Evidence for "O_{640}" precursor different from "M_{412}" in the bacteriorhodopsin photocycle. Flash photolysis investigations in the visible and mid-infrared range.

W. Mäntele, F. Siebert

Institut für Biophysik und Strahlenbiologie der Universität Freiburg, Albertstr.23, D-7800 Freiburg, Fed.Rep.of Germany

In flash photolysis measurements monitoring the product "O_{640}" we observed, that the amount of this intermediate is increased twofold in D_2O, while the amount of "M_{412}" and "L_{550}" remains unchanged.
At wavelengths between 640 nm and 700 nm a fast absorbance increase with a risetime shorter than 10 µsec is observed, whose amplitude also doubles in D_2O, but, in contrast to "O_{640}", is independent of temperature.
In addition we observed, that under all our experimental conditions the decay of "M_{412}" can be described by a single exponential decay function. From these three observations we conclude that "M_{412}" cannot be the precursor of "O_{640}". From the D_2O-dependence of the fast absorbance increase mentioned above, we suggest that it may represent a product of a pathway different from the "M_{412}"-containing cycle. Further evidence for this is obtained by kinetic infrared measurements, from which the bacteriorhodopsin-L, the BR-M and the L-M difference spectra will be presented.

In addition to the well known kinetics of the photocycle, we observe in the infrared region a slow component with a risetime of about 1 msec, which increases in D_2O by a factor of approximately two. The spectral distribution of this component will be shown. This slow component could reflect a change in the protein, that does not manifest in the visible spectral range, but may lead to subsequent chromophore reactions. In addition, the infrared difference spectra will be compared with resonance Raman data of the photoproducts taken from literature. For the C=C-vibration and the fingerprint region good agreement with this data is obtained.
The spectral feature in the region of the C=N-vibration is very complex, and the effects of deuteration are not easy to interpret. To us, however, it appears that the model of a simply protonated Schiff base cannot explain our experimental results.