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Communication

Photochemical properties of a Rhodopsin for Light Energy Conversion obtained from Yellow Sea in Korea

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ABSTRACT : Proteorhodopsin (PR) is a photoinduced proton pump found abundantly in ocean and fresh water habitat, and has an important role in photoenergy conversion to bioenergy in the living cells. Numerous sequences that encode PR protein variants were discovered by environmental genome sequencing and they indicated the high sequence similarity.

A new-type of PR (YS-PR) which had been discovered from the surface of Yellow Sea was found to have only 5 amino acid differences from the previously known green-light absorbing PR (GPR) protein, but showed different photochemical properties. This YS-PR showed a 10 nm red-shifted absorption maximum, when compared with GPR. It also showed slower photocycling rate than GPR. However, the photoconversion rate of YS-PR was fast enough to pump protons. Four different amino acids out of 5 were similar to Blue-light absorbing PR (BPR), suggesting that those residues might be responsible for the observed spectral and photoconverting properties.

Since four kinds of haloarcheal rhodopsin had been identified from *Halobacterium salinarum*, various microbial rhodopsins were discovered from marine and fresh water habitats. These microbial rhodopsins displayed several different photochemical properties serving as ion pump, light dependent ion channel or photosensory receptor for photosignal transduction[1-9].

Proteorhodopsin (PR), a photoactive proton pump, was identified in SAR86 group of γ-proteobacteria from environmental genome sequencing for the first time[10] and since then numerous PR variants and partial sequences of PR-like gene has been reported from the ocean and fresh water samples[4-6, 11-12]. Several subfamilies of PRs have been known to depend on their absorption maxima such as green-light absorbing proteorhodopsin (GPR) and blue-light absorbing proteorhodopsin (BPR). Their distribution was shown to be stratified with depth[3,13-14].

Here, we report that a novel PR gene was isolated from environmental genome sample, collected from the surface of Yellow Sea (37.26°N, 126.22°E) by degenerate PCR method with specific primer set (Table 1). This gene was found to be very similar to that of GPR, only differing in 5 amino acids (Y167, A188, T191, V194, V195) (Fig. 1). All of those differences were located in helix E and F of the protein, which is similar to BPR sequence. Among PR subfamilies, the amino acid sequence identity was more than 70% and it has been reported that a single residue difference in retinal binding pocket and elsewhere determined the absorbance and photochemical properties[13,15]. This might be responsible for why the novel-type PR showed the different spectral attribute from GPR.

Table 1. Primer sequences used in degenerate PCR.

	Primer name	Sequence
Forward	degen-PR-NdeI	5'-CATATGAAATTATTACTGATATTAGG-3'
Reverse1	degen-PR-NotI	5'-GCGGCCGCTTAAGCATTAGAAGATTCTTTAACAGC-3'
Reverse2	degen-PR-NotI-6his	5'-GCGGCCGCTTAGTGATGATGGTGGTGATGAGCATTAGAA GATTCTTTAACAGC-3'

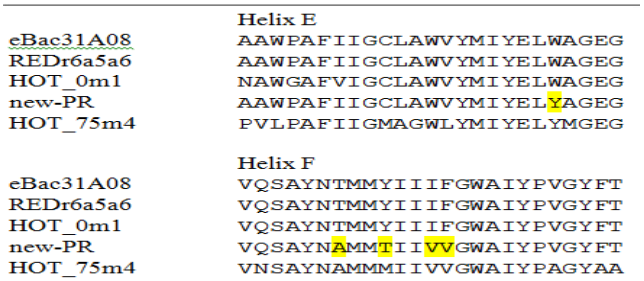

Figure 1. Amino acid sequence alignment of YS-PR with various GPR and BPR. The residues of new PR different from GPR were highlighted and those were similar with BPR. All the differences are located in helix E and F. (GPR: eBac31A08, REDr6a5a6, HOT_0m1, BPR: HOT_75m4)

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To verify the spectral property of new-PR, absorbance was measured under various pHs. Microbial rhodopsins exhibit two spectral forms depending on the protonation of proton acceptor residue[16]. At low pH, the spectrum is red shifted because of the proton acceptor protonation. Our YS-PR also showed the pH-dependent spectral property. When the absorption maximum at 535 nm was determined at neutral pH, it showed 10 nm red shift, compared with that of GPR (Fig. 2). The spectral values at acidic or alkaline pH were also slightly red shifted as compared to that of GPR. It was earlier reported that single amino acid replacement at 194th residue exhibited slightly red shifted spectrum[15]. Although there might be influences due to every single variant residue, the V194 residue (I194 in GPR) located in helix F seemed to play a role in the formation of red-shifted spectrum displayed by YS-PR.

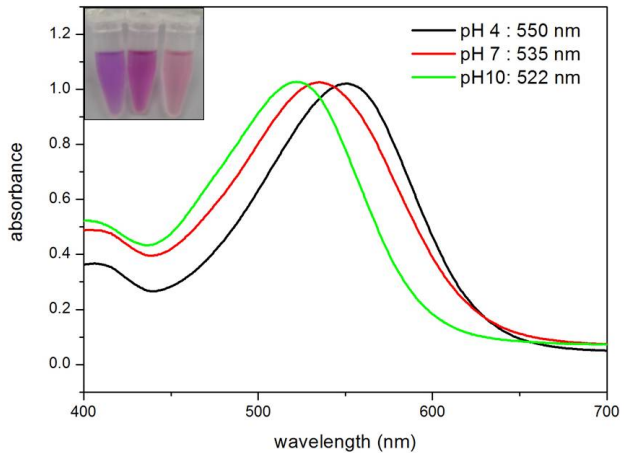

Figure 2. pH dependence of absorption spectra displayed by the novel- type PR. In general, microbial rhodopsins exhibit two spectral forms in a proton dependent equilibrium. Red shifted absorption maximum was indicated at low pH. YS-PR indicated 535 nm of absorption maximum at pH 7. (Inset: proteins at each pHs. left: pH4, middle: pH7, right: pH10)

The photoactivated PR pigment changes the conformation of chromophore and returns to its original form after sequentially passing through several photointermediates. The photo-intermediates of PR are similar to those of BR and are consistent with its proton transport function[17]. It is known that the photocycle of PR contains at least five intermediates termed K, M1, M2, N, and O[18]. To determine the rate of the formation of photointermediates, light-adapted different spectrum was measured. After 1-min illumination, the dark-adapted ground state pigment color was disappeared. And M intermediate like blue shifted pigment as well as O intermediate like red shifted photo-intermediates were accumulated during light adaptation (Fig. 3).

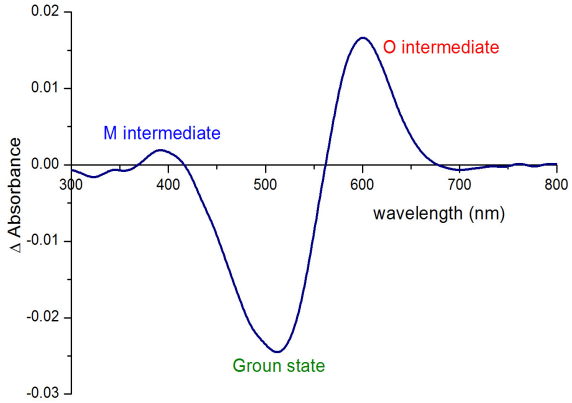

Figure 3. Different spectrum of YS-PR under light illumination. Purified YS-PR protein in 0.02% DDM solution was illuminated with white light. After illumination, dark-adapted pigment (Ground state) was disappeared and blue shifted M intermediate and red shifted O intermediate were accumulate.

PR is a light-driven proton pump[10,19-20] and has fast photo-conversion rate like other ion pumping rhodopsins. It was previously reported that the photocycle rate of GPR had been determined in 10 msec[15]. However, YS-PR was shown to have relatively slow photocycle. When the recovery rate of photoactivated ground state was measured in terms of two different $t_{1/2}$-values fitted in the second order exponential, it showed 23.3 msec and 130.1 msec, respectively, when monitored at 530 nm after laser flash (Fig. 4). The decay rate of O intermediate was 92.6 msec. The photo-conversion rate of new-PR was found to be nearly 10 times slower than that of GPR. However, the amount of M intermediate was too low to be detected.

The photocycling rate of BPR is relatively slower than GPR[14]. The overall photoconversion rate of YS-PR was similar to that of BPR. Earlier studies proposed that the single amino acid replacement in the 105th residue determined the photochemical and spectral properties of GPR and BPR. From those perspectives, we suggest that the amino acid residues located in the helix F, which are very similar to that of BPR, influenced the photointermediate conversion rate of the new-PR.

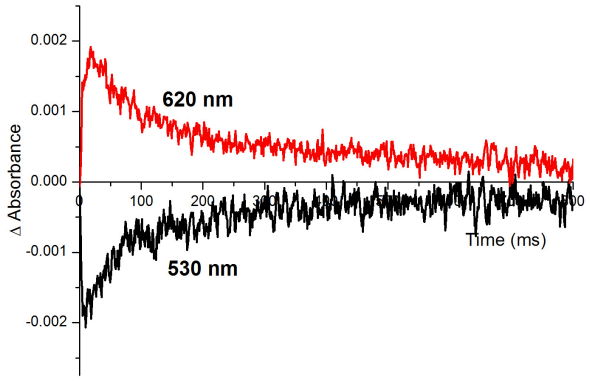

Figure 4. Photochemical reaction cycle of YS-PR. Absorption changes after a laser pulse were followed at 530 nm (Ground state) and 620 nm (O intermediate). Eight traces were averaged and $t_{1/2}$-values were calculated by exponential curve fits using Origin 7.0. Formation rate of ground state indicated second order exponential rate, 23.3 msec and 130.1 msec and decay rate of O intermediate was 92.6 msec.

In summary, the newly obtained PR protein had several amino acid sequence differences and displayed similar, but little different photochemical and photo-physical properties, when compared with other known GPR proteins. We suggest that those differences are attributed to those amino acid variations. However, how each alteration of those residues contributes to the observed photochemical attributes and the photoconversion mechanism in the living cells remains to be solved.

Experimental Procedures

Proteorhodopsin Protein Purification

Overnight cultures of his-tagged PR protein in *E. coli* UT5600 were induced with 5~10 μM all-trans-retinal (Sigma, USA) and 1 mM IPTG (Duchefa, Netherland) for *5* h at 30℃. *E. coli* cells were harvested and suspended in sonication buffer (50 mM Tris-HCl, pH 7.0, 150 mM NaCl). Cells were lysed by sonication (Branson sonifier 250) at 4℃ followed by low-speed (3,220 x g for 20 min) centrifugation (Eppendorf centrifuge 5810R) to remove cell debris. Cell membranes with rhodopsins were sedimented by centrifugation at 95,000 x g for 1 h at 4℃ (Ti70 rotor, Beckman XL-90 ultracentrifuge) and the pellets were suspended in sonication buffer. The rhodopsins were extracted from the membranes with gentle shaking in extraction buffer (1% n-dodecyl-β-D-maltopyranoside (DDM), 150 mM NaCl, 50 mM Tris-HCl, pH 7.0) overnight at 4℃. Insolubilized material was removed by centrifugation at 30,000 x g for 15 min at 4℃(Ti70 rotor, Beckman XL-90 ultracentrifuge). The supernatant was purified with Ni^{2+}-NTA agarose (Qiagen).

Light adapted different spectra

Purified PR protein at pH 7.0 was illuminated for 1 min and the time resolved spectrum was measured by using Scinco S-3100 spectrophotometer in every 5 sec for 30 sec under light illumination. Non-light illumination state sample at pH 7.0 were the reference.

Flash photolysis

Flash-induced absorption changes were measured with a laboratory-constructed cross-beam flash spectrophotometer, Olis-RSM (Rapid-Scanning Monochromator) 1000 (Olis, Inc. USA), with a Minilite Q-Switched Nd-YAG laser (532 nm, 3-5 ns, 25 mJ) providing the actinic flash. For calculation of the amplitude spectra of the exponentially decaying components, the measurements were performed at 530 nm and 620 nm, the species with initial state and O intermediate, respectively. Each trace was obtained by averaging 8 individual measurements. Amplitudes and $t_{1/2}$-values were calculated by fitting the traces with the single exponential curve-fitting program Origin 7.0.

KEYWORDS : proteorhodopsin, photoreceptor, proton pump

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