KINETICS OF THE BLUE LIGHT-INDUCED INHIBITION OF PHOTOELECTRIC ACTIVITY OF BACTERIORHODOPSIN

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1. Introduction

In previous photoelectron studies of bimolecular lipid membranes containing bacteriorhodopsin membrane sheets (BR) it was found that if this system is illuminated by green and blue light simultaneously, the latter is capable of decreasing the photopotential generated by green light [1]. It was proposed that the blue light induces fast conversion of $BR_{412} \rightarrow BR_{570}$ in a fashion which is not coupled with proton pumping. A kinetic model of this effect has been elaborated [2].

The study of photoelectric activity of BR incorporated into lipid-impregnated collodion film has revealed three electrogenic phases differing in time constants [3]. On the basis of flash-photolysis absorption measurements on BR water suspension, these phases were correlated with different steps in the photochemical cycle of BR. According to the comparison of the kinetic parameters, simultaneously with the formation of BR_{412} a rise in the photopotential indicates proton translocation from the Schiff-base in the pigment–protein complex. A further, slower rise in the photopotential following the regeneration of BR_{570} is due to the proton uptake from the opposite side of the membrane.

The present paper deals with fast kinetic aspects of the blue light-induced decrease of the photopotential in connection with the BR photochemical cycle. The quenching was induced by a blue flash, following a green one, after appropriate delay times. It has been shown, that the decreasing effect of the blue flash begins with a very fast drop ($\tau < 5$ μs) in the photopotential, which may be connected with either fast rebinding of the proton by excited BR_{412} and/or a charge displacement in the chromophore region (probably photoisomerization). Only the BR_{412} not excited by blue light regenerates via the basic, dark pathway, consequently only this part contributes further to develop the second part of the photopotential. This decrease in the number of the dark recombination of BR_{412} causes a further decrease in the maximum of photopotential.

In parallel with the photoelectric measurements, flash photolysis absorption measurements were carried out to compare photoelectric and photochemical properties of BR. Double flash excitation with variable delay times was used, and the lifetime of BR_{412} in a reconstituted model membrane was determined by photoelectric measurements. It was proved that the incorporation of BR into phospholipid-impregnated collodion film does not change the kinetics of BR_{412} formation and decay.

2. Materials and methods

In control measurements on the steady-state behaviour of two model systems: BR in bimolecular lipid membrane [2] and BR in lipid-impregnated collodion membrane (BR-CLM) [3] it turned out that as far as the effects under investigation are concerned both of them demonstrate qualitatively the

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same responses. The BR-CLM was chosen for the present work since it demonstrated higher stability and larger photopotential values. The procedure of formation of BR-CLM, the basic photoelectric measurement techniques used, were as in [3] except that in this case the following bathing solution was used: 100 mM NaCl; 2.5 mM EDTA; in water, at pH 6.

In flash-photolysis absorption measurements the light transmittance of the sample was followed (in a direction perpendicular to the exciting lights) by illuminating the sample with a monitoring beam from a tungsten lamp through a monochromator. The transmitted light was measured by a photomultiplier behind cut off filters and a second monochromator.

In some qualitative measurements green flashes were produced b a neodymium Q-switched laser of doubled frequency (λ = 530 nm; flash duration $\tau_{1/2}$ = 15 ns; I = 8 mJ). Due to the instability of the laser pulse energy, in quantitative experiments flashes containing green light (called green flash) were obtained with a commercial flash lamp (Regula Varian, FRG) with an OC 13 filter (USSR): flash duration $\tau_{1/2}$ = 0.4 ms; I = 16 mJ.

Short blue flashes were obtained from ISSH 110 flash tube (USSR): flash duration $\tau_{1/2}$ = 5 μs; I = 2 mJ. A dual beam oscilloscope (S8-13, USSR) was used for synchronization of flashes with the recording system and the delay time setting between two flashes. The time resolution of the measurements was about 5 μs. All measurements were carried out at room temperature.

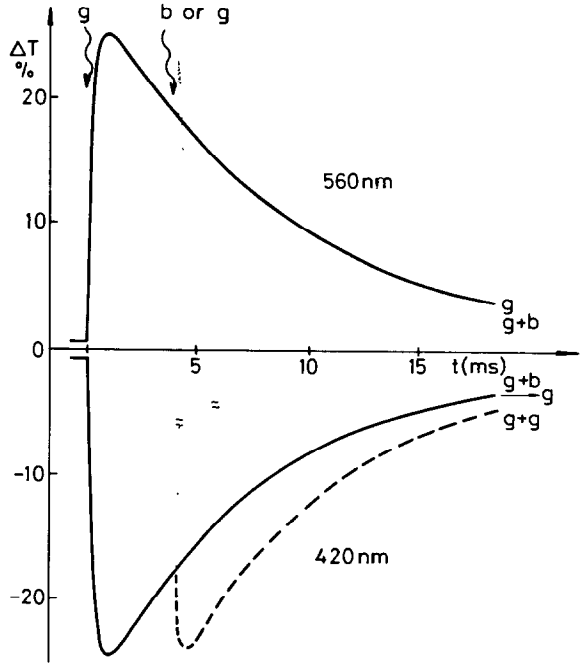

Fig.1. Change in the light transmittance (ΔT) following single and double flash excitations in water suspension of purple membrane monitored at 560 nm and 420 nm. The notations of the curves: g, single green flash excitation (——); g + g, green flash followed by a second green one after delay time t_d = 4 ms (- - -); g + b, green flash followed by a second blue one with the above delay (· · ·). The optical noise during the second excitation can be seen which is caused by direct scattering of the light to the measuring multiplier (in spite of the use of monochromator and cut off filters before the multiplier).

3. Results and discussion

The inhibiting effect of blue light was investigated by the described double-pulse technique in both absorption and photoelectric measurements. To investigate the fast regeneration of BR_{570} from BR_{412} the measuring wavelengths 560 nm and 420 nm were chosen. If after a green flash (inducing $BR_{570} \rightarrow BR_{412}$ conversion) the sample is illuminated by a second, blue flash, a fast ($\tau < 5$ μs) absorbance change takes place: decrease at 420 nm and increase at 560 nm (fig.1.). This proves that excitation of BR_{412} does in fact induce a fast $BR_{412} \rightarrow BR_{570}$ conversion as it was found earlier in low temperature spectroscopic measurements [4,5]. Respectively, recent experiments by Kalisky et. al. [6] also show that upon double (green and blue) flash excitation at room temperature the decay time of BR_{412} is in the ns range.

For detailed study of the kinetics of the photoelectric inhibition effect an optimum delay time of the blue flash (t_d) was chosen (t_d = 0.8 ms). The typical photoelectric response and the dependence of the decreasing effect on the intensity of the green light are shown in fig.2. The results are qualitatively the same as for steady-state measurements [1,2]: at low green intensity, i.e., when small amount of BR_{412} is produced the blue flash increases the photopotential, since BR_{570} absorbs also blue light to a

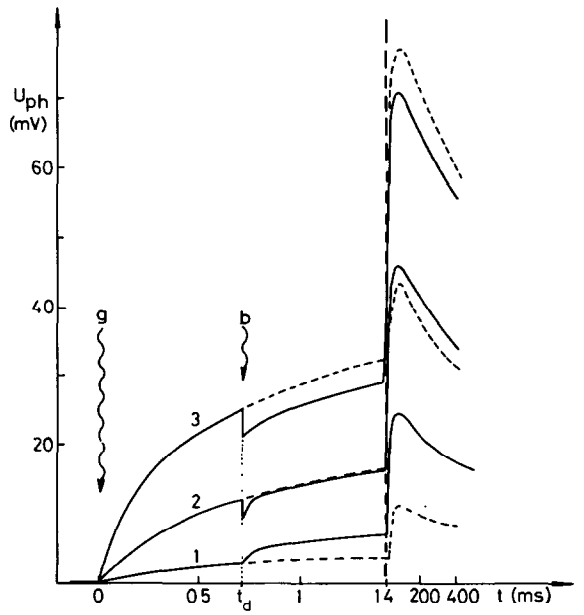

Fig.2. The time course of the photopotential (U_{ph}) of BR-CLM under successive green (g) and blue (b) flash excitations (t_d = 0.75 ms) at different green light energies; 1, light intensity, I = 0.15 mJ; 2, I = 1.0 mJ; 3, I = 16 mJ, at a constant blue light intensity (2 mJ). (——) The time course of the photopotential in an experiment with double excitation. (- - - - - -) The time course of the photopotential without second (blue) flash. The range on the time-coordinate is changed at 1.4 ms to allow full representation of the photosignal.

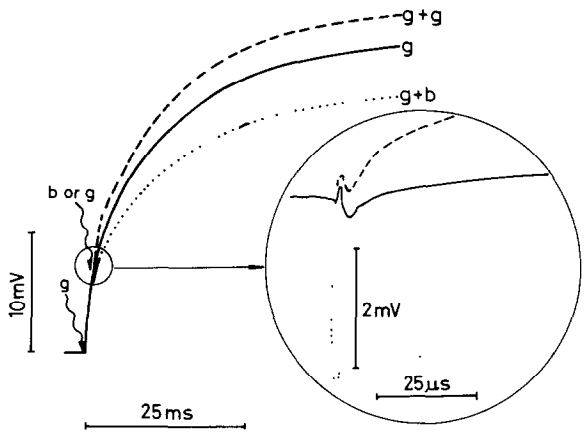

Fig.3. The time course of the effect of secondary excitation on the green pulse-induced photoelectric response at different time scales. Notations see fig.1 except t_d = 1 ms. At the higher time resolution (25 μs scale) rate of the changes of the photopotential was limited by the duration of the second flash ($\tau_{1/2} \simeq 5$ μs). The control curve (g; when the light of the second flash does not reach the collodion film) shows the electrical noise caused by the second flash.

certain extent. At high green light intensities blue light decreases the photopotential.

The blue light effect begins with a very fast decreasing step ($\tau < 5$ μs), remaining unresolved due to the duration of the blue flash (fig.3). This is explained as follows: Since the Schiff-base of BR is deprotonated in BR_{412} state, proton translocation occurs in the pumping direction during the formation of BR_{412}, observed as the fast rise ($\tau \simeq 50$ μs) in the photopotential [3]. It was proposed [2] that blue light excitation of BR_{412} either prevents proton release from the membrane, or causes proton rebinding from the side of the membrane to which it has been released. In both cases proton translocation to the Schiff-base, against the pumping direction (from either the intramolecular or the extramolecular space) takes place, which causes this fast fall in photopotential. This drop, however, is >10-times faster than the rise in the photopotential following the formation of BR_{412}, just as blue light-induced reconversion of BR_{412} into BR_{570} is much faster than its formation. Normal proton pumping is completed by proton uptake from the opposite side of the membrane during thermal reconversion of $BR_{412} \rightarrow BR_{570}$ which causes the second, slower phase in the rise of the photopotential [3]. Blue light-induced fast recombination decreases the number of the above mentioned proton uptakes, thus the slope and the maximum in the photopotential curve will be further decreased (fig.2,3). Besides this effect another can be present in parallel in the negative change in the photopotential. Excitation of BR_{412} may cause a fast transient change in the membrane potential due to displacement of charges caused by the possible *cis–trans* isomerization of retinal [7].

The dependence of the blue light effect on the duration of the time interval between the green and the blue flashes (delay time) has been investigated both by spectroscopic and photoelectric methods (fig.4,5). As seen in fig.4 in the case of very short interval between the flashes ($t_d < 0.16$ ms) the blue flash causes a further increase, instead of decrease in the photopotential. For longer delay times, the

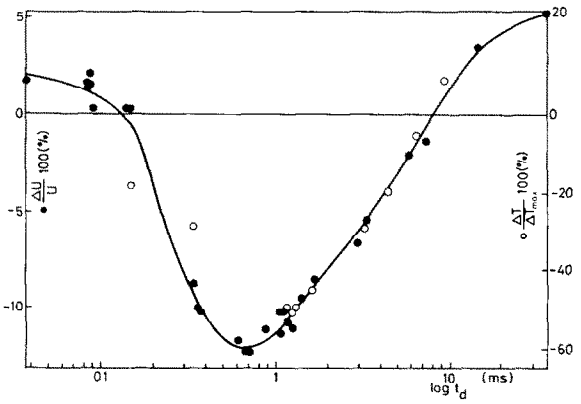

Fig.4. Dependence of the blue light-induced relative changes in photopotential (ΔU) of BR-CLM and in transmission of purple membrane water suspension (ΔT at 560 nm) on logarithm of the delay time (t_d) of the second (blue) flash. The first part of the curve is slightly distorted due to the duration of the first (green) flash ($\tau_{1/2}$ = 0.4 ms).

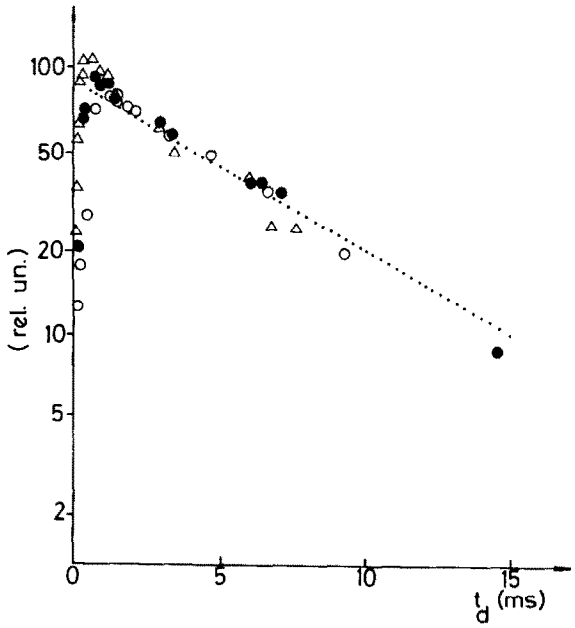

Fig.5. The logarithm of the data taken from fig.4 and plotted in linear delay time (t_d) scale. Notations are the same as in fig.4. (Δ) correspond to relative amplitude of the first fast drop in the photopotential caused by the blue light; (· · ·) spectral decay of BR_{412} measured at 420 nm after a 15 ns green pulse in water suspension of purple membranes at room temperature.

inhibiting effect appears, the optimal t_d at which the inhibition reaches its maximum is 0.8 ms. At t_d>10 ms the blue flash causes again a growth of the photopotential. In analogous experiments the absorption changes showed similar behaviour. In fig.5 the t_d is plotted in linear scale against the logarithm of the potential and transmission changes. All parameters have similar kinetics to that of the decay of BR_{412}. The decrease of the photopotential by blue light is proportional to the concentration of BR_{412}.

During the preparation of this paper, the work of San-Bao Hwang et al. [8] appeared, describing the negative photoelectric effect of the secondary blue light (after a green light) also in dried multilayer system. In such a system the kinetics of the photochemical cycle and its relation to the photoelectric activity of BR are dramatically changed compared to the negative or BR-CLM model system.

Using double pulse excitation technique the lifetime of photosensitive BR intermediates can be determined by photoelectric measurements in BR model systems. The fact that the effect of blue light on the photopotential and the spectral decay of BR_{412} have similar time dependence is a direct proof that:

1. BR_{412} is responsible for the blue light inhibition;
2. The life time of BR_{412} does not change when BR is incorporated into a collodion film impregnated with decane solution of phospholipid.

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