Physical Properties of the Transmembrane Segment 3 of Rhodopsin

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Abstract

Activation of G protein-coupled receptors (GPCRs) originates in ligand-induced protein conformational changes that are transmitted to the cytosolic receptor surface. In the photoreceptor rhodopsin, and possibly other rhodopsin-like GPCRs, protonation of a carboxylic acid in the conserved E(D)RY motif at the cytosolic end of transmembrane helix 3 (TM3) is coupled to receptor activation [1]. Here, we have investigated the structure of synthetic peptides derived from rhodopsin TM3. Polarized FTIR-spectroscopy reveals a helical structure of a 31-mer TM3 peptide reconstituted into PC vesicles and helical structure is also observed for the TM3 peptide in detergent micelles and depends on pH especially in the C-terminal sequence. In addition, the fluorescence emission of the single tyrosine of the D(E)RY motif in the TM3 peptide exhibits a pronounced pH sensitivity that is abolished when Glu is replaced by Gln demonstrating that protonation of the conserved Glu side chain affects the structure in the environment of the D(E)RY motif of TM3. The pH-regulation of the C-terminal TM3 structure may be an intrinsic feature of the E(D)RY motif in other class I receptors allowing the coupling of protonation and conformation of membrane-exposed residues in full length GPCRs.

Results and Discussion

In this study [2] the synthetic peptide TM3 (108-138), derived from helix 3 of rhodopsin was reconstituted in to large unilamellar PC vesicles and detergent micelles in which they exhibit typical alpha helical structure. The IR data gathered from the pH change of the TM3 peptide reveals that the amide 1 bond is found at $1655\,cm^{-1}$ with a slight shoulder at $1624\,cm^{-1}$ which is more pronounced at pH 8.8 than at pH 2. Acidic pH favors the helical content and the IR data imply the solvent accessibility of Glu^{134}, the only titratable group in the accessed range. The pH induced structural transition was further addressed by the fluorescence of Tyr^{136} in detergent solubilised TM3. The pH dependent spectral changes of Tyr^{136} were recorded and a pKa of 6 of the acid-induced decrease of tyrosine emission is found, whereas emission increases again below pH 4. The pH dependence is almost completely abolished when Glu^{134} is replaced by Gln. In combination with FTIR data, the results show that the conserved carboxylate is solvent-accessible in micelles and confers pH sensitivity to the TM3 peptide secondary structure specifically in the environment of the D(E)RY motif. In conclusion we have proved here that TM3 sequence forms an α-helix in the absence of helix packing interactions and the measured tilt of the helical axis argues that the C- and the N- termini localize at the lipid bilayer. The demonstrated protonation of Glu^{134} thus occurs at the hydrophobic / hydrophilic phase boundary in lipids or detergent micelles and in the latter the protonation state of Glu^{134} affects the neighboring tyrosine and stabilizes the helical TM3 structure. We hypothesize that the conserved hydrophobicity profile around the D(E)RY motif renders the carboxylate to be a critical side chain for the pH- dependent positioning of TM3 by generating a more contigious hydrophobic region at the TM3 C-terminus in the protonated site.

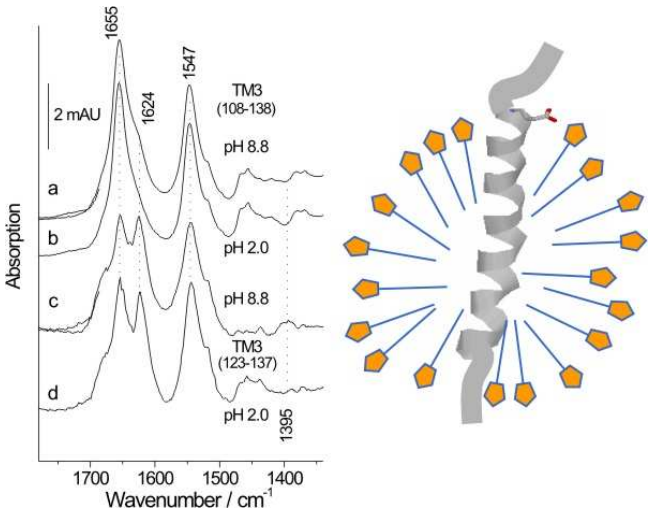

Fig. 1 *ATR-FTIR-spectra of detergent- solubilised TM3 peptides. TM3 of rhodopsin (108-138) in 5% dodecylmaltoside at pH 8.8 (a) and pH 2 (b). Absorption of a TM3 fragment (123-137) at pH 8.8 (c) and at pH 2 (d). The sketch to the right shows the proposed peptide structure in the micelle with the titratable carboxylate which induces local helicity upon protonation.*

[1] K. Fahmy, T.P. Sakmar, F. Siebert, Biochemistry 39 (2000) 10607-10612.

[2] S. Madathil, G. Furlinski, K. Fahmy, Biopolymers. 82 (4) (2006) 329-333