Stronger bonds between 25-hydroxyvitamin D 1 α -hydroxylase and pseudovitamin D-deficiency rickets

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The D vitamins (cholecalciferols) are 'pre-pro-hormones' which become metabolically active after a sequence of hydroxylations (1, 2). Ergocalciferol (vitamin D₂) is derived from plants, and cholecalciferol (vitamin D₃) is produced in humans and animals. Vitamin D refers to either or both compounds. Three principal enzymes are involved in the activation or inactivation of vitamin D, with respect to the vitamin D receptor, by hydroxylating carbon-25 (vitamin D 25-hydroxylase), carbon-1 in the α -position (25-hydroxyvitamin D 1 α -hydroxylase), or carbon-24 (25-hydroxyvitamin D 24-hydroxylase). The hydroxylases belong to a family of enzymes known as cytochrome P450 mixed function monooxygenase (3). Three distinct parts of the enzymes, ferredoxin reductase, ferredoxin, and cytochrome P450, make an electron transport chain that ends in a heme prosthetic group bound to the cytochrome P450 subunit. Reduction of the iron ion attached to the heme molecule enables the enzymes to carry out the hydroxylation reactions.

Vitamin D is initially hydroxylated in the liver by vitamin D 25-hydroxylase. The activity of this hydroxylase is not influenced by calcium or phosphate homeostasis. In renal tubular cells, 25-hydroxyvitamin D is either converted to 1 α ,25-dihydroxyvitamin D, which is the most potent naturally occurring vitamin D metabolite, or inactivated to 24,25-dihydroxyvitamin D by the enzymes 25-hydroxyvitamin D 1 α -hydroxylase or 24-hydroxylase respectively. These two enzymatic systems respond to many of the same regulators, but in opposite directions. Parathyroid hormone (PTH) is the most important stimulator of 1 α -hydroxylase activity, which is also stimulated by phosphate depletion, oestrogens, prolactin, growth hormone, insulin, and calcitonin. The product of the enzymatic reaction, 1 α ,25-dihydroxyvitamin D, attenuates enzyme activity via its receptor (1, 2).

Both rat 25-hydroxylase and 24-hydroxylase genes were cloned several years ago, whereas cloning of the 1 α -hydroxylase has been much more difficult due to its low level of expression (4, 5). Recently, two studies presented the cDNA sequences of the genes encoding mouse and rat 1 α -hydroxylase and the chromosomal location of the human counterpart of the gene. This represents a major break-through towards a genetic diagnosis of hereditary pseudovitamin D deficiency (vitamin D-dependent) rickets type I, a rare autosomal recessive disorder associated with defects in the 1 α -hydroxylase (6, 7).

In an elegantly designed study, Takeyama *et al.* (8) used kidney cells from mice lacking the vitamin D receptor in their approach to clone the 1 α -hydroxylase gene. These animals have abnormally high serum levels of 1 α ,25-dihydroxyvitamin D₃, indicating a high 1 α -hydroxylase activity and high levels of the 1 α -hydroxylase mRNA. Renal cell mRNA from these animals was used to make a cDNA expression library in an eukaryotic cell line (COS-1 cells). The cells were also transfected with a vector encoding a fusion protein constructed with the ligand-binding domain of the vitamin D receptor and the yeast GAL4 DNA-binding domain. In addition, the cells were transfected with a reporter plasmid expressing the enzyme β -galactosidase in response to GAL4 activation. Cells transfected with the 1 α -hydroxylase gene from the cDNA library converted 25-hydroxyvitamin D₃ added to the cell cultures to 1 α ,25-dihydroxyvitamin D₃, which subsequently activated the fusion protein and the expression of β -galactosidase. Cells expressing this enzyme were stained when incubated with X-Gal. Stained cells were harvested, and their cDNA originating from the renal cell library was amplified by PCR. The PCR products were purified, subcloned into a new vector and sequenced.

The cDNA sequences indicated that the mouse 1 α -hydroxylase has an open reading frame consisting of 507 amino acids and a molecular mass of 55 kDa. The protein has a mitochondrial target signal and is homologous to members of the cytochrome P450 family of enzymes. It has a putative sterol-binding domain and a heme-binding domain with between 60 and 93% sequence similarities to rat vitamin D 25-hydroxylase and mouse 25-hydroxyvitamin D 24-hydroxylase. Subsequent studies confirmed that the enzyme encoded by the cloned cDNA could convert 25-hydroxyvitamin D₃ to 1 α ,25-dihydroxyvitamin D₃.

Expression of the gene was limited to the kidneys in normal mice and was 50-fold higher in 7-week-old vitamin D receptor deficient mice. The transcript was not detected in cells and tissues previously reported to have 1 α -hydroxylase activity, such as macrophages and placenta. Interestingly, the cytochrome P450 enzyme CYP27 has also been shown to hydroxylate 25-hydroxyvitamin D₃ in the 1 α -position (9). Expression of the 1 α -hydroxylase gene was reduced in normal mice treated with 1 α ,25-dihydroxyvitamin D₃, but not in vitamin D receptor deficient mice, indicating that the

liganded vitamin D receptor down-regulates the level of 1α -hydroxylase.

St-Arnaud *et al.* (10) used a different approach and cloned the gene encoding 1α -hydroxylase from a cDNA library based on kidney mRNA from vitamin D deficient rats. Based on the assumption that the 1α -hydroxylase and 24-hydroxylase genes have similar sequences in the heme-binding domain, the library was screened with low stringency with a DNA probe encompassing this region of the rat 24-hydroxylase cDNA. The cDNAs obtained indicated an open reading frame of the rat 1α -hydroxylase of 1503 bp encoding a 501 amino acid protein of 55 kDa. Expression of the gene was upregulated in kidneys from mice treated with PTH and attenuated by treatment with 1,25-dihydroxyvitamin D₃.

A human genomic clone containing the 1α -hydroxylase gene was obtained using the rat cDNA as a probe. By fluorescent *in situ* hybridisation the labelled clone indicated that the human 1α -hydroxylase gene is located on the long arm of chromosome 12, region q13.1–q13.3. The locus for pseudovitamin D-deficiency rickets has been shown to be in the interval 12q13–q14 (11), which strongly suggests that mutations in the 1α -hydroxylase gene are responsible for this phenotype. The search for mutations that may link the gene with the disease has already started.

References

- 1 Henry HL, Dutta C, Cunningham N, Blanchard R, Penny R, Tang C *et al.* The cellular and molecular regulation of 1,25-(OH)₂D₃ production. *Journal of Steroid Biochemistry and Molecular Biology* 1992 **41** 401–407.
- 2 Bouillon R, Okamura WH & Norman AW. Structure–function relationships in the vitamin D endocrine system. *Endocrine Reviews* 1995 **16** 200–257.
- 3 Degtyarenko K. Structural domains of P450-containing monooxygenase systems. *Protein Engineering* 1995 **8** 737–747.
- 4 Usui E, Noshiro M & Okuda K. Molecular cloning of cDNA for vitamin D₃ 25-hydroxylase from rat liver mitochondria. *FEBS Letters* 1990 **262** 135–138.
- 5 Ohyama Y, Noshiro M & Okuda K. Cloning and expression of cDNA encoding 25-hydroxyvitamin D₃ 24-hydroxylase. *FEBS Letters* 1991 **273** 195–198.
- 6 Prader A, Illig R & Heierli E. Eine besondere Form der primären Vitamin D resistenten Rachitis mit Hypocalcämie und autosomal dominanten Erbgang: Die hereditäre Pseudomangelrachitis. *Helvetica Paediatrica Acta* 1961 **16** 452–468.
- 7 Fraser D, Kooh S, Kind P, Holick M, Tanaka Y & De Luca H. Pathogenesis of hereditary vitamin D-dependent rickets. *New England Journal of Medicine* 1973 **289** 817–822.
- 8 Takeyama K-I, Kitanaka S, Sato T, Kabori M, Yanagisawa J & Kato S. 25-Hydroxyvitamin D₃ 1α -hydroxylase and vitamin D synthesis. *Science* 1997 **277** 1827–1830.
- 9 Axén E, Postlind H, Sjöberg H & Wikvall K. Liver mitochondrial cytochrome-P450 CYP27 and recombinantly expressed human CYP27 catalyze 1- α -hydroxylation of 25-hydroxyvitamin D₃. *Proceedings of the National Academy of Sciences of the USA* 1994 **91** 10014–10018.
- 10 St-Arnaud R, Messerlian S, Moir J, Omdahl J & Glorieux F. The 25-hydroxyvitamin D 1- α -hydroxylase gene maps to the pseudovitamin D-deficiency rickets (PDDR) disease locus. *Journal of Bone and Mineral Research* 1997 **12** 1552–1559.
- 11 Labuda M, Labuda D, Korab-Laskowska M, Cole D, Zietkiewicz E, Weissenbach J *et al.* Linkage disequilibrium analysis in young populations: Pseudo-vitamin D-deficiency rickets and the founder effect in French Canadians. *American Journal of Human Genetics* 1996 **59** 633–643.

Note added in proof

The rat 1α -hydroxylase gene sequence was recently also reported by Shinki *et al.* (*Proceedings of the National Academy of Sciences of the USA* 1997 **94** 12920–12925). Monkawa *et al.* (*Biochemical and Biophysical Research Communications* 1997 **239** 527–533) cloned the human 1α -hydroxylase gene and showed that it consists of nine exons and is present as a single copy. Finally, Fu *et al.* (*Molecular Endocrinology* 1997 **11** 1961–1970) also cloned the human gene and established the molecular basis of pseudovitamin D-deficiency rickets by demonstrating that a patient with the disease was a compound heterozygote for two null mutations in the 1α -hydroxylase gene.