BACTERIORHODOPSIN: A MOLECULAR PHOTOELECTRIC REGULATOR

Quenching of photovoltaic effect of bimolecular lipid membranes containing bacteriorhodopsin by blue light

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1. Introduction

Most studies on the function of bacteriorhodopsin (BR), the purple protein–pigment complex forming the molecular basis of light-energy conversion for a new type of photophosphorylation, have been performed on purified membrane fragments, employing biochemical and photochemical methods [1–8]. BR works as a light-driven vectorial proton-pump mediated by its photochemical cycle: a proton is ejected by the illuminated BR into the extracellular medium while a proton is taken up, during the dark reformation of BR, from the intracellular space [2,9]. The reconversion of the bleached BR into purple-complex can be accelerated by 412 nm illumination, from which the acceleration of turnover number of the proton-pump was postulated [1,10–13]. That BR behaves as a molecular photoelectric generator was also demonstrated by using proteoliposomes and thin lipid membranes [14–16]. The reconstruction of certain functions of the *Halobacterium halobium* envelope in bimolecular lipid membranes (BLM) containing BR in an oriented fashion has quite recently been achieved by the present authors [17–19]. Although the action spectrum followed the absorption spectrum of BR, the steady-state photoelectric response observed could not be correlated to any elementary step in the photochemical cycle, but rather reflected the overall process. It was noticed that the photovoltaic response of a BR-BLM under white-light illumination was less than expected on the basis of monochromatic illumination with the 500 nm and 554 nm components of the same white light, respectively [17,19].

The present report is concerned with this unusual phenomenon. It is shown that the bleached BR (412 form, which we suggest to call leuco-BR = LBR) under blue-light excitation operates as a proton-sink (or partly as a light-driven reverse proton pump?) for protons produced and pumped through the membrane by the photo-excited BR. This effect is considered to cause the quenching of the photopotential generated by BR irradiated in its green absorption band and thereby, the reduced photoelectric efficiency of white-light. The phenomenon seems to be of fundamental importance in the cellular regulatory mechanism of Halobacteria.

2. Materials and methods

The BR used was obtained by standard procedures of extraction and purification from *H. halobium* strain NRL R_1M_1 [20]. The BLM serving as host for the purple membrane fragments was made from a modified membrane-forming solution under the same conditions as described earlier [18,19]. The dark resistance and capacitance of the BR–BLMs used were typically about $2 \cdot 10^8$ $\Omega.cm^2$ and 0.4 $\mu F.cm^{-2}$, respectively.

The optical arrangement used is given in fig.1. The photoelectric response was measured with a Keithley Type 603 differential electrometer amplifier connected to a Kipp and Zonen Type BD-5 fast micrograph. The

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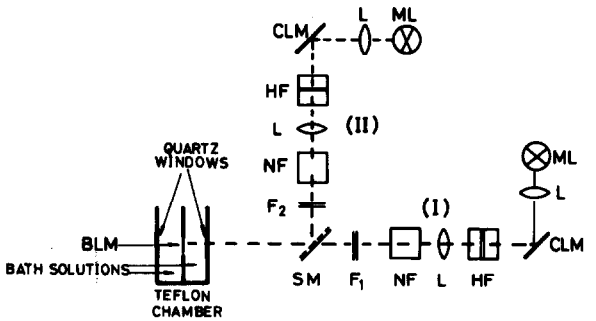

Fig.1. Optical arrangement used in the double-excitation experiments. ML high-pressure mercury vapor lamp, L glass lens, HF glass heat-filter in water jacket, CLM cold-light mirror, NF neutral density-filter set, F broad-band glass filter (F_1 VG 4, F_2 BG 12), SM semi-transparent mirror.

BR-BLM was illuminated either separately or simultaneously through appropriate heat filters by two 200 W high-pressure mercury vapor lamps fed by stabilized power supplies. Exciting light beams with different spectral compositions were selected by broad-band glass filters BG 12 and VG 4 (Carl-Zeiss, Jena), respectively. The filters were chosen so that the spectral distributions of the transmitted light beams match the main absorption bands of BR and/or LBR, respectively (fig.2). The direct excitation of the protein moiety of BR in the ultraviolet region was avoided by using glass optics. All the experiments were carried out at room temperature in a darkened room.

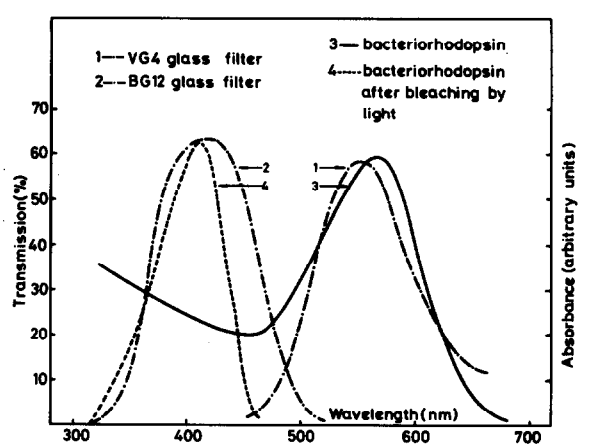

Fig.2. Absorption spectra of BR and LBR, and transmission spectra of the broad-band glass filters used (the absorption spectrum of the bleached BR is taken from ref. [1]).

3. Results and discussion

BR-BLMs exhibited a fast-rising, large amplitude photovoltaic effect (10–25 mV) if illuminated with either broad-band blue (340 nm $<\lambda<$ 490 nm) or green (490 nm $<\lambda<$ 640 nm) light, similarly to the white-light experiments [17–19]. The sign of the photoresponse suggests that possibly positively charged species, such as protons, crossed the membrane. Upon white-light illumination the photovoltaic response showed a sigmoid-like dependence on the logarithm of incident light-energy density [17,19]. It was found that, above a certain illumination level, the contribution of the 554 nm component of white-light to the photoresponse was systematically less than the photopotential generated alone by monochromatic 554 nm light beam with the same energy density. A closer inspection of the photochemical cycle of BR called attention to the possible involvement and role of LBR in controlling the photo-induced membrane potential and proton-gradient.

To prove this hypothesis the following experiments were carried out: A BR-BLM was illuminated in its green absorption band through a VG 4 filter. This illumination allowed a sufficiently high excitation level with green-light to bring the photoelectric response near to saturation. As seen in fig.3a, a further increase in the intensity of the green-light through light-pathway II (fig.1) did not lead to any increment in the photoelectric steady-state. If, instead of this additional green-light, blue-light matching the absorption spectrum of LBR hit the BR-BLM illuminated by high-intensity green-light, the steady-state level of the photovoltaic signal dropped (fig.3a). The removal of the superimposed blue exciting-light resulted in the restoration of the original steady-state level due to green-light, demonstrating the complete reversibility of this 'negative photoeffect' or quenching effect of blue-light. If the BR-BLM was first illuminated by blue-light and afterwards simultaneously exposed to blue- and green-light, the photovoltaic steady-state increased up to the level achieved by double excitation in the previous experiment (figs.3a and 3b). If now the blue component of the exciting light was removed, while the green one was left on, a further rise in the steady-state photosignal occurred (fig.3b). The dependence of the additional photoeffect due to the superimposed blue-

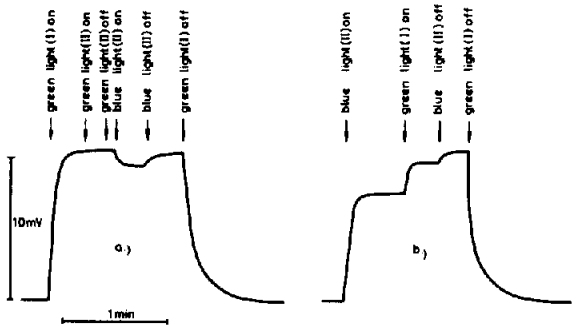

Fig.3. Time course of photoeffects of a BLM containing bacteriorhodopsin upon double excitation: (a) green-light as background illumination, superimposed green and blue-light illumination as indicated. (b) Blue-light as background illumination, superimposed green-light excitation as indicated. Incident light-energy density of green-light was 53 mW·cm^{-2}, that of blue-light 7 mW·cm^{-2} far from the saturation level for the LBR photoeffect.

light excitation on the intensity of the green 'background' illumination, obtained on another BR-BLM, is given in fig.4. It is clearly seen that the quenching of the green-light induced photoeffect, i.e., the superimposed photoresponse with opposite sign,

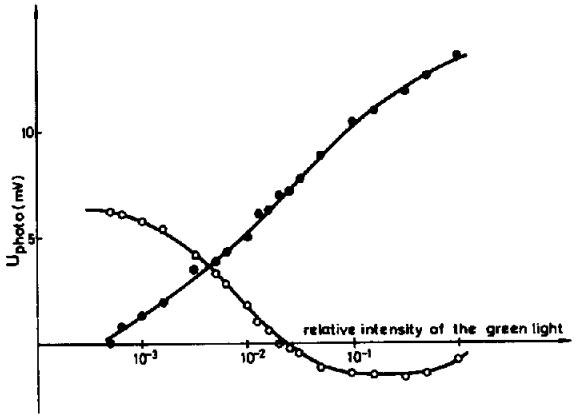

Fig.4. Dependence of blue-light induced additional photoeffect on the light-energy density of incident green-light (•——•) and the dependence of the green-light induced photoeffect on the light-energy density of incident green-light (o——o). The maximum incident energy-densities were the same as in fig.3. The blue-light starts controlling the green-light induced photovoltage at the incident energy-density 1.1 mW·cm^{-2}. Maximum number of incident photons: 10^{18} photons·cm^{-2}·s^{-1} for green-light, 10^{17} photons·cm^{-2}·s^{-1} for blue-light.

occurs when the intensity of the green light is high enough.

The interpretation of the above experimental findings is the following. According to the photochemical cycle of BR [1] the overall process of BR bleaching by light can be written in the form

$$h\nu + \mathrm{BR} \rightarrow \mathrm{BR}^* \rightarrow \mathrm{LBR} + \mathrm{H}^+ \quad \text{(pumped through the membrane)} \qquad (1)$$

($h\nu$ denotes light quantum, BR* is an electronically excited BR), while the following overall recombination process is enacted in the dark:

$$\mathrm{LBR} + \mathrm{H}^+ \rightarrow \mathrm{BR}. \qquad (2)$$

The present-day knowledge concerning the details of the dark reverse reaction does not tell us anything about the origin of the proton needed for re-protonation of LBR to BR, and all the possible pathways of reconversion of LBR into BR have not yet been cleared up [4–8,21]. On the basis of fig.3 and since BR incorporated into a BLM is photoelectrically active in its whole absorption spectrum above 375 nm [17–19], it is obvious that the inhibitory effect of blue-light cannot be accounted for by the excitation of BR in its short-wave region. Consequently, the quenching of the green-light induced photoeffect by blue-light must be connected with the only blue-light absorbing product of green-light excitation of BR, the LBR which absorbs light exactly in that spectrum region where quanta are transmitted by the BG 12 filter (fig.2). Thus, the experimental results presented in figs 3 and 4 coincide with Oesterhelt and Hess's observations obtained with consecutive green- and blue-light illuminations that the rebinding of protons by LBR, i.e., the overall reaction [2] can be accelerated by light if LBR is irradiated in its blue absorption band [1,10–13]. The light-induced reformation of BR can be visualized by the overall scheme

$$h\nu' + \mathrm{LBR} \rightarrow \mathrm{LBR}^* + \mathrm{H}^+ \rightarrow \mathrm{BR}. \qquad (3)$$

LBR first goes into an electronically excited state (LBR*), after which LBR* captures a proton, probably forming an excited complex which, following de-excitation, returns to ground state. The sign of the additional photoresponse attributed to the

blue-light excitation of LBR indicates that LBR* may:

(i) Pick up a proton from that compartment into which protons are pumped by green-light excited BR.

(ii) Inhibit the proton release into the aqueous phase.

In the latter and less probable case it is assumed that proton formed following the deprotonation of the Schiff-base of BR still resides within the protein moiety when blue-light excitation occurs. Both above processes suggest that the species LBR* has a fairly high proton-affinity, governing a proton movement opposite to the pump towards and into (but probably not through) the membrane.

The above phenomenon is a clear-cut evidence that LBR offers a pathway different from the dark process for the reformation of the purple-complex. There is no way at present to say anything about the details of this light-induced reconversion of BR. It appears rather certain that LBR and LBR* take up protons at opposite interfaces. It is clear that under high-intensity white-light illumination, besides overall processes (1) and (2), generating and maintaining protonic and electric gradients, the process given in the overall scheme (3) also takes place, which spoils and/or quenches in part the primary photoeffect. Whether LBR* is only a proton-sink or whether it can in part work as a reverse proton-pump is not known. The data presented favor the former mechanism, although theoretically, the latter cannot be completely excluded yet.

Beyond doubt, the blue-light excitation of LBR does speed up the regeneration of BR [1,10–13], but it does not accelerate the turnover number of the proton-pump. Consequently, concomitant illumination with green- and blue-light does not increase the attainable photoelectric power via speeding up the proton-pump even in model systems.

The remarkable deviations in the blue-regions of different (photophosphorylation, photosensory, photoelectric) action spectra [3,17–19,22,23] can be certainly attributed to the simultaneous excitation of BR and LBR. Our results further permit prediction of the intensity-dependences of the different photo-induced action spectra, especially in the blue spectral region.

It is easy to see that the combination of the overall processes (1), (2) and (3) represents a feed-back controlled system at molecular level. This mechanism may operate even in Halobacteria when the spectral composition of the illuminating light corresponds to the positions or at least to the overlap region of the absorption spectra of BR and LBR. In the molecular feed-back system process (3) plays the role of safety outlet valve or shunt. At low intensity of illumination the LBR concentration is also low therefore, the blue-light being mainly absorbed by BR drives the proton-pump and reaction (3) runs at a very low rate. At high excitation level the LBR concentration is also high, consequently the blue-light component is partially absorbed by LBR and the proton consuming process described by (3) is intensified, which reduces the light induced photopotential. Thus, the function of LBR in the proton-pump is two-fold: it makes complete the proton-pump on one hand, and moderates the influences of the intensity and spectral distribution of illuminating light on the light-induced electrochemical-gradient on the other hand.

The possible existence of such a regulatory mechanism in Halobacteria emerges from the fact that:

(i) The packing density of BR in *Halobacterium* membranes is certainly much higher than that in our model system.

(ii) Light-energy densities in the corresponding spectral regions of the solar spectrum are considerably higher than those used in these experiments (e.g., 20 $mW \cdot cm^{-2}$ for the solar spectrum and 0.25 $mW \cdot cm^{-2}$ for these studies in the green band of BR [24]).

Under these conditions BR may be involved in controlling the membrane potential, intracellular pH, forming a molecular moderator and regulator of vital importance and serving as a molecular basis of adaptiveness.

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References

[1] Oesterhelt, D. and Hess, B. (1973) Eur. J. Biochem. 37, 316–326.

[2] Oesterhelt, D. and Stoeckenius, W. (1973) Proc. Natl. Acad. Sci. USA 70, 2853–2857.
[3] Dannon, A. and Stoeckenius, W. (1974) Proc. Natl. Acad. Sci. USA 71, 1234–1238.
[4] Lozier, R. H., Bogomolni, R. A. and Stoeckenius, W. (1975) Biophys. J. 15, 955–962.
[5] Stoeckenius, W. and Lozier, R. H. (1974) J. Supramol. Struct. 2, 769–781.
[6] Dencher, N. and Wilms, M. (1975) Biophys. Struct. Mechanism 1, 259–271.
[7] Kung, M. C., de Vault, D., Hess, B. and Oesterhelt, D. (1975) Biophys. J. 15, 907–911.
[8] Chance, B., Porte, M., Hess, B. and Oesterhelt, D. (1975) Biophys. J. 15, 911–917.
[9] Racker, E. and Stoeckenius, W. (1973) Biochem. Biophys. Res. Commun. 55, 224–230.
[10] Oesterhelt, D., Hartmann, R., Fisher, U., Michel, H. and Schreckenbach, Th. (1975) Proc. 10th FEBS Meet. 40, 239–251.
[11] Hess, B. (1976) FEBS Lett. 64, 26–28.
[12] Oesterhelt, D., FEBS Lett. 64, 20–25.
[13] Hess, B., Kuschnitz, D. and Oesterhelt, D. (1976) 11th FEBS Meet. Abstr.
[14] Drachev, L. A., Jasaitis, A. A., Kaulen, A. D., Kondrashin, A. A., Liberman, E. A., Nemecek, I. B., Ostroumov, S. A., Semenov, A. Yu. and Skulachev, V. P. (1974) Nature 249, 321–324.
[15] Shieh, P. and Packer, L. (1976) Biochem. Biophys. Res. Commun. 71, 603–609.
[16] Herrmann, T. R. and Rayfield, G. W. (1976) Biochem. Biophys. Acta 443, 623–628.
[17] Dancsházy, Zs. and Karvaly, B. (1976) Int. Conf. Photochem. Storage and Conversion of Solar Energy, London/Ontario, Abstracts, p. F 5.
[18] Dancsházy, Zs. and Karvaly, B. (1976) FEBS Lett. 72, 136–138.
[19] Tien, H. T. and Karvaly, B. (1977) in: Photochemical Conversion and Storage of Solar Energy, (Boltón, J. ed) in press.
[20] Oesterhelt, D. and Stoeckenius, W. (1974) Methods Enzymol. 31, 667–678.
[21] Vsevolodov, N. N. and Kayushin, L. P. (1976) Studia Biophys. 59, 81–88.
[22] Drachev, L. A., Frolov, V. N., Kaulen, A. D., Liberman, L. A., Ostroumov, S. A., Plakunova, V. G., Semenov, A. Yu. and Skulachev, V. P. (1976) J. Biol. Chem. 251, 7059–7065.
[23] Dencher, N. (1974) in: Biochemistry in Sensory Functions (Jaenicke, L. ed) pp. 161–163, Springer Verlag, Heidelberg.
[24] Balzani, V., Moggi, L., Manfrin, M. F., Boletta, F. and Gleria, M. (1975) Science 189, 852–856.