ELSEVIER

FEBS Letters 340 (1994) 114–116

FEBS 13708

FEBS LETTERS

Phototactic behaviour of the archaebacterial *Natronobacterium pharaonis*

Birgit Scharf[a,*], Elmar K. Wolff[b]

[a]*Max-Planck-Institut für molekulaire Physiologie, Rheinlanddamm 201, 44139 Dortmund, Germany*
[b]*Universität Witten/Herdecke, 58453 Witten, Germany*

Received 3 December 1993; revised version received 13 January 1994

Abstract

Natronobacterium pharaonis can react tactically to photo- and chemostimuli. It moves by rotation of a flagellar bundle which is monopolarly inserted. Under sufficient oxygen supply the photophobic response of *N. pharaonis* has been measured. The resulting action spectrum matches the absorption spectrum of the purified retinylidene protein psR-II. Retical synthesis could be inhibited by nicotine. Cells grown in the presence of nicotine show a strongly reduced photoresponse, which could be restored by addition of retinal. These data identify psR-II as the receptor for negative phototaxis.

Key words: Phototaxis; Aerotaxis; Sensory rhodopsin; Halophilic archaebacteria; *Natronobacterium pharaonis*

1. Introduction

The phototactic response of the halophilic archaebacterium *Halobacterium salinarium* is mediated by two retinal-dependent proteins, sensory rhodopsin I (sR-I) and sensory rhodopsin II (sR-II). The combination of these pigments controlls the swimming behaviour in such a way that the organisms are repelled by gradients of light from shorter wavelengths and are attracted by light gradients above 520 nm (for a review see [1]).

An sR-II like pigment, psR-II, has been observed in another archaebacterial species *Natronobacterium pharaonis* [2]. A comparison of the biochemical and photochemical properties of both pigments sR-II and psR-II revealed similarities between the two proteins and it could also be shown that the cells exhibit a photophobic response towards blue-green light [3]. From these experiments it was concluded that psR-II acts as photoreceptor. However, a direct proof was not obtained. The measurements of the phototaxis have been hampered by the fact, that the cells have no ability to react in an anaerobic milieu. Under the experimental conditions the oxygen supply is rapidly depleted. Arginine, which has been described as a fermentative energy source for *H. salinarium* [4] and has also been used in a comparable analysis [5], could apparently not be utilized by *N. pharaonis* [3].

In this communication a method for the analysis of the photoresponse is presented which overcomes the problems of oxygen consumption so that an action spectrum could be measured. The data prove the function of the retinal pigment in *N. pharaonis* by the comparison of its absorption spectrum and the action spectrum for the photophobic response of the cells. This is confirmed by the observation that the photophobic behaviour is dependent on retinal synthesis.

2. Materials and methods

2.1. Strain and cell culture

N. pharaonis isolate SP1/28 (hR^+, $sR\text{-}II^+$ and car^+, kindly provided by W. Stoeckenius) was grown aerobically for 48–72 h in a synthetic growth medium [6] approximately to a density of 4×10^9 cells/ml.

Motile cells were selected by growing on synthetic medium soft agar plates (0.15%) similar to the procedure described in [7].

2.2. Nicotine-grown cells and retinal reconstitution

For blocking the biosynthesis of retinal, cells were grown for 72–84 h on synthetic medium containing 3 mM nicotine (Sigma, Deisenhofen, Germany) and then 1 ml was inoculated in 10 ml synthetic medium containing 2 mM nicotine. After 72 h the cells were grown to the end of the exponential phase.

For reconstitution of the photoreceptor one half of the culture was incubated for 14 h with 2 mM retinal from an ethanolic stock solution in a 25 ml Erlenmeyer flask at 40°C in the dark and shaking with 120 rpm. For comparison, the second half was treated with the same volume of ethanol under identical conditions.

2.3. Sample preparation and measuring conditions

The cell suspension was transferred directly from the culture flask to a 0.1 mm microscope slide chamber [5] filling it half and half by air. For stability of the air–cell-suspension interface and to avoid uncontrolled movement of the cells while focussing and positioning the sample, the chamber is sealed with molten paraffin wax. For aquiring action spectra the sealed cell suspension was adapted for at least 30 min to establish a stable oxygen gradient and a constant local cell concentration. The

*Corresponding author. Fax: (49) (231) 1206 389.

SSDI 0014-5793(94)00092-A

individual data for the action spectra were obtained at a defined distance to the suspension-air-interface at constant aerotactic conditions and cell concentrations.

An Orthoplan microscope at a primary magnification of 10- to 30-fold was used and the data were analyzed as described earlier [5,8].

2.4. Electron microscopy

A drop of cell suspension was placed for 1 min on a 200 mesh copper grid, which was covered by formvar. After removal of the drop with filter paper, the grid was dried at room temperature, and shadowed with a 2.5 mm carbon-platinum film at a 30°C angle, which was stabilized by a 10–15 mm carbon backing film. Micrographs were taken with a Zeiss EM 902 electron microscope at 80 kV.

3. Results and discussion

Blue-light-absorbing rhodopsins are found in at least two different archaebacterial species, *Halobacterium salinarium* and *Natronobacterium pharaonis*. While the function of the halobacterial pigment as receptor for photophobic response is well studied, the natronobacterial system has, scarcely been analyzed.

First the motility apparatus of *N. pharaonis* was studied. Morphology and flagellation of cells can be analyzed by electron microscopy as shown in Fig. 1 (upper part). The rod-shaped cell body is about 0.7 μm in width and 2 μm long. Cells with a length of 5 μm could also be observed. The cells are motile by flagella, which are usually inserted at one pole of the cell. About eight flagella are forming a bundle. In the lower part of Fig. 1 the insertion of the single flagella into the cell body is shown at a higher magnification. The morphology and flagellation of *N. pharaonis* is very similar to that of *H. salinarium* [9].

The chemotactic behaviour of the cells could be used for the isolation of highly motile strains. Cells swarming on soft agar plates are forming concentric rings after about two days of inoculation (data not shown). The cells respond chemotactically to spatial gradients generated by uptake and catabolism as already described for eubacteria [10].

For the analysis of the phototactic behaviour special experimental conditions had to be developed *N. pharaonis* became immotile after a few minutes under anaerobic conditions as they were found in the original experimental set-up for the analysis of action spectra for phototaxis [3]. In contrast to *H. salinarium* [4], arginine could not serve as fermentative energy source for *N. pharaonis* [3]. Thus, for the study of the phototactic behaviour the creation of an aerobic milieu is necessary. The measuring chamber was filled half with cell suspension leaving enough air in the sealed chamber to provide aerobic conditions for the cells in a window of about 100–400 μm for more than a day. A stable aerotactic profile developed after about 30 min. The cells accumulated in a distance of about 300 μm from the air–liquid interface. In the maximum of the profile the cells are adapted to the local aerobic conditions. The observed aerotactic response is similar to the behaviour of *H. salinarium* [5,11] but occurs faster. The cells in the aerotactic band that has formed in a distinct distance from the air zone are energized by oxidative phosphorylation. This aid allows now the analysis of phototaxis under aerobic conditions using the population method [5].

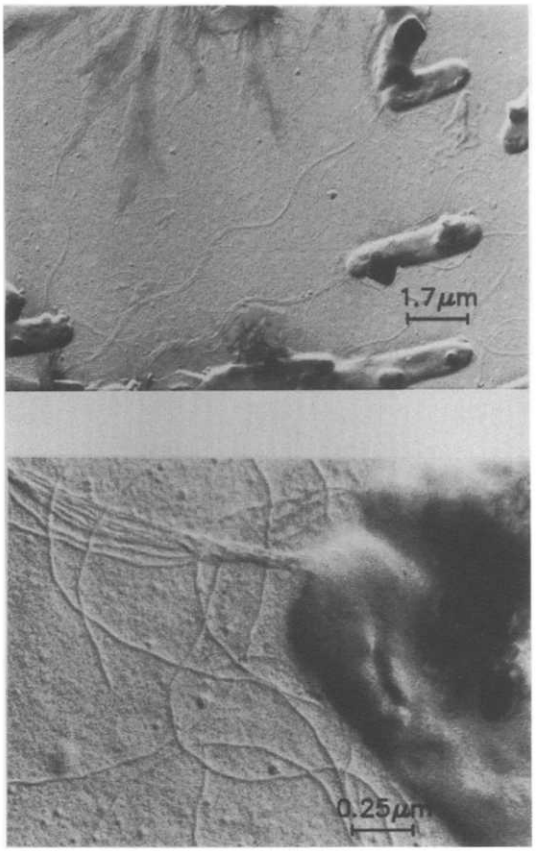

Fig. 1. Electron micrograph of *N. pharaonis*.

Natronobacterium are repelled by short-wavelength light [3]. The resulting depletion of cells in the measuring light spot can be recorded as increase in photomultiplier current. Non-saturating light conditions were checked using neutral density filters to ensure linearity of the reaction in the analyzed region. A complete action spectrum under aerobic conditions could be gained by measuring the response of the cells in a wavelength region from 440 to 560 nm in 10 nm steps. In Fig. 2 the action spectrum of the photophobic response of *N. pharaonis* is plotted and compared with the absorption spectrum of purified psRII-II [12]. The action spectrum corresponds well to the absorption spectrum of the pigment in most regions with a maximum at 500 nm and a shoulder at 470 nm. The intensity of the action spectrum in the range between 465 and 490 nm is higher than the photoreceptor absorption. The possibility that these points fall within the error range seems unlikely, because systematically five neighbouring points have higher intensities (as indicated by the bars which mark the absolute deviation;

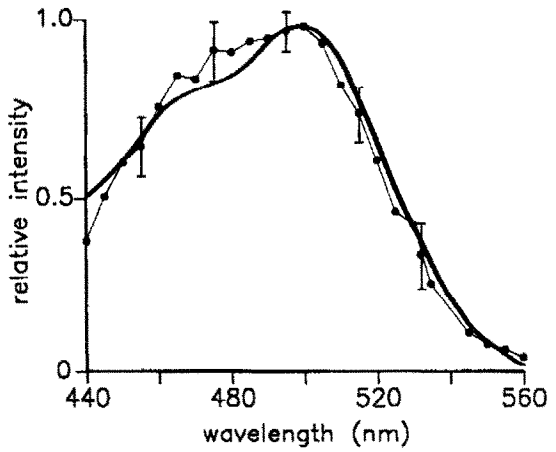

Fig. 2. Action spectrum for repellent response of *N. pharaonis*. The circles have been calculated as mean from four different measurements; the bars represent the maximal measured deviation. The absorption spectrum of psR-II (thick line) was taken from [12]; the spectrum was normalized at 500 nm.

Fig. 2). The source of this discrepancy has to be studied further. The absorption spectrum of psR-II in the short-wavelength region is slightly increased compared to the action spectrum because of interferences by the γ-band of remaining cytochromes.

The shape of the action spectrum provides evidence that the photophobic response is really mediated by psR-II. If this is true than the action spectrum should also be dependent on retinal synthesis, because psR-II has been identified spectroscopically and biochemically as retinylidene protein [3,12,13].

The biosynthesis of retinal can generally be suppressed by nicotine which has also been observed for *N. pharaonis*. If the active pigment is a retinal-dependent protein cells grown in a medium containing nicotine should therefore show a loss of their light sensitivity. The photoresponse should then be restored by exogenous addition of retinal, which allows a reconstitution of the receptor pigment. In Fig. 3 it is demonstrated that this is also observed for nicotine-grown *N. pharaonis* cells and retinal reconstituted ones. Addition of retinal indeed restores the phototactic response. Comparing the phototactic activity of nicotine-grown cells and control cells a reduction in responsiveness of about 70% could be achieved. The remaining answer is due to still existing psR-II molecules in the cell, because retinal synthesis could not be totally blocked without inhibiting important cell functions. The obtained reduced action spectrum for nicotine-growth cells has the same shape as the spectrum for untreated cells. This is an evidence, that no further retinal independent photosensor for phobic response is present.

In *H. salinarium* the retinylidene pigment sR-I mediates a positive response to orange-green light. Interestingly, *N. pharaonis* shows no photophilic reaction in the analyzed wavelength region. Furthermore, photochemical and biochemical analysis of membrane preparations gives no hint for the existence of a second retinal-dependent photoreceptor. Therefore, *N. pharaonis* seems to possess only one class of photoreceptor molecules, the retinylidene pigment psR-II.

Acknowledgements: We thank N. Adamek and A Scholz for technical help. Helpful discussions and critical reading of the manuscript by M. Engelhard are gratefully acknowledged.

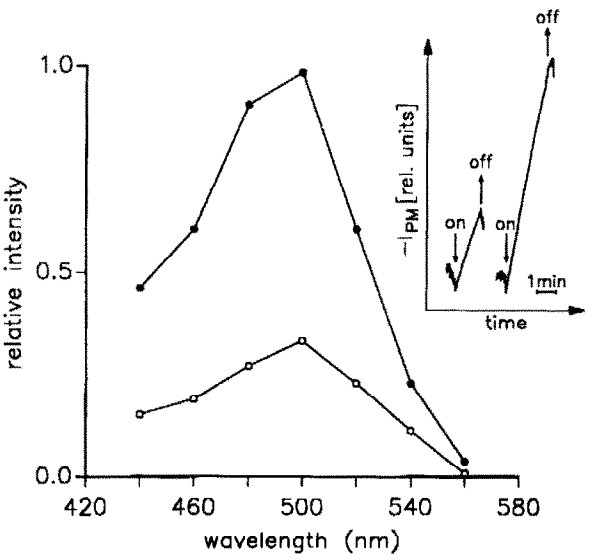

Fig. 3. Action spectrum of nicotine-grown *N. pharaonis* (open circles) and nicotine-grown cells reconstituted with retinal (closed circles). Insert: measuring traces of the photophobic response at 500 nm for nicotine-grown cells and reconstituted ones. I_{PM} = photomultiplier current. The arrows mark the illumination period.

References

[1] Oesterhelt, D. and Marwan, W. (1990) in: Biology of Chemical Response (Armitage, J.P. and Lackie, J.M., Eds.) Cambridge 46, pp. 220–239.
[2] Bivin, D.B. and Stoeckenius, W. (1986) J. Gen. Microbiol. 132, 2167–2177.
[3] Scharf, B., Pevec, B., Hess, B. and Engelhard, M. (1992) Eur. J. Biochem. 206, 359–366.
[4] Hartmann, R., Sickinger, H.D. and Oesterhelt, D. (1980) Proc. Natl. Acad. Sci. USA 77, 3821–3825.
[5] Stoeckenius, W., Wolff, E.K. and Hess, B. (1988) J. Bacteriol. 170, 2790–2795.
[6] Scharf, B. and Engelhard, M. (1994) Biochemistry, submitted.
[7] Spudich, J.L. and Stoeckenius, W. (1979) Photobiochem. Photobiophys. 1, 43–53.
[8] Wolff, E.K., Bogomolni, R.A., Scherrer, P., Hess, B. and Stoeckenius, W. (1986) Proc. Natl. Acad. Sci. USA 83, 7272–7276.
[9] Alam, M. and Oesterhelt, D. (1984) J. Mol. Biol. 176, 459–475.
[10] Adler, J. (1966) Science 153, 708–716.
[11] Bibikov, S.I. and Skulachev, V.P. (1989) FEBS Lett. 243, 303–306.
[12] Scharf, B., Engelhard, M. and Siebert, F. (1992) in: Structures and Function of Retinal Proteins (Rigaud, J.L., Ed.) Colloque INSERM 221, pp. 317–320.
[13] Imamoto, Y., Shichida, Y., Hirayama, J., Tomioka, H., Kamo, N. and Yoshizawa, T. (1992) Biochemistry 31, 2523–2528.