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EFFECTS OF ELECTRIC FIELD ON THE PHOTOCYCLE OF BACTERIORHODOPSIN

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The photoconversion of bacteriorhodopsin and the effects of an applied electric field ($5 \cdot 10^7$ V · m^{-1}) were studied in dry films of purple membranes from *Halobacterium halobium*. The electric field was found to cause at least two different effects: (1) it blocks in part the formation of the batho-bacteriorhodopsin (K), most probably due to electrically-induced dark transition of some bacteriorhodopsin molecules into the photochemically inactive form; (2) it decreases the rate of the intermediate M decay, the rise time of the M formation being unaffected by electric field. The observed phenomena may suggest a feedback control mechanism for the regulation of the bacteriorhodopsin photocycle in purple membranes.

It is well known that the transmembrane electric field is an important factor in the regulation of energy conversion processes in biological systems [1–3]. In view of this, it would be interesting to see how an electric field affects the photocycle of bacteriorhodopsin – the simplest known biological energy transducer [4,5].

We have described in our previous work the experimental approach used for studies of electric polarization processes in energy-transforming biomembranes and their active fragments [6]. The method is based on monitoring optical characteristics and dielectric parameters of films of preparations exposed to an external electric field.

In the present work we have investigated, using the same approach, the effects of applied electric field on the bacteriorhodopsin photocycle in films of purple membranes from *H. halobium*. At least two different field-induced effects were observed: (1) an electrically-induced dark transition of some bacteriorhodopsin molecules into the photochemically inactive form which blocks in part the formation of the batho-bacteriorhodopsin (K) and (2) a slowing of the decay kinetics of the intermediate, M. The data may suggest a field-dependent mechanism for regulation of the bacteriorhodopsin photocycle in purple membranes.

Preparation of air-dried films of purple membranes (relative humidity of 40–50%) and measurements of electrically induced absorption changes under steady-state illumination were performed as previously described [7,8]. Fast events of bacteriorhodopsin photocycle and effects of electric field were monitored on a single-beam differential spectrometer with laser excitation (at 530 nm; pulse width 15 ns; energy 3.5 mJ) and a time resolution of 25 ns.

In dry films of purple membranes, the characteristics of bacteriorhodopsin photocycle differ somewhat from those in the aqueous phase. Fig. 1 shows differential spectra of laser flash-induced absorbtion changes in film preparations at different times after the excitation. The maxima correspond to main phases of the bacteriorhodopsin cycle. There are three absorption peaks, at 565, 630 and 415 nm. The peak position at 565 nm corresponds to the initial form of light-adapted bacteriorhodopsin in dry films [9]; the long-wave

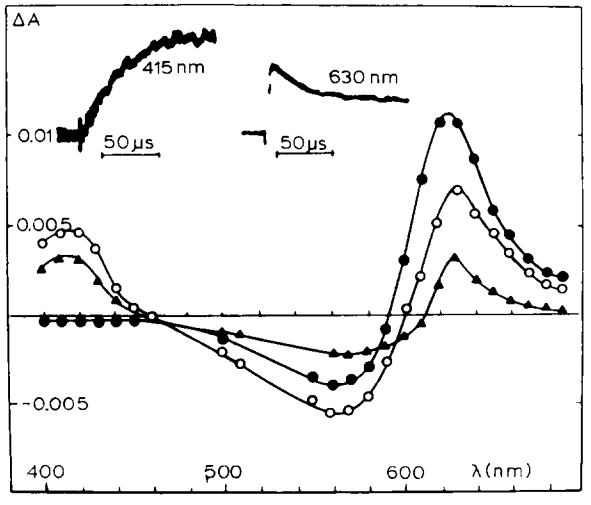

Fig. 1. The spectra of bacteriorhodopsin absorption changes induced by a laser flash (530 nm; 15 ns; 3 mJ) in dry films of purple membranes from *Halobacterium halobium* (a) (-●-●-) immediately after the flash; (b) (-○-○-) 100 μs; (c) (-▲-▲-) 25 ms after the flash; (insert) kinetics of absorption changes of the 415 and 630 nm absorption bands.

intermediate (maximum at 630 nm) and batho-bacteriorhodopsin (K) are very close in spectral position, the short-wave maximum corresponds to the intermediate M [10–12].

The rise-time of the photoinduced K formation cannot be resolved with our spectrophotometer ($t_{1/2} \leqslant 25$ ns). The decay kinetics of this intermediate is at least biphasic, with the components $t_{1/2} = 18 \pm 2$ μs and $t_{1/2} = 12 \pm 4$ ms. The half-time of intermediate M appearance is 20 ± 2 μs, as measured by absorbance increase at 415 nm (Fig. 1).

If one plots a differential spectrum of absorbance changes occurring in the 100 μs after the flash (this spectrum can be derived from Fig. 1 by subtracting spectrum (a) from spectrum (b)), it can be seen that the resultant spectrum has only two pronounced bands (a positive with a maximum at 415 nm and a negative with a maximum at 620 nm) and also some unresolved wide negative absorbance changes in the 480–580 nm region. The spectral width of the 620 nm band is approx. 40 nm, which differs from that expected for the intermediate K (its width is accepted to be approx. 100 nm).

The kinetic correspondence of intermediate M formation and fast component of intermediate K decay may suggest the direct transition of K into M. But narrow spectral width of the 620 nm band and the presence of absorption changes in the 480–580 nm region imply a more complex reaction path. To study the bacteriorhodopsin photocycle in dry films at length, special kinetic experiments under controlled humidity are needed. Spectral measurements on dry films of purple membranes actually show a decrease in some intermediates in the bacteriorhodopsin photo-cycle on dehydration [13,14].

The slow decay component of the 630 nm signal ($t_{1/2} = 12 \pm 4$ ms) is presumably due to the reversal of the batho-bacteriorhodopsin K into the initial bacteriorhodopsin$_{570}$. An indication of this is the kinetic component with $t_{1/2} = 11 \pm 3$ ms seen in the dark 570 nm absorbance changes. To understand these kinetic data, one may rely upon experimental results concerning the possible paths of the bacteriorhodopsin photocycle in dried purple membrane films [9,13]. It has been shown that the population of bacteriorhodopsin molecules did not remain homogeneous, at least as judged by its kinetic characteristics after dehydration. Moreover, a pronounced decrease in the yield of the photoinduced formation of the intermediate M has been observed. Thus, in dry films some of bacteriorhodopsin molecules may undergo photoconversion through the intermediate, but others may not. In our case we believe that in one of the bacteriorhodopsin populations, photoconversions proceed as

$$\text{bacteriorhodopsin}_{570} \rightarrow \text{K} \ldots \text{L(?)} \rightarrow \text{M}$$

and in the other population a shorter 'cycle' bacteriorhodopsin$_{570}$ K may occur. The kinetic component of K → bacteriorhodopsin transition with a $t_{1/2}$ of about 11 ms obviously corresponds to this latter population. Reverse transitions of the bacteriorhodopsin intermediates have been observed in Ref. 15. Under the conditions of our experiment the reversal may be induced by absorption of actinic or monitoring light by batho-product K. Such photoinduced conversions of bacteriorhodopsin and visual rhodopsin are well known [15,16].

When an electric field ($5 \cdot 10^7$–$6 \cdot 10^7$ V · m^{-1})

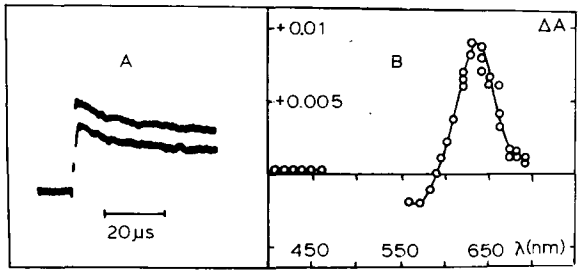

Fig. 2. Effect of applied electric field ($5 \cdot 10^7$ V·m^{-1}) on laser flash-induced absorption changes of bacteriorhodopsin in dry films of purple membranes from *H. halobium* (A) Kinetics of 630 nm absorption changes; 1, field off; 2, field on. (B) The spectrum of field-induced absorbance decrease measured as a difference between absorbance changes induced by laser flash before and after application of electric field.

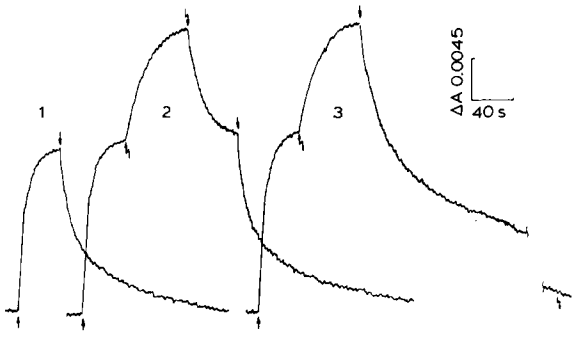

Fig. 3. Kinetics of photoinduced and electrically induced absorption changes of bacteriorhodopsin at 420 nm in dry films of purple membranes from *H. halobium*. (1) Photoinduced absorption changes in the absence of electric field; (2), (3) the same in the presence of the field; dark decay of the absorbance changes is recorded with the field off (2) and on (3); ↑ light on; ↓ light off (continuous illumination at 500–600 nm; 80 W·cm^{-2}) ↾↑ field on; ↿↓ field off ($5 \cdot 10^7$ V·m^{-1}).

is applied one observes a decrease in the amplitude of flash-induced absorption changes measured immediately after the flash (Fig. 2A). The spectrum of the field-induced absorption decrease is very close to that for intermediate K (Fig. 2B). The observation implies that the electric field may cause a reversible diminution of the amount of bacteriorhodopsin involved in photocycle.

At the same time, the electric field was found to produce no effect on the kinetics of the light-induced formation of the intermediate, M ($t_{1/2} = 20$ μs).

Another type of field-induced effect was seen in our experiments under continuous illumination. Application of an electric field in this case causes an increase in the photostationary level of intermediate M and a pronounced deceleration in its dark decay.

The decay kinetics of the 415 nm absorption changes of bacteriorhodopsin in dry films can be resolved into three exponential components with characteristic times of 70–80 ms (τ_1), 5–7 s (τ_2) and 40–60 s (τ_3). After application of the electric field, the total deceleration in M decay is mainly due to a 2-fold increase in τ_3, τ_1 and τ_2 being little affected.

The deceleration in M, with the rate of its production unchanged, may be the cause of the field-induced stimulation of its steady-state level seen under continuous illumination (Fig. 3; see also Ref. 8).

The multi-phase decay kinetics of M is in good agreement with data reported earlier in [13] for dry films of purple membranes. This character of the decay was concluded to be due to three conformational states of the intermediate M, and therefore to three different ways of its conversion into the initial bacteriorhodopsin$_{570}$.

It is worth noting that the number of kinetic components does not increase even after thorough desiccation of the purple membranes, though characteristic times of the decay become longer [13].

This complicated kinetic pattern may reflect the influence of the hydration state on the photoconversion of the trimers of bacteriorhodopsin, with the most slowly decaying component arising from sites with the lowest degree of hydration within the bacteriorhodopsin complex. If so, the observation that the electric field exerts its effect predominantly on the slowest component of M decay may be explained in terms of greater local electric field density at sites with less hydration within the membrane.

The known experimental data are not sufficient for exact quantitative description of the molecular mechanism of the action of an electric field on photoconversion of bacteriorhodopsin. However, it seems reasonable to suggest that these effects are due to electric field-induced conformational changes in chromophore/protein complexes within the bacteriorhodopsin molecule. Such changes have recently been observed [17]. The authors have

found that electric field causes structural changes in the bacteriorhodopsin molecule involving alteration of two types of proton binding site and pK shifts in opposite directions to the light-induced pK shifts [17].

It worth noting that samples studied in our experiments were not specially treated to obtain a preferential orientation of purple membranes. As has been mentioned earlier [8], these samples presumably contain two approximately equal fractions of purple membrane sheets, both being parallel to the electrode planes but having opposite orientation with each other. Therefore, we must expect that the electric field either affects one of these fractions or causes the same effects in both. Experiments with samples in which purple membranes are preferentially oriented in one direction may help us to understand the mechanism of the action of electric field on bacteriorhodopsin photoconversion. These experiments are now in progress.

It should be stressed that the observation of the above-mentioned field-dependent phenomena is not restricted to conditions realized in our model only. In recent studies with bacteriorhodopsin incorporated into liposomal membranes, a dependence of the phototransient M behaviour upon the electric component of the transmembrane electrochemical gradient, which is in agreement with our data, was observed [18]. Evidence for the occurrence of events of the kind discussed here also comes from studies reported in Refs. 15 and 19, in which the yield of formation of the intermediate M and its decay time in the whole cells of *H. halobium* were found to be sensitive to uncouplers.

Thus, it seems likely that the phenomena observed in our experiments may take place in the purple membranes in vivo, working as a feedback control mechanism of the bacteriorhodopsin photocycle.

We hope that our model has some experimental advantages as it allows variation of sample temperature, humidity, etc., which may be useful in further investigations of molecular mechanisms of bacteriorhodopsin functioning.

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