Non-chromophoric spectral changes during the bacteriorhodopsin photocycle probed by kinetic infrared spectroscopy

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The photocycle of light-adapted bacteriorhodopsin, BR_{570}, has been investigated by our recently developed method of kinetic infrared spectroscopy (1,2). From time-resolved absorbance changes IR-difference spectra were obtained at a time scale from microseconds to seconds. On the basis of IR-spectra of model compounds and the kinetics of these absorbance changes, some of them can be attributed to chromophore molecular changes due to the BR_{570}-L_{550} and the BR_{570}-M_{412}-transition.

For an interpretation on the molecular level, these difference spectra will be compared with resonance Raman data and with the IR-spectra of model compounds.

In addition, we observed spectral features that do not derive from the chromophore: their spectra do not agree with spectra of model compounds and their time courses do not coincide with those of the intermediates of the photocycle.

Since these spectral features also include components in the microsecond time scale, they must represent molecular changes that are uncoupled from the photocycle at an early intermediate.

It has been shown recently that the amplitude of the intermediate O_{640} doubles upon ^{1}H-^{2}H-exchange (3). This behaviour is also observed for several non-chromophoric components of the difference spectra (4). They may be attributed to a precursor of O_{640} which does not show up in the visible spectral range, and will be discussed in the light of the recent observation that the pH-dependence of the proton pumping efficiency parallels that of the O_{640}-amplitude (5,6).

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