the tendency depends on the substrate nanostructures.

1SE0900 MOLECULAR SIMULATIONS OF ENZYME CATALYSIS

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An important goal of computational and theoretical biochemistry is helping elucidate how enzymes achieve their catalytic efficiency. The differing length and time scales of processes that contribute to catalysis, however, makes this a challenging task for molecular simulation techniques. An approach that has proved particularly powerful for the investigation of the chemical steps in enzymatic and other condensed phase reaction processes is the use of hybrid quantum chemical and molecular mechanical potentials. This talk will describe the types of hybrid potentials used in the author's group and highlight recent methodological developments aimed at increasing their precision and utility. These improvements will be illustrated by a discussion of their application to specific enzyme systems.

1SE0920 チトクロム P450cam 触媒サイクルの最初のステップにおける水分子排出機構について Molecular Mechanism of Water Expelling System in the Initial Step of Cytochrome P450cam Catalytic Cycle

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Cytochrome P450cam responsible for d-camphor hydroxylation non-covalently binds protoheme IX, in which the two heme-propionate side chains, 6- and 7-propionate, form characteristic hydrogen-bonding interactions with nearby Arg112 and Arg229/Asp297 residues, respectively. To understand the role of interaction between those residues and the propionate side chain, we first prepared P450cam reconstituted with one-legged heme, where one of the propionate side chains is replaced with methyl group, and then characterized the protein structure by spectroscopic methods and crystal structure analysis. We also determined the activity of the reconstituted proteins toward d-camphor hydroxylation. From these studies, it is found that the removal of the 7-propionate side chain dramatically decelerates the d-camphor hydroxylation due to the decrease of the d-camphor affinity, because the water molecules in the substrate-binding site still remain even in the presence of the substrate. This result supports the fact that the 7-propionate side chain contributes to the water expelling system from the substrate-binding site to the bulk at the initial step of P450cam catalytic cycle. Furthermore, the replacement of Asp297 with L297 is also found to clearly decrease the apparent enzymatic activity, suggesting the hydrophobic lysine residue inhibits the water-expelling event. In this presentation, I discuss the molecular mechanism of the water-expelling system that initiates the P450cam catalytic reaction.

1SE0940 O₂ アナログ結合構造に基づくチトクロム酸化酵素の反応機構 Reaction mechanism of cytochrome c oxidase based on the O₂ analogues-bound structures.

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Cytochrome c oxidase in the respiratory chain catalyzes the O₂ reduction coupled to the proton pump across the membrane. The O₂ reduction site consists of heme a₃ and copper atom (Cu_B). O₂ binds to the reduced heme iron Fe_{a3}²⁺ resulting in the formation of the oxy-form, in which O₂⁻ is bound to the Fe_{a3}. The O-O bond of the oxy-form is rapidly broken. To investigate the O-O bond cleavage mechanism, the O₂ reduction site is probed by X-ray structural analyses of CO, NO and CN⁻ derivatives. Formation of the Fe_{a3}²⁺-CN⁻ derivative contributes to the trigonal planar coordination of Cu_B¹⁺ and displaces one of its three coordinated imidazole groups while a water molecule becomes hydrogen-bonded to both the CN⁻ ligand and the hydroxyl group of Tyr244. O₂⁻ in the oxy-form is expected to induce the same structural change induced by CN⁻. This allows O₂⁻ to receive three electron equivalents non-sequentially from Cu_B¹⁺, Fe_{a3}³⁺ and Tyr-OH, providing complete reduction of O₂ with minimization of production of active oxygen species (1).

To understand the O₂ reduction mechanism, X-ray structures of other reaction intermediates, including the P-, F- and O-forms, are currently analyzed. Furthermore, to determine accurate structural change at the O₂ reduction site and protonation state in the proton transfer pathway, X-ray structural analysis at 1.4 Å resolution is underway.

(1) Muramoto K, Ohta K, Shinzawa-Itoh K, Kanda K, Taniguchi M, Nabekura H, Yamashita E, Tsukihara T, Yoshikawa S, *Proc. Natl. Acad. Sci. USA.* 107, 7740-7745 (2010).

1SE1000 Mutagenesis studies on the functional mechanism of cytochrome c oxidase and cytochrome P450cam

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Cytochrome c oxidase catalyzes reduction of molecular oxygen to water coupled to proton pumping across the membranes. This enzyme utilizes protons from membrane potential-negative side of the membrane for water formation and pumping. A proton transfer pathway, D-pathway located in the bacterial and bovine enzymes has been proposed to convey both protons. Mutations of specific sites in the bacterial D-pathway abolish the O₂ reduction activity, while those of the other sites abolish the proton-pumping activity without decelerating the O₂ reduction. These mutation results support the D-pathway function. Functional amino acid residues of this pathway are conserved between the bacterial and bovine enzymes, suggesting functional conservation. The D-pathway proton pumping contradicts to the H-pathway mechanism suggested by the X-ray structural and mutagenesis analyses of the bovine enzyme. Our recent mutations of the bovine D-pathway, which corresponds to those of the bacterial enzymes leading to decoupled proton pumping, do not change the enzymatic activity. These results imply the two enzymes pump proton through different pathway. Here, mutagenesis study on proton transfer pathway of the bovine enzyme and on the catalytic reaction mechanism of P450cam, heme b-containing monooxygenase of D-camphor will be presented. Structure-based mutagenesis and biochemical, spectroscopic and crystallographic (if possible) characterization of the mutant enzymes has important roles in elucidation of the functional mechanism of the enzymes.

1SE1020 Genetic code extension and establishment by aminoacyl-tRNA synthetase and tRNA modification enzymes

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Recent inspired protein engineering has added non-canonical amino acids to the genetic code by using the orthogonality of aminoacyl-tRNA synthetase (aaRS) and tRNA pair. However, natural evolutionary process already developed such a system some 3 billion years ago. We present here the structure basis of how aaRSs have evolved to extend the genetic code to incorporate 21st and 22nd non-canonical amino acid (pyrrolysine¹ and selenocysteine^{2,3}) based on the orthogonality to any other aaRS/tRNA pair.

Some tRNA modifications not only reinforce the canonical L-shaped tertiary structure but also ensure the tRNA specificity for codon by ensuring the substrate specificity of tRNA and an aaRS. We show here the structural basis for how a modification enzyme establishes the genetic code by correctly determining the tRNA specificity for codon and amino acid⁴⁻⁶.

References

1. K. Nozawa *et al.* *Nature* **457**, 1163-1167 (2009).
2. Y. Araiso *et al.* *Nucleic Acids Res.* **36**, 1187-1199 (2008).
3. Y. Araiso *et al.* *Proc. Natl. Acad. Sci. USA.* **106**, 16215 (2009).
4. K. Nakanishi *et al.* *Nature* **461**, 1144 (2009).
5. Y. Suzuki *et al.* *Nucleic Acids Res.* **37**, 2910-2925 (2009).
6. M. Umitsu *et al.* *Proc. Natl. Acad. Sci. USA.* **106**, 15616 (2009).

1SE1040 遺伝暗号リプログラミング：その技術と応用 Genetic Code Reprogramming

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The lecture describes a new means to reprogram the genetic code, which enables us to express "non-standard" peptides containing multiple non-proteinogenic amino acids. To execute the genetic code reprogramming, we developed an artificial RNA enzyme (ribozyme), referred to as "flexizyme" capable of aminoacylating tRNAs. The most notable feature of flexizyme is its versatility; it is able to charge virtually any amino acids onto tRNAs bearing various anticodons. Moreover, the flexizyme also charges amino acids with non-proteinogenic sidechain, D-amino acids, N-alkyl-amino acids, and hydroxy acids onto tRNA. Thus, the reassignment of such non-proteinogenic amino acids to We also integrated this unique enzyme system with a specially reconstituted E. coli cell-free transcription-translation coupled system, from which certain amino acids (occasionally their cognate aminoacyl-tRNA synthetases) were withdrawn to vacant the corresponding codons. By the integration of these two systems, any desired amino and hydroxy acids can be reassigned to the vacant codons, and we have recently showed that a wide variety of non-standard peptides can be expressed from mRNAs under the reprogrammed genetic code. The lecture also describes applications of the methodology to the ribosomal generation of cyclic peptides closed by a linkage between the N-terminus and Cys sidechain or a linkage between the