AB022. Membrane binding properties of the C-terminal segment of retinol dehydrogenase 8

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Background: Retinol dehydrogenase 8 (RDH8) is a 312-amino acid (aa) protein involved in the visual cycle. Bound to the outer segment disk membranes of photoreceptors, it reduces all-trans-retinal to all-trans-retinol1 as one of the rate-limiting steps of the visual cycle2. RDH8 is a member of the short-chain dehydrogenase/reductase family. Its C-terminal segment allows its membrane-anchoring through the postulated presence of an amphipathic α-helix and of 1 to 3 acyl groups at positions 299, 302 and 3043. The secondary structure and membrane binding characteristics of RDH8 and its C-terminal segment have not yet been described.

Methods: To evaluate the membrane binding of RDH8, the full-length protein (aa 1–312), a truncated form (aa 1–296), its C-terminal segment (aa 281–312 and 297–312) as well as different additional variants of this segment were used. The truncated protein binds membranes less efficiently than the full-length form. Thus, the C-terminal segment of RDH8 is essential for the binding and has thus been further examined. The intrinsic fluorescence of tryptophan residues at positions 289 and 310 of the wild-type C-terminal segment of RDH8 and the mutants W289F, W310F and W310R have thus been used to determine their extent of binding to lipid vesicles and to monitor their local environment. Unilamellar lipid vesicles composed of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) or a mixture of POPC and 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-L-serine (POPS) were used to mimic the phospholipid content of the outer segment disk membranes of photoreceptors.

Results: An increase in fluorescence intensity and in fluorescence lifetime is observed upon increasing the concentration of lipid vesicles. These data allowed calculating values of partition coefficient of the C-terminal segment of RDH8 varying between Kp =1.1 E6 to 1.7 E6. It is noteworthy that the observation of a more intense shift to lower wavelengths upon membrane binding of the mutant W310R and W310F indicates a deeper incorporation of the remaining tryptophan residue at position 289 into the lipid bilayer. The secondary structure of the C-terminal segment of RDH8 observed by circular dichroism and infrared spectroscopy shows a superposition of α-helical, β-turn and unordered structures.

Conclusions: The peptides derived from the C-terminal segment of RDH8 show a strong binding to lipid vesicles. These strength of binding is independent of the type of lipid and the presence of a mutation.

Keywords: Peptide-membrane binding; electrostatic interactions; hydrophobic interactions; fluorescence spectroscopy

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