Deuterium Isotope Effects on Channelrhodopsin-2

*T. Resler, B. Schultz, V. Lórenz-Fonfría, R. Schlesinger, J. Heberle

Freie Universität Berlin, Physics, Berlin, Germany

Channelrhodopsin-2 (ChR2) is a light-gated cation channel from the unicellular algae Chlamydomonas reinhardtii.1 It is a promising tool in optogenetics, where it is used to induce action potentials in neuronal cells by light.2 ChR2 is a seven transmembrane protein with retinal as a chromophore. After blue light excitation the retinal isomerizes and the protein undergoes a photocycle with different structural intermediates characterized in the visible and infrared (IR).3-5 Here, we observe transient absorbance changes in the IR region recorded by a new quantum cascade laser (QCL) setup. The setup has a time resolution of 10 ns, a spectral resolution of 0.001 cm-1 and an emission power of 200 mW/cm-1. With this technical advancement we measured the deuterium kinetic isotope effect (KIE) on ChR2-wt. In the carboxylic region we observe a large KIE (> 2) for the negative band at 1735 cm-1 which corresponds to the primary proton donor (D156).5 Under H/D exchange this C=O stretching vibration should down shift in frequency but this could not be observed.6,7 To clarify this unusual frequency shift we investigate the ChR2-D156E variant where the negative band of the D156 band is already up shifted by 26 cm-1 wavenumbers to 1763 cm-1. In this spectral range we can observe a clear deuterium isotope effect which results in a spectral down shift by 8 cm-1. We demonstrate the H/D exchangeability of the residue at position 156 which supports the assignment of D156 as the primary proton donor to the retinal Schiff base.5

References:

[1] Nagel, G.; Szellas, T.; Huhn, W.; Kateriya, S.; Adeishvili, N.; Berthold, P.; Ollig, D.; Hegemann, P.; Bamberg, E. Proc Natl Acad Sci U S A 2003, 100, 13940.

[2] Berndt, A.; Schoenenberger, P.; Mattis, J.; Tye, K. M.; Deisseroth, K.; Hegemann, P.; Oertner, T. G. Proc Natl Acad Sci U S A 2011, 108, 7595.

[3] Ritter, E.; Stehfest, K.; Berndt, A.; Hegemann, P.; Bartl, F. J. J Biol Chem 2008, 283, 35033.

[4] Nack, M.; Radu, I.; Schultz, B. J.; Resler, T.; Schlesinger, R.; Bondar, A. N.; del Val, C.; Abbruzzetti, S.; Viappiani, C.; Bamann, C.; Bamberg, E.; Heberle, J. FEBS Lett 2012, 586, 1344.

[5] Lórenz-Fonfría, V. A.; Resler, T.; Krause, N.; Nack, M.; Gossing, M.; Fischer von Mollard, G.; Bamann, C.; Bamberg, E.; Schlesinger, R.; Heberle, J. Proc Natl Acad Sci U S A 2013, 110, E1273.

[6] Ito, S.; Kato, H. E.; Taniguchi, R.; Iwata, T.; Nureki, O.; Kandori, H. J Am Chem Soc 2014, 136, 3475.

[7] Neumann-Verhoefen, M. K.; Neumann, K.; Bamann, C.; Radu, I.; Heberle, J.; Bamberg, E.; Wachtveitl, J. J Am Chem Soc 2013, 135, 6968.