Article Addendum

A photochromic photoreceptor from a eubacterium

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Sensory rhodopsin I (SRI) is one of the most interesting photosensory receptors because of its function in using the photochromic reaction to mediate opposing signals which depend on the color of light. It was initially thought that SRI exists only in the archaea, but we recently reported for the first time a newly functional SRI from a eubacterium, Salinibacter ruber (SrSRI). The amino acid sequence of SrSRI shows 43% identity with the well-known SRI (HsSRI) and contains most of the amino acid residues identified as necessary for SRI function. The photochemical properties of SrSRI are similar to those of HsSRI. In addition, SrSRI is a highly stable protein, even in dilute salt conditions. Thus, SrSRI could be a key protein for characterizing its association with the SrSRI transducer protein, SrHtrI, and for elucidating structural changes of SRI and HtrI that occur during their function. Recently, new approaches to manipulate cellular functions with rhodopsins have been established. SRI can activate and deactivate a kinase, CheA, by the photochromic reaction. Kinases are key molecules for signal transduction in various organisms, and SRI could potentially manipulate their cellular functions.

Many organisms utilize light not only as an energy source but also as a signal. Light is one of the most important signals that provide critical information to biological systems. In addition to vision in vertebrates, many microorganisms can sense light and show avoidance or attractive behavior from or toward light, behaviors that are collectively called phototaxis. These responses are regulated by photoactive membrane-embedded molecules (rhodopsins), which are 7-transmembrane helix proteins, which contain retinal (vitamin A-aldehyde) as a chromophore. Bacteriorhodopsin (BR) and halorhodopsin (HR) work as light-driven ion pumps to produce energy.1,2 While visual pigments3 and signal transduction molecules, such as sensory rhodopsin-I (SRI) and sensory rhodopsin-II (SRII, also called phoborhodopsin), work as sensors to transfer the signals to the cytoplasm.4,5 Rhodopsin molecules had been believed only to exist in the archaea and in some higher organisms such as humans but not in fungi or other eukaryotic microorganisms. However, the nop-1 gene, which encodes a protein homologous to archaeal rhodopsins, was identified in a eukaryotic filamentous fungus Neurospora crassa in 1999.6 Additionally, a light-driven H+ pump (proteorhodopsin, PR) from γ-proteobacteria,7 channelrhodopsins-1, 2 (ChR1 and ChR2, also called Chlamydomonas sensory rhodopsins A, B) from Chlamydomonas reinhardtii,8,9 a sensory rhodopsin (ASR) from Anabaena sp. PCC7120,10 a light-driven H+ pump (Gloeobacter rhodopsin, GR) from a cyanobacterium Gloeobacter violaceus11 and a rhodopsin (AR) from a marine alga Actetabularia acetabulum12 were reported from 2000 through 2007. Further, new rhodopsin molecules have been found in various organisms over the past few years.13,14 Thus, rhodopsins are widespread in biological species, indicating that photo-responses by rhodopsin activation are important for living cells.

Among the rhodopsins, SRI shows quite unique features. SRI from an archaea Halobacterium salinarum (HsSRI) can be activated by orange light (560–580 nm).15,16 A kinase, CheA, can be activated during the photocycle, which induces the counterclockwise rotation of the flagellar motor, resulting in positive phototaxis to orange light.17 The absorption spectrum of SRI overlaps the spectra of BR and HR. Thus, SRI functions as an attractant sensor for energy production by BR and HR. Interestingly, SRI also functions as a repellent sensor to avoid harmful near-UV light.15,16 SRI absorbs orange light and forms the long-lived photo-intermediate, S373, which then forms the P510 intermediate upon a second photon absorption by the near-UV light.18 Only when the photocycle signals are produced through the P510 intermediate, can SRI stimulate CheA to induce clockwise rotation of the flagellar motor, resulting in a negative phototaxis to near-UV light.17 Thus, SRI has received considerable attention because of its function to mediate opposing signals (On/Off switching of a kinase) depending on different colors of light by the photochromic reaction.19 However, its inherent instability hampers elucidation of its molecular mechanism.

The year 2005 marks more than two decades since HsSRI was functionally implicated in haloarchaea. The genome sequence of the eubacterium Salinibacter ruber predicts that bacterium has two genes encoding SRI-like proteins, which were the first eubacterial sensory rhodopsin-I genes identified.20 To determine whether these genes encode functional proteins, we cloned and expressed them in...
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Escherichia coli. We successfully produced one of them (SRII) as a recombinant protein, which has an absorption maximum at 558 nm. SRI shows 43% amino-acid sequence identity to HsSRI and contains all-trans retinal as a chromophore. The turnover rate of the SRIII photocycle is slower than those of the light-driven ion-pumping rhodopsins, BR, HR, PR and GR. In addition, the S373-like intermediate (SRII[S373]) forms a P510-like intermediate (SRII[S150]) upon the second photon absorption (Fig. 1A), indicating similarities to the well-known SRI, HsSRI. Interestingly, SRI is more than 10,000 times more stable than HsSRI in dilute salt conditions (Fig. 1B) over a wide pH range (pH 2.5–10) and at high temperature (−60°C). The rhodopsins, except for a vertebrate rhodopsin, have almost the same three-dimensional structures, implying that functional differences in those proteins originate from differences in their amino acid side chains. Sudo and Spudich successfully engineered BR into an SRII-like photosensor by replacing only three residues and Sasaki et al., also engineered BR into a HR-like light-driven chloride pump by replacing only one residue. Thus, the high stability of SRI might be achieved by very small differences in its amino acid side chains. In any case, highly stable SRI could be a key protein to understand the molecular mechanisms of SRI because the stable sensory rhodopsin-II (SRII, blue light receptor for 500 nm) from Natronomonas pharaonis (NpSRII) allowed more detailed information to be obtained about the function and structural changes of SRII. In fact, we have already demonstrated the unique structure and structural changes of SRI during the photocycle by Fourier Transform Infrared spectroscopy (manuscript submitted).

Photoactive proteins, including rhodopsins, are activated and controlled by light and work as respective functional photoreceptors. Thus, one could precisely regulate various phenomena in living cells using photoactive proteins. In fact, Zhang and coworkers succeeded in controlling Caenorhabditis elegans locomotion using the light-gated ion channeling ability of channelrhodopsin-2 (ChR2) combined with the light-driven Cl− permeability of HR. Recently, their group also reported that locomotion can be controlled using the other channel rhodopsin, which has a different absorption maximum. As described above, SRI can activate and deactivate a kinase, CheA, by the photochromatic reaction. Kinases are key molecules for signal transduction in various organisms, thus it should be possible to manipulate cellular function using SRI to activate other kinases.

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