1P-220 ロドプシンの構造を安定化する分子内相互作用

Intramolecular interaction of rhodopsin stabilizing the native structure

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Rhodopsin is a photoreceptor protein located in the rod outer segments. Rhodopsin is embedded in the membrane with seven transmembrane helices, which is a typical motif of G-protein coupled receptors (GPCRs). To investigate the intramolecular interactions which maintain the native structure of rhodopsin, we tried to assemble functional rhodopsin molecule from rhodopsin fragments split at the loop region. Among the loops of rhodopsin, the second extracellular loop (ECL2), connecting helices IV and V, appears to be the most important. ECL2 is in contact with 11-*cis*-retinal chromophore and contains Cys187 which forms disulfide bond with Cys110. Thus we designed the gene of rhodopsin fragments which are yielded by split of rhodopsin at the various positions of ECL2. The fragments were co-expressed and reconstituted into pigments by adding 11-*cis*-retinal.

When rhodopsin was split at the C-terminal end of ECL2, enough amount of rhodopsin (SR-1) was obtained. In SR-1, packing of fragments is sufficiently stabilized by interhelical interaction and chromophore/protein interaction. However, if ECL2 was split at the N-terminal end of ECL2, the amount of rhodopsin (SR-2) was significantly reduced, indicating that the loss of peptide bond between helix IV and ECL2 cannot be compensated by the noncovalent interactions. However, since the presence of Trp175 in C-terminal fragment of SR-2 improved the expression level of rhodopsin, the hydrophobic interaction of Trp175 partially substitutes for peptide bond. Both SRs showed the normal absorption spectra, photobleaching process and resistance to hydroxylamine, indicating that their structures are comparable to native rhodopsin. These findings provide the insights into the structure/function relationships of GPCRs.

1P-221 桿体・錐体視物質における活性中間体の pH 依存性の比較研究

Comparative studies on the pH-dependency of the active intermediate between rod and cone visual pigments.

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see ITP2-03

1P-222 青色光センサータンパク質 PixD の光誘起構造変化および分子間相互作用変化の研究

Study on light-induced conformational change and intermolecular interaction change of a blue light sensor protein PixD

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PixD is a blue light sensor protein with BLUF domain, and involves phototaxis of a cyanobacterium *Synechocystis*. According to the crystal structure as well as molecular weight in solution, PixD forms a characteristic decamer. This oligomeric structure could play an important role in light signal transduction. To understand signal transduction mechanism, it is necessary to investigate dynamics of conformational change and intermolecular interaction change of the sensor protein. The transient grating method enables us to follow the time development of diffusion coefficient (*D*) of photoexcited species with high time resolution. Therefore, since *D* is sensitive to conformation and volume of a molecule, it is expected to clarify these dynamics associated with the oligomeric state. We could detect light-induced *D*-change derived from a conformational change of the PixD dimer with a time constant of 40 ms. Interestingly, we also observed light-induced dissociation of the PixD decamer into the dimer at higher excitation intensity. It was reported that, in the presence of a response regulator, PixD forms a decamer-based complex, and this complex disassembled upon light irradiation. The reduced dimer-dimer interaction could destabilize the decamer, and this interaction change would occur at its interface loop region including a conserved Met residue which is revealed to be indispensable for the signal transduction. We will discuss the photoreaction dynamics and the role of the Met residue based on the measurement of the M93A mutant.

1P-223 対イオンの位置を変えたロドプシン変異体におけるレチナル発色団光異性化効率

Photoisomerization efficiency of the retinal chromophore in

rhodopsin mutants with displaced counterion

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see ITP2-04

1P-224 代謝型グルタミン酸受容体のリガンド結合に伴う二量体配置転換の比較解析

Comparative analyses of ligand-induced dimeric rearrangements of metabotropic glutamate receptors

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see ITP2-05

1P-225 バリエトプシンの分子機能の探索

Exploring Molecular Functions of Parietopsin

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see ITP2-06

1P-226 高速原子間力顕微鏡 (AFM) を用いたバクテリオロドプシンの光励起に伴う動態観察

High-speed atomic force microscopy visualization reveals dynamic molecular processes in photo-activated bacteriorhodopsin

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see IYP1-03

1P-227 プロトンポンプを目指したバクテリアのセンサーロドプシンの変異体研究

The mutation study to create proton pumps from a bacterial sensor rhodopsin

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see ITP2-07

1P-228 古細菌型ロドプシンのカーボンナノチューブへの内包

Encapsulation of ion-pumping rhodopsins into multi-wall carbon nanotubes

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see ITP2-08

1P-229 霊長類色覚視物質の赤外分光解析

Low-temperature FTIR study of primate color pigments

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