Synthesis of retinylidene-peptides of bacteriorhodopsin from Halobacterium Halobium

Martin Engelhard and Benno Hess
Max-Planck-Institut für Ernährungsphysiologie, 4600 Dortmund, FRG.

The purple membrane from Halobacterium Halobium contains a single protein to which retinal is bound via a Schiff-base linkage to a lysine residue. On light excitation, the chromophore undergoes a photocycle during which protons are pumped across the membrane (for a review see (1)). Three observations distinguish the ground state of bacteriorhodopsin from that of protonated retinylidene azomethines: 1. the bathochromic shift of the absorption maximum (440 ⟶ 570 nm), 2. the stabilization of the 13-cis over the all-trans conformation and 3. the induction of chirality in the chromophore. In order to elucidate these protein-retinal interactions we synthesized retinylidene-peptides with sequences simulating the retinal binding site: (TfaGly-Lys(Tfa)-Lys-Phe-Tyr-Ala (I), TfaGly-Asp-Ala-Lys-(Tfa)-Lys-Phe-Tyr-Ala (II) and TfaGly-Val-Ser-Asp-Pro-Asp-Ala-Lys(Tfa)-Lys-Phe-Tyr-Ala (III).

To insure the specific binding of retinal to Lys-41 all other amino functions were protected by the trifluoroacetyl group which is stable under the final HF-cleavage conditions. The spectroscopic properties of the retinylidene peptide III are strikingly different from other azomethines of retinal. This peptide, on reaction with retinal reveals a strong red shift in the absorption spectrum indicating a negatively charged group in the neighbourhood of the azomethine. Additionally an interaction of the bound retinal with tyrosine can be shown. Furthermore, the induction of chirality, induced by these interactions is indicated by the results of circular dichroic measurements. Our experiments demonstrate that the peptides II and III, being only 5% of the amino acid sequence of bacteriorhodopsin display already some features of the intact bacteriorhodopsin molecule.

(1) Hess, B., Kuschmitz, D. and Engelhard, M., Bacteriorhodopsin. Plenum Publishing Corporation, in press.