Room-temperature FTIR spectroscopy of active internal water molecules in the bacteriorhodopsin photocycle

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Introduction. Bacteriorhodopsin (bR) is a membrane protein located in the purple membrane of *H. salinarum*. The light-induced all-trans to 13-cis isomerization of its retinal chromophore starts a series of physicochemical and structural changes, finally leading to a net proton translocation from the cytoplasmic to the extracellular side, and the recovery of bR to the initial state (the bR photocycle).

The proton pumping mechanism has attracted the interest of many researchers. Nowadays, the important role of internal water molecules in the proton pump mechanism is generally accept, and strongly supported by experimental results. Fourier transform infrared (FTIR) difference spectroscopy and X-ray structures of temperature trapped photocycle intermediates have provide the main experimental contributions for the understanding of the role of internal water molecules during the bR photocycle.[1,2] However, the concern of whether low temperature trapped intermediates may differ from the intermediates at room temperature is always present among researches.

Here we present room temperature FTIR difference spectra for the L and M intermediates, which correspond to the states before and after the primary proton transfer, respectively.

Materials & Methods. bR photocycle was studied at 293 K and pH 7. Time resolved infrared absorbance changes of a bR film hydrated with the vapour phase of a water/glycerol mixture (relative humidity of 93%) upon 532 nm laser excitation were recorded from 2.5 μs to 10 ms and 800 cm^{-1} to 4000 cm^{-1}. TR-IR difference spectra from 5-30 μs were dominated by L-bR difference spectra, whereas the M-bR spectra dominate from 100 μs on.

Results. Room temperature M-bR ($M\text{-}bR_{293K}$) spectrum was obtained by averaging time-resolved spectra between 0.5-2 ms, and it was estimated to represent pure M-bR spectra with a minor contribution of N-bR (≈10%). Comparison of $M\text{-}bR_{293K}$ with low temperature M-bR ($M\text{-}bR_{230K}$) spectrum revealed almost identical bands in the 1900-800 cm^{-1}, where retinal, peptide backbone, Schiff base and many amino acids contribute. The 4000-2700 cm^{-1}

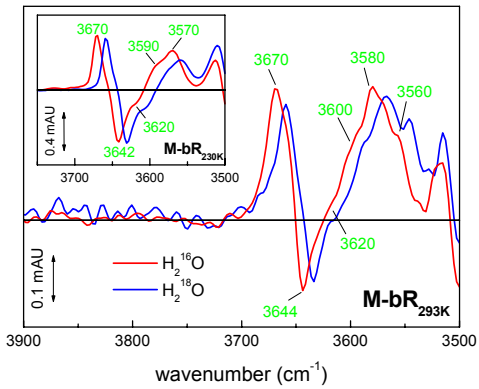

Fig. 1. Room temperature and low temperature M-bR for water molecules under weak hydrogen conditions.

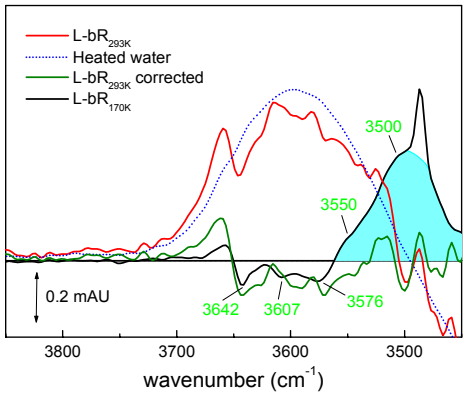

Fig. 2. Room temperature and low temperature L-bR.

region, where the O-H, N-H and C-H stretching vibrations appears, was also quite similar between M-bR_{293K} and M-bR_{230K}. The isotopic induced shifts when the bR film is hydrated with either $H_2{}^{16}O$ or $H_2{}^{18}O$ was used as a criterion to assign O-H vibrations of internal water molecules under weak hydrogen conditions, which were found to be very similar between M-bR_{293K} and M-bR_{230K} (see Fig.1).

L-bR was obtained averaging the transient absorbance changes between 5-50 μs. The resulting L-bR_{293K} was estimated to contain, after correction for M-bR contribution, 75% L-bR and 25% K-bR. Comparison of L-bR_{293K} with L-bR_{170K}, revealed differences in the 1900-800 cm^{-1} region, especially for retinal vibrations, peptide backbone, and carboxylic acids. Unfortunately, the 4000-2700 cm^{-1} region for L-bR_{293K} showed a contribution of transiently heated bulk water, hampering the direct observation of O-H, N-H and C-H stretching vibrations for this intermediate. However, when the shape of heated water was taken into account, the absence in L-bR_{293K} of a intense band assigned to internal water molecules in the cytoplasmic domain changing in the L intermediate became more evident (see Fig. 2).

Conclusions. The M intermediate trapped at 230K is equivalent to the M intermediate present at physiological conditions. However, the L intermediate trapped at 170K may not report accurately the actual retinal, protein and internal water disposition, or changes, for the L intermediate at a physiological temperature.

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References.

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