Archives of Biochemistry and Biophysics 523 (2012) 9–18

Contents lists available at SciVerse ScienceDirect

Archives of Biochemistry and Biophysics

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

Review

25-Hydroxyvitamin D-24-hydroxylase (CYP24A1): Its important role in the degradation of vitamin D

Glenville Jones *, David E. Prosser, Martin Kaufmann

Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, Canada K7L 3N6

ARTICLE INFO

Article history:
Available online 12 November 2011

Keywords:
CYP24A1
25-Hydroxyvitamin D_3
1,25-Dihydroxyvitamin D_3
Catabolism
Gene mutations
Idiopathic infantile hypercalcemia
CYP24A1 null-mouse
FGF-23

ABSTRACT

CYP24A1 is the cytochrome P450 component of the 25-hydroxyvitamin D_3-24-hydroxylase enzyme that catalyzes the conversion of 25-hydroxyvitamin D_3 (25-OH-D_3) and 1,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$) into 24-hydroxylated products, which constitute the degradation of the vitamin D molecule. This review focuses on recent data in the CYP24A1 field, including biochemical, physiological and clinical developments. Notable among these are: the first crystal structure for rat CYP24A1; mutagenesis studies which change the regioselectivity of the enzyme; and the finding that natural inactivating mutations of CYP24A1 cause the genetic disease idiopathic infantile hypercalcemia (IIH). The review also discusses the emerging correlation between rising serum phosphate/FGF-23 levels and increased CYP24A1 expression in chronic kidney disease, which in turn underlies accelerated degradation of both serum 25-OH-D_3 and 1,25-$(OH)_2D_3$ in this condition. This review concludes by evaluating the potential clinical utility of blocking this enzyme with CYP24A1 inhibitors in various disease states.

Introduction

The mitochondrial enzyme, 25-hydroxyvitamin D_3-24-hydroxylase was first described in the early 1970s and initially believed to be involved solely in the renal 24-hydroxylation of 25-OH-D_3 [1]. Work performed over the last 35 years has shown that the enzyme activity is the result of a combination of three components: ferredoxin, ferredoxin reductase and cytochrome P450, only the latter component, referred to as CYP24A1, being specific for this reaction [2,3]. It is now known that CYP24A1 catalyzes the conversion of both 25-OH-D_3 and 1,25-$(OH)_2D_3$ into 24-hydroxylated products targeted for excretion along well-established pathways. We review current knowledge of the structure and function of this protein as well as providing an update on the latest information regarding the physiological and clinical importance of CYP24A1.

CYP24A1: biochemistry and catalytic properties

Though, CYP24A1 was initially referred to as the 25-hydroxyvitamin D_3-24-hydroxylase, work with the recombinant enzyme has shown that it is able to catalyze multiple hydroxylation reactions at carbons C-24 and C-23 of the side chain of both 25-OH-D_3 and its hormonal form, 1,25-$(OH)_2D_3$ [2,3]. Indeed, our view of the role of CYP24A1 has expanded greatly to suggest that this single P450, alone, is responsible for the 5-step, 24-oxidation pathway from 1,25-$(OH)_2D_3$ to produce calcitroic acid, a known biliary catabolite [4,5], as well as catalyzing a similar pathway which starts with 23-hydroxylation and culminates in the 1,25-$(OH)_2D_3$-26,23-lactone (Fig. 1) [6,7]. In addition, CYP24A1 also efficiently hydroxylates the vitamin D_2 side chain of 25-OH-D_2 and 1,25-$(OH)_2D_2$ to give a more limited series of polyhydroxylated products [8,9]. The 24- and 23-products of the vitamin D_3 side chain appear in a specific order, reinforcing the concept of two distinct pathways initiated by a species-dependent C-24 or a C-23 hydroxylation step. Fig. 2 depicts a partial amino acid sequence alignment of CYP24A1 from 57 species covering bony fish to man and showing an impressive conservation of residues for at least a good part of the protein. Of particular note, is the dichotomy that exists at residue 326 where most species of CYP24A1 have Ala326 and exhibit 24-hydroxylation to calcitroic acid while a number of more primitive organisms have Gly326 and show predominantly 23-hydroxylation to give a 26,23-lactone product. The functional significance of two distinct pathways in different species is unknown [10].

CYP24A1: crystal structure, homology modeling and mutagenesis studies

In 2010, the crystal structure of the rat CYP24A1 was elucidated but in the presence of the detergents Cymal and CHAPS [11]. Although the active site of rat CYP24A1 did not contain its natural substrate, for the most part the crystal structure did confirm the

* Corresponding author. Address: Department of Biomedical and Molecular Sciences, Botterell Hall, Room 651, Queen's University, Kingston, ON, Canada K7L 3N6. Fax: +1 613 533 2022.
E-mail address: gj1@queensu.ca (G. Jones).

0003-9861/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.abb.2011.11.003

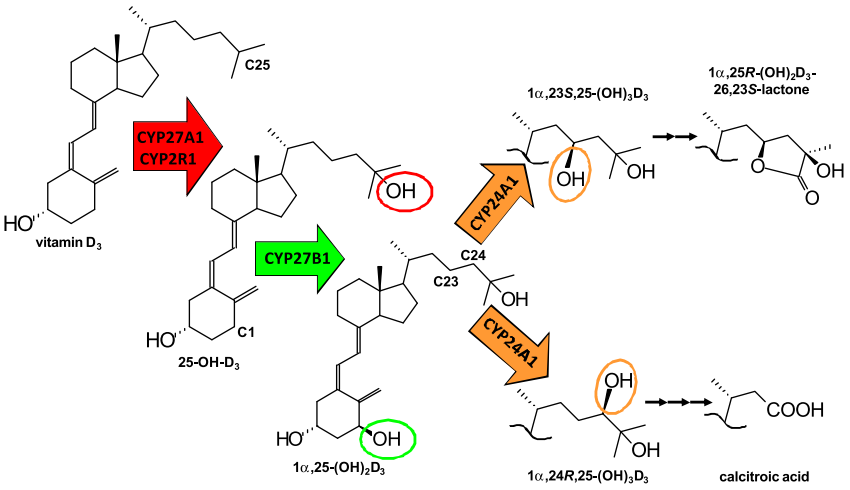

Fig. 1. Important steps in the pathway of vitamin D metabolism.

predicted tertiary structure of the protein, as well as the putative active-site residues from previous homology models and mutagenesis studies [10,12–15]. The crystal structure of rat CYP24A1 reveals a canonical cytochrome P450 structure of helices and β-sheets surrounding a prosthetic heme group and a substrate binding cavity. Virtually all of the protein is required to maintain the shape, structure, heme-binding, and function of the enzyme. The crystal structure of the rat CYP24A1 enzyme is shown in Fig. 3 with 1,25-$(OH)_2D_3$ (sticks and spheres; purple) positioned using state-of-the-art docking software into the wide-open cleft that constitutes the substrate binding cavity [11].

Even before the crystal structure of CYP24A1 was determined, mutagenesis studies were initiated based upon the remarkable conservation of structure across cytochrome P450s. Sakaki and colleagues who had shown that rat CYP24A1 is primarily a C24-hydroxylase, as compared to the human enzyme which is capable of both C24- and C23-hydroxylation [7], performed a follow-up study in which they mutated T416M and I500T in the β3b- and β5-sheets, respectively to try to change the properties of the rat enzyme by substituting amino acids to those found in the human enzyme [12]. They postulated that these residues interact with A-ring and cis-triene moieties of the 1α,25-$(OH)_2D_3$-docked substrate. The T416M and I500T caused significant changes in the C24:C23 hydroxylation ratio from 100:1 to 12.5:1 or 6.3:1, respectively, whereas, in their hands, the wild-type human enzyme had a ratio of 3.7:1 [12]. The observation that these modifications did not completely shift the regioselectivity of the rat enzyme from being a 24-hydroxylase to that of the human ortholog with significant 23-hydroxylase activity suggests that other species-based differences might act individually or in concert with residues at 416 and 500 to explain the observed enzymatic differences between human and rat CYP24A1 forms.

Prosser et al. [10] adopted a somewhat similar approach to study differences between the human and opossum CYP24A1, the latter being representative of the orthologs that are predominantly 23-hydroxylases, with a C24:C23 hydroxylation ratio of 0.04:1. Using the human CYP24A1 as a starting point, they noted 80 amino acid differences between human and opossum CYP24A1 but focused on mutating Ala326 to Gly326 found in the opossum and many other 'primitive' CYP24A1s (see Fig. 2) because this residue occupied a critical position directly above the heme in the I-helix that abuts the side chain of 1α,25-$(OH)_2D_3$ docked within a homology model of hCYP24A1 (see Fig. 3). The single A326G substitution radically changed the metabolic pattern observed for the resultant enzyme (Fig. 4A and B) by changing the enzyme properties from a 24-hydroxylase with a C24/C23 hydroxylation ratio = 8.1:1 to a 23-hydroxylase with a C24/C23 hydroxylation ratio = 0.12:1, a value that closely resembled opossum CYP24A1 (0.04:1). Thus it appeared that amino acid residue 326 alone was likely responsible for much of the regioselectivity difference observed between human and opossum CYP24A1 orthologs. Further docking studies comparing the positions of 1α,25-$(OH)_2D_3$ docked for optimal C24- versus C23-hydroxylation suggested that the loss of a methyl group from the amino acid at 326 in the I-helix by substituting Gly for Ala, provides extra space for the side chain of 1α,25-$(OH)_2D_3$ to slide deeper into the substrate-binding cavity in order to optimally place C23 as opposed to C24 above the heme (Fig. 4C and D), and committing catabolism through to 1α,25-$(OH)_2D_3$-26,23-lactone. The striking impact of A326G on regioselectivity is logical, given its direct contact with the substrate side-chain directly above the heme – as compared with Ile500 and Met416 located in the distal substrate access channel.

Mutations at other sites in human CYP24A1 that have been shown to modulate the regioselectivity of the enzyme include Ile131, Leu148, Met246, and Val391 [13]. In mutagenesis studies of residues over a single turn of the F-helix forming the top of the substrate binding cavity of rat CYP24A1 performed by Annalora and colleagues [14,16], it was shown that mutations at sites facing away from the cavity (Met245, Ser247, Thr248) retained 1α,25-$(OH)_2D_3$ binding affinity similar to the wild-type, whereas mutations at sites Phe249 and Met246 directly protruding into the cavity, impaired substrate binding to different degrees. Based upon the work of Annalora et al. [14,16], CYP24A1 is a 1α,25-$(OH)_2D_3$-binding protein first, and a catabolic enzyme second. All of these residues including Ala326 and Ile500, originally selected on the basis of homology modeling [10,12–15] as putative substrate contact points, have been implicated in forming the CHAPS-containing substrate-binding site in the crystal structure of rat CYP24A1 [11]. A recent report [17] suggests that a V391L mutation in the human CYP24A1 also changes enzymatic properties by introducing 1α-OH-D_3-25-hydroxylase activity absent in the wild-type enzyme and ascribes this to a combination of a change in the position of substrate within the active site and altered substrate binding affinity [10,16].

Taken together, the generation of homology models for CYP24A1 and mutagenesis studies have led to an unprecedented understanding of the amino-acid architecture of the substrate-binding pocket, many aspects of which have been confirmed by