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Structure of highly active T107A mutant of cytochrome P450 Vdh in complex with vitamin D_3

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1 Introduction

Vitamin D_3 (VD_3) is a secosteroid that plays an important role in many physiological systems in our human body. VD_3 is obtained from the diet or is synthesized from 7-dehydrocholesterol within the skin in response to sunlight. VD_3 is further converted to the active forms of VD_3: 25-hydroxyvitamin D_3 (25(OH)VD_3) in the liver, and then to 1α,25-dihydroxyvitamin D_3 (1α,25$(OH)_2VD_3$) in the kidney. Both steps are catalyzed by cytochrome P450s. The hydroxylated VD_3 and its derivatives are currently used as drugs for treating the symptoms of many diseases, such as rickets, osteomalacia, osteoporosis, hypoparathyroidism and psoriasis. Currently, microbial conversion system using actinomycete *Pseudonocardia autotrophica* cells is a practical use for the production of 25(OH)VD_3 and 1α,25$(OH)_2VD_3$ [1]. *P. autotrophica* possesses a vitamin D hydroxylating cytochrome P450 (P450 Vdh (CYP107BR1)). However, the catalytic activity of Vdh is very low, because VD_3 is a nonnative substrate for Vdh. To develop the more efficient biocatalytic production of hydroxylated VD_3 by using the recombinant system, we have obtained the highly active mutant of Vdh (Vdh-T107A) by engineering the putative ferredoxin binding site. The Vdh-T107A exhibited exceptionally high VD_3 25-hydroxylating activity and was ~80-fold more active than the wild-type Vdh. To investigate the structural basis for the activity enhancement by such a trivial mutation on the molecular surface, we have undertaken the crystallographic analysis of the Vdh-T107A.

2 Methods

Vdh-T107A was overexpressed by *Escherichia coli*, and purified by Ni-affinity and anion-exchanging chromatography. Crystals of Vdh-T107A mutant were obtained by hanging-drop vapor diffusion method. Plate-shaped crystals (0.20 × 0.40 × 0.02 mm) were obtained in the solution containing 0.1 M Bis-Tris, pH 6.0–7.0, 0.2 M NaCl, and 15–20% PEG 3350. X-ray diffraction data were collected on the beamline BL-5A at the Photon Factory, by using the Quantum210r detector (ADSC). Prior to data collection, the crystal was soaked in a cryoprotectant solution supplemented with 20% glycerol and flash-cooled under a nitrogen gas stream at 100 K. The crystal of the Vdh-T107A belongs to the space group $C222_1$, with unit-cell dimensions a = 61.4, b = 105.5, and c = 142.0 Å. The structure of Vdh-T107A was determined by the molecular replacement method with the program MOLREP. The X-ray model of Vdh-K1 (PDB code, 3A50 [2]) was used as a search model.

3 Results and discussion

The structure of Vdh-T107A complexed with VD_3 was determined by the molecular replacement to a resolution of 2.57 Å. The final refined model consists of amino-acid residues 2-403, heme cofactor, VD_3, and 36 water molecules with a crystallographic R factor and R_{free} factor of 0.187 and 0.231, respectively. The data collection and refinement statistics are summarized in Table 1. Atomic coordinates of Vdh-T107A were deposited in PDB under accession code 2VRM.

Table 1. Data collection and refinement statistics.

Data collection statistics	
Beamline	BL-5A, PF
Wavelength (Å)	1.0000
Resolution (Å)	50–2.57 (2.6–2.5)[a]
Unit-cell dimensions	
a, b, c (Å)	61.4, 105.5, 142.0
α, β, γ (°)	90.0, 90.0, 90.0
Space group	$C222_1$
Unique reflections	14,156
R_{sym}[b]	0.048 (0.557)[a]
Completeness (%)	98.6 (92.3)[a]
Redundancy	5.8 (5.6)
Mean $I/\sigma(I)$	24.2 (3.1)[a]
Refinement	
Resolution range (Å)	50–2.57
R_{work}[c]	0.187
R_{free}[d]	0.231
Total number of atoms	3,212
Average B-factor (Å^2)	76.7
r.m.s.d. bond distances (Å)	0.008
r.m.s.d. bond angles (°)	1.93

We have previously reported the structure of wild-type Vdh and its highly active quadruple mutant Vdh-K1 generated by directed evolution [2]. Crystal structure analyses showed that wild-type Vdh forms an open conformation, while the Vdh-K1 exhibits a closed conformation, both in the presence and absence of substrate. Interestingly, the structure of Vdh-T107A was

well superimposed on that of Vdh-K1 with a root mean square deviation (rmsd) of 0.7 Å for 390 Cα atoms (Figure 1). The results clearly show that Vdh-T107A also forms a closed conformation similar to Vdh-K1. The conformational dynamics between an open and closed state of P450 is considered to exist in solution, and thus, similar to Vdh-K1, the T107A mutation might lead to the shift in the conformational equilibrium towards a closed state. The molecular dynamic simulation is currently underway to demonstrate how such a trivial mutation contributes to the conformational dynamics of P450 fold.

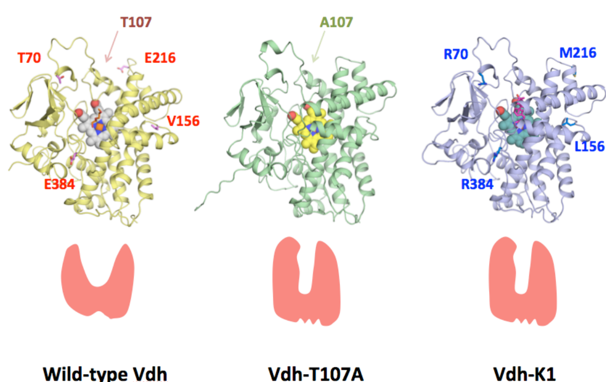

Figure 1. Comparison of structures between wild-type Vdh (open form), Vdh-T107A (closed form) and Vdh-K1 (closed form).

4 Conclusion

The crystal structure of Vdh-T107A showed a closed state similar to that of Vdh-K1, which is a highly active quadruple mutant generated by directed evolution. It is likely that the T107A mutation is responsible for the shift in the conformational equilibrium in solution from the open to the closed state of P450 fold. Highly efficient biocatalytic synthesis of hydroxylated VD_3 is expected by using the Vdh-T107 mutant.

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References

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