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Structures of cytochrome P450 vitamin D_3 hydroxylase (Vdh) before and after directed evolution

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Introduction

Vitamin D_3 hydroxylase (Vdh) isolated from the *Pseudonocardia autotrophica* is a cytochrome P450 catalyzing regio- and stereo-selective sequential hydroxylation of vitamin D_3 (VD_3): the conversion of VD_3 to 25-hydroxyvitamin D_3 ($25(OH)VD_3$) and then of $25(OH)VD_3$ to 1α,25-dihydroxyvitamin D_3 ($1\alpha,25(OH)_2VD_3$). The $1\alpha,25(OH)_2VD_3$ functions as a hormone with a critical role in maintaining calcium and phosphate homeostasis as well as in controlling the differentiation and proliferation of multiple cell type. Many symptoms associated with VD_3 deficiency and the VD metabolic disorder, which include psoriasis, osteoporosis, rickets, and hypoparathyroidism, are treated using $1\alpha,25(OH)_2VD_3$. Vdh is currently in practical use for the industrial production of $1\alpha,25(OH)_2VD_3$ by bioconversion. To realize the more effective bioconversion, a highly active Vdh mutant (Vdh-K1) was generated by directed evolution [1]. Vdh-K1 is a quadruple mutant (T70R/V156L/E216M/E384R) that is about 22 times more active in the hydroxylation of VD_3 to $25(OH)VD_3$ than wild-type Vdh (Vdh-WT) in *in vivo* bioconversion. Intriguingly, the four mutational points do not lie in the active site, but are scattered throughout the molecule. To address how these mutations effect the significant enhancement in Vdh activity and why the Vdh is able to catalyze sequential hydroxylation of VD_3, we have undertaken the crystallographic studies.

Methods

Vdh and its mutants were expressed by *Escherichia coli* or *Rhodococcus erythropolis*, and purified by Ni-affinity chromatography. All crystals were obtained by the hanging-drop vapor-diffusion method at 20°C. The structures were solved by the molecular replacement method. The detail experimental procedures were previously described [2].

Results and discussion

We have determined the structures of Vdh-WT in the substrate-free form and of the quadruple mutant Vdh-K1 generated by directed evolution in the substrate-free, VD_3-bound, and $25(OH)VD_3$-bound forms. Vdh-WT exhibits an open conformation with the distal heme pocket exposed to the solvent both in the presence and absence of a substrate, whereas Vdh-K1 exhibits a closed conformation in both the substrate-free and substrate-bound forms. The results suggest that the conformational equilibrium was largely shifted toward the closed conformation by four amino-acid substitutions scattered throughout the molecule. The substrate-bound structure of Vdh-K1 accommodates both VD_3 and $25(OH)VD_3$ but in an anti-parallel orientation. The occurrence of the two secosteroid binding modes accounts for the regio-selective sequential VD_3 hydroxylation activities.

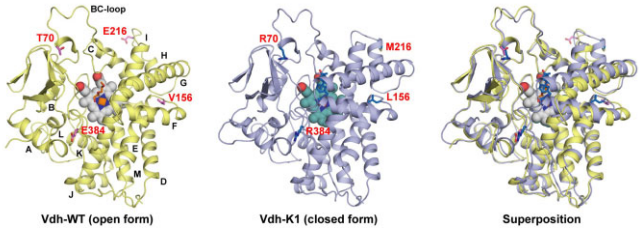

Fig. 1. Open Vdh-WT and closed Vdh-K1 structures. Superposition of these structures is also shown.

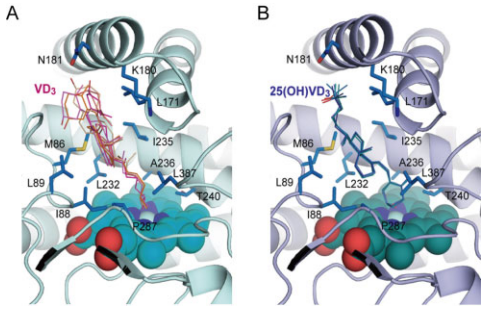

Fig. 2. VD_3 (A) and $25(OH)VD_3$ binding (B) to Vdh-K1. All five refined substrate models in the asymmetric unit are superposed and shown as thin sticks. Residues creating the substrate-binding pocket are shown in sticks and are labeled. The heme cofactor is the sphere.

References

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