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Crystallographic analysis of complex structures of fungal denitrifying enzyme P450nor

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Introduction

P450nor (CYP55) functions as nitric-oxide reductase to reduce NO to N_2O, in the denitrification system of a fungus, *Fusarium oxysporum* [1].

$2NO + NAD(P)H + H^+ \rightarrow N_2O + NAD(P)^+ + H_2O$

P450nor can perform this reaction without the aid of other redox partners, directly transferring two electrons from NAD(P)H to bound heme [2]. The reaction is unusual as compared with other monooxygenase cytochrome P450s. Electron transfer from NAD(P)H to an one-electron redox center usually requires the aid of other redox partner(s) such as a flavoprotein. Therefore, the electron transfer catalyzed by P450nor apparently contradicts the common sense of biological electron transfer. Moreover, P450nor is shown to catalyze N_2O-forming "codenitrification" reaction [3].

To elucidate the reaction mechanism of this unusual P450 enzyme, we tried to obtain complex structures with the dinucleotide-cofactor or its analogues.

Manuscript preparation

We succeeded in crystallizing a complex form of P450nor with an NADH analogue, nicotinic acid pyridine dinucleotide (NAAD), utilizing a double mutant (Ser73Gly/Ser75Gly) of the enzyme [4], whose affinity for NAD(P)H and its analogues was remarkably improved. The dataset up to 1.8 Å resolution was collected at BL6A, and the crystal structure was refined to R = 20.6% and R_{free} = 24.3% [5] (Fig. 1). The complex structure provided conclusive evidence for the mechanism of the unprecedented electron transfer. Comparison of the structure with those of dinucleotide-free forms [6] revealed a global conformational change, not a hinge motion, accompanied by local movements caused by the binding of the pyridine nucleotide into the heme-distal pocket. Arg64 and Arg174 [7] fix the pyrophosphate moiety upon the dinucleotide-binding. Most of the charged residues in the pocket are clustered on the 'Arg64-side', whereas Arg174 is the sole charged residue on the opposite side. A salt bridge network (Glu71-Arg64-Asp88) at the 'Arg64-side' is shown to be crucial for a high catalytic turnover of this enzyme [8, 9]. Electron donor specificity for NADH and NADPH is maintained by the size of the side chains of the residues in the B'-helix, which is located on the top of the 'Arg64-side'. Stereo-selective hydride transfer from NADH to NO-bound heme [7] was strongly suggested from the structure, the nicotinic acid ring being fixed near the heme by the conserved Thr residue in the I-helix and the upward-shifted propionate side chain of the heme. A proton channel near the NADH channel is formed upon the dinucleotide-binding, which should direct continuous transfer of the hydride and proton.

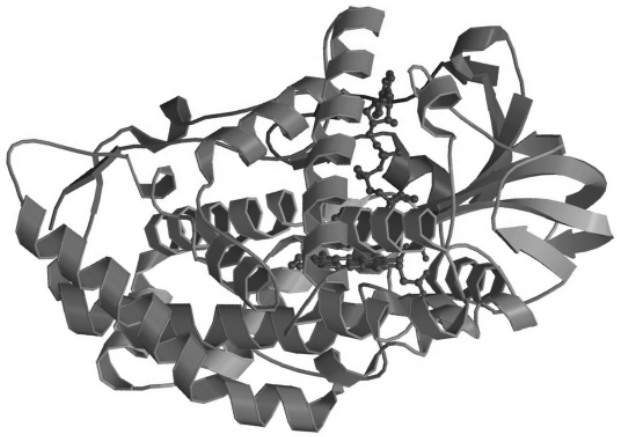

Figure 1 Ribbon diagram of the crystal structure of P450nor complexed with NAAD.

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