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The incipient structural and vibrational energy relaxation process of photolyzed carbonmonoxy myoglobin (MbCO) was analyzed by the perturbation ensemble molecular dynamics (PEMD) method, in which many pairs of perturbed and unperturbed MD simulations are executed for ensemble-averaging to obtain statistically significant results by canceling out thermal fluctuations. First, the structural relaxation including the “clamshell rotation” in E and F helices was successfully analyzed. The clamshell rotation starts at His64, Val68, and His93 following both the heme doming and the dissociated CO ligand collision. The good agreement with previous experimental results indicates that the PEMD method can predict those changes driven by perturbations even if the changes might be subtle and smaller than thermal fluctuations. Next, the vibrational energy relaxation from the heme to the globin matrix was elucidated not only temporally but also spatially. This is the first “thorough” report of the spacetime-resolved excess kinetic energy redistribution of photolyzed MbCO in the globin matrix with a statistically significant precision, ± 1 K. The incipient anisotropic vibrational relaxation occurs clearly within a picosecond in the direction perpendicular to the heme plane by the “through-bond” and “through-projectile” pathways, and the isotropic relaxation then follows by the “through-space” pathway. Finally, it is concluded that the PEMD method is a powerful tool to understand the incipient relaxation process driven by perturbations.

111512 電子顕微鏡画像に基づいた拘束付き分子動力学法：シトクロム P450 還元酵素・ヘムオキシゲナーゼ-1 複合体の原子モデル構築
Atomic modeling of a complex of cytochrome P450 reductase and hemoxygenase-1 by molecular dynamics restricted EM 3D maps

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Heme oxygenase-1 (HO-1) catalyzes the physiological degradation of heme at the expense of molecular oxygen using electrons donated by NADPH-cytochrome P450 reductase (CPR). Details of electron transfer from CPR to HO-1 are, however, not understood, because an atomic model of CPR/HO complex is not identified. To understand the molecular mechanism of electron transfer, we got 3D reconstruction of CPR/HO complex by transmission electron microscopy. However, since the spatial resolution is 2.5 nm, it is impossible to evaluate CPR/HO complexes at the atomic level. In this study, we propose a novel atomic model constructed based on a three-dimensional electron microscopic map, and estimate a binding manner between CPR and HO-1. At first, we manually fitted each of atomic models of CPR and HO, which were proposed from X-ray crystallography, into EM map to construct a rough model of CPR/HO complexes. Next, we refined this atomic model using the SRMD method, which is an extension of steered molecular dynamics (MD) simulation techniques; cryo-EM maps were used as an external potential for MD. We furthermore, extended SRMD for a complex in success. Using different initial parameters and models, we calculated MD many times with SRMD method to obtain more than 10 atomic models well-explaining the EM map. Although they showed over all same structure, some their regions showed structural differences. We would like to discuss a molecular mechanism of the electron transfer between CPR and HO from RMSD (Root Mean Square Deviation) and stabilities.

111524 ヘムオキシゲナーゼによるヘム分解の環境適応
Fine tuning of the heme catabolism by heme oxygenase in response to the reaction milieu

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Heme oxygenase (HO) catabolizes heme into carbon monoxide, biliverdin, and free iron. The HO reaction proceeds successively by three oxygenation reactions which require electrons and molecular oxygen. The first active species of the heme conversion, hydroperoxy heme, essentially requires proton transfer to the oxyheme for its formation. HO functions in different milieu depending on the species so that HO chemistry must be finely tuned by respective active site. In this study, fish (puffer fish, Tfr) and rat HO1-reactions were compared under various pH and oxygen concentration. The heme degradation by Tfr HO1 and rat HO1 showed distinctly different pH dependences. In the cytochrome P450

reductase (CPR)/NADPH coupled reaction, the heme conversion rate by Tfr HO1 is twice faster than that by rat HO1 at neutral-basic conditions. In the ascorbate coupled reactions, pH dependence of the heme-conversion rate is more contrastive between Tfr HO1 and rat HO1: the former exhibited the maximum rate at pH ~ 8 and the higher rate was kept up to pH 8.5 but the rate is relatively slow at the acidic pH, whereas the latter showed the maximum rate at pH ~ 7 and relatively high rate at acidic pH, but the rate decreased largely at basic pH. These differences can be explained as due to the differences in the CPR-HO interactions and to the differences in the axial coordination as well as in the pKa of acid-base transition of respective heme-HO1 complexes. Oxygen concentration also affected the heme degradation rate by Tfr HO1 and rat HO1, differently.

111536 基質存在下 CO 型インドールアミン 2,3 ジオキシゲナーゼの共鳴ラマンスペクトル

Resonance Raman Study on CO-bound Indoleamine 2,3-Dioxygenase with Tryptophan

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Indoleamine 2, 3-dioxygenase (IDO) incorporates two oxygen atoms from molecular dioxygen into tryptophan (Trp) yielding N-formylkynurenine, in various tissues in our body except liver. This reaction is the first step of the kynurenine pathway, which is the main Trp metabolism. It has been known that L-Trp is the best substrate with a characteristic reactivity so-called substrate inhibition, in which velocity of dioxygenation reaction first increases with increasing L-Trp concentration up to ca. 100 μ M and then it decreases with increasing the L-Trp concentration. In the case of D-Trp, larger K_m and comparable V_{max} compared to those of L-Trp are observed without substrate inhibition. The mechanism of the substrate inhibition has to be clarified. In this study, we have examined the interaction of L- and D-Trp with CO-bound IDO as a good model of IDO-O₂-Trp ternary complex intermediate, in order to elucidate the different reactivity of the enantiomers by using resonance Raman technique. When L-Trp binds to CO-bound IDO, Raman modes of heme as well as those of CO have changed significantly, whereas binding of D-Trp to CO-bound IDO causes little or no change in CO related or heme Raman modes. These results indicate that the binding of L-Trp induces structural change of heme, which is favorable for enzyme reaction. Different rate limiting steps in the catalytic reaction between the enantiomers likely causes their distinct reactivity.

111548 リガンド結合型インドールアミン 2,3-ジオキシゲナーゼの共鳴ラマンスペクトル

Resonance Raman Study on Ligand-Bound Forms of Indoleamine 2,3-Dioxygenase

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Indoleamine 2, 3-dioxygenase (IDO) is a heme-containing enzyme. This enzyme incorporates two oxygen atoms into L-tryptophan (Trp), yielding N-formylkynurenine. This is in the major catabolic pathway of Trp. Resonance Raman (RR) studies have revealed coordination geometries of some key reaction intermediates, the oxygenated (Fe-O₂), ternary complex (Fe-O₂-Trp) and ferryl-oxo (Fe=O) intermediates. However, the overall catalytic mechanism remains unclear. Crystal structure of IDO has been determined for the 4-phenylimidazole (4-PI) ligated form at a resolution of 2.3 Å. This is the only crystal structure reported at a reasonably good resolution. In the present study, we have measured RR spectra of 4-PI ligated IDO and those of imidazole (Im) ligated IDO as a control of six-coordinate IDO. The RR spectra of 4-PI and Im ligated IDO displayed six-coordinate and low-spin characters for ferric and ferrous states. In the spectra of 4-PI ligated IDO, alteration of heme side chain modes and activation of out-of-plane mode have been detected relative to those of Im ligated IDO. These results indicate that the phenyl group of 4-PI affects the heme distal environment and the heme side chain. The authentic substrate of Trp does not ligate to heme iron but binds to distal heme pocket. We will compare RR spectra of four forms of IDO, 4-PI-ligated, Im-ligated, Trp-bound and free IDO, and discuss about their structural differences, which may provide a clue to understand the catalytic mechanism of IDO.

111612 Precise Crystal Structure and Spectroscopic Analyses of a Heme Protein, Cytochrome c'