The Kinetics of Formation of Metarhodopsin in Intact Photoreceptors of the Fly

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The formation of metarhodopsin in fly photoreceptor no. 1–6 occurs at room temperature with a time constant of 125 μs ($Q_{10} \approx 2.5$). The formation of rhodopsin is faster by factor of 1/10 to 1/100 at least.

The visual pigment of the photoreceptors no. 1 – 6 of the fly is bistable, whereby the rhodopsin (R) has an absorption maximum close to 490 nm, the metarhodopsin (M) has one at 570 nm [1 – 3]. The difference spectrum can be characterised by the minimum at 470 nm, an isosbestic point at 500 – 510 nm and a maximum at 570 nm. We measured the kinetics of transitions between both pigment states by means of flash photolysis at intact flies (*Drosophila*, white-eyed mutant), using the "deep pseudopupil" technique [4].

The virtual images of rhabdomeres no. 1 – 3 from the "deep pseudopupil" were imaged onto a diaphragm placed in front of a photomultiplier, and the light intensity transmitted by the rhabdomeres, was measured. Averaging techniques were used in order to improve the signal to noise ratio. The fly was mounted in a chamber that allows cooling down of the animal from room temperature to $-5\,^{\circ}$C. The flashes were generated by two photoflashes (Braun, type F 900, Frankfurt, Germany), which were triggered at appropriate times by a microprocessor. The light from the photoflashes was filtered and conducted through light guides to the eye of the fly.

The changes in transmission, induced by short wavelength ("blue") flashes (cut off filter, Melles Griot, type 03 SW PO 11, $T_{1/2} = 460$ nm) that shift rhodopsin into metarhodopsin, as well as by long wavelength ("orange") flashes (cut on filter, Schott, type OG 530, $T_{1/2} = 530$ nm) that shift metarhodopsin back into rhodopsin were measured at the three characteristic points of the difference spectrum: the minimum, the isosbestic point and the maximum.

Fig. 1 demonstrates the decrease in transmission following the blue flash, and the increase in transmission following the orange flash. As can be seen at 9.5 °C metarhodopsin is formed or decomposed after the blue and orange flash respectively, within a fraction of a second.

If instead of orange (590 nm) measuring light blue (480 nm) light is used, transmission increases and decreases again after the blue and orange flashes, respectively. At the isosbestic point no significant changes can be observed. The transitions described correspond to the characteristic points of the difference spectrum.

If the time resolution is increased to approximately 1 ms, at room temperature all transitions are still too fast to be resolved. A time constant of 40 ms as reported by Lo and Pak [5], indicative of a "darkening effect", as distinct from pure pigment conversion, could not be found under our experimental conditions.

In order to get information on the kinetics of the transition we cooled an animal down to 5 °C. As can be seen in Fig. 2 the kinetics of formation of metarhodopsin after a blue flash is now resolved. Analysis of records as presented in Fig. 2 shows that the decay is exponential with a time constant of

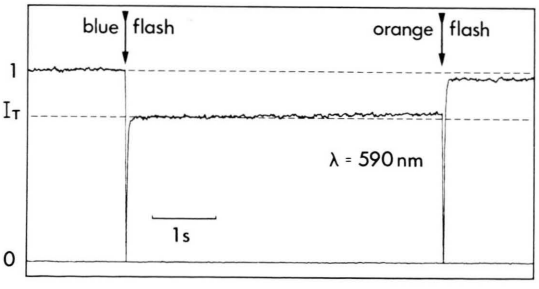

Fig. 1. The formation and the decomposition of metarhodopsin in *Drosophila* photoreceptors R 1–3 after a blue and an orange flash, respectively, as monitored by means of orange ($\lambda = 590$ nm) measuring light (ordinate: transmitted intensity I_T). The dashed lines have been drawn parallel to the zero-line (bottom) in order to illustrate 1. that the measuring light slowly shifted metarhodopsin back to rhodopsin during the time between the two flashes and 2. that the orange flash was not sufficient to shift all metarhodopsin back into rhodopsin. During the flashes an electronic gate device connected the in- and output of the amplifier of the photomultiplier to ground in order to prevent its overloud due to straylight of the flashlights. Temperature: 9.5 °C, $N = 8$ records averaged, amplifier band width 0–10 Hz.

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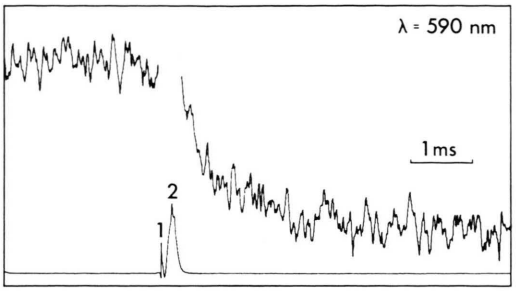

Fig. 2. Kinetics of the formation of metarhodopsin after a blue flash on a fast timescale, as monitored by means of orange ($\lambda = 590$ nm) measuring light (top trace). The bottom trace shows the stimulus flash as recorded by means of a photomultiplier (1: trigger artifact, 2: light induced signal). Temperature: 5 °C, $N = 64$ traces averaged, amplifier band width 0–10 000 Hz.

$\approx$ 1 ms at 5 °C. From the temperature dependence of the time constants ($Q_{10} \approx 2.5$), a time constant of 1.25×10^{-4} s for the formation of metarhodopsin can be extrapolated for room temperature (25 °C)*. The temperature dependence corresponds to an activation energy of approximately 10 kcal/mol.

Whereas the formation of metarhodopsin can be resolved at temperatures below room temperature, the formation of rhodopsin as measured at 480 nm after a red flash is faster than 300 μs at 0 °C, and hence beyond the time resolution of the setup.

The results gave no detectable indication of an intermediate formed at the transition from rhodopsin to metarhodopsin within the time resolution analysed here. This is concluded from the fact that there is no change in transmission at the isosbestic point, as well as from the fact that the formation of metarhodopsin follows a simple exponential law.

The time constant of the transition from R to M of 1.25×10^{-4} s at 25 °C (1.9×10^{-4} at 20 °C) is slower by a factor of 10 than the transition of lumi- to metarhodopsin I in bovine rod outer segments (halflive $t_{1/2} = 1.6 \times 10^{-5}$ s at 20 °C [6]); it is faster than the suggested transition from prelumi- to metarhodopsin in the *Limulus* ventral eye ($t_{1/2}$, 25 °C $= 10^{-2}$ s [6]) and also faster than transitions measured in isolated rhabdoms of the crayfish ($t_{1/2}$, 25 °C $\approx 5 \times 10^{-2}$ s [7]).

* At the II European Neurosciences Meeting, Florence, September 5 to 8, 1978, Hamdorf and Razmjoo as well as Muijser and Stavenga presented evidence that in Calliphora the transition M → R is faster than 0.5 ms at room temperature.

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