Low Temperature FTIR Difference Spectroscopy Reveals New Insights on the Proton Pathway of Proteorhodosin at Different pH Values

G. Schäfer[1], M.-K. Verhoefen[2], S. Shastri[3], I. Weber[3], C. Glaubitz[3], J. Wachtveitl[1,2], W. Mäntele[1]

[1] *Institute of Biophysics, Max von Laue-Str.1, Johann Wolfgang Goethe-University Frankfurt , 60438 Frankfurt, Germany,* [2] *Institute of Physical and Theoretical Chemistry, Johann Wolfgang Goethe-University Frankfurt, Max von Laue-Str.7, 60438 Frankfurt, Germany,* [3] *Institute of Biophysical Chemistry and Center of Biomolecular Magnetic Resonance, Johann Wolfgang Goethe-University Frankfurt, Max von Laue-Str.9, 60438 Frankfurt, Germany*

Proteorhodopsin (PR) discovered in the g-proteobacterium SAR86 is believed to be a lightdriven proton pump [1] Since the first description of PR several studies have uncovered evidence of intermediates comparable to the photoproducts of the BR photocycle. However, there is an ongoing discussion about the inversion of the proton pumping direction due to pH-change and the general function of PR. Until now, no M intermediate could be detected kinetically at acidic pH [2, 3], but recently low temperature visible spectroscopy demonstrated the existence of a band typical for the M state at pH 4 [4].

To further investigate the photocycle of PR, we have used FTIR difference spectroscopy at low temperature between 77 and 250 K. Low-temperature spectroscopy has widely been used to trap and investigate intermediates in the photocycle of proteins. In combination with FTIR spectroscopy, it is a powerful tool to gather information about the involvement of amino acids and about structural changes of the chromophore concomitant with the light reaction. Until now, only the K intermediate of the PR photocycle has been characterized using this technique [5].

We have used various samples ranging from PR reconstituted with lipids to PR 2Dcrystals. Each sample provides different advantages, such as high concentration and stability in the case of the crystals or the observable lipid-protein interactions in reconstituted samples.

We started our experiments with a comparison of the K-state at 77 K for our samples at pH 9, 8.5, 5.5 and 5. For pH 8.5 and 5.5 an increased amount of L-intermediate was monitored, which was absent in the spectra at pH 9 and 5. This hints to lowered reaction barriers for PR between pH 5.5 and 8.5. In agreement with this finding the samples at pH 5.5 and pH 8.5 show a similar kinetic behavior and identical spectral characteristics differing from either higher or lower pH values at higher temperatures. They both lack the characteristic positive band at 1755 cm^{-1} indicative for protonation of Asp-97. This band in combination with amid II changes was clearly observable at 242 K, showing M state formation for pH 9. However, the pH 5 sample showed an M-like state at 227 K, which involves aspartic or glutamic acid side chain modes at 1726 cm^{-1} and 1730 cm^{-1}.

Our results hint at different proton translocation pathways at pH 9, pH 8.5 to 5.5 and pH 5. This finding further supports the assumption of variable vectoriality proposed by Friedrich et al. [2].

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