Comparative Study of the Absorption Change at 387 nm, the Alkalisation Effect, and Light Scattering Changes as Indicators for Light-Induced msec Rhodopsin Transitions.

Detlef Emeis and Klaus Peter Hofmann

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

In bovine rod outer segment (ROS) preparations we observe three light-induced msec-events which are taken as indicators for rhodopsin transitions:

1) The absorption change at 387 nm due to the metarhodopsin I/ metarhodopsin II (MI/MII) transition.
2) The absorption change at 595 nm of the pH-indicator bromcresolpurple due to the proton uptake.
3) The intensity change of scattered light at 950 nm due to disk membrane contraction (P-signal) (1,2).

We carried out simultaneous measurements of the light scattering signal and the absorption changes at either 387 nm or 595 nm. With our two-wavelength differential device (3,4) absorption signals appear free of scattering contributions. The ΔpH-Signal is obtained as the difference of the signals before and after buffering of the sample and is therefore free of pigment absorption contributions. The signals are fitted by a Marquardt program (5).

The following results are obtained:

1) Concerning the ΔpH-signal, we confirmed our earlier result (6) that, dependent on preparation, up to three kinetic components are contained in the signal time course.
2) The MI/MII-signals are, as in our previous investigation (7), in most cases best represented by a sum of two exponentials.
3) Neither the ΔpH-signal (6) nor the MI/MII-signal shows the behaviour of a consecutive reaction at any temperature.
4) The P-signal is described as a modified consecutive reaction (8). The description with two and only two time constants is sufficient at all temperatures. We are able to determine the time constants up to a relation of 20 between them.
5) Comparing the Arrheniusplots of P, MI/MII, and ΔpH, no congruence between the various time constants is found.

We have to conclude that under our conditions

1) none of the observable metarhodopsin species is parallel or precursor to the alkalisation effect
2) neither the proton uptake nor the MI/MII transition represents the molecular precursor of the structure effect visualized in the P-signal.

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