一酸化窒素還元酵素の構造と機能：呼吸酵素の分子進化と機能変換

Structure and Function of Bacterial Nitric Oxide Reductase; Conversion of Function and Molecular Evolution of Respiratory Enzymes

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Respiration is a bio-process to gain bio-energy ATP for our life. In aneriobic respiration, a molecular oxygen (O_2) is reduced with four electrons and four protons to two water molecules, catalyzed by metal-enzymes, cytochrome c oxidase (C*c*O);

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$$

The reaction is catalyzed by Heme (Fe) - Cu binuclear center of CcO. Coupled with the O_2 reduction, C*c*O makes a proton gradient between the cellular membrane by the pumping mechanism. The proton gradient is eventually utilized for the ATP synthesis by ATPase. Molecular structures of mitochondrial and bacterial CcO have been already determined, and the catalytic and the proton pumping mechanisms have been extensively studied by many biochemical and physico-chemical techniques.

C*c*O in the aerobic respiration corresponds to nitric oxide reductase (NOR) in denitrification (anaerobic respiration) of bacteria, in which nitrate (NO_3^-) and/or nitrite (NO_2^-) are converted into nitrogen (N_2): $NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$. In this bio-process, nitric oxide (NO) is intermediately produced, but should be rapidly decomposed into nitrous oxide (N_2O) because of its high toxicity to cells. NOR, which contains the heme (Fe) - non-heme Fe binuclear center, can catalyze the conversion of NO to N_2O with two protons and two electrons, as follows;

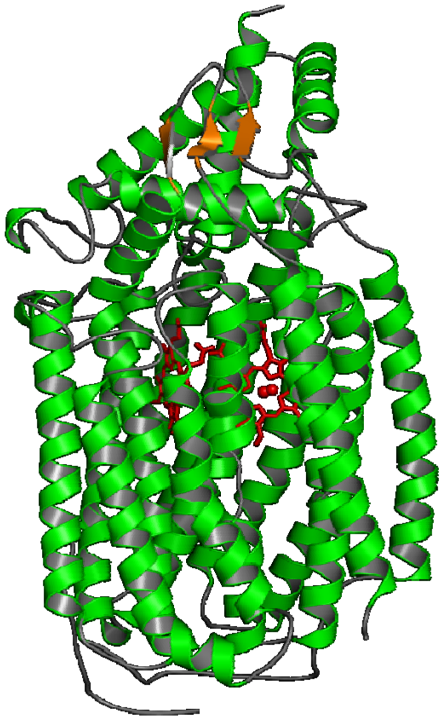

Figure 1: Crystal Structure of NOR from *Bacillus stearothermophilus*

$$2NO + 2H^+ + 2e^- \rightarrow N_2O + H_2O$$

However, NOR does not have the proton pumping ability. The primary structure of NOR is identical by about 20% to those of C*c*O. Thus it has been believed that NOR shares a common ancestor with C*c*O in the evolution.

Most recently, we succeeded in structural determination of bacterial NOR, which is a first structure of the ancestral respiration enzyme. The overall fold of the transmembrane region (Figure 1) and the position of the binuclear (catalytic) center are similar to those of CcO. However, the coordination of the non-heme metal is much different from that of the Cu in CcO, as shown in Figure 2. The difference might reflect difference of the catalytic function between both respiration enzymes. We will discuss the reaction mechanism of the NO reduction we proposed (Figure 3)[1] on the basis of our crystal structure of NOR.

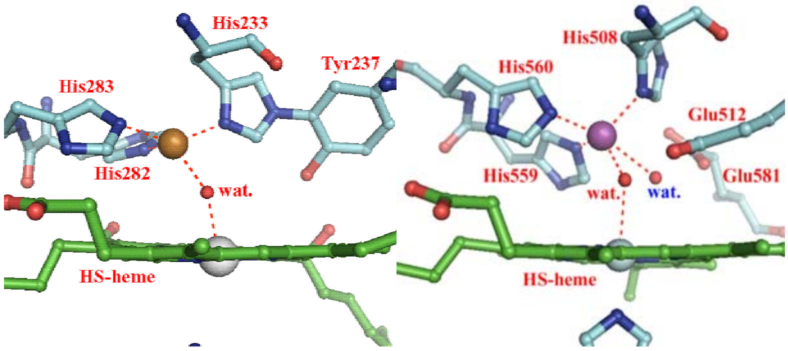

Figure 2: Structures of catalytic sites of NOR (left) and C*c*O (right)

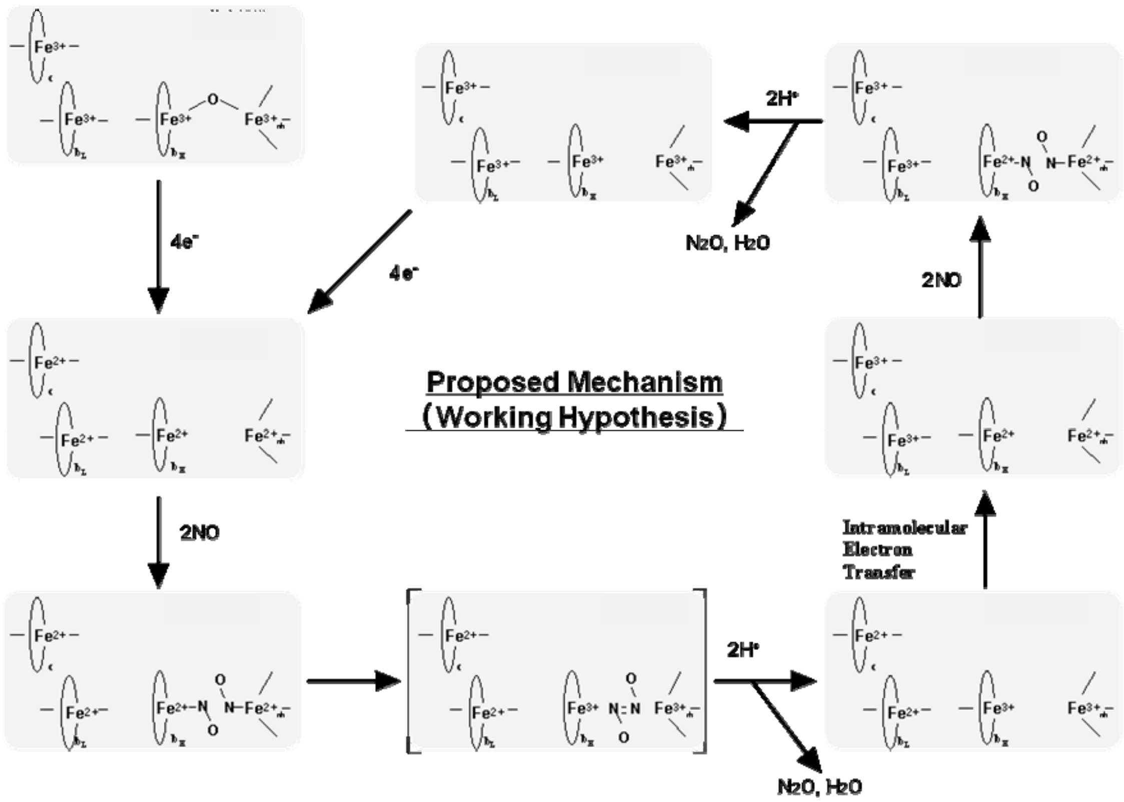

Figure 3: NO Reduction Mechanism Catalyzed by NOR, Which We Proposed as a Working Hypothesis[1]

Reference

1. H. Kumita, K. Matsuura, T. Hino, S. Takahashi, H. Hori, Y. Fukumori, I. Morishima, Y. Shiro, *J. Biol. Chem.* **279**, 55247-55254 (2004)