Interaction between the Retinal Chromophore and Amino Acid Residues in Bacteriorhodopsin

Dietrich Kuschmitz, Martin Engelhard and Benno Hess

Max-Planck-Institut für Ernährungsphysiologie, Rheinlanddamm, GFR-4600 Dortmund.

The transition from a noncrystalline to a crystalline protein state when retinal binds to bacterio-opsin, and the spectral shift of the retinal-Schiff-base linkage to 570 nm, indicate strong interaction between the retinal chromophore and amino acid side chains of the protein in bacteriorhodopsin (bR) (1). In order to define the physico-chemical nature of these protein-chromophore interactions we have investigated: a. the behaviour of proton binding groups and aromatic groups of the protein upon light activation of the retinal chromophore and b. the basic structural requirements for such an interaction by testing chemically sythesized peptides with sequences containing the retinal binding site for their interaction with retinal.

The results are: a. in the transition from the dark to the light state of bR, pK shifts of several proton binding groups occur (2), 1-2 negative sites (deprotonation of tyrosine) are formed, and the micro-dielectric within the protein is changed (3). b. the difference spectra of the retinylidene-peptide revealed only an enhancement of the absorption in the aromatic region and a peak at 460 nm, if a proton binding group (aspartate) can interact with the nitrogen of the Schiff's base.

The results show the importance of proton binding groups for the structure of the active site of bR, as well as for the photocycle-proton translocation process, which involves cyclic pK shifts. This may also be the basis for a proton to photocycle stoichiometry up to 2 (4), which cannot be explained by a protonated-deprotonated retinal-Schiff-base linkage alone.

(1) Schreckenbach, T., Walckhoff, B and Oesterhelt, D. (1977) Eur. J. Biochem., 76, 499-551.
(2) Hess, B., Korenstein, R. and Kuschmitz, D. (1978) in: "Energetics and Structure of Halophilic Microorganisms" Eds. R. Caplan and M. Ginzburg, Elsevier/North Holland, Biomedical Press, pp. 89-103.
(3) Hess, B. and Kuschmitz, D. (1979) FEBS Lett. 100, 334-340.
(4) Kuschmitz, D. and Hess, B. (1977) 11th FEBS Meeting, Copenhagen, Abstr. A4-13/708;
Ort, D.R. and Parson, W.W. (1979) Biophys. J., 25, 341-353; Govindjee, R., Ebrey, T.G. and Crofts, A.R. (1980), Biophys. J., 30, 231-242; Bogomolni, R.A., Baker, R.A., Lozier, R.H. and Stoeckenius, W. (1980) Biochemistry, 19, 2152-2159.