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