bio-active molecules. However, since so far developed caged compounds require high energetic UV light for their activation, cytotoxicity and poor permeability of the irradiation light restrict the range of their applications. Here, we report a widely known fluorophore BODIPY as a novel PRPG which can be photolyzed with extremely longer wavelength visible light. Through the investigation of photo-chemical properties of BODIPY derivatives, BODIPY derivatives bearing phenol derivatives at 4 position whose fluorescence are quenched due to its intramolecular photo-induced electron transfer released the phenols upon irradiation of visible light. Based on this finding, we developed novel caged glutamic acid which can be activated with far less cytotoxic and more tissue-permeable visible light irradiation. In addition, since the activating efficiency of the caged compounds is comparable to the existing ones, we can use them in much the same way as the widely used caged compounds with less cytotoxicity and more permeability of the irradiation.

1P-242 二種の大腸菌走化性受容体による混合クラスター形成と受容体間相互作用

Lateral communication between distinct types of bacterial chemoreceptors in a mixed cluster

Hiroki Irieda (1,2), Michio Homma (1) and Ikuro Kawagishi (2,3,4,5). (1: Div. of Biological Science, Grad. Sch. of Science, Nagoya Univ.; 2: Research Center for Micro-Nano Technology, Hosei Univ.; 3: Dept. of Frontier Bioscience, Fac. of Bioscience and Applied Chemistry, Hosei Univ.; 4: Dept. of Frontier Bioscience, Fac. of Engineering, Hosei Univ.; 5: Dept. of Materials Chemistry, Grad. Sch. of Engineering, Hosei Univ.)

The chemotaxis of *Escherichia coli* is one of the most extensively studied sensory systems. Environmental stimuli are sensed by methyl-accepting chemotaxis proteins (MCPs), including Tar for aspartate and Tsr for serine, which regulate the cytoplasmic histidine kinase CheA. MCP homodimers cluster at a cell pole together with CheA and the adaptor protein CheW. This clustering is supposed to contribute signal amplification by facilitating interactions between MCP dimers. It is believed that three MCP dimers interact with each other in the periplasmic and cytoplasmic domains to form a hexagonal array. However, the molecular mechanism underlying such MCP communications is obscure. Using *in vivo* disulfide crosslinking assays, we examined the dimer-to-dimer interactions between Tar and Tsr. Co-expression of Cys-mutant versions of Tar and Tsr resulted in crosslinked Tar-Tsr heterodimer, which is predicted to result from crosslinking between Tar and Tsr homodimers. Moreover, aspartate binding to Tar affected not only Tar-Tar crosslinking but also Tar-Tsr and Tsr-Tsr crosslinking, a result which is consistent with horizontal signal propagation via interaction between MCP dimers. To understand the precise mechanism of signal amplification, more detailed structural information of the cluster is indispensable. We therefore set out to purify and analyze trimers of dimers of MCP that are crosslinked at either periplasmic or cytoplasmic positions.

1P-243 コレラ菌・海洋ビブリオ菌のアミノ酸走化性に関する受容体ホモログの解析

Cloning and characterization of chemoreceptor homologs involved in chemotaxis toward amino acids in *Vibrio cholerae* and related species

So-ichiro Nishiyama (1)(2), Daisuke Suzuki (3), Michio Homma (3) and Ikuro Kawagishi (1)(2) (1: Dept of Frontier Bioscience, Faculty of Bioscience and Applied Chemistry, Hosei Univ.; 2: Research Center for Micro-Nano Technology, Hosei Univ.; 3: Division of Biological Science, Graduate School of Science, Nagoya Univ)

Vibrio cholerae, an etiological agent of cholera, shows chemotaxis, which is implicated in pathogenicity as well as survival in various environments. The genome of a classical biotype strain of *V. cholerae* is deduced to encode 45 chemoreceptor homologs, which we name methyl-accepting chemotaxis protein-like proteins (MLPs). Our recent studies revealed that Mlp24 and Mlp37 mediate attractant responses to similar but distinct sets of amino acids. Sequence analyses suggest that these MLPs constitute a subfamily with Mlp8 that has been implicated in colonization of *V. cholerae* in host intestine and MLPs of *Pseudomonas aeruginosa* that are involved in amino acid taxis. The amino acid sequences and the deduced secondary structures suggest that their periplasmic domains are structurally different from the amino acid chemoreceptors of enteric bacteria such as Tsr and Tar. Among the subfamily members, Mlp37 is unique in that it mediates responses to taurine (2-aminooethane sulfonate). We found that marine *Vibrio species* (*V. alginolyticus* and *V. parahaemolyticus*) do not respond to taurine. Their genomes are deduced to encode 30 MLPs, but only one of them (tentatively named MlpA) belongs to the subfamily. Interestingly, expression of *V. alginolyticus* mlpA restored taurine taxis of the mlp37 deletion mutant of *V. cholerae*. This result implies involvement of component(s) in *V. cholerae* other than Mlp37. Analyses are in progress to elucidate mechanisms underlying sensing amino acids and other compounds by the members of this subfamily.

1P-244 コレラ菌走化性関連シグナリングシステムIIIタンパク質の局在制御

Localization control of components of the chemotaxis-related signaling system III in *Vibrio cholerae*

Geetha Hiremath (1)(2), Tatsuki Ebisawa (1)(2), Akihiro Hyakutake(3), Daisuke Suzuki(4), So-ichiro Nishiyama(2)(5), Michio Homma(4), Ikuro Kawagishi(1)(2)(5)(6)(1: Department of Materials Chemistry, Graduate School of Engineering, Hosei University; 2: Research Center for Micro-Nano Technology, Hosei University; 3: Research Institute for Microbial Diseases, Osaka University; 4: Division of Biological Science, Graduate School of Science, Nagoya University; 5: Department of Frontier Bioscience, Faculty of Bioscience and Applied Chemistry, Hosei University; 6: Department of Frontier Bioscience, Faculty of Engineering,

Hosei University)

Vibrio cholerae, the causative agent of cholera, with a single polar flagellum regulates its movement and chemotaxis. The genome sequence of El Tor and classical strains predicts that the bacterium has three sets of chemotaxis-related signaling proteins or Che proteins (each consisting of a distinct system). Our laboratory previously showed that components of system II (CheA2 and CheY3) directly involved in chemotaxis localize to the flagellated pole constitutively, whereas components of system I (CheA1 and CheY2) localize to a pole under anaerobic (or microaerobic) conditions. However, localization of system III components have not been explored. To get an insight into the functions of system III, we examined their subcellular localization by using GFP fusions. GFP-CheA3 and GFP-CheW2 of system III localized to a pole when incubated without shaking, but their polar localization was lost after vigorous shaking, suggesting that polar localization of the components is controlled in response to changes in energy metabolism, a phenomenon similar to system I. Further analyses of GFP-CheA3 deletion mutants revealed that the carboxy terminal region is responsible for the anaerobic localization of CheA3. CheY4-GFP and GFP-CheY4, however, did not localize even with standing culture. This might result from steric hindrance of GFP or low affinity of CheY4 to CheA3. The polar localization of systems I and III also propose that their components assemble to function in anaerobic environments, such as host intestine.

1P-245 細菌化学感覚受容体のフェノール応答に関与する領域の同定

Identification of the regions involved in phenol-sensing by the bacterial chemoreceptors

Ken Takeuchi, Tomohiro Tokunaga, Tomonori Iwama and Keiichi Kawai. (Faculty of Applied Biological Sciences, Gifu University)

Bacteria such as *Escherichia coli* and *Salmonella typhimurium* show chemotaxis by modulating their swimming pattern. The chemotactic stimuli are sensed by methyl-accepting chemoreceptors embedded in the cytoplasmic membrane. Phenol is more complicated chemotactic stimulus than the others. Many of chemoreceptors recognize this compound as a repellent, but some chemoreceptors recognize as an attractant. Citrate receptor Tcp mediates a repellent response to phenol and serine receptor Tsr mediates a weaker repellent response. But aspartate receptor Tar mediates an attractant response to phenol. Thus, the same chemical stimulus induces opposite responses. However, the molecular mechanism underlying the sensing of phenol is unknown. In this study, we constructed various kinds of chimeras and investigated their phenol-sensing properties by the capillary assay. At first, we examined the phenol-sensing abilities of the chimeras between Tcp and Tar to identify the region determining the polarity of receptor signaling in response to phenol. These experiments showed that a short sequence within the cytoplasmic region of the receptor (Gly245-Glu308 of Tar and Gly244-Glu308 of Tcp) is important for the decision of signaling polarity. Currently, we are analyzing the chimeras between Tcp and Tsr to identify the region responsible for the phenol recognition.

1P-246 細菌走化性受容体のセリン認識メカニズムの解析

Serine recognition mechanism of bacterial chemotactic receptors

Hirokata Tajima(1,4), Katsumi Imada(2), Mayuko Sakuma(1), Michio Homma(1), Ikuro Kawagishi(3,4,5,6). (1: Dept Biological Science, Graduate School of Science, Nagoya Univ; 2: Graduate School of Frontier Biosciences, Osaka Univ; 3: Dept Materials Chemistry, Graduate School of Engineering, Hosei Univ; 4: Research Center for Micro-Nano Technology, Hosei Univ; 5: Dept Frontier Bioscience, Faculty of Bioscience and Applied Chemistry, Hosei Univ; 6: Dept Frontier Bioscience, Faculty of Engineering, Hosei Univ)

Escherichia coli has closely related amino acid chemoreceptors with strict ligand specificity: Tar for aspartate and Tsr for serine. We have determined the apo and serine-bound structures of the periplasmic domain of Tsr. The structures revealed that some of the ligand-binding residues of Tsr are arranged differently from the conserved ligand-binding residues of Tar. Furthermore, many negatively charged residues were arranged around the ligand-binding pocket of Tsr, although positively charged residues were arranged around that of Tar. It is concluded that these alterations of arrangements of residues may be necessary for the ligand specificities of Tsr and Tar. To further understand the ligand recognition mechanism, we prepared and characterized periplasmic fragments of mutant Tsr and Tar as well as those of Tsr-Tar chimeras. Serine binding is supposed to cause a subtle structural change in the Tsr periplasmic domain, which would eventually cause structural change(s) in the cytoplasmic signaling domain to regulate the CheA kinase activity. The three-dimensional structures of the periplasmic domain of Tar and the cytoplasmic domain of Tsr and the HAMP domain of a sensor of unknown function from an extremely thermophilic bacterium have already been determined. However, the full-length structure is prerequisite for our understanding of the most basic question, i.e. how the protein transmits signals across the membrane. We therefore set out to purify and crystallize full-length Tar and Tsr.

1P-247 コレラ菌走化性関連システムIシグナル伝達複合体の嫌気条件下における構築

Assembly of the components of a chemotaxis-related signaling system of *Vibrio cholerae* under anaerobic conditions

Tatsuki Ebisawa (1,2), Akihiro Hyakutake (3), Daisuke Suzuki (4), So-ichiro Nishiyama (2,5), Michio Homma (4) and Ikuro Kawagishi (1,2,5,6)

The chemotaxis of *Vibrio cholerae* has been implicated in pathogenicity and survival in various environments. The *V. cholerae* genome has three gene clusters

that encode three distinct sets of proteins (systems I, II and III), each of which are homologous to signaling components responsible for bacterial chemotaxis. Only system II is essential and sufficient for chemotaxis. To gain an insight into cellular functions of the other two systems, we are examining subcellular localization of their components by using GFP-fusion constructs. System II components (CheA2 and CheY3) localize to the flagellated pole constitutively, whereas System I components (CheA1 and CheY2) also localize to the same pole, but only under anaerobic (or microaerobic) conditions. Here we observed that two other system I components, the transmembrane transducer protein Mlp13 and the adaptor protein CheW, localize at the pole only under anaerobic conditions, suggesting that the anaerobic/aerobic of control is exerted at the level of the assembly/disassembly of the MLP-CheW-CheA ternary complex. We constructed and characterized a series of deletions of CheA1 and found that P1[2] domains of CheA1 is sufficient for polar localization under anaerobic conditions. The assembly of the System I components under anaerobic condition could be involved in response to specific signals in anaerobic environments such as host intestine.

1P-248 プロトンポンプへの機能転換をめざしたハロロドプシンの変異導入

Trial to convert halorhodopsin into a proton-pump

Keisuke Nakashima (1), Mikihiro Shibata (1),(2), Makoto Demura (3) and Hideki Kandori (1). (1: Department of Frontier Materials, Nagoya Institute of Technology; 2: School of Mathematics and Physics, College of Science and Engineering, Kanazawa University; 3: Division of Molecular Life Sciences, Faculty of Advanced Life Science, Hokkaido University)

Bacteriorhodopsin (BR) and Halorhodopsin (HR) are light-driven proton and chloride-ion pumps, respectively. We previously converted BR into a chloride-ion pump by a single amino acid replacement (D85T) [1]. In the BR mutant, the Schiff base counterion (Asp85) was replaced to the corresponding amino acid (Thr) in HR. This finding implied that BR and HR share a common transport mechanism, and the ionic specificity is determined by the Schiff base region. However, it has been shown that the T-to-D mutant (such as T126D in *pharaonis* HR) does not convert HR into a proton pump, suggesting that the ion-pumping mechanism is not so simple. Proton pump may be more difficult to be designed, where the pKa of the proton donor and acceptor has to be considerably changed in the water-containing hydrogen-bonding network [2].

In BR, Asp96 and Glu204 constitute proton pathway, whose corresponding residues in pHR are Ala137 and Thr244, respectively. BR and HR possess highly conserved residues near the retinal Schiff base, such as Ala53 in BR and Ser78 in pHR. Gly220 in BR and Ala260 in pHR are also characteristic amino acids. In this study, we try to convert pHR into a proton pump based on the above mutations. In the poster, we will present various properties of these mutant proteins together with their proton-pump activities.

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1P-249 PYP の N 末端領域における構造変化についての理論研究

A theoretical study on the structural change of the N-terminal domain of PYP

Motoshi Kamiya (1), Shinji Saito (2), Iwao Ohmine(1). (1: Chemistry Department, Faculty of Science, Nagoya Univ.; 2: Institute for Molecular Science)

Photoactive yellow protein (PYP) from *Halorhodospira halophila* is believed to be the photosensor for the negative phototaxis. PYP has a *p*-coumaric acid as the chromophore. The photocycle of PYP is initiated by the photoisomerization of the chromophore in the pG state. After the isomerization, the protonation of the chromophore and the deprotonation of Glu46 take place. This chromophore protonated state is called pB'. After the alteration in the protonation state of the chromophore and Glu46, the N-terminal domain undergoes structural changes, which may involve the unfolding of the N-terminal domain. As a consequence of these structural changes, the pB state, which is believed to be the active structure for the signal transduction, appears.

Recently, Hoshihara *et al.* demonstrated that the decrease in the diffusion coefficient is coupled with the formation of pB'. (Biophys. J. 94, 2187) Since the decrease in the diffusion coefficient is closely related with the disappearance of the helices in the N-terminal region, they concluded that the unfolding of the N-terminal region and the formation of pBp (protonation of the chromophore) take place simultaneously. Their result is very interesting and attractive, although it is different from the conventional idea. We thus calculated the self diffusion coefficient (D_s) and the radius of gyration (R_g) of PYP structures. We will discuss about the structure of pB' and pB on the basis of D_s , R_g , and the free energy.

1P-250 全反射赤外分光法によるファラオニスフォボロドプシンのプロトン放出機構の研究

Early proton release from *pharaonis* phoborhodopsin studied by ATR-FTIR spectroscopy

Yuya Kitade (1), Yuji Furutani (1), Naoki Kamo (2) and Hideki Kandori (1). (1: Department of Frontier Materials, Nagoya Institute of Technology; 2: Graduate School of Pharmaceutical Sciences, Hokkaido University)

pharaonis phoborhodopsin (ppR, also called *pharaonis* sensory rhodopsin II; pSRII) is a photoreceptor protein for negative phototaxis in *Natronomonas pharaonis*. In the absence of transducer protein, pHtrII, ppR can pump protons across the membrane like bacteriorhodopsin (BR), the best-studied proton pump. Previously, early proton release from the extracellular side upon formation of the M state of ppR was observed in the presence of chloride at acidic pH, but not in

their absence at any pH [1]. No such proton release for the mutant at Asp193 (Glu204 in BR) strongly suggested that the proton was released around this residue. In the case of BR, a protonated water cluster near Glu194 and Glu204 was identified as a proton releasing group by time-resolved FTIR spectroscopy [2, 3]. In this study, we applied attenuated total reflection (ATR) FTIR technique to investigate effect of chloride on the structure of ppR and the M intermediate. We found that Asp193 is protonated in the presence of chloride ions, while it deprotonates upon formation of the M intermediate. Double difference of the M minus ppR spectra with/without chloride coincides with the difference spectrum of chloride binding to ppR, indicating that the M formation accompanies proton and chloride releases from the Asp193 region. Difference in the proton releasing mechanism between ppR and BR will be discussed.

References

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1P-251 アナバセンサリーロドプシンとトランスデューサータンパク質との相互作用の全反射赤外分光法による研究

Protein-protein interaction between Anabaena sensory rhodopsin and 14kD soluble protein studied by ATR-FTIR spectroscopy

Yuji Furutani(1), Aya Nakatsuma(1), Akira Kawanabe(1), Kwang-Hwan Jung(2), Hideki Kandori(1)(1: Department of Frontier Materials, Nagoya Institute of Technology; 2: Department of Life Science and Interdisciplinary Program of Integrated Biotechnology, Sogang University)

Anabaena Sensory Rhodopsin (ASR) is an archaeal-type rhodopsin found in cyanobacteria. The gene encoding ASR forms a single operon with a 14kDa soluble protein, suggesting that ASR might function as a photoreceptor coupled with the soluble transducer. We previously revealed that ASR exhibits a 100 % photochromic reaction between the all-trans and 13-cis chromophores [1]. We also reported the early structural changes of the all-trans and 13-cis forms of ASR [2,3]. The X-ray crystal structures of ASR and the 14kD protein were reported, where the 14kDa protein forms homo-tetramer. The previous isothermal titration calorimetry (ITC) measurement determined the dissociation constant between ASR and the tetrameric 14kDa protein to be 17 μ M. However, little has been known about the molecular mechanism of the protein-protein interaction between them. Here we study the structural changes upon interaction between ASR and the 14kDa protein by use of Attenuated total reflection (ATR)-FTIR spectroscopy. ATR technique allows measurement of infrared spectra of samples soaked in desired buffer solution. In the meeting, we will show the effect of the 14kDa transducer protein on the photochromic reaction of ASR and discuss the signal transduction mechanism between them.

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1P-252 Rhodobacter capsulatus 由来 PYP の複合体状態における分光学的特長

Spectral characterization of the complex form of the photoactive yellow protein of Rhodobacter capsulatus

Yoichi Yamazaki, Hironari Kamikubo and Mikio Kataoka (Graduate School of Materials Science, Nara Institute of Science and Technology)

Photoactive yellow protein (PYP) is a blue light photo-receptor firstly obtained from purple sulfur photosynthetic bacteria, *Halorhodospira halophila* (Hh). PYP absorbs blue light by use of a covalently attached chromophore, *p*-coumaric acid. PYP-homologous proteins classified three types, the first group is represented by Hh-PYP which has clear single absorbance maximum around 440nm, and the second group is represented by PYP obtained from purple non-sulfur bacterium such as *Rhodobacter capsulatus* (Rc) which shows two absorbance peaks at around 370 and 440nm. In the rest group, PYP is a part of multi domain proteins as a sensor domain.

The Rc-PYP absorbs UV light and binds with its associate protein. This complex form is suggested to be an active state in the signal transduction process. To characterize this protein-protein complex form, we determined its spectral features and dissociation constant. UV light illumination to the Rc-PYP causes trans-cis isomerization on the chromophore. The cis-form chromophore is in equilibrium of protonation and deprotonation states with a pKa 6.4. In the complex form, the deprotonated cis-form chromophore showed about 20nm red-shifted absorption maximum. In acidic condition, complex form also changes its chromophore protonation state with a pKa about 6. Thus chromophore protonation state is not influence in binding capability; actually it showed same Kd value for binding in acidic condition with neutral condition.

1P-253 ロドプシンのカーボンナノチューブへの内包

Encapsulation of a proton-pumping rhodopsin into carbon nanotubes

Takayoshi Kawashima (1), Kazuhiro Yanagi (2), Yuji Furutani (1), Hiromichi Kataura (2), Hideki Kandori (1). (1: Department of Frontier Materials, Nagoya Institute of Technology; 2: Nanotechnology Research Institute (NRI), National Institute of Advanced Industrial Science and Technology (AIST))

Encapsulation of small molecules into carbon nanotubes (CNT) has been extensively studied in chemistry and material sciences, because molecular