Biochemical Pharmacology 83 (2012) 149–163

Contents lists available at SciVerse ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

Comparison of the function and expression of CYP26A1 and CYP26B1, the two retinoic acid hydroxylases

Ariel R. Topletz [a,1], Jayne E. Thatcher [a,1], Alex Zelter [b], Justin D. Lutz [a], Suzanne Tay [a], Wendel L. Nelson [c], Nina Isoherranen [a,*]

[a] Department of Pharmaceutics, University of Washington, Seattle, WA, United States
[b] Department of Biochemistry, University of Washington, Seattle, WA, United States
[c] Department of Medicinal Chemistry, University of Washington, Seattle, WA, United States

ARTICLE INFO

Article history:
Received 9 September 2011
Accepted 7 October 2011
Available online 14 October 2011

Keywords:
Vitamin A
Cytochrome P450
Retinoic acid
Metabolism
Tissue expression

ABSTRACT

All-trans-retinoic acid (*at*RA) is an important signaling molecule in all chordates. The cytochrome P450 enzymes CYP26 are believed to partially regulate cellular concentrations of *at*RA via oxidative metabolism and hence affect retinoid homeostasis and signaling. CYP26A1 and CYP26B1 are *at*RA hydroxylases that catalyze formation of similar metabolites in cell systems. However, they have only 40% sequence similarity suggesting differences between the two enzymes. The aim of this study was to determine whether CYP26A1 and CYP26B1 have similar catalytic activity, form different metabolites from *at*RA and are expressed in different tissues in adults. The mRNA expression of CYP26A1 and CYP26B1 correlated between human tissues except for human cerebellum in which CYP26B1 was the predominant CYP26 and liver in which CYP26A1 dominated. Quantification of CYP26A1 and CYP26B1 protein in human tissues was in agreement with the mRNA expression and showed correlation between the two isoforms. Qualitatively, recombinant CYP26A1 and CYP26B1 formed the same primary and sequential metabolites from *at*RA. Quantitatively, CYP26B1 had a lower K_m (19 nM) and V_{max} (0.8 pmol/min/pmol) than CYP26A1 (K_m = 50 nM and V_{max} = 10 pmol/min/pmol) for formation of 4-OH-RA. The major *at*RA metabolites 4-OH-RA, 18-OH-RA and 4-oxo-RA were all substrates of CYP26A1 and CYP26B1, and CYP26A1 had a 2–10-fold higher catalytic activity towards all substrates tested. This study shows that CYP26A1 and CYP26B1 are qualitatively similar RA hydroxylases with overlapping expression profiles. CYP26A1 has higher catalytic activity than CYP26B1 and seems to be responsible for metabolism of *at*RA in tissues that function as a barrier for *at*RA exposure.

1. Introduction

Retinoic acid (RA), the active metabolite of vitamin A, is a critical signaling molecule in animals. RA exists as at least four different isomers: *all-trans*-RA (*at*RA), 9-*cis*-RA, 13-*cis*-RA, and 9,13-*dicis*-RA. Of these, *at*RA is considered to be the biologically active isomer, but 9-*cis*-RA and 13-*cis*-RA also have activity and are marketed as drugs [1]. *at*RA acts by binding to retinoic acid receptors (RARs) regulating gene transcription, thereby having effects on cell cycle and cell survival [1–3]. The observed effects of *at*RA are dependent on its concentrations in the cell as well as on expression levels of RAR isoforms. During development, deficiency or overexposure to RA has detrimental effects resulting in fetal malformations [4,5]. In children and adults, RA signaling is associated with maintenance of immunity and of the epithelia, reproduction and spermatogenesis, regulation of glucose homeostasis, cell cycle and apoptosis, and maintenance of neurogenesis and neuronal cell survival [1,6–8].

Dietary intake of *at*RA precursors, synthesis of *at*RA from retinol and retinal, and the metabolism of *at*RA collectively determine the concentration of *at*RA in specific tissues and cells. The CYP26 enzymes appear to be responsible for metabolism of *at*RA in all chordates and hence are believed to contribute to regulation of *at*RA homeostasis and signaling [9–11]. The CYP26 family consists of three isoforms: CYP26A1, CYP26B1 and CYP26C1, which fulfill different function but their specific roles are not well understood [9]. Recombinant CYP26A1 forms three primary hydroxylated products, 4-OH-RA, 18-OH-RA and 16-OH-RA, and a number of sequential metabolites from *at*RA [12–14]. Based on studies in transfected cell lines, both CYP26A1 and CYP26B1 are hydroxylases that form 4-OH-RA and 18-OH-RA from *at*RA [14–16], whereas CYP26C1 appears to

* Corresponding author at: Department of Pharmaceutics, University of Washington, Box 357610, Seattle, WA 98195-7610, United States.
Tel.: +1 206 543 2517; fax: +1 206 543 3204.
E-mail address: ni2@u.washington.edu (N. Isoherranen).
[1] These two authors contributed equally to this work.

0006-2952/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2011.10.007

prefer 9-*cis*-RA as a substrate [16]. The similar proposed catalytic characteristics of these isozymes are of interest because the amino acid sequence identity between the CYP26 proteins is only 42–51% [15,16], suggesting that there are structural differences between these proteins. However, no studies comparing the catalytic activities or the metabolites formed by recombinant CYP26A1 and CYP26B1 have been published. Since many oxidative metabolites, such as 4-OH-RA, 4-oxo-RA and 18-OH-RA, formed from *at*RA by P450s have pharmacological activity and bind to RARs [17], it is possible that different metabolites formed by the CYP26 isoforms contribute to their *in vivo* functional differences.

During mouse development, the expression of the CYP26 isoforms is distinct in a spatio-temporal manner [18,19]. In human fetal tissues CYP26A1 seems to be expressed exclusively in the brain whereas CYP26B1 was not present in the brain but found in all other tissues tested. *Cyp26a1*$^{-/-}$ and *Cyp26b1*$^{-/-}$ mice have distinct phenotypes and CYP26A1 and CYP26B1, but not CYP26C1, are essential for healthy development [19–22]. The requirement of CYP26A1 and CYP26B1 for healthy development is likely due to specific expression patterns and different regulation. Whether the same applies to adult tissues is not known and the expression pattern of CYP26A1 and CYP26B1 in adult tissues is not well characterized. mRNA and protein data suggest that CYP26A1 expression is high in the liver whereas CYP26B1 mRNA is low or undetected in human liver [23–25]. Based on single donor mRNA, CYP26B1 has ubiquitous expression in adult human tissues [15]. Despite the detection of mRNA, no data on corresponding protein expression are available. However, *at*RA metabolism has been shown in rat testes, kidney, liver and lung microsomes [26], although the metabolite ratios were different between tissues suggesting different CYPs contribute to the metabolism in different tissues. Based on these data, it was hypothesized that CYP26A1 and CYP26B1 have different catalytic characteristics and different tissue distribution in adult tissues.

To test whether CYP26A1 and CYP26B1 are functionally similar *in vitro* and have distinct tissue expression patterns, CYP26A1 and CYP26B1 mRNA and protein expression were characterized in selected human tissues, CYP26A1 and CYP26B1 were expressed using baculovirus infected insect cells, the kinetics of formation of 4-OH-RA and 18-OH-RA were examined, and the elimination of 4-OH-RA, 4-oxo-RA and 18-OH-RA by CYP26A1 and CYP26B1 were determined. In addition, the sequential metabolites formed from *at*RA by the two CYPs were identified. The results provide the first characterization of recombinant CYP26B1 and the first comparison of the function and expression patterns of CYP26A1 and CYP26B1.

2. Materials and methods

2.1. Chemicals and enzymes

*at*RA, acitretin, cholic acid, imidazole and NADPH were purchased from Sigma–Aldrich (St. Louis, MO). 4-OH-RA and 4-oxo-RA (Fig. 1) were synthesized as previously described [13,27]. Rat P450 reductase was expressed in *Escherichia coli* and purified as previously reported [28]. CYP26A1 was expressed in Sf9 cells and microsomes were prepared as previously described [13]. All Supersomes® were co-expressed with reductase and, with the exception of CYP4A11, CYP1A1, CYP1A2, CYP2C18 and CYP2D6, with cytochrome b5 and purchased from BD Gentest (BD Biosciences, Woburn, MA). Nonidet P40, phenymethanesulfonyl fluoride (PMSF) and complete EDTA-free protease inhibitor cocktail tablets were purchased from Roche (Indianapolis, IN). Potassium chloride was purchased from Fisher Scientific (Pittsburgh, PA). Sodium phosphate and glycerol were obtained from JT

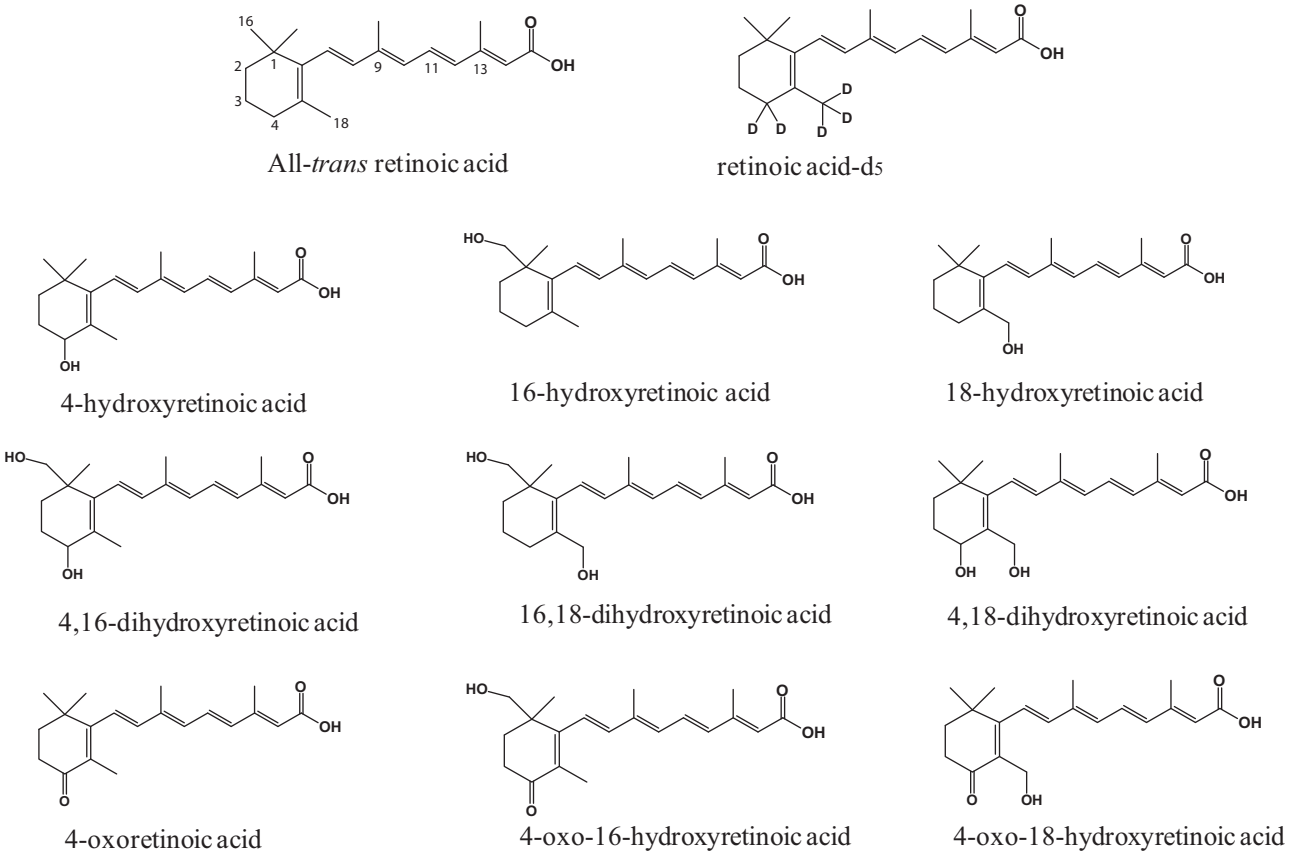

Fig. 1. Chemical structures of *at*RA, *at*RA-d_5, and detected or proposed *at*RA metabolites.