Lipid Protein Interactions underlie an Evolutionary Conserved Proton Switch in a Transmembrane Peptide derived from Helix-3 of Class-1 GPCRs

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Abstract

The visual photoreceptor rhodopsin is a prototypical class-I (rhodopsin-like) G protein-coupled receptor (GPCR). Photoisomerization of the covalently bound ligand 11-*cis*-retinal leads to restructuring of the cytosolic face of rhodopsin. The ensuing protonation of Glu-134 in the class-conserved D(E)RY motif at the C-terminal end of transmembrane helix-3 promotes the formation of the G-protein-activating state. Glu-134 acts as an autonomous proton switch also in synthetic transmembrane peptides, where lipid protein interactions couple protonation to helix extension and hydrophobic burial of the side chain resulting in an elevated side chain pKa. The implied change in "helical-end-solvation" by interfacial water has been investigated by pulsed hydration experiments. we observed within several seconds (i) lipid/peptide conformational changes by time-resolved Fourier transform infrared (FTIR) difference spectroscopy and (ii) water penetration into the lipidic phase using the simultaneous recording of the fluorescence of a tryptophan (trp) inserted 3 amino acids N-terminally from the ERY motif. Cross-correlation of both monitors reveals the linkage of lipid ester carbonyl hydration to helical end unwinding followed by water penetration into the bilayer one helical turn N-terminally of the conserved ERY motif. Trp-emission further shows that the indole ring is more water-exposed in the ionized than in the protonated state of the adjacent ERY motif evidencing a shift of the lipid/water phase boundary relative to the transmembrane peptide upon proton uptake. The proton-dependent reorganisation of the lipid/peptide/water microdomain N-terminally of the ERY motif is further supported by a pH-sensitive Förster-resonance-energy-transfer from the peptidic trp to 5-(dimethylamino)naphthalene-1-sulfonyl (DANSYL)-labelled lipids. In conclusion, the data reveal a conserved hydration site at the membrane water interface of GPCRs that attracts or repels water in response to protonation, thereby, linking proton uptake to protein conformation and rearrangement of the lipid/peptide/water phase boundary independently of helix-helix interactions.

Biography

Dr. Fahmy completed his doctorate in biophysics studying the molecular mechanism of proton translocation in bacteriorhodopsin by Fourier transform infrared (FTIR) spectroscopy at Freiburg University, Germany, in 1991. After his spectroscopic studies on rhodopsin mutants at the Rockefeller University, New York (USA) as a post doctorate, he habilitated and became group leader at the Institute of Zoology at Freiburg University in 1999, focusing on FTIR and fluorescence spectroscopy of receptor G-protein interactions. This led to the development of fluorescence-IR-cross-correlation spectroscopy to analyse the allosteric coupling between distant protein domains during receptor activation. Since 2003, Dr. Fahmy leads the Biophysics Division at the Helmholtz-Zentrum Dresden-Rossendorf, where he served on the scientific advisory board and is a faculty of the Dresden-International-PhD-Programme (DIPP).

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