Journal of Photoscience (2002), Vol. 9(2), pp. 114–117

Ultraviolet Resonance Raman Spectroscopy of Bacteriorhodopsin and Its Photointermediates

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Ultraviolet resonance Raman (UVRR) spectroscopy was used to elucidate the dynamic change of the protein structure of bacteriorhodopsin (BR) during the photocycle. The photointermediates minus light-adapted (LA) BR difference spectra show Trp difference signals, which are assigned to Trp189 or Trp182 on helix F by using the mutants, W182F and W189F. The Difference signals of Trp182 indicates an increase in hydrogen bonding strength at the indole nitrogen and a large change in the side chain conformation ($\chi^{2,1}$ torsion angle) in the $M_1 \rightarrow M_2$ transition. On the other hand, Trp189 shows an increased hydrophobic interaction. These results suggest that the tilt of helix F occurs in the $M_1 \rightarrow M_2$ transition. In the $M_2 \rightarrow N$ transition, the hydrophobic interaction of Trp182 decreases drastically. The decrease in hydrophobic interaction of Trp182 in the N state suggests an invasion of water molecules that promote the proton transfer from Asp96 to the Schiff base. Structural reorganization of the protein after the tilt of helix F may be important for efficient reprotonation of the Schiff base.

Key words : UVRR Spectroscopy, bacteriorhodopsin, intermediate, tryptophan, helix F

INTRODUCTION

Bacteriorhodopsin (BR)[1], a membrane protein of *Halobacterium salinarium*, utilizes light energy to transport protons across the cell membrane. BR consists of seven transmembrane α-helices, A through G with a chromophore, *all-trans* retinal, covalently linked to Lys216 near the middle of helix G via a protonated Schiff base. When visible light illuminates BR, retinal is isomerized from the all-trans to 13-cis cofiguration and the photo-induced isomerization drives a sequential photocycle including intermediates J, K, L, M, N, and O. During the photocycle, a proton is translocated from the cytoplasmic side to the extracellular side of the cell membrane. Molecular models for the proton translocation have been

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[1]Abbrebiations: UVRR, ultraviolet resonance Raman; BR, bacteriorhodopsin; LA, light adapted; WT, wild type

proposed on the basis of the results of various spectroscopic and mutational studies. According to a model, one of the key components in the transport is the tilt of helix F, which enables the deprotonation of Asp96 and reprotonatoion of the retinal Schiff base in the M → N transition.

UV resonance Raman (UVRR) spectroscopy is a useful tool to elucidate structures and environments of aromatic residues in proteins. Previous UVRR studies have provided some pieces of information on the conformation, hydrogen bonding, and environmental hydrophobicity of Trp residues in BR [1,2]. However, each Trp Raman bands are superposition of bands of eight Trp residues and give only information averages about these residues. Point mutation is an effective method to extract a spectrum of single Trp residue. In this study, attention focuses on the structures and environments of two Trp residues of helix F, Trp182 and Trp189, which are located on the cytoplasmic and extracellular side of retinal, respectively [3]. Dynamic structural changes of these Trp side chains have examined in the L-N time domain by time-resolved UVRR spectroscopy [4,5].

MATERIALS AND METHODS

Purple membranes of WT and mutant BR were purified by the standard method. UVRR spectra were excited with a continuous radiation (244 or 229 nm) from an intra-cavity frequency-doubled Ar^+ laser (Coherent, Innova 300 FReD). Time-resolved UVRR spectra were recorded by using a dual-beam flow apparatus. A line-focused visible beam (514.5 nm) from an Ar^+ laser (NEC, GLG-3302) was used to initiate the photocycle. The shortest delay time achievable with the apparatus was 20 μsec. Raman scattered light was collected with a quartz lens and introduced in a fore-prism UV Raman spectrometer [6] equipped with a liquid-nitrogen-cooled CCD detector (Princeton Instruments, LN/CCD-1752).

RESULTS AND DISCUSSION

Structures of Trp182 and Trp189.

The UVRR spectra of Trp182 and 189 were obtained by subtracting the spectrum of the Trp mutant from that of WTBR. The Raman bands of Tyr are almost canceled out in the difference spectra, indicating that the structures of Tyr residues are not affected by the mutation. The frequency of the W17 band, a measure of the strength of hydrogen bonding at the indole nitrogen [7], indicates that the side chains of both Trp residues are moderately hydrogen bonded to acceptors. The frequency of the W3 band is known to be sensitive to the absolute value of the torsion angle, $|\chi^{2,1}|$, about the C_β-C_3 bond of the side chain [8]. The W3 frequencies of Trp182 and 189 show that the side chains are fixed at $|\chi^{2,1}|$ angles of 100° and 27°, respectively. These results are in agreement with an atomic model of WTBR (PDB code, 1C3W). The W7 band of Trp182 exhibits an unusual triplet feature with an additional peak at 1370 cm^{-1}. The W7 splitting is due to the Fermi resonance of the W7 fundamental with two combination of out-of-plane vibrations, W25 + W33 and W28 + W29. The appearance of the third component of the W7 Fermi resonance is attributed to an upshift of the W28 frequency, which results from a strong steric interaction between Trp182 and the methyl groups of retinal.

Structural changes in the $L(M_1) \rightarrow M_2$ transition.

The third component of the W7 triplet appears as a negative peak at 1370 cm^{-1} in the L state and the intensity decrease persists in the M_1 and M_2 states. The intensity decrease of the 1370 cm^{-1} component is ascribed to partial reduction of the steric repulsion between Trp182 and retinal associated with the retinal isomerization. A large wavenumber upshift of the W3 band of Trp182 was observed for the M_2 intermediate, showing that the torsion angle, $|\chi^{2,1}|$, decreases in the M_2 intermediate. The W17 band of Trp182 shows a frequency downshift in the L(M_1) → M_2 transition and the shifts indicate an increase in hydrogen bonding strength of the indole ring. According to the atomic model of LA BR, a water molecule bridges Trp182 and the amide C=O of Ala215 of helix G through a inter-helical hydrogen bond. The hydrogen bond of the water molecule with Ala215 C=O is disrupted and a new hydrogen bond is formed with the hydroxyl group of Thr178 on helix F in the M state model (PDB code, 1CWQ). The intra-helical hydrogen bond between the water and Trp182 may be stronger than that of inter-helical hydrogen bond. This is consistent with the UVRR finding that the hydrogen bond of Trp182 strengthened in the M_2 state.

The 244-nm UVRR intensities of the W16 and W18 bands of Trp189 increase in the M_2 state. The increases in intensity can be explained by a red shift of the B_b absorption maximum of Trp189. The B_b transition undergoes a red shift when the strength of hydrogen bonding at the indole nitrogen or hydrophobic interaction of the indole ring with the environment increases [9]. The 229-nm UVRR difference spectra have shown that the W17 wavenumber, which is sensitive to the hydrogen bonding, does not change for Trp189. Therefore, the intensity increases of W16 and W18 are ascribed to a red-shift of the B_b transition caused by an increase in hydrophobic interaction of Trp189 in the M_1→M_2 transition.

A large outward tilt of helix F from the center of the protein was proposed by electron and x-ray diffraction studies of the M and N intermediate. The present study shows the large conformational change of the Trp182 side chain and the disruption of the inter-helical hydrogen bonding connecting helix F and G. Moreover, the hydrophobic interaction of Trp189 is significantly increased in the M_2 state. These structural and environmental changes probably indicate that the outward tilt of helix F starts in the M_1 → M_2 transition.

Structural changes in the M_2 → N transition.

At neutral pH, the N intermediate of WT BR decays rapidly and its molar fraction rises no more than 0.3 during the photocycle. However, the N intermediate accumulates to a significant amount at alkaline pH because delayed recovery of the BR state. Time-resolve difference spectrum (delay time, 10 msec) measured at pH 9.5 show a large intensity decrease of the Trp182 bands on going from M_2 to N, while the hydrogen bonding strength of the Trp182 indole ring does not change because the W17 difference feature in the M_2 state is maintained in the N state. Therefore the large intensity decreases are attributed to a large change of the Trp182 environment from hydrophobic to hydrophilic. On the other hand, positive peaks assigned to Trp189 in the M_2 state are not observed in the N difference spectrum, indicating that the protein structure around Trp189 is very similar to that in the initial BR state.

One of the major events during the M_2 → N transition is the proton transfer from Asp96 to the retinal Schiff base, which is promoted by lowering the pK_a of Asp96. The large decrease in hydrophobicity of the Trp182 environment in N probably means an entry of water molecules that may constitute a proton conducting pathway from the

cytoplasmic surface to Asp96. The increased hydration is likely to promote deprotonation of Asp96. The opening of cytoplasmic side initiated by the tilt of helix F in the $M_1 \rightarrow M_2$ transition may be completed by a further conformational change including a rearrangement of the E-F inter-helical loop on the cytoplasmic side in the $M_2 \rightarrow N$ transition.

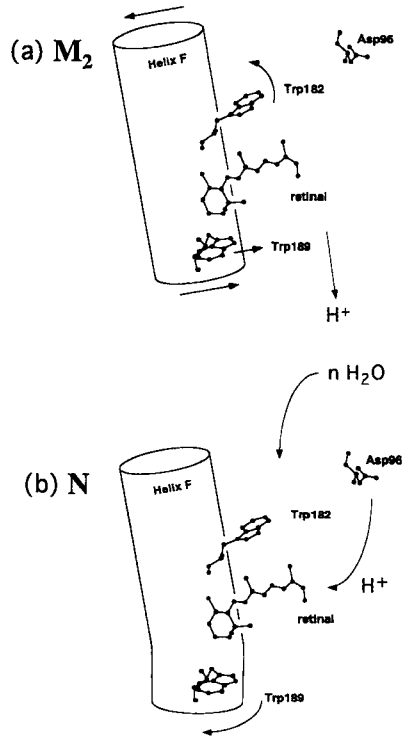

Fig. 1. Structural models of M_2 and N based on the time-resolved UVRR study. Arrows indicate motions in the BR $\rightarrow$ M_2 (a) and $M_2 \rightarrow$ N (b) transition.

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