

【Grant-in-Aid for Scientific Research (S)】
Science and Engineering (Chemistry)



Title of Project : Molecular Science of NO Dynamics in Biological System

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Research Project Number : 26220807 Researcher Number : 70183051

Research Area : Bio-related Chemistry, Bioinorganic Chemistry

Keyword : Nitric oxide, Enzymatic Reaction, Respiratory enzymes, Nitrous oxide

【Purpose and Background of the Research】

Nitric oxide (NO) plays a crucial role on the signal-transduction in mammalian cells. On the other hand, since it has a radical character, it readily reacts with many bio-molecules, giving a sever cell damage. In this project, we will study on the NO dynamics (generation, propagation and decomposition) in cell by using bacterial denitrification system as a model. The denitrification is a kind of microbial anaerobic respiration, in which NO_3^- and NO_2^- are converted into N_2 in the stepwise reduction. In this process, nitrite reductase (NiR) can reduce NO_2^- to NO ($\text{NO}_2^- + 2\text{H}^+ + \text{e}^- \rightarrow \text{NO} + \text{H}_2\text{O}$), followed by reduction to N_2O ($2\text{NO} + 2\text{H}^+ + 2\text{e}^- \rightarrow \text{N}_2\text{O} + \text{H}_2\text{O}$) by nitric oxide reductase (NOR). NiR and NOR are targets of this projects.

【Research Methods】

1. Molecular Mechanism of NO Reduction by NOR: Based on the molecular structure of NOR we determined previously, we will study on the NO reduction mechanism by NOR. Especially, using the time-resolved techniques, we will challenge to determine the structure and electronic states of the short-lived intermediate (NO-bound form) in the enzymatic reaction ($\tau_{1/2} \sim 1$ ms).

2. Molecular Mechanism of NO Propagation: To propagate NO from NiR (NO-generating enzyme) to NOR (NO-decomposing enzyme), we assume that these two enzymes must interact with each other. The NiR-NOR complex formation will be studied for the purified enzymes by crystallographic and biophysical techniques. The complex formation in cell will be

also visualized using fluorescent-microscope. Structural determination of super-complex of NiR-NOR

with the electron donor will be also challenged, which will show presence of the continuous

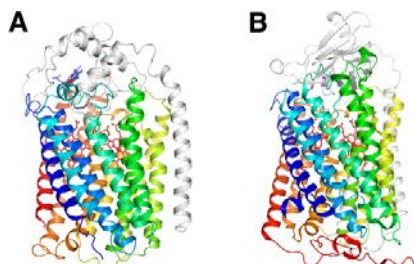


Figure 1: Crystal Structures of A. Nitric Oxide Reductase & B. Cytochrome Oxidase

reaction system for conversion from NO_2^- to N_2O in cell.

3. Molecular Evolution of Respiratory Enzymes in Laboratory: NOR has been believed to share the same ancestor with cytochrome oxidase (CcO), aerobic respiratory enzyme, in their molecular evolution (Figure 1). We will challenge evolve NOR to CcO on the laboratory; e.g., the enzymatic activity of NOR will be converted from NO-reduction to O_2 -reduction by using the site-directed mutagenesis.

【Expected Research Achievements and Scientific Significance】

Dynamic of NO in cell will be elucidated on molecular basis of the related proteins. The knowledge will be extended to the study of mammalian system, i.e., NO synthase and NO receptor. In addition, this will provide bases for designing compounds which can regulate the function of NOR and/or NiR-NOR complex. Such study will contribute to medicinal science and environmental science, since NOR is also essential for survival of some pathogen, and the product of the NOR reaction, N_2O , is a green-house gas as well as the ozone-depleting substance.

【Publications Relevant to the Project】

- T. Hino, et al.: "Structural Basis of Biological N_2O Generation by Bacterial Nitric Oxide Reductase" *Science* **330**, 1666-1670 (2010)
- Y. Matsumoto, et al.: "Crystal Structure of Quinol-Dependent Nitric Oxide Reductase from *Geobacillus Stearothermophilus*" *Nat. Strl. Mol. Biol.* **19**, 238-245 (2012)

【Term of Project】 FY2014-2018

【Budget Allocation】 150,100 Thousand Yen

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