2P101 高圧力下での酵素分解による鶏卵白アレルゲンタンパク質のアレルギー性の低減化の試み
Attempt at reduction of allergenic potency for allergenic protein from hen egg white by proteolysis under high pressure

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A common strategy for eliminating allergenic properties from allergenic protein is to destroy their tertiary structures permanently using proteolysis. Nonetheless, in practice because past investigations have carried out randomly to explore crucial digestive condition without care for conformational specificity of proteins, it has been hard to find a crucial condition for digestion of allergenic proteins. These ways are not efficiently, therefore we need to establish new method based on understanding structural property of a protein. In principle since proteolysis is happened against unfolded structure of protein, increasing of population of unfolded structure in allergenic protein is important to achieve the effective digestion. However, most allergenic proteins have high resistance against proteolysis due to the presence of quite stable folded structure. In addition, structural information of them had been rarely reported until now. In contract, previous our study revealed that proteolysis of ubiquitin, which have high resistance against proteinase under physiological condition, could be significantly accelerated under pressure due to dramatic enhancement of population of unfolded structure by pressure. Here, we study of ovomucoid that is well-known as major allergenic protein in hen egg white, to clarify its thermodynamic property using high pressure fluorescence spectroscopy probed tyrosine and to establish the effective method for eliminating allergenic properties based on proteolysis under pressure.

2P102 In vitro selection for covalent binding via disulfide interchange with ribosome display

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In vitro protein evolution is a powerful method that enables rapid selection for protein molecules with high affinity and specificity. These molecules would be of experimental and eventually therapeutic interest. Here we report what is, to our knowledge, the first in vitro selection for covalent binding by using ribosome display, a method which does not include living cells at any steps. A small protein domain called WW domain and a disulfide-containing biotinylated peptide were used as a starting protein and its binding target of ribosome display, respectively. After five rounds of the selection, we obtained Cys variants of WW domain that bind covalently and specifically to the target by disulfide interchange, and results in biotinylated. Such Cys variants may be useful for a polypeptide tag which allows for covalent labeling of a protein of interest with chemically diverse compounds. We have thus showed ribosome display, a technique that combines the advantages of very rapid selection with the use of large libraries, can be applied to the selection of covalent-binding proteins.

2P103 高感度多周波数 EPR による還元型デオキシ Hb 高スピンヘム鉄の低準位電子状態の研究
Low Lying Electronic States of the Ferrous High-Spin ($S = 2$) Heme in Deoxy-Hb Studied by High-Sensitive Multi-Frequency EPR

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Low-lying electronic states of the ferrous high spin ($S = 2$) heme in deoxy-hemoglobin (Hb) have not been definitely characterized. We reported previously a peculiar broad EPR signal at zero field measured with our high-sensitive EPR system with cavity resonators (34-122 GHz). The EPR spectra are not simulated by any set of parameters for the $S = 2$ spin Hamiltonian assuming five spin states in the 5B_2 ground state. We did not observe any other signals at temperatures of up to 30 K and magnetic fields of up to 14 Tesla. Since EPR measurements at frequencies over 130 GHz are limited due to the resonator size, we try to measure EPR signals at higher frequencies (90-220 GHz) using a transmission probe which is not very sensitive as compared with the resonators. We could tide over the insensitivity by using a large volume (100 μ l) sample with high concentration (27 mM/heme base).

In this presentation, we report a new broad EPR signal of deoxy-Hb over 100 GHz, the resonant peak of which shifts to higher field upon increasing frequency. The asymptote of frequency as a function of resonance field intersects 110 GHz at zero field. This means that the energy gap between the ground and the first excited states is about 3.7 cm^{-1} , which is in good agreement with the broad absorption band at 3.5 cm^{-1} reported from the far-infrared experiments of deoxy-Hb.

Mössbauer and MCD experiments evidenced for the 5E_g and 5B_2 terms as the ground multiple states. Theoretical analyses beyond the spin Hamiltonian are in progress.

2P104 ヘモグロビンのアロステリーは MWC モデルと調和できるか?
Can hemoglobin allostery be reconciled with the MWC model?

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The Monod-Wyman-Changeux (MWC) two-state allosteric model continues to be the most practical theory that relates the structure and function of hemoglobin and other allosteric proteins in general. However, it is also true that the quantitative understanding of hemoglobin cooperativity based on the MWC two-state model has not yet been successful. Perhaps the most serious problem is that the oxygen affinity of T-state deoxyhemoglobin in solution varies widely with solution conditions even though all the crystal structures of deoxyhemoglobin determined to date (over a wide range of crystallization conditions and in different crystal lattices) adopt essentially the same quaternary structure (classical T). To solve these puzzling observations, we have investigated the oxygen equilibrium properties of unliganded, fully-liganded and partially-liganded conformations of hemoglobin in crystals and in wet porous silica gel matrices. Our results are not inconsistent with the MWC concerted mechanism but suggest the existence of two (at least two) distinct T-states with widely different oxygen affinities; one is the classical T-state with a very low affinity for oxygen and the other is a high-affinity T-state (or a new allosteric P-state) whose structure remains undetermined. The functional properties of this P-state and our attempts to crystallize it will be presented.

2P105 ウシ心筋シトクロム c 酸化酵素 O 型中間体の X 線構造解析
1G1420 X-ray structural analysis of catalytic O intermediate in bovine cytochrome c oxidase

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Cytochrome *c* oxidase (CcO) catalyzes oxygen reduction coupled the proton pump across the membrane. The oxygen reduction site consists of heme and copper atom. In the oxygen reduction cycle, several intermediate forms have been identified by visible absorption and resonance Raman spectroscopy. In the F-form, the ferryl-oxo species is formed in the catalytic site. In next step, delivery of an electron and a proton to the catalytic site generates the O-form, in which the ferric-hydroxy species is formed. X-ray structures of the O-form have not been determined yet. In this study, we prepared the crystals of bovine CcO containing the O-form and performed X-ray structural analysis. The bovine CcO crystal in the fully reduced state was treated by molecular oxygen, which caused the absorption change at 604 nm. Four electrons transfer from the fully reduced CcO to molecular oxygen is expected to generate the fully oxidized CcO, i.e. the O-form. However, the absorption spectral measurement around 580 nm showed that the F-form also exist in the crystal. X-ray structural analysis showed that the electron density is located near Fe_{a3} and Cu_B. The result indicates that, one oxygen atom binds to Fe_{a3} and another oxygen atom binds to Cu_B in the O-form. Structure of the proton transfer pathway in the O-form is currently analyzed.

2P106 ウシ心筋シトクロム c 酸化酵素 P 型中間体の X 線構造解析
X-ray structural analysis of catalytic peroxy intermediate in bovine cytochrome c oxidase

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Å Cytochrome *c* oxidase (CcO) catalyzes oxygen reduction coupled with electron transfer in the Å mitochondrial membrane. In the oxygen reduction cycle, O₂ binds on the reduced heme *a*₃-Cu_B site in the oxygen reduction, which results in the formation of the oxy intermediate. Next, O-O bond is broken forming the peroxy intermediate. Previous X-ray structural analysis of bovine cytochrome *c* oxidase showed that the peroxide ligand was bound to the heme *a*₃-Cu_B site in the resting oxidized state, forming the peroxo complex (Fe_{a3}-O-O-Cu_B) (1). However, X-ray structural analysis of catalytic intermediates was not studied except four-electron reduced state. So we proposed to determine the structure of the peroxy intermediate. The addition of hydrogen peroxide to the resting oxidized enzyme caused absorption change at 607 nm. The single peak at 607nm was formed (peroxy intermediate). X-ray analysis