Constitutive Expression of Class 3 Aldehyde Dehydrogenase in Cultured Rat Corneal Epithelium*

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Mammalian Class 3 aldehyde dehydrogenase (ALDH) is normally associated with neoplastic transformation or xenobiotic induction by aromatic hydrocarbons in liver. However, Class 3 ALDH is constitutively expressed at it's highest specific activity in corneal epithelium. Tissue-specific, differential gene expression is often controlled by alternative, independent molecular pathways. We report here the development of an in vitro corneal epithelium culture system that retains constitutive high expression of the ALDH3 gene. This model system was used to establish, by enzymatic assays, Western and Northern analyses, histochemical and immunocytochemical staining, and 5' RACE methodologies that constitutive and xenobiotic induction of Class 3 ALDHs occurs from a single gene. Our results also provide a plausible explanation for the very high Class 3 ALDH activity in mammalian cornea, as the primary mechanism of oxidation of lipid peroxidation-derived aldehydes. Further studies with corneal epithelium suggest the presence of additional mechanisms, other than Ah-receptor-mediated, by which the ALDH3 gene can be differentially regulated in a tissue-specific manner.

Aldehyde dehydrogenases are a family of NAD-dependent enzymes that catalyze the oxidation of cellular aldehydes to carboxylic acids. Physiological substrates include ethanol-derived acetaldehyde, aldehydes from membrane lipid peroxidation and aldehydes from neurotransmitter, drug, and xenobiotic metabolism (1). Aldehyde dehydrogenases are both constitutively expressed or inducible under a variety of conditions. Of the three major groups of mammalian ALDHs,1 Class 1 enzymes are cytosolic and either constitutive or drug-inducible. Class 1 isozymes are NAD-specific and prefer aliphatic aldehydes as substrate. Class 2 ALDH is localized to the mitochondria. This isozyme appears to be primarily responsible for oxidizing acetaldehyde, as well as several aldehydes generated by lipid peroxidation. Like Class 1 ALDHs, Class 2 ALDH uses NAD and preferentially functions at micromolar concentrations of small aliphatic aldehydes. Both Class 1 and Class 2 ALDHs are tetramers of identical subunits, the monomers being approximately 500 amino acids long with molecular masses of 55 kDa.

Class 3 aldehyde dehydrogenase is cytosolic and appears to be either constitutively produced or inducible, depending on the tissue (1). Class 3 ALDH prefers NAD as coenzyme, but this isozyme can use NADP effectively, in vitro (2). Class 3 ALDH preferentially catalyzes the oxidation of aromatic aldehydes, such as benzaldehyde, and medium chain length aliphatic aldehydes, as such hexanal. Consequently, in vitro assays using benzaldehyde and NADP serve as a marker for Class 3 ALDH activity (1, 3). Both the induced and constitutive forms of Class 3 ALDH are dimers of identical monomers, each 453 amino acids in length (4). The subunit molecular weight is approximately 50 kDa.

Cloning and characterization of rat ALDH3 indicates the gene spans 9 kb and has 11 exons, the first of which is noncoding (5). Southern and Northern analyses indicate that Class 3 ALDH from induced and constitutively expressing liver cell lines is derived from a single gene (6). Furthermore, current evidence indicates that regulation of ALDH3 occurs at the level of transcription (1).

Among the aldehyde dehydrogenases, Class 3 ALDH is most clearly expressed in a tissue-specific manner. It is found at its highest constitutive levels in mammalian cornea (1, 7, 8). Class 3 ALDH is believed to play a protective role in corneal tissue as either an NAD binding protein with UV absorption capabilities or as a catalytic enzyme involved in oxidizing lipid aldehydes generated during UV assault (7, 8). Given the enzyme’s constitutive abundance in the cornea, it has also been suggested that Class 3 ALDH functions as a structural protein. While not detectable in normal liver, Class 3 ALDH is expressed in liver following exposure to certain xenobiotics or during neoplastic transformation (1, 9). Xenobiotics are believed to induce Class 3 ALDH activity via increased ALDH3 gene transcription by an Ah-receptor mediated process (10, 11). Constitutive expression in cornea and stomach, however, suggests that an independent mechanism may underlie tissue-specific expression.

We are interested in the mechanisms controlling high constitutive, tissue-specific ALDH3 expression. Because of its extremely high Class 3 ALDH activity, we have chosen the rat cornea as our model. This required the development of an in vitro corneal epithelium culture system that expresses near in vivo levels of Class 3 ALDH for extended periods. Here we report a modified method (12) for successfully culturing rat corneal epithelium that maintains its differentiated properties, including high Class 3 ALDH activity. This in vitro system is then used to begin examining constitutive expression of the ALDH3 gene. In doing so we address the following questions. 1) Does rat corneal epithelium produce Class 3 ALDH constitutively in culture? Does expression continue at near in vivo levels for an extended period? 2) How is Class 3 ALDH distributed intracellularly? 3) What characteristics are similar between corneal Class 3 ALDH and xenobiotic-induced or transformed liver Class 3 ALDH? 4) Are the constitutively expressed and xenobiotic-activated Class 3 ALDHs expressed from a single
gene? 5) What mechanisms may be involved in constitutive ALDH3 expression?

MATERIALS AND METHODS

Reagents—Unless otherwise specified, all tissue culture media, supplements, growth factors, assay reagents, buffers, developing reagents, and colloids were from Sigma. Tissue culture dishes came from Corning (Catalog number 25000-35). In our hands, only Corning dishes supported corneal epithelial growth and differentiation. The following reagents were purchased from the supplier indicated: nitroblue tetrazolium (Bio-Rad), goat anti-rabbit IgG horseradish peroxidase (Sigma), [3H]dCTP (Amersham Corp.), DNA Random Priming Kit (U. S. Biochemical Corp.), GeneCLean DNA purification (Intermountain Scientific), Ultrapure agarose (Life Technologies, Inc.), Hybond-N membrane (Amersham), 5' RACE kit (Life Technologies, Inc.), Sequenase sequencing kit (U. S. Biochemical Corp.), [35S]dATP (Amersham), Sequenase (Stratagene), T4A cloning vector (InVitrogen), Taq polymerase (Perkin-Elmer), Sprague-Dawley rats (Sasco), AE3 and AE5 antibodies to cytokeratins (ICN), polyvinylidene difluoride 0.2 μm membrane (Bio-Rad), Spurr’s resin (EM Sciences), Unicryl (Goldmark Biologicals), colloidal-gold goat anti-rabbit IgG antibody (British BioCell). A 1.5-kb cDNA fragment from the ALDH5 gene was a generous gift from Dr. David Crabbe (Indiana University School of Medicine, Indiana).

Collagen Coating Dishes—Thirty-five millimeter Corning culture dishes were aseptically coated with 0.1% rat tail collagen type I in 0.1 M acetic acid at 37°C for 24 h prior to explant attachment. Collagen coating solution was removed following incubation, and the plates were rinsed once with sterile phosphate-buffered saline (PBS). pH 7.5. Dish was then adequately aseptically air-dried.

Cornea Explantation—Corneas were removed from whole, propulsed rat eyes by circumcision of the tissue just internal to the limbus. Following dissection, corneas were sectioned into 1-mm² explants. A single explant was transferred, epithelial side up, to the center of a 35-mm², collagen-coated Petri dish. Explants were allowed to anchor to the dish for 5 min before media was added. One milliliter of complete medium (F-12, high glucose with phenol red; 2% fetal calf serum, 10 mM sodium bicarbonate, 0.005 mM histidine, 0.1 mM choline chloride, 10 μM ascorbic acid, 5 μM thymidine, 2 μg/ml insulin, 100 μg/ml streptomycin, 100 U/ml penicillin, and 100 μg/ml ampicillin) was added to each plate. Explants were then incubated at 37°C, 5% CO₂ environment, in continuous dark. Medium was changed daily. At 4 days post-explantation, tissue explants were removed and suspended in fresh medium. Five milliliters of complete medium was added to the dish, and the explants were transferred to a 15-ml conical tube for further processing. Films were dried in a speed vacuum concentrator and stored at −80°C until use. 

ALDH Histochemistry and Immunocytochemistry—Cultures were oxidized in 0.5% benzaldehyde (4 mM), NADP (1.3 mM), phenazine methosulfate (1.3 mM), and Tris-buffered saline, pH 7.2. A histochemical staining solution (13) containing benzaldehyde (4 mM), NADP (1.3 mM), phenazine methosulfate (0.13 mM), and nitro blue tetrazolium (0.48 mM) in PBS was applied to 2 ml/dish. The reaction was allowed to proceed in the dark for 15 min. The tissues were then rinsed with PBS. Cultures were examined and photographed by phase contrast microscope.

Cells were fixed as described for ultrastructure analysis. Following dehydration, cells were infiltrated and embedded in Unicryl and sectioned. The grids were rinsed in 10% ovalbumin/PBS (O/PBS) for 10 min. Grids were incubated for 4 h at room temperature in a humid chamber with rabbit polyclonal anti-Class 3 ALDH antibody diluted 1:100 in PBS. After incubation, the grids were rinsed in 2 ml of O/PBS. The tissues were then transferred to 0.05 M Tris-buffered saline for 10 min. Grids were then incubated with colloidal gold-labeled secondary goat anti-rabbit IgG for 1 h at room temperature in a humid chamber. Grids were rinsed in a series of washes with PBS and with PBS and with PBS with 0.1% aqueous uranyl acetate for 4 min. The grids were then air-dried before viewing on an OLY J EM-1210 transmission electron microscope.

Determination of ALDH Activity—At various days of growth, five dishes of cultured epithelium were rinsed once with cold PBS. Anchored cells were scraped with a rubber policeman, pooled, and pelleted at 10,000 rpm for 5 min in the cold. The pellets were resuspended in 200 μl PBS and pelleted at 10,000 rpm for 5 min in the cold. The pellets were incubated at 37°C for 2 h with 200 μl of 10% SDS/PBS. The supernatants were transferred to a glass microhemogenezizer and dounced 10 times on ice. Following 30 min on ice, the slurry was centrifuged at 14,000 rpm for 15 min at the cold. Supernatants were assayed for ALDH activity as described previously (14). Briefly, 25 μl of cell extract was added to an assay mixture containing either 1 mM benzaldehyde and 1 mM NADP for Class 3 ALDH activity or 20 mM propionaldehyde and 1 mM NAD for Class 1 and Class 2 activity in assay buffer (60 mM phosphate, 1 mM EDTA, 1 mM β-mercaptoethanol, pH 8.5). The increase in absorbance due to NADP(H) or NAD(H) production oxidation of the respective substrate was monitored at 340 nm. Protein determinations were by the method of Bradford (15).

Western Blot Analysis—Ten micrograms of whole epithelial protein extract prepared for enzymatic assays (see above) were denatured with SDS, separated by polyacrylamide gel electrophoresis, transferred to nitrocellulose, and incubated with rabbit polyclonal antibodies to Class 1 ALDH (1:3500), Class 2 ALDH (1:5000), or Class 3 ALDH (1:7500) (7, 16) respectively. Membranes were then incubated with goat anti-rabbit IgG horseradish peroxidase conjugated to horseradish peroxidase (1:1000) and developed with 9-aminonaphtho-carbazole and hydrogen peroxide as described (7, 16).

Northern Analysis—Total RNA from tissue samples (e.g. whole cornea, cultured cells, normal liver, normal testes, 3-methylcholanthrene (3-MC) treated liver etc.) was prepared by the guanidinium isothiocyanate (17) method. RNA concentrations were calculated from 260 determinations. Equivalent concentrations of each RNA were electrophoresed under nondenaturing conditions and stained with ethidium bromide to assess RNA integrity and loading amounts for subsequent Northern analysis (based on 18 and 28 S ribosomal RNA banding). Comparable levels of 18 and 28 S ribosomal RNA were detected in all samples. Five micrograms of each RNA were then denatured in formaldehyde and separated by formaldehyde-agarose electrophoresis. RNA was transferred to Hybond-N membrane and UV-cross-linked. Membranes were hybridized with [32P]dCTP labeled cDNA probes for 24 h at 42°C. Membranes were washed under progressively more stringent conditions and stained with ethidium bromide to assess RNA integrity and loading amounts for subsequent Northern analysis.

Clustered exon probes were prepared by digesting the 3.4-kb cDNA with the appropriate restriction enzymes and labeled as above. A cDNA fragment containing the 5'-UTR and exons 1 and 2 was isolated from an EcoR I/Rsal digest. A 284-bp Rsal fragment containing exons 3 and 4 was isolated from an EcoR I/Bgl II digest. An Rsal/Bgl II digest released cDNA sequences corresponding to exons 6, 7, and 8. Finally, sequences for exons 9, 10, 11, and the 3'UTR were obtained from a Bgl II/Xho digest.

Human ALDH5 cDNA was digested with Xho and CiaI to release a 5'UTR and 3'UTR.
We have successfully cultured rat corneal epithelium by explanting corneal tissue plugs onto collagen-coated plastic dishes. The culture conditions have consistently produced confluent, near-homogenous populations of rat corneal epithelium virtually devoid of stromal fibroblasts and endothelium. Outgrowth of epithelia is encouraged by supplementing with growth factors that selectively stimulate epithelial mitosis (e.g. epidermal growth factor) (19). Fibroblasts remain trapped within the stromal collagen (20). Endothelium, which demonstrates limited viability in vitro (21), is suppressed by anchoring the explant endothelial side down.

The characteristic “pavement” morphology of epithelium growing in sheets down off of the top of the explant is apparent (Fig. 1A). That the cultures are predominantly epithelial was confirmed by in situ immunofluorescence and Western analysis of cell type-specific cytokeratins. Cytokeratins are cellular matrix proteins that serve as markers for both cell type classification and differentiation state. The basic/acidic cytokeratin pair (K3/K12) is specifically expressed in differentiated, corneal-like epithelia (22). Monoclonal antibody, AE5, recognizes the 64-kDa basic cytokeratin, K3 (23). In situ immunofluorescence of 10-day cultured rat corneal epithelium using monoclonal antibody AE5 discerns intracellular keratin fibers throughout the cultured cell population (Fig. 1B). At the ultrastructural level, the appearance of desmosomes confirms that the cultured cells are epithelial (Fig. 1C). Compared with other normal epithelial cells (Fig. 1, inset), few mitochondria or other organelles (e.g. Golgi, endoplasmic reticulum, lysosomes) are observed in cultured rat corneal epithelium (Fig. 1D). We estimate the number of mitochondria in corneal epithelium to be approximately 10% that of keratinocytes. These results are consistent with those observed for rabbit corneal epithelium (20, 22).

Western analysis of protein extracts from cultured corneal epithelium and whole cornea, using monoclonal antibody AE3, which detects a range of Type II basic cytokeratins (24), indicates the presence of a variety of cytoskeletal proteins (Fig. 2A).
Corneal epithelium Class 3 ALDH activity was determined with benzaldehyde and NADP at various times from 24 h to 25 days post-explantation, for cultures maintained in continuous dark (Fig. 4). Initially, the level of Class 3 ALDH in epithelial cultures is comparable with that of intact cornea. Activity in corneal cultures decreases over the following 4 days, despite active cell division. By the 5th day in culture, Class 3 ALDH activity has declined to approximately 25% of the original activity. Interestingly, Class 3 ALDH activity then begins to rise, peaking again at 10 days growth. By 10 days, activity in dark-maintained cultures is greater than 70% of intact cornea. From 11–25 days, Class 3 ALDH activity in cultured corneal epithelia steadily declines to a level approximating that of the moderately Class 3 ALDH expressing hepatoma cell line HTC (Fig. 4) (25, 26, 28). This decrease and subsequent resurgence of a protein associated with differentiated cell function is not uncommon. Frequently, primary cultures exhibit extensive declines in the levels of noncell cycle proteins as differentiated cells de-differentiate to enter a highly proliferative state ($G_0 \rightarrow G_1$) (29). During this time (1–5 days), culture conditions (e.g. epidermal growth factor, insulin, collagen matrix) stimulate transcription of cell cycle proteins at the expense of differentiated gene expression. However, as proliferation slows (5–10 days), transcription resumes for genes expressed in fully differentiated cells (30).

Determination of ALDH activity with an aliphatic substrate (propionaldehyde) and NADP yields approximately one-third the activity as compared with assays with an aromatic substrate and NADP (data not shown). This 3-fold higher activity from the aromatic aldehyde:NADP assay is diagnostic of Class 3 ALDH as the major aldehyde dehydrogenase isoform in a tissue (3). Additionally, aldehyde dehydrogenase activity at micromolar aliphatic aldehyde concentrations is not detectable in cultured corneal epithelium (Fig. 4, inset) or in whole corneal homogenates (data not shown). These data suggest that mitochondrial Class 2 ALDH, which has a $\mu M$ $K_m$ for aliphatic aldehydes (9), is present at very low levels in corneal epithelium. This is consistent with the reduced number of corneal mitochondria noted earlier.

Due to overlapping substrate preferences, it is difficult to assess the levels of specific ALDH isoforms simply by activity
assays. Therefore, cell extracts from various stages of corneal cultures were also analyzed by Western analysis using monospecific antibodies to the Class 1, 2, and 3 isoforms. Class 1 ALDH is easily detected in normal rat liver and to a lesser extent appears in 3-methylcholanthrene (3-MC) treated liver and whole, uncultured corneas (Fig. 5A). Class 1 ALDH is not detectable in either cultured corneal epithelium, rat hepatoma cells (HTC), or a normal rat liver epithelial cell line (Clone 9). Class 2 ALDH is present in normal liver, HTC cells, and 3-MC-treated liver (Fig. 5B). Interestingly and consistent with the assay data, Class 2 ALDH protein is absent from whole cornea and cultured corneal epithelia. This again correlates with the apparent lack of mitochondria in corneal epithelium. Compared with HTC cells, 3-MC treated liver and normal liver, Class 3 ALDH is clearly the most abundant ALDH isoform in whole cornea and cultured corneal epithelium (Fig. 5C). Furthermore, the enzyme appears to be the same size, 50 kDa, as authentic Class 3 ALDH from HTC cells. Consistent with the assay data, Western analyses detect the changes in Class 3 ALDH protein levels with increased time in culture. As noted previously, Class 3 ALDH from 3-MC-treated liver appears to be slightly smaller than the enzyme derived from cornea and HTC cells (31). Whether this represents partial proteolysis or an induction-associated post-translational modification event is yet to be determined.

Since the size of the protein, substrate/coenzyme preferences, subcellular localization, and histochemical expression patterns are identical for Class 3 ALDH constitutively expressed in cornea and induced in liver; and since previous evidence indicates regulation of the ALDH3 gene is controlled at the level of transcription (1, 9), corneal transcripts were compared with liver transcripts. In liver, ALDH3 transcription is from a major start site 45 bp 5' to the initiator codon and produces a 1.7-kb message (5, 6, 9). Northern analysis indicates ALDH3 transcripts in both whole cornea and cultured corneal epithelium are identical in size to that of ALDH3 transcripts from HTC cells or xenobiotic-treated liver (Fig. 6). Consistent with both assay and Western analysis data, ALDH3 transcript levels decline from the 1st to the 5th day of growth, peak at 10 days, and subsequently decline steadily for the remainder of the culture's viability. This is also consistent with the decrease and subsequent increase in expression of differentiated-function genes in cultured primary cells noted above. Northern hybridization under extremely stringent conditions with a series of cluster probes spanning the ALDH3 exons and untranslated regions indicates corneal transcripts possess all known ALDH3 exons (Fig. 7). Additional or deleted sequences were not evidenced by any obvious differences in transcript size.

RACE methodologies were employed to identify the transcriptional start site and polyadenylation signal sequence for corneal ALDH3 transcripts. First pass 5' RACE generated an anticipated 600-bp PCR fragment from cultured corneal mRNA (Fig. 8). Nested amplification of this fragment then yielded the expected 350-bp piece. If the transcriptional start site for the ALDH3 gene in cornea was dramatically different from that of ALDH3 in liver, then more or less 5'-UTR sequence would first be evident by the 5' RACE product size. Dideoxy sequencing of the cloned corneal 5'-UTR indicated 100% sequence identity through the first 150 bp of cornea and liver ALDH3 transcripts. This confirms that identical transcriptional start sites are used in both cornea and liver. For both tissues, the transcriptional start site defines the same first noncoding exon, 45 bp up-
stream of the translational initiation codon, which itself resides in exon two. 3΄ RACE also produced appropriately sized amplification products from cultured corneal mRNA, relative to HTC cell ALDH3 transcripts (18), 700 and 300 bp, respectively (Fig. 9). Dideoxy sequencing likewise confirmed the use of the same polyadenylation signal sequence for both liver and corneal transcripts. Finally, termination codons for the constitutively expressed and inducible ALDH3 transcripts are in the same nucleotide sequence and are located at the same distance from the polyadenylation signal.

If the gene is the same and the protein is the same, but expression is tissue-dependent, then perhaps the reason for differential gene expression is the occurrence of multiple regulatory mechanisms. Previous studies have indicated that activation of the ALDH3 gene in xenobiotic-treated liver is Ah-receptor mediated and controlled at the level of transcription (32–34). Our observations from corneal epithelium exposed to light suggest an additional pathway of ALDH3 regulation also exists. The data presented earlier on ALDH3 activity in cultured corneal epithelium was obtained from cultures grown in continuous dark. Corneal epithelium cultured under cycling light conditions consistently maintains higher levels of Class 3 ALDH, earlier in culture, and without as severe a decline and subsequent rebound in enzyme activity (Fig. 10). Although there is some variability in specific activity which reflects the difficulties of biochemical analysis of organ cultured cells, the results from several light versus dark grown corneal cultures are qualitatively identical. Steady-state levels of ALDH3 mRNA similarly mirror continued expression of the protein in 5–15-day lighted cultures (Fig. 10, inset). These observations are consistent with reports of increased Class 3 ALDH activity correlated with eye opening in newborn mice (35). Until the eyes are open and exposed to light, Class 3 ALDH activity is barely detectable in whole cornea. While Class 3 ALDH detection correlates to eye opening, this observation does not imply a cause-effect relationship. Presumably, however, if constitutive corneal ALDH3 gene expression is light-influenced, then Class 3 ALDH levels would decrease in corneal epithelium grown in continuous dark. Conversely, cultures grown under light conditions could anticipate a steady-state production of Class 3 ALDH. How dark grown cultures re-establish high Class 3 ALDH activity, and why light-exposed corneal cultures do not exhibit higher steady-state specific activities, remains under investigation. However, it is tempting to speculate that light maintains corneal epithelium in a highly differentiated state and that maintenance of relatively high levels of Class 3 ALDH reflect this.

These observations suggest an activation or maintenance pathway in ALDH3 constitutively expressing tissue that is independent from the Ah-receptor mediated pathway utilized during xenobiotic induction. Spectral analysis of the light
source (a cool white fluorescent bulb) documents that 95% of energy emissions are above 400 nm (36). While the major source (a cool white fluorescent bulb) documents that 95% of scripts in human and bovine cornea, may also oxidize aldehyde the molecular basis for possible light-regulated ALDH3 responsive-element consensus sequences within the first 1 kb of particular interest given the location of two putative UV-responsive regions of mitochondrial ALDH2. Corneas were processed from adult rats subjected to normal 12-h cycles of light/dark. Prior to Northern analysis, adult rat corneas were demonstrated to have high levels of Class 3 ALDH activity as determined by enzymatic assays (data not shown).

Class 3 aldehyde dehydrogenase activity and dark conditions. Compared with 10, 15, and 20 day epithelial cultures grown under cycling light conditions, cultured epithelium; dark cultured epithelium; broken lines, open circles

**Fig. 10.** ALDH activity in rat corneal epithelium cultured under lighted conditions. Class 3 aldehyde dehydrogenase activity (benzaldehyde and NADP) of rat corneal epithelium grown under light and dark conditions. Patterns: solid lines, closed circles = continuous dark cultured epithelium; broken lines, open circles = cycled light cultured epithelium; dash/dot lines, solid triangles = growth curve for both light and dark conditions. Inset, ALDH3 mRNA detected in 0, 5, 10, 15, and 20 day epithelial cultures grown under cycling light conditions. Compared with ALDH3 mRNAs from dark grown cultures (Fig. 6), note steady-state production of transcript from 5–15 days (in the light), which is consistent with enzyme activity levels for the same period. Error bars denote mean ± S.D.; n = 3.

**Fig. 11.** Northern analysis of ALDH transcripts in rat cornea, testes, and liver. 10 μg total RNA from rat cornea (lanes 1, 4, and 7), testes (lanes 2, 5, and 8), and liver (lanes 3, 6, and 9) probed with radioactively labeled cDNA fragments of ALDH3 (A), ALDH2 (B), and ALDH5 (C), see “Materials and Methods” for cDNA probe preparation. ALDH3 transcript (1.7 kb) is expressed only in cornea. ALDH2 message (2.0 kb) is found primarily in normal liver and to a lesser extent in testes. The 3.0-kb transcript for ALDH5 is not detectable in any rat tissue RNAs tested. The 2.0-kb band in lanes 8 and 9 (testes and liver, respectively) reflects cross-hybridization of ALDH3 cDNA to homologous regions of mitochondrial ALDH2. Corneas were processed from adult rats subjected to normal 12-h cycles of light/dark. Prior to Northern analysis, adult rat corneas were demonstrated to have high levels of Class 3 ALDH activity as determined by enzymatic assays (data not shown).

(Km = 17 μM), respectively. To maintain transparency, however, corneal epithelium has limited cellular organelles, including mitochondria (25). Consequently, mitochondrial Class 2 ALDH is not available at sufficient levels to function effectively in lipid aldehyde oxidation. Recent studies also indicate that Class 2 ALDH is inactivated by suicide adduct formation when exposed to physiologically relevant (nm to μM) concentrations of cytotoxic lipid aldehyde substrates (38, 39). Furthermore, Western analysis of corneal epithelium indicates only minimal levels of Class 1, compared with Class 3 ALDH. Given the absence of Class 2 ALDH and limited amounts of Class 1 ALDH in corneal epithelium, high levels of Class 3 ALDH obtain. Although somewhat less efficient than other ALDH’s in oxidizing some lipid aldehydes (40) (e.g., malondialdehyde, 4-hydroxynonenal), Class 3 ALDH is far less sensitive to adduct inactivation by substrate aldehydes (39). Moreover, Class 3 ALDH preferentially oxidizes medium chain length aliphatic aldehydes (C\textsubscript{6} to C\textsubscript{10}) produced by lipid peroxidation (Km values = 1–20 μM) (40). Finally, Class 3 ALDH may function as a UV-sink by simply binding NAD and absorbing ultraviolet light (41). In either capacity, Class 3 ALDH appears to perform a major role in protecting the cornea from ultraviolet damage.

Summarily, constitutive corneal Class 3 ALDH is identical to xenobiotic-induced liver Class 3 ALDH in subcellular localization, culture expression patterns, substrate preferences, polypeptide size, and antibody cross-reactivity. Furthermore, identical transcriptional start sites, polyadenyelation signals, termination codons, and transcript lengths indicate that a single ALDH3 gene is indeed differentially expressed in a tissue-specific manner. However, constitutive expression of the ALDH3 gene in corneal epithelium may be controlled by a light inducible-light maintenance pathway, separate from the Ah receptor mediated process found in liver. Given the reduced numbers of mitochondria in corneal epithelium and the resultant low levels of Class 2 and putative Class 5 ALDHs, Class 3 aldehyde dehydrogenase may be a key enzyme in protecting the cornea from the cytotoxic effects of UV radiation.

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