a superfamily of NAD(P)$^+$-dependent enzymes which catalyze the oxidation of a wide variety of endogenous and exogenous aldehydes to their corresponding acids. Some ALDHs have been identified as corneal crystallins and thereby contribute to the protective and refractive properties of the cornea. ALDH3A1 is highly expressed in the cornea of most mammals with the exception of rabbit that abundantly expresses ALDH1A1 in the cornea instead of ALDH3A1. In this study, we examined the gene expression of other ALDHs and found high messenger levels of ALDH1B1, ALDH2 and ALDH7A1 in mouse cornea and lens. Substantial evidence supports a protective role for ALDH3A1 and ALDH1A1 against ultraviolet radiation (UVR)-induced oxidative damage to ocular tissues. The mechanism by which this protection occurs includes UVR filtering, detoxification of reactive aldehydes generated by UVR exposure and antioxidant activity. We recently have identified ALDH3A1 as a nuclear protein in corneal epithelium. Herein, we show that ALDH3A1 is also found in the nucleus of rabbit keratocytes. The nuclear presence of ALDH3A1 may be involved in cell cycle regulation.

1. Introduction

The human eye is exposed daily to solar ultraviolet radiation (UVR). UVR is subdivided into three wavebands: UVA (315–400 nm), UVB (280–315 nm) and UVC (100–280 nm). The cornea absorbs all UVC and most UVB, whereas UVA is primarily absorbed by the lens. No UVC or UVB (and very little UVA (<1%)) reach the retina [4]. According to "Draper's law", only the fraction of energy that is absorbed by a tissue can change or damage the tissue. High level UVR exposure may lead to thermal damage in the eye; chronic low level UVR exposure, particularly that associated with the shorter and higher energy wavelengths of UVC and UVB, damages the ocular tissues by photochemical reactions that induce oxidative stress through the generation of reactive oxygen species (ROS) [4]. ROS have the capacity to attack important macromolecules including proteins, DNA and lipids and thereby cause protein modification, DNA damage and lipid peroxidation, the sequelae of which is cellular damage or death [57]. It is known that UVR-induced formation of ROS is involved in various types of eye pathologies including cataract formation, corneal and retinal degeneration [55].

The cornea, the outermost layer of the eye, serves two fundamental functions: (i) it is the initial barrier that protects the inner ocular tissues against environmental insults and (ii) it forms the "refraction" unit (together with lens) to permit light entry and focusing on the retina [54]. The cornea consists of a stratified squamous epithelium, a thick stroma containing collagen fibers, proteoglycans, glycosaminoglycans and keratocytes, and a posterior single layer of endothelium. Due to its ability to absorb UVC and UVB, the cornea plays a pivotal role in protecting internal ocular tissues (lens and retina) from UVR-induced damage and in so doing makes itself more vulnerable to damage. Seemingly to withstand this challenge, the cornea is equipped with robust antioxidant systems. Enzymatic antioxidants present in the cornea include catalase (CAT), glucose-6-phosphate dehydrogenase (G6PDH), glutathione peroxidase (GPX), glutathione reductase (GR) and superoxide dismutase (SOD). Non-enzymatic antioxidants in the cornea include ascorbate, glutathione (GSH), α-tocopherol and NAD(P)H [40].

Several studies have shown that a few water-soluble enzymes are abundantly expressed in the cornea in a taxon-specific manner. These enzymes are given the name "corneal crystallins" to emphasize their similarity to the lens crystallins [52] in that: (i) they are in most cases diverse, cytoplasmic proteins with metabolic functions, (ii) they display taxon-specificity, and (iii) they accumulate to high levels in transparent tissues, while they are present at lower levels in other tissues. These crystallins are believed to contribute to the transparent and refractory properties of the cornea and lens [33]. In addition to such physicochemical roles, corneal and lens crystallins may, through their metabolic properties, serve to protect against UVR-induced damage through detoxification, chaperone activity

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Table 1
ALDH isozymes identified as corneal and lens crystallins.[a]

	ALDHs	Species
Corneal cystallins	ALDH3A1/BCP54	Most mammals
	ALDH1A1	Rabbit, human, pig, chicken, fish
	ALDH2	Rabbit, fish
Lens crystallins	ALDH1A1	Mammals
	ALDH1A8/η-crystallin	Elephant shrew
	ALDH1A9/Ω-crystallin	Scallops
	ALDH1C1/2/Ω-crystallin	Cephalopods

[a] See text for references.

and generation of the antioxidant NAD(P)H [40]. Such "enzyme" crystallins include the aldehyde dehydrogenase isozymes (ALDHs).

ALDHs represent a superfamily of $NAD(P)^+$-dependent enzymes that catalyze the oxidation of a wide variety of endogenous and exogenous aldehydes to their corresponding acids. Some aldehydes play vital physiological roles, including vision, embryonic development and neurotransmission. Others, such as the major products of lipid peroxidation *viz.* 4-hydroxy-2-nonenal (4-HNE) and malondialdehyde (MDA), are cytotoxic and carcinogenic [40]. 4-HNE and MDA possess terminal carbonyl groups, making them strong electrophiles and highly reactive. These aldehydes are relatively long-lived, allowing them to react with cellular components more distant from their site of generation [20]. The ALDH superfamily (and other enzymes involved in aldehyde metabolism) plays critical roles in protecting cells from these toxic metabolites by regulating their levels. The pathophysiological significance of this protective role is exemplified by human diseases associated with mutations and polymorphisms of *ALDH* genes [62].

Some members in the ALDH superfamily have been identified as crystallins in the cornea and lens of both vertebrates and invertebrates (Table 1). In vertebrates, it was first reported that bovine corneal protein 54 (BCP54) comprised 20–40% of total soluble protein of the bovine cornea; later studies identified BCP54 as ALDH3A1 [65]. It is now well established that ALDH3A1 is a major soluble protein in the cornea of most mammalian species including human; by contrast, rabbits express ALDH1A1 in the cornea [28,34]. Other vertebrates, including chicken, frog, and fish, also express ALDH1A1 and, in some cases, ALDH2 rather than ALDH3A1 in the cornea [47]. Another member of ALDH class 1 proteins, ALDH1A8 (η-crystallin), is a lens crystallin in the elephant shrew [26]. In invertebrates, ALDH1A9 (Ω-crystallin) and ALDH1C1/2 (Ω-crystallins) are lens crystallins in scallops and cephalopods, respectively. Interestingly, ALDH enzyme activities is extremely low in lens extracts from these invertebrate species [66]. Although expressed in a taxon-specific fashion, members of ALDH class 1 and ALDH3A1 are well conserved in mammals, showing ~90% homology in amino acid sequence among human, rabbit, cow, sheep, mouse and rat [43]. The essential residues for $NAD(P)^+$-binding (Lys-192, Gly-245, Gly-250, Glu-399, and Phe-401; numbering based on human ALDH1A1) and for catalytic activity (Cys-302 and Glu-268) are strictly conserved in these species [43].

In this paper, we review the current knowledge on corneal ALDH3A1 and ALDH1A1, report the mRNA expression profile of nine ALDH isozymes in mouse cornea and lens and present experimental data showing the novel nuclear localization of ALDH3A1 in rabbit corneal keratocytes.

2. Materials and methods

2.1. Animals

New Zealand white rabbits (2–4 kg) were purchased from Western Oregon Rabbit Company (Philomath, Oregon) and male C57BL/6J mice of approximately 8-wk old were purchased from Jackson Laboratory (Bar Harbor, Maine). All procedures involving animals were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Colorado Denver and the University of California, Irvine.

Table 2
PCR primers used in Q-PCR analysis.

mRNA	Primer ID	Product size (bp)	Gene number
ALDH1A1	Mm00657317_m1	116	NM_013467.3
ALDH1A2	Mm00501306_m1	68	NM_009022.3
ALDH1A3	Mm00474049_m1	86	NM_053080.3
ALDH1B1	Mm00728303_s1	68	NM_028270.4
ALDH2	Mm00477463_m1	59	NM_009656.3
ALDH3A1	Mm00839312_m1	87	NM_001112725.1; NM_007436.2
ALDH3A2	Mm00839320_m1	71	NM_007437.4
ALDH4A1	Mm00615268_m1	105	NM_175438.3
ALDH7A1	Mm00519645_m1	85	NM_138600.4; NM_001127338.1

2.2. ALDH3A1 stably transfected cell lines

The development and characterization of the rabbit corneal keratocytes (TRK43) stably transfected with human ALDH3A1 has been previously described [39]. Cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and 100 U/ml penicillin–100 μg/ml streptomycin solution and maintained at 37 °C in a humidified 5% CO_2 incubator.

2.3. RNA isolation, reverse transcription and quantitative real-time PCR (Q-PCR)

Mice were euthanized by CO_2 inhalation followed by cervical dislocation. Corneas and lenses were removed directly from enucleated eyes and pooled from 3–5 animals. Total RNA was isolated from respective ocular tissues using RNeasy Mini kit (Qiagen, Valencia, CA) according to manufacturer's protocol. cDNA was synthesized using Superscript III RT kit (Invitrogen, Carlsbad, CA) according to manufacturer's instructions, using 5 μg total RNA in a 20 μl reaction volume. Q-PCR reactions were carried out using 30 ng cDNA by the Taqman gene expression assay (ABI, Foster City, CA) according to manufacturer's protocol. Gene-specific Q-PCR primers were purchased from ABI (Foster City, CA) and are summarized in Table 2. Relative mRNA levels were reported as fold of control (=1), which showed the least amount after normalization to β-actin (ACTB).

2.4. Generation of chicken anti-rabbit ALDH1A1 antibody

Immunization of chickens using recombinant rabbit ALDH1A1 [43] and collection of eggs were performed by Calbiochem (San Diego, CA, USA). Egg yolks were carefully separated from the white by washing with deionised water and collected without the yolk skin in a graduated cylinder. Two volumes of 100 mM phosphate buffer (pH 7.2) containing 0.02% (w/v) sodium azide (Sigma–Aldrich) were added and mixed thoroughly. 3.5% (w/v) polyethylene glycol (PEG 6000, Sigma–Aldrich) was introduced and stirred until the PEG was completely dissolved. After incubation at 4 °C overnight, the sample was centrifuged at 4500 *g* for 20 min at room temperature. The supernatant was filtered through n.4 Whatman paper and solid PEG was added to a final concentration of 12% (w/v). Following incubation overnight at 4 °C, the mixture was centrifuged at 12,000 *g* for 20 min. The pellet containing IgY was resuspended in KPO_4 (1/6 of the initial volume of yolk) and dialyzed against the same buffer at 4 °C with 10 K molecular-weight Dialysis Cassettes (Pierce). The purity and specificity of anti-rabbit ALDH1A1 IgY was tested using whole cell lysates and recombinant ALDH1A1 by Western immunoblotting as previously described [49]. The pre-immune IgY, which was prepared in the same manner as for anti-ALDH1A1 IgY, was used as the serum control. The purified anti-rabbit IgY recognized a single band running approximately at a MW 55 kDa (data not shown).

2.5. Immunohistochemistry (IHC)

IHC staining was carried out on 5 μm thick paraffin embedded rabbit eye sections mounted on microscope slides (Fisher). Sections deparaffinized in xylol and rehydrated in graded ethanol solutions were pretreated in 10 mM sodium citrate in a microwave at 95 °C for 30 min. Slices were then treated with 3% hydrogen peroxide (Sigma–Aldrich) to block peroxidase activity and, after washing in PBS-Brij 0.2% (Sigma–Aldrich), incubated in blocking buffer (Zymed) at room temperature in a humidified chamber for 1 h (to minimize non-specific binding). Samples were incubated with anti-ALDH1A1 antibody at a dilution of 1:50 for 1.5 h. Antibody binding was detected using horseradish peroxidase-conjugated anti-chicken antibody (1:100 dilution) for 45 min. Sections were rinsed three times in PBS-Brij and incubated with avidin–biotin complex reagents (Dako-cytomation) for 1 h at room temperature. Sections were then rinsed three times in PBS-Brij and diaminobenzidine (DAB) was used as a chromophore. Sections were then counterstained with