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Fungal denitrification and nitric oxide (NO) reductase P450nor

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Denitrification, together with nitrogen fixation and nitrification, forms the global nitrogen cycle that plays important roles in maintaining the global environmental homeostasis.

$N_2 \rightarrow NH_3$ (nitrogen fixation)

$NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^-$ (nitrification)

$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$ (denitrification)

Formerly, it was thought that only prokaryotes (bacteria) participate in the nitrogen cycle. However, it was found in my lab. about 10 years ago that many fungi can perform denitrification[1, 2]. Up to date the denitrifying systems of several fungal strains have been characterized[3-5]. It was shown by these studies that the fungal system is localized at the respiring organelle of eukaryote, the mitochondrion, and acts as anaerobic respiration to produce ATP like the bacterial systems do[4, 6]. One of the most characteristic properties of the fungal denitrifying system is the involvement of cytochrome P450 (P450) as nitric oxide reductase (P450nor)[7] that catalyzes the following reaction.

$$2NO + NADH + H^+ \rightarrow N_2O + H_2O + NAD^+$$

P450 usually acts as a monooxygenase as represented by the following reaction scheme, and two electrons from NAD(P)H are transferred to P450

$$S + O_2 + NAD(P)H + H^+ \rightarrow SO + H_2O + NAD(P)^+$$

via other protein components such as flavoprotein reductase. In case of P450nor, however, the electrons from NADH are transferred to the bound heme directly, and thus P450nor can catalyze the above reaction without the aid of other proteinaceous components. In spite of its exceptional function as P450 the tertiary structure of P450nor revealed by X-ray crystallography was fundamentally

the same as those of other P450s and thus it was conclusively shown to belong to the P450 superfamily[8]. The reaction scheme of P450nor can be devided into 3 steps as below[9].

step 1: Fe^{3+} + NO -> Fe^{3+} - NO

step 2: Fe^{3+} - NO + NADH -> *I* + NAD+

step 3: *I* + NO + H^{+} -> Fe^{3+} + N_2O + H_2O

The resting ferric enzyme (Fe^{3+}) binds the first substrate (NO) (step 1), and then the formed Fe^{3+}-NO complex is reduced by NADH to form an intermediate (*I*) that exhibits its Soret peak at 444 nm (step 2). Finally, *I* reacts with the second NO to form the product N2O (step 3). Although the overall structure is similar to those of other P450s the unique function of P450nor should be reflected to its structure. We found that a positive charge cluster that is comprised of many Lys and Arg residues and distributed outside and inside the heme-distal pocket plays an important role in the direct interaction with NADH[10]. Further, B'-helix was shown to play an important role in discriminating NADH and NADPH11.

Our results[12] along with recent progress in genome analyses revealed that P450nor is widely distributed among fungi, showing that denitrification generally occurs in fungi. These results along with our recent finding of fungal ammonia fermentation[13] first revealed that many fungi are facultative anaerobes, showing a sharp contrast to the up to date recognition that fungi are generally aerobic organisms.

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