TWO COMPONENT FAST PHOTO-SIGNALS DERIVED FROM ROD OUTER SEGMENT MEMBRANES ATTACHED TO POROUS CELLULOSE FILTERS

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1. Introduction

Light absorption by the visual pigment rhodopsin gives rise to transient electric photo-responses in the millisecond range. Fast, two component photosignals* have been observed in different rhodopsin-containing systems: the eye in situ [1,2] or the excised, live retina extracellularly [3,4] as well as intracellularly [5,6]. To a degree these responses could also be obtained from artificial systems: e.g., from a digitonin solution of rhodopsin in a dialysis membrane system [7] or from membrane fragments attached to a Teflon film [8]. In the latter case, being placed in a polar/apolar interface region the rhodopsin produces only the slow second component of the photosignal.

For studying the molecular origin of the photo-signal it would be more useful to have a simple system in which the aqueous medium has access to each membrane side. Here, a model system is described which meets this requirement. This system shows a light-stimulated transient two-component response which is largely comparable to the photo-voltage signal with R1 and R2 components obtained from the live retina. A thorough kinetic analysis of the photo-signals obtained from the artificial membrane system as well as from the intact photoreceptor could establish no direct kinetic coupling between the electric photo-signal R2 and the spectroscopically defined transition *meta* I to *meta* II of rhodopsin.

* Originally called 'early receptor potential' (ERP) [1], but being a rhodopsin (rather than a physiological receptor signal the term) 'fast photosignal' or 'voltage' (FPV) is preferred [4]

2. Materials and methods

Bovine rod outer segments were prepared according to [9] and stored at liquid nitrogen temperature until use. All experiments were carried out under dim red light. To form the electrically active system a suspension of bovine rod outer fragments was highly concentrated by centrifugation (rhodopsin $\sim 10^{-3}$ M) and applied by a micropipette to one side of a 5 × 5 mm^2 cellulose acetate or cellulose nitrate membrane filter. No organic solvent was necessary for this procedure. In most experiments filters with 0.2 μm pore size were used (Sartorius SM11307). As shown in fig.1, the membrane filter was placed between two compartments of a thermostatted chamber filled with Ringer solution. White light from a 15 μs xenon flash

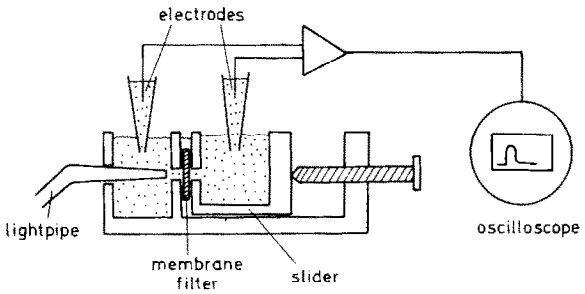

Fig.1. Experimental set up. Both compartments of the cell are filled with Ringer solution before the filter covered with rod membrane fragments is inserted into the gap between both compartments and clamped together by the slider. Light flashes of 15 μs duration are delivered through a light pipe to the cell. Electric responses are measured by Ag/AgCl electrodes, shielded against scattered light. Amplifier: Tektronix AM 502, Oscilloscope: Tektronix 5103N. The thermostating is achieved by thermistor controlled peltier elements.

(EGG, FX193) was applied through a light pipe directly onto the active system (fig.1). Photo-signals were measured by Ag/AgCl electrodes (~ 5 kΩ) amplified with variable bandwidth (input: 1 M/47 pF) and recorded on a storage oscilloscope.

3. Results

On illuminating the membrane filter covered with bovine rod outer fragments by an intense white flash, a fast photo-voltage was generated which was similar to that recorded extracellularly from the intact bovine retina. The response of the artificial membrane system at pH 7 is shown for different temperatures in fig.2. The signal at 50°C (fig.2a) is monophasic showing a polarity which is positive on that side of the chamber where the membrane fragments are attached to the filter. The polarity does not depend on the direction of the light input. By applying successive saturating flashes the amplitude of the response is decreased by 50%/flash (fig.2c) due to the photolysis of rhodopsin [10]. Lowering the temperature of the system reduces the amplitude of the positive signal (R2) and a second faster component of opposite polarity (R1) appears (fig.2b). The action spectrum of the response is centered around 500 nm and shows no participation of cone pigments (fig.3). The temperature dependence of the R2-decay constant is given in fig.4. The activation energy of 100 ± 10 kJ/

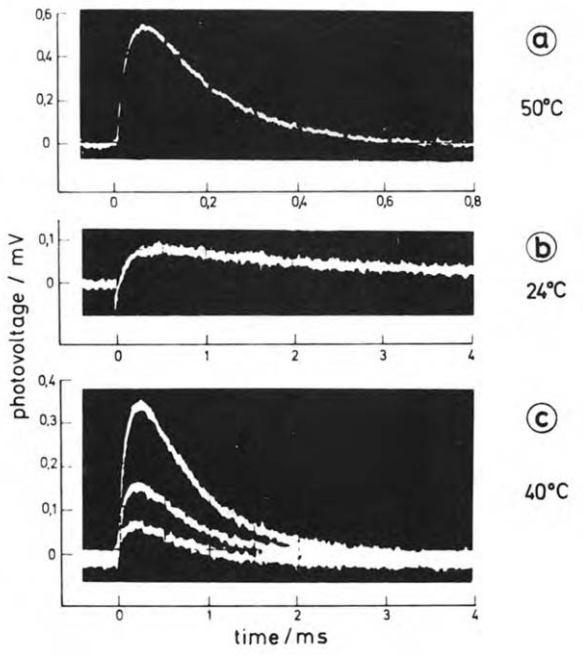

Fig.2. Electric photo-signals of the artificial membrane system evoked by saturating white flashes of 15 μs duration at different temperatures given in different time scales: (a) 50°C; (b) 24°C; (c) responses to 3 subsequent saturating flashes at 40°C; Bandwidth, 10 Hz–100 kHz; ringer solution (pH 7.0).

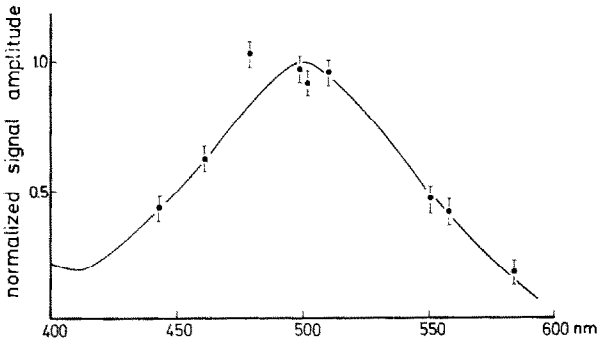

Fig.3. Action spectrum of the photo-signal measured in the artificial rod membrane system at 45°C. For each data point the signal amplitude of a low intensity first spectral flash relative to that of a second white flash of constant intensity is normalized to equal numbers of the spectral photons of the first flash. For comparison the action spectrum is fitted to the maximum of the absorption spectrum of bovine rhodopsin (corrected according to Heller from [17]).

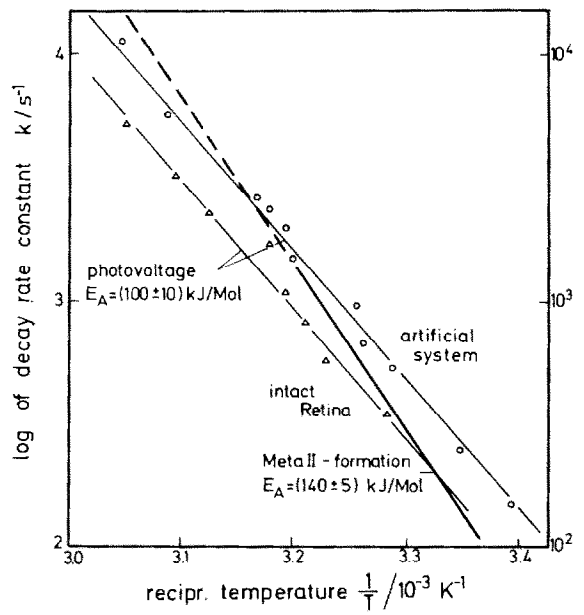

Fig.4. Arrhenius plot of the photo-signal decay rate constant for (○) artificial system, (△) intact retina, both in Ringer solution (pH 7.0). For comparison also the Arrhenius plot of the reciprocal of the mean reaction time τ_2 of the *meta* II formation (absorption A_{380} increase) is given (heavy line).

mol agrees well with that derived from the R2-decay measured in the intact bovine retina [11].

4. Discussion

4.1. *Structure of the active filter membrane system*

The formation of the electrically active system was observed with cellulose acetate and cellulose nitrate but not with polyamide filters. It is supposed that some special interactions exist between the surface of the first two materials and the rod lipid membrane. The interaction would seem to be relatively strong since intact frog rods have been shown to be easily disrupted on close contact with the filter surface [12]. Therefore the formation of the active system might be explained in the following way: a considerable amount of filter pores is sealed off parallel to the filter surface by a membrane containing oriented rhodopsin. In this way, the two component response of the active system is actually measured when both sides of the rod lipid membrane are in contact with the aqueous medium. In contrast, even the low temperature photoresponses obtained [8] from rod fragments in a polar/apolar interface region did not show any R1 component except at pH <2.3 [8]. The difference might be due to the missing contact of the rhodopsin containing layer with the aqueous phase on one side which could be necessary for the generation of the R1 component. In both systems the decay time of the R2 responses is of the same order of magnitude. In the Teflon film system, however, the reciprocal decay time shows a non-linear Arrhenius plot. This might indicate that the R2 generating mechanism is also influenced by the medium surrounding the rhodopsin.

4.2. *The generation of the photo-signal*

It has been shown, that the extracellular response of the intact photoreceptor roughly reflects the first derivative of the intracellularly recorded signal [5]. This implies that the time constant for the charge redistribution between outer and inner segments is significantly shorter than the rise time of the intracellularly measured charge displacement. In this case the decay of the extracellular R2 response is given by the rise of the transmembrane signal [11]. If the active system is indeed formed by small single membrane pieces all in parallel on the filter surface, its electric response should correspond to the intracellularly recorded FPV. The measured response, however, has nearly the same time course as the extracellular signal (fig.2). Therefore, in the artificial system the effective discharge time constant must also be shorter than the rise of the primary charge displacement, and the response of the artificial system reflects the displacement current during the conformational changes of the oriented rhodopsin. Consequently the artificial system must be described by a current source (rhodopsin conformational change?) and a simple parallel combination of a capacitance (filter pores sealed off by rod membranes) and a high conductance (open pores) which together represent the short time constant of the system.

4.3. *Coupling of photo-signals to transitions of rhodopsin intermediates*

For the rat retina the rise of the R2 component was compared initially at physiological temperature with the spectroscopically monitored *meta* I to *meta* II transition of rhodopsin [13]. In this temperature range the appearance of the R2 component agreed with the A_{380}* increase within a factor of 2. The kinetic coupling between the *meta* II formation and the R2 rise was presupposed also in calculating the space distribution of the rat retina FPV [14]. Later on, however, it was found that the activation energies for both processes were quite different and that the accordance at physiological temperatures was just a coincidental crossover of the different temperature dependencies [11]. Therefore, a kinetic coupling was proposed between the *meta* II formation and the increase in the intracellularly recorded FPV [5] or, if the integral/differential relation holds, the decay of the extracellularly recorded R2 component of the FPV (see above and [11]). According to the Arrhenius plot in fig.4, however, the activation energy of the R2 decay in the artificial system is smaller than the 120–150 kJ/mol activation energy usually obtained from the kinetics of the *meta* II-formation of rhodopsin. The signal from the artificial system is generally faster than that from the intact retina. This result is consistent with the finding that the A_{380} increase indicating conformational changes of rhodopsin is also faster in disrupted membranes than in the intact retina [15].

The results of electrical and spectroscopic studies indicate that the electric photo-signal in the rhodopsin-

* In [13] actually measured at 404 nm.

containing membrane is closely related to the spectroscopically-defined transitions of rhodopsin intermediates. However, the R2 component of the electric signal cannot yet be directly identified with the *meta* I to *meta* II transition of rhodopsin. It is evident that there is still no satisfactory answer to the kinetic coupling problem. However, it might be emphasized that the electric signal is well characterised whereas on the other hand the *meta* I to *meta* II transition appears to be a spectroscopic pattern of a highly complicated reaction [16].

Acknowledgements

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