Understanding Retinol Metabolism: Structure and Function of Retinol Dehydrogenases*

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Retinoids (vitamin A derivatives) have dual functions in physiology. 11-cis-Retinal serves as the universal chromophore of the visual pigments in the eye, and the hormonal retinoids, mainly all-trans- and 9-cis-retinoic acid (RA),2 regulate the expression of target genes via activation of two classes of nuclear retinoid receptors, the retinoic acid receptors (RARs), and the retinoid X receptors (RXRs) (1). The dietary sources of retinoids are the proforms of the vitamin, mainly esterified retinol, or the ultimate precursors for retinoids, various carotenoids. Thus, the biosynthesis of active forms of retinoids is of crucial importance for many physiological processes, including embryonic development, reproduction, postnatal growth, differentiation and maintenance of various epithelia, immune responses, and vision (2–4).

During fasting conditions the common precursor of the active retinoids, all-trans-retinol (vitamin A), is transported in plasma in a 1:1 complex with retinol-binding protein (RBP) (5). Retinol, in the form of retinyl esters, can also be transported to target cells using the common lipoproteins in plasma (6). Following uptake by target cells, retinol is converted, through several enzymatic steps, into its active derivatives (Fig. 1). Retinol is oxidized once to generate retinal and twice to generate RA. For the generation of the 9-cis- or 11-cis-retinoids, an isomerization reaction takes place prior to the first oxidation (7). The enzymes for most of these activities have been identified. To date some 20 different enzymes displaying all-trans- or cis-trans-retinol dehydrogenase (RDH) activities have been isolated and partially characterized by standard biochemical methods (Table 1). However, whether all of these enzymes truly function as retinol dehydrogenases in vivo remains to be determined. To this end it will be of great importance to analyze the enzymatic characteristics of the identified RDHs in intact cells, isolated organs, and in animal models. Here we discuss current knowledge regarding the biological roles, and structure-function relationships of RDHs, focusing on the microsomal members of the short chain dehydrogenase/reductase (SDR) family.

Two Families of Mammalian Retinol Dehydrogenases

Two different classes of retinol dehydrogenases have been proposed to carry out the oxidative conversion of retinol into retinal, namely the classical cytosolic alcohol dehydrogenases (ADHs), belonging to the medium chain dehydrogenases/reductase family, and some microsomal members of the SDR superfamily (RDHs) (Table 1) (8). ADHs are well known to catalyze the oxidation of a wide range of substrates in vitro (or reduction of the corresponding aldehyde forms), including ethanol, retinol, other aliphatic alcohols, steroids, and lipid peroxidation products (9). At present it is unclear whether members of the ADH family contribute to retinol oxidation under normal physiological conditions. Mice lacking Adh1 or Adh4 are viable and fertile and show no abnormalities unless challenged with excess doses of retinol (10). Targeted deletion of Adh3 indicates a potential role for this ubiquitously expressed enzyme in retinoid metabolism (11). However, evidence supporting a direct role of this enzyme in RA biosynthesis is lacking.

In conclusion, data accumulated until now do not provide strong support for a role of the ADHs in physiological RA biosynthesis.

The microsomal RDHs of the SDR family generally display higher substrate specificity as compared with the ADHs, catalyzing the oxidation of different isomers of retinol as well as some steroids (12, 13). Genetic evidence, mainly from different eye diseases and studies of zebrafish development, provide strong support for a key role of these enzymes in physiological oxidation of retinol. The biological roles of this class of RDHs are discussed in more detail below.

Biological Roles of Retinol Dehydrogenases of the SDR Family

The prototypic microsomal retinol dehydrogenase, RDH5 (11-cis-retinol dehydrogenase), was identified in the retinal pigment epithelium (RPE) of the eye and was shown to catalyze the oxidation of 11-cis-retinol into 11-cis-retinal in the visual cycle (14). Missense mutations in rdh5 are associated with the rare recessive eye disease known as fundus albipunctatus, characterized by stationary night blindness, accumulation of white spots in the retina, and development of cone dystrophy among elderly (15). This observation provided the first genetic evidence for the involvement of microsomal RDHs in retinoid metabolism in vivo. Interestingly, targeted deletion of murine rdh5 generates a much milder phenotype, suggesting a higher degree of redundancy in mice (16).

Indeed, other retinol dehydrogenases have recently been implicated in the generation of visual chromophore in mice (RDH11–14) (17, 18). Most notably, mutations in rdh12 have been shown to cause a retinal disease known as Leber’s congenital amaurosis, demonstrating that this enzyme is responsible for the reduction of all-trans-retinol into retinal in the photoreceptor cells (19). The proposed role of RDH11 in the visual cycle has, however, been questioned (20), and others (21) have suggested that this enzyme (also referred to as RalR1 or PSDR1) serves as a retinal reductase rather than an oxidizing enzyme, although purely based on in vitro data. This same enzyme has also been implicated in retinoid homeostasis in normal and neoplastic prostate, and its expression seems to be regulated by androgens (22).

Recent data also show that RDHs play important roles in RA signaling during gut development and differentiation and maybe in pathological conditions. Thus, RDH5 and RDH12 (also known as hRodH-E2) are down-regulated in colon adenomas and carcinomas, leading to reduced synthesis of RA (23). Moreover, the same report demonstrates that adenomatous polyposis coli (APC) and the colon-specific transcription factor CDX2 can control the production of RA via regulated expression of RDH12. Further investigations have identified two zebrafish RDHs, rdh12zRDH and rdh11zRDH, that are necessary for proper development of the intestine, as well as other organs, and demonstrate a genetic link between RDH-mediated RA synthesis and the upstream regulator APC (24, 25).

Taken together, several RDHs have been ascribed functional roles in the generation of 11-cis-retinal and RA. Further genetic dissection is clearly needed to pinpoint the exact roles of the different enzymes. However, given the possible redundancy among the RDHs, it is likely that different combinations of RDHs need to be inactivated to generate clear morphological and/or functional phenotypes in mice.

Domain Organization and Topology of Retinol Dehydrogenases

The RDHs of the SDR family have an overall amino acid sequence similarity of at least 30%. Among the conserved features is the cofactor binding site (amino acid residues G-X-X-G-X-G-X, representing any amino acid residue) and the catalytic site (amino acid residues Y-X-X-K) (8). In addition, many of them display a similar domain organization and most likely share a common folding and membrane topology. Particularly, the catalytic core SDR domain seems very conserved within the SDR superfamily, with an almost identical domain fold in most of the crystallized enzymes. Nevertheless, researchers exploring the membrane topology of the RDHs have come to different conclusions regarding the topology of these enzymes and the number of transmembrane domains (TMDs) that anchor these enzymes in the ER membrane (Fig. 2).

Initial studies conducted in 1999 demonstrated that the prototypic RDH5 is anchored to the ER membrane by two TMDs, which flank the conserved SDR core domain (27). It was shown by proteolysis K protection experiments, as well as endoglycosidase H treatment of an RDH5 mutant carrying an ectopic gly-
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FIGURE 1. Schematic overview of the retinoid activating pathways. A, chemical structure of all-trans-retinol, and its active derivatives, all-trans-retinoic acid, 9-cis-retinoic acid, and 11-cis-retinal. B, outline of the retinoid activation pathways. Target cells take up all-trans-retinol from retinol-binding protein (holo-RBP) or from retinyl esters transported in either chylomicrons (CM) or lipoproteins (LP). A putative receptor activity has been reported for RBP-mediated uptake. Intracellularly retinol may be isomerized into 9-cis- or 11-cis-retinol, of which the former activity is yet to be identified, followed by sequential oxidation into retinal and RA. RDHs and retinal dehydrogenases (RalDH) catalyze the first and second oxidations, respectively. The two isoforms of RA activate the nuclear retinoid receptors, RARs and RXXs, to control target gene transcription. 11-cis-Retinal is regenerated in the eye by a process referred to as the visual cycle (Unaded).

TABLE 1

<table>
<thead>
<tr>
<th>Species</th>
<th>Enzyme name(s)</th>
<th>Substrate(s) (retinoid isomers)</th>
<th>Cofactor preference</th>
</tr>
</thead>
<tbody>
<tr>
<td>m</td>
<td>CRAD1/rdh6</td>
<td>9-cis/11-cis</td>
<td>NAD+</td>
</tr>
<tr>
<td>m</td>
<td>CRAD2/rdh7</td>
<td>11-cis (all-trans)</td>
<td>NAD+</td>
</tr>
<tr>
<td>m</td>
<td>CRAD3/rdh9</td>
<td>9-cis/11-cis</td>
<td>NAD+ + NADP+</td>
</tr>
<tr>
<td>m</td>
<td>mRDH1/rdh1</td>
<td>All-trans/9-cis</td>
<td>NAD+ + (NADP+)</td>
</tr>
<tr>
<td>b/h/m</td>
<td>11-cis-RDH5/RDH4</td>
<td>9-cis-11-cis</td>
<td>NAD+</td>
</tr>
<tr>
<td>h/m</td>
<td>RDH10</td>
<td>All-trans</td>
<td>NADP+</td>
</tr>
<tr>
<td>h/m</td>
<td>RDH11/RalR1/PSDR1</td>
<td>All-trans/9-cis/11-cis</td>
<td>NADP+</td>
</tr>
<tr>
<td>h/m</td>
<td>RDH12</td>
<td>All-trans/9-cis/11-cis</td>
<td>NADP+</td>
</tr>
<tr>
<td>h/m</td>
<td>RDH13</td>
<td>Dual trans/cis</td>
<td>NA</td>
</tr>
<tr>
<td>h/m</td>
<td>RDH14</td>
<td>All-trans/9-cis/11-cis</td>
<td>NADP+</td>
</tr>
<tr>
<td>m/r</td>
<td>ERolDH*</td>
<td>All-trans</td>
<td>NA</td>
</tr>
<tr>
<td>b/h</td>
<td>hiRoDH-E2/RDHL*</td>
<td>All-trans</td>
<td>NADP+</td>
</tr>
<tr>
<td>r</td>
<td>RoDH1</td>
<td>All-trans</td>
<td>NADP+</td>
</tr>
<tr>
<td>r</td>
<td>RoDH2</td>
<td>All-trans</td>
<td>NADP+</td>
</tr>
<tr>
<td>r</td>
<td>RoDH3</td>
<td>NA*</td>
<td>NA</td>
</tr>
<tr>
<td>h</td>
<td>RoDH4/hiRoDH-E</td>
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<td>NADP+</td>
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<td>NADP+</td>
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<tr>
<td>z</td>
<td>rdh11/r2RDHA</td>
<td>All-trans</td>
<td>NA</td>
</tr>
<tr>
<td>z</td>
<td>rdh1/r2RDHB</td>
<td>All-trans</td>
<td>NA</td>
</tr>
</tbody>
</table>

* a, bovine; h, human; m, mouse; r, rat; z, zebrafish.
* eRolDH and hiRoDH-E2/RDHL are orthologs.
* NA, not available.

Unfortunately, the data summarized here do not allow a simple consensus interpretation, a fact that has hampered the progress of the field during recent years. It appears unlikely, but not possible to fully exclude, that these related enzymes display different membrane topologies. Instead, the experimental design may influence the outcome. It is notable that the analyses of the wild-type proteins, at least concerning the prototypic RDH5 and CRAD1, give identical results using several different techniques. Considering that membrane translocation, topology, and domain folding are highly context-dependent processes, the use of fusion proteins containing distinct peptide segments may introduce artifacts (36–38) and are in the absence of careful analyses of the intact proteins only indicative.

The topology of this class of enzymes has several important cell biological implications with regard to substrate and cofactor delivery, interplay with other components of the retinoid signaling pathway, as well as possible regulatory events taking place to control the enzymatic activity. Such issues are discussed below.

Cell Biological Perspectives on Retinol Dehydrogenases

Although the number of identified RDHs belonging to the SDR family has increased considerably during the past few years, most of them have only been characterized by means of traditional biochemical approaches and not in cells. Consequently, detailed knowledge of the molecular function of these enzymes is limited. The development of cellular assays should provide the tools necessary for functional studies of these enzymes in vivo. While fundamental biochemical work has given important data, e.g., on the substrate specificities and cofactor preferences of the different enzymes (Table 1), in vitro studies might not accurately reflect their relevance in vivo, as exemplified below.

cosylation site, that the catalytic ectodomain is facing the lumen of the ER and that only a short C-terminal tail of seven amino acids protrudes into the cytosol (Fig. 2B, model a). Studies on the related SDR member CRAD1 have confirmed, by independent techniques, that this enzyme has a lumenal topology of the catalytic domain (28, 29). Moreover, genetic and biochemical data, as well as computer-based modeling, suggest that these enzymes occur as functional homodimers (29, 30).

Other groups have presented different results regarding the topology of related enzymes, based on computer predictions and biochemical analyses of truncated variants of RoDH1 and RoDH4 (Fig. 2B, model b (31, 32) and model c (33)) and also by searching for topological signals in the various domains of mRDH1 by fusing them to the fluorescent reporter protein GFP (model c (34)). As shown in Fig. 2, the models differ not only in general membrane topology but also with regard to the number of TMDs. In model b, hydrophobic segments within the conserved core SDR domain has been proposed to serve as TMDs. However, the three-dimensional structure of the crystallized members of the SDR family would argue against this model as these hydrophobic stretches of amino acid residues in all cases are confined to the central core region of the SDR domain. In model c, the SDR domain has a cytosolic orientation and the C-terminal hydrophobic segment has not been included as a TMD. This model has also been supported by studies on the topology of RDH11/RalR1 (35). However, recent data show that the corresponding C-terminal region in CRAD1 can serve as a bona fide TMD in CD4 fusion proteins, retaining the proteins in the ER membrane and orienting with the C terminus facing the cytosol (29). Yet another report demonstrates that RDH12 carries endoglycosidase H-sensitive glycans (19), which is consistent only with the luminal topology represented by model a.
The use of reporter assays for studies of retinoid receptor activation and target genes, established in the 1990s, has inspired the development of coupled enzyme/reporter assays. These assays enable reconstitution of the biochemical retinoid pathways generating RA in vivo. Using this approach, it was shown that deletion of the cytosolic tail (see Fig. 2B, model a) in RDH5 and CRAD1 resulted in abolished enzymatic activity in vivo but not in a traditional in vitro assay (28). Further studies have demonstrated that the cytosolic tail is necessary for the functional activity of these enzymes, which might reflect that this region is involved in interaction(s) with some type of effector molecule(s) (29). Several possible functions of such interactions can be envisaged, including a role in substrate and/or co-factor delivery, coupling with other functional events upstream or downstream in the pathway, or it may represent a control point in the pathway, ultimately regulating the generation of RA. These results underscore the necessity of analyzing RDH function in a cellular context to clarify their roles in physiological retinol oxidation.

Regulation of RDH Activity — The possible regulation of the enzymatic activities of the RDHs is still largely unexplored. As the concentration of all-trans-retinol in plasma is ~2–3 μM, whereas the concentration of RA ligands required for receptor activation is a 1000-fold lower (i.e. in the nanomolar range), target cells should require some means to control the generation of these highly potent receptor ligands. Apart from possible regulation at the protein level, as implied by the data summarized above, recent work shows that some RDHs are regulated at the transcriptional level. As mentioned above, RDHL is under the control of APC and CDX2 (24), and the expression of RDH11/RalR1/PSDR1 seems to be regulated by androgens (22). Yet, other RDHs are regulated at the transcriptional level. As mentioned above, RDH5 has been proposed to reduce cis-11-retinal to trans-11-retinal. RDH5 has been shown to interact with retinal G protein-coupled receptor (47). This RPE protein is an opsin, which mediates photoisomerization of its visual cycle enzymes RDH5 and RDH12, are likely to be constitutively active and may not be subject to such regulation.

Subcellular Localization of RDHs — Despite the controversy regarding the membrane topology of RDHs, it is well established that these enzymes localize to the smooth ER. Although the molecular mechanism underlying ER localization remains to be explained, recent work on CRAD1 demonstrates that its putative C-terminal TMD mediates ER retention of the enzyme (29). Possible reasons for this include lateral interaction(s) with other ER resident components within the membrane or exclusion from the lipid bilayer of forward transporting vesicles of the secretory pathway. The visual cycle enzyme RDH5 is also known to localize in the smooth ER of RPE cells or in transfected cells (14, 27). In light of this, the isolation of RDH5 in a protein complex localized to the apical membranes of RPE cells is intriguing. This is described further below.

One of the RDHs expressed in photoreceptors, prRDH1/rdh8, differs from other RDHs, both with regard to the domains flanking the SDR domain and the mode of membrane interaction and localization. Thus, prRDH localizes to the rod and cone outer segments by means of a C-terminal localization signal and is peripherally associated to the membranes via fatty acylation of one or more conserved cysteine residues (39). However, any functional implications this distinct localization mechanism might have are yet to be resolved.

Interactions of RDHs with Other Proteins — It is widely believed that retinol bound to cellular retinol-binding protein I (CRBPI) is the physiological substrate for all-trans-specific RDHs, as evidenced by cross-linking experiments and in vitro kinetics (reviewed in Ref 40). It follows that this view also requires a cytosolic orientation of the catalytic domain of these RDHs (Fig. 2B, model c). However, at present no functional data exist to support this role for CRBPI in a cellular context. On the contrary, studies on mice deficient in CRBPI indicate that this carrier protein is mainly involved in retinyl ester storage, rather than playing a vital role in RA biosynthesis (41, 42). More profound effects have been seen upon deletion of cellular retinaldehyde-binding protein (CRALBP), which operates in the visual cycle, in the regeneration of 11-cis-retinal (reviewed in Ref 43). CRALBP has been suggested to facilitate the generation of 11-cis-retinal by mediating the transfer of 11-cis-retinol from the retinoid isomerase to RDH5, which catalyzes oxidation of 11-cis-retinol into 11-cis-retinal. The mechanism of substrate delivery, whether it involves retinoid-binding proteins directly or not, needs to be determined at the molecular level in the context of living cells.

A few additional reports also demonstrate protein interactions involving RDH5. For instance, RDH5 was first discovered through its association with RPE65 (14), a protein that has been implicated in retinol uptake (44, 45) and recently was shown to be the retinoid isomerase in the visual cycle (46). However, any functional roles of this interaction are yet unknown. Furthermore, RDH5 has been shown to interact with retinal G protein-coupled receptor (47). This RPE protein is an opsin, which mediates photoisomerization of its bound all-trans-retinal into 11-cis-retinal. RDH5 has been proposed to reduce the product into 11-cis-retinol, although the functional significance of such a reaction remains unknown. Recent studies also suggest that RDH5 occurs in a larger retinoid processing complex present in the apical processes of RPE cells (48, 49). This complex includes CRALBP, which binds to the PDZ domain protein EB50/NHERF1, which in turn interacts with ezrin. Ezrin binds to various plasma membrane components and links them physically to the actin cytoskeleton. It was suggested that this retinoid processing complex might facilitate localized synthesis and release of an 11-cis-retinoid in the apical area of the RPE.

Concluding Remarks

The RDHs are well characterized with regard to their enzymatic properties in vitro. However, to learn more about their roles in physiological retinol oxidation, functional aspects need to be explored at the cellular and organism levels. Tomorrow’s challenges are thus to investigate the cell biological architecture of the retinoid metabolic pathways and to understand possible regulatory mechanisms that control the spatially and temporally restricted generation of active retinoids in vivo. Such analyses are likely to involve different kinds of in vivo reporter strategies to monitor enzyme function, either established in cell lines, organ cultures, or in transgenic animals carrying reporter constructs.

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