is small and physiologically less important, indicating that these relaxed conformations simply reflect a structural polymorphism of a high-affinity relaxed state.

111336 ヘテロトロピックなエフェクター存在下におけるオキシヘモグロビンのR-T構造変化

R-T quaternary structure transition of oxy hemoglobin in the presence of heterotropic effectors

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Heterotropic effectors of IHP and L35 have been shown to interact not only with deoxy- but also oxy-Hbs causing significant reduction in their O₂-affinities and the modulation of cooperativity. Under oxygen pressure of 760 mmHg, ¹H-NMR spectra of oxy-Hb in the presence of L35 and IHP below pH 7 showed a marker of the T-quaternary structure at 14 ppm, originated from inter-dimeric $\alpha_1\beta_2$ -hydrogen-bonds. The midpoint of the pH transition in the presence of L35 and IHP was determined to be 7.4. The pH transition was also observed for oxy-Hb with L35 and other anionic compounds such as hexacalboxybenzene and 2,3-BPG, and the midpoint of the pH transition was closely related to the number of their negative charges. The L35-concentration dependence of the T-marker in the presence of IHP indicates that there are at least kinds of L35-binding sites in the ligated T-quaternary structure. Based on previous X-ray crystal structures of Hb-L35 complex, the strong binding site is probably intra-dimeric binding sites between α_1 G- and β_1 G-helices, and the weak L35-binding site near the IHP binding site may cause the R-T transition without release of O₂. The tertiary structure of Hb around heme pockets seems to be fluctuated by the L35 and IHP binding to the central cavity. The tertiary structural fluctuation induced by the effectors may contribute to the significant reduction of the O₂-affinity of oxy-Hb, which hardly depends on the quaternary structures.

111348 ヒトヘモグロビンのリガンド結合による四次構造変化を理解する新しい考え；紫外共鳴ラマン分光法による解析

A New Way for Understanding Quaternary Structure Changes of Hemoglobin upon Ligand Binding on the Basis of UV-Resonance Raman Spectroscopy

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The single residue vibrational spectra of tryptophan (Trp) and tyrosine (Tyr) residues in human adult hemoglobin (HbA) which play important roles in cooperative oxygen binding, were determined for the deoxy and CO-bound forms by applying UV resonance Raman spectroscopy to various variant Hbs. It was found that Trp β 37, Tyr α 42, Tyr α 140, and Tyr β 145 at the α^1 - β^2 subunit interface underwent transitions between two contact states (named as T and R) upon ligand binding, while Trp α 14, Trp β 15, and Tyr β 35 displayed little changes. The corresponding spectral changes were identified only for the $\alpha_2\beta_2$ tetramer, but not for isolated α and β chains, and therefore, were exclusively attributed to a quaternary structure change. Ligand binding as well as allosteric effectors and pH altered only the number of the T-contacted Tyr and Trp residues without varying the two contact states themselves. A new method to evaluate semi-quantitatively the amount of T-contacted Tyr and Trp residues in a given liganded form is here proposed, and with it a quaternary structure was determined for various symmetrically half-liganded forms obtained with ligand-hybrid, metal-hybrid and valency-hybrid Hbs. It was found that ligand binding to the α or β subunits yielded different subunit contacts and that the contact changes of the Trp and Tyr residues were not always concerted. The contact changes at the α^1 - β^2 interface is related to the proximal strain exerted on the Fe-His(F8) bond, which is noted to be much larger in the α than β subunits in the deoxy $\alpha_2\beta_2$ tetramer.

111412 逆分子系統解析法による硬骨魚類の祖先型ヘモグロビンの設計及び合成

Design and synthesis of ancestral Osteichthyes hemoglobins by reverse molecular phylogenetic analyses

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The vertebrate hemoglobin (Hb), the oxygen carrier contained in red blood cells, is tetrameric composed of α and β chains. Terrestrial vertebrates are considered to have evolved from ancestors common to those of fish which resides in different water environments. Hbs from teleost show the Root effect in which the oxygen capacity of Hb is halved upon lowering of blood pH to an acidic range. In the present study we intend to make clear how fish Hb acquired cooperativity and the Root effect through molecular evolution by a reverse molecular evolution technique based on phylogenetic analyses using more than 1,200 registered sequences of fish Hbs. We designed the ancestral Hb which might exist about 2.5 million years ago and constructed an expression system for the α and β chains of this Hb. We confirmed that these chains are actually produced in *E. coli* cells.

111424 MD simulation of the temperature-dependent structural changes of fish myoglobin

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Objectives: Myoglobin (Mb) is a heme protein, usually functioning as an oxygen reservoir in muscle. It is generally accepted that fish Mbs are less stable compared to mammalian counterparts, as demonstrated by fast autooxidation rate and melting under low temperature conditions. In order to understand the precise labile profiles of fish Mbs, MD simulation was performed on tuna Mb at several different temperatures in comparison with mammalian (whale) Mb.

Materials & methods: The AMBER 11 suite of programs was used in this study. The crystal structures of blackfin tuna Mb (PDB: 2NRL) and sperm whale Mb (1U7R) were used as templates. The structure was solvated with a 7Å buffer between the protein surface and the cube boundary. Periodic boundary conditions were used, and the ff99 force-field was employed. The systems underwent 100 steps of minimization, followed by two steps of equilibration, each 200 ps long under NPT conditions. The temperature range was from 273 to 300 K. All the production MD runs were performed at constant pressure for 100 ns.

Results and discussion: RMSF values were found to be higher in the loop regions than the helical regions. The helical regions except Helix C showed lower values. The N and C termini were also flexible. In the 2D RMSD, major and minor clusters were recognized. Clear differences were observed in the temperature effect on RMSD and RMSF between the two Mbs, namely, tuna Mb showed fluctuations to a larger extent.

111436 ヘム側鎖がミオグロビンの円偏光二色性(CD)に与える影響

Effects of side chains of the heme on circular dichroism (CD) of myoglobin

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Hemoglobin (Hb) and myoglobin (Mb) exhibit mostly positive CD bands in the Soret region. A negative CD band is exceptionally observed only for *Chironomus* and lamprey Hbs. Since heme itself is optically inactive, the CD spectra derived from the heme in the globin reflects its interactions with asymmetric environments. The CD activity in the Soret region has been proposed to arise from an allowed π - π^* transition in aromatic side chains of globin coupled with the heme Soret transition. The 180° rotation of the heme about the 5,15-meso-carbon axis (reversed heme) causes an interexchange of the 2,7-methyl groups with the 8,3-vinyl groups, respectively. Recently we found that the Hb containing the reversed heme exhibits a prominent negative CD band in the Soret region. This suggests that the Soret optical activity of heme derives from the interaction of the heme side chains with globin. In order to examine this possibility, we reconstituted Mb with unnatural hemes lacking the vinyl and/or the propionate, and compared their CD spectra with that of native Mb. Replacement of the vinyl side chains with methyls induced a little change to the CD in the Soret region. In contrast, the modification of the propionate side chains resulted in almost complete disappearance of the CD band. These results demonstrate that the interaction of heme propionate groups with globin evidently contributes to the appearance of the prominent Soret CD band of Mb.

111448 摂動アンサンブル MD 法による光解離 MbCO の初期緩和過程の高分解能解析

High precision analysis of the incipient relaxation process of photolyzed carbonmonoxy myoglobin by perturbation ensemble MD method

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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'