

X-ray structures of cytochrome P450 Vdh (CYP107BR1) inactive mutants

Yoshiaki Yasutake^{1*} and Tomohiro Tamura¹¹ Bioproduction Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Sapporo 062-8517, Japan

1 Introduction

Vitamin D₃ hydroxylase (Vdh) from the *Pseudonocardia autotrophica* is a cytochrome P450 monooxygenase (CYP107BR1) that catalyzes regio- and stereo-specific sequential hydroxylation of vitamin D₃ (VD₃) to produce 25-hydroxy VD₃ and 1 α ,25-dihydroxy VD₃. Hydroxylated VD₃ is currently used as a pharmaceutical for the treatment of symptoms associated with VD₃ deficiency and the VD metabolic disorder such as osteoporosis, rickets, and hypoparathyroidism. We have previously performed the directed evolution and site-directed mutagenesis studies to improve the activity level of Vdh, and successfully obtained the approximately 80 times more active Vdh-K1 mutant (T70R/V156L/E216M/E384R) and the T107A mutant [1, 2]. Crystal structures of those highly active mutants revealed the overall conformation was changed from open to closed states, thereby enabling high substrate binding affinity and catalytic activity. As juxtapositions, we have also obtained the complete inactive mutants F106V and L348M in the course of the site-directed mutagenesis study around the ferredoxin-binding site. These mutations are trivial but located in the vicinity of T107, a hot-spot residue enabling the dramatic activity improvement as described [2]. To investigate the structural mechanism of drastic increase/decrease of activity level of Vdh, we have undertaken the crystallographic studies of those inactive mutants.

2 Experiment

Vdh mutants were expressed by *Escherichia coli* and purified by Ni-affinity and anion-exchanging chromatography. All crystals were obtained by the hanging-drop vapor-diffusion method at 20°C. The data were processed by HKL2000 or iMosflm/SCALA. The structures were solved by the molecular replacement method using the structure of the wild-type Vdh (Vdh-WT) as a search model with the program MOLREP. The model refinement was performed by phenix.refine. The data of Vdh-L348M mutant was hemihedral twinning, so that we have performed the model refinement by REFMAC5 with twinning operators (-H, -K, L) and the fraction 0.35 to obtain the reasonable *R* and *R*_{free} values (below 30%). The data collection and refinement statistics are summarized in Table 1.

3 Results and Discussion

We have crystallized the Vdh-F106V in the same crystallization condition to that of Vdh-WT (Bis-tris pH 7.5, NaCl, CaCl₂ and PEG400). The space group and the unit-cell parameters of the Vdh-F106V crystal are also

same to those of Vdh-WT (*P*3₁; *a* = *b* = 62, *c* = 98 Å). Asymmetric unit contains one Vdh-F106V monomer. In contrast, Vdh-L348M crystallized in the different crystallization condition (Bis-tris pH7.5, NaCl, CaCl₂ and PEG 2000MME). The data was initially processed as a orthorhombic system *P*2₁2₁2₁, but the model refinement stuck at around *R*_{free} = 40%. We found that the L-test in the data merging stage suggested that the data was twinning. Therefore we reduced the symmetry with the space group *P*2₁, and the *R* and *R*_{free} values converged at below 30%. There are four Vdh-L348M monomers in the asymmetric unit.

Table 1: Data collection and refinement statistics

	Vdh L348M	Vdh F106V
BL	BL-17A	BL-17A
Wavelength (Å)	0.9800	0.9800
Resolution (Å)	50-2.70 (2.85-2.70)	50-2.10 (2.15-2.10)
Unit-cell		
<i>a, b, c</i> (Å)	81.2, 107.7, 88.4	61.7, 61.7, 98.2
α, β, γ (°)	90, 90, 90	90, 90, 120
Space group	<i>P</i> 2 ₁	<i>P</i> 3 ₁
<i>R</i> _{merge}	0.142 (0.482)	0.066 (0.463)
Completeness (%)	98.8 (98.8)	99.6 (99.3)
Redundancy	3.1 (3.1)	8.6 (8.5)
Mean <i>I</i> / σ (<i>I</i>)	8.4 (3.0)	51.2 (8.2)
Refinement		
<i>R</i> _{work}	0.220	0.177
<i>R</i> _{free}	0.280	0.224
Twinning op. (Twinning fraction)	-H, -K, L (0.35)	-
Average B (Å ²)	23.3	21.3

The overall structures of Vdh-F106V and L348M are well superimposed on that of Vdh-WT in open conformation. However, there is a notable rearrangement of the hydrogen-bond near the mutational residue. In the structures of Vdh-WT and highly active Vdh-K1 and Vdh-T107A mutants, main-chain N of F106 forms hydrogen-bond with main-chain O of G103 as a 3₁₀ helix manner. In contrast, the hydrogen-bond is broken in the structures of inactive Vdh-F106A and Vdh-L348M mutants, and alternatively, the main-chain N of F/V106 forms hydrogen-bond with main-chain O of V102, leading to the extension of α -helix C to residue 106 (Fig.

1). The conformational change from open to closed state is mostly made by relocation of α -helices D, E, F, G and H and thus the stabilization of the C-terminal end of α -helix C might suppress the mobility of the residues 106-108, and hinder the conformational dynamics of the Vdh molecule.

References

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- [2] Y. Yasutake *et al.*, ChemBioChem., 14, 2284–2291 (2013).

* y-yasutake@aist.go.jp

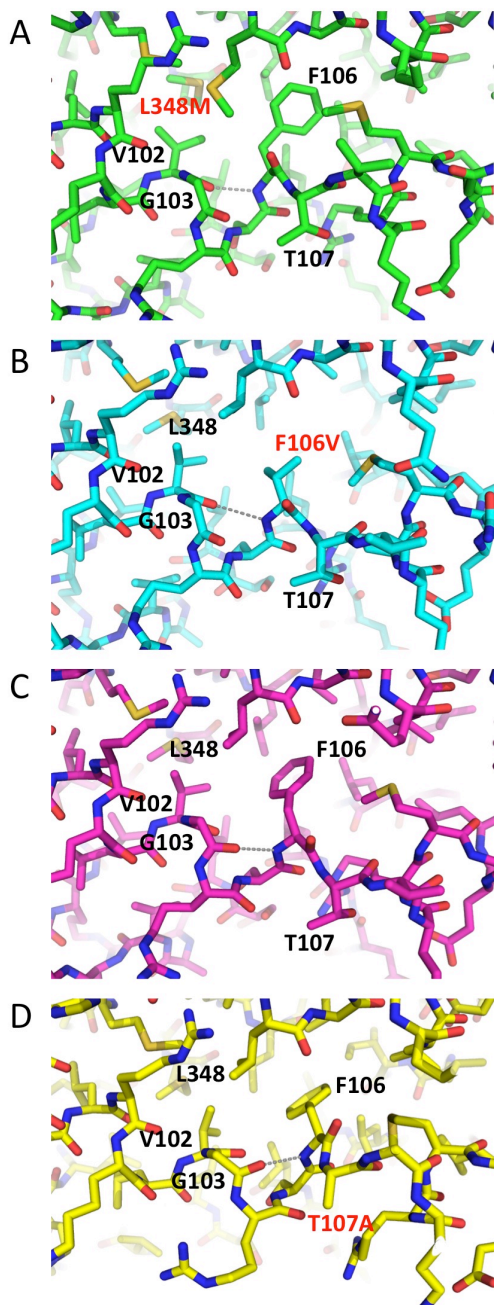


Fig. 1: Hydrogen-bonding rearrangements. (A) F106V, (B) L348M, (C) Vdh-WT and (D) Vdh-T107A.

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