Structure and function of retinol dehydrogenases of the short chain dehydrogenase/reductase family

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Abstract

All-trans-retinol is the common precursor of the active retinoids 11-cis-retinal, all-trans-retinoic acid (atRA) and 9-cis-retinoic acid (9cRA). Genetic and biochemical data supports an important role of the microsomal members of the short chain dehydrogenases/reductases (SDRs) in the first oxidative conversion of retinol into retinal. Several retinol dehydrogenases of this family have been reported in recent years. However, the structural and functional data on these enzymes is limited. The prototypic enzyme RDH5 and the related enzyme CRAD1 have been shown to face the lumen of the endoplasmic reticulum (ER), suggesting a compartmentalized synthesis of retinal. This is a matter of debate as a related enzyme has been proposed to have the opposite membrane topology. Recent data indicates that RDH5, and presumably other members of the SDRs, occur as functional homodimers, and need to interact with other proteins for proper intracellular localization and catalytic activity. Further analyses on the compartmentalization, membrane topology, and functional properties of microsomal retinol dehydrogenases, will give important clues about how retinoids are processed.

Abbreviations: atRA, all-trans-retinoic acid; 9cRA, 9-cis-retinoic acid; ER, endoplasmic reticulum; RalDH, retinal dehydrogenase; RAR, retinoic acid receptor; RDH, retinol dehydrogenase; RPE, retinal pigment epithelium; RXR, retinoid X receptor; SDR, short chain dehydrogenase/reductase

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1. Introduction

Retinoids (vitamin A derivatives) are essential for a wide range of physiological processes, including embryonic development, reproduction, vision, immune responses, and differentiation and maintenance of various epithelia. 11-cis-retinal serves as the chromophore of the visual pigments in vertebrate retinas, and the hormonal retinoids, all-trans-retinoic acid (atRA) and 9-cis-retinoic acid (9cRA), regulate the non-visual functions via activation of two classes of nuclear retinoid receptors, retinoic acid receptors (RARs) and retinoid X receptors (RXRs). Recent results suggest that retinoids are also involved in the maintenance of circadian rhythms, mediated by specialized photoreceptor cells (Lucas et al., 2003; Van Gelder et al., 2003).

Retinol is converted into retinal and retinoic acid, by one or two rounds of oxidation, respectively. Two classes of enzymes have been implicated in the first oxidation of retinol into retinal, the microsomal members of the shortchain dehydrogenases/reductases (SDRs), and the cytosolic medium chain alcohol dehydrogenases (ADHs) (Duester, 2000; Napoli, 1999). However, not much is known about the contribution of the individual enzymes to retinoid biosynthesis in vivo, nor is it clear how they function at the cell biological level, i.e. in terms of compartmentalization of the enzymes and their substrates, and how they function in concert with other proteins. Here we review recent data on the structure-function relationships of retinol dehydrogenases of the short chain dehydrogenase/reductase (SDR) family.

2. Retinol dehydrogenases of the short chain reductase/dehydrogenase family

In recent years, several retinol dehydrogenases (RDHs) have been added to the family of SDRs. In common to the SDRs is a well conserved domain structure with an N-terminal coenzyme—binding motif (consensus GXXXGXG) and an active site sequence (consensus YXXXK). Substrates for the SDR family of enzymes include steroids, fatty acids, and lipid alcohols, such as retinol. Several isozymes able to oxidize various isomers of retinol have been described, displaying different tissue distribution and substrate specificities in vitro.

The first retinol dehydrogenase of the SDR family to be identified and cloned, was the bovine 11-cis-retinol dehydrogenase (RDH5), abundantly expressed in the retinal pigment epithelium (RPE) of the eye (Simon et al., 1995). Mutations in the RDH5 gene has been shown to cause fundus albipunctatus, a rare form of stationary night blindness, characterized by a delay in the regeneration of cone and rod photopigments, and accumulation of white spots in the retina (Yamamoto et al., 1999). This provided the first genetic evidence that members of the SDR family are involved in retinoid metabolism in vivo, and that RDH5 plays a crucial role in the production of 11-cis-retinal. However, the photoreceptor cells ultimately do recover, indicating that other enzymes or biochemical pathways can produce 11-cis-retinal at a slower rate than normal. Likewise, studies on RDH5-deficient mice show that alternative
pathways must exist (Driessen et al., 2000). These mice have a normal dark adap-
tation and show no signs of fundus albipunctatus, and only at high bleaching levels
delayed dark adaptation can be detected. Recently, a new subfamily of dual-sub-
strate enzymes (RDH11-14) were described, which could account for this redun-
dancy in mice (Haeseleer et al., 2002). An all-trans-retinol dehydrogenase, denoted
RDH10, has also been identified in the RPE, although its function remains to be
clarified (Wu et al., 2002). In the photoreceptor outer segments, two enzymes,
retSDR1 and prRDH, have been implicated in the reduction of all-trans-retinal into
all-trans-retinol, which is the rate limiting step in the visual cycle (Haeseleer et al.,
1998; Rattner et al., 2000).

In addition to 11-cis-retinol, RDH5 oxidizes 9-cis-retinol. Taken together with the
extra-ocular expression pattern, it was suggested early on that the enzyme may have
dual functions, catalyzing chromophore production in the eye, and the first oxidative
step in 9cRA synthesis at other sites (Romert et al., 1998). However, the mild
phenotype seen in RDH5-deficient mice does not support an essential role of the
enzyme in 9cRA biogenesis in mice (Driessen et al., 2000). The related enzymes, cis-
retinol/androgen dehydrogenase 1-3 (CRAD1-3), efficiently metabolize 9-cis and/or
all-trans-retinol, and are highly expressed in the liver, kidney, and lung (Chai et al.,
1997; Su et al., 1998; Zhuang et al., 2002). RoDH1, RoDH2 and RoDH3, cloned
from rat liver (Chai et al., 1995a,b; Chai et al., 1996), and their human homologues,
RoDH4 and RoDH-like 3α-HSD (Gough et al., 1998; Kedishvili et al., 2001), have
been shown to metabolize all-trans-retinol, as well as 3α-hydroxysteroids. Yet an-
other SDR, active with all-trans and cis-retinols, as well as steroids, is the mRDH1
identified in mouse (Zhang et al., 2001).

3. Structure and function of RDHs

Biochemical data on the structure, topology and localization of the RDHs has
only been reported for a few of the RDHs. RDH5 and CRAD1 are attached to the
endoplasmic reticulum (ER) membrane by two membrane anchoring helices, with
the bulk of the enzyme, including the co-factor binding and the active site, facing the
luminal side, and a C-terminal tail of 6–7 amino acids protruding into the cytosol
(Fig. 1). This is evidenced by a number of independent observations, i.e. by pro-
teinase K protection of wild type and HA epitope-tagged RDH5; Endo H trea
tment of N-glycosylated mutants; immunofluorescence at conditions of selective membrane
permeabilization; and electron microscopy (Simon et al., 1999; Tryggvason et al.,
2001, and unpublished data). However, other authors have come to different con-
clusions regarding the related enzyme RoDH1/RoDH4 (Belyaeva et al., 2003;
Lapshina et al., 2003; Wang et al., 2001).

There is experimental evidence that the cytosolic tail is of functional importance
in vivo. Deletion of the cytosolic tail in murine RDH5 and CRAD1, abolishes en-
yzymatic activity in a cellular reporter assay (Fig. 2), but not in vitro, and mislocalizes
the enzyme intracellularly (Tryggvason et al., 2001). These results may reflect the
need for interactions with other components for proper localization and catalytic
A common structural feature of SDRs is homodimer and tetramer formation (Jörnvall et al., 1995). RDH5, and presumably CRAD1, occur as functional homodimers, as indicated by genetic and biochemical data. A patient with fundus albipunctatus was found to be compound heterozygote, expressing one active RDH5 mutant (A294P) and one inactive (R280H), leading to a trans-dominant negative

*Fig. 1.* A molecular model of RDH5. (A) Schematic model showing the topology of microsomal RDHs. (B) A molecular model of dimeric RDH5, generated based on the coordinates of the related enzyme 17β-hydroxysteroid dehydrogenase. Residues 27-287 were fit into the known structure, and the N- and C-terminal membrane-anchoring segments (residues 1–26, and 288–317, respectively), have been added to the model.

![Image of a molecular model of RDH5](image)

*Fig. 2.* Metabolic pathways of retinoids, and the principles of the cell reporter assay. (A) All-trans-retinol, derived from dietary retinoids, is the ultimate precursor for the biologically active retinoids 11-cis-retinal, all-trans-retinoic acid and 9-cis-retinoic acid. (B) A reporter system that can be used to study the function of retinol dehydrogenases in intact cells. JEG3 cells are cotransfected with plasmids encoding retinol and retinal dehydrogenases (RDH and RalDH), as well as reporter plasmids encoding a Gal4-RXR (retinoid X receptor) fusion protein and a fusion of Gal4 DNA response elements with a TK-promoter driven luciferase gene. By addition of 9-cis-retinol as substrate for RDH, 9cRA is ultimately produced, thus activating transcription of the luciferase gene. The luciferase activity can be quantified luminometrically, and used as a measure of the activities of the retinoid metabolizing enzymes.

![Image of metabolic pathways of retinoids](image)
effect on the active mutant exerted by the inactive mutant. Cross-linking data and molecular modelling suggested that RDH5 occurs as functional homodimers. Taken together, this implied that formation of inactive heterodimers of the RDH5 mutants caused the phenotype seen in this patient (Lidén et al., 2001).

The fact that RDH5 and CRAD1 are facing the lumen of the ER has several important functional implications. For instance, the cofactor NAD+, required for the oxidative reaction, must either be transported from the cytosol, which is rich in cofactor, or produced in the lumen by as yet unknown pathways. Measurements in isolated microsomes confirm that sufficient concentration of NAD+ is present in the lumen (Bublitz and Lawler, 1987), and the milieu in the lumen is oxidative (Hwang et al., 1992), which would favor retinol oxidation. Likewise, the substrates need to be transported into the lumen of the ER by some means, and the product 9-cis-retinal is somehow translocated to the cytosol where retinal dehydrogenases (RdLDHs) catalyze the formation of 9cRA. Since the pathway of 9cRA biosynthesis can be reconstituted in different cell lines, using the reporter system mentioned above, these functions may be carried out by general cellular mechanisms, in common to many cell types. Interestingly, the cytosolic enzyme ADH4, reported to oxidize 9-cis-retinol in vitro, was much less efficient than the microsomal enzymes in transfected cells, despite the fact that it is localized in the same compartment as the retinal dehydrogenase (Tryggvason et al., 2001). Perhaps the lumenal milieu is necessary for efficient retinol oxidation in vivo, and cytosolic enzymes may have other functions, possibly acting as retinal reductases.

4. Interactions of RDHs with other components involved in retinoid metabolism

As discussed above, the requirement of an intact cytosolic tail in RDH5 and CRAD1 for function in intact cells implies that interaction with other protein(s) is necessary for correct localization and/or catalytic activity. The nature and identity of such binding proteins remains to be determined. Interactions of RDH5 with other proteins have been shown in a few cases, although the exact functions of these are unknown. For instance, RDH5 was first noticed through its association with RPE65 (Båvik et al., 1992). This protein is highly expressed in the RPE, localized to both the plasma membrane and the ER membranes, and has been implicated in various events of retinoid metabolism, including retinol uptake (Bavik et al., 1992), and isomerization (Redmond et al., 1998). Another RPE protein reported to interact with RDH5 is the retinal G protein-coupled receptor (RGR) (Chen et al., 2001). This rhodopsin-like opsin mediates photoisomerization of its bound all-trans-retinal into 11-cis-retinal, and RDH5 is proposed to reduce the product into 11-cis-retinol, although the function of this backward reaction is unclear.

5. Concluding remark

In summary, more data on the expression, compartmentalization and membrane topology of microsomal retinol dehydrogenases, as well as functional characterization
in intact cells and animals, should clarify the role of the individual enzymes in retinoid biosynthesis.

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