Recombinant Class I Aldehyde Dehydrogenases Specific for All-trans- or 9-cis-Retinal*

The molecular basis for the specificity of aldehyde dehydrogenases (ALDHs) for retinal, the precursor of the morphogen retinoic acid, is still poorly understood. We have expressed in Escherichia coli both retinal dehydrogenase (RALDH), a cytosolic aldehyde dehydrogenase originally isolated from rat kidney, and the highly homologous phenobarbital-induced aldehyde dehydrogenase (PB-ALDH). Oxidation of propionaldehyde was observed with both enzymes. On the other hand, recombinant RALDH efficiently catalyzed oxidation of 9-cis- and all-trans-retinal, whereas PB-ALDH was inactive with all-trans-retinal and poorly active with 9-cis-retinal. A striking difference between PB-ALDH and all other class I ALDHs is the identity of the amino acid immediately preceding the active nuleophile Cys<sup>301</sup> instead of Cys<sup>301</sup> (12). Nevertheless, these amino acids could be exchanged in either RALDH or PB-ALDH without affecting substrate specificity. Characterization of chimeric enzymes demonstrates that distinct groups of amino acids control the differential activity of RALDH and PB-ALDH with all-trans- and 9-cis-retinal. Of 52 divergent amino acids, the first 17 are crucial for activity with all-trans-retinal, whereas the next 25 are important for catalysis of 9-cis-retinal oxidation. Recombinant enzymes with specificity for all-trans- or 9-cis-retinal were obtained, which should provide useful tools to study the relative importance of local production of all-trans- versus 9-cis-retinoic acid in development and tissue differentiation.

Vitamin A (retinol) plays an important role in normal embryonic development and epithelial cell differentiation (1, 2). It exerts its effects by conversion to the biologically active all-trans and 9-cis-retinoic acids (3), which function as specific ligands for nuclear receptors (retinoic acid receptors and retinoid X receptors) (3, 4). All-trans-RA binds and activates retinoic acid receptors, whereas its 9-cis-isomer binds with high affinity to both retinoic acid receptors and retinoid X receptors (4, 5). The metabolic pathway for the synthesis of RA involves oxidation of retinal to retinoic acid, followed by oxidation of retinal to RA (6, 7). Numerous enzymes able to catalyze the oxidation of retinal to retinal and of retinal to RA in vitro have been identified and include members of the alcohol and aldehyde dehydrogenase families, respectively (7, 8).

Aldehyde dehydrogenases (ALDHs; EC 1.2.1.3; also called aldehyde: NAD(P) oxidoreductase) are a superfamily of NAD(P)-dependent enzymes that oxidize a variety of aldehydes to their corresponding acids. They are divided into three classes based on sequence similarity (9). Class I ALDHs (the ALDH1 family) are expressed in the cytosolic fraction of mammalian tissues. Several class I ALDHs purified or cloned from human, bovine, or murine tissues (RALDH<sup>1</sup>*, RALDH<sup>2</sup>*, and RALDH<sup>3</sup>*, also known as ALDH1A1**, ALDH1A2**, and ALDH1A3**) have been shown to catalyze the oxidation of all-trans-retinal to retinoic acid, whereas class II (ALDH2) and class III (ALDH3) enzymes have no such activity (10–17). These enzymes can also catalyze conversion of other aldehydes to the corresponding acids and are considered broad specificity enzymes. It is thus possible that all class I ALDHs may have a similar range of substrates but may not be redundant because of different patterns of tissue expression. On the other hand, some degree of specificity for natural aldehyde substrates may exist within this family, allowing each enzyme to play a defined physiological role. In particular, the degree and the molecular mechanisms of specificity for all-trans-retinal and its isomers within the class I enzymes are not well characterized.

RALDH (ALDH<sub>1A1**</sub>) was initially purified and cloned from rat kidney (18, 19), based on its capacity to catalyze the oxidation of all-trans- and 9-cis-retinal, but not 13-cis-retinal, to the corresponding acids with high efficiency (20). Furthermore, we have also shown that RALDH is highly expressed and developmentally regulated in the kidney (21) and in other tissues such as trachea, intestine, and stomach epithelia (22, 23), where its expression is regulated by vitamin A status. On the other hand, PB-ALDH (ALDH<sub>1A7**</sub>) is expressed mainly in rat liver after induction by phenobarbital (24). Like all members of the class I ALDH family, the two ALDHs share a high degree of amino acid identity (89.6%). However, results from our laboratory and others (25–27) indicate that PB-ALDH and RALDH have different substrate specificities because PB-ALDH does not catalyze all-trans-retinal conversion. Furthermore, we show that PB-ALDH is also poorly active with 9-cis-retinal.

We have used point mutants and chimeric enzymes to characterize...
amino acids important for activity with retinal isomers. We find that amino acids of RALDH that are necessary and sufficient for catalysis of all-trans-retinal differ from those that are required for catalysis of 9-cis-retinal. The molecular basis underlying the differential specificity of RALDH and PB-ALDH for retinal substrates is discussed.

**EXPERIMENTAL PROCEDURES**

**Reagents**—Restriction enzymes were purchased from New England Biolabs and Promega. Retinoids were obtained from Sigma. The PB-ALDH cDNA and the polyclonal antibodies raised against PB-ALDH were generous gifts from Dr. Ronald Lindahl (University of South Dakota) and Dr. James J. Lipsky (Mayo Clinic/Foundation, Rochester, MN).

**Bacterial Expression Vectors**—cDNAs coding for the open reading frame of RALDH and PB-ALDH were cloned into the Nde I and HindIII sites of the bacterial expression plasmid pT7-7 by PCR amplification. Primers corresponding to the N terminus and the C terminus of RALDH were designed according to the published cDNA sequences (19). The N-terminal primer contains a NdeI site, an initiation ATG codon, and nucleotides 1–21 of the RALDH open reading frame. The C-terminal primer contains a HindIII site and the reverse complement of nucleotides 1479–1506 of RALDH including the termination codon TAA. Because the nucleotide sequences of PB-ALDH N terminus and C terminus are identical to those of RALDH (28), the same primers were used for PCR amplification of PB-ALDH cDNA. Correct insertion of the RALDH and PB-ALDH cDNAs in pT7-7 was verified by DNA sequencing.

Mutations at position 301 were introduced by two rounds of PCR amplification using primers hybridizing with nucleotides 892–916 of RALDH or PB-ALDH but containing mutations converting Cys301 into Ile or Ile301 into Cys, respectively, and the N-terminal and C-terminal primers. The corresponding cDNAs were digested with NdeI and HindIII and cloned into the corresponding sites of pT7-7, yielding expres-

**FIG. 1.** RALDH, but not PB-ALDH or PB-ALDH301C, catalyzes retinal oxidation. A Western analysis of bacterially expressed RALDH and PB-ALDH. Extracts were prepared from BL21-DE3 bacteria transformed with pT7-7-RALDH (lane 1), pT7-7-PB-ALDH (lane 2), or pT7-7-PB-ALDH301C (lane 3). Crude protein extracts were separated by SDS-PAGE (8% polyacrylamide gel) and subjected to immunoblotting with an antibody directed against PB-ALDH. Extracts were then adjusted for enzyme expression levels and for the amount of total protein using an extract from bacteria transformed with the parental expression vector pT7-7 and submitted again to Western blotting to monitor for uniform expression levels. B–D, enzymatic reactions were performed with 10 μM all-trans-retinal (B), 10 μM 9-cis-retinal (C), or 100 μM propanal (D), respectively, using extracts standardized for expression of RALDH (●), PB-ALDH (▲), or PB-ALDH301C (◇).

**TABLE I**

Kinetic constants for RALDH and chimeric enzymes active with retinal substrates

Kinetic parameters were calculated using Prism software from saturation experiments performed twice in triplicate as described in Figs. 4 and 5. Variation between experiments was less than 10% for all Km and Vmax values except for the Vmax values of RALDH and RALDH-343 for all-trans-retinal, which were ±20%. Units of velocity are expressed per mg of crude protein extracts standardized for enzyme expression levels.

|           | Km (μM) | Vmax (nmol/min/mg) | Vmax/Km | 9-cis |
|-----------|---------|--------------------|---------|-------|
|           | All-trans | 9-cis  | All-trans | 9-cis  | All-trans | 9-cis |
| RALDH     | 10       | 8.7    | 3.2      | 6.3    | 0.32      | 0.72  |
| RALDH-343 | 4.9      | 3.9    | 2.4      | 2.8    | 0.48      | 0.72  |
| RALDH-131 | 3.0      | ND     | 1.5      | 8.5    | ND        | ND    |
| PB-131    | ND       | 29     | ND       | ND     | ND        | ND    |

*ND, not determined.
sion vectors for RALDH301I and PB-ALDH301C. The presence of the mutations was confirmed by sequencing. Two clones corresponding to independent PCR amplification reactions were characterized for each mutant with similar results.

Chimeras RALDH-131, PB-131, RALDH-343, and PB-343 were constructed by exchange of cDNA fragments between the RALDH and PB-ALDH cDNAs using ScaI or ApaI sites (nucleotides 392–397 and 1026–1031, respectively) and the NdeI and HindIII cloning sites. Note that fragments of PB-ALDH flanked by ApaI sites were produced by PCR amplification, whereas the endogenous ApaI site in RALDH was used. Chimeras RALDH-131 and RALDH-343 contain the RALDH cDNA to the ScaI or ApaI site, respectively, followed by the complementary sequences of the PB-ALDH cDNA. Conversely, chimeras PB-131 and PB-343 contain the PB-ALDH cDNA to the ScaI or ApaI site, respectively, ligated to the complementary fragments of the RALDH cDNA.

**Bacterial Expression of RALDH and PB-ALDH**—Parental and recombinant plasmids were transformed into *Escherichia coli* BL21-DE3 cells, which express the T7 polymerase under the control of a lac promoter. Expression was induced with 0.5 mM isopropyl-β-D-thiogalactopyranoside in exponentially growing bacteria and continued for 3 h. Bacterial pellets were sonicated in 2 mM Tris-HCl, pH 7.2, containing 1 mM EDTA, 100 mM NaCl, and 0.5% Tween 20, and protein extracts were collected after elimination of debris by centrifugation. Expression of RALDH and PB-ALDH was monitored by Western blot using rabbit polyclonal antibodies raised against PB-ALDH that cross-react with RALDH as described previously (22, 23).

**Enzyme Assays**—At least two clones originating from independent PCR reactions and coding for RALDH, PB-ALDH, or mutants thereof were expressed in bacteria and characterized in enzyme assays. Similar results were obtained in all cases using duplicate constructs, indicating that activity of the recombinant enzymes was not affected by potential mutations introduced during PCR amplification. Enzymatic activity was assayed in a final volume of 250 μl containing 0.02% Tween 80, 1.61 mM dithiothreitol, 603 μM NAD, and crude protein extracts containing RALDH, PB-ALDH, or mutant enzymes. In all experiments, extracts were standardized for enzyme expression levels, as assessed by Western blotting, and for total protein concentration. Varying concentrations of substrate (all-trans, 9-cis, or propanal) were added in 2.5 μl of dimethyl sulfoxide. After incubation with retinal at 25 °C for 60 min, the reaction was terminated by cooling at 4 °C in ice-cold water. Retinoic acid was quantified by high performance liquid chromatography (29). All-trans-retinoic acid production was undetectable using protein extracts from bacteria carrying the parental pT7-7 plasmid. Low levels of 9-cis-retinoic acid production were observed with extracts from bacteria carrying the parental pT7-7 plasmid and subtracted from the values obtained with extracts expressing RALDH or PB-ALDH. Controls performed without protein extract or without substrate indicated that retinoic acid production and NADH formation required both enzyme and substrate. When propanal was the substrate, enzyme activity was quantified by measuring the NADH formed 10 min after initiating the reaction by absorption at 340 nm (Beckman DU-40 spectrophotometer). Kinetic data for all substrates were obtained by performing saturation curves under conditions in which enzyme activity was linear with respect to protein concentration and reaction time, and by fitting the data from the saturation curves to Michaelis-Menten and

**FIG. 2. Comparison of the amino acid sequences of RALDH and PB-ALDH.** The amino acid sequence of RALDH is shown, and amino acids that differ in PB-ALDH are indicated. The position of the main structural elements in the substrate entrance tunnel as identified in sheep ALDH1 (33) is indicated above the corresponding amino acid regions (highlighted in bold). The nucleotide binding domain of ALDH1 comprises amino acids 8–135 and 159–270, the catalytic domain comprises residues 271–470, and the oligomerization domain comprises residues 140–158 and 486–495 (Ref. 33; numbering is identical for both ALDH1 and RALDH). Amino acid 301, which was mutagenized in PB-ALDH and RALDH, is indicated.

| RALDH | MBSPAQPAVP | APLNLKIQH | TKIPINNSWH | DSVGKRFPPV |
|-------|------------|-----------|------------|------------|
| PB-ALDH | ----------- | ----------- | ------------ | ----------- |
| RALDH | LNPA7E7VIC | HVEEGKADV | DKAVALARQ | PQIGSPKRTM |
| PB-ALDH | I-    | -        | -         | -         |
| RALDH | DASEGRGNN | KLADLMDKR | LLLATIEIN | GKVNPANAYL |
| PB-ALDH | A-   | -      | -         | -         |
| 135
| Helix αC | ▼
| RALDH | SDCGSGSIAL | KYCAGWADKI | HGGQTIPSDD | IFTFTRREPI |
| PB-ALDH | L-TEV- | -F-        | -          | -         |
| 170
| Helix αD | ▼
| RALDH | GVCQGIDIPS | FPLLFIWIKI | GPALSQGNTV | VVVAQYQPTPL |
| PB-ALDH | -N-    | -G-IL-     | -A-        | -I-        |
| RALDH | TALHMSLIM | EAGFPFGVNO | IVPQYQPTAG | AAISHMMDVD |
| PB-ALDH | -Y-    | -Y-        | -V-        | -S-        |
| RALDH | KVATPSTGQV | GGLIAKRAK | SNLKVYTLNL | GKSPECIVFA |
| PB-ALDH | -S-    | -S-        | -E-        | -         |
| 282
| Helix αH | ▼
| RALDH | DADLDDAVBF | AHGTVFYHGQ | QCQAARASRF | VEBSVYDSFV |
| PB-ALDH | -S-    | -Q-        | -F-        | -I-        |
| RALDH | RKSVERAKKY | VLNPLTQGI | NQGFQIDKEQ | HQLLEILIES |
| PB-ALDH | -R-    | -R-        | -B-        | -S-        |
| RALDH | KKEFGAKLEC | GGRWGNKGF | FQDPTVFSNV | TDMRIAKER |
| PB-ALDH | -    | -          | -         | -         |
| RALDH | IPFGVQQIMK | FKSIDDIKVR | ANNTTYGLAA | GVPTKLDRA |
| PB-ALDH | -      | -          | -E-        | -P-        |
| 455
| Loop | ▼
| RALDH | ITVSDDLQAG | VVWVNCYML | SAQCFPGSFK | MSGNNGELGE |
| PB-ALDH | -      | -          | -T-        | -          |
| RALDH | HGLYETFELK | TVANKISQKN | S           |
| PB-ALDH | Q-V- | -         | -         | -         |
RESULTS

Recombinant RALDH, but Not PB-ALDH, Catalyzes Efficient Conversion of Retinoids—To investigate the mechanisms of specificity for retinal substrates within class I ALDHs, we expressed two members of this family, rat RALDH and PB-ALDH, in E. coli with the aim of comparing their enzymatic properties with retinal isomers. To ascertain that expression levels of the two enzymes were similar, immunoblots were performed with crude extracts from BL21-DE3 bacteria transformed with expression vectors for RALDH (Fig. 1A, lane 1) or PB-ALDH (Fig. 1A, lane 2) or with the parental pT7-7 expression vector (data not shown), using an antibody raised against PB-ALDH. No bands were detected in extracts from control bacteria transformed with pT7-7 (data not shown), whereas a single band with a molecular mass of 55 kDa could be detected in both RALDH- and PB-ALDH-expressing extracts (Fig. 1A, lanes 1 and 2). The molecular mass of the expressed enzymes is identical to the one reported for both purified enzymes (18, 24).

In addition, the antibody recognized both enzymes equally well because the relative intensities of the bands were comparable after [35S]methionine incorporation.

Purified RALDH was previously shown to catalyze the conversion of all-trans- and 9-cis-retinal, but not 13-cis-retinal, to their respective retinoic acids (20). We therefore sought to determine whether recombinant RALDH retained these enzymatic properties. Increasing concentrations of bacterial extracts containing similar levels of recombinant RALDH or PB-ALDH were assayed with all-trans- or 9-cis-retinal at 25 °C. All-trans-retinoic acid production increased with RALDH concentration, but no significant levels of retinoic acid production were detected with PB-ALDH (Fig. 1B). In addition, RALDH also catalyzed oxidation of 9-cis-retinal (Fig. 1C). On the other hand, high amounts of PB-ALDH were necessary to observe production of low levels of 9-cis-retinoic acid, suggesting that this enzyme possesses low activity for 9-cis- but not all-trans-retinal.

To test whether the kinetic properties of the recombinant RALDH for retinal substrates are similar to those of the purified enzyme from rat kidney, we assessed the saturation kinetics of RALDH with all-trans- and 9-cis-retinal (Fig. 4; Table I). The $K_m$ values obtained (8.7 and 10.9 μM for 9-cis- and all-trans-retinal; see also Ref. 20). In addition, a higher catalytic efficiency was obtained with 9-cis- versus all-trans-retinal (2.3-fold ratio), whereas 13-cis
distinct regions of RALDH control specificity for all-trans- and 9-cis-retinal substrates—To further delineate regions of RALDH that are important for catalysis of all-trans- and 9-cis-retinal, we analyzed the activity of the RALDH-131 and PB-131 chimeras with these substrates. As observed with PB-343, PB-131 was inactive with all-trans-retinal but active with propanal (Fig. 3B). Note that conversion of propanal could be observed with PB-343, demonstrating that this chimera is not catalytically inactive with all substrates (Fig. 3C). Kinetic parameters measured for the RALDH-343 chimera with all-trans- and 9-cis-retinal (Fig. 4B) indicate that catalytic efficiency is slightly increased for all-trans-retinal, whereas that for 9-cis-retinal is unchanged compared with RALDH (Table I). Together, these results suggest that the 10 amino acids that differ between RALDH and PB-ALDH from residues 131 to 344 (see Fig. 3B), the replacement of Cys301 to Ile in RALDH did not affect the capacity of the enzyme to catalyze all-trans-retinal oxidation (data not shown), suggesting that the identity of the amino acid at position 301 (Cys or Ile) does not influence enzymatic activity with all-trans-retinal.

Distinct Regions of RALDH Control Specificity for All-trans- and 9-cis-Retinal Substrates—To further delineate regions of RALDH that are important for catalysis of all-trans- and 9-cis-retinal, we analyzed the activity of the RALDH-131 and PB-131 chimeras with these substrates. As observed with PB-343, PB-131 was inactive with all-trans-retinal but active with propanal (Fig. 3B), suggesting that amino acids 1–131 of RALDH are necessary for catalysis of all-trans-retinal. Note that PB-131, like RALDH and RALDH-343, was more active than PB-ALDH with propanal (Fig. 3C). This suggests that the region from 131 to 343 of RALDH contains amino acids that are
responsible for increased activity with propanal. Furthermore, chimera RALDH-131 was active with all-trans-retinal (Fig. 3B), with a $V_{\text{max}}/K_m$ comparable to that of RALDH (Fig. 5C; Table I), suggesting that these amino acids are sufficient to confer activity with all-trans-retinal.

Surprisingly, however, chimera RALDH-131 had very little activity for 9-cis-retinal (Fig. 5A). We therefore investigated whether the reciprocal chimera had activity with this substrate. Indeed, although PB-131 was inactive with all-trans-retinal, it was active with 9-cis-retinal (Fig. 5, B and D), with a modest decrease in catalytic efficiency (about 2.5-fold) compared with the parental enzyme (Table I). These results, together with the observation that PB-343 is not active with 9-cis-retinal (data not shown), suggest that amino acids 131–343 of RALDH are important for catalysis of 9-cis-retinal.

Thus, amino acids of RALDH that can confer activity to PB-ALDH with all-trans- and 9-cis-retinal are different, allowing the generation of enzymes selective for each retinal isomer (Fig. 5; Table I).

**DISCUSSION**

We have previously demonstrated the existence of a class I aldehyde dehydrogenase that oxidizes retinal in rat kidney and purified this activity to homogeneity (18). Results described here demonstrate that the characteristics observed with the enzyme purified from rat kidney are retained by the recombinant RALDH enzyme. The good correlation in $K_m$ values and the absence of activity with 13-cis retinal and the higher efficiency of 9-cis- versus all-trans-retinal conversion provide strong evidence that RALDH encodes the purified kidney enzyme (20). Our results also demonstrate that posttranslational modifications specific to eukaryotes are not needed for RALDH enzymatic activity, a result consistent with previous observations made with bacterially expressed ALDHs (27, 35, 36).

Contrary to what was observed with RALDH and to the high activity for retinal substrates reported for other class I ALDHs (10–17), PB-ALDH was inactive for all-trans-retinal oxidation, although its activity for propanal was in good agreement with previously published data (24). This is in keeping with the previously reported lack of all-trans-RA synthesis in vivo in a Xenopus embryo assay with mouse PB-ALDH (26) and with results obtained with bacterially expressed PB-ALDH (27). In addition, we report here that PB-ALDH has only very low activity with 9-cis-retinal. Therefore, these two class I rat aldehyde dehydrogenases have differential activities with retinal substrates despite a high amino acid sequence identity (89.6%).

Amino acids involved in binding of the NAD$^+$ cofactor are nearly all conserved between RALDH and PB-ALDH, and the active site nucleophile Cys$^{302}$ and general base Glu$^{268}$ (30) are also conserved in the two enzymes. Interestingly, however, the amino acid immediately upstream of Cys$^{302}$ is Ile in rat and mouse PB-ALDH, instead of the Cys seen in RALDH and all other class I ALDHs. In addition, the side chain of Cys$^{301}$ is in a strikingly different position in the structures of ALDH1 and ALDH2 enzymes, possibly resulting in altered accessibility of...
helix
tural elements that are part of both the nucleotide binding
t-ALDH also differ in regions corresponding to the ALDH1
cences in amino acid identity in the substrate entrance tunnel as
differences in the structure of side chains of conserved
ences in amino acid identity in the substrate entrance tunnel as
various substrates to other members of this family. We recently
ary acid identity in the substrate entrance tunnel as
25 divergent amino acids between positions 131 and 343 are
nactivities among 9-, all-, and 13-retinoid isomers, and of the
pecific roles played by each isomer of retinoic acid in develop-
Specificity of Class I ALDHs for Retinal Isomers

Some of the amino acids that may impart substrate speci-
city in the aldehyde dehydrogenase family are located in the
C-terminal loop of the substrate entrance tunnel (31–34). How-
ever, characterization of chimeras in which PB-ALDH and
RALDH sequences have been exchanged at position 343 dem-
strates that the 10 amino acids that differ between the two
enzymes in this region, including three substitutions in the
C-terminal loop, are not crucial for activity with retinal sub-
strates. On the other hand, exchange of the N-terminal 131
amino acids demonstrates that structural determinants found
in this region are both necessary and sufficient to confer to
PB-ALDH the capacity to convert all-trans-retinal. This region
includes 7 of the 8 amino acids that differ between the two
enzymes in helix αC. Interestingly, several of the changes in
helix αC involve replacement of large amino acids in PB-ALDH
with smaller ones in RALDH. It is thus possible that steric
hindrance in this region, which corresponds to the left wall of
the substrate entrance tunnel, may explain the lack of reac-
tivity of PB-ALDH with all-trans-retinal.

Surprisingly, the N-terminal region of RALDH, although suf-
icient for activity with all-trans-retinal when linked to the
complementary regions of PB-ALDH, did not confer activity with
9-cis-retinal. On the other hand, chimera PB-131, but not PB-
343, was active with 9-cis-retinal. These results suggest that the
25 divergent amino acids between positions 131 and 343 are
 crucial for activity with 9-cis-retinal, but not all-trans-retinal.
Because these amino acids include residues in helices αD and αH,
differential binding of 9-cis-retinal between RALDH and PB-
ALDH may also explain their different substrate specificity.

It will be of interest in future studies to extend our direct
comparison of the enzymatic properties of class I ALDHs with
retinal substrates to other members of this family. We recently
observed that purified human ALDH1 (ALDH1A1**5) catalyzes
conversion of all three retinal isomers with comparable effi-
ciences (37), whereas RALDH does not catalyze 13-cis-retinal
conversion and is more active with 9-cis-retinal versus all-
trans-retinal. Thus, our results suggest that evolution within
the class I ALDHs has led not only to different patterns of
spatio-temporal expression but also to differences in substrate
specificity and, possibly, differences in their roles in the control
of local concentrations of retinoic acid isomers. Generation of
transgenic mice expressing different class I ALDHs under the
control of the same promoter should clarify the specific func-
tions of each enzyme in different tissues. In addition, the avail-
ability of isomer-specific enzymes as described here should
prove useful in the study of the mechanisms of interconversion
among 9-cis-, all-trans-, and 13-cis-retinoid isomers, and of the
specific roles played by each isomer of retinoic acid in develop-
ment and differentiation in vivo.

Acknowledgments—We are grateful to Drs. Ronald Lindahl and
James J. Lipicky for the generous gift of polyclonal antibodies directed
against PB-ALDH. We also thank Sharmeen Naurast for excellent tech-
nical assistance.

REFERENCES
1. Smith, S. M., Pang, K., Sundin, O., Wedden, S. E., Thaller, C., and Eichele, G.
(1989) Development 107, suppl. 121–131
2. Roberts, A. B., and Sporn, M. B. (1984) in The Retinoids (Sporn, A. B., Roberts,
M. B., and Goodman, D. S., eds), pp. 209–286, Academic Press, Orlando, FL
3. Delarue, L. M. (1991) FASEB J. 5, 2635–2643
4. Chambon, P. (1996) FASEB J. 10, 940–954
5. Mangesdorff, D. J., Umeesono, K., and Evans, R. M. (1994) in The Retinoids:
Biochemistry and Medicine (Sporn, A. B., Roberts, M. B., and Goodman,
D. S., eds), pp. 319–349, Raven Press, New York
6. Bhat, P. V., Poissant, L., Falardeau, P., and Lacroix, A. (1988) Biochim. Cell
Biochemistry 66, 735–740
7. Duester, G. (1996) Biochemistry 35, 12221–12227
8. Blaner, W. S., and Olson, J. A. (1994) in The Retinoids: Biology, Chemistry and
Medicine (Sporn, A. B., Roberts, M. B., and Goodman, D. S., eds), pp.
229–256, Raven Press, New York
9. Lindahl, R. (1992) Crit. Rev. Biochem. Mol. Biol. 27, 283–335
10. Lee, M.-O., Manthey, C. L., and Sladek, N. E. (1991) Biochem. Pharmacol.
42, 1279–1285
11. Yoshida, A., Hsu, L. C., and Dave, V. (1992) Enzyme (Basel) 46, 239–244
12. El-Akawi, Z., and Napoli, J. L. (1994) Biochemistry 33, 1938–1943
13. Saari, J. C., Champer, R. J., Asson-Batres, M. A., Garwin, G. G., Huang, J.,
Crahil, J. W., and Milian, A. H. (1995) Visual Neurosci. 12, 263–272
14. Bhat, P. V., Poissant, L., and Wang, L. (1996) Biochemistry 74, 695–700
15. Wang, X., Penzes, P., and Napoli, J. L. (1996) J. Biol. Chem. 271, 16288–16293
16. Zhao, D., McCaffrey, P., Ivens, K. J., Nave, R. L., Hogan, P., Chin, W. W., and
Drager, U. C. (1996) Eur. J. Biochemistry 230, 15–22
17. Li, H., Wagner, E., McCaffrey, P., Smith, D., Andreadis, A., and Drager, U. C.
(2000) Mech. Dev. 95, 263–269
18. Labrecque, J., Bhat, P. V., and Lacroix, A. (1993) Biochim. Biophys. Acta 71, 85–89
19. Bhat, P. V., Labrecque, J., Boutin, J.-M., Lacroix, A., and Yoshida, A. (1995)
Gene (Amst.) 166, 303–306
20. Labrecque, J., Dumas, P., Lacroix, A., and Bhat, P. V. (1995) Biochem. J. 305, 681–684
21. Bhat, P. V., Marcinkevicius, M., Li, Y., and Mader, S. (1998) J. Histochem.
Cytochem. 46, 1025–1032
22. Bhat, P. V., Bader, T., Nettesheim, P., and Jetten, A. M. (1998) Biochim. Cell
Biochemistry 76, 59–62
23. Bhat, P. V. (1998) FERB Lett. 426, 260–262
24. Labrecque, J., and Evers, S. (1984) J. Biol. Chem. 259, 11991–11996
25. Bhat, P. V., Chow Lan, N., Guimond, J., and Mader, S. (1998) FASEB J. 12,
A4790
26. Ha, L. C., Chang, W.-C., Hoffman, I., and Duester, G. (1999) Biochem. J. 338, 387–385
27. Kathmann, E. C., Naylor, S., and Lipsky, J. J. (2000) Biochemistry 39, 1170–1176
28. Dunn, T. J., Kuleske, A. J., Lindahl, R., and Pitot, H. C. (1989) J. Biol. Chem.
264, 13057–13065
29. Bhat, P. V., Poissant, L., and Lacroix, A. (1988) Biochim. Biophys. Acta 967,
211–217
30. Perozich, J., Nicholas, H., Wang, B.-C., Lindahl, R., and Hempel, J. (1999) Protein
Sci. 8, 137–146
31. Liu, Z.-J., Sun, Y.-J., Rose, J., Chung, Y.-J., Hsiao, C.-D., Chang, W.-R., Kuo,
J., Perozich, J., Lindahl, R., Hempel, J., and Wang, B.-C. (1997) Nat. Struct.
Biochemistry 4, 317–326
32. Steinmetz, C. G., Xie, P., Weiner, H., and Hurley, T. D. (1997) Structure 5,
701–711
33. Moore, S. A., Baker, H. M., Blythe, T. J., Kitson, K. E., Kitson, T. M., and
Baker, E. N. (1998) Structure 6, 1541–1551
34. Lamb, A. L., and Newcomer, M. E. (1999) Biochemistry 38, 6003–6011
35. Hsu, L. C., Chang, W.-C., Shibuya, A., and Yoshida, A. (1992) J. Biol. Chem.
267, 3030–3037
36. Penzes, P., Wang, X., and Napoli, J. L. (1997) Biochim. Biophys. Acta 1342,
175–181
37. Bhat, P. V., and Samaha, H. (1998) Biochem. Pharmacol. 57, 195–197
Recombinant Class I Aldehyde Dehydrogenases Specific for All-trans- or 9-cis-Retinal
Veronique Montplaisir, Nathaly Chow Lan, Julie Guimond, Céline Savineau, Pangala V. Bhat and Sylvie Mader

J. Biol. Chem. 2002, 277:17486-17492.
doi: 10.1074/jbc.M112445200 originally published online March 6, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M112445200

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

This article cites 34 references, 7 of which can be accessed free at http://www.jbc.org/content/277/20/17486.full.html#ref-list-1