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Iron is an absolute requirement for nearly all organisms. Bacterial pathogens have evolved the iron acquisition systems because iron is not readily available in human body. *Staphylococcus aureus* employs the iron-regulated surface determinant (Isd) system as its primary heme-iron uptake pathway. In other words, this system is a promising target to battle *S.aureus*. Heme molecule is first extracted from humans' hemoglobin and captured by IsdH or IsdB. IsdA and IsdC relays heme to IsdE on the plasma membrane. Although the crystal structures of Isd proteins have been solved with and without heme, the mechanism of the one-way transport is still unclear because of the close similarity of these structures. We examined the heme-binding and heme-transport mechanisms of Isd proteins not only in vitro, but also using in silico methodologies. Isothermal titration calorimetry (ITC) experiments showed that the downstream Isd proteins have higher affinities against heme than the upstream proteins. Qualitatively consistent results were obtained from the free-energy calculations with the MM-PBSA method, which revealed enthalpic contributions of the residues to the affinity to heme for each Isd protein. Based on these results, we designed and synthesized a heme derivative. We will examine its growth inhibition activity in vivo. In addition, the QM/MM (ONIOM) calculation is being performed for a model of a heme-transport complex composed of two Isd proteins and one heme. We will discuss the transport mechanism between the Isd proteins.

2PT152 ヒトヘモグロビンの酸素親和性の制御における本質：四次構造、三次構造変化に共通して起こる Fe-His 結合の変化

Essence in oxygen affinity regulation of human adult hemoglobin: Change of Fe-His bond length by quaternary or tertiary structure change

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Human adult hemoglobin (HbA) possessing $\alpha_2\beta_2$ tetramer structure exhibits cooperativity in oxygen binding. X-ray crystallographic studies have demonstrated the presence of two distinct quaternary structures, called T (tense) and R (relaxed) states, which correspond to the low- and high- O_2 affinity forms, respectively. Both the quaternary structures of T and R exhibit different Fe-His bond lengths: they are shorter in R state than T state in both the α and β subunits.

Extensive examinations of HbA in the presence of the allosteric effector bezafibrate (BZF) showed a reduction of the oxygen affinity of oxyHbA in the R structure and the importance of tertiary structure change for oxygen affinity is stressed. Recently, one of authors reported that the decrease in oxygen affinity of oxyHbA upon BZF addition accompanies the elongation of Fe-His bond in α subunit [1]. Our finding is consistent with the molecular dynamics calculation of Yonetani and Laberge [2], in which the main chain in E and F helices of HbA fluctuates more in the presence than in the absence of BZF. This enhanced fluctuation in the E and F helices results in weakening of the Fe-His bond.

Thus, the elongation of Fe-His bond length is caused by not only quaternary structure but also tertiary structure change. This suggests that the elongation of Fe-His bond is essential in oxygen affinity regulation of human adult hemoglobin. [1] S. Nagatomo, H. Hamada and H. Yoshikawa, *J. Phys. Chem. B* **115**, 12971 (2011). [2] T. Yonetani and M. Laberge, *Biochim. Biophys. Acta* **1784**, 1146 (2008).

2PT153 Structural fluctuation and change around heme pockets of liganded Hb induced by L35 binding

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Hemoglobin (Hb) takes two structures, depending on oxygen binding; T-quaternary structure with low oxygen-affinity and R-quaternary structure with high oxygen-affinity. Heterotropic effectors decrease the oxygen-affinity and its cooperativity. In the presence of two potent effectors, L35 and inositol hexaphosphate (IHP) at pH 6.8, 1H-NMR spectra of human adult Hb showed oxy-T-quaternary structure. The NMR signals around heme pockets of the oxy-T structure were rather broad, indicating that some structural fluctuations occur around the heme, and the fluctuations may be related with the reduction of the oxygen-affinity. In order to clarify the structural fluctuations in the heme-pocket induced by L35 and IHP, NMR spectra of liganded met-Hbs were measured in the presence of the effectors. It has been known that the N3-metHb

shows some IHP-induced hyper fine shifted (hfs) signals besides the heme methyl signals around 15-35 ppm. The addition of L35 to the N3-metHb with IHP showed that the heme signals were shifted and broadened, but the IHP-induced hfs signals were increased. The pH dependence of the hfs signals was also examined and the pH increase from 6 to 8 showed that the IHP-induced signals disappeared, suggesting that the spectral change should be related to the change in the oxygen affinity. The quaternary structural change of metHb will be discussed based on hydrogen bond signals around 12-13 ppm.

2PT154 ヒトヘモグロビンの β 145Tyr の Thr または Leu への置換が機能と構造に与える影響

Effects of the substitutions of Thr or Leu for β 145 Tyr on the structure and oxygen binding properties of human hemoglobin

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In all the vertebrate hemoglobins and myoglobins, the penultimate position (HC2) is invariably occupied by tyrosine (Tyr). The natural and artificial mutants of β 145Tyr studied so far, show the extremely high oxygen affinity and low cooperativity. To examine the role of β 145Tyr on the structure and oxygen binding properties, we expressed two new artificial mutant Hbs in *E. coli*, rHb(β Y145L) and rHb(β Y145T) and purified them as described previously [1]. These mutant Hbs exhibited a markedly high oxygen affinity and diminished cooperativity (P_{50} =0.6-0.9 mmHg, Hill's n =1.3-1.4). In the presence of inositol hexaphosphate (IHP), however, oxygen affinity of these mutant Hbs decreased and exhibited significant cooperativity; P_{50} = 1.7 mmHg, n = 1.9 for Hb (β Y145T); P_{50} =2.4 mmHg, n =2.2 for Hb(β Y145L). From the oxygen binding properties of these mutant Hbs, the replacement of β 145Tyr by Leu seems to stabilize the T-conformation more than that of Thr. DeoxyHb (β Y145L) exhibited a smaller negative CD band at 286 nm (a T-state marker band) than that of deoxyHbA, but exhibited an increase in intensity of the negative CD band upon the addition of IHP. These results indicate that the residue at HC2 of β subunits contributes to stabilization of the T-conformation through hydrophobic interactions with the surroundings in addition to hydrogen bonding to β FG5Val carbonyl group. [1]Nagai,M., Nagai,Y., Aki,Y., Imai,K., Wada, Y., Nagatomo,S., and Yamamoto,Y. *Biochemistry*, **47**, 517-525 (2008).

2PT155 *cbb3*型チトクロム酸化酵素の酸素消費活性

Oxygen consumption activity of *cbb3*-type cytochrome c oxidase

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Terminal oxidases of the aerobic respiratory chain in inner mitochondrial membrane and bacterial cell membrane catalyze oxygen reduction to water coupling to electron and proton transfer. Terminal oxidases containing heme and copper atom at catalytic sites are called as heme-copper oxidases, which are classified into A-, B-, C- and several types of subfamilies. Although structures of the oxygen reduction sites are similar between subfamilies, structures of the proton pathways and energy transduction efficiency are different. To understand energy transduction mechanism, in this study, we compared oxygen consumption activity of cytochrome *aa3* (A-type) and *cbb3* (C-type) oxidases purified from bovine heart mitochondria and *Vibrio cholera* cells, respectively. The measurement was performed by using ascorbate as electron donor and TMPD as mediator. In the presence of *aa3* or *cbb3*, oxygen concentration was almost linearly decreased. When the enzyme concentration was changed, the rate varied as *aa3* up to 5 μ M and *cbb3* up to 0.2 μ M. The rate increased with increasing concentration TMPD, indicating that electron transfer from TMPD to the enzyme is rate limiting step. In ascorbate/TMPD system, the specific activity of *cbb3* was estimated to be 50 to 60 times higher than that of *aa3*. Inhibitory effect by nitric monoxide was measured. Addition of nitrite decreased oxygen consumption rate of both *aa3* and *cbb3*, but in the case of *cbb3* the rate was dependent on the oxygen concentration suggesting change of rate limiting step.

2PT156 *cbb3*型チトクロム酸化酵素の精製

Purification of *cbb3*-type cytochrome c oxidase

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