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Photosensory Retinal Pigments in *Halobacterium halobium*

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Abstract. In *Halobacterium halobium,* nicotine is known to block the synthesis of retinal. Cells grown in the presence of nicotine do not show any photophobic response. Addition of retinal$_1$ or retinal$_2$ restored the photophobic responses to light-increase in the UV and to light-decrease in the green-yellow part of the spectrum. The action spectra of the two retinal$_2$-photosystems were red-shifted by 15–20 nm, compared with the corresponding retinal$_1$ systems. We conclude that each of the two photosystems, PS 370 and PS 565, has its own photosensory pigment with retinal as the chromophoric group.

Key words: *Halobacterium halobium* – Photosensory pigments – Retinal$_1$ – Retinal$_2$ – Action spectra.

Introduction

The movements of *Halobacterium halobium* are affected by light (Berg and Stoeckenius 1973): The bacteria reverse their direction of swimming when the intensity of light is increased in the UV (photosystem PS 370) or decreased in the green-yellow part of the spectrum (PS 565) (Dencher 1974; Hildebrand and Dencher 1975). Bacteria grown under conditions where no retinal is synthesized, don't show any photophobic response. Addition of retinal to the culture medium restores the two photosystems (Dencher 1978; Dencher and Hildebrand 1979). This suggests that retinal-containing proteins are the photosensory pigments responsible for these reactions (Dencher 1978; Dencher and Hildebrand 1979). Bacteriorhodopsin (BR) was proposed to be the pigment for the photosystem 565, a different retinal-containing protein for the system 370 (Dencher 1978; Dencher and Hildebrand 1979).

In this paper we describe experiments with bacteria whose retinal$_1$ was substituted by retinal$_2$ (3,4-dehydroretinal). Retinal$_2$ was chosen because it is interchangeable with retinal$_1$ both in rhodopsin and in bacteriorhodopsin. The properties of retinal$_1$ and retinal$_2$ pigments are usually nearly identical with the exception that all absorption spectra, including those of the transients, are red-shifted by about 15–20 nm – in ethanol the absorption maximum of trans retinal$_1$ is at 383 nm, that of trans retinal$_2$ at 399 nm.

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Methods

Halobacterium halobium, strain R_1L_3, was grown in the presence of 1.4 mM L-nicotine in 20 ml sterile culture medium at 37° C semiaerobically on a rotary shaker (100 rev/min) under illumination with a fluorescent lamp (100–300 $\mu W/cm^2$ near the Erlenmeyer flasks). Nicotine hinders the conversion of lycopene to β-carotene and retinal by blocking the synthesis of the ring in carotenoids (Howes and Batra 1970; Sumper et al. 1976). Growth to the stationary phase was repeated until no further phototophobic response was to be seen (6–7 times).

After addition of 10 µl of a 10^{-3} M ethanolic trans retinal solution to 1 ml suspension of bacteria, the photosystems 370 and 565 were restored within 40 and 80 min, respectively. The apparatus used for observing the photophobic responses was described by Hildebrand and Dencher (1975). To observe the bacteria under the microscope, white background illumination of an intensity of 4 $\mu W/cm^2$ was applied (xenon lamp XBO 150). For the light stimuli additional light of a 200 W Hg-lamp, connected to a monochromator, was used. The number of photons was adjusted to a constant value for each wavelength by varying the slitwidth of the monochromator. This implied that the slitwidth had to be varied between 0.1 and 0.3 mm, the bandwidth changed between 1.5 and 4.5 nm. The length of the flashes was determined by opening or closing a shutter. The latency time, defined as the time elapsing between the onset of the stimulus and the first observable response (a stop of swimming), was taken as a measure of the sensitivity of the photosystems. To measure the step-up reaction (PS 370), light flashes of a duration of 2 s and an intensity of 2.2×10^{10} photons/mm^2s were used. To measure the step-down reaction (PS 565), the cells were illuminated with a permanent light intensity of 3.4×10^{11} photons/mm^2s (in addition to the background light), which was blocked by a shutter (negative flash) for 2 s. In an alternative way to determine the sensitivity of the system 370, the number of photons necessary to get a standard response was measured at different wavelengths (see Fig. 4). The standard response was a latency time of 2.1–2.2 s.

The absorption spectra were measured with a Cary 118 C spectrophotometer. For the BR suspensions the scattered transmission compartment was used. Trans $retinal_2$ was obtained by oxidation of $retinol_2$ with MnO_2, and after high performance liquid chromatography its purity was better than 98%. $Retinol_2$ was a gift of Hoffmann-La-Roche & Co. Ltd, Basel, Switzerland.

Results and Discussion

The latency time can be used as a measure for the sensitivity of the photophobic responses of *Halobacterium halobium.* It depends on the temperature and the wavelength, intensity and duration of the stimulating light flash. Spontaneous reactions, i.e., stop and reversal of the swimming direction without a change in light intensity, occur under our conditions with a frequency of 1 in 40 s. Both in spontaneous and light-induced reactions, the time between a stop and the reversal is about 0.2 s.

Figure 1 shows for both photosystems the dependence of the latency time on the intensity of the light change. To measure the latency time of the two systems restored with $retinal_1$ or its 3,4-dehydro-analogon at different wavelengths (Fig. 3), we chose an

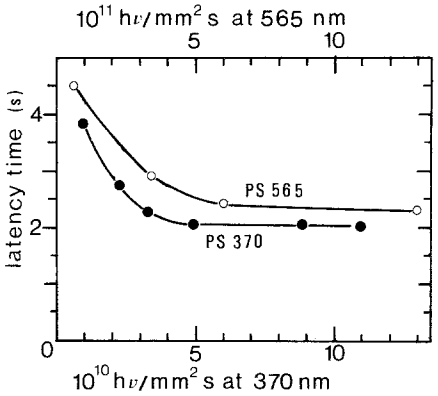

Fig. 1. Dependence of the latency time of the photophobic reactions of halobacteria on the light intensity. PS 370: duration of light flash 2 s, wavelength 370 nm, light intensity given on the lower abscissa; PS 565: permanent ilumination at 565 nm, whose intensity is given on the upper abscissa, was interrupted for 2 s (negative flash). Background illumination was always 4 μW/cm², temperature 24° C, each point is the mean of 20 measurements, s. D. was less than 10%

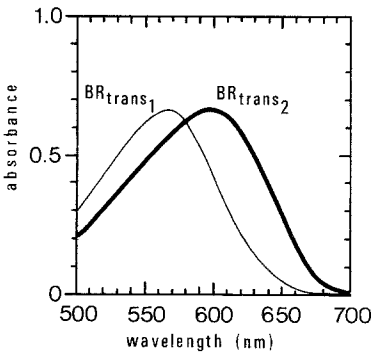

Fig. 2. Absorption spectra of *trans*$_1$ bacteriorhodopsin (thin line) and *trans*$_2$ bacteriorhodopsin (thick line), regenerated from bacterioopsin with *trans* retinal$_1$ and *trans* retinal$_2$, respectively. The spectra were normalized to equal maximum absorption by appropriate dilution of the sample and corrected for baseline. Light pathlength 10 mm, room temperature

intensity of 2.2 × 10^{10} hν/mm² s for PS 370 and of 3.4 × 10^{11} hν/mm² s for PS 565. These intensities are high enough to observe light-induced responses without interference of spontaneous reversals, but low enough to avoid a 'flattening' of the action spectrum because of saturation.

The absorption spectra of trans$_1$ bacteriorhodopsin (BR_{trans1}) (Dencher et al. 1976) and *trans*$_2$ bacteriorhodopsin (BR_{trans2}), each measured with a suspension of BR regenerated from bacterioopsin and the corresponding retinal isomers, are shown in Fig. 2. The red shift is comparable to that of the two retinals in solution. Figure 3 describes the action spectra of the two photosystems 370 and 565 in bacteria grown in nicotine-containing medium whose photosystems were restored by the respective trans retinals. From this figure can be seen that retinal$_1$ as well as retinal$_2$ is able to reconstitute both photosystems in the living bacteria.

The following features of the curves in Figs. 3 and 4 are remarkable: In both photosystems the maxima of the action spectra for the retinal$_2$-treated bacteria are shifted by about 15–20 nm to the red. This corresponds to the shift one would expect from the spectra of the two retinals in solution and from the spectra of the two bacteriorhodopsins. From Fig. 4 can be seen that the photoefficiency of the retinal$_1$ and the retinal$_2$ system is about the same (ε_{max} of retinal$_1$ and retinal$_2$ is equal within less than 10%).

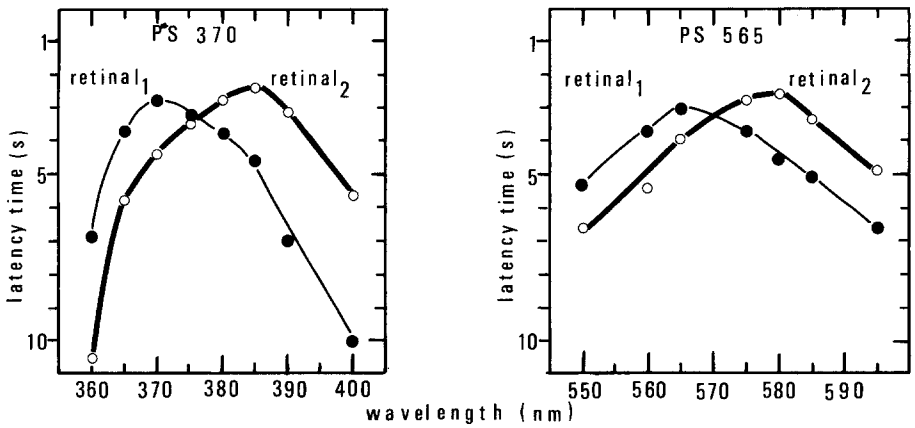

Fig. 3. Action spectra of the photophobic responses of *Halobacterium halobium,* grown in the presence of nicotine and then incubated with *trans* retinal$_1$ and *trans* retinal$_2$, respectively. Step-up response (PS 370): Sensitivity of the system at different wavelengths is given as the latency time after the onset of the light flash of 2 s duration and an intensity of 2.2×10^{10} photons/mm^2 s. Step-down response (PS 565): Sensitivity is given as the latency time after the onset of the negative flash of 2 s duration, i.e., interruption for 2 s of a permanent illumination of 3.4×10^{11} photons/mm^2 s. Temperature 24° C. Each point is the mean of 20 measurements, s. D. was less than 10%. Curves were four times reproduced with new cultures

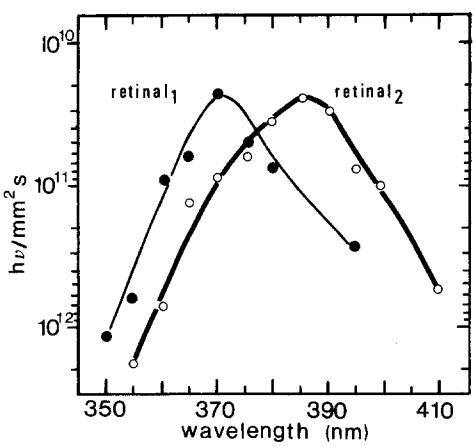

Fig. 4. Action spectra of the step-up response (PS 370) of *Halobacterium halobium,* grown in the presence of nicotine and then incubated with *trans* retinal$_1$ and *trans* retinal$_2$, respectively. Sensitivity at different wavelengths is defined as the number of photons necessary to evoke a standard response. A latency time of 2.1–2.2 s at 30° C was chosen as the standard response. Each point is the mean of 20 measurements, s. D. was less than 5%. Curves were reproduced four times with new cultures

The fact that two different retinals with different absorption spectra lead to different action spectra, points to retinal being the chromophore of the photosensory pigments in both photosystems. These sensory pigments are most likely protein retinal complexes, the protein components of which are not yet known.

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