Primary Step in Bacteriorhodopsin Photosynthesis: Bond Stretch Rather than Angle Twist of Its Retinal Excited-State Structure

Li Song† and M. A. El-Sayed*

Laser Dynamics Laboratory
School of Chemistry and Biochemistry
Georgia Institute of Technology
Atlanta, Georgia 30332-0400

Received March 19, 1998

Bacteriorhodopsin (bR) is a transmembrane protein which spans the cell membrane of *Halobacterium salinarium* and functions as a light-driven proton pump. Upon the absorption of light, bR undergoes a photocycle which involves several intermediate states[1−4] that are identified by the maxima of their absorption spectra. An accepted kinetic scheme for this photocycle is given as

$$bR_{568} \xrightarrow{h\nu} I_{460} \xrightarrow{0.5ps} J_{625} \xrightarrow{3ps} K_{610} \xrightarrow{2\mu s} L_{550} \underset{-H^+}{\overset{\sim 60\,\mu s}{\rightleftharpoons}} M_{412} \xrightarrow[+H^+]{\sim ms} N_{520} \rightarrow O_{640} \rightarrow BR_{568}$$

The decay time of the excited state of retinal (0.50 ps) is found to correspond to the formation time of the primary photoproduct J which decays in 3 ps to give K. This has been confirmed by many groups.[5−8] Subpicosecond time-resolved spontaneous resonance Raman studies[9] suggested that the photoisomerization occurs on the picosecond time scale. Anti-Stokes picosecond time-resolved resonance Raman studies[10] showed that J is the vibrationally hot retinal of the K intermediate.

According to this photocycle, the first step is believed to be retinal photoisomerization. It was proposed[6,7] that the exit from the Franck−Condon region occurs rapidly (200 fs) along the $C_{13}-C_{14}$ bond rotation to attain the 90° configuration from which either isomerization to the 13-*cis* retinal or the return to the ground state takes place in 500 fs. Recently,[11,12] spectral dynamic studies showed no change in the frequency of the emission within the 500 fs and thus questioned the 90° twist model. A new model was then proposed (the three-state model)[13] which suggested strong mixing of the lowest electronic state (the ^{1}Bu-type state) with the nearby ^{1}Ag-type state along the $C_{13}-C_{14}$ torsional coordinate. Due to the mixing, the energy of the mixed state remains constant within the protein environment. At the angle (time) at which the two states cross, rapid photoisomerization occurs.

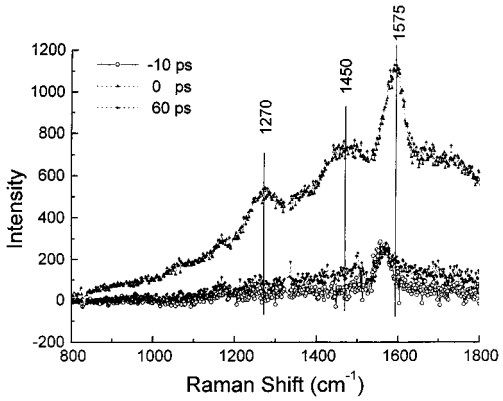

Figure 1. Corrected resonance Raman spectra of the excited state of retinal in deionized blue bR recorded with pump and monitoring laser pulses overlapping in time (top spectrum) and at −10- or 60-ps delays (bottom spectra). The latter two spectra are those of the ground state before excitation and after the excited state has decayed, respectively. The zero delay spectrum results from scattering of structures formed in the excited state within the 1.0-ps laser pulse width. In this spectrum, the presence of an intense highly shifted band due to the C═C stretching vibration at 1575 cm^{-1}, and the absence of strong bands resulting from the HOOP vibrations in the 850−980 cm^{-1} region suggest that the excited state of retinal in deionized bR relaxes primarily by changing its C═C (and C−C) bond distance.

More recently, results[14] on the femtosecond transient absorption of modified bR, whose retinal has its $C_{13}-C_{14}$ bond-locked, have questioned twisting around this bond. The important dynamics observed on this system were comparable to those observed in native bR. These results, and others using atomic force sensing techniques,[15] raised the possibility that retinal photoisomerization is not the primary step in bR photosynthesis.

A recent ab initio calculation[16] on the photoisomerization of a triene protonated Schiff base showed that bond stretch precedes bond twist in this process. This confirms a previous qualitative valence bond picture that was proposed[17] to explain the catalytic effect of the negative charge of aspartate 85 on the retinal photoisomerization of bR.

To determine the exact dynamics of the retinal excited state, the changes in its structure during its lifetime need to be determined. Unfortunately, time-dependent X-ray structural determination on the femtosecond time scale is not presently possible. In this report, we give the results of the resonance Raman spectroscopy of vibration frequencies of the *retinal excited states* in bR and in acid blue bR averaged over their lifetimes. We then compared these frequencies with those observed in the ground-state absorption spectrum determined from hole burning experiments.[18] The latter gives the excited-state vibration frequencies *at zero time*, i.e., in the Franck−Condon region. From the comparison, we concluded that the changes in the vibration frequencies (and thus the structure) of the retinal occurred during its excited-state lifetime.

Figure 1 shows the difference between the spectrum recorded with the double laser beams and the control spectrum for deionized blue bR. Three intense peaks are observed in the zero delay time spectrum: 1575, 1450, and 1270 cm^{-1}. It should be

* To whom correspondence should be addressed.
† Present address: Lucent Technology, Norcross, GA 30092.

(1) Stoeckenius, W.; Lozier, R. H.; Bogomolni, R. A. *Biochim. Biophys. Acta* **1979**, *505*, 215−78.
(2) Birge, R. R. *Annu. Rev. Biophys. Bioeng.* **1981**, *10*, 315−54.
(3) Callender, R. H.; Honig, B. *Annu. Rev. Biophys. Bioeng.* **1977**, *6*, 33.
(4) Mathies, R. A.; Lin, S. W.; Ames, J. B.; Pollard, W. T. *Annu. Rev. Biophys. Biophys. Chem.* **1991**, *20*, 491−518.
(5) Petrich, J. W.; Breton, J.; Martin, J. L.; Antonetti, A. *Chem. Phys. Lett.* **1987**, *137*, 369−75.
(6) Mathies, R. A.; Cruz, C. H. B.; Pollard, W. T.; Shank, C. V. *Science* **1988**, *240*, 777−9.
(7) Nuss, M. C.; Zinth, W.; Kaiser, W.; Koelling, E.; Oesterhelt, D. *Chem. Phys. Lett.* **1985**, *117*, 1−7.
(8) Dobler, J.; Zinth, W.; Kaiser, W.; Oesterhelt, D. *Chem. Phys. Lett.* **1988**, *144*, 215−20.
(9) Van den Berg, R.; Jang, D. J.; Bitting, H. C.; El-Sayed, M. A. *Biophys. J.* **1990**, *58*, 135−41.
(10) Brack, T. L.; Atkinson, G. H. *J. Phys. Chem.* **1991**, *95*, 2351−6.
(11) Hasson, K. C.; Gai, F.; Anfinrud, P. A. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 15124−15129.
(12) Haran, G.; Wynne, K.; Xie, A.; He, Q.; Chance, M.; Hochstrasser, R. M. *Chem. Phys. Lett.* **1996**, *261*, 389−395.
(13) Schulten, K.; Humphrey, W.; Logunov, I.; Sheves, M.; Xu, D. *Isr. J. Chem.* **1995**, *35*, 447−464.
(14) Zhong, Q.; Ruhman, S.; Ottolenghi, M.; Sheves, M.; Friedman, N.; Atkinson, G. H.; Delaney, J. K. *J. Am. Chem. Soc.* **1996**, *118*, 12828−12829.
(15) Rousso, I.; Khachatryan, E.; Gat, Y.; Brodsky, I.; Ottolenghi, M.; Sheves, M.; Lewis, A. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 7937−7941.
(16) Garavelli, M.; Celani, P.; Bernardi, F.; Robb, M. A.; Olivucci, M. *J. Am. Chem. Soc.* **1997**, *119*, 6891.
(17) Song, L.; El-Sayed, M. A.; Lanyi, J. K. *Science* **1993**, *261*, 891−4.
(18) Kamalov, V. F.; Masciangioli, T. M.; El-Sayed, M. A. *J. Phys. Chem.* **1996**, *100*, 2762−5.

S0002-7863(98)00390-4 CCC: $15.00
Published on Web 08/12/1998

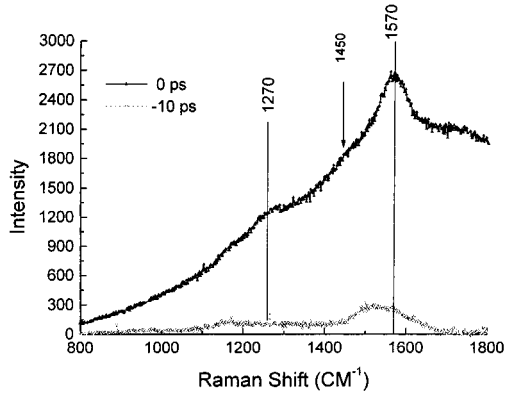

Figure 2. Corrected time-resolved resonance Raman spectrum of the excited state of retinal in purple membrane bR_{568} at 0-(top spectrum) and −10-ps (bottom) delay. The −10-ps delay spectrum is that of the ground state prior to excitation. The zero delay spectrum resulting from excited-state structures formed within the 1.0-ps laser pulse width shows a large shift in the C═C stretching frequency (at 1570 cm^{-1}) from that observed[20] in the ground-state absorption (i.e. at zero time). This, together with its large bandwidth, *suggests time dependent changes in the* C═C bonding during the excited-state lifetime within the 1.0-ps laser pulse width used. Because of this, one concludes that the excited state exits the Franck−Condon region along the C═C stretching modes.

mentioned that since the laser pulses have 1.0 ps pulse width, zero delay spectra actually record the scattering from structures formed in the excited state during the full width of the laser pulses. On the basis of the ground-state Raman assignment,[19,20] the observed excited-state peaks are assigned to the C═C stretching, CH_3 deformation, and C−C−H in-plane rocking, respectively. The assignment of the 1575 cm^{-1} peak to C═C stretching vibration is the most certain due to both its high intensity and since no other possible vibration is expected to show with this intensity in this frequency range). It has a full width at half-maximum (fwhm) of 40 cm^{-1}, similar to the laser line width of 35 cm^{-1}. *No strong scattering is observed, corresponding to the HOOP vibrations in the 850−980 cm^{-1} region.*

The time dependence of the Raman intensity at 1575 cm^{-1} as a function of delay time between the pump and probe pulses shows biexponential decay with time constants of 2 and 12 ps are in good agreement with the previously reported[17] excited-state lifetimes of deionized bR samples determined from the decay of the optical absorption. This supports the assignment of this band to the Raman scattering of the excited state of deionized bR.

Figure 2 shows the corrected time-resolved resonance Raman spectrum of the purple membrane (bR_{568}) sample. Since wt BR has a shorter lifetime, its Raman intensities are weaker than those of deionized bR. The peak at 1570 cm^{-1} is clearly observed, but the HOOP vibrations are not. Notice that the band at 1570 cm^{-1} has a fwhm of ∼60 cm^{-1}, much broader than the laser line width. It is also much broader and less symmetric on the low energy side when compared with the corresponding band for deionized bR. This result suggests that a change larger in wt bR than in deionized bR in the C═C stretching frequency of the excited state has taken place within the laser pulse width.

The important observed results for bR_{568} and possible conclusions can be summarized as follows: (1) In the Franck−Condon region, i.e., upon light absorption, the C═C stretching vibration frequency in the excited state is found previously[18] (from hole burning experiments) to be lower than that in the ground state; (2) during the retinal excited-state lifetime, the C═C stretching vibration frequency increases to a value higher than that in the ground state; (3) the width of the C═C stretching vibration band in the excited state of wt bR is broader and more asymmetric on the low energy side when compared with that for deionized bR, suggesting larger changes in the retinal structure in the excited state along the C═C stretching normal coordinate within the 1 ps laser pulse width; and (4) no Raman scattering corresponding to the HOOP vibrations is observed in the excited state.

Results 1 and 2 strongly suggest that retinal excitation leads to changes in the π-electron density and bond order, which changes the single into double C−C bonds and vice versa. This is consistent with the Raman results[19,20] of the ground state of bR_{568} which showed that the strongest resonance enhancement was observed for the carbon−carbon stretching vibrations. A simple valence bond picture[17] shows that if the initial excitation leads to the displacement of the positive charge from the protonated Schiff base nitrogen to the different odd-numbered carbon atoms on the polyene chain, but otherwise keeps the delocalization of the π-electrons over the whole system, the average C═C bond order and thus its frequency is expected to decrease. This results from the fact that the six double bonds in the ground state become five (delocalized over the same length of the retinal chain). This explains Result 1.

Due to the electrostatic interaction with the protein charges (e.g., Asp85), localization of the positive charge on C_{13} could take place (as was previously proposed.)[17] Within a few vibrations, the bond distances relax to accommodate the new π-charge distribution in the electronic excited state. As a result of the nuclear relaxation, C_{13}^{+} finds itself surrounded with two single bonds and the π-system is divided into two parts, one having four conjugated double bonds (C_5 to C_{12}) and the other has one double bond on C_{14}−C_{15}. If the observed resonance Raman scattering results from the conjugated system of four double bonds, instead of the six double bonds present in the ground state, an increase in the C═C bond order and frequency is expected. This can account for Result 2. Result 3 can be explained by the fact that the laser pulse width is twice that of the excited-state lifetime of bR but much shorter than that of deionized bR. Thus, all the retinal structural changes can be reflected in the averaged Raman signal of bR, but only a small fraction of the change can be recorded for blue bR.

The fact that the signature of nonplanarity (the HOOP bands) was not observed (Result 4) could be due to one of the following causes: (1) photoisomerization (nonplanarity) occurs rapidly toward the end of the excited-state lifetime (e.g. as a result of curve[13] or conical[16] crossing); (2) photoisomerization does not occur from the excited state[14,15] but rather from vibrationally hot ground state; or (3) nonplanarity around the C_{13}−C_{14} bond might not show resonance Raman enhanced HOOP vibrations if the Raman scattering occurs from the planar C_5 to C_{12} conjugated system which is not electronically coupled to the C_{13}−C_{14} system around which twisting occurs. Independent of which reason might explain the absence of the HOOP vibration, our results suggest that changes in the carbon−carbon bond distances are what occur *first* upon the excitation of retinal in bacteriorhodopsin. This is consistent with the fact that the excitation process itself involves a $\pi \rightarrow \pi^*$ promotion, which changes the carbon−carbon bond orders of the different bonds of the conjugated system.

Acknowledgment. The authors wish to thank Professors M. Olivucci and M. Robb for valuable discussions. The assistance of Mr. Victor Volkov in the experimental work and Ms. Tina Masciangioli for the careful reading of the manuscript are greatly appreciated. The support of the Department of Energy, Office of Basic Energy Research under Grant No. DE-FG02-97 ER-14799 is greatly appreciated.

Supporting Information Available: Experimental details and analysis of the raw spectrum (4 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

JA980390D

(19) Smith, S. O.; Mathies, R. A. *Biophys. J.* **1985**, *47*, 251−4.
(20) Smith, S. O.; Braiman, M. S.; Myers, A. B.; Pardoen, J. A.; Courtin, J. M. L.; Winkel, C.; Lugtenburg, J.; Mathies, R. A. *J. Am. Chem. Soc.* **1987**, *109*, 3108−25.