Biochemistry

 Volume 35, Number 38 September 24, 1996

*New Concepts in Biochemistry*

# Involvement of Alcohol Dehydrogenase, Short-Chain Dehydrogenase/Reductase, Aldehyde Dehydrogenase, and Cytochrome P450 in the Control of Retinoid Signaling by Activation of Retinoic Acid Synthesis†

Gregg Duester*

*The Burnham Institute, 10901 North Torrey Pines Road, La Jolla, California 92037*

*Received May 17, 1996*⊗

ABSTRACT: The effects of vitamin A (retinol) on growth and development are mediated by the active metabolite retinoic acid which controls a nuclear receptor signaling pathway. While elegant work on the retinoic acid receptor family has focused attention upon how the receptor controls this pathway, there now exists a relatively large gap in our understanding of how retinol is activated to form the ligand. During vertebrate embryogenesis and in adult organs retinoic acid is detected in a distinct spatiotemporal pattern, suggesting that it is produced from retinol in a regulated fashion. Enzymes involved in retinol and retinal metabolism are likely candidates for regulators of tissue retinoic acid levels. Members of the alcohol dehydrogenase and short-chain dehydrogenase/reductase enzyme families catalyze the reversible interconversion of retinol and retinal, the rate-limiting step, whereas members of the aldehyde dehydrogenase and cytochrome P450 enzyme families catalyze the irreversible oxidation of retinal to retinoic acid. The identification of enzymes likely to catalyze retinol oxidation *in vivo* has been particularly controversial, and this is made even more difficult by the reversible nature of this reaction. Taking into account enzymatic properties and coenzyme preferences, a case can be made that class IV alcohol dehydrogenase catalyzes retinol oxidation to provide retinal for retinoic acid synthesis, whereas microsomal retinol dehydrogenase (a short-chain dehydrogenase/reductase) catalyzes the reduction of retinal to retinol to promote retinoid storage. Further studies on these enzyme families will allow this layer of control in the retinoid signaling pathway to be understood.

It is clear from recent studies that vitamin A (retinol) regulates growth, development, and epithelial maintenance by conversion to an active form, retinoic acid (RA),[1] which functions as a ligand controlling a nuclear receptor signaling pathway [reviewed by Kastner *et al.* (1994a)]. Two families of retinoid receptors have been identified, i.e., the retinoic acid receptor (RAR) and the retinoid X receptor (RXR) families. In most instances it appears that the active receptor is an RAR/RXR heterodimer which binds DNA regulatory sequences and regulates gene transcription in response to ligand binding [reviewed by Mangelsdorf and Evans, (1995)]. RAR binds *all-trans*-RA and the closely related isomer 9-*cis*-RA, whereas RXR binds only 9-*cis*-RA, suggesting that it

† This work was supported by National Institutes of Health Grants AA07261 and AA09731.

* To whom correspondence and reprint requests should be addressed. Phone: (619) 646−3138. FAX: (619) 646−3195. E-mail: greggd@ljcrf.edu.

⊗ Abstract published in *Advance ACS Abstracts,* September 15, 1996.

[1] Abbreviations: ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; CRBP, cellular retinol-binding protein; CRABP, cellular retinoic acid-binding protein; P450, cytochrome P450; RA, retinoic acid; RAR, retinoic acid receptor; RBP, serum retinol-binding protein; RXR, retinoid X receptor; SDR, short-chain dehydrogenase/reductase.

S0006-2960(96)01176-2 CCC: $12.00 

may play a different role in retinoid signaling than RAR. Generation of mice carrying mutations in RARs or RXRs have provided proof that these receptors indeed control retinoid signaling since the defects observed mimic essentially all those previously seen during vitamin A deficiency (Lohnes *et al.*, 1994; Mendelsohn *et al.*, 1994; Sucov *et al.*, 1994; Kastner *et al.*, 1994b; Luo *et al.*, 1996).

In contrast to this great explosion of information regarding retinoid receptors, our understanding of how retinol is physiologically activated to form the ligand for this signaling pathway is much more limited. To unravel this next layer of biological control in the retinoid signaling pathway, a firm understanding of the enzymes involved in production of RA is necessary. We know that retinol is transported via the serum throughout the body at relatively high levels where it is available to essentially all cells for potential conversion to RA [reviewed by Soprano and Blaner (1994)]. Studies now underway are beginning to reveal a complex network of enzymes involved in the metabolism of retinol to RA. Discussed below is evidence that RA is produced in a unique spatiotemporal pattern as well as evidence for the existence of several distinct families of metabolic enzymes which may participate in the conversion of retinol to RA.

## RETINOIC ACID IS PRESENT IN A SPATIOTEMPORAL PATTERN

Studies performed on vertebrate embryos have revealed that endogenous RA is detectable in a distinct spatiotemporal pattern, suggesting that the conversion of retinol to RA is regulated. Studies on mouse embryos using a RA bioassay have shown that RA is undetectable at E6.5 during the egg cylinder stage but that it is first detectable at E7.5 during the primitive streak stage localized in embryonic, but not extraembryonic, tissues (Ang *et al.*, 1996a). RA in E8.5 and E9.5 mouse embryos is preferentially localized in posterior tissues (trunk) as well as craniofacial tissues and the retina, with much less RA detected in the brain (Ang *et al.*, 1996a; McCaffery *et al.*, 1993). Similar results have been obtained using a transgenic mouse strain, in which expression of a *lacZ* transgene linked to a RA response element is initially detected at E7.5 limited to posterior tissues, and then by E8.5 in trunk tissues posterior to the first somite as well as in the craniofacial tissues and retina, but not the brain (Rossant *et al.*, 1991). Also, a posterior preference for RA synthesis has been demonstrated in cultured tissues of E7.75 mouse embryos (Hogan *et al.*, 1992). Later in mouse embryonic development RA is found at high levels in the spinal cord (McCaffery & Dräger, 1994), and by E16.5 RA is also abundant in the adrenal gland, kidney, and intestine but at much lower levels in the liver, heart, and brain (Ang *et al.*, 1996b). HPLC analysis of E10.5−13.5 mouse embryonic tissues has indicated that RA is present at significantly higher levels in spinal cord, hindbrain, somites, and limb buds than in the forebrain and midbrain (Horton & Maden, 1995).

RA measurements performed on adult tissues have also revealed tissue-specific differences. HPLC measurements of several rat tissues have shown that *all-trans*-RA is present in the range of 1.8−29 pmol/g (Kurlandsky *et al.*, 1995). The amount of tissue RA derived from uptake via the serum as opposed to *de novo* synthesis was measured, and it was found that certain tissues such as brain and liver obtain most of their RA via plasma uptake, but that other tissues such as the testis, epididymis, and pancreas synthesize most of their RA *in situ* (Kurlandsky *et al.*, 1995). Measurements using a bioassay have shown that RA is easily detectable in several adult mouse tissues including the intestine, liver, adrenal gland, lung, testis, epididymis, and uterus (all lying in the range 6−138 pmol/g) but below the limit of detection (1 pmol/g) in other tissues including heart, brain, and spleen (Ang *et al.*, 1996b). Thus, the retinoid signaling pathway is likely to involve a tightly controlled mechanism for regulating endogenous RA levels in specific cell types.

## ENZYMES CATALYZING RETINOL AND RETINAL METABOLISM

The pathway for conversion of retinol to RA involves first the oxidation of retinol to retinal, then the oxidation of retinal to retinoic acid. Thus, in order to fully understand RA synthesis both reactions must be considered. Over the years numerous enzymes involved in retinol and retinal metabolism have been identified, purified, and characterized. These enzymes are members of four distinct families; i.e., alcohol dehydrogenase (ADH), short-chain dehydrogenase/reductase (SDR), aldehyde dehydrogenase (ALDH), and cytochrome P450 (P450). A mechanism for RA synthesis and retinoid storage involving these enzyme families is proposed (Figure 1). Each family is composed of several evolutionarily-related forms, some of which can utilize retinoids as substrates. Since each of these enzyme families has an ancient origin (Jörnvall *et al.*, 1995; Hempel *et al.*, 1993; Nelson *et al.*, 1993; Persson *et al.*, 1995), it is likely that divergence of ancient forms involved in the metabolism of other compounds has given rise to more recent forms which participate in retinoid metabolism needed for retinoid signaling, a relatively recent signaling pathway known to exist only in vertebrate animals (Kastner *et al.*, 1994a) and possibly other chordates (Kawamura *et al.*, 1993). A summary of the presently known retinol and retinal metabolizing enzymes grouped into their respective families is shown (Table 1).

*Alcohol Dehydrogenase (ADH).* Early studies indicated that liver ADH (now known as class I ADH) catalyzes the oxidation of retinol to retinal in the presence of NAD (Bliss, 1951; Zachman & Olson, 1961). This enzyme is also able to oxidize ethanol to acetaldehyde, and can in fact utilize many alcohols as a substrate (Vallee & Bazzone, 1983). In mammals, only class I ADH has evolved to efficiently oxidize ethanol, suggesting that the additional ADH classes have evolved to oxidize other alcohols. Since alcohol dehydrogenation is a reversible reaction, ADHs can also catalyze the reduction of aldehydes such as retinal when assayed with NADH *in vitro*. ADH is a cytosolic dimeric zinc-dependent enzyme with a subunit molecular weight of 40 kDa (Vallee & Bazzone, 1983). More recent studies have shown that mammalian ADH is a complex enzyme family composed of seven evolutionarily-related classes, each with unique properties and sites of gene expression (Jörnvall *et al.*, 1995; Duester *et al.*, 1995; Kedishvili *et al.*, in press). Amino acid sequence comparisons between the classes have revealed interclass sequence identities in the 57−69% range, indicating divergence from a common progenitor about 450 million years ago during early vertebrate evolution (Cederlund *et al.*, 1991). Since only class III ADH has been found in organisms outside of the vertebrates, all other classes of vertebrate ADH have arisen from duplication and divergence of a primordial class III ADH gene which encodes a

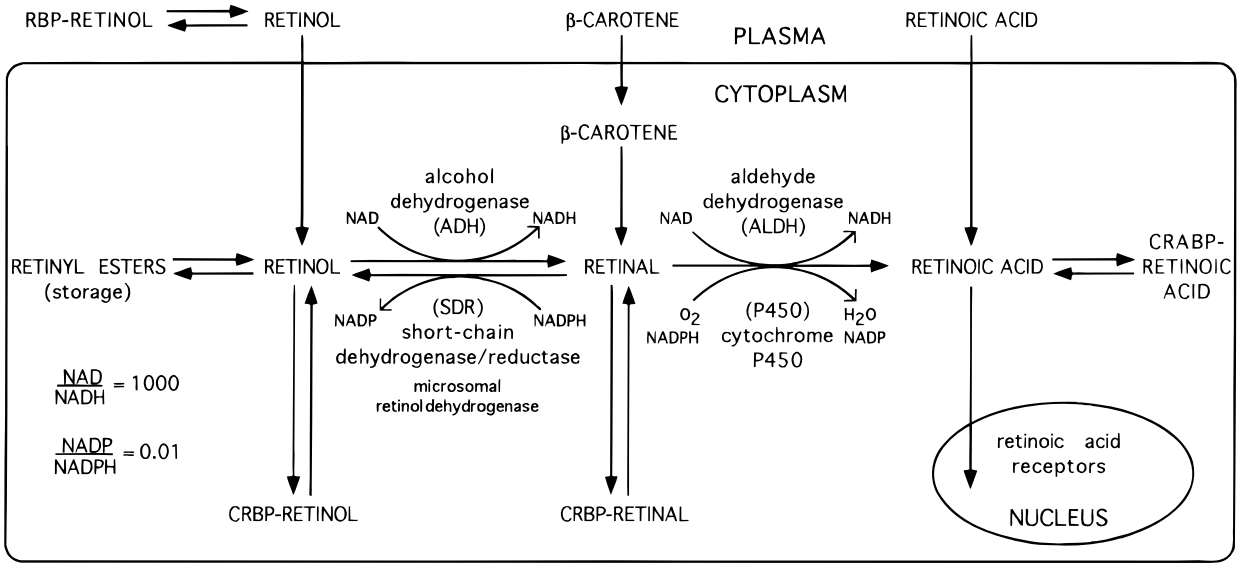


FIGURE 1: Proposed mechanism for RA synthesis and retinoid storage. Cellular uptake of retinol, carried by serum retinol-binding protein (RBP), $\beta$-carotene, and RA is indicated. Many cells perform local RA synthesis for signaling as well as synthesis of retinyl esters for retinoid storage. Inside the cell, retinol or retinal may bind cellular retinol-binding protein (CRBP) and RA may bind cellular retinoic acid-binding protein (CRABP); these proteins have been proposed to regulate metabolism, but mutations of CRABP have no critical effect on retinoid signaling (Lampron *et al.*, 1995). The reversible interconversion of retinol and retinal, the rate-limiting step in RA synthesis, is catalyzed by some members of the ADH and SDR enzyme families. The pyridine nucleotide coenzyme preferences of ADH and the SDR enzyme microsomal retinol dehydrogenase are indicated, and the proposed physiological directions catalyzed by each enzyme are inferred from the known ratios of oxidized and reduced forms of the coenzymes as indicated (Veech *et al.*, 1969). The irreversible oxidation of retinal to RA is catalyzed by some members of the ALDH and P450 enzyme families.

Table 1: Enzyme Families with Members Catalyzing Retinol or Retinal Metabolism

| enzyme family | retinoid activity |
|---|---|
| **Reversible Retinol/Retinal Interconversion** | |
| alcohol dehydrogenase | |
| class I | yes |
| class II | yes |
| class III | no |
| class IV | yes |
| class V | ? |
| class VI | ? |
| class VII | yes |
| short-chain dehydrogenase/reductase | |
| microsomal retinol dehydrogenase | yes |
| 11-cis-retinol dehydrogenase | yes |
| 11$\beta$-hydroxysteroid dehydrogenase | ? |
| 17$\beta$-hydroxysteroid dehydrogenase | ? |
| **Oxidation of Retinal to Retinoic Acid** | |
| aldehyde dehydrogenase | |
| class I | yes |
| class II | no |
| class III | no |
| new class (embryonic) | yes |
| cytochrome P450 | |
| P450 1A1 | yes |
| P450 1A2 | yes |
| P450 2B4 | no |
| P450 2C3 | no |
| P450 2E1 | no |
| P450 3A6 | no |

glutathione-dependent formaldehyde dehydrogenase found ubiquitously in all organisms analyzed (Koivusalo *et al.*, 1989; Danielsson & Jörnvall, 1992; Danielsson *et al.*, 1994). Class III ADH does not function in either retinol or ethanol metabolism (Beisswenger *et al.*, 1985), but other vertebrate ADHs which have evolved from it have evidently acquired this ability via mutation, adaptation, and selection.

Several purified ADHs have been demonstrated to catalyze retinol oxidation *in vitro* including human class I, II, and IV ADHs (Yang *et al.*, 1994; Kedishvili *et al.*, 1995), rat class I and IV ADHs (Boleda *et al.*, 1993), mouse class IV ADH (Connor & Smit, 1987), and chick class VII ADH (Kedishvili *et al.*, in press). These ADHs are able to metabolize *all-trans*, 9-*cis*, and 13-*cis* retinoid isomers (Boleda *et al.*, 1993; Yang *et al.*, 1994). Class III ADH does not catalyze retinol oxidation (Boleda *et al.*, 1993; Yang *et al.*, 1994), and neither class V nor class VI ADHs have been examined for retinol activity.

The expression of ADH genes is suggestive of a role in RA synthesis. For example, expression of class I and class IV ADHs in adult mouse tissues occurs primarily in epithelial cell populations, many of which are known to require RA for proper differentiation (Holmes, 1978; Vonesch *et al.*, 1994; Zgombic-Knight *et al.*, 1995; Ang *et al.*, 1996b). Expression of class IV ADH in mouse embryos coincides with sites of RA detection, suggesting that this ADH isozyme may play a role in regulating the initial turn-on of RA synthesis during embryogenesis (Ang *et al.*, 1996a; Deltour *et al.*, 1996).

*Short-Chain Dehydrogenase/Reductase (SDR).* An enzyme activity distinct from ADH that can oxidize retinol to retinal or reduce retinal to retinol has been found in rat liver microsomes, i.e., microsomal retinol dehydrogenase (Leo *et al.*, 1987; Napoli & Race, 1990). This enzyme, unlike ADH, prefers the phosphorylated coenzyme NADP(H) over NAD(H) and is membrane-bound, whereas ADH is cytosolic (Napoli *et al.*, 1992; Boerman & Napoli, 1995a). Microsomal retinol dehydrogenase is not inhibited by ADH inhibitors such as ethanol, 4-methylpyrazole, or citral (Boerman & Napoli, 1995a). This enzyme has also been found to utilize *all-trans*-retinol or *all-trans*-retinal either free or bound to cellular retinol-binding protein I (CRBP-I), but is unable to

oxidize 9-*cis*-retinol or 13-*cis*-retinol in either the free or bound form (Posch *et al.*, 1991; Boerman & Napoli, 1995b). CRBP-I-bound retinol has been reported to cross-link to a microsomal protein the approximate size of microsomal retinol dehydrogenase, leading to the hypothesis that CRBP-I may deliver retinol directly to the enzyme following protein−protein interaction (Boerman & Napoli, 1995b). The ability of CRBP-I-bound retinol to act as a substrate for purified ADHs has not been examined.

Cloning of cDNAs for three related forms (types I, II, and III) of rat liver microsomal retinol dehydrogenase indicated sequence homology with members of the short-chain dehydrogenase/reductase (SDR) family (Chai *et al.*, 1995a,b, 1996). The ADH and SDR enzyme families are related evolutionarily, sharing a similar coenzyme-binding domain, but differ in that ADH has a greater subunit molecular weight of about 40 kDa and is zinc-dependent whereas SDR has a shorter subunit of 25−35 kDa (i.e., short-chain) and no metal requirement (Persson *et al.*, 1995). All three types of microsomal retinol dehydrogenase are expressed predominantly in liver with only very low levels in kidney, brain, lung, and testis with the exception of the type II form which is expressed significantly in kidney (Chai *et al.*, 1995b; Chai *et al.*, 1996).

Cloning of a cDNA for the retinal pigment epithelium-specific 11-*cis*-retinol dehydrogenase revealed that it is also a member of the SDR family (Simon *et al.*, 1995). Physiologically, this enzyme is known to produce the visual pigment 11-*cis*-retinal by oxidizing 11-*cis*-retinol, and is absolutely specific for the 11-*cis* isomer of retinol as substrate and NAD as coenzyme (Simon *et al.*, 1995). This enzyme and microsomal retinol dehydrogenase provide the first examples of SDR enzymes able to participate in retinoid metabolism. It will be interesting to see whether other members of the SDR family metabolize retinol/retinal. Also, since many members of the SDR family are known to metabolize hydroxysteroids, it will be interesting to determine if the microsomal retinol dehydrogenases described above are able to metabolize hydroxysteroids as well as retinoids.

*Aldehyde Dehydrogenase (ALDH).* A role for liver ALDH (now known as class I ALDH) in the NAD-linked oxidation of retinal to RA has been described (Futterman, 1962; Lee *et al.*, 1991; McCaffery *et al.*, 1992). Just as ADH is able to utilize a wide variety of alcohols as substrates, ALDH is able to utilize a wide variety of aldehydes as substrates including acetaldehyde and retinal [reviewed by Lindahl (1992)]. However, unlike retinol oxidation, the oxidation of retinal is essentially irreversible. This explains why the administration of RA to vitamin A deficient animals does not result in the production of retinal and retinol needed for retinoid storage nor in the production of the visual pigment 11-*cis*-retinal (Dowling & Wald, 1960). Also, like ADH, it has been shown that ALDH can metabolize both *cis* and *trans* retinoid isomers, oxidizing *all-trans*-retinal and 9-*cis*-retinal to the corresponding RA isomers (Labrecque *et al.*, 1995).

A family of ALDHs exist which have been categorized into at least three classes (Lindahl, 1992). Class I ALDHs are cytosolic enzymes with 55 kDa subunits, whereas class II ALDHs are mitochondrial enzymes with 55 kDa subunits, and class III ALDHs are cytosolic or microsomal enzymes with 50 kDa subunits. Among these ALDHs, class I and class II are much closer evolutionarily, having polypeptides of nearly the same length (about 500 residues) which share 60−70% interclass sequence identity and having genes composed of 13 exons (Hsu *et al.*, 1995). On the other hand, class III ALDHs have shorter polypeptides (about 450 residues) sharing only 30% sequence identity with the class I and II forms, and have a much different gene structure consisting of only nine or ten exons (Hsu *et al.*, 1995). Current studies indicate that additional ALDH forms exist which may eventually be placed in different classes.

Class I ALDH has been reported to have high activity for retinal oxidation as described above, with class II and III ALDHs having no significant retinal-oxidizing activity (Lee *et al.*, 1991). Studies in vertebrate embryos link class I ALDH to retinal oxidation for RA synthesis, and provide evidence for an additional class which uses retinal efficiently. A very high level of class I ALDH expression has been observed in the retina of the embryonic mouse, a site which is particularly high in endogenous RA (McCaffery *et al.*, 1992). Class I ALDH resides in the dorsal portion of the retina, whereas a different ALDH with higher specificity for retinal resides in the ventral retina where a higher amount of RA exists (McCaffery *et al.*, 1993). A new class of ALDH also resides in the mouse embryonic spinal cord, another site particularly high in endogenous RA (McCaffery & Dräger, 1994), and ALDH has been reported to colocalize with endogenous RA in zebrafish embryos (Marsh-Armstrong *et al.*, 1994). The tunicate, an invertebrate chordate, has also been reported to have endogenous RA as well as an ALDH which oxidizes retinal suggesting that organisms as ancient as primitive chordates may have evolved a retinoid metabolic pathway designed to produce RA for retinoid signaling (Kawamura *et al.*, 1993).

*Cytochrome P450 (P450).* Some members of the P450 family have been discovered to participate in RA synthesis. P450 1A1 and 1A2, are able to oxidize retinal to RA, with P450 1A1 by far the most active form (Roberts *et al.*, 1992; Raner *et al.*, 1996). Other forms of P450 tested (2B4, 2C3, 2E1, 3A6) have negligible activity for retinal oxidation to RA (Raner *et al.*, 1996). P450 1A1 and 1A2 were able to oxidize both *all-trans*-retinal and 9-*cis*-retinal to the corresponding RA isomers (Raner *et al.*, 1996). This indicates that two mechanisms exist for the irreversible oxidation of retinal isomers to RA isomers, one involving ALDH and another involving P450. P450 1A1 gene expression has been observed in mouse embryos as early as day 7 of gestation (Kimura *et al.*, 1987), indicating that it could play a role in the onset of RA synthesis during embryogenesis.

In addition to catalyzing the oxidation of retinal to RA as described above, involvement of the P450 enzyme family in RA metabolism was suggested from early studies indicating that RA could be oxidized to 4-oxo-RA by a microsomal enzyme requiring NADPH and oxygen, but that this was inhibited by carbon monoxide and other P450 inhibitors (Roberts *et al.*, 1979). Further studies have identified numerous P450 enzymes which can oxidize carbon 4 in the $\beta$-ionone rings of RA, retinal, and retinol (Raner *et al.*, 1996). This reaction may lead to retinoid turnover and excretion since 4-hydroxy and 4-oxo retinoids are more hydrophilic but may also play a role in retinoid signaling since 4-oxo-RA binds to RAR and leads to activation nearly as efficiently as *all-trans*-RA (Pijnappel *et al.*, 1993). Cellular retinoic acid-binding proteins I and II (CRABP-I and CRABP-II) have been hypothesized to play a role in regulating the metabolism of RA by P450s or other enzymes (Ong *et al.*,

1994). However, these proteins may not function in this manner. Gene knockout studies have shown that mice lacking both CRABP-I and CRABP-II are essentially normal, indicating that these binding proteins are not essential for normal retinoid metabolism or signaling (Lampron *et al.*, 1995). Instead it was suggested that the function of these binding proteins may be to sequester RA during vitamin A deficiency to help maintain retinoid signaling.

## PHYSIOLOGICAL ROLES OF ENZYMES CATALYZING RETINOL/RETINAL INTERCONVERSION

As described above, two families of enzymes have been discovered which catalyze the reversible interconversion of retinol/retinal (ADH and SDR) as well as two families of enzymes catalyzing irreversible oxidation of retinal to RA (ALDH and P450). Since the latter reaction is irreversible, it is likely that forms of ALDH or P450 able to catalyze this reaction *in vivo* will participate in the synthesis of RA. On the other hand, the reversibility of the retinol/retinal interconversion makes it more difficult to ascertain whether an enzyme performing this reaction is likely to be participating physiologically in RA synthesis (retinol oxidation) or retinol storage (retinal reduction). In addition, studies on the conversion of retinol to RA in homogenates of either a mammalian cell line (Napoli, 1986) or rat embryos (Chen *et al.*, 1995) have shown that retinol oxidation is the rate-limiting step. This points out the importance of determining the physiological direction of enzymes catalyzing this reversible step.

As an example, there exists a controversy over whether class IV ADH or microsomal retinol dehydrogenase, or both, catalyze retinol oxidation *in vivo*. This is a very difficult question to answer and may require mutational studies to resolve. However, the biochemical properties of these two enzymes can be used to make an educated guess as to their overall relative rates of retinol oxidation or retinal reduction. The catalytic efficiency ($k_{cat}/K_m$) of purified human class IV ADH for retinol oxidation with NAD is reported to be 1900 $min^{-1}$ $mM^{-1}$, with the catalytic efficiency for retinal reduction with NADH being 1200 $min^{-1}$ $mM^{-1}$ (Yang *et al.*, 1994). A purified preparation of rat microsomal retinol dehydrogenase has reported $V_{max}$ values of 115 pmol $min^{-1}$ $mg^{-1}$ for retinol oxidation with NADP, and 613 pmol $min^{-1}$ $mg^{-1}$ for retinal reduction with NADPH (Boerman & Napoli, 1995a). An estimate of $k_{cat}$ can be made from these values using a molecular weight of 88 kDa (34 and 54 kDa subunits) for the purified preparation of microsomal retinol dehydrogenase (Boerman & Napoli, 1995a,b). Thus, the catalytic efficiencies for microsomal retinol dehydrogenase can be calculated as 17 $min^{-1}$ $mM^{-1}$ for retinol oxidation and 93 $min^{-1}$ $mM^{-1}$ for retinal reduction. Even without a cross-comparison of the values for these two enzymes it is evident that microsomal retinol dehydrogenase is approximately 5-fold more efficient in retinal reduction than in retinol oxidation, whereas class IV ADH is 1.6-fold more efficient in retinol oxidation than retinal reduction. These values also suggest that ADH is approximately 100-fold more efficient in retinol oxidation than microsomal retinol dehydrogenase. However, the difference is likely to be less since the preparation of microsomal retinol dehydrogenase, (which contains primarily the 34 and 54 kDa subunits) is not as pure as that of class IV ADH, and is less stable.

The coenzyme preferences of ADH and SDR enzymes further help identify whether a particular enzyme is likely to perform retinol oxidation or retinal reduction physiologically. All classes of vertebrate ADH are specific for NAD(H). Class IV ADH uses NAD to perform retinol oxidation and can use NADH to perform retinal reduction, but only the former reaction can be considered physiologically significant in animals since the level of NAD in aerobically-growing cells vastly exceeds the level of NADH (Veech *et al.*, 1969). The vertebrate SDR family contains a mixture of forms, some preferring NAD(H) and some preferring the phosphorylated coenzyme NADP(H). In particular, 11-*cis*-retinol dehydrogenase is specific for NAD(H) which helps direct it, like ADH, to perform retinoid oxidation due to the high ratio of NAD to NADH. In contrast, microsomal retinol dehydrogenase prefers NADP(H) which might be expected to help direct it to perform retinal reduction due to the high ratio of NADPH to NADP physiologically (Veech *et al.*, 1969). Future studies on enzymes performing retinol/retinal interconversion should address coenzyme preference and physiological coenzyme ratios in order to make predictions about the potential *in vivo* role of a particular enzyme.

Despite the name ascribed to microsomal retinol dehydrogenase, a role for it as a retinal reductase converting retinal to retinol is likely as discussed above. This would suggest that microsomal retinol dehydrogenase functions in retinol storage. The reductive reaction is known to occur in the intestine where the breakdown of $\beta$-carotene (the ultimate source of all retinoids) produces *all-trans*-retinal which is then reduced to *all-trans*-retinol by a microsomal retinal reductase activity that functions best with *all-trans*-retinal bound to cellular retinol binding protein type II (CRBP-II) present at particularly high levels in the intestine (Kakkad & Ong, 1988; Li *et al.*, 1986). Conversion of $\beta$-carotene to retinal and retinol is also known to occur to a lesser extent in the liver and other organs (Olson, 1989). Thus, microsomal retinol dehydrogenase, which is abundant in liver, is a likely candidate for the retinal reductase suspected to exist in liver; a lack of expression studies in the intestine prevent a determination of whether it or a related SDR enzyme is also a candidate for the intestinal retinal reductase. The activity of microsomal retinol dehydrogenase with *all-trans*-retinol (as a retinol dehydrogenase) or with *all-trans*-retinal (as a retinal reductase) is stimulated in both cases by CRBP-I, a protein which has been shown to bind both of these retinoids (Levin *et al.*, 1988). It is clear that CRBP-I can also bind *all-trans*-retinol to facilitate conversion to retinyl esters, another reaction designed for retinoid storage (Yost *et al.*, 1988), and CRBP-I is particularly abundant in the liver (Ong *et al.*, 1982), the main organ for retinoid storage. Since there appears to be substantial experimental support of a role for both CRBP-I and CRBP-II in retinoid storage, it seems unlikely that these proteins also function in RA synthesis. Thus, the reported preference of microsomal retinol dehydrogenase for CRBP-I-bound retinoids suggests that CRBP-I and microsomal retinol dehydrogenase may perform essentially the same function in liver that CRBP-II and retinal reductase perform in the intestine, i.e., conversion of *all-trans*-retinal to *all-trans*-retinol for retinoid storage.

The ability of class I, II, and IV ADHs to metabolize 9-*cis* and 13-*cis* retinoid isomers as well as the *all-trans* form (Boleda *et al.*, 1993; Yang *et al.*, 1994) provides further support for this enzyme catalyzing retinol oxidation. Whether

or not retinoid configurations other than *all-trans*-retinol, *all-trans*-retinal, and *all-trans*-RA are actually needed for retinoid signaling, they are produced by non-enzymatic isomerizations which can produce *cis*-forms (Urbach & Rando, 1994; Kojima *et al.*, 1994) or by oxidations involving P450s which can modify the $\beta$-ionone ring (described above) and other enzymatic conversions. This suggests that an enzymatic pathway for their turnover must exist if nothing else. That pathway is likely to involve oxidation since this produces more hydrophilic retinoids that can be more easily excreted. Thus, ADH may serve a role in retinoid turnover as well as retinoid activation by converting various retinol configurations to the corresponding retinal configurations. Since ALDH and P450 also have broad substrate specificities, they may be able to metabolize various retinal configurations to the corresponding RA configurations, providing either an active retinoid or a turnover product. In contrast, microsomal retinol dehydrogenase is very specific for the *all-trans* configuration (Boerman & Napoli, 1995b) suggesting that it is not designed to oxidize or reduce the wide variety of retinoids that exist *in vivo*. However, there may exist other SDR family members which can utilize alternative retinoid forms. This specificity of microsomal retinol dehydrogenase suggests that its role is to convert *all-trans*-retinal to *all-trans*-retinol for retinol storage. In contrast to retinoid turnover which must be able to accommodate any retinoid forms which have accumulated in a cell, storage need only be designed to produce *all-trans*-retinol and *all-trans*-retinyl esters, the basic configurations known to be able to produce all the active retinoids for both signaling and vision.

Studies on ADH inhibitors has provided yet further evidence that this enzyme functions in retinol oxidation for RA synthesis. The ADH inhibitor 4-methylpyrazole has been shown to inhibit the conversion of retinol to RA in mouse embryos *in vivo* (Collins *et al.*, 1992). Microsomal retinol dehydrogenase is not inhibited by 4-methylpyrazole (Chai *et al.*, 1995a). Citral, an inhibitor of ADH but not microsomal retinol dehydrogenase, essentially eliminates *in vitro* retinol oxidation in rat embryo homogenates (Chen *et al.*, 1995). Also, ethanol is able to inhibit ADH-catalyzed retinol oxidation *in vitro* (Mezey & Holt, 1971; Van Thiel *et al.*, 1974; Julià *et al.*, 1986), and ethanol treatment of mouse embryos has been demonstrated to reduce endogenous RA levels (Deltour *et al.*, 1996). Since ethanol is not an inhibitor of microsomal retinol dehydrogenase (Chai *et al.*, 1995a), this provides evidence that an ADH is responsible for embryonic retinol oxidation to promote RA synthesis.

## CONCLUSION

Already it has been established that four enzyme families (ADH, SDR, ALDH, and P450) are involved in retinol and retinal metabolism. Individual members of these families that participate in retinoid metabolism have been identified. On the basis of enzymatic properties one can assign specific potential functions to several of these enzymes. As these enzymes and their genes are further examined the regulatory mechanisms for RA synthesis, retinoid storage, and retinoid turnover will be determined. Increased knowledge of the enzyme families involved in retinol and retinal metabolism will soon allow this layer of control in the retinoid signaling pathway to be deciphered.

## REFERENCES


Ang, H. L., Deltour, L., Hayamizu, T. F., Zgombic-Knight, M., & Duester, G. (1996a) *J. Biol. Chem. 271*, 9526−9534.

Ang, H. L., Deltour, L., Zgombic-Knight, M., Wagner, M. A., & Duester, G. (1996b) *Alcohol. Clin. Exp. Res. 20* (in press).

Beisswenger, T. B., Holmquist, B., & Vallee, B. L. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 8369−8373.

Bliss, A. F. (1951) *Arch. Biochem. 31*, 197−204.

Boerman, M. H. E. M., & Napoli, J. L. (1995a) *Arch. Biochem. Biophys. 321*, 434−441.

Boerman, M. H. E. M., & Napoli, J. L. (1995b) *Biochemistry 34*, 7027−7037.

Boleda, M. D., Saubi, N., Farrés, J., & Parés, X. (1993) *Arch. Biochem. Biophys. 307*, 85−90.

Cederlund, E., Peralba, J. M., Parés, X., & Jörnvall, H. (1991) *Biochemistry 30*, 2811−2816.

Chai, X., Boerman, M. H. E. M., Zhai, Y., & Napoli, J. L. (1995a) *J. Biol. Chem. 270*, 3900−3904.

Chai, X., Zhai, Y., Popescu, G., & Napoli, J. L. (1995b) *J. Biol. Chem. 270*, 28408−28412.

Chai, X., Zhai, Y., & Napoli, J. L. (1996) *Gene 169*, 219−222.

Chen, H., Namkung, M. J., & Juchau, M. R. (1995) *Biochem. Pharmacol. 50*, 1257−1264.

Collins, M. D., Eckhoff, C., Chahoud, I., Bochert, G., & Nau, H. (1992) *Arch. Toxicol. 66*, 652−659.

Connor, M. J., & Smit, M. H. (1987) *Biochem. J. 244*, 489−492.

Danielsson, O., & Jörnvall, H. (1992) *Proc. Natl. Acad. Sci. U.S.A. 89*, 9247−9251.

Danielsson, O., Atrian, S., Luque, T., Hjelmqvist, L., Gonzàlez-Duarte, R., & Jörnvall, H. (1994) *Proc. Natl. Acad. Sci. U.S.A. 91*, 4980−4984.

Deltour, L., Ang, H. L., & Duester, G. (1996) *FASEB J. 10*, 1050−1057.

Dowling, J. E., & Wald, G. (1960) *Proc. Natl. Acad. Sci. U.S.A. 46*, 587−608.

Duester, G., Ang, H. L., Deltour, L., Foglio, M. H., Hayamizu, T. F., & Zgombic-Knight, M. (1995) *Adv. Exp. Med. Biol. 372*, 301−313.

Futterman, S. (1962) *J. Biol. Chem. 237*, 677−680.

Hempel, J., Nicholas, H., & Lindahl, R. (1993) *Protein Sci. 2*, 1890−1900.

Hogan, B. L. M., Thaller, C., & Eichele, G. (1992) *Nature 359*, 237−241.

Holmes, R. S. (1978) *Comp. Biochem. Physiol. B 61*, 339−346.

Horton, C., & Maden, M. (1995) *Dev. Dyn. 202*, 312−323.

Hsu, L. C., Chang, W.-C., Lin, S. W., & Yoshida, A. (1995) *Adv. Exp. Med. Biol. 372*, 159−168.

Jörnvall, H., Danielsson, O., Hjelmqvist, L., Persson, B., & Shafqat, J. (1995) *Adv. Exp. Med. Biol. 372*, 281−294.

Julià, P., Farrés, J., & Parés, X. (1986) *Exp. Eye Res. 42*, 305−314.

Kakkad, B. P., & Ong, D. E. (1988) *J. Biol. Chem. 263*, 12916−12919.

Kastner, P., Chambon, P., & Leid, M. (1994a) in *Vitamin A in Health and Disease* (Blomhoff, R., Ed.) pp 189−238, Marcel Dekker, Inc., New York.

Kastner, P., Grondona, J. M., Mark, M., Gansmuller, A., LeMeur, M., Decimo, D., Vonesch, J.-L., Dollé, P., & Chambon, P. (1994b) *Cell 78*, 987−1003.

Kawamura, K., Hara, K., & Fujiwara, S. (1993) *Development 117*, 835−845.

Kedishvili, N. Y., Bosron, W. F., Stone, C. L., Hurley, T. D., Peggs, C. F., Thomasson, H. R., Popov, K. M., Carr, L. G., Edenberg, H. J., & Li, T.-K. (1995) *J. Biol. Chem. 270*, 3625−3630.

Kedishvili, N. Y., Gough, W. H., Chernoff, E., Hurley, T. D., Stone, C. L., Popov, K.M., Bosron, W. F., & Li, T.-K. *Adv. Exp. Med. Biol.* (in press).

Kimura, S., Donovan, J. C., & Nebert, D. W. (1987) *J. Exp. Pathol. 3*, 61−74.

Koivusalo, M., Baumann, M., & Uotila, L. (1989) *FEBS Lett. 257*, 105−109.

Kojima, R., Fugimori, T., Kiyota, N., Toriya, Y., Fukuda, T., Ohashi, T., Sato, T., Yoshizawa, Y., Takeyama, K., Mano, H., Masushige, S., & Kato, S. (1994) *J. Biol. Chem. 269*, 32700−32707.

Kurlandsky, S. B., Gamble, M. V., Ramakrishnan, R., & Blaner, W. S. (1995) *J. Biol. Chem. 270*, 17850−17857.
Labrecque, J., Dumas, F., Lacroix, A., & Bhat, P. V. (1995) *Biochem. J. 305*, 681−684.
Lampron, C., Rochette-Egly, C., Gorry, P., Dollé, P., Mark, M., Lufkin, T., LeMeur, M., & Chambon, P. (1995) *Development 121*, 539−548.
Lee, M.-O., Manthey, C. L., & Sladek, N. E. (1991) *Biochem. Pharmacol. 42*, 1279−1285.
Leo, M. A., Kim, C., & Lieber, C. S. (1987) *Arch. Biochem. Biophys. 259*, 241−249.
Levin, M. S., Locke, B., Yang, N. C., Li., E., & Gordon, J. I. (1988) *J. Biol. Chem. 263*, 17715−17723.
Li, E., Demmer, L. A., Sweetser, D. A., Ong, D. E., & Gordon, J. I. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 5779−5783.
Lindahl, R. (1992) *Crit. Rev. Biochem. Mol. Biol. 27*, 283−335.
Lohnes, D., Mark, M., Mendelsohn, C., Dollé, P., Dierich, A., Gorry, P., Gansmuller, A., & Chambon, P. (1994) *Development 120*, 2723−2748.
Luo,J., Sucov, H. M., Bader, J., Evans, R. M., & Giguère, V. (1996) *Mech. Dev. 55*, 33−55.
Mangelsdorf, D. J., & Evans, R. M. (1995) *Cell 83*, 841−850.
Marsh-Armstrong, N., McCaffery, P., Gilbert, W., Dowling, J. E., & Dräger, U. C. (1994) *Proc. Natl. Acad. Sci. U.S.A. 91*, 7286−7290.
McCaffery, P., Lee, M.-O., Wagner, M. A., Sladek, N. E., & Dräger, U. C. (1992) *Development 115*, 371−382.
McCaffery, P., Posch, K. C., Napoli, J. L., Gudas, L., & Dräger, U. C. (1993) *Dev. Biol. 158*, 390−399.
McCaffery, P., & Dräger, U. C. (1994) *Proc. Natl. Acad. Sci. U.S.A. 91*, 7194−7197.
Mendelsohn, C., Lohnes, D., Décimo, D., Lufkin, T., LeMeur, M., Chambon, P., & Mark, M. (1994) *Development 120*, 2749−2771.
Mezey, E., & Holt, P. R. (1971) *Exp. Mol. Pathol. 15*, 148−156.
Napoli, J. L. (1986) *J. Biol. Chem. 261*, 13592−13597.
Napoli, J. L., & Race, K. R. (1990) *Biochim. Biophys. Acta, Gen. Subj. 1034*, 228−232.
Napoli, J. L., Posch, K. C., & Burns, R. D. (1992) *Biochim. Biophys. Acta Protein Struct. Mol. Enzymol. 1120*, 183−186.
Nelson, D. R., Kamataki, T., Waxman, D. J., Guengerich, F. P., Estabrook, R. W., Feyereisen, R., Gonzalez, F. J., Coon, M. J., Gunsalus, I. C., Gotoh, O., Okuda, K., & Nebert, D. W. (1993) *DNA Cell Biol. 12*, 1−51.
Olson, J. A. (1989) *J. Nutr. 119*, 105−108.
Ong, D. E., Crow, J. A., & Chytil, F. (1982) *J. Biol. Chem. 257*, 13385−13389.
Ong, D. E., Newcomer, M. E., & Chytil, F. (1994) in *The Retinoids: Biology, Chemistry, and Medicine*, 2nd ed. (Sporn, M. B., Roberts, A. B., & Goodman, D. S., Eds.) pp 283−317, Raven Press, New York.
Persson, B., Krook, M., & Jörnvall, H. (1995) *Adv. Exp. Med. Biol. 372*, 383−395.
Pijnappel, W. W. M., Hendriks, H. F. J., Folkers, G. E., Van den Brink, C. E., Dekker, E. J., Edelenbosch, C., Van der Saag, P. T., & Durston, A. J. (1993) *Nature 366*, 340−344.
Posch, K. C., Boerman, M. H. E. M., Burns, R. D., & Napoli, J. L. (1991) *Biochemistry 30*, 6224−6230.
Raner, G. M., Vaz, A. D. N., & Coon, M. J. (1996) *Mol. Pharmacol. 49*, 515−522.
Roberts, A. B., Nichols, M. D., Newton, D. L., & Sporn, M. B. (1979) *J. Biol. Chem. 254*, 6296−6302.
Roberts, E. S., Vaz, A. D. N., & Coon, M. J. (1992) *Mol. Pharmacol. 41*, 427−433.
Rossant, J., Zirngibl, R., Cado, D., Shago, M., & Giguère, V. (1991) *Genes Dev. 5*, 1333−1344.
Simon, A., Hellman, U., Wernstedt, C., & Eriksson, U. (1995) *J. Biol. Chem. 270*, 1107−1112.
Soprano, D. R., & Blaner, W. S. (1994) in *The Retinoids: Biology, Chemistry, and Medicine*, 2nd ed. (Sporn, M. B., Roberts, A. B., & Goodman, D. S., Eds.) pp 257−281, Raven Press, New York.
Sucov, H. M., Dyson, E., Gumeringer, C. L., Price, J., Chien, K. R., & Evans, R. M. (1994) *Genes Dev. 8*, 1007−1018.
Urbach, J., & Rando, R. R. (1994) *Biochem. J. 299*, 459−465.
Vallee, B. L., & Bazzone, T. J. (1983) *Curr. Top. Biol. Med. Res. 8*, 219−244.
Van Thiel, D. H., Gavaler, J., & Lester, R. (1974) *Science 186*, 941−942.
Veech, R. L., Eggleston, L. V., & Krebs, H. A. (1969) *Biochem. J. 115*, 609−619.
Vonesch, J.-L., Nakshatri, H., Philippe, M., Chambon, P., & Dollé, P. (1994) *Dev. Dyn. 199*, 199−213.
Yang, Z.-N., Davis, G. J., Hurley, T. D., Stone, C. L., Li, T.-K., & Bosron, W. F. (1994) *Alcohol.: Clin. Exp. Res. 18*, 587−591.
Yost, R. W., Harrison, E. H., & Ross, A. C. (1988) *J. Biol. Chem. 263*, 18693−18701.
Zachman, R. D., & Olson, J. A. (1961) *J. Biol. Chem. 236*, 2309−2313.
Zgombic-Knight, M., Ang, H. L., Foglio, M. H., & Duester, G. (1995) *J. Biol. Chem. 270*, 10868−10877.

BI961176+