

Sensory Transduction in *Halobacterium halobium*: Retinal Protein Pigment Controls UV-Induced Behavioral Response

Norbert A. Dencher

Dept. of Biophysical Chemistry, Biocenter, University of Basel, CH-4056 Basel
and

Eilo Hildebrand

Institut für Neurobiologie, Kernforschungsanlage, D-5170 Jülich

Z. Naturforsch. **34 c**, 841 – 847 (1979); received June 5/July 6, 1979

Bacteria, Photophobic Response, Bacteriorhodopsin, Carotenoids, Accessory Pigment

Both photosystems, PS 370 and PS 565, controlling behavioral responses in *Halobacterium halobium* [E. Hildebrand and N. Dencher, Nature **257**, 46 – 48 (1975)] are reversibly inhibited when bacteria are grown in the presence of 1 mM nicotine which is known to block biosynthesis of retinal.

Photobehavior can be restored within some minutes to hours by adding retinal to nicotine-treated bacteria, PS 370 thereby reappearing earlier than PS 565. The reconstitution rate depends on the concentration and on the kind of retinal isomers applied. All-*trans* retinal is most effective. PS 370 becomes fully sensitive if reconstituted in the presence of nicotine. This rules out the possibility that the alkaloid may directly inhibit steps of signal transmission following photoreception.

The action spectrum of PS 370 regenerated with retinal alone of *H. h.*, strain R₁L₃ (a mutant deficient in carotenoids), fails to show all secondary peaks around 450 nm which in strain R₁ occur besides the prominent maximum at 370 nm. Addition of carotenoids (mainly α -bacterioruberin) to reconstituted cells of R₁L₃ restores the sensitivity in that spectral region. Carotenoids or flavin solely added to nicotine-treated bacteria cannot restore photobehavior.

We conclude that the active pigment of PS 370, which mediates the photophobic response to increase of light intensity (step-up response), represents a retinal protein complex and that carotenoids participate in photoreceptor function as accessory pigments. The biochemical relation of the UV-absorbing retinal protein complex to bacteriorhodopsin is discussed.

Halobacteria became interesting after the role of bacteriorhodopsin (BR) in light energy conversion had been discovered by Oesterhelt and Stoekenius [1, 2]. It turned out that they are suitable objects also for studying photosensory processes in bacteria. A characteristic behavioral response of *Halobacterium* is controlled by two photosystems (PS) which are different with respect to spectral sensitivity, adaptation and other important features [3]. Halobacteria are bipolarly flagellated and swim equally well in both directions parallel to their long axis. Temporal changes of incident light trigger a sudden reversal of swimming direction which, from time to time, also occurs spontaneously. Decrease of yellow-green light elicits the “step-down photophobic response” (for terminology see ref. [4]) which is mediated by PS 565, whereas increasing light intensity in the ul-

traviolet (UV) and blue triggers the “step-up photophobic response” through PS 370.

Several lines of evidences led to the assumption that the active pigment of PS 565 is BR [3], a chromoprotein which contains retinal (vitamin A aldehyde) covalently linked to a lysine residue of a protein, bacterio-opsin (BO) [1]. The nature, however, of the UV-absorbing pigment of PS 370 was not known up to now. A flavin protein together with carotenoids, the latter acting as shielding pigments, has been considered as well as a retinylidene protein [3]. The experiments reported here show that most likely the latter is true and that carotenoids act mainly as accessory pigments thereby participating in the quantum catch of PS 370.

Materials and Methods

Bacteria

Halobacterium halobium (*H. h.*), strains R₁ and R₁L₃, were grown in sterile culture medium [5] semiaerobically at 40 °C under illumination with white light of 1 mW cm⁻² as described elsewhere

Abbreviations: BR, bacteriorhodopsin; PS, photosystem; UV, ultraviolet; BO, bacterio-opsin; *H. h.*, *Halobacterium halobium*; PM, purple membrane.

Reprint requests to N. A. Dencher.

0341-0382/79/0900-0841 \$01.00/0



Dieses Werk wurde im Jahr 2013 vom Verlag Zeitschrift für Naturforschung in Zusammenarbeit mit der Max-Planck-Gesellschaft zur Förderung der Wissenschaften e.V. digitalisiert und unter folgender Lizenz veröffentlicht: Creative Commons Namensnennung-Keine Bearbeitung 3.0 Deutschland Lizenz.

Zum 01.01.2015 ist eine Anpassung der Lizenzbedingungen (Entfall der Creative Commons Lizenzbedingung „Keine Bearbeitung“) beabsichtigt, um eine Nachnutzung auch im Rahmen zukünftiger wissenschaftlicher Nutzungsformen zu ermöglichen.

This work has been digitalized and published in 2013 by Verlag Zeitschrift für Naturforschung in cooperation with the Max Planck Society for the Advancement of Science under a Creative Commons Attribution-NoDerivs 3.0 Germany License.

On 01.01.2015 it is planned to change the License Conditions (the removal of the Creative Commons License condition “no derivative works”). This is to allow reuse in the area of future scientific usage.

[6]. Bacteria of the stationary growth phase (about 10^9 cells/ml) were used for experiments.

Retinal-free cells were obtained by culturing bacteria in the presence of 1 mM nicotine until photophobic responses could no longer be evoked. For this purpose 500 ml nicotine-containing growth medium was inoculated with 1 ml suspension of bacteria. With bacteria grown under these conditions this procedure was repeated every 5 to 6 days, altogether usually 5 times. Bacteria treated in this way, which will be referred to as "nicotine cells", were used directly from the culture and without further dilution tested immediately after the transfer to the experimental chamber.

Chemicals

Retinal isomers (all-*trans* and 13-*cis*) were obtained from Fluka and purified by high pressure liquid chromatography. 11-*cis* retinal was a gift of Dr. W. Sperling. L-nicotine and riboflavin were purchased from Fluka and Merck, respectively.

Reconstitution of photoresponses

Restoration of photosystems after nicotine treatment was examined either after removal of nicotine or after addition of different chromophores. In the first case nicotine cells were harvested by 1.5 min centrifugation at $10,000 \times g$ (Eppendorf type 5412), washed and resuspended in nicotine-free culture medium. Thereafter bacteria were stored at 38 °C in the dark until use. Artificial regeneration of photopigments was studied as follows: Ethanolic solution of different retinal isomers and/or carotenoids or riboflavin, respectively, was added to a suspension of nicotine cells in nicotine-containing culture medium at 32 or 38 °C in the dark. Samples used for determination of action spectra were incubated for at least 12 h under these conditions.

If not otherwise stated the retinal concentration in the samples was 1.8×10^{-6} M. Riboflavin in aqueous and ethanolic solution was used in a similar final concentration. Carotenoids were extracted from *H. h.*, strain R₁, with methanol/acetone (1/1, v/v) after the purple membrane (PM) had been separated and discarded, and added in a 2- to 3-fold excess (as compared to the content of R₁) to the sample. The ethanol concentration in all samples tested was usually 0.1%, but never exceeded 0.5%.

Observation and measurements

Bacteria were observed in a small quartz chamber of about 4 × 6 mm width and 0.2 mm depth covered by a quartz slide under a phase contrast microscope (Leitz, Ortholux, objective: Zeiss, Neofluar 63 × or Ultrafluor 100 ×, respectively). Background illumination of $\lambda > 645$ nm which is not absorbed by retinal was applied through the condensor. Monochromatic stimulating light (half width ≤ 10 nm), variable with respect to wavelength and intensity could be generated either by a xenon lamp (Hanovia, 150 W) in connection with a high intensity grating monochromator (Bausch and Lomb, UV/visible range) or a high pressure mercury lamp (Osram, HBO 200) connected with a quartz prism monochromator (Zeiss, M 4 QIII). Light stimuli were applied by an incident light illuminator through the objective lens of the microscope and controlled by an electronic shutter. Usually the stimulus was kept on until the response occurred. In order to measure sensitivity of bacteria towards light, single cells were observed and the latency, the time elapsed between onset of stimulus and the first observable response (stop response), was determined by a stop watch. Either the latency measured upon a constant stimulus or the stimulus intensity necessary to elicit a standard response (constant latency) was used as a measure of sensitivity. Light intensity was measured by means of a light detector (UDT 40) connected with a calibrated pin 10 UV. Each measurement was repeated several times with different specimens (see figure legends).

In the reconstitution experiments every sample or at least area of the sample was exposed only once to a light stimulus to avoid photoisomerization of retinal prior to the measurement.

Temperature of the preparation was kept constant (± 0.2 °C) by means of a thermostatic microscope stage equipped with Peltier elements. Kinetic measurements and determination of action spectra were performed at 32 °C.

Absorption spectra were measured either by a Cary 17 spectrophotometer or a Cary 118 with scattered transmission accessory.

Results

Effect of nicotine on photophobic responses

Nicotine is known to inhibit retinal synthesis by blocking cyclization of lycopene to β -carotene [7].

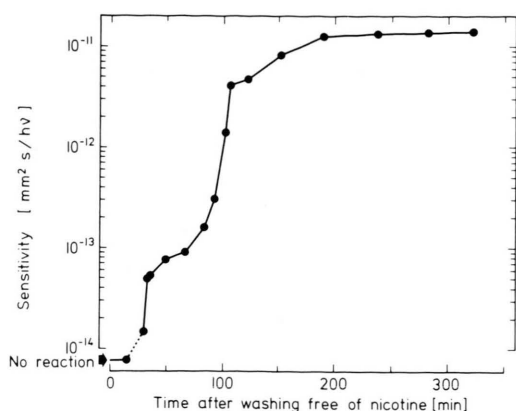


Fig. 1. Recovery of PS 370 in nicotine-grown bacteria after removal of the alkaloid. Ordinate: sensitivity given as the reciprocal of light intensity which evokes a standard response. Each data point was determined after the stimulus intensity had been readjusted several times until at least 19 out of 20 specimens examined showed the response within a given latency of 1.9 to 2.0 s. Stimulating light, $\lambda = 370$ nm. Temperature, 38 °C. Sensitivity of R_1L_3 cells before nicotine treatment was $8.3 \times 10^{-12} \text{ mm}^2 \text{ s/hv}$.

Consequently halobacteria grown in the presence of nicotine did not form PM which contains BR as the only protein.

Examination of light-dependent behavioral responses revealed that both photosystems became inactive after nicotine treatment. However, normal movement, swimming rate, and the ability to carry out spontaneous reversals were not influenced. Photophobic responses could be evoked neither by decreasing the light intensity at 565 nm nor by a sudden illumination with UV-light of 280 or 370 nm, respectively. Even stimuli which were about 1000-fold as strong as normally required were found to be ineffective.

The inhibitory effect of nicotine was completely reversible in the case of PS 370 (Fig. 1). After removal of nicotine the photophobic response reappeared. The sensitivity of PS 370 was monitored at 370 nm, and the first response could be detected 30 min after the washing procedure. Maximum sensitivity was restored after about 200 min and was as large as before nicotine treatment and as in untreated R_1 cells (Fig. 4).

Reconstitution of photosystems after nicotine treatment

In a series of experiments different isomers of retinal were added to suspensions of *H. h.*, strain R_1L_3 ,

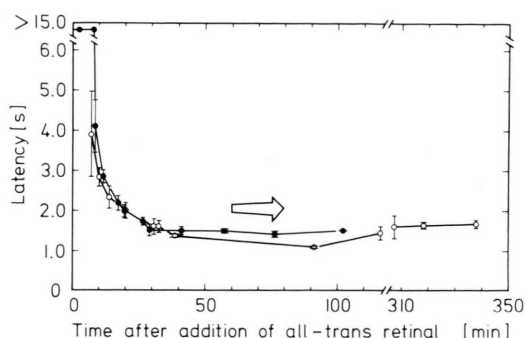


Fig. 2. Reconstitution of PS 370 after addition of all-*trans* retinal (final concentration $1.8 \times 10^{-6} \text{ M}$) to nicotine cells. Ordinate: latency between onset of stimulus ($4.7 \times 10^{11} \text{ h} \nu \cdot \text{mm}^{-2} \cdot \text{s}^{-1}$, $\lambda = 370 \text{ nm}$) and stop response of bacteria. Different symbols belong to two series of experiments. Data points and vertical bars represent mean \pm SD of 4 to 14 measurements. Arrow depicts reappearance and final latency of PS 565 ($1.5 \times 10^{13} \text{ h} \nu \cdot \text{mm}^{-2} \cdot \text{s}^{-1}$, $\lambda = 565 \text{ nm}$). Temperature, 32 °C.

after the photobehavior had been blocked by nicotine treatment as described above. To simplify the procedure recovery of PS 370 occurring under these conditions was measured by monitoring the decrease of latency of the photophobic response elicited by a constant light stimulus of $\lambda = 370 \text{ nm}$.

Addition of all-*trans* retinal completely restored PS 370. After a delay of less than 10 min a fast increase in sensitivity (decrease in latency) occurred which levelled off after 30 to 40 min (Fig. 2). Sensitivity of PS 565 could first be detected 60 to 80 min following retinal incubation (depicted by the arrow in Fig. 2). Its final sensitivity, however, was only about one tenth of the value measured in control experiments, *i.e.* $0.6 \times 10^{-13} \text{ mm}^2 \text{ s/hv}$ instead of $5 \times 10^{-13} \text{ mm}^2 \text{ s/hv}$ at a given latency of 2.0 s.

Varying the concentration of all-*trans* retinal resulted in a change of the time elapsed between incubation and the reappearance of PS 370. A response with a latency of 3 s was observed about 6, 12, and 45 min after incubation with 9.0×10^{-6} , 1.8×10^{-6} , and $3.6 \times 10^{-7} \text{ M}$, respectively, all-*trans* retinal. In the latter case the original sensitivity was not completely restored even after 150 min.

A similar increase of the lag period was observed if 13-*cis* retinal or 11-*cis* retinal was used instead of all-*trans* retinal. At equal concentrations of these isomers and constant stimulus strength a response with a

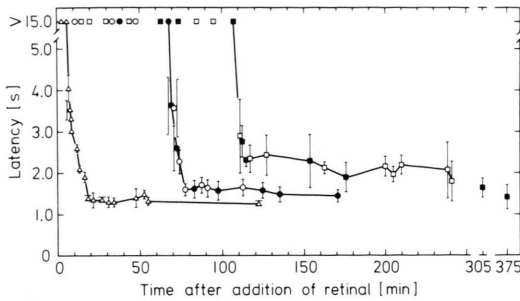


Fig. 3. Reconstitution of PS 370 after addition of all-*trans* (Δ), 13-*cis* (\circ , \bullet), and 11-*cis* retinal (\square , \blacksquare), respectively (final concentration 1.8×10^{-6} M). Open and solid symbols represent different series ($n = 4 - 12$). Ordinate as in Fig. 2. Stimulating light, $8 \times 10^{11} \text{ h} \nu \cdot \text{mm}^{-2} \cdot \text{s}^{-1}$, $\lambda = 370 \text{ nm}$. Temperature, 38°C .

latency of 3 s could be evoked after about 70 or 110 min, respectively, instead of 10 min in the case of all-*trans* retinal (Fig. 3). All reconstitution experiments were successful in the presence of 1 mM nicotine (besides retinal).

Addition of riboflavin and of carotenoids extracted from *H. h.*, strain R_1 , to nicotine-treated cells did not restore either PS 370 or PS 565: No photophobic responses could be evoked under these conditions.

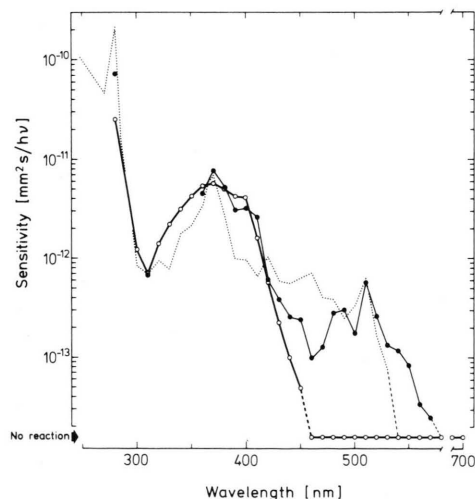


Fig. 4. Action spectra of step-up photophobic response (PS 370) obtained from strain R_1L_3 nicotine cells incubated with all-*trans* retinal (\circ) and incubated with all-*trans* retinal and carotenoids (\bullet). For comparison action spectrum obtained from carotenoid-rich R_1 cells (dotted line, from Ref. [3]). Ordinate as in Fig. 1. Background illumination, $\lambda > 645 \text{ nm}$. Data points were determined as described in the legend to Fig. 1.

Action spectra

The sensitivity at 370 nm of *H. h.*, mutant strain R_1L_3 , was almost unchanged as compared to that of R_1 . However, in contrast to the latter no step-up photophobic response could be elicited at wavelengths beyond 450 nm.

The action spectrum obtained from nicotine cells, strain R_1L_3 , after reconstitution of PS 370 is given in Fig. 4 (open circles) as compared to the action spectrum of the untreated strain R_1 (dotted curve). It shows a marked band with a maximum sensitivity at 370 nm, as in strain R_1 , but no response beyond 450 nm. Moreover, the 370 band appears much broader than in the case of R_1 and sensitivity at 280 nm is significantly reduced.

The light intensity required to elicit a standard response (latency 2 s) at 370 nm was $1.8 \pm 0.2 \times 10^{11} \text{ h} \nu \text{ mm}^{-2} \text{ s}^{-1}$ (SD, $n = 5$) in reconstituted nicotine cells as compared to $1.5 \pm 0.2 \times 10^{11} \text{ h} \nu \text{ mm}^{-2} \text{ s}^{-1}$ ($n = 7$) in strain R_1 .

Effect of carotenoids

Halobacteria, strain R_1L_3 , with reconstituted PS 370 as described above were incubated with carotenoids from strain R_1 . As a result of this treatment bacteria were markedly sensitized against light of wavelengths between 450 and 570 nm. The action spectrum obtained under these conditions (Fig. 4, solid circles) resembles fairly well that of R_1 , although some distinct deviations are obvious around 450 nm and beyond 550 nm. Sensitivity at 280 nm is somewhat enhanced as compared to reconstituted R_1L_3 without carotenoid treatment but not as high as in R_1 .

Discussion

Receptor pigment of PS 370

The results reported here indicate that the active pigment in PS 370 of *H. h.* is a UV-absorbing retinal protein complex. Moreover, they support our assumption [3] that the step-down photophobic response (PS 565) is mediated by BR.

Inhibition of PS 370 by nicotine which is known to block retinal synthesis at the stage of β -carotene formation from lycopene [7] and the ability of retinal to restore this PS point towards a retinylidene protein as the active receptor molecule. The alternative explanation that some step of signal transmission follow-

ing photoreception by PS 370 requires BR or retinal seems unlikely though this possibility cannot be ruled out with certainty. The action spectrum obtained from retinal-reconstituted R_1L_3 cells (Fig. 4) excludes a flavin protein which has been suggested as the only alternative candidate for the primary photoreceptor of PS 370 [3]. Moreover, no reasonable mechanism is conceivable by which retinal or BR may function in the following process of signal transmission. The fact that the photobehavior can be restored in the presence of nicotine excludes the possibility that the alkaloid itself might block certain steps of signal transmission between stimulus reception and flagellar activation.

Although it seems evident that a retinal protein complex is responsible for sensory transduction of PS 370 the molecular structure of the pigment remains obscure. We were so far unable to detect the pigment spectroscopically or biochemically. However, it is not an uncommon feature of sensory pigments of microorganisms to be present only in very small amounts making it easier to characterize them by means of action spectra than to isolate them.

The action spectrum obtained from bacteria whose PS 370 was reconstituted by retinal (Fig. 4, open circles) matches fairly well the absorption spectrum of a retinylidene protein which can be obtained *in vitro* after treatment of PM with 10 mM CTAB * at alkaline pH [1] (Fig. 5, solid curve). In this complex those non-covalent interactions between chromophore and protein moiety which are thought to cause the bathochromic shift of the absorption spectrum of BR and visual pigments are obviously absent. The pronounced band at 370 nm in the action spectrum of PS 370 is probably due to the absorption by the unprotonated Schiff-base linkage.

In nicotine-treated cells no behavioral response could be induced at 280 nm even at strongly increased light intensity. This observation indicates that a protein by itself cannot trigger the response. Retinal is obviously necessary for sensory transduction and only that energy which is absorbed by a specific protein coupled to the chromophore contributes to excitation.

Reappearance of photoresponses 30 min after washing the cells free of nicotine (Fig. 1) indicates a fast resumption of retinal synthesis. In the reconstitution experiments all-*trans* retinal was most effective

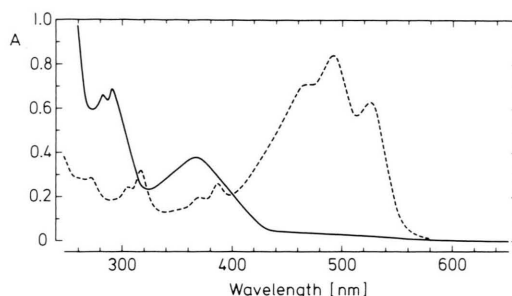


Fig. 5. Absorption spectra of a retinylidene protein obtained after treatment of PM with 10 mM CTAB at pH \approx 8.5 (solid curve) and of carotenoids extracted from *H. h.*, strain R_1 in ethanol (dashed curve).

tive in restoring PS 370. We conclude from this that the apoprotein preferentially or solely combines with this isomer and that catalytic isomerization of 13-*cis* and 11-*cis* retinal to the *trans* configuration may take place prior to reconstitution as indicated by the lag time observed in the case of *cis* isomers (Fig. 3). Photoisomerization by background or stimulating light can be excluded (see Materials and Methods) as well as noncatalytic dark-isomerization, which is negligible at temperatures below 60 °C.

Relation to bacteriorhodopsin

In the growing culture of *H. h.*, strain R_1 , PS 370 appears about 10 h earlier than PS 565. It shows maximal sensitivity immediately, whereas PS 565 develops more slowly paralleling the formation of PM [3]. Also in the reconstitution experiments the reappearance of PS 370 preceded that of PS 565 (Fig. 2), which attained only about one tenth of its original sensitivity. The latter is in agreement with the finding that BO inhibits its own synthesis [8]. Therefore, in nicotine cells the concentration of regenerable BO is much smaller than the concentration of BR in untreated cells. The sequence of appearance of photosystems might be a hint that the pigment of PS 370 represents a precursor of BR. This would imply that the protein part of both pigments is identical or principally identical. But also different pathways of biosynthesis are possible.

The result that PS 370 can be fully restored by all-*trans* retinal although a much smaller concentration of regenerable BO was found in nicotine cells as compared to the amount of BR in untreated halobacteria [8] argues against an identical protein of both retinal protein complexes.

* Cetyltrimethyl-ammoniumbromide, a detergent.

The rate of sensitivity increase of retinal-reconstituted PS 370 is comparable to the formation rate of BR from BO and all-*trans* retinal *in vitro*. However, reappearance of the first inducible step-down photophobic response, mediated by PS 565, is slower than regeneration of BR [9], but faster than PM assembly [8]. The latter fact might indicate that a hexagonal crystalline lattice of BR is not a prerequisite of PS 565.

Role of carotenoids

The mutant strain R_1L_3 of *H. h.* used in our experiments contains a considerably smaller amount of carotenoids (mainly α -bacterioruberin) as compared to strain R_1 . The missing sensitivity around 500 nm of R_1L_3 cells points to an accessory function of carotenoids in PS 370. Another mutant, R_1M_1 , which lacks carotenoids too, also does not show the step-up photoresponse at wavelengths beyond 460 nm [10].

As demonstrated above (Fig. 4, solid circles) carotenoids extracted from *H. h.*, strain R_1 , (Fig. 5, dashed curve) cause a distinct increase in sensitivity to light of wavelengths between 420 and 570 nm if added to a suspension of bacteria after PS 370 has been restored with retinal. This result supports the conclusion that carotenoids, at least in part, contribute to PS 370 as accessory pigments provided that the retinylidene protein is present. Carotenoids by themselves cannot trigger the behavioral response, but transfer the absorbed energy to the retinal protein complex. This energy transfer from a compound of longer wavelength absorption towards a compound of shorter wavelength absorption may result from a triplet-triplet or exciton interaction. With regard to the relative concentrations of both substances in the living bacterium the efficiency of energy migration seems to be low.

Qualitatively, the absorption spectra of the *in vitro* modified BR (retinylidene protein) and of extracted carotenoids (Fig. 5) agree with the action spectrum of PS 370 of strain R_1 (Fig. 4, dotted line). The slight differences in the position of maxima around 500 nm are in accordance with the spectral blue shift of carotenoids observable in ethanolic solution. Furthermore, selective loss of membrane-bound carotenoids by the extraction procedure applied can explain the differences between action spectra of R_1 and reconstituted cells (Fig. 4, solid circles). Absorption spectra of nicotine cells reveal that also synthesis of carotenoids absorbing beyond 380 nm is

inhibited under these conditions. As can be seen in Fig. 4 the band at 370 nm in the action spectrum of R_1 and carotenoid-reconstituted cells of R_1L_3 is sharper as compared with that of cells solely reconstituted with retinal. One possible explanation is that part of the carotenoids act as shielding pigments in this spectral region. However, at present we have no direct experimental proof for this assumption.

Collaboration of a retinal protein complex and carotenoids in photoreceptor function is not a unique feature. The majority of ommatidia of the fly compound eye contains, besides rhodopsin, a photostable dichroic pigment, possibly a carotene, embedded in the membrane of the central rhabdomere. It is suggested that this carotene acts as antenna pigment transferring energy to the UV-sensitive visual pigment [11].

Localization of PS 370

The PM contains BR as the only protein embedded in the lipid matrix of the cell membrane of *Halobacterium* [1, 12]. Neither carotenoids nor a UV-absorbing retinylidene protein have been detected in this membrane fraction. With respect to its sensory function PS 370 is most likely also an integral part of the cytoplasmic membrane. We therefore conclude that the active retinal protein complex together with accessory carotenoids, in a special molecular arrangement, are located in membrane areas not occupied by the PM. With respect to the high content of carotenoids in the so-called red membrane [13] we suppose that PS 370 is part of this membrane fraction.

Sensory transduction

We have so far no direct experimental proof how excitation of the photoreceptor molecule might be coupled to flagellar activity in *H. h.* The similarity of behavioral responses elicited by both photosystems suggests a similar mechanism at least of late steps of signal transmission. A sudden decrease of membrane potential, in different ways caused by the photosystems, could well serve as a common signal which triggers flagellar activation [14]. Membrane depolarization is also thought to be involved in behavioral responses of bacteria towards chemical stimuli [15].

In the case of PS 565 depolarization could result from a decrease in electrogenic proton pumping activity of BR after a sudden decrease in light intensity. A similar drop in potential across the membrane

may be generated by PS 370 if the retinylidene protein acts as a membrane pore or mediates the opening of a pore for a certain ion species [14, 16]. In contrast to PS 565 the sensitivity of PS 370 was found to be independent of the pH in the medium (Dencher and Janković, unpublished results). This may indicate that in the latter case protons are not directly involved in the transduction mechanism. Increasing the external K^+ concentration so as to depolarize the membrane [17] inhibits both photosystems equally well (Hildebrand and Janković, unpublished results).

While the predominant function of PS 565 is obviously *photocoupling*, i. e. energy conversion, PS 370 acts only as *sensory transducer*. However, the biological relevance of PS 370 is not clear. It may, together with PS 565, enable the organism to find optimal places for utilization of light energy and to stay

there. The aqueous environment of *H. h.* may act as a light filter due to selective absorption by pigment-containing microorganisms. This determines the spectral composition of sunlight depending on the depth of water. By means of both photosystems the organisms possibly accumulate in a certain layer which corresponds to light conditions favorable for their survival [16]. PS 370 might prevent the cells from damaging irradiation [18] or may represent only an evolutionary remnant [14].

Acknowledgements

We thank Dr. Lily Jan for a gift of the mutant strain R_1L_3 . We are grateful to Dr. Angelika Schimz for critically reading the manuscript. This work was supported by Deutsche Forschungsgemeinschaft (SFB 160).

- [1] D. Oesterhelt and W. Stoeckenius, *Nature New Biol.* **233**, 149 – 152 (1971).
- [2] D. Oesterhelt and W. Stoeckenius, *Proc. Nat. Acad. Sci. USA* **70**, 2853 – 2857 (1973).
- [3] E. Hildebrand and N. Dencher, *Nature* **257**, 46 – 48 (1975).
- [4] B. Diehn, M. Feinleib, W. Haupt, E. Hildebrand, F. Lenci, and W. Nultsch, *Photochem. Photobiol.* **26**, 559 – 560 (1977).
- [5] D. Oesterhelt and B. Hess, *Eur. J. Biochem.* **37**, 316 – 326 (1973).
- [6] P.-J. Bauer, N. A. Dencher, and M. P. Heyn, *Biophys. Struct. Mechanism* **2**, 79 – 92 (1976).
- [7] C. D. Howes and P. P. Batra, *Biochim. Biophys. Acta* **222**, 174 – 179 (1970).
- [8] M. Sumper and G. Herrmann, *FEBS Letters* **69**, 149 – 152 (1976).
- [9] M. E. Keen and N. A. Dencher, *EMBO Workshop on Transduction Mechanism of Photoreceptors*, Oct. 4 – 8 (1976), Jülich/Germany, abstract p. 64.
- [10] W. Nultsch and M. Häder, *Ber. Dtsch. Bot. Ges.* **91**, 441 – 453 (1978).
- [11] K. Kirschfeld and N. Franceschini, *Biophys. Struct. Mechanism* **3**, 191 – 194 (1977).
- [12] R. Henderson, *Ann. Rev. Biophys. Bioeng.* **6**, 87 – 109 (1977).
- [13] M. Sumper, H. Reitmeier, and D. Oesterhelt, *Angew. Chem. Int. Ed. Engl.* **15**, 187 – 194 (1976).
- [14] E. Hildebrand, *Taxis and Behavior* (ed. G. L. Hazelbauer), pp. 35 – 73, Chapman and Hall, London 1978.
- [15] J. B. Miller and D. E. Jr. Koshland, *Proc. Nat. Acad. Sci. USA* **74**, 4752 – 4756 (1977).
- [16] N. A. Dencher, *Energetics and Structure of Halophilic Microorganisms*, (eds. S. R. Caplan and M. Ginzburg), pp. 67 – 88, Elsevier/North Holland, Amsterdam 1978.
- [17] G. Wagner, R. Hartmann, and D. Oesterhelt, *Eur. J. Biochem.* **89**, 169 – 179 (1978).
- [18] J. D. Dundas and H. Larsen, *Arch. Microbiol.* **44**, 233 – 239 (1962).