Volume 243, number 2, 303–306 FEB 06739 January 1989

Mechanisms of phototaxis and aerotaxis in *Halobacterium halobium*

S.I. Bibikov and V.P. Skulachev

A.N. Belozersky Laboratory of Molecular Biology and Bioorganic Chemistry, Moscow State University, Moscow 119899, USSR

Received 29 November 1988

DCCD and cyanide, the H^+-ATPase and respiratory chain inhibitors, increase the sensitivity of *Halobacterium halobium* to the attractant effect of light. This phenomenon requires the presence of bacteriorhodopsin and occurs at high light intensities (about 100 W/m^2). Strains lacking both bacteriorhodopsin and halorhodopsin exhibit an increased sensitivity of aerotaxis. These observations are in good agreement with the hypothesis that $\Delta\tilde{\mu}H$ reception is one of the mechanisms responsible for phototaxis and aerotaxis in *H. halobium*.

Protonmotive force reception; Phototaxis; Aerotaxis; (*Halobacterium halobium*)

1. INTRODUCTION

Two photoreactions are properties of halobacterial cells: they are attracted by the long wavelength light and repelled by the blue light [1]. It was shown that different retinal pigments contribute to a phototactic response, namely, bacteriorhodopsin [2], halorhodopsin [3], sensory rhodopsin [4], and the recently discovered phoborhodopsin, or pigment P480 [5,6].

Bacteriorhodopsin was thought for some time to be the best candidate for the receptor of attractant light because the maximum of its absorption spectrum coincides with the maximum of the action spectrum for this light [1]. It was suggested earlier in our group that operation of bacteriorhodopsin as the light-driven H^+ pump results in a $\Delta\tilde{\mu}H$ increase which is sensed by a special $\Delta\tilde{\mu}H$ receptor ('protometer') [8,9]. The same $\Delta\tilde{\mu}H$ receptor may be involved in the halorhodopsin-mediated attractant effect of the light since the halorhodopsin-produced $\Delta\psi$ must, according to definition, be monitored by the protometer. Later, however, it was found that mutants lacking both bacteriorhodopsin and halorhodopsin respond to the attractant as well as to the repellent photostimuli [10]. Sensory and phoborhodopsins were assumed to be responsible for these effects.

We present evidence here that the bacteriorhodopsin-mediated $\Delta\tilde{\mu}H$-dependent mechanism of photoreception contributes to phototactic reactions only at high light intensities. As to the sensory and phoborhodopsins, they apparently serve as specialized receptors for dim light. We also suggest that the $\Delta\tilde{\mu}H$ reception is involved in aerotaxis in halobacteria.

2. MATERIALS AND METHODS

A list of strains is presented in table 1. Abbreviations of genetical indices are adopted partially from [12]. R_1M_X is a spontaneous mutant of R_1M_1 strain lacking bacteriorhodopsin. Its colonies appeared as bright yellow isolates among the purple-brown colonies of R_1M_1.

Cells were harvested, as in [9], in a peptone medium and were resuspended before experiments in a basal salt solution containing 4.3 M NaCl, 27 mM KCl, 81 mM $MgSO_4$ and 25 mM Mops, pH 7.0.

Microscopical observations were carried out using a Univar photomicroscope (Reichert) equipped with a thermostatted

Correspondence address: S.I. Bibikov, A.N. Belozersky Laboratory of Molecular Biology and Bioorganic Chemistry, Moscow State University, Moscow 119899, USSR

This paper is dedicated to the 60th birthday of Professor W. Nultsch

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

Table 1

Halobacterial strains

Strain	Genotype	Phenotype
R_1M_1	bop$^+$ hop$^\pm$ sop$^+$ rub$^-$ vac$^-$ ret$^+$	bR$^+$ hR$^\pm$ sR$^+$
R_1M_x	bop$^-$ hop$^\pm$ sop$^+$ rub$^-$ vac$^-$ ret$^-$	bR$^-$ hR$^\pm$ sR$^+$
ET1001	bop$^+$ hop$^+$ sop$^+$ rub$^+$ vac$^-$ ret$^+$	bR$^+$ hR$^+$ sR$^+$
F1x3	bop$^-$ hop$^-$ sop$^+$ rub$^+$ vac$^-$ ret$^+$	bR$^-$ hR$^-$ sR$^+$
L-07	bop$^-$ hop$^+$ sop$^+$ rub$^-$ vac$^-$ ret$^-$	bR$^-$ hR$^-$ sR$^-$

Genetical markers: bop, bacterio-opsin; hop, halo-opsin; sop, sensory opsin; rub, bacterioruberin; vac, gas vacuoles; ret, retinal

stage kept at 37°C. Observations were made under phase contrast at 1000 ×.

In a phototaxis study, photosensitivity was estimated (i) as the fraction of cells that reversed within 3 s after the onset of a light stimulus, i.e. a rapid decrease of intensity of background light ($\lambda > 540$ nm), or (ii) as the average time needed for reversion of 75 cells after the stimulus. A stimulating light beam was delivered through the microscope condenser, and its intensity was varied by neutral filters. The fraction of cells that reversed was corrected for spontaneous reversals.

In aerotaxis experiments, cells were kept under argon in a peptone medium for 30 min. Then 20 μl of cell suspensions were applied to a microscope slide and closed carefully with a top coverslip so as to trap air bubbles in a chamber. Prepared slides were hermetized with vaseline oil and observed at room temperature. Photographs were taken on an Opton photomicroscope.

3. RESULTS

The previous results obtained in our group have shown that DCCD and cyanide, the inhibitors of light-independent $\Delta\tilde{\mu}$H generators in *H. halobium* strongly increased the sensitivity of the cells to the attractant effect of the long wavelength light [8,9]. This fact was interpreted as indicating a $\Delta\tilde{\mu}$H-dependent mechanism of reception in the green light response. In the study described below we reproduced this experiment on different strains of *H. halobium*. As shown in table 2, the addition of DCCD and cyanide induces (i) an increase in the fraction of cells reversing faster than 3 s after the stimulus and (ii) a reduction of the average time of response when the initial intensity of the background light is high (near 100 W/m^2). This effect is pronounced in the R_1M_1 strain but is not expressed at all in the R_1M_X strain lacking bacteriorhodopsin. The halorhodopsin content was found to be negligible in this strain.

Addition of DCCD and cyanide at low background intensity causes cessation of motility of some halobacterial cells, others continue to move slowly, probably being energized by cyanide-insensitive respiration and/or by a potassium diffusion potential [13]. These cells exhibit a characteristic photoreaction but its sensitivity is the same with and without DCCD and cyanide.

Thus bacteriorhodopsin-dependent photoreception can be observed only at a high intensity of stimulating light. As to the dim light, it seems to be sensed by a more sensitive specialized photoreception system. Sensory rhodopsin is a good candidate for such a system.

It was shown earlier that different stimuli affecting the value of $\Delta\tilde{\mu}$H, such as uncouplers of oxidative phosphorylation, were sensed by halo-

Table 2

Influence of DCCD and sodium cyanide on phototactic sensitivity of *H. halobium* cells at different intensities of the light stimulus

Strain	Light intensity (W/m^2)	Reversion (%)		Average time of response (s)	
		Control	+ DCCD + NaCN	Control	+ DCCD + NaCN
R_1M_1	97	33	80	4.64 ± 0.34	3.00 ± 0.30
R_1M_1	14	24	26	5.94 ± 0.45	5.80 ± 0.57
R_1M_x	97	24	28	6.29 ± 0.93	6.39 ± 1.02

Cells were prepared as described in section 2. Light ($\lambda > 540$ nm) from the 200 W mercury lamp (HBO-101) was projected through the cut-off orange filter. 40% decrease in the light intensity by a neutral filter was used as a stimulus. Additions: 0.1 mM DCCD and 1 mM NaCN bacteria in a $\Delta\tilde{\mu}$H-dependent manner [9]. The studies on various species of bacteria demonstrated that aerotaxis and $\Delta\tilde{\mu}$H reception were controlled by similar, if not identical, mechanisms [14,15]. In order to test this observation on halobacteria we measured the aerotaxis sensitivity of different *H. halobium* strains.

As shown in fig.1, cells of Flx3 and L-07 strains of *H. halobium* readily form aerotactic bands around the small air bubbles on the microscope slide under green light illumination. It usually takes from 15 min to 1 h, depending on the state of the culture. On the other hand, cells of ET1001 and R_1M_1 strains with both bacteriorhodopsin and halorhodopsin accumulate near the air bubbles much slower under green background light. It takes from 1.5 to 3 h, and the aerotactic bands which they form are faint and diffuse. This observation is easily explained if we assume that the protometer is involved in the oxygen reception by *H. halobium*. By analogy with our former experiments, the presence of the active bacteriorhodopsin and halorhodopsin reduces the respiratory chain contribution to the generation of $\Delta\tilde{\mu}$H, thus decreasing the sensitivity of the $\Delta\tilde{\mu}$H receptor-mediated system of aerotaxis.

4. DISCUSSION

The recent progress made in the studies on photoreception in *H. halobium* is connected, first of all, with the finding of new, very sensitive, specialized rhodopsin-like pigments, such as sensory rhodopsin [4] or phoborhodopsin [6]. Several photons per cell seem to be sufficient to actuate a photoresponse mediated by these pigments. However, such a high sensivity may create some problems for sensing bright light which is of

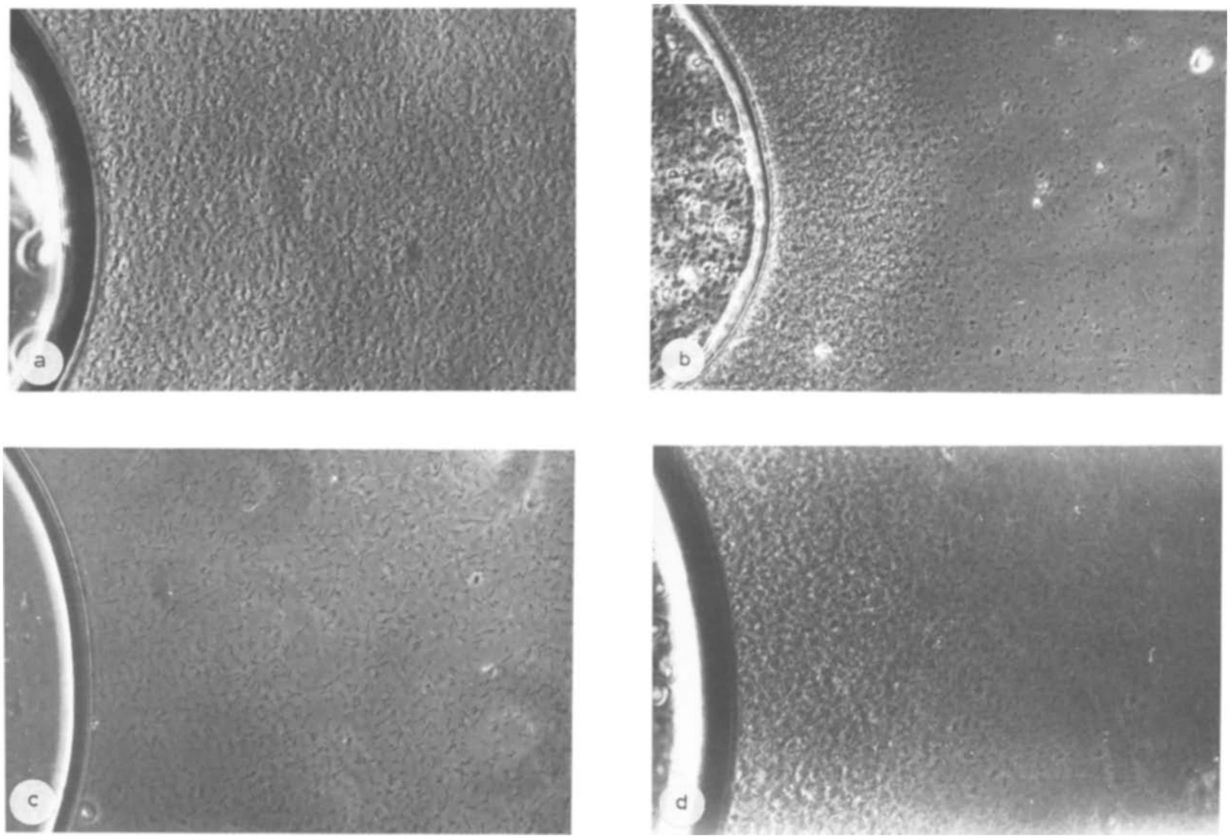

Fig.1. Aerotaxis of *H. halobium* cells. Microscope slides were prepared as described in section 2. 300 ×. (a) R_1M_1 strain, (b) F1x3 strain, (c) ET1001 strain, (d) L-07 strain (1 h after slide preparation).

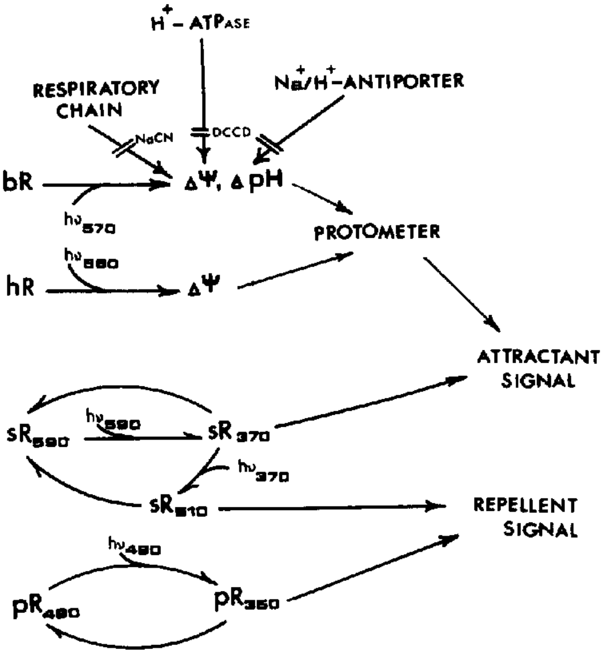

Fig.2. Interrelations of photo- and aerotaxis systems in *H. halobium*. bR, bacteriorhodopsin; hR, halorhodopsin; sR, sensory rhodopsin; pR, phoborhodopsin.

several orders of magnitude stronger than the minimal one still inducing a photoreaction of the cell. Here the bacteriorhodopsin-mediated $\Delta\bar{\mu}$H-dependent mechanism of reception may operate. Since halobacteria choose bright solar salterns for living, a sensing of high intensity light could be useful. On the other hand, situations where bacteriorhodopsin (and halorhodopsin) does not contribute to $\Delta\bar{\mu}$H generation and, consequently, is not involved in phototaxis, may also occur in the natural environment. (i) Bacterio- and halorhodopsin are absent from well aerated cultures at the early stages of development [16]. (ii) In the dark, $\Delta\bar{\mu}$H is generated by respiration and probably also by H^+-ATPase utilizing ATP produced by arginine fermentation [17]. (iii) Nearly 0.1% of all cells in the culture have insertions in the bacteriorhodopsin gene [12]. Mechanism of $\Delta\bar{\mu}$H reception under these conditions appears to be responsible for aerotaxis of halobacterial cells (fig.2).

Acknowledgements: Thanks are due to Professor D. Oesterhelt, Drs J. Spudich, Z. Danczshazy and A. Mankin for providing the *H. halobium* strains.

REFERENCES

[1] Hildebrand, E. and Dencher, N. (1975) Nature (London) 257, 46–48.
[2] Schimz, A., Sperling, W., Ermann, P., Bestman, H.J. and Hildebrand, E. (1983) Photochem. Photobiol. 38, 417–423.
[3] Traulich, B., Hildebrand, E., Schimz, A., Wagner, G. and Lanyi, J. (1983) Photochem. Photobiol. 37, 577–579.
[4] Spudich, J.L. and Bogomolni, R.A. (1984) Nature (London) 312, 509–513.
[5] Oesterhelt, D., Blanck, A., Hegemann, P., Kolling, E., Marwan, W., Schertler, G., Tittor, J. and Vogelsang, H. (1986) Biol. Chem. Hoppe-Seyler 367, suppl., 33.
[6] Tomioka, H., Takahashi, T., Kamo, N. and Kobatake, Y. (1986) Biochem. Biophys. Res. Commun. 139, 389–395.
[7] Hildebrand, E. and Schimz, A. (1986) Trends Biochem. Sci. 11, 402.
[8] Baryshev, V.A., Glagolev, A.N. and Skulachev, V.P. (1981) Nature (London) 292, 338–340.
[9] Baryshev, V.A., Glagolev, A.N. and Skulachev, V.P. (1983) J. Gen. Microbiol. 129, 367–373.
[10] Bogomolni, R.A. and Spudich, J.L. (1982) Proc. Natl. Acad. Sci. USA 79, 6250–6254.
[11] Ehrlich, B.E., Schen, C.R. and Spudich, J.L. (1984) J. Membrane Biol. 82, 89–94.
[12] Betlach, M., Friedman, J., Boyer, H.W. and Pfeiffer, F. (1984) Nucleic Acids Res. 12, 7949–7959.
[13] Arshavsky, V.Yu., Baryshev, V.A., Brown, I.I., Glagolev, A.N. and Skulachev, V.P. (1981) FEBS Lett. 133, 22–26.
[14] Taylor, B.L. (1983) Annu. Rev. Microbiol. 37, 551–573.
[15] Glagolev, A.N. and Sherman, M.Yu. (1983) FEMS Microbiol. Lett. 17, 147–150.
[16] Oesterhelt, D. and Stoeckenius, W. (1971) Nature New Biol. 233, 149–152.
[17] Hartmann, R., Sickinger, H.-D. and Oesterhelt, D. (1980) Proc. Natl. Acad. Sci. USA 77, 3821–3825.