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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.

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

Results and Discussion

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 [3], 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% [4] (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 [5] 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 [6] 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]. 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.

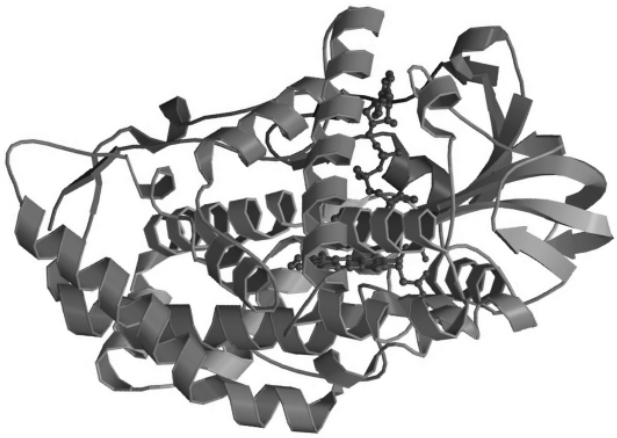

Figure 1 Ribbon diagram of the crystal structure of P450nor complexed with NAAD.

Reference

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