1SC-02 緑藻類における好気および嫌気呼吸酵素の特徴と遺伝子発現制御

Characterization and regulation of aerobic and anaerobic respiratory enzymes of Pseudomonas aeruginosa

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Pseudomonas aeruginosa, a ubiquitously distributed opportunistic pathogen, has a highly branched respiratory chain terminated by multiple terminal oxidases and denitrification enzymes. At least five terminal oxidases for aerobic respiration have been identified in the P. aeruginosa cells. Three of them, the cbu-1 oxidase, the cbu-2 oxidase, and the aor oxidase, are cytochrome c oxidases and the other two, the bo3 oxidase and the cyanide-insensitive oxidase, are quinol oxidases. Each oxidase has a specific affinity for oxygen, efficiency of energy coupling, and tolerance to various stresses. These terminal oxidases are used differentially according to the environmental conditions. P. aeruginosa also has a set of the denitrification enzymes that reduce nitrate to molecular nitrogen via nitrite, nitric oxide, and nitrous oxide. These nitrosogen oxidases function as alternative electron acceptors and enable P. aeruginosa to grow under anaerobic conditions. The control of the expression of these aerobic and anaerobic respiratory enzymes contributes to the adaptation of P. aeruginosa to a wide range of environmental conditions. Enzymatic characteristics and evolutionary implications of these respiratory enzymes and the regulatory system that controls the expression of the respiratory genes in the P. aeruginosa cells are overviewed in this presentation.

1SC-03 一酸化窒素還元酵素の立体構造から探る呼吸酵素の分子進化

Crystal structure of nitric oxide reductase, a key enzyme in the molecular evolution of respiratory complex


Respiration is a fundamental process in which electrons generated by glycolysis and other energy metabolic pathways are utilized for the reduction of terminal electron acceptors. In aerobic respiratory chain, cytochrome c oxidase (COX) catalyzes the reduction of O2 molecule as a terminal electron acceptor, whereas, in anaerobic respiration, several kinds of metalloproteins reduce inorganic molecules like oxynitrogen or sulfide compounds as final electron acceptors. Denitrification, a representative anaerobic respiratory chain of facultative microbes, is composed of four reductases, each of which utilizes oxynitrogen as terminal electron acceptor. Of these, nitric oxide reductase (NOR) catalyzes the reduction of NO with one equivalent of electron to form N2O. Interestingly, because catalytic subunit of NOR (NorB) shares characteristic features with subunit I of COXs, NOR has been thought to be an ancestor of or have the same progenitor with COXs in the molecular evolution of anaerobic to aerobic respiration.

We solved the crystal structure of c-type NOR (cNOR) from a denitrifying bacterium Pseudomonas aeruginosa at 2.7 Å resolution. From the structural comparisons between cNOR and COXs, we will discuss the molecular evolutionary history of terminal respiratory enzymes, that could be important for the adaptation to the drastic environmental changes occurred during the emergence of oxygenic photosynthesis.

1SC-04 末端酸化酵素のプロトンポンプ機能の多様性

Diversity in proton pumping mechanisms of the terminal oxidases

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D-pathway of cytochrome oxidase has been proposed to transfer both protons for pumping and water formation. One of the experimental supports for the proposal is that D-pathway is well conserved from mammalian to bacteria. However, recently it has been found that the D-pathway is not conserved in certain bacterial terminal oxidases. The conserved structures across all families of the heme-copper oxygen reductases include the heme α3CuA dinuclear site, the four histidine imidazole groups that coordinate the metals and the covalently linked His-Tyr moiety. It has been proposed that the O2 reduction site and proton pumping site are restricted to the conserved structure, based on the proposition that the structures of the proton-pumping system and the O2 reduction site are conserved completely among the terminal oxidases of cell respiration. An alternative interpretation is as follows: the O2 reduction reaction without release of reactive oxygen species is a set of well organized complex chemical reactions. No structure for the O2 reduction site better than the Fe/Cu system has been found in the history of evolution of all life. Therefore the structure of the O2 reduction site is conserved completely among the terminal oxidases. On the other hand, proton-pumping involves a set of chemically simple processes, protonation/deprotonation of polar functional groups. Many amino acid residues are expected to be involved in this process. Diversity among the proton pumping system is therefore not surprising.

1SC-05 固所作動型プロトコロロフィド還元酵素の結晶構造—蛋白質の構造と分子互変—

X-ray crystal structure of the dark-operative protorhophyllididide oxidoreductase - Evolutionary implications-


The greening ability of plants in the dark is attributed to the activity of dark-operative protorhophyllididide (Pchlide) oxidoreductase (DPOR) catalyzing the stereo-specific reduction of C17 = C18 double bond of Pchlide to form chlorophyllide a, the direct precursor of chlorophyll a. We show a crystal structure of the DPOR catalytic component from Rhodobacter capsulatus at 2.3 Å resolution. The overall structure with two copies each of homologous BchN and BchB subunits is similar to that of nitrogense MoFe protein. Each catalytic BchN-BchB unit contains one Pchlide and one iron-sulfur cluster (NB-cluster). Intriguingly, NB-cluster and Pchlide are arranged spatially as almost identical to the P-cluster and FeMo-cofactor in MoFe protein. There are many genes encoding unidentified nitrogenase-like enzymes in the genomes of methanogens and some nitrogen-fixing bacteria. This type of versatile metalloenzymes has an intrinsic weakness, the extreme sensitivity to oxygen. At the early phase of the evolution, the environment was strictly anaerobic, and this type of metalloenzymes could be recruited as many reductases with various substrate specificities accommodating large substrates such as Pchlide and FeMo-co. Once oxygenic photosynthesis evolved, the oxygen levels began to increase. DPOR provided a base for oxygenic photosynthesis by formation of chlorin, the Chl parental structure, and ironically the resultant aerobic environment eventually prevented the operation of DPOR.

1SD-01 SAIL - NMR 法によるタンパク質質構造の研究

Dynamic study of proteins by the SAIL-NMR method

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There is growing interest in the field of protein dynamics. Many people envisage that the motion of a protein is linked to its functional aspects. We have been developing stereo-array isotope labeling (SAIL) methods to study protein dynamics. The SAIL method utilizes a protein with an optimal isotope labeling pattern, with respect to the intended analysis. In the present paper, we will show some of our latest data focusing on protein dynamics. The first topic is an analysis of the ring-flipping motion of an aromatic ring about its C(beta)-C (gamma) axis in Phe and Tyr residues embedded in the hydrophobic core. While the aromatic rings appear to be tightly packed by other side-chain atoms in protein crystal structures, NMR observations have revealed that they certainly flip even in the core, thus showing that proteins undergo large-amplitude slow breathing motions. The second topic is disulfide bond isomerization. While a disulfide bond is generally considered to stabilize the tertiary structure of a protein, it can adopt different conformations, thereby providing structural elastivity to some extent. Here we discuss these two topics by presenting the application of the SAIL-NMR method to an analysis of the interactions between BPTI, a protease inhibitor, and trypsin.

1SD-02 T 細胞受容体はダイナミックかつ協調的な 4 次元構造変化により活性化する — NMR および光ビンセット法による解析—

A T-cell receptor quaternary structure change revealed by NMR and optical twizzer is important for T cell activation

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The T cell receptor (TCR) mediates antigen recognition and T cell activation via its dimeric αβ, CD3γδ, CD3ε, and CD3ζ subunits, however, a structural mechanism relating both functions has remained elusive. Here, we determine the NMR footprints on CD3ζ of one non-agonist and two agonist anti-CD3 monoclonal antibodies (mAbs). NMR cross-saturation mapping showed that

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