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FTIR spectroscopy of the two-photon product of sensory rhodopsin I

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A halophilic archaeon, *Halobacterium salinarum*, exhibits phototactic behaviors, by which the organism is guided to red-orange light and evades shorter wavelengths of light. The phototaxis is mediated by two retinal proteins, sensory rhodopsin I and II (SRI and SRII), whose structures are analogous to the cognate protein bacteriorhodopsin, a light-driven proton pump. SRI mediates both attractant and repellent swimming behaviors to orange light and near-UV light, respectively. The two different signaling through the single photoreceptor have been ascribed to the presence of two active structures of SRI (S_{373} and P_{520}), which are produced upon orange light illumination of SRI and upon subsequent near-UV illumination of S_{373}, respectively.

In the present study, we have measured the difference FTIR spectra of S_{373} and P_{520} states. In P_{520}, the isomeric structure of the chromophore is assignable to all-*trans*, and the Schiff base of the chromophore is protonated with concomitant deprotonation of Asp76, a combination which allows for the formation of a salt bridge between them. It was suggested that the way of interaction between the Schiff base and the counterion, which is different among SRI_{587}, S_{373} and P_{520} and which has been shown to drive the conformational changes in the cognate protein, bacteriorhodopsin, is the key to controlling conformational changes for the attractant and the repellent signaling by SRI.

key words: sensory rhodopsin, Fourier transform infrared spectroscopy, signaling state, conformational changes

INTRODUCTION

Halobacterium salinarum, an archaeon, living under extremely halophile conditions utilizes light as the source of energy by use of retinal containing membrane protein, bacteriorhodopsin (BR) and halorhodopsin (HR), which generate electrogenic membrane potential by pumping protons and chlorides, respectively, across the plasma membrane.

This organism uses the same types of retinal proteins, sensory rhodopsin I (SRI), sensory rhodopsin II (SRII), as sensors for the phototactic swimming behaviors (1, 2). SRII mediates only a photophobic response of the cells to evade blue-green light, while SRI mediates both an attractant response to orange light and a repellent response to near-UV light. The response of the cells to the two different wavelengths of light is rationalized by the presence of two signaling states of SRI, S_{373} and P_{520}, which are produced upon photoreaction of SRI with red-light and upon photoreaction of S_{373} with near-UV light, respectively (3).

These four retinal proteins share a common architecture in which a retinal chromophore is linked to a lysine residue in the middle of the 7th helix via a protonated Schiff base, which is stabilized by forming a salt bridge with the negatively charged aspartic acid on the 3rd helix or with a chloride in the case of HR. In the proton pump BR, photo-isomerization of the chromophore from all-*trans* to 13-*cis* and subsequent disruption of the salt-bridge upon proton transfer from the Schiff base to Asp85 plays a key role in inducing a vectorial proton movement and the opening of the cytoplasmic channel that follows. This machinery, which is conserved in the SRs, may also be relevant for the conformational changes for the signaling by the SRs (2).

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The mechanism of SRI to produce two different signaling states may well be explained by analyzing the active site structure, the isomeric state of the chromophore and the protonation state of the Schiff base counteion, Asp76. In the dark state, this residue is protonated in SRI, which is supposed to express a conformation attributable to the O intermediate of BR. S_{373}, a signaling state for the attractant swimming behavior, is thought to correspond to the M intermediate of BR having a 13-*cis* chromophore with unprotonated Schiff base.

In the present study, we performed Fourier transform infrared (FTIR) spectroscopy for the purpose of characterizing P_{520} state in terms of the active site structure, which is believed to define overall protein structure.

RESULTS & DISCUSSION

SRI was overproduced in a *H. salinarum* strain Pho81 transformed with a plasmid vector pSO12, which contains the *sopI* gene under the control of the *bop* promoter. Purification of SRI and the subsequent reconstitution into halobacterial lipid membrane were carried out as described in (4). The SRI-containing membrane was pelleted by centrifugation at 10,000 rpm and squeezed between two BaF_2 windows spaced with a 25-μm thick Teflon sheet O-ring. The specimen was set in a cryostat placed in a FTIR spectrometer.

S_{373} was produced at 243 K by illumination of SRI_{587} with >510-nm light. Figure 1a presents the difference spectrum in the UV-visible region before and after the illumination. The maximum absorption near 373 nm substantiates the formation of S_{373} at the expense of SRI_{587} upon the photoreaction. S_{373} was further light-converted at 243 K with a near-UV light ($\sim$ 40-nm band width centered at 390 nm) to a photoproduct, which absorbs near 530 nm, as is shown in the difference spectrum versus S_{373} (Fig. 1b). Since at 243 K this product was unstable and decayed to SRI_{587} in minute time scale (Fig. 1b), even the spectrum measured immediately after the illumination was suspected to be contributed to substantially by SRI_{587}. To obtain the spectrum without the significant contribution of SRI_{587}, we conducted another set of measurement in which S_{373} produced at 243K was light-converted at 223 K, where the decay of the photoproduct was prevented. Because the maximum absorption of the photoproduct was near 520 nm (not shown), we denote it as P_{520}.

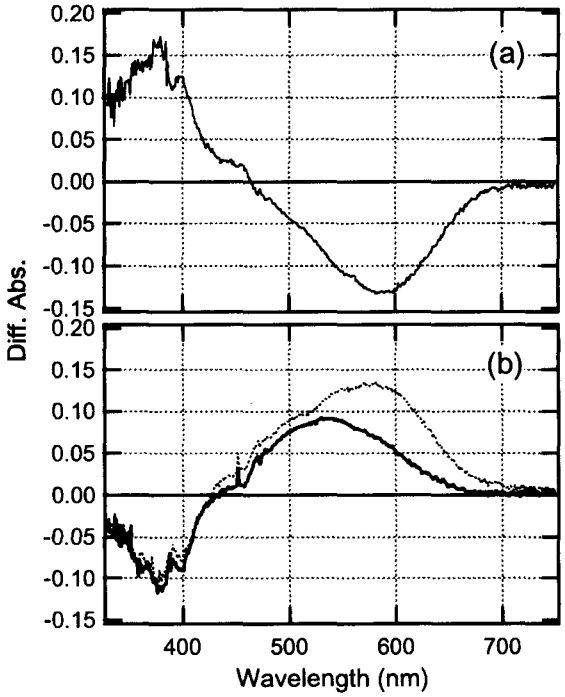

Figure 1: Difference spectra in the UV-visible region at 243 K. (a) S_{373} was produced upon illumination of SRI_{587} with >510-nm light. The difference spectrum before and after the illumination represent the difference of S_{373} and SRI_{587}. (b) Difference between the spectra measured immediately after illumination of with 390-nm light vs. that before the illumination(bold line) and the spectrum measured 1 hr after the illumination vs. that before the illumination (dotted line).

The difference FTIR spectrum of S_{373} *minus* SRI as shown in Fig. 2a reproduced the previous measurement of the corresponding spectrum (5). The 1762 and 1747 cm^{-1} bands in the negative and the positive side of the C=O stretching frequency region have been assigned to those of Asp76 in the SRI and S_{373}, respectively, demonstrating that Asp76 is protonated in both SRI and S_{373}. In the finger print region (1250 - 1100 cm^{-1}), coupled C-C stretching and bending modes exhibit characteristic patterns of bands depending on the isomeric state

of the chromophore. The absence of the bands in the positive side indicates that the Schiff base is deprotonated in S_{373}, whereas in the negative side the 1197 and 1164 cm^{-1} bands are ascribable to those of the all-*trans* chromophore in SRI_{587}.

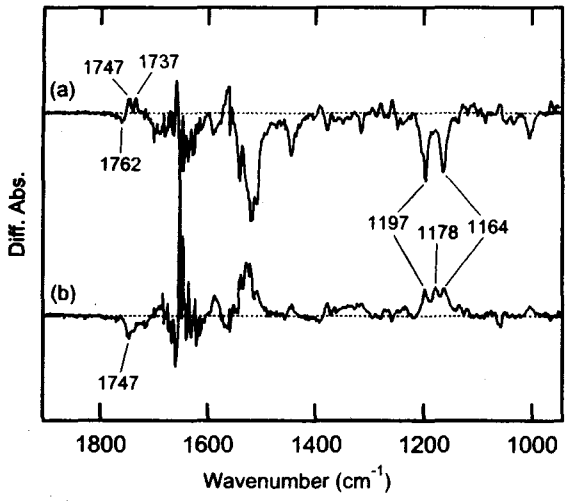

Figure 2: Difference FTIR spectra of S_{373} *minus* SRI_{587} (a) and P_{520} *minus* S_{373} (b) at 243 K.

S_{373} was further illuminated with the near-UV light at 243 K to produce P_{520}. The difference FTIR spectrum of P_{520} *minus* S_{373} (Fig. 2b) shows a new positive band in the finger print region at 1178 cm^{-1} as well as at 1197 and 1164 cm^{-1}. Although the assignment of the 1178 cm^{-1} band has yet to be conducted, we assume that the chromophore in P_{520} is all-*trans*, because the latter two bands are attributable to those of the all-*trans* chromophore. Perhaps, also in SRI_{587}, the vibrational mode near 1178 cm^{-1} exists, though with lower intensity, in view of the presence of the negative amplitude near 1178 cm^{-1} in Fig. 2a. These changes in the amplitudes of the bands in the finger print region between SRI_{587} and P_{520} is ascribable to the difference in the electronic structure on the chromophore or in the distortion of the chromophore

In the C=O stretching mode region, only the negative band at 1747 cm^{-1} is seen, indicating that Asp76 deprotonated in P_{520}.

Our conclusion, drawn from these results, is that, in going from S_{373} to P_{520}, the chromophore is photoisomerized from 13-*cis* to all-*trans* with concomitant proton transfer from Asp76 to the Schiff base. Since this active site structure would be relevant for establishing the salt bridge between the protonated Schiff base and the Asp76, the conformation for the repellent signaling is thought to correspond to the dark state structure of BR.

If the conformations of the cognate halobacterial rhodopsins are to undergo conformational changes in the same manner as in the case of BR, the signaling conformation of SRI for the attractant swimming behavior is likely to be the results of the cytoplasmic half channel, which is opened by tilting 6th and 7th helices, whereas the conformation for the repellent signaling is thought to be more compact as the result of the salt bridge between the protonated Schiff base and Asp76. The dark state SRI presumably has a conformation poised in between the two extremes, in view of the fact that the protonated Asp76 could weaken the interaction with the protonated Schiff base, without completely abolishing it.

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