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Review

Vitamin D 25-hydroxylase – Four decades of searching, are we there yet?

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ABSTRACT

Bioactivation of vitamin D_3 involves 25-hydroxylation and subsequent 1α-hydroxylation to produce 1α,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$], the active hormone. Six cytochrome P450 enzymes (CYP27A1, CYP2R1, CYP2J2/3, CYP3A4, CYP2D25 and CYP2C11) catalyzing the initial 25-hydroxylation step are reviewed, and their physiological relevance as vitamin D 25-hydroxylases *in vivo* is discussed.

Vitamin D_3 (Fig. 1), the inert secosteroid precursor, is synthesized in the skin from 7-dehydrocholesterol in response to ultraviolet irradiation followed by thermal isomerization, or can be obtained from dietary intake. Vitamin D_2 (Fig. 1), a plant derived vitamin D, is not naturally produced in the body and has to be acquired exogenously. Both vitamin D_3 and vitamin D_2 play a central role in calcium homeostasis and maintaining bone health.

Bioactivation of vitamin D_3 involves two sequential hydroxylation steps catalyzed by 25-hydroxylase and 1α-hydroxylase to produce 25-hydroxyvitamin D_3 [25$(OH)D_3$] in the liver and subsequently, the hormonally active 1α,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$][1] mainly in the kidney, respectively (Fig. 2). The serum level of 1,25$(OH)_2D_3$ (25–60 pg/mL) [1] is tightly controlled through transcriptional regulation of 1α-hydroxylase modulated by a vast range of cellular agents as well as autoregulatory feedback. The concentration of 25$(OH)D_3$ (20–60 ng/mL) [1,2], the major circulating form of vitamin D_3, does not seem to be under much metabolic control and reflects intake of vitamin D_3, thus serving as indicator of vitamin D status.

Since the isolation and identification of 25$(OH)D_3$ and 1,25$(OH)_2D_3$ four decades ago [3–6], tremendous effort has been expended on searching the enzymes responsible for vitamin D activation. It was not until 1997 when CYP27B1 was cloned from various species including human [7–11] and later confirmed as the sole 25$(OH)D_3$ 1α-hydroxylase in all tissues. The vitamin D 25-hydroxylase, on the other hand, remains elusive to this day. This review will focus on the potential candidates of vitamin D 25-hydroxylase in perspective of their evolutionary conservation, enzymatic properties, and physiological and pathological relevance.

Tissue distribution and subcellular localization

Liver was established as the major site of 25-hydroxylation using radiolabeled vitamin D_3 and based on the fact that production of 25$(OH)D_3$ in hepatectomized rats was significantly reduced [12,13]. Although extrahepatic activities of 25-hydroxylase were evident in a number of tissues such as kidney and intestine [13–16], their endocrine contribution is not clear, and the physiological implications are likely limited to the autocrine/paracrine functions to regulate tissue specific processes. Earlier work reported contradictory subcellular localization of 25-hydroxylase in the liver [17–20] mostly due to cross contamination of microsomal and mitochondrial fractions as well as low and labile activities of the crude preparations. It is now generally believed that both microsomes and mitochondria contain 25-hydroxylases with different catalytic properties.

General enzymatic properties

Initial studies on the 25-hydroxylation of vitamin D_3 using fractioned liver homogenate suggested that the enzyme was NADPH-dependent and required cytosolic component for maximal activity [17,18]. Incorporation of ^{18}O from molecular oxygen provided strong evidence for a mixed-function monooxygenase [18,21]. Finally, marked inhibition of the reaction was observed in the presence of CO [18,19]. All these findings demonstrated the participation of a cytochrome P450.

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[1] *Abbreviations used*: 1,25$(OH)_2D_3$, 1α,25-dihydroxyvitamin D_3; 25(OH)D3, 25-hydroxyvitamin D_3; 1α$(OH)D_3$, 1α-hydroxyvitamin D_3; 1α$(OH)D_2$, 1α-hydroxyvitamin D_2; CTX, cerebrotendinous xanthomatosis.

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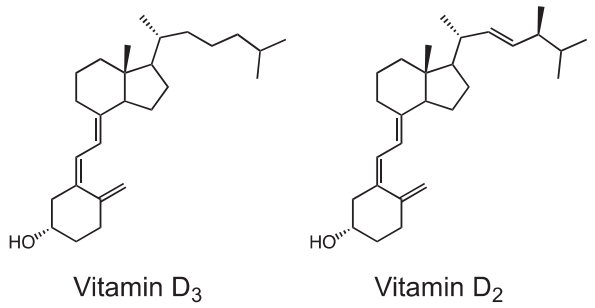

Fig. 1. Structures of vitamin D_3 and vitamin D_2.

The majority of mammalian P450s are associated with intracellular membranes. The microsomal 25-hydroxylases are anchored in the endoplasmic reticulum and the mitochondrial ones are located in the inner membrane of mitochondria [22]. Both activities are part of the electron transfer systems, involving either a single P450 reductase (microsomal) or a relay chain comprising ferredoxin reductase and ferredoxin (mitochondrial) (Fig. 3).

Candidates for vitamin D 25-hydroxylase

CYP27A1

CYP27A1 is the only mitochondrial enzyme exhibiting vitamin D 25-hydroxylase activity. Studies on CYP27A1 in the vitamin D activation pathway coincided with previous work of sterol 27-hydroxylase, which was detected in the early 1970s [23–25] and implicated in cholesterol metabolism and bile acid synthesis. Purification of sterol 27-hydroxylase and vitamin D 25-hydroxylase was achieved separately from rabbit and rat liver mitochondria [26–30]. It was quickly demonstrated that both enzymatic activities were purified concomitantly although 27-hydroxylation activity of 5β-cholestane-3α,7α,12α-triol was much higher than 25-hydroxylation of vitamin D_3, with turnover numbers of 25–36 min^{-1} and 0.35–0.395 min^{-1}, respectively [27–29,31]. Additional evidence that heat-induced denaturation and chemical modification resulted in reduction of the hydroxylation activities accordingly, and that the substrates acted as inhibitors of the parallel reactions, suggested that a common enzyme was responsible for both activities [31]. However, a monoclonal antibody raised against the 27-hydroxylase did not inhibit 25-hydroxylation of vitamin D_3 [28].

Molecular cloning of *cyp27a1* gene in rabbit [32], rat [33,34], and human [35,36] confirmed that the previously purified sterol 27-hydroxylase and vitamin D 25-hydroxylase were indeed the same enzyme of ~500 amino acids and with a molecular weight of 50–55 kDa for the mature protein. CYP27A1 is found in many tissues including kidney, intestine, ovary, lung, and skin as well as liver [37]. Heterologous expression of CYP27A1 using transfected mammalian COS cells, yeast, and *Escherichia coli* revealed CYP27A1 as a multifunctional P450 with broad substrate specificity and able to catalyze multiple oxidation reactions *in vitro* [35,36,38–42]. It was highly active in the pathway of bile acid

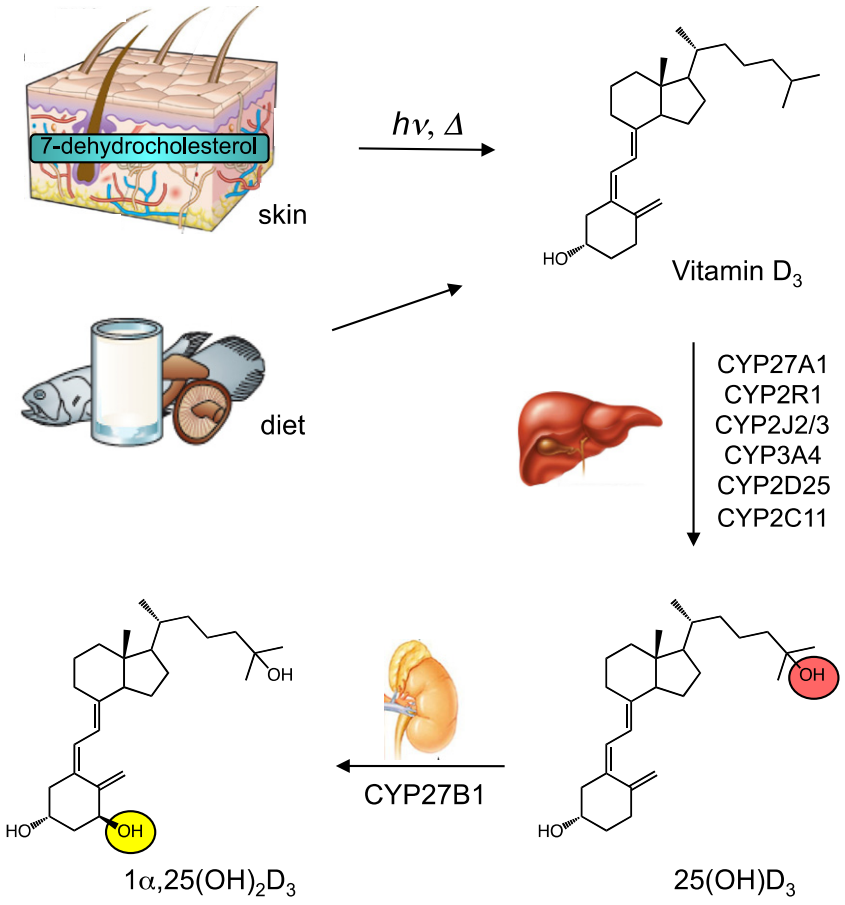

Fig. 2. Vitamin D_3 activation pathway. Vitamin D_3 is converted to the active metabolite $1,25(OH)_2D_3$ by sequential 25-hydroxylation and 1α-hydroxylation catalyzed by vitamin D 25-hydroxylase and 1α-hydroxylase (CYP27B1), respectively. One mitochondrial (CYP27A1) and five microsomal (CYP2R1, CYP2J2/3, CYP3A4, CYP2D25, and CYP2C11) P450 isoforms are potential candidates of the 25-hydroxylase.