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# ACTION SPECTRA OF THE PHOTOPHOBIC RESPONSE OF BLUE AND RED FORMS OF *Blepharisma japonicum*

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**Abstract**—When exposed, in the presence of molecular oxygen, to light intensities of the order of 3–30 W $m^{-2}$, the ciliate *Blepharisma japonicum* changes its color from red to blue, because of the photooxidation of the photoreceptor pigment, blepharismin, to oxyblepharismin. Both red- and blue-pigmented cells show step-up photophobic responses. The action spectra of the light-dependent behavior of the red and the blue form of *Blepharisma* have been determined; their structure is very similar to that of the absorption spectra of the red and blue pigment, respectively. These findings suggest that the photosensing and phototransducing properties of blepharismin are maintained in its photooxidized form, oxyblepharismin.

## INTRODUCTION

When exposed to light irradiances above about 30 W $m^{-2}$, in the presence of oxygen, the heterotrichous ciliate *Blepharisma japonicum* is readily killed by photodynamic reactions in which the endogenous red pigment, blepharismin, closely related to hypericin,[1] acts as photosensitizer.[2]

Upon a sudden increase in light intensity, *Blepharisma* also exhibits an avoiding motile reaction (step-up photophobic response), with a threshold of about 0.02 W $m^{-2}$ for dark-adapted cells. Action spectroscopy indicates that blepharismin is the photoreceptor pigment responsible also for this photoresponse.[3,4]

In addition to photoavoiding reactions, there is another light-induced process that favors the survival of *Blepharisma*

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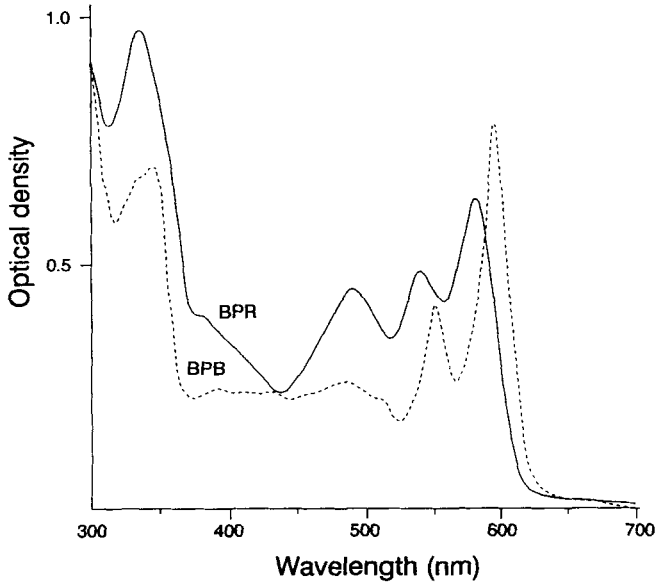
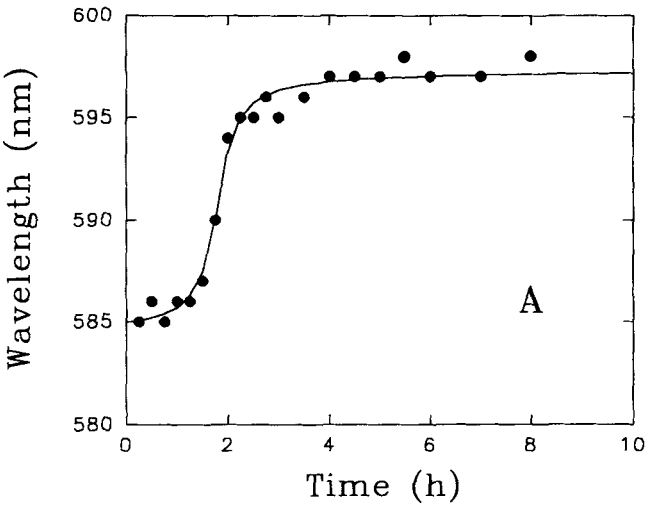



Figure 1. Absorption spectra of ethanol solutions of acetone-extracted blepharismin (BPR) and oxyblepharismin (BPB).



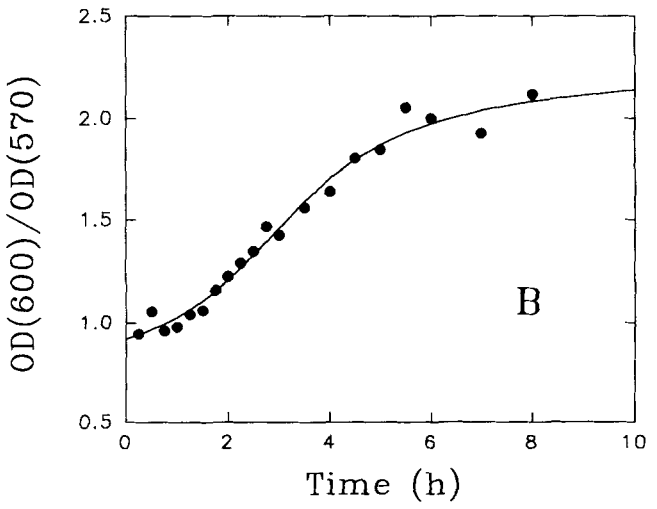




Figure 2. A: Shift of the absorption maximum of *Blepharisma* pigments (crude extract in acetone solution) *vs* irradiation time in the presence of oxygen. B: Ratio of optical densities at 600 nm and 570 nm of *Blepharisma* pigments (crude extract in acetone solution) *vs* irradiation time in the presence of oxygen. Fluence rate, 8 W $m^{-2}$.

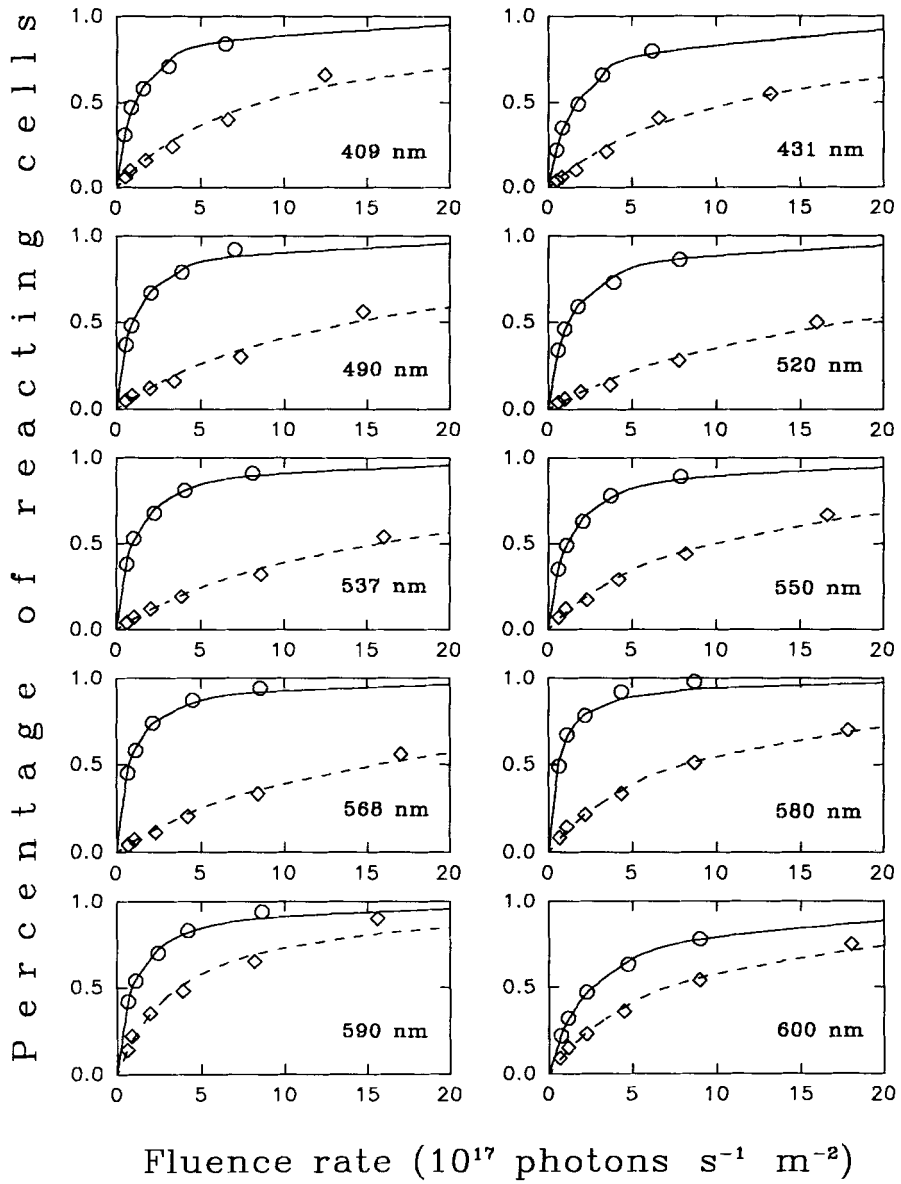



Figure 3. Fluence–response curves for red (full line) and blue (broken line) cells. Data points fitted with a hyperbolic function of the type $y = x/(x + a)$.

cells: if the cells are exposed to fluence rates between about 3 and 30 W $m^{-2}$, in the presence of oxygen, the native red form of blepharismin is progressively converted into a blue form, which is not phototoxic for the cells.[2] These blue *Blepharisma* cells can, in fact, be exposed to fluence rates of the order of 1500 W $m^{-2}$ for times up to 20 min, without any detectable viability impairment.[5]

In agreement with Giese and Zeuthen,[6] this photomodified blepharismin can be identified as an oxidized blepharismin, oxyblepharismin, on the basis of the following evidences: (1) no light effect on red cells in the absence of oxygen; (2) absorption spectra of extracts from blue cells are identical to those of red cell extracts treated with oxidant agents like tetranitromethane (unpublished results) and ozone.[7] This process seems not to be reversible. In fact, cells in a resting medium remain blue, whereas in nutrient medium they are able to resynthetize the red pigment. Moreover, in agreement with Giese and Grainger,[7] we have ascertained that blue extracts treated with reductant agents do not revert to the red form.

In a series of preliminary experiments, it was shown that, upon sudden increases of fluence rates blue cells also exhibit step-up photophobic response.[8]

A central point to clarify is the nature of the photoreceptor pigment responsible for the photoresponse of the blue cells. In fact, even though the absorption spectra of pigment extracts from light-treated *Blepharisma* are definitely different from those of red cell extracts (Fig. 1), trace amounts of red blepharismin could be still present in blue cells and still capable of acting as light detector and transducer.

To ascertain the nature of the photoreceptor pigment for blue *Blepharisma,* the action spectrum of the photophobic response of these cells has been determined and its structure compared with that of the action spectrum of the photomotile response of red *Blepharisma.*

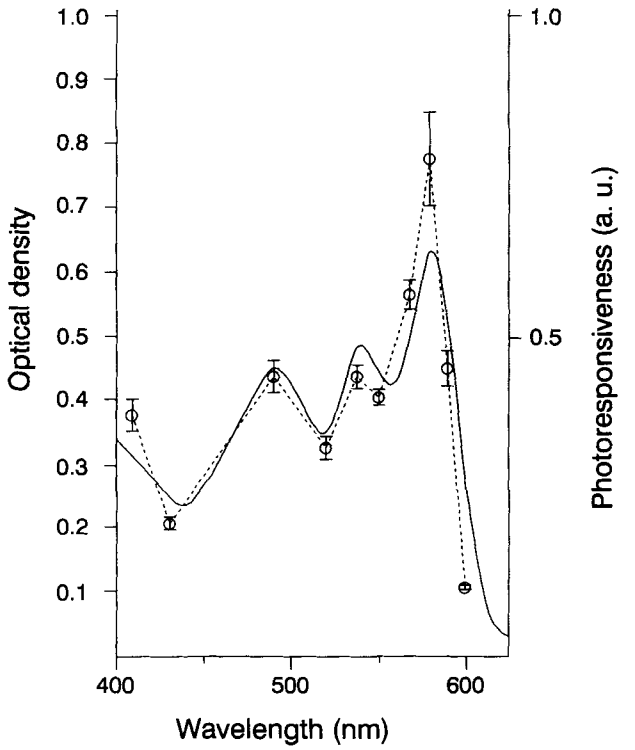



Figure 4. Action spectrum (broken line) of the photophobic response of red cells and absorption spectrum of blepharismin extracts in ethanol (full line).

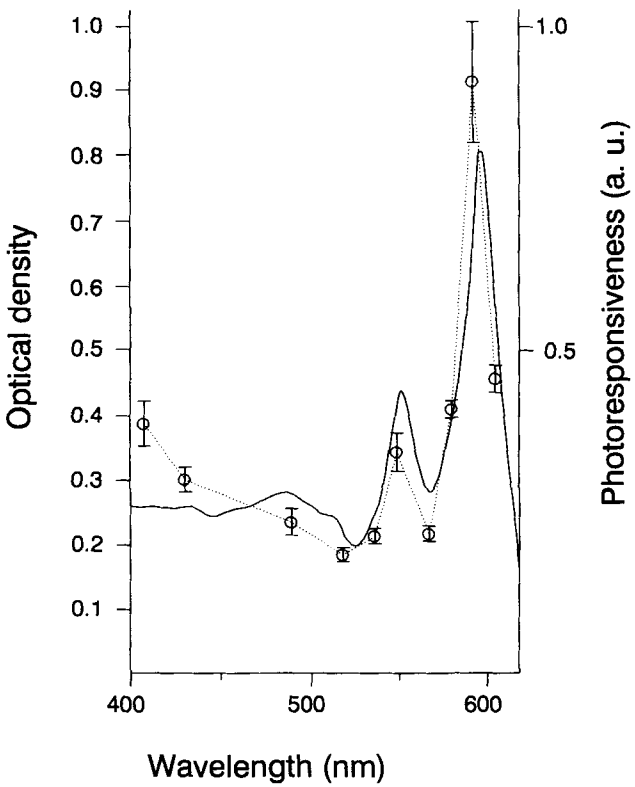



Figure 5. Action spectrum (broken line) of the photophobic response of blue cells and absorption spectrum of oxyblepharismin extracts in ethanol (full line).

## MATERIALS AND METHODS

*Blepharisma japonicum* has been grown as previously described.[3,9] In order to photooxidize blepharismin *in vivo*, cells were gently centrifuged, washed and resuspended in a resting medium, and then exposed to cold white light (fluence rates, 8 W $m^{-2}$) in a thermostatted room at 296°K. Low density cellular suspensions were used to avoid cell-to-cell screening and facilitate homogeneous exposure to light for all the cells.

To assess the degree of photooxidation of the pigment, absorption spectra of extracts in acetone solution were measured at different irradiation times, collecting 0.5 mL volumes from a 20 mL cell suspension. As shown in Fig. 2A, after 3 h of irradiation the absorption maximum in the visible shifts from 585 nm to 598 nm (respective blepharismin and oxyblepharismin peaks in ethanol as well as in acetone solution). In contrast, the ratio between the absorbances at 600 nm and 570 nm increases up to 7–8 h of irradiation (Fig. 2B). In all our experiments cells were kept under illumination for 36 h in order to fully oxidize blepharismin into oxyblepharismin.

After such an irradiation period, cells were motile and viable; even after 10 days of light treatment cells were blue-colored and pigment acetone extracts had an optical absorption spectrum typical of oxyblepharismin. Our results do not agree with the observations of Giese,[2] who reported that after 96–150 h of irradiation *Blepharisma* was fully bleached.

Red cells were kept in the same resting medium, in the dark, for the same time period as the blue ones. Photomotile responses of individual cells have been recorded and analyzed by means of a microvideorecording system previously described.[10]

Irradiation wavelengths were selected by means of Balzers (Lichtenstein) interference filters (about 10–12 nm bandwidth at half-height); light intensities were varied by means of Balzers neutral density filters.

To determine action spectra, for each actinic wavelength the percentage of responding cells was plotted *vs* fluence rate. Experimental data points of each dose–response curve were fitted with a hyperbolic function of the type suggested by Lipson[11]:

$$y = x/(x + a)$$

where $y$ is the response (percentage of cells showing step-up photophobic response) and $x$ is the fluence rate. The parameter $a$ has the dimension of a fluence rate. Its inverse, equal to $dy/dx$ for $x = 0$ and corresponding to the slope of the dose–response curve in the origin, was chosen as photoresponsiveness index and plotted on the ordinate in the action spectrum.

Acetone crude extracts of red and blue blepharismin chromophores were prepared according to Sevenants[1] and Ghetti *et al.*[5]

## RESULTS AND DISCUSSION

As shown in Fig. 3, the dose–response curves reach saturation at fluence rates significantly lower for red cells than for blue ones; as a matter of fact, for blue cells fluence rates up to about 15–20 × $10^{17}$ photons $m^{-2}$ $s^{-1}$ have been used, whereas for red cells the highest fluence rate used was about 10 × $10^{17}$ photons $m^{-2}$ $s^{-1}$.

In Figs. 4 and 5, the action spectra of the photophobic response of red and blue cells, respectively, are shown, together with the absorption spectra of blepharismin and oxyblepharismin in ethanol solution.

These data unambiguously indicate that the photoreceptor pigment for *Blepharisma* photobehavior is blepharismin for red cells and oxyblepharismin for blue cells.

This means that the molecular transformation of blepharismin, following light-induced oxidation, does not modify its reactivity as far as its photoperception and photosignalling function is concerned. Moreover, such a molecular modification, as mentioned above, reduces the photodynamic potency of oxyblepharismin as an endogenous sensitizer in blue *Blepharisma* cells.[5] The fact that the endogenous pigment, after oxidation, retains its phototransducing capability but

loses the photodynamic properties of the native form, is in agreement with our previous data showing that photosensitized reactions do not play any significant role in triggering photomotile responses in *Blepharisma*.[10,12]

Notwithstanding the capability of oxyblepharismin to function as photoreceptor pigment, its efficiency in triggering the motile response seems to be lower than that of blepharismin, as may be inferred from the dose–response curves reported in Fig. 3.

Work is in progress to assess whether this difference can be correlated with spectroscopic parameters, such as fluorescence lifetimes and quantum yields, of blepharismin and oxyblepharismin. It appears that blepharismin shows a lower fluorescence quantum yield and shorter fluorescence lifetime than oxyblepharismin (Bisi *et al.*, in preparation). Thus, it is reasonable to associate higher phototransducing efficiency of blepharismin with its overall lower radiative transition rate constant compared to oxyblepharismin.[13,14] However, phenomena such as physiological light adaptation of the cells and/or photoinduced loss of pigment must also be investigated to fully account for the structure and differential functions of blepharismin and oxyblepharismin.

## REFERENCES


1. Sevenants, M. R. (1965) Pigments of *Blepharisma undulans* compared with hypericin. *J. Protozool.* **12**, 240–245.
2. Giese, A. C. (1981) The photobiology of *Blepharisma*. *Photochem. Photobiol. Rev.* **6**, 139–180.
3. Scevoli, P., F. Bisi, G. Colombetti, F. Ghetti, F. Lenci and V. Passarelli (1987) Photomotile responses of *Blepharisma japonicum* I: action spectra determination and time-resolved fluorescence of photoreceptor pigments. *J. Photochem. Photobiol. B Biol.* **1**, 75–84.
4. Ghetti, F. (1991) Photoreception and photomovement in *Blepharisma japonicum*. In *Biophysics of Photoreceptors and Photomovements in Microorganisms* (Edited by F. Lenci, F. Ghetti, G. Colombetti, D.-P. Häder and P.-S. Song), pp. 257–265. Plenum, New York.
5. Ghetti, F., P. F. Heelis, G. Checcucci and F. Lenci (1992) A laser flash photolysis study of the triplet states of the red and the blue forms of *Blepharisma japonicum* pigment. *J. Photochem. Photobiol. B Biol.* **13**, 315–321.
6. Giese, A. C. and E. Zeuthen (1949) Photooxidation in pigmented *Blepharisma*. *J. Gen. Physiol.* **32**, 525–535.
7. Giese, A. C. and R. M. Grainger (1970) Studies on the red and the blue forms of the pigment of *Blepharisma*. *Photochem. Photobiol.* **12**, 489–503.
8. Checcucci, G., F. Ghetti and F. Lenci (1991) Action spectra of the photophobic response of the blue and red *Blepharisma japonicum*. IV Congress Eur. Soc. Photobiol., Abstract E-2, Amsterdam, 1–9 Sept. 1991.
9. Miyake, A., T. Harumoto, B. Salvi and V. Rivola (1990) Defensive function of pigment granules of *Blepharisma japonicum*. *Eur. J. Protistol.* **24**, 310–315.
10. Checcucci, G., F. Lenci, F. Ghetti and P.-S. Song (1991) A videomicroscopic study of the effect of a singlet oxygen quencher on *Blepharisma japonicum* photobehavior. *J. Photochem. Photobiol. B Biol.* **11**, 49–55.
11. Lipson, E. D. (1991) Action spectroscopy. In *Biophysics of Photoreceptors and Photomovements in Microorganisms* (Edited by F. Lenci, F. Ghetti, G. Colombetti, D.-P. Häder and P.-S. Song), pp. 293–309. Plenum, New York.
12. Ghetti, F., G. Checcucci and F. Lenci (1992) Photosensitized reactions as primary molecular events in photomovements of microorganisms. *J. Photochem. Photobiol. B Biol.* **15**, 185–198.
13. Lenci, F., F. Ghetti, G. Colombetti, D.-P. Häder and P.-S. Song (eds.) (1991) *Biophysics of Photoreceptors and Photomovements in Microorganisms*. Plenum, New York.
14. Song, P.-S., S. Suzuki, I. D. Kim and J. H. Kim (1991) Molecular properties of biological light sensors. In *Biophysics of Photoreceptors and Photomovements in Microorganisms* (Edited by F. Lenci, F. Ghetti, G. Colombetti, D.-P. Häder and P.-S. Song), pp. 21–41. Plenum, New York.