

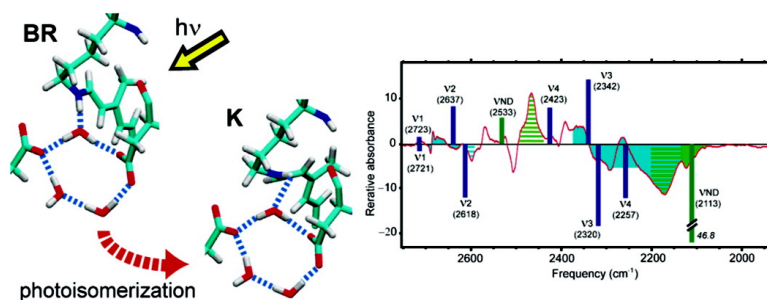
Communication

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Role of Hydrogen-Bond Network in Energy Storage of Bacteriorhodopsin's Light-Driven Proton Pump Revealed by *ab Initio* Normal-Mode Analysis

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Bacteriorhodopsin (bR) is a membrane protein that resides in *Halobacterium salinarum*.¹ It converts light energy absorbed at its chromophore, retinal, into a transmembrane proton gradient by actively transporting protons. Upon photoabsorption, bR's retinal chromophore isomerizes from an all-trans configuration to a 13-cis one. The photochemical reaction at the chromophore triggers a photocycle involving protein conformational changes that are coupled to the active proton transport.

Recently, X-ray crystallographic^{2,3} and theoretical⁴ studies have independently proposed structural models of the early intermediate state, K, which arises 3 ps after photoabsorption and can be trapped at liquid nitrogen temperatures. Figure 1 depicts the structures of the binding pocket of bR before excitation (resting BR) and in the K state. Upon formation of K, the retinal chromophore is clearly observed to have isomerized around the C₁₃=C₁₄ bond, whereas no significant movement is seen in the rest of the binding pocket. The three structures proposed²⁻⁴ are similar to one another, but show distinct differences from a previously proposed K structure,⁷ which has been suggested to be of a late K (so-called KL) state by theoretical modeling.⁴

The availability of the K intermediate structures has permitted one to address an important question, namely, how bR stores the light energy absorbed by the chromophore. The theoretical study⁴ has suggested that energy storage is achieved through destabilization of the binding pocket, mainly due to a delocalized torsion of the Schiff base half of the chromophore and weakened interactions between the Schiff base and nearby polar groups, Wat402 and Asp85. These two contributions almost equally comprise the overall energy storage, as previously suggested by Birge and Cooper on a theoretical basis.⁸ However, one of the X-ray crystallographic studies proposed a strong, localized distortion of the C₁₂-C₁₃=C₁₄ angle observed in the X-ray structure, but absent in the theoretical model, to be the primary mechanism of storing energy after isomerization.²

One clue to elucidate the energy storage mechanism is given by Fourier transform infrared (FTIR) spectroscopy, which has precisely measured strength of hydrogen bonds in the binding pocket.⁹⁻¹³ Despite its high sensitivity and accuracy, however, the FTIR observations do not directly link to the protein atomic structures, and assignment of the signals based on the structural models has to be attained. In this communication, we present an *ab initio* quantum mechanical/molecular mechanical (QM/MM) vibrational normal-mode analysis for the binding pocket of the proposed K intermediate structure, to link protein structure and FTIR spectra.

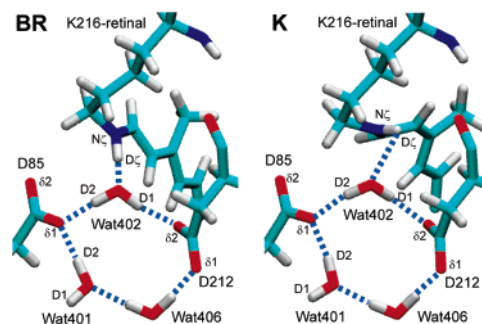


Figure 1. Structures of the binding site in BR (left) and K (right) refined and modeled theoretically.^{4,5} Dashed lines indicate hydrogen bonds. In BR, the N_c-D_c bond of the Schiff base points down to Wat402, whereas in K it points horizontally toward D212 because of a twisted 13-cis structure of retinal. The molecular image was created with VMD.⁶

We focus on the O-D and N-D stretching modes of the key water molecules, Wat401 and Wat402, and the Schiff base, respectively (see Figure 1), which have been measured in FTIR experiments.¹⁰⁻¹³ In the BR resting state, these modes have been analyzed by an earlier *ab initio* QM/MM study.⁵ The normal-mode analysis was carried out at the Hartree-Fock level of theory with Dunning's¹⁴ double- ζ basis set with anion functions on O atoms of aspartates for the same QM/MM system as that in ref 5 (see Supporting Information for a detailed description of the method used). The method has successfully predicted assignment of the vibrational modes in the BR resting state¹⁰⁻¹³ despite small computational errors due to several limitations (see Supporting Information). A QM/MM routine⁵ with the AMBER force field¹⁵ implemented in HONDO¹⁶ was used.

Figure 2 compares the calculated IR spectrum with the experimental one.¹² In BR, the ν_3 and ν_4 modes assigned to stretching vibrations of the O-D₂ bonds of Wat401 and Wat402 hydrogen bonding to O _{δ 1} of Asp85, respectively (see Figure 1), possess low frequencies with large intensities. Thus, those two modes have been theoretically proposed to constitute the low-frequency bands observed in the FTIR experiments.^{10,12,13}

The N-D stretching mode of the Schiff base, ν_{ND} , also exhibits a significantly low frequency with a strong intensity. A previous QM/MM study has therefore predicted that ν_{ND} also gives a large component of the low-frequency bands.⁵ Recently, Kandori et al.¹¹ have successfully assigned the N_c-D_c stretching mode of the Schiff base, which lies in the low-frequency bands, by an FTIR measurement using a site-specific isotope labeled bR, and the predictions have supported the FTIR results.

Upon the formation of K, the frequency of ν_{ND} considerably upshifts to 2533 cm⁻¹ by 420 cm⁻¹. A significant reduction of the intensity (46.8 to 5.8) is accompanied by the upshift. The angle of

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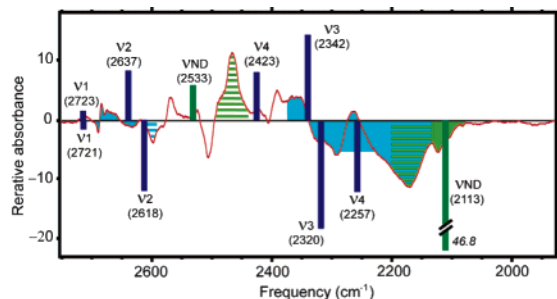


Figure 2. Calculated IR signals of the O–D and N–D stretching modes, shown in blue and green vertical lines, respectively. The signals in negative and positive are those of BR and K, respectively. The calculated absorbance values are relative to that of the O–D stretching mode of D₂O in vacuo evaluated at the same level of theory. The calculated IR signals are compared to the experimentally observed K–BR spectrum,¹² drawn as a red line. The experimental signals painted in blue and green are of the O–D and N–D stretching modes.^{10,11} Horizontal stripes indicate partial contributions of the stretching modes to the bands.

the transition dipole moment is almost perpendicular (89°) to the membrane normal (see Supporting Information). The upshift of frequency, decrease of intensity, and angle of transition dipole moment of the ν_{ND} mode are in good agreement with the observed spectra in the FTIR measurements.¹¹

In the K intermediate, the ν_4 band (2423 cm⁻¹) assigned to O–D₂ of Wat402 exhibits a significant upshift of the frequency from BR's one (166 cm⁻¹). This originates from the weakened hydrogen bond of Wat402 to Asp85. In K, the Schiff base is pulling Wat402, rendering the distance between O of Wat402 and O_{δ1} of Asp85 longer by 0.05 Å. Therefore, the ν_4 band is also responsible for part of the aforementioned high-frequency shift of the prominent low-frequency bands upon K formation observed in the FTIR experiments. Unfortunately, no clear IR signal due to a water molecule has been observed in the frequency region, although there is a complex spectral change around a sharp peak at 2468 cm⁻¹ resulting from the use of D₂¹⁸O, indicating the possible existence of a water band.

The ν_3 band (2320 cm⁻¹[BR]/2342 cm⁻¹[K]) is attributed to the stretching mode of O–D₂ of Wat401, and possibly assigned to bands at 2323 (BR) and 2359 cm⁻¹ (K) in the FTIR spectrum. The frequency upshift of the band upon K formation is considerable (22 cm⁻¹), even though Wat401 does not undergo significant conformational change (see Figure 1). The angle of the IR transition dipole moment also changes considerably (48° in BR to 15° in K; see Supporting Information). Apparently, the weakened hydrogen bond of Wat402 to Asp85 underlying the large high-frequency shift of ν_4 described above gives rise to nonlocal perturbation to ν_3 through a reduced vibrational mode coupling of ν_3 with ν_4 .

The frequency of ν_2 (2618 cm⁻¹[BR]/2637 cm⁻¹[K]), assigned to the O–D₁ stretching mode of Wat402, is high because the O–D₁ bond is only weakly hydrogen-bonded to O_{δ2} of Asp212 (see Figure 1). The asymmetric hydrogen-bond strengths of two O–D bonds of Wat402 indicated by the large frequency difference between ν_2 and ν_4 has been predicted previously⁵ and confirmed by a recent FTIR study.¹³ Upon the formation of K, the frequency of ν_2 exhibits an upshift of 19 cm⁻¹. These ν_2 modes can be assigned to the experimental IR bands at 2636 cm⁻¹[BR]/2662 cm⁻¹[K],¹⁰ since those bands show a similar spectral change in the high-frequency region.

The highest frequency mode ν_1 (2721 cm⁻¹[BR]/2723 cm⁻¹[K]) is assigned to the O–D₁ stretching mode of Wat401. The absence of hydrogen bonding of the O–D₁ bond of Wat401 (see Figure 1) results in the high frequency of the stretching motion. The ν_1 modes presumably correspond to the IR signals observed at 2690 and 2684

cm⁻¹ in BR and K,¹⁰ respectively, since those signals have been assigned to the highest frequency modes of water in the spectrum, and only slight shifts of the frequencies has been observed in the BR-to-K transition (–6 cm⁻¹ in the IR experiments, and 2 cm⁻¹ in the present study).

The QM/MM vibrational normal mode calculation using theoretically determined structural models has succeeded in reproducing prominent features of the K–BR IR spectrum experimentally observed, namely, the large upshift of frequencies of ν_{ND} and ν_4 . The spectral change implies that hydrogen bonds among the Schiff base, Wat402, and Asp85 become significantly weaker upon formation of K, and therefore energy after photoabsorption is partially stored in the form of the weakened hydrogen bonds. A previous QM/MM study⁴ has estimated the contribution of weakened hydrogen bonds to the energy storage to be 11 kcal/mol, which is more than half of the total energy storage of 16 kcal/mol.^{4,8,17} Hence, the QM/MM calculations through the assignment of the experimentally observed vibrational signals suggest that besides delocalized retinal torsion weakened hydrogen bonds are an important means of the energy storage in bR.

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Supporting Information Available: Methodological details of the QM/MM vibrational analysis and a table summarizing vibrational frequencies, intensities, and angles of transition dipole moments of the O–D and N–D stretching modes in the BR and K states. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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