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Vitamin D_3 (VD3) is a steroid hormone that plays a crucial role in bone metabolism, control of cell proliferation and differentiation in mammals. VD3 is activated by sequential hydroxylation at C25 and C1α, catalyzed by cytochrome P450 monooxygenases. In humans, three species of P450, CYP27A1, CYP27B1 and CYP2R1, are involved with the activation of the VD3 in liver and kidney. The activated form of VD3 (1α,25-dihydroxy VD3) is currently used as a pharmaceutics for osteoporosis, psoriasis, rickets and hypoparathyroidism. We have recently isolated the VD3 hydroxylating enzyme (VDH), a novel cytochrome P450 from actinomycete *Pseudonocardia autotrophica*. To investigate the substrate binding mechanisms enabling the sequential hydroxylation of VD3, we have undertaken the crystallographic studies. The recombinant VDH was overexpressed using *Rhodococcus erythropolis*, purified and crystallized. The crystals belong to the trigonal space group $P3_1$, with unit-cell parameters $a = b = 61.7$ and $c = 98.8$ Å. The structure of VDH in substrate-free form was solved by molecular replacement at 1.75 Å resolution, using the P450eryF structure as a search model. The VDH exhibits a typical P450 fold, and the clear electron density indicates that polyethylene glycol is bound to the active site. Possible substrate-binding residues of VDH were identified, which could be targeted for protein engineering to enhance the substrate affinity and catalytic activity. The crystallization experiments for the substrate complexes are currently under way. This work was supported by the Project on Development of Basic Technologies for Advanced Production Methods Using Microorganism Functions by the New Energy and Industrial Technology Development Organization (NEDO).

Keywords: crystal structure, cytochrome P450, vitamin D3

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Structure of the *E.coli* amidase AmiD and implications for the enzymatic mechanism of related enzymes

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AmiD is the fourth and last identified N-acetylmuramyl-L-Alanine amidase of *Escherichia coli*. This lipoprotein is anchored in the outer membrane and is not involved in cell separation during the bacterial division like the 3 other cytoplasmic amidases AmiA, AmiB and AmiC. AmiD's function is not clearly established but it could be part of the perpetual remodelling and recycling of the peptidoglycan in the eubacterial cells. The breaking down of peptidoglycan fragments could also reduce the innate immune responses triggered by the recognition of these fragments. Peptidoglycan Recognition Proteins (PGRPs) which share a common fold with AmiD are receptor proteins precisely fulfilling this function in a wide range of organisms. We present three structures of the *E.coli* N-acetylmuramyl-L-Alanine amidase AmiD: the active enzyme, the EDTA inactivated enzyme in complex with the substrate anhydro-N-acetylmuramic acid-L-Ala-D-γGlu-L-Lys and the active enzyme in complex with the L-Ala-D-γGlu-L-Lys peptide, a product of AmiD's activity. The AmiD structure shows two specific features of this enzyme compared to the rest of the family. It has a quite flexible N-terminal extension allowing for an easier reach of the peptidoglycan while inserted into the outer membrane. AmiD also has an extra C-terminal domain providing an extended geometrical complementarity for the substrate. The role of this domain for the specific activity of AmiD is not clear. Thanks to the structures of the complexes we propose a new slightly modified mechanism for the N-acetylmuramyl-L-Alanine amidases of this family.

Keywords: amidase complex, peptidoglycan degradation, N-acetylmuramyl-L-Alanine amidase

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Crystal structures of [NiFe] hydrogenase maturation proteins: HypC, HypD and HypE

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[NiFe] hydrogenases catalyze the reversible oxidation of molecular hydrogen and carry a $NiFe(CO)(CN)_2$ center at the active site. The assembly of the metal center requires specific auxiliary proteins: Hyp proteins (HypABCDFE). HypA and HypB are involved in the insertion of the Ni atom. HypE and HypF are involved in the synthesis of the cyanide ligand. HypC, HypD are required in the insertion and cyanation of the Fe atom. The whole pathway of the maturation of [NiFe] hydrogenase has been elucidated, but each step in the maturation is not fully understood. In order to elucidate the maturation process at an atomic resolution, we have determined the crystal structures of HypC, HypD, and HypE from *Thermococcus kodakaraensis* KOD1 [1]. The overall structure of HypC consists of an OB-fold like β barrel domain and a C-terminal α helix. Comparison of HypC molecules in the asymmetric unit shows that the C-terminal α helix is very flexible. The structure of HypE consists of two α/β domains and is similar to other PurM superfamily proteins. The C-terminal tail of HypE shows ATP-dependent large conformational changes. The structure of HypD is composed of two α/β domains and an Fe-S cluster binding domain. Conserved regions of HypD show its probable iron-binding and active sites for cyanation. Furthermore, the [4Fe-4S] cluster environment of HypD is shown to be quite similar to that of ferredoxin:thioredoxin reductase (FTR), indicating the existence of a redox cascade similar to the FTR system. These results provide deep insights into the cyanation reaction mechanism via thiol redox signaling in the HypCDE complex.

[1] Watanabe, S., Matsumi, R., Arai, T., Atomi, H., Imanaka, T., Miki, K., *Mol. Cell*, 2007, 27, 29

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