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Low-Temperature FTIR Spectroscopy of Bacteriorhodopsin and Phoborhodopsin

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Archaeal rhodopsins possess retinal molecule as their chromophores, and their light-energy and light-signal conversions are triggered by all-trans to 13-cis isomerization of the retinal chromophore. Relaxation through structural changes of protein then leads to functional processes, proton pump in bacteriorhodopsin (bR) and transducer activation in phoborhodopsin (pR). It is known that sensory rhodopsins can pump protons in the absence of their transducers. Thus, there should be common and specific features in their protein structural changes for function. In this paper, our recent studies on pR from *Natronobacterium pharaonis* (*p*pR) by means of low-temperature Fourier-transform infrared (FTIR) spectroscopy are compared with those of bR. In particular, protein structural changes upon retinal photoisomerization are studied. Comparative investigation of *p*pR and bR revealed the similar structures of the polyene chain of the chromophore and water-containing hydrogen-bonding network, whereas the structural changes upon photoisomerization were more extended in *p*pR than in bR. Extended protein structural changes were clearly shown by the assignment of the C=O stretch of Asn105. FTIR studies of a *p*pR mutant with the same retinal binding site as in bR revealed that the Schiff base region is important to determine their colors.

Key words : archaeal rhodopsin, signal transduction, proton pump, retinal, photoisomerization, vibrational spectroscopy, internal water molecules, IR difference spectra

INTRODUCTION

We have studied protein structural changes in the proton pump of bR by means of low-temperature FTIR spectroscopy. In particular, accurate measurement in the high frequency region (4000-800 cm^{-1}) allowed to obtain direct information of hydrogen-bonding alteration [1]. As the consequence, we revealed how protein responds to light through retinal isomerization.

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The results include structural changes of water molecules [2, 3], the Schiff base [4], and threonine side-chains [5, 6]. In addition, protein structural changes in late intermediates were also studied [7]. In this study, we extended the IR study to *p*pR, and spectral changes in *p*pR were compared with those of bR.

MATERIALS & METHODS

The wild-type and mutant proteins of *p*pR were expressed in *Escherichia coli*, solubilized with 1.5 % *n*-dodecyl β-D-maltoside, and purified with a Ni column, followed by reconstitution into the PC liposomes.

RESULTS & DISCUSSION

Low-Temperature FTIR Spectroscopy of ppR and bR. Infrared spectral changes of *p*pR before and after photoisomerization are compared with those of bR at 77 K [8]. Spectral comparison of the C-C stretching vibrations of the retinal chromophore shows that chromophore conformation of the polyene chain is similar between *p*pR and bR. This fact implies that the unique chromophore-protein interaction in *p*pR, such as blue-shifted absorption spectrum with vibrational fine structure, originates from both ends, β-ionone ring and the Schiff base regions. In fact, less planer ring structure and stronger hydrogen bond of the Schiff base were suggested for *p*pR. Similar frequency changes upon photoisomerization are observed for the C=N stretch of the retinal Schiff base and the stretch of neighboring threonine side chain (Thr79 in *p*pR and Thr89 in bR), suggesting that photoisomerization in *p*pR is driven by the motion of the Schiff base like bR. Nevertheless, structure of the K state after photoisomerization is different between *p*pR and bR. In bR, chromophore distortion is localized in the Schiff base region, as shown in its hydrogen out-of-plane vibrations. In contrast, more extended structural changes take place in *p*pR in view of chromophore distortion and protein structural changes. Such structure of the K intermediate of *p*pR is probably correlated with its high thermal stability. In fact, almost identical infrared spectra are obtained between 77 and 170 K in *p*pR.

Internal Water Molecules of ppR and bR. In the Schiff base region of bR, three internal water molecules are involved in a pentagonal cluster structure [2]. These water molecules constitute a hydrogen-bonding network consisting of two positively charged groups, the Schiff base and Arg82 and two negatively charged groups, Asp85 and Asp212. Previous infrared spectroscopy of bR revealed stretching vibrations of such water molecules under strong hydrogen-bonding conditions using spectral differences in D_2O and $D_2{}^{18}O$ [3]. The present study extends the infrared analysis to *p*pR. Despite functional difference between *p*pR and bR, similar spectral features of water bands were observed before and after photoisomerization of the retinal chromophore at 77 K [9]. This implies that the structure and the structural changes of internal water molecules are similar between *p*pR and bR. Higher stretching frequencies of the bridged water in *p*pR suggest that the water-containing pentagonal cluster structure is considerably distorted in *p*pR. These observations are consistent with the crystallographic structures of *p*pR and bR.

Assignment of the Vibrational Band of Asn105 in ppR. Low-temperature FTIR spectroscopy of *p*pR and bR revealed that protein structural changes upon retinal photoisomerization are more extended in *p*pR. The FTIR spectroscopy of the N105D mutant protein of *p*pR assigns the vibrational bands at 1704 and 1700 cm^{-1} as C=O stretches of Asn105 in *p*pR and ppR_K, respectively [10]. A comparative investigation between *p*pR and bR further reveals that the structure at around position 105 in *p*pR is similar to that of the corresponding position (Asp115) in bR; this observation is supported by the recent X-ray crystallographic structures of *p*pR. Nevertheless, structural changes upon photoisomerization at position 105 in *p*pR are greater than those at position 115 in bR. As a consequence of a unique chromophore-protein interaction in *p*pR extended protein structural changes occur accompanying retinal photoisomerization, and these include Asn105 which is about 7 Å from the retinal chromophore.

A ppR Mutant with the Same Retinal Binding Site Residues as in bR. *p*pR has a blue-shifted absorption maximum (500 nm) relative those of other archaeal rhodopsins such as bR (570 nm). Among the 25 amino acids that are within 5 Å from the retinal chromophore, 10 are different in bR and *p*pR, and they are presumed to be crucial in determining the color of their chromophores. However, the spectral red-shift in a multiple mutant of *p*pR, in which the retinal binding site was changed similar to that of bR (BR/*p*pR), was less than 40 % (λmax = 524 nm) than expected [11].

In this study, we report on low-temperature FTIR spectroscopy to BR/*p*pR, and compare the infrared spectral changes before and after photoisomerization with those for *p*pR and bR [12]. The C-C stretch and hydrogen out-of-plane (HOOP) vibrations of BR/*p*pR were similar to those of bR, suggesting that the surrounding protein moiety of BR/*p*pR becomes like bR. However, BR/*p*pR exhibited a unique IR band regarding the hydrogen bond of the protonated Schiff base. It has been known that *p*pR has stronger hydrogen bond of the Schiff base than bR as judged from the frequency difference between their C=NH and C=ND stretches. We found that replacement of the 10 amino acids of bR to *p*pR (BR/*p*pR) does not weaken the hydrogen bond of the Schiff base. Rather, the hydrogen bond in BR/*p*pR is stronger than that in the native *p*pR. We conclude that the principal factor of the smaller than expected opsin-shift in BR/*p*pR is the strong association of the Schiff base with the surrounding counterion complex.

CONCLUSION

Comparative FTIR study of *p*pR and bR before and after photoisomerization provided various common and different features between them [8-10, 12]. It appears likely that functional difference originates from fine architecture of each protein, and thus FTIR spectroscopy is a powerful tool to reveal their molecular mechanisms. Further efforts, including the analysis of late intermediates and complex with transducer, will lead to better understanding of light-energy and light-signal conversions in archaeal rhodopsins.

REFERENCES

1. Kandori, H., N. Kinoshita, Y. Shichida and A. Maeda (1998) Protein structural changes in bacteriorhodopsin upon photoisomerization as revealed by polarized FTIR spectroscopy. *J. Phys. Chem. B* 102, 7899-7905.
2. Kandori, H. (2000) Role of internal water molecules in bacteriorhodopsin. *Biochim. Biophys. Acta* 1460, 177-191.
3. Kandori, H. and Y. Shichida (2000) Direct observation of the bridged water stretching vibrations inside a protein. *J. Am. Chem. Soc.* 122, 11745-11746.
4. Kandori, H., M. Belenky and J. Herzfeld (2002) Vibrational frequency and dipolar orientation of the protonated Schiff base in bacteriorhodopsin before and after photoisomerization. *Biochemistry* 41, 6026-6031.
5. Kandori, H., N. Kinoshita, Y. Yamazaki, A. Maeda, Y. Shichida, R. Needleman, J. K. Lanyi, M. Bizounok, J. Herzfeld, J. Raap and J. Lugtenburg (1999) Structural change of threonine 89 upon photoisomerization in bacteriorhodopsin as revealed by polarized FTIR spectroscopy. *Biochemistry* 38, 9676-9683.
6. Kandori, H., N. Kinoshita, Y. Yamazaki, A. Maeda, Y. Shichida, R. Needleman, J. K. Lanyi, M. Bizounok, J. Herzfeld, J. Raap and J. Lugtenburg (2000) Local and distant protein structural changes on photoisomerization of the retinal in bacteriorhodopsin. *Proc. Natl. Acad. Sci. USA* 97, 4643-4648.
7. Kandori, H., Y. Yamazaki, Y. Shichida, J. Raap, J. Lugtenburg, M. Belenky and J. Herzfeld (2001) Tight Asp85-Thr89 association during the pump switch of bacteriorhodopsin. *Proc. Natl. Acad. Sci. USA* 98, 1571-1576.
8. Kandori, H., K. Shimono, M. Iwamoto, Y. Sudo, Y. Shichida and N. Kamo (2001) Structural changes of *pharaonis* phoborhodopsin upon photoisomerization of the retinal chromophore: Infrared spectral comparison with bacteriorhodopsin. *Biochemistry* 40, 9238-9246.
9. Kandori, H., Y. Furutani, K. Shimono, Y. Shichida, and N. Kamo (2001) Internal water molecules of *pharaonis* phoborhodopsin studied by low-temperature infrared spectroscopy. *Biochemistry* 40, 15693-15698.
10. Kandori, H., K. Shimono, Y. Shichida and N. Kamo (2002) Interaction of Asn105 with the retinal chromophore during photoisomerization of *pharaonis* phoborhodopsin. *Biochemistry* 41, 4554-4559.
11. Shimono, K., Y. Ikeura, Y. Sudo, M. Iwamoto and N. Kamo (2001) Environment around the chromophore in *pharaonis* phoborhodopsin: mutation analysis of the retinal binding site. *Biochim. Biophys. Acta* 1515, 92-100.
12. Shimono, K., Y. Furutani, H. Kandori and N. Kamo (2002) A *pharaonis* phoborhodopsin mutant with the same retinal binding site residues as in bacteriorhodopsin. *Biochemistry* 41, 6504-6509.