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BACTERIORHODOPSIN (BR_{570}) BATHOCHROMIC BAND SHIFT IN AN EXTERNAL ELECTRIC FIELD

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Summary

In dry films of bacteriorhodopsin-containing purple membranes from *Halobacterium halobium* the external electric field (10^4—10^5 V · cm^{-1}) induces the appearance of a product spectrally close to the initial intermediate of bacteriorhodopsin (BR) photochromic cycle (batho form, K). This result and also preliminary data of the electret-thermal analysis of the preparations suggest that the dielectric polarization in chromophore-protein-lipid complexes might be an essential step of the primary stabilization of light energy in photo-bioenergetic processes.

The application of new physical methods [1,2] makes it possible to observe the effects of electric polarization in photosynthetic membranes and pigment-protein complexes [3,4]. Polarization manifests itself through the electrochromism of pigments and reversible conformational changes of macromolecular components. The latter are believed to be involved in the stabilization of photochemically separated charges.

The electrogenic stage in the photocycle of bacteriorhodopsin (BR) was found to exist and its characteristics were studied by Skulachev et al. [5].

It thus seemed reasonable to investigate the photoenergetic reactions of BR by the methods used for studies of polarization properties of photosynthetic preparations.

Experiments were done with the fraction of purple membranes from *Halobacterium halobium*, R_1, provided by the Bioenergetics Department of A.N. Belozersky Laboratory at Moscow State University. Thin layers of purple membranes were prepared by drying concentrated water suspension (5—7 mg

Abbreviation: BR, bacteriorhodopsin.

BR per 1 ml, pH 7.2) on a glass slide covered with a translucent aluminum film. Drying was carried out at room temperature and at 10^{-1} torr pressure. The second translucent aluminum electrode was evaporated in vacuum on the upper surface of the dry preparation. After that the samples were equilibrated with room humidity (65—75%) for 24 h. Sample thickness as calculated from their electric capacity was $2—8 \cdot 10^{-6}$ m. Total absorbance of the sample was about 2 absorbance units. The absorbance of the purple membrane film itself at λ = 570 nm was 2 units. Stabilized d.c. voltage applied to the aluminum electrodes varied up to 1000 V. The main results were obtained with a field strength of approx. 10^5 V·cm^{-1}, the value characteristic of the transmembrane potential in vivo. Field-induced absorbance changes were measured by a single-beam differential spectrophotometer. For details see Refs. 2 and 3. Photochromic conversions were monitored in parallel at room and low (80 K) temperatures. Samples were activated by continuous light (λ = 400—600 nm) or single flash light (40 ms in duration, $\lambda > 520$ nm).

Light-adapted BR_{570} in partly dehydrated purple membrane films seems to be capable of undergoing the whole photochromic cycle [6,7]. At room temperature the formation of the photoproduct BR_{412} is observed in our preparations (Fig.1a). In dried films, however, kinetics differ somewhat from

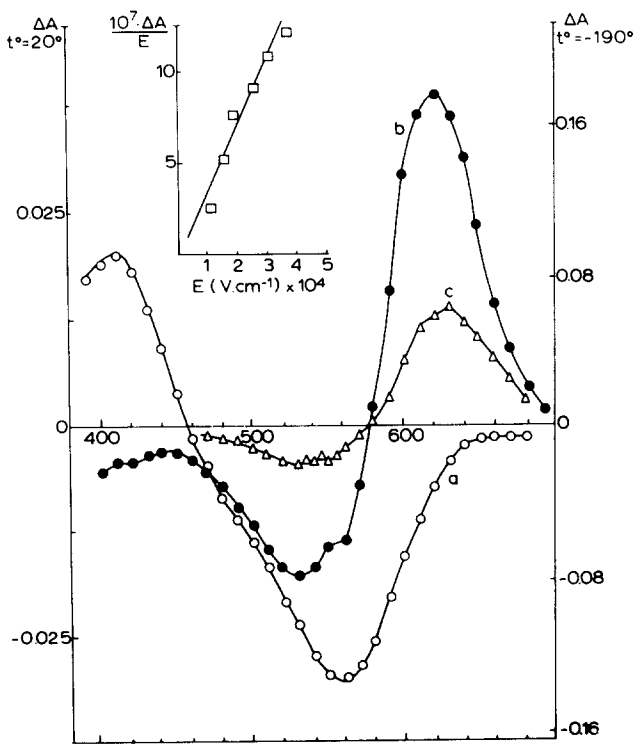

Fig.1. The spectra of the absorbance changes in dry films of purple membranes from *H. halobium*.
a. Absorbance changes induced by actinic light with 400—600 nm at 20°C; b. The same at t° = —190°C; c. Field-induced absorbance changes at t° = 20°C, $E = 2 \cdot 10^4$ V·cm^{-1}. Inset: Field-strength dependence of + ΔA_{630} for a layer.

those in the liquid phase. The half-time of BR_{412} decay ($t_{1/2}$) is 2—5 ms in aqueous suspension and 45—50 ms in dried films which is in good agreement with the data of Korenstein and Hess [8]. The measurements of linear dichroism with photoselective excitation indicate that in our films the dichroic ratio $\Delta A_{\parallel}/\Delta A_{\perp}$ at 570 and 410 nm is near 2.2. This implies that BR transition dipole moments are strongly oriented in the film. It seems natural, taking into account the geometry of the purple membrane and spatial arrangement of retinal chromophores in BR molecules. A transient dichroism of the decay kinetics of BR_{412} in dry films was constant within milliseconds thus showing that BR is immobilized in the purple membrane under these conditions.

Electric field ($E = 5 \cdot 10^4\ V \cdot cm^{-1}$) applied to the films in the dark at 20°C causes reversible absorbance changes of BR_{570} (Fig.1c). A bleaching in spectral range of 430—575 nm and an appearance of a broad intensive band centred at $\lambda \leqslant 630$ nm are observed. The kinetics of rise and decay of the field-induced signal are not monophasic. The initial fast phases ($\tau_r < 1$ s; $\tau_d < 1$ s) are followed by slow changes (τ_r = 60—80 s; τ_d = 80—100 s) reaching the steady-state level. The amplitude of the signal is quadratic in field strength (Fig.1, inset). The field-induced differential spectrum indicates the formation of an intermediate that corresponds spectrally to a so called batho-BR(K), the first photoproduct in BR_{570} cycle, which has been observed at room [9] and low temperatures [6] and with high-time resolution flash photolysis [10]. The spectrum of the photo-induced absorbance changes measured in our samples at 80 K (Fig.1b) is similar to that reported previously and close to the observed field-induced spectrum (Fig.1c). The photoinduced batho-BR (K) is stable at low temperatures but can be reconverted to the initial BR_{570} by light with λ = 630 nm [6]. This was also observed in our experiments. We have also studied the simultaneous effect of electric field and red light ($630 < \lambda < 1100$ nm). The latter was observed to cause reversible decrease of the field-induced signal though red light alone had no effect. In some separate series of measurements the effect manifested at room temperature. However, in other experiments with preparations isolated from different batches of cells (though made by the same procedure) it occurred only after freezing the sample in the field down to —120°C. The reason of such variance is unclear at present.

Nevertheless the electric field action seems to be similar to that of blue-green irradiation, which induces the photochromic reactions of BR_{570}.

It is probable that the electric field may affect the electron density in the BR_{570} molecule and the purple membrane resulting in a red shift of BR absorption spectrum in a way it occurs under actinic light exposure. The fact that the electrically-induced red shift is large and slow kinetic components are present suggests that the polarization is occurring in the purple membrane. One can expect this, because the retinal chromophore is located in the protein-lipid matrix which can easily be polarized by an external electric exposure. Due to this some effective reactive field may arise in the vicinity of retinal.

The preliminary electret-thermal analysis of the preparations under study gives support to this view. In fact, samples frozen to 150 K in the presence of electric field ($10^4\ V \cdot cm^{-1}$) and then gradually heated, after the removal

of the field, showed an electric current of $10^{-9} - 10^{-8}$ A flowing in the external circuit (maximum observed at 260 K). This current of thermodepolarization is a result of the relaxation of different charged groups following the changes in their positions induced by the electric field.

We do not try at this stage of our investigation to make a conclusion about the identity of the photo- and field-induced batho-BRs. BR is supposed to pump protons against pH gradients in the range of 3—12 [10], at the expense of the light energy absorbed. If so at the initial step of the photoprocess when the bathochromic BR shift occurs, at least 1 eV of the absorbed light quantum energy should be stored taking into account concomitant energy losses. Meanwhile, the field-induced bathochromic shift observed occurs in a field of not more than 0.1 eV. The estimation is made assuming the homogeneity of the field $5 \cdot 10^4$ V·cm^{-1} applied across a dry film prepared from 0.005-μm thick membrane fragments. However, one cannot exclude the possibility of local field concentration on microinhomogeneities [11].

The observed effect gives some clue to an eventual understanding of the physical nature of the batho-BR intermediate. The formation of the batho-intermediate in the photocycle of BR_{570} might be closely related to the redistribution of the electron density in the molecule with simultaneous polarization in its vicinity during the lifetime of the chromophore excitation. The polarization may include the shift of the protons near the chromophore group as the first step in their translocation.

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