2P336 粘土層間におけるロドプシン発色団の性質
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2aD14 Characteristics of the Rhodopsin Chromophore in Clay Interlayers
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Color vision is achieved by cone pigments which regulate their absorption maxima by the amino acids surrounding their chromophore, an 11-*cis* retinal Schiff base. Although it has been nearly impossible to reproduce such color tuning by other materials, we recently found that clay acts as a novel model matrix to mimic color tuning of the chromophore [1]. Protonated 11-*cis* and all-*trans* retinal Schiff bases exhibit different colors when adsorbed into the clay minerals (montmorillonite, MMT) from three mining places, which entirely covers the visible light region. Thus, silicate-based clay can mimic the carbon-based protein for color tuning of the chromophore in our vision.

In this study, we analyzed the mechanism of color tuning and photochemistry of the rhodopsin chromophore in clay interlayers. MMT consists of negatively charged silicate layers and readily exchangeable interlayer cations, such as Na⁺, K⁺, Ca²⁺ and etc. We found that interlayer cations strongly influence colors, but Ca²⁺-exchanged clays still show three different colors dependent on the mining places. The photoisomerization processed will be also discussed based on the ongoing HPLC measurements.

[1] Furutani et al., submitted.

2P338 霊長類ロドプシンから見た光受容蛋白質のヒドロキシルアミン安定性の違い
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Difference in hydroxylamine susceptibility between primate and bovine rhodopsins.
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Most vertebrate retinas contain two types of photoreceptor cells, rods and cones, which are responsible for twilight and daylight vision, respectively. The rod and cone visual pigments exhibit different molecular properties before and after light absorption. That is, cone pigments show rapid formation and decay of G protein-activating states upon absorption of light, while they show high susceptibility to hydroxylamine in the dark. The amino acid residues responsible for the former difference have been identified; however, the residue(s) for the latter difference have not been identified due to the vast divergence of amino acid sequence between rod and cone pigments. We have cloned and expressed primate rhodopsin and compared its susceptibility to hydroxylamine with bovine rhodopsin. As a result, primate rhodopsin showed significantly higher susceptibility than bovine rhodopsin. We also designed and expressed site directed mutants of primate and bovine rhodopsin to identify the amino acid residue(s) responsible for the difference. Some mutants in which the hydrophobic residues in the transmembrane regions are changed showed altered susceptibility to hydroxylamine. These results suggest that the susceptibility to hydroxylamine is regulated by the hydrophobic interactions in transmembrane regions. Because the residues in cone pigments are different from bovine rhodopsin, these findings would give a clue to elucidating the mechanism for the difference in the susceptibility to hydroxylamine between rod and cone visual pigments.

2P337 ウシロドプシンのレチナール結合部位の構造安定性の解析-Thr118 の H/D 交換実験
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Structural fluctuations affecting the retinal-binding pocket in bovine rhodopsin studied by hydrogen/deuterium exchange of Thr118
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Visual rhodopsins possess an 11-*cis* retinal as a chromophore, and its photoisomerization to the all-*trans* form initiates our vision. It is believed that thermal isomerization seldom occurs in the binding pocket (~200 kJ/mol), and such property of 11-*cis* retinal as an inverse agonist is highly advantageous for inhibiting occasional activation in dark (dark events). However, the reported activation enthalpy of the dark events is ~90 kJ/mol, being much lower than that of thermal isomerization, and this paradox has been never solved. Protein dynamics of rhodopsin must be taken into account for better understanding.

In this study, we characterized structural fluctuations affecting the retinal-binding pocket in bovine rhodopsin by hydrogen/deuterium exchange method with FTIR spectroscopy. Thr118 is located in the retinal-binding pocket and isolated from hydrogen-bonded network connecting to the outside of protein. As expected from the crystal structure, the O-H group is not deuterated in D₂O at room temperature [1]. Nevertheless, we found that the hydrogen/deuterium-exchange takes place at 30-70 °C in dark and estimated the exchange ratio by monitoring the O-H and O-D stretching vibration of Thr118. As a result, the activation enthalpy was obtained to be ~95 kJ/mol, which is equivalent to that of the dark events. Structural fluctuation in rhodopsin and possible correlation with the dark events will be discussed.

[1] T. Nagata et al., *J. Phys. Chem. A* 106, 1969-1975 (2002).

2P339 メタロドプシン I と II における pH 依存性平衡状態の直接観測
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Direct observation of the pH dependent equilibrium between metarhodopsin I and II
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Vertebrate rhodopsins convert to a G protein-activating intermediate metarhodopsin II (MII) through a series of thermolabile intermediates upon absorption of a photon. It is generally implicated that MII forms pH-dependent equilibrium with its precursor metarhodopsin I (MI). This implication is based on the facts that the amounts of MI and MII in the mixture changed when rhodopsin was irradiated at different pH conditions. But we cannot deny an another possibility that this pH-dependency might originate not from an equilibrium between MI and MII but from a stage prior to them. To discriminate between these two possibilities and to obtain detailed information about the chemical nature of the structural changes from MI to MII, it is necessary to directly observe the pH-dependent equilibrium by changing pH of the MI/MII mixture. In the present study, we have developed a method to change a sample pH without disturbing other conditions such as temperature and, and compared the amounts of MI and MII between the samples whose pH were adjusted before and after irradiation, respectively. We confirmed that the amounts of MI and MII were the same between these samples. This is the first experimental confirmation of the equilibrium between MI and MII. Based on these results, we will discuss the relationship between pH-dependent MI/MII equilibrium and the global protein conformational change. We will also discuss a potential key amino acid residues involved in the relationship.