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EVIDENCE THAT THE LONG-LIFETIME PHOTOINTERMEDIATE OF S-RHODOPSIN IS A RECEPTOR FOR NEGATIVE PHOTOTAXIS IN HALOBACTERIUM HALOBIUM

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SUMMARY The effect of blue background light on behavioral response of Halobacterium halobium to step-like stimulation with green-orange attractant light was examined. The results strongly support the previously proposed hypothesis that a long-lifetime photointermediate of s-rhodopsin is the photoreceptor for repellent light: the step-like increase in green-orange light was convertible from attractant stimulus to repellent one, when the cells were constantly illuminated with blue light. No difference of the threshold intensity of the blue background light was observed between the mutant strain that lacks both bacteriorhodopsin and halorhodopsin and the wild type strain, suggesting that the two light-driven ion pumps are not participant in sensing attractant light.

Two sensory photosystems apparently control the behavior of Halobacterium halobium (1). One, named PS565, causes the cells to be attracted to green-orange light that is effective for energizing the cell membrane through a light-driven H^+ pump, bacteriorhodopsin (bR) (2), and a light-driven Cl^- pump, halorhodopsin (hR) (3,4). Avoidance of the cells from blue-near ultraviolet light is mediated by the other photosystem, PS370, which has maximal sensitivity at 370 nm (1). Receptors for both photosystems are still ambiguous (5), although it was established earlier that both systems require retinal (6,8). A step-like increase in blue-near UV light or a step-like decrease in green-orange light causes a reversal of the swimming direction of the cells (step-up or step-down behavioral response, respectively) after a short latent period that depends on the intensity of the stimulus. Inverse stimulus either with the repellent light or with the attractant light causes a prolonged period of smooth swimming.

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The signals from PS370 and PS565 are integrated inside the cells: when a step-like increase in repellent light and a step-like decrease in attractant light are simultaneously introduced into the cells, the cells respond more quickly than to one of the stimulus alone (9). In addition, Dencher (6) reported that PS370 obeys Weber's law; i.e., the sensitivity for the step-up increase in repellent light is weakened by continuous illumination with the repellent light.

Therefore, as a possibility, one can expect that the attractant response should be emphasized if PS370 is inhibited by continuous illumination with blue-near UV light.

On the other hand, if the photoreceptor for repellent light is a long-lifetime photointermediate (denoted as sR_{373} after the wavelength of absorption maximum) of s-rhodopsin (sR) (10-12) as suggested by Bogomolni and Spudich (11), presence of the blue-near UV background light should inhibit the step-down response of the cells to attractant light, because the attractant light functions so as to generate the photoreceptor for the repellent light.

Therefore the effect of blue background light on photoattractant system (PS565) is a key to distinguish between the two possibilities described above. In this communication, we describe that the result shows that the latter is the case. Furthermore, the experimental design is presented so as to give a clue to the question what is the receptor for PS565 or whether bR or hR contribute the photosystem. The results obtaned suggest that the photoreceptor is sR.

MATERIALS AND METHODS

The strains used were Flx3 (bR^-, hR^-, sR^+), OD2w (bR^-, hR^+, sR^+)—generous gifts from J. L. Spudich, R1 (bR^+, hR^+, sR^+)—a generous gift from E. Hildebrand and A. Schimz, and S9 (bR^+, hR^+, sR^+). All these cells were used after the selection for motility (9). Cells were grown in 10 ml culture tubes. At stationary growth phase, 40-50 µl of the cell culture was transferred into 2.2 ml of pepton medium (pH 7.0) enriched with trace metals (9), and incubated for 2 hours prior to measurement.

Essence of the automated method for the measurement of phototactic response was described earlier (7). To introduce the second

background light (from 100W high-pressure mercury lamp), the microscope (Nikon XB-Ph-11) was modified with a half mirror so that the sum of the observing light (from 12V-50W tungsten halogen lamp) and the second beam passed into the specimen through a phase-contrast condensor.

Monochromatic light for actinic irradiation or for the second background illumination was obtained with use of narrow band interference filters (KL-series, Toshiba).

All measurement were performed at 37 °C.

RESULTS AND DISCUSSION

Fig.1 shows the effect of blue constant background illumination on step-down response of the cells to attractant light. The blue background illumination inhibited the response almost completely when 50 % lower intensity (photons/mm^2s) than that of the green actinic light was applied. The result solely argues against the idea that PS370 has an independent photoreceptor other than sR_{373}, if following three possibilities can be excluded experimentally. [1] The blue light as well as any other background light could cause the adaptation of the cells to attractant light. [2] Since we might always observe the response to the integrated signals from PS370 and PS565, the blue continuous illumination leads to enhancement of PS370 which gives rise to an apparent decrease in PS565. [3] The blue light blocks the sensory transduction.

Possibility [2] is unlikely because we also observed that the step-up response to blue-near UV light was inhibited or unaffected by

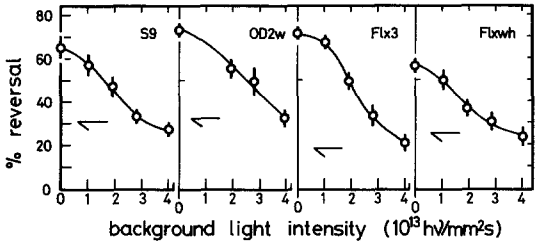
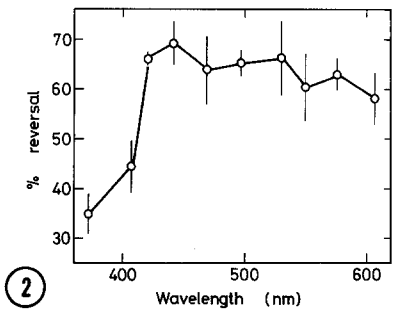


Figure 1. Effect of blue (407±14 nm) background light on step-down responses of *H. halobium* strain S9 (bR^+,hR^+,sR^+), OD2w (bR^-,hR^+,sR^+), Flx3 (bR^-,hR^-,sR^+), and Flxwh (bR^-,hR^-,sR^+) to attractant light. Flxwh is a spontaneous mutant which contains less carotenoids obtained from Flx3. Ordinate is the per cent of the reversed cells during 3 sec after sudden interruption of actinic irradiation (565±15 nm, 7.3×10^{13} hν/mm^2s). Arrows indicate the levels of the spontaneous reversal of the cells. Each point represents average and standard error (vertical bars) of the results from 3-4 measurements, during each of which (15 min) 100-150 tracks of the cells were counted. Actinic light was interrupted for 4 sec in every 15-20 sec.



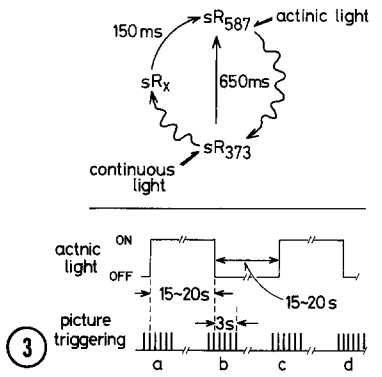


Figure 2. Wavelength dependence of the background light effect on step-down response of *H. halobium* strain Flx3 to attractant light. The intensity of the background light was adjusted to 16 W/m^2 with neutral density filters. The intensity of the light for observation (λ>600 nm) and the actinic light (576±15 nm) were 44 W/m^2 and 6.2×10^{13} hν/mm^{2}s), respectively. Bars represent the standard errors from three measurements (100-150 tracks of the cells, each).

Figure 3. UPPER: Simplified scheme of the photocycle of sR (11). LOWER: Timing for the automated measurement of the step-up and the step-down responses. Tracks of the cells were automatically detected from 6 consecutive digitized pictures a, b, c, ---, recorded with a video-computer system (7). The step-up responses were obtained from a, c, ---. and the step-down responses were from b, d, ---.

blue background illumination. In order to test the possibility [1], the background effect was measured at various wavelength (Fig.2). Although red background light inhibited the photoattractant response to some extent, the effect was further enhanced in blue-near UV region indicating that possibility [1] is not the case.

In order to examine whether the effect comes from sR photocycling as suggested in ref.12 or is due to the possibility [3], we measured the step-down and the step-up responses simultaneously, subjecting repeated pulses of green-orange actinic light (see Fig.3 lower). The result with bR,hR-deficient strain Flx3 is shown in Fig.4-A. The green-orange light was converted from an attractant stimulus to a repellent one for the cells, when the intensity of the blue background light was increased.

The result not only excludes the possibility [3] , but strongly supports the hypothesis that the long-lived photointermediate (sR_{373}) of sR is the photoreceptor for repellent light. The mechanism of the conversion was simply explained as follows. When the photo-

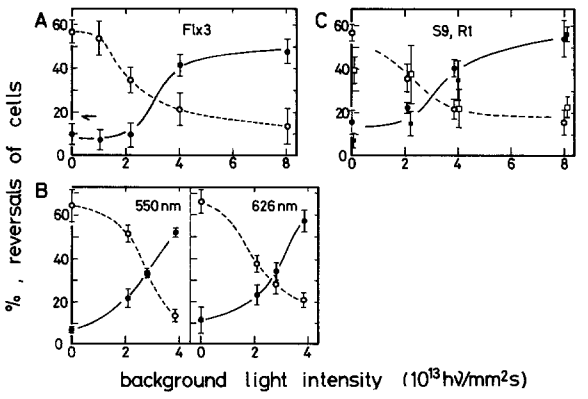


Figure 4. Effect of blue (407±14 nm) background light on both step-up and step-down responses of *H. halobium* to intermittently irradiated actinic light. --○-- , --□-- : step-down responses. —●— , —■— : step-up responses. Each point represents the average from 4-6 measurements (40-50 cell tracks, each) with standard errors. Abscissa: Intensity of the blue background light (407±14 nm). A) Responses of strain Flx3 to 583nm actinic light (half width 15nm, intensity 6×10^{13} hν/mm^{2}s). Light for observation was red (λ>600 nm, 44 W/m^2). Note that the red background light may cause the adaptation of PS565. B) Responses of the strain Flx3 to 550 nm and 626 nm actinic light (half width 15 nm, intensity 6×10^{13} hν/mm^{2}s). Light for observation was dark red (λ>650 nm, 32 W/m^2). C) Responses of strain S9 to 583nm actinic light (half width 15nm, intensity 6×10^{13} hν/mm^{2}s)(○,●) and strain R1 to 565nm actinic light (half width 15 nm, 6×10^{13} hν/mm^{2}s) (□,■). Light for observation was red (λ>600 nm, 44W/m^2).

intermediate is generated upon absorbing the actinic light, it immediately acts as a photoreceptor for blue light so as to elicit repellent response of the cells.

The conversion induced by blue backeground light can be used as a clue to the survey of photoreceptor pigment(s) for PS565. At medium intensity of the blue light, the green-orange actinic light contains the two opposite signals for the cells: attractant signal and the repellent one.

Because the repellent signal in this case is caused by photoactivation of sR, the dependence of the repellent signal—if it is distinguished from the integrated signal—on actinic wavelength should be equal to the absorption spectrum of sR. On the other hand, one can not predict the genuine action spectrum of PS565, until the evidence is obtained which shows what is the photoreceptor of PS565 (5). Therefore, the dependence of the conversion of the step-up and the step-

down responses on actinic wavelength is expected to reflect the difference spectrum between the action of PS565 and the absorption of sR.

In this point of view, we compared the background intensities that causes the crossing of the step-up and the step-down responses at the two actinic wavelengths, 550nm and 626nm, where sR has the similar extinction coefficient across the absorption maximum (13). As shown in Fig.4-B, these are almost identical. We considered the result as a support for the hypothesis that sR is the photoreceptor of PS565.

Since the lifetime of sR_{373} is at least about two orders of magnitude longer than photointermediates of bR or hR, it is unlikely that the effect of blue light is also caused by the photointermediate of bR or hR. Therefore the effect of blue background light on PS565 in bR,hR-containing strain can be used as a measure of the possible contribution of bR or hR to PS565, because the contribution of sR to PS565 and that to PS370 should be cancelled under the blue background illumination that causes the crossing of the step-up and the step-down responses of bR,hR-deficient strain Flx3.

The effects of blue background light on the response of H. halobium S9 (bR^+, hR^+, sR^+) and of R1 (bR^+, hR^+, sR^+) are shown in Fig.4-C. These were almost indistinguishable from that of bR,hR-deficient strain Flx3. At present, it seemed less likely juding from Fig.4 that bR or hR participates in photoattractant sensing. Our data on aerotaxis of the cells also supported that there is no $\Delta\tilde{\mu}H^+$ sensing mechanism in H. halobium (to be published). Further studies including the refinement of the action spectrum of PS565 are in progress in this laboratory.

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