ALDH6, a cytosolic retinaldehyde dehydrogenase prominently expressed in sensory neuroepithelia during development.

Felix Grün†*, Yukihiro Hirose*, Shimako Kawauchi†, Toshihiko Ogura§ and Kazuhiko Umesono.

Institute for Virus Research, Kyoto University, 53 Kawaramacho Shogoin, Kyoto 606-8507, Japan;
* Institute of Basic Medical Science, University of Tsukuba, Tsukuba 305-8575, Japan;
§ Nara Institute of Science and Technology, Nara 630-0101, Japan.

† Corresponding author:
Department of Developmental & Cell Biology,
University of California Irvine,
5207 Biosci II, Irvine,
CA 92697-2300, USA.
Tel. (949) 824-5385; E-mail: fgrun@uci.edu

* Authors contributed equally.

Running title - Aldehyde dehydrogenase 6

Key words: aldehyde dehydrogenase, retinal, retinoic acid, neuroepithelium
Aldehyde dehydrogenase 6

Summary

We have isolated the chick and mouse homologs of human ALDH6 that encode a third cytosolic retinaldehyde-specific aldehyde dehydrogenase. In both chick and mouse embryos, strong expression is observed in the sensory neuroepithelia of the head. *In situ* hybridisation analysis in chick shows compartmentalised expression primarily in the ventral retina, olfactory epithelium and otic vesicle; additional sites of expression include the isthmus, Rathke’s pouch, posterior spinal cord interneurons and developing limbs. Recombinant chick ALDH6 has a \( K_{0.5} = 0.26 \, \mu M \), \( V_{\text{max}} = 48.4 \, \text{n mole/min/mg} \) and exhibits strong positive cooperativity (\( H = 1.9 \)) towards *all-trans* retinaldehyde; mouse ALDH6 has similar kinetic parameters. Expression constructs can confer 1000-fold increased sensitivity to retinoic acid receptor dependent signalling from retinol in transient transfections experiments. The localisation of ALDH6 to the developing sensory neuroepithelia of the eye, nose and ear and discreet sites within the CNS suggests a role for RA signalling during primary neurogenesis at these sites.
Aldehyde dehydrogenase 6

Introduction

Genes with promoters that contain retinoic acid response elements (RARE) are subject to regulation by the ligand dependent nuclear transcription factors - the retinoic acid receptors RARs and RXRs. Disturbances in Vitamin A signalling, either by Vitamin A deficiency, through teratogenic excess of the receptor ligand retinoic acid (RA) or by retinoic acid receptor knockout studies (1-3), have shown that retinoid signals participate in vertebrate morphogenesis within specific temporal windows and target tissues. Affected tissues include the eye, craniofacial structures, heart, circulatory, urogenital, respiratory system, limbs and the A-P axis of the CNS (4-8).

Precise control over the effective concentration of the receptor ligands, all-trans and 9-cis RA, is therefore a central requirement for proper receptor function and is maintained by the balance between synthesis and degradation. In retinoic acid sensitive target tissues, ligands could be derived either by uptake from the low levels circulating in serum, or through the in situ metabolism of the pro-hormone Vitamin A (retinol) by alcohol dehydrogenases (including members of the short-chain dehydrogenase/reductase and medium chain alcohol dehydrogenase families) and aldehyde dehydrogenases - reviewed in (9,10). Such an intracrine mechanism has the advantage that ligand synthesis and signalling can be tightly coupled through the cell specific regulation of the respective enzymes - a property that would be desirable in various developmental models where RA is thought to act locally as a morphogenic signal. Degradation of RA proceeds primarily through further oxidative metabolism by members of the cytochrome P450 family of enzymes such as CYP26 (11).

In vertebrates, phylogenetic analysis indicates 13 families of aldehyde dehydrogenases that fall into two main clades. The “class 3” group, which consists mostly of substrate-specific dehydrogenases, and the “class 1/2” dehydrogenases that have broader substrate specificity (12). The “class 1/2” group contains members that can utilise retinaldehyde at submicromolar concentrations (13-16). Recent reports confirm the essential role of ALDH1 and RALDH2 in RA signalling in vivo. For instance, premature expression of ALDH1 or RALDH2 by mRNA injection
Aldehyde dehydrogenase 6

into *Xenopus* embryos results in the induction of premature RA synthesis and teratogenic effects (17), whilst the targeted disruption of RALDH2 in mice has shown its essential and specific role in RA signalling during axial rotation (body turning), heart and limb morphogenesis (18).

Studies using retinoic acid sensitive β-Gal reporter transgenic mice or indicator cell lines (19-21) have demonstrated that RA signalling is restricted to specific tissues or regions during vertebrate embryogenesis and that many of these sites, but not all, co-localise to the expression pattern of ALDH1 and RALDH2. One notable discrepancy is the ventral neural retina where evidence from *in situ* hybridisation and zymographs of isoelectric focusing gels in both mouse and chick support the presence of an additional high activity retinaldehyde dehydrogenase that is distinct from ALDH1 or RALDH2 (22,23).

Here we describe the cloning and characterisation of a third cytosolic aldehyde dehydrogenase, ALDH6, that can synthesise RA and is expressed in the sensory neuroepithelia including the ventral retina. We also show that ALDH6 is able to specifically transactivate the RAR-dependent signalling pathway when transfected into cells and can sensitise cells to retinol by shifting the dose-response curve 1000-fold. Comparisons to other ALDHs suggest that ALDH6 is equivalent in efficacy to RALDH2 and 10-fold better than ALDH1 in supporting RA-dependent signalling.

**Experimental Procedures**

*Cloning of mouse and chick ALDH6 cDNAs-* PCR amplicons of aldehyde dehydrogenases (1025bp for mouse; 713bp for chick) were amplified from 100 ng oligo-d(T) primed mouse organ or chick embryonic day 4 (E4) ventral retina cDNA using 2.5 Units ExTaq polymerase (TaKaRa Shuzo, Kyoto, Japan) with 50 pmoles each of forward and reverse degenerate primers: mouse ALDHdegF, 5’ GCW GGI TGG GCI GAY AAR ATY CAY GG 3’; mouse ALDHdegR, 5’ CCR TKI CCW GAC ATY TTR AAS CC 3’; chick ALDHdegF, 5’ CAR ATH ATH CCI TGG AAY TT 3’; chick ALDH6degR, 5’ AAI ATY TCY TCY TTI GCI AT 3’. Cycle conditions were 5 mins
Aldehyde dehydrogenase 6

at 95 °C, then 30 cycles of 95 °C for 30 secs, 60 °C for 30 secs and 72 °C for 90 secs. Amplicons were cloned into pCRII-TOPO vector (Invitrogen, Carlsbad, CA), screened by restriction enzyme digestion pattern and four clones sequenced.

The full length ORF (1539 bp) of mouse ALDH6 (mALDH6) was amplified with 20 pmoles of the gene specific primers ALDH6ATG (5’ ATG GCT ACC ACC AAC GGG GCT 3’) and ALDH6STOP (5’ TCA GGG GTT CTT CTC CTC GAG 3’) deduced from mouse EST sequences AA790530 and AA499064; four independent clones were sequenced. In addition three independent 3.26 kb ALDH6 cDNA clones which included 5’ and 3’ UTR regions were amplified with 20 pmoles each of ALDH6ESTA (5’ CCG GAG AGT GCG AAC CAG TTA 3’) and ALDH6ESTC (5’ CAC ACC ACA GGG GTA AAC CTT 3’) derived from EST sequences AA790530, AA560389 and AA848809. The sequence for mouse ALDH6 is available under GenBank Accession Number AF152359.

Chick ALDH6 5’ cDNA sequences were cloned with a 5’ RACE kit (GIBCO Life Technologies, Grand Island, NY). 600 ng of E4 ventral retina RNA was reverse transcribed with the gene specific primer YHR3 (5’ TCA TCC ACA GGG ATA GTC CTG 3’), oligo-d(C) tailed, then amplified by two rounds of PCR with 20 pmoles of the kit oligo-d(G) adapter primer and the nested gene specific primers YHR2 (5’ CCT GGT GAA ACA GAC 3’) and YHR1 (5’ CAG AGA GCC AAT GTA CAG TGA TG 3’); 30 cycles each, annealing temperature was 55 °C for 30 secs. Four independent 0.6 kb clones were sequenced. Chick ALDH6 3’ cDNA sequences (approximately 3 kb) were cloned by sequential PCR with 20 pmoles of oligo d(T)-M13-M4 primer and the nested gene specific primers YHF4 (5’ GCC ATT GAA GAC AGA GCC CTG 3’), YHF6 (5’ TAC CGA GTA TGG ACT CAC 3’) and YHF7 (5’ AGC TTC TGC TTT GCA GTC G 3’). The sequence for chick ALDH6 (cALDH6) is available under GenBank Accession Number AF152358.

Transient Transfections and Reporter Gene Assays- The RARß retinoic acid response element-thymidine kinase luciferase reporter construct (RARY2-tk-Luc), thymidine kinase-luciferase (tk-
Aldehyde dehydrogenase 6

Luc) control plasmid, MH-100x4-Luc reporter plasmid, GAL4-nuclear receptor effector constructs (GAL-RAR, GAL-RXR, GAL-VDR, GAL-PPAR) and β-galactosidase (pCMX-βGal) plasmid have been previously described (24,25). The ORFs of chick aldehyde dehydrogenases were subcloned into the mammalian expression vector pCAGGS (26) to generate pCAGGS-cALDH1, -cRALDH2 and -cALDH6. JEG-3 cells were transfected by the calcium phosphate method in 24-well tissue culture plates for 8 hrs with ALDH constructs and reporter plasmids as indicated in the figure legends, then washed with PBS and transferred to serum-free ITLB (5 µg/ml insulin, 5 µg/ml holo-transferrin, 1X Gibco’s Defined Lipid Mix and 1.2 g/L bovine serum albumin) supplemented DMEM (GIBCO BRL, Grand Island, NY). Retinoids were added and cells incubated for 24-36 hrs before being assayed for luciferase and β-galactosidase activities.

In situ hybridisation for ALDH6 expression- cDNA fragments encoding chick ALDH6 (nucleotides 433-1458 in sequence AF152358), chick ALDH1 (nucleotides 597-1178 (27)), mouse ALDH1 (AHD-2) and ALDH6 (nucleotides 433-1458 in sequence AF152359) were used to prepare digoxygenin-labelled antisense RNA probes. Whole-mount in situ hybridisation was performed as described by Wilkinson (28) and section in situ hybridisation was performed as described by Ishii et al. (29), except that both hybridisation and post-hybridisation washes were carried out at 69 °C and RNAse treatment was omitted. Chick embryos were embedded in O.C.T compound (Sakura Finetechnical Co., Ltd., Tokyo) for frozen sections. Mouse embryos and E4-E7 chick embryos were embedded in paraffin. The sections were cut at 6-12 µm thickness.

Aldehyde dehydrogenase assays- Recombinant chick ALDH6 enzyme was prepared from the expression vector pBAD-cALDH6 in DH5α bacterial cultures induced with 0.2 % arabinose. Purification from lysates was by sequential column chromatography with a 100x2.5 cm Sephacryl S-300 gel filtration column (Pharmacia Biotech, Uppsala, Sweden) developed in PBS. Active fractions were dialysed against 20 mM HEPES pH 7.5, applied to a preparative 10x2.5 cm Macroprep High Q column (Biorad, Hercules, CA) and eluted with a gradient from 0-1 M NaCl in
Aldehyde dehydrogenase 6

60 mins. Final purification was by a TSK-Gel DEAE-5PW (Tosoh, Tokyo, Japan) anion exchange column with a gradient of 0-0.3 M NaCl in 30 mins. Aldehyde dehydrogenase activity was monitored by NADH-dependent formazan dye formation at 566 nm. Briefly, 1-10 µg protein were assayed in 200 µl enzyme buffer (50 mM Tris-Cl pH 8.5, 200 mM KCl, 250 µM NAD, 250 µM nitroblue tetrazolium (NBT), 8 µM phenomethyl sulfonate (PMS) and 0.26 % gelatin) with varying concentrations of substrate aldehydes. Retinaldehyde dehydrogenase activity was confirmed by HPLC assay as described below. Protein concentrations were assayed with the Biorad Protein Kit and purity determined by SDS-PAGE analysis.

Kinetic parameters for aldehyde substrates and NAD were determined by HPLC quantitation of retinoic acid formation or NADH production at 340 nm. Retinaldehyde dehydrogenase activity was assayed with 0.1-0.4 µg protein and 2 µM all-trans retinaldehyde in 1 ml enzyme buffer (50 mM HEPES pH 8.0, 200 mM KCl, 2 mM NAD, 1 mM MgCl₂ and 1 mM DTT) at 37°C for 10 mins. Retinoids were extracted by addition of 250 µl acetonitrile:butanol (1:1) and 200 µl saturated potassium phosphate buffer according to the method of McLean (30), separated using a 5x4.1mm C18 reverse phase TSK-Gel SuperODS column (Tosoh, Tokyo, Japan) and quantitated by photodiode array detection. HPLC gradient conditions were as follows. Flow rate 1.5ml/min, 100 % 50 mM ammonium acetate pH 6.9; linear change from 0-60 % acetonitrile in 3.0 mins; isocratic at 60 % acetonitrile for 3.0 mins; linear change from 60-100% acetonitrile in 1.5 minutes; isocratic at 100 % acetonitrile for 2.5 mins.

Kinetic data were fitted using the nonlinear regression analysis program Prism (GraphPad Software, Inc., San Diego, CA).

Results

Screening of aldehyde dehydrogenase PCR amplicons from mouse and chick tissues

In the developing chick and mouse retina, ALDH1 is restricted to the dorsal neural retina, whilst RALDH2 is expressed in the pigmented retinal epithelium (31). However an additional
Aldehyde dehydrogenase 6

retinaldehyde dehydrogenase activity, named V1 in mouse and C-V in chick, that is biochemically distinct from ALDH1 and RALDH2 has been detected in the ventral neural retina (22,23). Since the small tissue sample size makes it impractical for classical protein purification, we isolated novel ALDH cDNA clones by designing degenerate oligonucleotide PCR primers to regions of high sequence conservation in an alignment of published human, mouse, and chick ALDH sequences for the non-allelic ALDH1/2/5/6 and RALDH2 genes. In addition to detecting the known aldehyde dehydrogenases, 4 of 26 clones from mouse and 5 of 120 clones from chick represented a new ALDH that by BLAST search of GenBank database sequences shared closest homology with human ALDH6 (32) and the recently described murine RALDH3 cloned from the lateral ganglionic eminence (33). On the basis of nucleotide and amino acid similarity we assign this new ALDH as the mouse and chick homolog of human ALDH6. Fig. 1A and B shows the mouse and chick nucleotide sequences and deduced open reading frame (ORF) of the longest cDNAs obtained by PCR as described in the Methods section.

The phylogenetic relationship between various aldehyde dehydrogenases is shown in Fig. 2 and Table 1. Human, mouse and chick ALDH6 sequences share 85-94 % amino acid conservation, equivalent to that seen between species for sequences of ALDH1 (83 %) or RALDH2 (96%). ALDH6 is only 69% conserved between either ALDH1 or RALDH2. The murine ALDH6 is essentially identical (99%) to RALDH-3 ((33); GenBank Accession Number AF253409). The ALDH6 ORF contains the NAD binding motif (GxGxxxG) at residues 235-241 and the conserved cysteine (Cys314) which acts as the active-site nucleophile (34). In addition residues that are found in all catalytically active ALDHs (12) are present in equivalent positions in an optimal alignment suggesting that this enzyme is functional.

**ALDH6 is expressed in sensory neuroepithelia during development**

We performed *in situ* hybridisation of chick and mouse embryos with antisense RNA probes of ALDH6. Figures 3-4 show the expression pattern of ALDH6 in whole mount and sections in chick from stage 10 to 18. No signal was detected with sense probes. ALDH6 is first
detected at stage 10 in the surface epithelium anterior to the optic vesicle but is absent from ectoderm directly overlying the lens vesicle (Fig. 3A and Fig. 4F). By contrast the expression of ALDH1 is first detectable as the optic vesicle makes contact with the overlying ectoderm and the lens placode begins to thicken at stage 12. ALDH1 expression is initiated in the proximal layer of the vesicle directly opposite the lens placode (Fig. 4A). This region of strong ALDH1 expression involutes during optic cup formation and expands to form the dorsal neural retina (Fig. 4A-E). In contrast ALDH6 expression extends to both the proximal and distal ventral sides of the vesicle that are destined to become respectively the ventral halves of the neural and pigmented retina (Fig. 4F-J). By embryonic day 4, ALDH1 and ALDH6 exhibit complementary non-overlapping domains of expression in the dorsal and ventral neural retina (Fig. 4 E, J). Separating these two expression domains is a region that transects the dorso-ventral axis of the retina where neither ALDH1 or ALDH6 is expressed. Interestingly this stripe represents the expression domain of CYP26, a cytochrome P450 enzyme that is involved in RA breakdown (11,35,36).

In later stages of eye development, neural retina differentiates into a well organized layered structure in which various types of cells reside at specific positions. At E10, both ALDH1 and ALDH6 were found to be expressed in the outer side of the inner nuclear layer where amacrine cells are differentiating (Figs. 5A, B, E and F) (37). Both transcripts were also found in the ganglion cell layer, albeit faintly. By E16, expression of both ALDH1 and ALDH6 have become faint (Fig. 5C, D, G and H).

Concurrent with its strong expression in retina, the posterior olfactory neuroepithelium and dorsal epithelium of the otic vesicle are early sites of expression (Fig. 3C, 6D-E). Additional sites in the developing head include a band of expression in the isthmus joining the mid and hindbrain and Rathke’s pouch (Fig. 6C, D). Although we could not detect expression during early limb bud formation (stages 16-21), ALDH6 was observed later at E4 as diffuse staining in mesenchymal limb tissue and at E7 in the hindlimb and forelimb interdigital zones and the perichondrial membranes of wing and leg buds (Fig. 6 A, B and G). In the posterior trunk, ALDH6 staining was
Aldehyde dehydrogenase 6

found in somites and a subset of spinal cord interneurons opposite the hindlimb field (Fig. 6G, H). This spinal cord expression co-localised with En-1, a specific marker for interneurons (38).

The localisation of ALDH6 expression to the sensory neuroepithelia is conserved between chick and mouse (Fig. 7A), although some minor differences were observed. In mouse embryos, ALDH6 was not detected before E8.5 but became clearly visible by E9.5 in the dorsal and ventral margins of the optic vesicle and in a large area of overlying surface head ectoderm (Fig. 7B). The dynamic nature of the expression pattern is identical to that reported for RALDH3 (33). By E10.5 ALDH6 expression in surface ectoderm is rapidly reduced (Fig. 7A,C), but is reexpressed at E11.5 where the ectoderm invaginates at the dorsal and ventral margins of the optic cup (Fig. 7D). During this period expression in the ventral neural retina becomes more pronounced (Fig. 7A, C, D) and in contrast to the chick, some staining is also transiently seen in the dorsal neural retina through E11.5 (Fig. 7D). However by E12.5 ALDH1 and ALDH6 are separated into distinct dorsal and ventral domains in the neural retina (Fig. 7E, F). Expression of ALDH6 in retina weakens slightly by E15.5 (Fig. 7G).

The conservation of expression between mouse and chick ALDH6 extended to the olfactory placode and otic vesicle which give rise to the sensory neuroepithelia of the nose and ear. Whole mount and section in situ hybridisation of mouse embryos showed initially strong uniform expression in the developing olfactory epithelium at E10.5 (Fig. 7A, C). Starting at E11.5 ALDH6 expression became progressively more restricted to the dorso-lateral neuroepithelium (Fig. 7D). By E15.5, transverse sections of olfactory structures exhibited a punctated cellular expression pattern in the sensory epithelium and the mesenchymal stroma directly underlying it that was restricted to discreet zones within the developing turbinates (Fig. 7H). Expression in the otic vesicle was transient and not detected after E10.5. In coronal sections of brain at E15.5 we also confirmed expression in the lateral ganglionic eminence as described in detail by Li et al. (33).

Mouse and chick ALDH6 can utilise retinaldehyde and are inhibited by citral.
In order to confirm that ALDH6 is indeed the retinaldehyde-specific dehydrogenase of ventral retina, we constructed inducible bacterial expression vectors containing the open reading frame. Conversion of \textit{all-trans} retinal to retinoic acid was only detected in lysates from bacterial cultures containing pBAD-ALDH6 and was dependent on induction of the protein (Fig. 8). Purified chick and mouse ALDH6 enzymes were used for determination of kinetic parameters and substrate specificity (Table 2). Activity was found to be strongly stimulated with increasing pH between 7-8 but was constant above pH 8.0. Enzyme assays were therefore carried out at pH 8.0. ALDH6, like other dehydrogenases in the “class 1/2” clade, can utilise a variety of aldehyde substrates preferring aliphatic longer chain aldehydes; $K_m$ values for acetaldehyde, benzaldehyde and octanal were respectively 2 mM, 4.3 µM and 0.24 µM. By comparison ALDH6 used \textit{all-trans} retinal as substrate with a $V_{\text{max}}$ of $48.4 \pm 0.4$ nmoles/mg/min, $K_{0.5}$ of $0.26 \mu M \pm 0.033$ and displayed positive cooperativity ($H = 1.9$) (Fig. 9A). Mouse ALDH6 had very similar kinetic parameters; $V_{\text{max}} = 58.1 \pm 1.0$ nmoles/mg/min, $K_{0.5} = 0.33 \pm 0.040 \mu M$ and $H = 1.8$ (n=3). The submicromolar affinity for retinaldehyde and substantial $V_{\text{max}}$ support the conclusion that ALDH6 can function as a retinaldehyde-specific dehydrogenase \textit{in vivo}.

We also determined the kinetic parameters for citral which acts as a high affinity slow turnover substrate of aldehyde dehydrogenases that has been used as a selective competitive inhibitor of RA synthesis. By inference, tissues disturbed by citral treatment are considered to be sites of RA synthesis and signalling. The ventral retina appears to be particularly sensitive to disruption by citral (39). Inhibition curves with citral at constant retinal concentrations were determined and used to calculate Ki values. Citral effectively inhibits RA synthesis of chick ALDH6 with a Ki of 98 nM and $V_{\text{max}}$ of 4.2 nmoles/mg/min (Table 2). By comparison, rat RalDH(I) and RalDH(II) have reported $K_m$ values of 1 and 12 µM (14), whilst human ALDH1 has a $K_m$ and $V_{\text{max}}$ of 4.2 µM and 73.2 nmol/min/mg respectively (40). Although RA synthesis from all three aldehyde dehydrogenases (ALDH1, RALDH2 and ALDH6) can be inhibited by citral, the lower affinity and higher turnover of citral by ALDH1 (40) suggests it is less sensitive to
disruption. The selective loss of the ventral retina by citral may therefore be a consequence of the intrinsic kinetic properties of ALDH1 and ALDH6.

Comparison of ALDHs for activation of RA signalling

Since aldehyde dehydrogenases exhibit broad substrate specificities, in vitro activity towards retinal may not necessarily imply functional significance in vivo. One consideration is the effective concentration of substrate encountered within the cell; the high affinity of retinal for CRBPs (41) suggests that free retinal is present only in nanomolar concentrations, although the high turnover of retinaldehydes during the visual cycle within the mature retina may represent a special case. We therefore tested chick ALDH6 for its ability to activate RA-dependent signalling under more physiologically relevant conditions. GAL-NR fusion constructs can be used to quantitate specific ligand dependent signalling in transient transfections. Various GAL-NR constructs were therefore transfected either with or without chick ALDH6 expression plasmid into the cell line JEG-3. JEG-3 is a human choriocarcinoma cell line that does not synthesise RA from 10 % FBS or 10 nM retinol in serum-free medium and that, by Northern hybridisation and PCR, does not express ALDH1, RALDH2 or ALDH6 (data not shown). RA-dependent transactivation through GAL-RAR could be detected either by exogenous addition of RA or in the presence of ALDH6 and a source of retinol (Fig. 10). The ability to support transactivation from retinol with ALDH6 indicates that aldehyde dehydrogenase activity is limiting in JEG-3 cells. Activation was specific to the GAL-RAR pathway as no specific activation with ALDH6 was observed with other GAL-NR constructs: Gal-RXR, -VDR, -GR or -PPAR. Identical results were observed with mouse ALDH6 (data not shown).

We also directly compared the efficacy of the various ALDHs to transactivate RA signalling under conditions of uniform promoter strength (Fig. 11). In transient transfections the dose-response curve for retinol was shifted 100-fold in the presence of chick ALDH1 and 1000-fold with chick RALDH2 or ALDH6. We also noted some increased GAL-RAR activity in ITLB media (retinol free) with ALDH6 or RALDH2 versus vector-only transfected cells (Fig. 10 and 11). This
residual activity is likely to represent the mobilisation and conversion into RA of the endogenous cellular pools of retinoids i.e. retinyl esters or CRBP-bound retinol, that could not be effectively washed out during removal of serum-containing medium following transfection. Either longer incubation in retinol free media or an additional wash at a later time point could reduce this effect. Hence in JEG-3 cells RA signalling is dependent on the expression of a retinaldehyde dehydrogenase activity and furthermore the specific ALDH expressed can determine the level of RA signalling from an equivalent retinol source.

**Discussion**

In this paper we have described the chick and murine homologs of human ALDH6 which correspond to the ventral retina chick C-V and murine V1 (also recently cloned as RALDH3 (33)) retinaldehyde dehydrogenase activities.

The kinetic parameters of chick and mouse ALDH6 for all-trans retinaldehyde (K\(_{0.5}\) = 0.26 µM, V\(_{\text{max}}\) = 48 nmol/min/mg and K\(_{0.5}\) = 0.33 µM, V\(_{\text{max}}\) = 58 nmol/min/mg respectively) compare favorably to published values for RALDH2 (rat RalDH(II) K\(_{m}\) = 0.7 µM, V\(_{\text{max}}\) = 105 nmol/min/mg (15)) and are equal or exceed those for rodent ALDH1 (rat RalDH(I), K\(_{0.5}\) = 1.4 µM, V\(_{\text{max}}\) = 52 nmol/min/mg, H = 1.7 (42); mouse ALDH1 (AHD-2), K\(_{m}\) = 0.7 µM (43)). Although a direct comparison is difficult without further evaluation of chick ALDH1 and mouse ALDH1, our kinetic data for ALDH6 in general supports the model that the ventral retina ALDH6 is likely to be the more efficient retinaldehyde dehydrogenase.

Further evidence in support of this conclusion is the differential ability of ALDH expression constructs to confer increased sensitivity to retinol for RAR-dependent signalling in transient transfections of JEG-3 cells. Both mouse and chick ALDH6 constructs were able to shift the dose-response curve of the RAR-signalling pathway by 1000-fold with retinol and are equally effective as RALDH2; ALDH1 is 10-fold less effective than ALDH6 or RALDH2 but nevertheless able to elicit a robust signalling response within the physiological range of retinol concentrations.
These data are consistent with the efficacy of ALDH1 and RALDH2 to synthesise moderate and high amounts of RA when mRNA is injected into *Xenopus* oocytes (17).

These data now raise the question whether different ALDH enzymes are functionally redundant within the RA signalling pathway or whether they impart additional signalling information such as setting boundaries, thresholds and/or gradients of RA? These concepts are most clearly visualised in the neural retina, where three distinct domains can be distinguished along the dorso-ventral axis on the basis of the expression pattern of ALDH1, CYP26 and ALDH6 that parallel functional RA-dependent signalling as observed in activation of RARE-βGal reporter cells and animals (36,44). The juxtapositioning of opposing synthetic and degradative enzyme activities suggests that steep gradients in RA concentrations could be generated over short distances that would radically alter the transcriptional regulation of RA sensitive target genes, essentially establishing sharp boundaries, but that within the dorsal and ventral ALDH domains the response is largely uniform.

Yet significant differences in kinetic properties of retinaldehyde dehydrogenases would also provide an intrinsically robust mechanism to quantitatively control RA synthesis and thereby regulate unique transcriptional responses encoded by threshold levels of RA. Measurement of RA synthetic ability in whole retina explants detects differences between the dorsal and ventral halves (36), indicating that although the dorsal and ventral halves of the retina are competent to synthesise the same active ligand, quantitative differences may define their positional identity; moderate levels equate with dorsal, high equals ventral. In this manner, dorsal and ventral phenotypes can be discriminated and regulated within the same signalling pathway. Consistent with such a model are observations reported with Zebrafish embryos where elevated levels of RA produced by systemic exposure has been shown to enhance early ventral eye characteristics, to expand dorsally expression of the ventral marker Pax2.1 and when applied locally on the dorsal side, to induce duplication of the choroid fissure (45). Whether in fact alternate retinaldehyde dehydrogenases elicit different RA signalling outputs in tissues, such as the neural retina, which are largely homogenous in structure and function, has not yet been experimentally addressed but provides a plausible mechanism of how...
Aldehyde dehydrogenase 6
to encode positional information or direct subtype cellular specialisation within RA sensitive tissues.

It is therefore intriguing to speculate if the prominent expression of ALDH6 in the developing neuroepithelia of the eye, nose and ear indicates a potential selective requirement for early sensory neurogenesis pathways. In observations similar to those described above for the retina, a failure of localised RA synthesis and signalling in the invaginating olfactory placode and dorso-lateral olfactory epithelium observed in citral treated and homozygous Pax6\textsuperscript{Sey} mutant mice coincides with severe broad disruption of the olfactory pathway (46). Later during olfactory epithelium development, retinoid signalling becomes restricted and defines a subset of olfactory receptor neurons interspersed with non-responsive cells (47). Reminiscent of these patterns are the changes seen in ALDH6 expression. Shifts in the A-P axis by either RA deficiency or excess also severely effects otic development (6,48,49).

Furthermore, retinoid sensitivity is seen in sensory pathways in the spinal cord. Elevated RAR/RXR expression and RA treatment increases, in contrast to citral treatment which decreases, the number of Isl1+ primary sensory neurons in \textit{Xenopus} tadpoles (50), and a sonic hedgehog independent retinoid-mediated pathway appears to control ventral spinal cord progenitor identity and promote interneuron diversity (including En1 and En2 positive neurons) in chick (51). Since ALDH6 localises to a subset of ventral spinal cord interneurons, but is distinct from the adjacent expression of RALDH2 present in the paraxial mesoderm and motor neurons arising from the most ventral lateral horn of the spinal cord (31), it may contribute to the synthesis of this local RA source and these specific neuronal subsets.

Of note also is the localisation of chick ALDH6 to the isthmus and Rathke’s pouch. The isthmus is a source of graded diffusible factors with organiser activity that specify the boundary of the mesencephalon from the segmented patterning of the rhombencephalon (52). Whilst RA acts as a posteriorizing factor in determination of the A-P axis and misregulation in signalling alters specification of rhombomeres within the hindbrain (8,49,53), RA synthesis from RALDH2 and ligand-dependent signalling extends only to the level of the first somite in developing chick and
Aldehyde dehydrogenase 6

mouse embryos (54,55). The expression of chick ALDH6 in a sharp, narrow line of demarcation across the anterior margin of the isthmus suggests that RA signalling may also influence a boundary rostrally of the hindbrain. Chick ALDH6 is also a specific marker of Rathke’s pouch and defines the oral ectoderm where it contacts the neural ectoderm of the diencephalon that are destined to fuse and form respectively the glandular and neural portions of the pituitary. Localised RA synthesis by ALDH6 is likely to affect aspects of pituitary development and has the potential to impact the hypothalamic-pituitary-gonadal axis, through the synergistic action between retinoid receptor signalling and pituitary specific transcription factors such as Pit-1 (56,57).

In conclusion, we have shown that ALDH6 is strongly expressed in the ventral retina, is a functional NAD-dependent aldehyde dehydrogenase, has high activity towards all-trans retinaldehyde and is sensitive to citral inhibition. Hence, the expression pattern and biochemical characteristics fit well with the mouse V1 (RALDH3) and chick C-V activities. The discreet localisation of ALDH6 highlights additional domains of RA synthesis during embryogenesis that are spatially distinct from sites of RALDH2 and ALDH1 expression and suggests a role in the development of a restricted set of neural tissues.

Acknowledgements

This work is dedicated in fondest memory to Prof. K. Umesono whose influence as mentor and friend will be greatly missed and without whom this work would not have been possible.

We thank R.T. Yu, K. Hara, K. Kosiba-Takeuchi, J.K. Takeuchi, M. Yamamoto and in particular B. Blumberg for critical reading and support of this work. This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan and Research for the Future Program (JSPS) and US EPA (STAR) grant G9D1-0090.
Aldehyde dehydrogenase 6

References:

1. Kastner, P., Grondona, J. M., Mark, M., Gansmuller, A., LeMeur, M., Decimo, D., Vonesch, J. L., Dolle, P., and Chambon, P. (1994) Cell 78(6), 987-1003.

2. Lohnes, D., Mark, M., Mendelsohn, C., Dolle, P., Dierich, A., Gorry, P., Gansmuller, A., and Chambon, P. (1994) Development 120(10), 2723-48.

3. Mendelsohn, C., Lohnes, D., Decimo, D., Lufkin, T., LeMeur, M., Chambon, P., and Mark, M. (1994) Development 120(10), 2749-71.

4. Wilson, J. G., Roth, C. B., and Warkany, J. (1953) Am J Anat 92, 189-217.

5. Morriss-Kay, G. M., and Sokolova, N. (1996) Faseb J 10(9), 961-8.

6. Maden, M., Gale, E., Kostetskii, I., and Zile, M. (1996) Curr Biol 6(4), 417-26.

7. Dickman, E. D., Thaller, C., and Smith, S. M. (1997) Development 124(16), 3111-21.

8. Blumberg, B., Bolado, J., Jr., Moreno, T. A., Kintner, C., Evans, R. M., and Papalopulu, N. (1997) Development 124(2), 373-9.

9. Napoli, J. L. (1999) Prog Nucleic Acid Res Mol Biol 63, 139-88.

10. Duester, G. (1999) Adv Exp Med Biol 463, 311-9.

11. White, J. A., Beckett-Jones, B., Guo, Y. D., Dilworth, F. J., Bonasoro, J., Jones, G., and Petkovich, M. (1997) J Biol Chem 272(30), 18538-41.

12. Perozich, J., Nicholas, H., Wang, B. C., Lindahl, R., and Hempel, J. (1999) Protein Sci 8(1), 137-46.

13. Yoshida, A., Hsu, L. C., and Dave, V. (1992) Enzyme 46(4-5), 239-44.

14. Penzes, P., Wang, X., and Napoli, J. L. (1997) Biochim Biophys Acta 1342(2), 175-81.

15. Wang, X., Penzes, P., and Napoli, J. L. (1996) J Biol Chem 271(27), 16288-93.

16. Zhao, D., McCaffery, P., Ivins, K. J., Neve, R. L., Hogan, P., Chin, W. W., and Drager, U. C. (1996) Eur J Biochem 240(1), 15-22.

17. Haselbeck, R. J., Hoffmann, I., and Duester, G. (1999) Dev Genet 25(4), 353-64.

18. Niederreither, K., Subbarayan, V., Dolle, P., and Chambon, P. (1999) Nat Genet 21(4), 444-8.
19. Balkan, W., Colbert, M., Bock, C., and Linney, E. (1992) Proc Natl Acad Sci U S A 89(8), 3347-51.
20. Colbert, M. C., Linney, E., and LaMantia, A. S. (1993) Proc Natl Acad Sci U S A 90(14), 6572-6.
21. Moss, J. B., Xavier-Neto, J., Shapiro, M. D., Nayeem, S. M., McCaffery, P., Drager, U. C., and Rosenthal, N. (1998) Dev Biol 199(1), 55-71.
22. McCaffery, P., Lee, M. O., Wagner, M. A., Sladek, N. E., and Drager, U. C. (1992) Development 115(2), 371-82.
23. Mey, J., McCaffery, P., and Drager, U. C. (1997) J Neurosci 17(19), 7441-9.
24. Umesono, K., Murakami, K. K., Thompson, C. C., and Evans, R. M. (1991) Cell 65(7), 1255-66.
25. Forman, B. M., Umesono, K., Chen, J., and Evans, R. M. (1995) Cell 81(4), 541-50.
26. Niwa, H., Yamamura, K., and Miyazaki, J. (1991) Gene 108(2), 193-9.
27. Godbout, R. (1992) Exp Eye Res 54(2), 297-305.
28. Wilkinson, D. G. (1992) (D.G., W., ed), pp. 75-83, IRL Press, Oxford.
29. Ishii, Y., Fukuda, K., Saiga, H., Matsushita, S., and Yasugi, S. (1997) Dev Growth Differ 39, 643-653.
30. McLean, S. W., Rudel, M. E., Gross, E. G., DiGiovani, J. J., and Pede, G. L. (1982) Clin. Chem. 28,, 693-696.
31. Berggren, K., McCaffery, P., Drager, U., and Forehand, C. J. (1999) Dev Biol 210(2), 288-304.
32. Hsu, L. C., Chang, W. C., Hiraoka, L., and Hsieh, C. L. (1994) Genomics 24(2), 333-41.
33. Li, H., Wagner, E., McCaffery, P., Smith, D., Andreadis, A., and Drager, U. C. (2000) Mech Dev 95(1-2), 283-289.
34. Blatter, E. E., Abriola, D. P., and Pietruszko, R. (1992) Biochem J 282(Pt 2), 353-60.
35. Swindell, E. C., Thaller, C., Sockanathan, S., Petkovich, M., Jessell, T. M., and Eichele, G. (1999) Dev Biol 216(1), 282-96.
Aldehyde dehydrogenase 6

36. McCaffery, P., Wagner, E., O’Neil, J., Petkovich, M., and Drager, U. C. (1999) Mech Dev 85(1-2), 203-14.
37. Milam, A. H., Possin, D. E., Huang, J., Fariss, R. N., Flannery, J. G., and Saari, J. C. (1997) Vis Neurosci 14(3), 601-8.
38. Burrill, J. D., Moran, L., Goulding, M. D., and Sauveressig, H. (1997) Development 124(22), 4493-503.
39. Marsh-Armstrong, N., McCaffery, P., Gilbert, W., Dowling, J. E., and Drager, U. C. (1994) Proc Natl Acad Sci U S A 91(15), 7286-90.
40. Kikonyogo, A., Abriola, D. P., Dryjanski, M., and Pietruszko, R. (1999) Eur J Biochem 262(3), 704-12.
41. Levin, M. S., Locke, B., Yang, N. C., Li, E., and Gordon, J. I. (1988) J Biol Chem 263(33), 17715-23.
42. Penzes, P., Wang, X., Sperkova, Z., and Napoli, J. L. (1997) Gene 191(2), 167-72.
43. Lee, M. O., Manthey, C. L., and Sladek, N. E. (1991) Biochem Pharmacol 42(6), 1279-85.
44. Wagner, E., McCaffery, P., and Drager, U. C. (2000) Dev Biol 222(2), 460-70.
45. Hyatt, G. A., Schmitt, E. A., Marsh-Armstrong, N., McCaffery, P., Drager, U. C., and Dowling, J. E. (1996) Development 122(1), 195-204.
46. Anchan, R. M., Drake, D. P., Haines, C. F., Gerwe, E. A., and LaMantia, A. S. (1997) J Comp Neurol 379(2), 171-84.
47. Whitesides, J., Hall, M., Anchan, R., and LaMantia, A. S. (1998) J Comp Neurol 394(4), 445-61.
48. Frenz, D. A., Liu, W., Galinovic-Schwartz, V., and Van De Water, T. R. (1996) Teratology 53(5), 292-303.
49. White, J. C., Highland, M., Kaiser, M., and Clagett-Dame, M. (2000) Dev Biol 220(2), 263-84.
50. Sharpe, C., and Goldstone, K. (2000) Mech Dev 91(1-2), 69-80.
51. Pierani, A., Brenner-Morton, S., Chiang, C., and Jessell, T. M. (1999) Cell 97(7), 903-15.
Aldehyde dehydrogenase 6

52. Crossley, P. H., Martinez, S., and Martin, G. R. (1996) *Nature* **380**(6569), 66-8.
53. Papalopulu, N., Clarke, J. D., Bradley, L., Wilkinson, D., Krumlauf, R., and Holder, N. (1991) *Development* **113**(4), 1145-58.
54. Niederreither, K., McCaffery, P., Drager, U. C., Chambon, P., and Dolle, P. (1997) *Mech Dev* **62**(1), 67-78.
55. Maden, M., Sonneveld, E., van der Saag, P. T., and Gale, E. (1998) *Development* **125**(21), 4133-44.
56. Lira, S. A., Kalla, K. A., Glass, C. K., Drolet, D. W., and Rosenfeld, M. G. (1993) *Mol Endocrinol* **7**(5), 694-701.
57. Rhodes, S. J., Chen, R., DiMattia, G. E., Scully, K. M., Kalla, K. A., Lin, S. C., Yu, V. C., and Rosenfeld, M. G. (1993) *Genes Dev* **7**(6), 913-32.
58. Cheng, Y., and Prusoff, W. H. (1973) *Biochem Pharmacol* **22**(23), 3099-108.
Aldehyde dehydrogenase 6

Footnotes:

Abbreviations: RAL, retinaldehyde; ROL, retinol; RA, retinoic acid; ALDH, aldehyde dehydrogenase; mALDH6, mouse aldehyde dehydrogenase 6; cALDH6, chick aldehyde dehydrogenase 6; RAR, retinoic acid receptor; RXR, retinoid X receptor.

Figure Legends

Fig. 1 Sequence of mouse and chick ALDH6

(A) Mouse, (B) Chick. Underlined nucleotide sequences represent regions corresponding to the degenerate primers used during PCR cloning. The NAD-binding site (GxGxxxG), putative catalytic cysteine (Cys314) and a polyadenylation motif (AATAAA) in the chick 3’ untranslated region are indicated in bold type. Amino acid residues are numbered starting from the first ATG codon in the deduced open reading frame.

Fig. 2 Phylogenetic analysis of aldehyde dehydrogenases.

Aldehyde dehydrogenase sequences were analysed by the CLUSTAL V algorithm.

Fig. 3 Whole mount in situ hybridisation of chick ALDH6.

(A) Whole mount stage 10 embryo: cALDH6 expression in the anterior head ectoderm. (B-C) Whole mount stage 18 embryo: strong cALDH6 expression in ventral retina and optic fissure, nasal pit, dorsal otic vesicle, isthmus and caudal somites. (D-E) Lateral view at stage 18 contrasting ventral cALDH6 and dorsal cALDH1 expression in the eye cup. Key: a, anterior; p, posterior; d, dorsal; v, ventral.

Fig. 4 Chick ALDH6 and ALDH1 expression in the eye

The expression of cALDH1 (A-E) and cALDH6 (F-J) during early eye development in chick. At st.12 (A, F): cALDH6 is expressed in the surface epithelium ventral to the optic vesicle.
Aldehyde dehydrogenase 6

The dorsal limit corresponds roughly with the ventral limit of the lens placode (lp) (indicated by arrow). This expression persists into the later stages (G-I). In the neural tissue at this stage, cALDH6 is expressed only at the junction of the eye vesicle and forebrain (arrowhead). cALDH1 is expressed in the lateral side of the eye vesicle underlying the lens placode. At st.13 (B, G): cALDH6 is expressed in the prospective pigmented retina and ventral neural retina. cALDH1 is expressed broadly in the region of the eye cup beneath the lens placode. At st.16 (C, H): cALDH6 expression remains basically the same as that at st.13. cALDH1 expression becomes restricted to the dorsal portion of the neural retina. At st.18 (D, I): cALDH6 and cALDH1 expression increase in intensity. cALDH6 expression can also be observed in the optic stalk. At E4 (E, J): expression of both cALDH6 and cALDH1 remain basically unchanged, but the cALDH6 expression in the optic stalk is weaker than at st.18 (indicated by arrowhead). The central retina region where neither cALDH1 nor cALDH6 expression is detected represents the expression domain of CYP26, a P450 enzyme involved in RA breakdown.

Fig. 5 Chick ALDH6 and ALDH1 expression in the neural retina.

cALDH6 (A-D) and cALDH1 (E-H) expression in E10 and E16 retina sections. At E10 (A, B, E, F): cALDH6 is expressed in the outer side of the inner nuclear layer (inl) in the ventral retina and weakly in the ganglion cell layer (gcl) of both ventral and dorsal retina. cALDH1 and cALDH6 expression resemble mirror images of each other along the dorso-ventral axis. At E16 (C, D, G, H): both cALDH6 and cALDH1 are expressed, albeit weakly. ipl, inner plexiform layer; opl, outer plexiform layer; pgl, pigmented epithelial layer.

Fig. 6 Expression of chick ALDH6 in limb bud and other tissues

In sections of wing (A) and leg (B) buds of E7 chick embryos, cALDH6 expression was observed in the interdigital necrotic zones and perichondrial membranes. (C-F) Sagittal sections of E4 chick embryo. cALDH6 is expressed in the isthmus (C), dorsal side of the otic vesicle (otv) (D), posterior side of nasal pit (np) (E), and Rathke’s pouch (F). (G) A transverse section of trunk
region around hind limb (hl) of E4 chick embryo; (H) is a higher magnification of (G). cALDH6 is expressed in the mesenchymal region of the limb bud and in glial cells and interneurons (indicated by arrowheads) in the posterior trunk. fb, forebrain; hb, hindbrain; mb, midbrain; nc, notochord.

**Fig. 7** *Mouse ALDH6 expression in sensory neuroepithelia.*

(A) Whole mount E10.5 mouse embryo: mALDH6 expression in ventral and dorsal retina, olfactory placode and dorsal margin of otic vesicle (arrow). Transverse sections of mouse head at (B) E9.5, (C) E10.5 and (D) E11.5: mALDH6 is expressed in head ectoderm overlying the optic vesicle, the dorsal and ventral margins of the neural retina and throughout the developing olfactory neuroepithelium. Serial sections of E12.5 eye hybridised for (E) ALDH1 (AHD-2) and (F) mALDH6: expression has become restricted to the dorsal and ventral neural retina respectively. (G) E15.5 transverse section of eye: mALDH6 continues to be expressed in the ventral neural retina but is weaker. (H) E15.5 transverse section of olfactory structures: mALDH6 is expressed in a punctate expression pattern in the neuroepithelium and underlying stroma. Orientation: dorsal (d) is top, ventral (v) is bottom and medial (m) is to the left, except as indicated in panel (H).

**Fig. 8** *Retinaldehyde dehydrogenase activity of recombinant chick ALDH6 in bacterial lysates.*

Bacterial cultures of pBAD or pBAD-cALDH6 were induced with 0.2 % arabinose, sonicated, centrifuged at 10000 x g for 10 mins and 1 µl supernatant aliquots assayed for retinaldehyde dehydrogenase activity as described in *Methods*. Panels are UV absorbance traces at 345 nm from HPLC runs of (A) pBAD vector control and (B) pBAD-cALDH6 samples. Retention times of authentic standards of *all-trans* RA and RAL were: RA - 5.4 mins, RAL - 7.1 mins.

**Fig. 9** *Kinetics of RA synthesis and inhibition by citral*

(A) RA synthesis from *all-trans* RAL by chick ALDH6. (B) Inhibition of RA synthesis by citral at 4 µM RAL. Activity was assayed using 0.1-0.3 µg recombinant protein at 37 °C, pH 8.0 as described in *Methods*. Data are representative of assays performed with 8-12 substrate data.
Aldehyde dehydrogenase 6

points and quantitated by peak integration of HPLC chromatograms. Run-to-run quantitation errors were determined to be less than ±3%.

**Fig. 10** Chick ALDH6 and retinol or serum mediate RA-dependent transactivation through the RAR pathway.

JEG-3 cells were transfected with 600 ng DNA/well of a mixture of MH100x4-Luc: GAL4, GAL4-RARα, -RXRα, -VDR, -ER, or -PPARγ: pCMX-βGal and pCAGGS or pCAGGS-cALDH6 plasmids (5:1:4:5) as indicated for 8 hrs, then washed twice with PBS and incubated for 24 hrs in serum free ITLB/DMEM with or without 10 nM ROL, 10 % FBS or the respective receptor specific agonist ligands *all-trans* RA (10 nM), LG1069 (10 nM), 1,25-OH-VitD₃ (10 nM), dexamethasone (10 nM) and BRL49653 (100 nM). GAL4 showed no significant response to 10 nM RA or any of the other specific ligands. Lysates were assayed for luciferase and β-galactosidase activity. Data is expressed as relative luciferase units of the means of triplicates ± S.E.M. from a representative experiment.

**Fig. 11** RA-dependent transactivation by chick aldehyde dehydrogenases.

JEG-3 cells were plated at 20-40 % confluency, transfected with 600 ng DNA/well RAREx2-tk-Luc: pCMX-βGal: pCAGGS vector or chick ALDH constructs (1:1:1) for 8 hrs, washed twice with PBS and incubated for 24 hrs in ITLB/DMEM medium with the indicated concentrations of retinoids. Dose-response curves are: open squares - pCAGGS vector with retinol (ROL), closed squares - pCAGGS vector with *all-trans* RA, closed circles - ALDH1 with ROL, open circles - RALDH2 with ROL, closed triangles - ALDH6 with ROL. Dose-response curves for RA with ALDHs are omitted for clarity but were essentially the same as the response observed with pCAGGS vector. Data points represent the means of triplicates; the S.E.M. was less than 15 % for all points.
Aldehyde dehydrogenase 6

Table 1 *Sequence Pair Distances of Human, Mouse and Chick ALDHs*

| Percent Divergence | ALDH | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|-------------------|------|---|---|---|---|---|---|---|---|---|
| h. ALDH6          | 1    | **93.6** | **86.0** | 69.7 | 67.9 | 69.4 | 69.2 | 69.8 | 69.6 |
| m. ALDH6          | 2    | 6.2 | 85.0 | 68.9 | 66.7 | 68.4 | 68.8 | 67.8 | 69.2 |
| c. ALDH6          | 3    | 13.9 | 14.8 | 71.5 | 69.1 | 70.2 | 68.6 | 69.0 | 68.4 |
| h. RALDH2         | 4    | 29.7 | 30.5 | 27.9 | 86.9 | 83.1 | 72.1 | 71.9 | 70.5 |
| m. RALDH2         | 5    | 31.3 | 32.5 | 30.3 | 13.0 | 80.1 | 70.9 | 70.9 | 70.7 |
| c. RALDH2         | 6    | 30.0 | 31.0 | 29.2 | 16.6 | 19.6 | 73.5 | 73.1 | 72.5 |
| h. ALDH1          | 7    | 30.3 | 30.7 | 30.5 | 27.5 | 28.7 | 26.1 | 97.3 | 95.6 |
| m. ALDH1          | 8    | 29.7 | 31.6 | 30.3 | 27.7 | 28.7 | 26.5 | 2.5  | 94.2 |
| c. ALDH1          | 9    | 29.9 | 30.3 | 30.7 | 29.1 | 28.9 | 27.1 | 4.2  | 5.6  |
### Table 2 Kinetic parameters of Chick and Mouse ALDH6

| Enzyme | Substrate      | Km (µM)\(^a\) | Vmax (nmoles/mg/min)\(^c\) | H   | N   |
|--------|----------------|----------------|-----------------------------|-----|-----|
| Chick  | Retinal        | 0.26 ± 0.033   | 48.4 ± 0.4                  | 1.9 | 4   |
| Mouse  | Retinal        | 0.33 ± 0.040   | 58.1 ± 1.0                  | 1.8 | 3   |
| Chick  | Octanal\(^b\)  | 0.24 ± 0.046   | 163 ± 14                    | -   | 4   |
| Chick  | Benzaldehyde\(^b\) | 4.3 ± 0.54     | 23 ± 1.3                    | -   | 3   |
| Chick  | Acetaldehyde\(^a\) | 3200 ± 300     | ND                          | -   | 3   |
| Chick  | Citral\(^b\)   | 0.098 ± 0.018  | 4.2 ±0.72                   | -   | 4   |
| Chick  | NAD            | 0.23 ± 0.019   | -                           | -   | 4   |

\(^a\) Assays were performed at pH 8.0 and 37 °C. Data represent means ± S.E.M.

\(^b\) For alternate substrates, Ki equals Km. Values were determined from inhibition curves of RA synthesis at fixed RAL concentrations in the range of 2-5 µM according to the method in (58).

\(^c\) Vmax values for aldehyde substrates (except RAL) were determined from rates of NADH formation at substrate concentrations 15 x Km
Fig. 2

Key:
h, human
m, mouse
c, chick
Fig. 6

A  wing

B  hind limb

C  isthmus  mb  hb

D  otic vesicle  otv

E  olfactory vesicle  np

F  Rathke's pouch

G  trunk/hindlimbs  hl

H  interneuron  nt  nc
Fig. 8

A

RAL

B

RA
Fig. 9

(A) Graph showing the relationship between RAL (µM) and RA (nmoles/min/mg).

(B) Graph showing the relationship between Log[Citral] (µM) and RA (nmoles/min/mg).
Fig. 11

![Graph showing the relationship between Retinoid (nM) and R.L.U.](image-url)
ALDH6, a cytosolic retinaldehyde dehydrogenase prominently expressed in sensory neuroepithelia during development
Felix Grün, Yukihiro Hirose, Shimako Kawauchi, Toshihiko Ogura and Kazuhiko Umesono

J. Biol. Chem. published online September 29, 2000

Access the most updated version of this article at doi: 10.1074/jbc.M007376200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts