Vol. 85, No. 1, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 14, 1978 Pages 383-390

PHOTOELECTRIC ACTIVITY OF DRIED, ORIENTED LAYERS OF PURPLE MEMBRANE FROM HALOBACTERIUM HALOBIUM

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Received September 15,1978

SUMMARY

Dried layers of purple membranes containing bacteriorhodopsin oriented by an electric field during drying were investigated by photoelectric and spectroscopic methods. There is evidence to show that the photochemical cycle of bacteriorhodopsin in a low hydration state is intact not only spectroscopically but also electronically in these layers. It has been observed that due to the blue-light excitation of the bleached form an intermediate absorbing at 520 nm is enriched. Thus this form seems to occur in the inhibitory pathway of bacteriorhodopsin.

INTRODUCTION

Bacteriorhodopsin /BR/ from Halobacterium halobium is a small protein having the unique properties of a light-driven proton pump (1-5). Upon illumination the protein undergoes a series of conformational changes, which lead to proton transfer through the cell membrane. During the photocycle the molecule has several intermediates with different life-times and absorption characteristics (4), two of which are relatively stable: the protonated ground state /BR_{570}/, and the deprotonated bleached form /M_{412}/ (2,4). The proton uptake can be accelerated by blue-light excitation of the bleached form (2,6,7).

Photopotential measurements on a bimolecular lipid membrane-- BR system indicate that blue light additional to green-light excitation causes a decrease in the photopotential induced by the green light (8,9). This inhibition effect has been interpreted as a shunt in the proton pump caused by the blue-light excitation of the M_{412} form (10-12).

0006-291X/78/0851-0383$01.00/0

The protein-pigment complex located in the purple membrane /PM/ of H. halobium is very stable under extreme conditions (13-18). These adventages of BR have been used to construct a new, simple model system: dried layers of PM fragments oriented by a electric field. The stability /for months/ and the high photoresponse /up to 500 mV/ of this system make it suitable for parallel photoelectric and spectroscopic investigations.

MATERIALS AND METHODS

The PM fragments used in the investigations were obtained by a standard procedure from H. halobium strain NRL R_1M_1 (19). One drop of a dense PM suspension in water was put on a glass plate having a conducting surface. For the orientation of the PM fragment a metal electrode was placed above the drop, and a high DC voltage was applied between the conducting surface of the glass plate and the metal electrode during drying. The electric field strength was varied up to 1000 V/cm, where the orientation /controlled by measuring the photoresponse/ was the highest. Illumination of the suspension during the drying promoted the orientation of the PM fragments.

The photopotential of the dried PM layers was measured with a vibrating plate contact-potential measuring equipment (20) /home-made, sensitivity 0.5 mV, response time 0.1 s/. The scheme of the set-up is outlined in Fig. 1. The dark surface potential of the layers was always compensated.

The humidity of the layers was kept constant with saturated salt solutions (21). The system was left to equilibrate for 24 hours before measurements. The data presented here were obtained with LiCl solution, which gives 12% relative humidity.

Difference absorption spectra after excitation of the layer were taken point by point at 25 nm intervals with a Perkin-Elmer Fluorescence Spectrophotometer Model MPF-3. After a two-minute excitation of the PM layers, the exciting beam was switched off, the measuring beam was switched on and the change of absorption of the layers was measured as a function of time. A constant relative humidity was also maintained in the photometer chamber by the method mentined above. All the results presented here were obtained on the same sample; similar results were obtained with a number of other samples.

RESULTS AND DISCUSSIONS

Simple air-drying of the PM suspension causes a parallel positioning of the membrane sheets on a glass surface (22). However, it is not a vectorial orientation and, as the BR has a vectorial proton pump, the vectorial orientation is the preliminary condition to get a photoresponse on PM layers. An

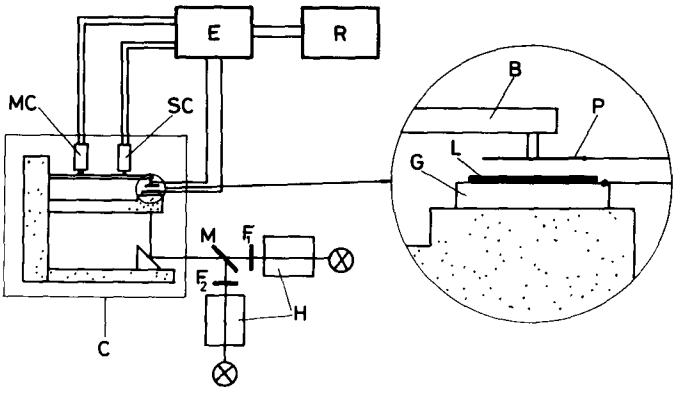


Fig. 1. Schematic diagram of surface potential measuring system used for photopotential measurements. E: electrometer, MC: moving coil, SC: sensory coil, R: recorder, C: closed chamber, B: vibrating bar, P: platinum plate, L: PM layer, G: glass plate with conducting surface, M: semitransparent mirror, H: heat filters, F_1: green band pass filter /T_{max}: 540 nm/, F_2: blue band pass filter /T_{max}: 405 nm/. Light sources: 200 W mercury arc lamps /HBO 200, Carl-Zeiss, Jena/.

electric field orients the PM fragments vectorially (23,24) and in the above method the electric field was combined with drying of the PM suspension. In this way vectorially-oriented, dried PM layers were obtained.

The absorption maximum of PM layers was around 560 nm /O.D. 0.4-0.8/. The absorption maximum is in line with the results of ref. (17), as in a low hydration state the BR is in dark-adapted form (17,25). The sign of the photopotential could be influenced by altering the direction of the applied electric field during drying, but the photopotential was much higher if the upper electrode was positive. Simple air-drying without an electric field causes a small vectorial orientation /small photopotential/, indicating a preferential orientation of the PM on the glass plate /probably induced by the hydrophilic effect of the glass surface/. The magnitude of the photopotential on PM layers depended on the electric field strength, the light intensity applied during

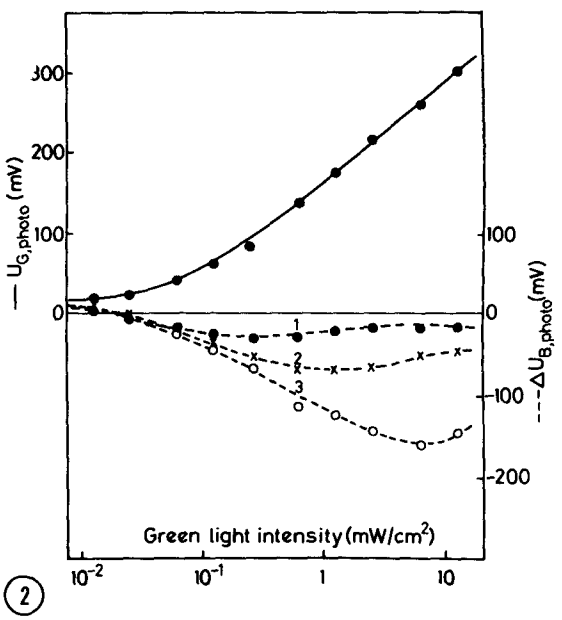
drying, the concentration of the suspension and the rate of drying. The difference observed in the magnitudes of photopotentials of different samples indicate that not all the relevant parameters in sample preparation have been brought under full control.

In layers made as described above the photopotential could be reproduced for more than half a year. Samples preserve their orientation in wet air as well /the highest relative humidity was 75%, where the photopotential was some mV/. The highest photoresponse was measured on the dried sample /over P_2O_5/, 500 mV at a green-light intensity of 18.5 mW/cm^2, which decreased when the sample was wetted.

The main intermediates of the photochemical cycle of BR are the same in dried PM layers as in aqueous suspensions of PM (16-18,26). Upon excitation of the layers by intense light, an increase in absorption occurs around 412 nm, which indicates the deprotonation of BR. The deprotonation followed by charge separation causes a change in the surface potential of the PM layers. This parameter was measured and a typical curve of photopotential dependence upon the exciting green-light intensity is shown in Fig. 2.

At high green-light intensities the photopotential decreases if an additional blue light was used simultaneously to illuminate the layers /dotted lines in Fig. 2/. The transient photoelectric activity of PM multilayers has been proved by flash excitation at relative humidities of 55-70% (26). The results presented here prove that the photochemical cycle of BR in these layers is totally intact electronically /regarding the charge displacement/ in low hydration state too.

The time-dependence of photopotentials at two light intensities are shown in Fig. 3. When the green light is swiched off the photopotential decreases exponentially with time. The decay



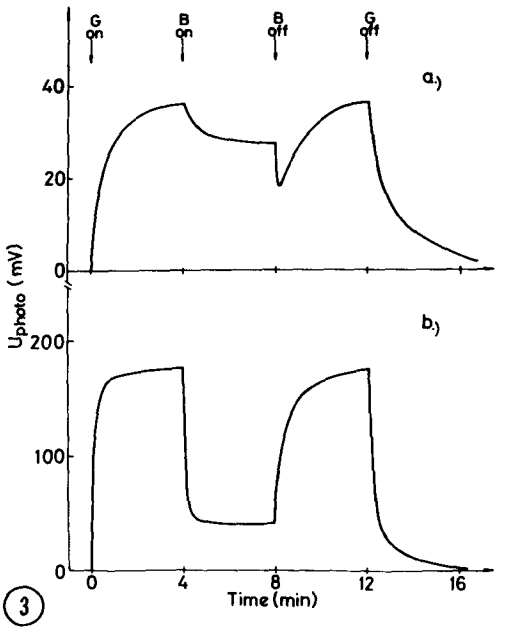


Fig. 2. Dependence of the photopotential on the green-light intensity varied with neutral glass filters /continuous curve/. The dotted lines indicate the changes in the photopotential induced by green excitation due to the blue-light excitation. The blue light intensities were: /1/ 0.7 mW/cm^2, /2/ 2.6 mW/cm^2 and /3/ 13.2 mW/cm^2.

Fig. 3. The time-dependence of the increase and decrease of photopotential following changes in illumination. G and B indicate green and blue light, respectively. The green-light intensities were: /a/ 9.2 x 10^{-2} mW/cm^2 and /b/ 1.8 mW/ cm^2. The blue-light intensity was 13.2 mW/cm^2 in both cases.

can be separated into three phases with life-times: τ_1<10 s, $\tau_2 \simeq 60$ s and $\tau_3 \simeq 100$ s. Practically the same values were obtained from spectroscopic measurements for the decrease of the M_{412} concentration. Accordingly the photopotential, which is caused by molecular charge displacement, as in ref. (26), indicates the presence of M_{412} intermediates.

Difference absorption spectra of PM layers are presented in Fig. 4. The effect of green bleaching light is obvious: it transforms BR_{570} /through other intermediates/ to M_{412} /curve 1 in Fig. 4/. There is no shoulder in the spectrum near 520 nm, i.e.

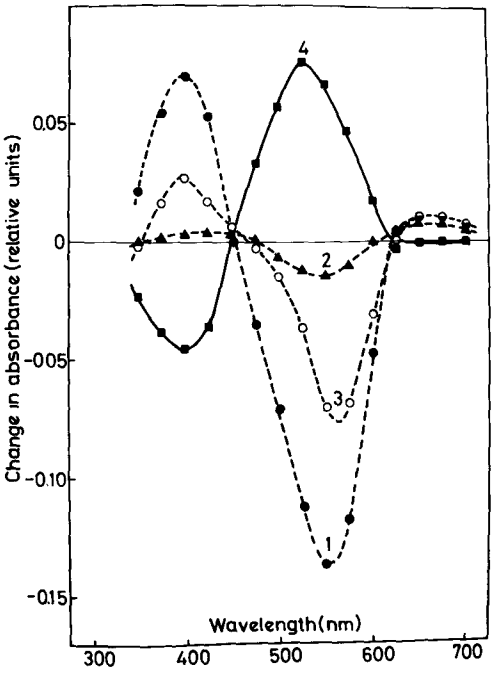


Fig. 4. Difference absorption spectra 3.6 s after excitation: curve 1: change after green-light excitation; curve 2: change caused by blue-light excitation; curve 3: change after green--plus-blue-light excitation; curve 4: difference in absorption of the bleached layer caused by blue light /the difference between curve 3 and 1/.

N_{520} has not developed at that time. On the other hand, a broad peak can be seen around 640 nm, indicating the appearance of O_{640} as shown in ref. (26). The absence of N_{520} is in contrast with the results presented in ref. (26), as O_{640} should follow N_{520}. The interpretation of this may be that N_{520} might not follow M_{412} in the all-trans cycle of BR, but /in accordance with other preliminary observations too/ appears in the 13-cis cycle of BR.

Blue-light excitation causes a similar but smaller effect to green-light excitation /curve 2 in Fig. 4/. However, the common effect of green-plus-blue light /curve 3/ as compared to the single green absorption change, results in an absorption peak

around 520 nm, i.e. the concentration of N_{520} is enriched by the blue-light excitation of M_{412}. N_{520} could also be observed in the absorption spectra of the PM suspension when it was excited by blue light after bleaching (7).

According to the model presented in refs. (10-12), the process of inhibition is that modification of the regular photochemical cycle of BR occurs due to the blue-light excitation of M_{412}. As blue light accelerates the dark reaction (2,6,7), the transmitted proton does not reach the extracellular surface of the PM, so the molecule can regain it, i.e. the proton pump is shunted. The results presented here show that the model of inhibition should be connected with the intermediate N_{520}, through which the inhibition pathway must be closed.

The results suggest that the intermediate N_{520} might work in the 13-cis cycle, and as the M_{412} works in the all-trans cycle of BR, the blue-light excitation of M_{412} would make a close connection between the two independent cycles of BR isomers.

ACKNOWLEDGEMENTS

The author is grateful to Dr.Zs. Dancsházy for preparation of the purple membrane fragments and to Prof.L. Keszthelyi for helpful discussions. Thanks are also due to Mr.K. Frank and Mrs.E. Nagy for their technical assistance.

This work was supported by the Hungarian Academy of Sciences, UNESCO/UNDP Grant to Hungary No. HUN/71/506/B/01/13, and by the National Committee of Technical Development /OMFB/.

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