2Q1436 バイオメトリ学における一方向ブロット移動をもたる構造の再構成: 時間分割FTIR法による研究
structural rearrangement for the unidirectional proton transfer in the bacteriorhodopsin photocycle: a time-resolved FTIR study

The photocycle of bacteriorhodopsin at neutral pH, the proton transfer from the Schiff base to Asp85 in the L-to-M transition causes deprotonation of the proton release group (PRG) which is located close to the extracellular surface. This leads to unidirectional proton transfer by preventing the reverse proton flow. This transition is lifed at pH 4.5 below the pKa ~5.7 of the PRG in M where proton release occurs later after proton uptake. High proton affinity to Asp85 at neutral pH is supposed to be resulted from structural rearrangement induced by the deprotonation of the PRG, in addition to a simple electrostatic interaction with the PRG. In this way, possible structural changes were explored in time-resolved FTIR spectral changes that persisted through the M, N, and O intermediates at pH 7, but not at pH 5 (Morgan et al., Biochemistry 49, 3273, 2010).

Two changes were observed. One is depletion of a broad continuum band, probably due to the deprotonation of a protonated water dimer (proton release from the PRG). The other is uncoupling of the N-C stretching vibration of the side chain of Lys216 from the C15-H bending vibration of the chromophore. Decrease in the intensity of the N-C bond may be responsible for the proton affinity to Asp85, which is disposed close to the N-C bond in Lys216 in M. Other changes that occur only in M are the perturbations of Arg82 and of a string of backbone carbonyl groups in helix G. These changes may link the deprotonation of the PRG to the change in Lys216.

2Q1446 アミノ酸変換活性の変容型セリノリドプロトン系の構造変化解析
structural changes during the photocycle of Salmonella senso rhodopsin I in the absence and presence of salt

Sensory rhodopsin I (SR1) is a dual photoreceptor which regulates both negative and positive phototaxis in microbial organisms. All known SR1s have been isolated from highly halophilic organisms, such as the eubacteria Halobacterium halobacter or the archaeon Halobacterium salinarum. Therefore, salt is expected to affect greatly the properties of SRI, and its structural changes during the photocycle. Due to the remarkable stability of SRI from S. ruber (sSR1) even in the absence of salt, the effect of various salts could be studied for the first time. Using low temperature FTIR spectroscopy, we could study the structural changes upon M-intermediate formation and presence of NaCl. Using a range of mutants we found, i.e., that, in the absence of salt, Asp102, a conserved residue, interacts with the beta ionone ring of the retinal chromophore, is protonated and deprotonated in the dark and M-intermediate, respectively. In contrast, in the present of salt, it is suppressed. We also studied the structural changes upon formation of the K-intermediate in the presence of various salts which, i.e., confirm the suppression of the perturbation of Asp102. Using D2O and D218O labelled samples, we could record changes in structure of bound water molecules near the chromophore. On the basis of the obtained results, we will discuss the structural changes around the retinal during the photocycle in the presence and absence of salt.

2Q1512 ハロロドプシン十変異体の研究
tenfold mutant of pharaoins halorhodopsin
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Nature created two kinds of light-driven ion pumps, bacteriorhodopsin (BR) and halorhodopsin (HR) functioning as outward proton and inward chloride pumps, respectively. We previously reported that BR can be converted to a chloride pump by a D-to-T mutation at position 85 [1]. This finding implied that BR and HR share a common transport mechanism, and the intrinsic specificity is determined by the Schiff base region. However, it has been shown that HR cannot pump protons by the reverse T-to-D mutation, indicating that the ion-pumping mechanism is not so simple. Our previous trial using multiple mutants was not successful to convert HR into a proton pump. Here we selected 10 amino acids that are highly conserved in each pump, and prepared the tenfold mutant of pharaoins HR (N41G/A44S/M578A/T126D/S130T/A137D/T224E/S249M/Y257T/A260G). Since the tenfold mutant HR still did not pump protons, we next study the mechanism of the asymmetric functional conversion between BR and HR. Our FTIR analysis of the Schiff base N-D and water O-D stretches in D2O showed that both H-bonds are strong in BR, while both are weak in HR, and all proton-pumping rhodopsins have strongly H-bonded waters [2]. The present FTIR analysis of the tenfold mutant revealed that the H-bond of the Schiff base becomes strong, while the water H-bond is still weak. We thus concluded that the internal water structure, the determinant of proton pump, is not sufficient for functional conversion.

2Q1524 フラボプロテインに作用するプロトン転送型のin situでの光活性化プロトンポンプの測定
in situ measurement of light-driven proton pump by proteorhodopsin of marine Flavobacteria

Bacteriorhodopsin (BR) is a light-driven proton pump in halophilic archaea, and BR-like rhodopsins have been also discovered in Bacteria and Eucaryota. Proteorhodopsin (PR) genes are widely distributed among marine prokaryotes [1]. When PR is expressed heterologously in E. coli, they show proton-pump activity, implying that PR functions for light-energy conversion in the marine environment. On the other hand, little effect of light has been reported for native cells, and it has been thus suggested that PR may have another function [2]. What is the real function of >5000 PRs in marine bacteria? In the present study, we successfully isolated 38 PR-containing strains of Flavobacteria, and examined the proton-pump activity for the selected 8 different strains. Upon illumination, the cell suspensions of all 8 strains showed marked pH drop that was abolished by the addition of protonophore, which provides the first experimental evidence that PR pumps protons under physiological conditions. Although the native cells contain huge amounts of carotenoids, we were able to obtain the light-induced different spectra between PR and retinal oxime in the presence of hydroxyamine. As a result, we quantitatively show the light-driven proton pump activity (124 ± 73 proton/PR/min), from which total solar energy that passes through PR can be estimated in the open ocean. The role of PR photophorylase will be discussed.

2Q1536 A Mutational Study of Anabaena Sensory Rhodopsin
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Anabaena Sensory Rhodopsin (ASR) is a microbial rhodopsin found in Anabaena (Nostoc) sp. PCC7120, a freshwater cyanobacterium. As it forms a single operon with a soluble protein of 14 kDa, it was suggested that ASR is a photosensory chromophore interacting with the 14 kDa transducer protein at the cytoplasmic surface. Microbial rhodopsins possess all-trans and 13-cis retinal as the chromophore, but only the former is important for function. In bacteriorhodopsin (BR), the stable product of the all-trans of the all-trans is 100% all-trans. Nevertheless, we found that the photoactivation of ASR is completely photochromic; the stable product of the all-trans is 100% all-trans, and that of the 13-cis form is 100% all-trans [1]. It is intriguing that BR and ASR exhibit similar protein architecture, but photoactivation is entirely different. A key amino acid may be Pro206 of ASR, because the corresponding amino acid of other microbial rhodopsins is Asp (Asp212 in BR). In the present study, we mutated Pro206 of ASR and examined their molecular properties. HPLC analysis showed that when Pro206 is mutated to Asp, the protein contains only