Volume 295, number 1,2,3, 223–226 FEBS 10596 December 1991

The proton pump bacteriorhodopsin is a photoreceptor for signal transduction in *Halobacterium halobium*

Sergei I. Bibikov[1], Ruslan N. Grishanin[1], Wolfgang Marwan[2], Dieter Oesterhelt[2] and Vladimir P. Skulachev[1]

[1]*A.N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow 119899, USSR* and [2]*Max-Planck-Institut für Biochemie, 8033 Martinsried, Germany*

Received 19 November 1991

Halobacterium halobium swims by rotating its polarly inserted flagellar bundle. The cells are attracted by green-to-orange light which they can use for photophosphorylation but flee damaging blue or ultraviolet light. It is generally believed that this kind of 'colour vision' is achieved by the combined action of two photoreceptor proteins, sensory rhodopsins-I and -II, that switch in the light the rotational sense of the bundle and in consequence the swimming direction of a cell. By expressing the bacteriorhodopsin gene in a photoreceptor-negative background we have now demonstrated the existence of a proton-motive force sensor (protometer) and the function of bacteriorhodopsin as an additional photoreceptor covering the high intensity range. When the bacteriorhodopsin-generated proton-motive force drops caused by a sudden decrease in light intensity, the cells respond by reversing their swimming direction. This response does not occur when the proton-motive force is saturated by respiration or fermentation.

Bacteriorhodopsin; Proton-motive force sensor; Photophobic response

1. INTRODUCTION

When bacteriorhodopsin (BR) was described in 1971 as the first prokaryotic retinal protein, it was suggested to be a photoreceptor in *Halobacterium* [1]. Bacteriorhodopsin was further characterized as a light-driven proton pump that converts light into chemical energy by powering the ATP-synthase [2]. A photosensory function was postulated at the same time on the basis of action spectra for the photophobic response of swimming cells [3]. Meanwhile, the existence of three additional retinal proteins, each of which occurring in much lower copy number than bacteriorhodopsin, has been demonstrated. Besides BR, halorhodopsin as a light-driven anion pump was isolated and characterized [4,5]. In mutants lacking bacteriorhodopsin and halorhodopsin the two non-electrogenic sensory rhodopsins (SR-I and SR-II) have been shown to control the swimming behavior [6–9] so efficiently that dark-adapted cells are single photon counters [10]. The sensory rhodopsins and the flagellar motor are linked by a chemical signalling path without the involvement of a membrane potential change [11].

AT high irradiance, cyanide enhances the sensitivity of the cells to a sudden decrease of green light intensity. This observation in combination with some other evidence led to the suggestion that bacteriorhodopsin in co-operation with a so-called protometer (a molecular device, generating a repellent signal upon a drop in proton-motive force) mediates the response to high irradiance [12,13]. Because of the nearly congruent absorption spectra of bacteriorhodopsin and SR-I which by itself triggers the response to green light, it was difficult to check the validity of this proposal. The recent development of a transformation protocol for halobacteria allowed an experimental approach to prove or disprove signal transduction between proton pumping and the flagellar motor by expressing the bacterio-opsin gene in a photoreceptor-negative background.

Correspondence address: S.I. Bibikov, A.N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow 119899, USSR. Fax: (7) (089) 8578 3555.

2. RESULTS AND DISCUSSION

H. halobium strain Pho81 [14] lacks the four retinal proteins and therefore does not respond to light. These cells were transformed according to a protocol for *H. volcanii* [15] that was adopted for *H. halobium* [16] with plasmid constructs containing the wild-type bacterio-opsin gene (*bop*) or alternatively a point-mutated derivative (Asp-96 to Asn, BR D96N). In the mutated bacteriorhodopsin, reprotonation of the Schiff base by aspartate is impaired resulting in a drastically reduced turnover number and proton pumping activity [17–19]. The drug mevinolin, an inhibitor of the eukaryotic and archaebacterial HMG-CoA reductase was employed as selection marker. Transformants that formed purple colonies were used for the experiments.

For observation of motility and behavior, the cells were placed on a slide, covered with a slip and the specimen sealed with a paraffin mixture to avoid evaporation and diffusion of oxygen into the cell suspension. In the dark, the cells gradually became immotile, if not supplemented with arginine which serves as a fermentative energy source [20]. Immotile cells regained motility upon irradiation with light when bacteriorhodopsin was present (Fig. 1). The effectiveness of BR D96N was reduced compared to wild-type but was enhanced by addition of azide which functionally replaces Asp-96 as a proton donor during the catalytic cycle [21]. Hence, the ability of bacteriorhodopsin to reconstitute motility depends on its proton pumping activity. The experiments demonstrate that anaerobic, starved cells can swim with light or arginine as the only energy source.

When light-adapted cells containing bacteriorhodopsin were exposed to a step-down in irradiance, they switched the flagellar motor, whereas the bacteriorhodopsin-deficient control did not respond (Fig. 2). The average response time of 3 s is in the range of that found for the sensory rhodopsin-mediated photophobic response [9]. However, in contrast to cells containing the sensory rhodopsins, the blue light, like the green one, proved to be attractive for the transformant (Fig. 2).

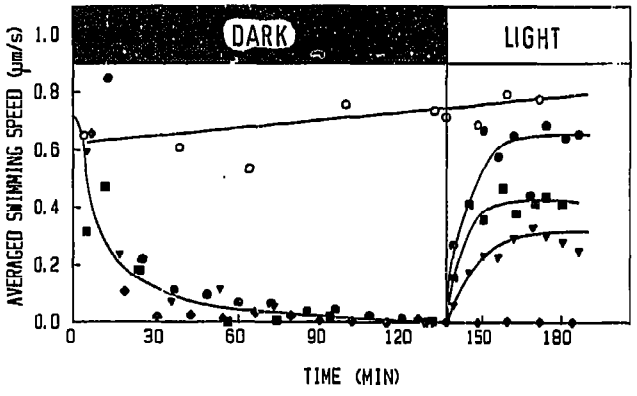

Fig. 1. Starvation and photokinesis of *H. halobium* strain Pho81 containing BR or BR D96N (aspartic acid in position 96 replaced by asparagine) as the only photoreceptors. The wild-type and D96N *bop* gene were isolated as 6.2 kbp *Pst*I fragments from *Halobacterium sp.* GRB genomic DNA and cloned [17] into pGEM4 (Metton, Promega, Biotech). This construct was fused with plasmid pH455 [15] via the common *Hin*dIII site to give the shuttle vectors p319WT and p319D96N [16,26]. The clones Pho81-B4 (with WT BR) and Pho81-D96N were used for the experiments. Cells were grown in the presence of mevinolin for 4 days under standard conditions [27] to the stationary phase and prepared after appropriate dilution with used growth medium (obtained by centrifugation of the culture) for microscopic observation. Motility was recorded in infrared light with an automated cell tracking system at 22°C as described [27]. Green light was generated by a xenon lamp and filtered through 2 cm 10% (w/v) $CuCl_2$ combined to an OG570 cut-off filter. The irradiance was 40 $W \cdot m^{-2}$. The swimming speed is plotted as a function of time for cells of (♦) Pho81, (●) Pho81B4, (○) Pho81B4 + 29 mM arginine, (▼) Pho81-D96N, (■) Pho81-D96N + 10 mM sodium azide. Each data point represents the average of the least 200 observed cells. The solid lines were drawn by eye.

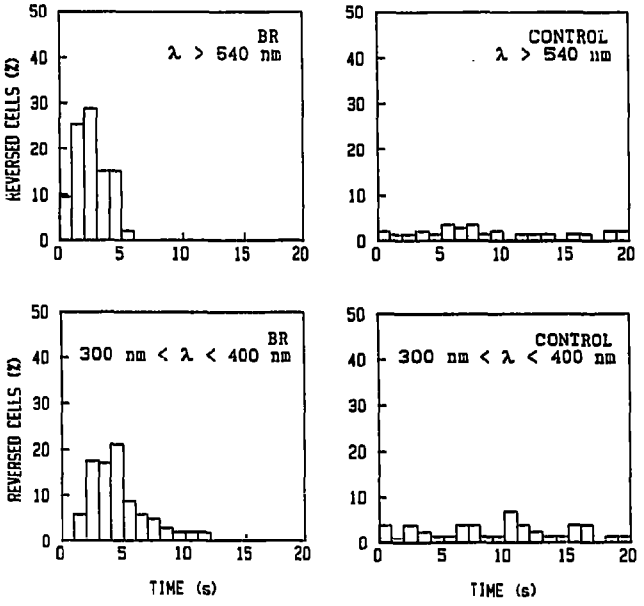

Fig. 2. Bacteriorhodopsin-mediated photophobic response to a step-down stimulus by orange or blue light. The cells of strains Pho81 (control) and Pho81B4 (BR-containing) were adapted to the background light of 300 $W \cdot m^{-2}$. At time $t = 0$ the irradiance was decreased to 40% by inserting a neutral density filter. In each experiment, 75 cells were evaluated by visual inspection. Orange light, >540 nm; blue light, 300–400 nm.

The percentage of cells responding to a step-down stimulus was a linear function of the step size (Fig. 3). The response was suppressed when the cells were supplemented with arginine which saturates the pmf (Fig. 1). On the other hand, the light sensitivity of the cells was increased when respiration was blocked by cyanide under semi-aerobic conditions thereby enhancing the contribution of bacteriorhodopsin to the pmf (not shown; see also [13,22]. Both results demonstrate that

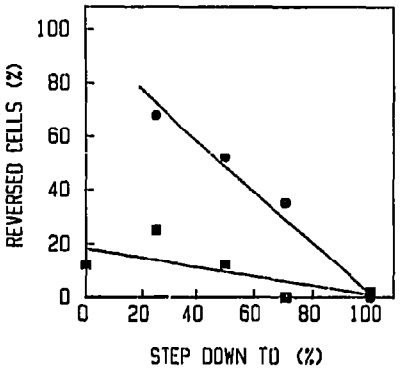

Fig. 3. Percentage of cells reversing as a function of the size of step-down in irradiance. The experiment was carried out in the absence (●) or precence (■) of 29 mM arginine. Step down was from 40 W/m^2 (equals 100%) down to the indicated values by insertion of neutral density filters. After each stimulus application, the cells were allowed to adapt to the original background irradiance for 60 s. For each data point, 30–100 cells were evaluated by automated cell tracking (see Fig. 1). The data were corrected for spontaneous reversals.

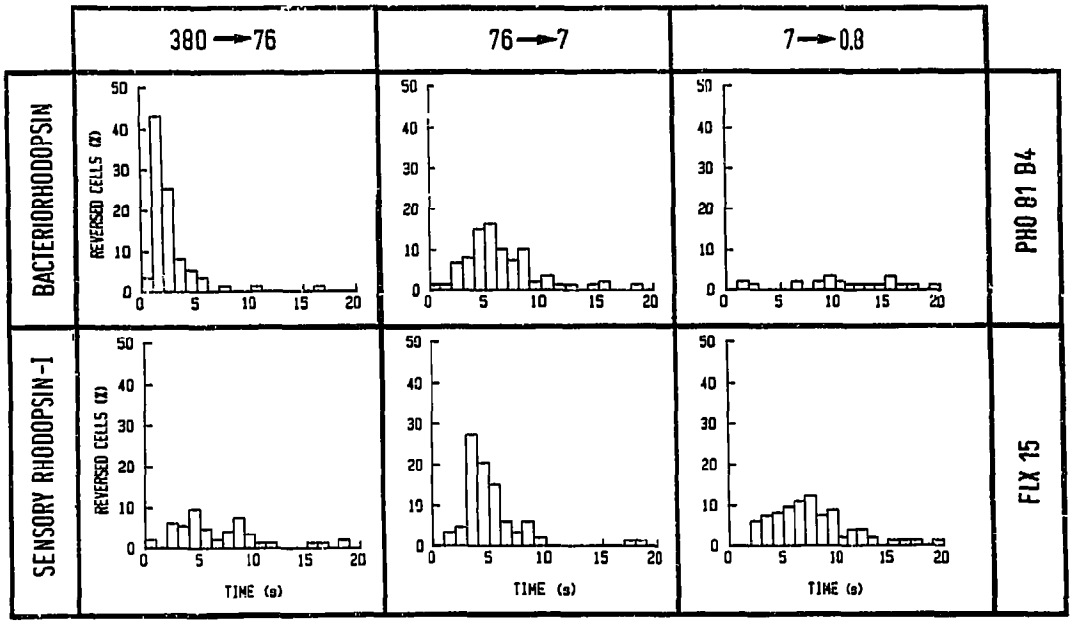

Fig. 4. Bacteriorhodopsin and sensory rhodopsin as high and low irradiance photoreceptors. The reaction patterns of cells containing exclusively BR (Pho81-B4) or the sensory rhodopsins (Flx15 [28]) are shown as a function of step-down stimuli at various irradiance levels (indicated at the top of the figure in 10^{18} hν · $m^{-2} \cdot s^{-1}$. Since monochromatic light of 565±10 nm was used, SR-I was selectively excited in Flx15. For each experiment 75 cells were evaluated.

the response is caused by a drop in proton-motive force rather than by a conformational state of bacteriorhodopsin. In addition a pmf sensor (protometer) has to be postulated that signals the flagellar motor switch [23].

Bacteriorhodopsin in combination with the pmf-sensor prevents phototrophically growing cells from entering the dark by reversing the swimming direction when the light intensity decreases. On the other hand, sensory rhodopsin-I also is known to reverse the swimming direction upon a decrease in green light irradiance [6] (maximal absorbance of BR at 570 nm compared to SR-I of 590 nm). The experiment shown in Fig. 4 excludes an unnecessary duplication of efforts by nature. While bacteriorhodopsin allows the cells to respond to a change in intensity at high irradiance, SR-I mediates the response in the flow irradiance range. Thus halobacteria developed a primitive but efficient adaptation mechanism to cover a large range of light intensities for response. Although not as sophisticated as the human eye in this respect, it seems an appreciable evolution in a 'primitive' archaebacterium.

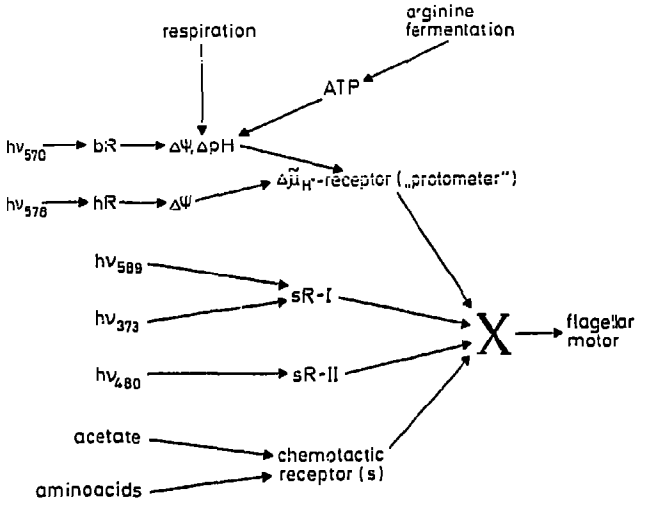

Fig. 5. Information flow in halobacterial signal processing. bR, bacteriorhodopsin; hR, halorhodopsin; sR, sensory rhodopsins; X, a component affecting the flagellar motor switch.

To check whether the flagellar motor is directly switched by a drop in pmf, we expressed bacteriorhodopsin in the mutant strain M415. Cells of this strain cannot switch unless somatically complemented with fumaric acid [24] which was shown to be released by light stimulation in wild-type cells [25]. Under conditions where light was the only energy source, the strain did not respond to step-down stimulation (not shown). This seems to exclude direct interaction of membrane potential and switch and at the same time suggests that the protometer might be linked to the motor by fumarate. Fig. 5 schematizes our current knowledge on the information flow in halobacterial signal processing. The sensory input emerging from chemoreceptors, photoreceptors, respiratory electron flow and light-driven ion

pumps is integrated by a branched signal chain to tune the behavior of a bacterial cell.

REFERENCES

[1] Oesterhelt, D. and Stoeckenius, W. (1971) Nature New Biol. 233, 149–154.
[2] Oesterhelt, D. and Stoeckenius, W. (1973) Proc. Natl. Acad. Sci. USA 70, 2853–2857.
[3] Hildebrand, E. and Dencher, N. (1975) Nature 257, 46–48.
[4] Steiner, M. and Oesterhelt, D. (1983) EMBO J. 2, 1379–1385.
[5] Schobert, B. and Lanyi, J.K. (1982) J. Biol. Chem. 257, 10306–10313.
[6] Spudich, J.L. and Bogomolni, R.A. (1984) Nature 312, 509–513.
[7] Takahashi, T., Tomioka, H., Kamo, N. and Kobatake, Y. (1985) FEMS Microbiol. Lett. 28, 161–164.
[8] Wolff, E.K., Bogomolni, R.A., Scherrer, P., Hess, B. and Stoeckenius, W. (1986) Proc. Natl. Acad. Sci. USA 83, 7272–7276.
[9] Marwan, W. and Oesterhelt, D. (1987) J. Mol. Biol. 195, 333–342.
[10] Marwan, W., Hegemann, P. and Oesterhelt, D. (1988) J. Mol. Biol. 199, 663–664.
[11] Oesterhelt, D. and Marwan, W. (1987) J. Bacteriol. 169, 3515–3520.
[12] Baryshev, V.A., Glagolev, A.N. and Skulachev, V.P. (1981) Nature 292, 338–340.
[13] Baryshev, V.A., Glagolev, A.N. and Skulachev, V.P. (1983) J. Gen. Microbiol. 129, 367–373.
[14] Sundberg, S.A., Bogomolni, R.A. and Spudich, J.L. (1985) J. Bacteriol. 164, 282–287.
[15] Cline, S.W., Schalkwyk, L.C. and Doolittle, W.F. (1989) J. Bacteriol. 171, 4987–4991.
[16] Ni, B., Chang, M., Duschl, A., Lanyi, J.K. and Needleman, R. (1990) Gene 90, 169–172.
[17] Soppa, J., Otomo, J., Straub, J., Tittor, J., Meeßen, S. and Oesterhelt, D. (1989) J. Biol. Chem. 264, 13049–13056.
[18] Butt, H.J., Fendler, K., Bamberg, E., Tittor, J. and Oesterhelt, D. (1989) EMBO J. 8, 1657–1663.
[19] Holz, M., Drachev, L.A., Mogi, T., Otto, H., Kaulen, A.D., Heyn, M.P., Skulachev, V.P. and Khorana, H.G. (1989) Proc. Natl. Acad. Sci. USA 86, 2167–2171.
[20] Hartmann, R., Sickinger, H.-D. and Oesterhelt, D. (1980) Proc. Natl. Acad. Sci. USA 77, 3821–3825.
[21] Tittor, J., Soell, C., Oesterhelt, D., Butt, H.-J. and Bamberg, E. (1989) EMBO J. 8, 3477–3482.
[22] Bibikov, S.I. and Skulachev, V.P. (1987) FEBS Lett. 243, 303–306.
[23] Glagolev, A.N. (1980) J. Theor. Biol. 82, 171–185.
[24] Marwan, W., Schäfer, W. and Oesterhelt, D. (1990) EMBO J. 9, 355–362.
[25] Marwan, W. and Oesterhelt, D. (1991) Naturwiss. 78, 127–129.
[26] Matuszak, A. (1990) Diploma thesis, Munich.
[27] Marwan, W. and Oesterhelt, D. (1990) J. Mol. Biol. 215, 277–285.
[28] Spudich, E.N. and Spudich, J.L. (1982) Proc. Natl. Acad. Sci. USA 79, 4308–4312.