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Effects of UV-B irradiation on motility and photoresponsiveness of the coloured ciliate *Blepharisma japonicum* ☆

Antonella Sgarbossa [a], Sabina Lucia [a], Francesco Lenci [a,*], Domenico Gioffré [a], Francesco Ghetti [a], Giovanni Checcucci [a,b]

[a] *Istituto di Biofisica del CNR, Via San Lorenzo 26, Pisa, Italy*
[b] *Dipartimento di Scienze dell'Ambiente e del Territorio dell'Università, Via A. Volta 4, Pisa, Italy*

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Abstract

The effects of artificial UV-B irradiation on motility, viability, photomotile responses and photoreceptor spectroscopic properties of the red ciliate *Blepharisma japonicum* were investigated. Both the mean velocity of the cells and the percentage of motile cells exhibiting step-up photophobic responses significantly decreased, increasing UV-B irradiation time. No marked difference with dark-kept control samples was observed in cells irradiated with visible light. Absorption and fluorescence measurements on cell suspensions indicated that no UV-B induced bleaching or spectroscopically detectable damage of the photoreceptor chromophore took place. The experimental results suggest that the UV-B target is a component of the photosensory transduction chain different from the photopigment itself and that cell vitality is damaged by a direct effect of UV-B, without any significant contribution from a pigment-sensitized photodynamic reaction.

Keywords: Blepharisma japonicum; Motility; Photomovement; Photoreceptor; Sensory transduction; UV-B

1. Introduction

The effects of ultraviolet radiation are currently investigated in a variety of aquatic microorganisms and ecosystems, in order to study both the UV-induced stress on single unicellular organisms (reduction of photosynthetic activity, decrease in growth and metabolic rates, DNA damage, impairing of photo- and graviorientation in the water column) and its repercussions on a population scale. For this purpose, artificial light sources in the laboratory as well as solar radiation in diverse natural habitats have been used [1–3].

From the vast amount of findings reported in the literature, a few examples of recent works in progress [4] are reported in Table 1. These data show that both solar and artificial UV-B irradiation significantly affect viability, growth rate, cell division processes, photosynthetic capability in all the examined systems, even though at different rates and with different efficiency. UV-A irradiation, on the contrary, can cause severe damages in some biological systems, but may also have minor effects, or no effect at all, in other ones [5–9].

Of particular interest are those aquatic microorganisms which are provided with light-sensing and signal-transducing apparatuses which enable them to migrate, vertically and/or horizontally, toward the best lit regions for their metabolism and growth. UV-B irradiation could be, in fact, perceived and transduced as a repelling sensory signal, but can also have a spoiling effect, possibly significantly faster and prevailing, on the photosensing apparatus. As a consequence, even after short UV-B exposures, cells may become unable to swim toward, or gather into, a shadowed niche, and thus to escape from harmful illumination conditions [10–19] (Table 2).

This paper reports the results of a comparative study of the effects of short term irradiations with UV plus visible light as well as with visible light only on the red coloured ciliate *Blepharisma japonicum*. This mi-

☆ Dedicated to our friend Prof. Dr. Donat-Peter Häder on the occasion of his 50th birthday.

* Corresponding author.

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Table 1
Effects of solar and artificial UV-A and UV−B irradiation on some biological systems described in the literature

Biological system	Light sources	Techniques	Main observations and/or conclusions	Refs.
Cyanobacteria	Tropical solar (Ghana, 4.30° N)	Cell motility measurements	UV-B, UV-A and visible all effective in motility impairment Only partial recovery and only after short exposure times	[5]
Dunaliella and *Ochromonas*	Solar radiation with and without UV-B component Artificial	O_2 photosynthetic evolution PAM Fluorescence	Specific effect of UV-B on photosynthetic efficiency	[6]
Euglena	Solar radiation at: 200 m a.s.l. 2957 m a.s.l.	Pigment optical absorption Cell motility measurements	Faster pigment bleaching and motility damage at 2957 m than at 200 m Solar at 2957 m+filter for UV-B→ Some protection Increased UV-B levels may have serious effects on phytoplankton populations	[7]
Periphytic algal assemblages	Artificial, in laboratory microcosm coral reef Solar tropical radiation, coral reef (Caribbean)		UV-B affects productivity and community structure — No significant effect of UV-A Unfiltered solar radiation reduces of 40% primary productivity of assemblages, with respect to those UV-B protected — No inhibitory effect of UV-A	[8]
Phytoplankton in coastal lagoons of Southern Baltic Sea	Solar radiation	Direct measurements of solar UV irradiation	UV-B decrease to 1% after penetration of water columns some dm; UV-A penetrates for some meters ↓ UV damage to phytoplankton closer to surface	[9]

Table 2
Photomotile responses of a few biological systems described in the literature

Microorganism	Light source	Motility damage	Photoresponse inhibition	Refs.
Cryptomonas sp. (freshwater)	Artificial	+ +	+ + +	[10]
Cryptomonas sp. (freshwater)	Solar	+ +	+ + +	[11]
Euglena gracilis	Artificial	+	+	[12]
Euglena gracilis	Solar	+	+	[13]
Fabrea salina	Artificial	–	+ + + +	[14]
Peridinium gatunense	Artificial	+	+ +	[15]
Peridinium gatunense	Solar	+	+ +	[16]
Phormidium uncinatum	Artificial	+ + + +	+ + +	[17]
Phormidium uncinatum	Solar	+ + + + +	+ + +	[18]
Volvox aureus	Solar	–	+ + + + +	[19]

croorganism reacts to light stimuli exhibiting step-up photophobic responses, that, according to action spectroscopy experiments, are mediated by the endogenous pigment blepharismin [20–22].

The effects of irradiation have been tested on viability, motility and photoresponsiveness of single cells. Absorption and fluorescence spectra of cell suspensions have also been measured, in order to ascertain the occurrence of UV-induced molecular alterations of blepharismin.

As blepharismin is also a photosensitizer, responsible for cell photokilling at high light intensities [23], a further question to clarify was the possibility that the observed consequences of UV-B irradiation were merely due to the photosensitising properties of the pigment, rather than to a direct effect of ultraviolet exposure.

2. Materials and methods

Blepharisma japonicum was grown as previously described [24]. The cells were collected by gentle centrifugation at about 100*g*, resuspended in a saline resting medium (SMB, Saline Medium for *Blepharisma*, [25]) and kept in the dark overnight to get them fully adapted to the new medium.

Cell suspensions in SMB, at a concentration of about 1500 cells ml^{-1}, were irradiated by means of three TL40W/12 Philips UV-B lamps. For every irradiation, each sample was covered with a new cellulose acetate film in order to remove the UV-C wavelengths. Besides experiments without additional coloured or pass-band filter, irradiations were performed with a Schott WG 1 filter ($T<0.1\%$ for $\lambda \leqslant 340$ nm, $T=50\%$ at 370 nm and $T \geqslant 85\%$ for $\lambda \geqslant 400$ nm) to eliminate the UV-B range and to use only the light emitted by the lamps in the visible. In both cases the total irradiance (UV-B + UV-A + visible and UV-A + visible) was kept about the same (~ 5 W m^{-2}) by means of UV-transparent neutral density filters. The spectral distributions were approximately 52% UV-B, 27% UV-A and 21% visible, and 0% UV-B, 16% UV-A and 84% visible with the filter. For the sake of simplicity, in what follows the two irradiation conditions will be referred to as "UV-B" and "visible" irradiation, respectively.

In the "UV-B" conditions, the artificial UV-B irradiance (~ 2.5 W m^{-2}) is comparable to that in the natural environment. UV-A and visible irradiances, on the contrary, are approximately 30 and 400 times lower than the natural ones.

Light irradiance was measured by means of two United Detector Technology detector heads (222UV and 211, equipped with a radiometric filter) and of a Newport 840 Power Meter. As the angular light acceptance of the UV detectors and of the samples was virtually the same, the measured irradiance values were not corrected.

Samples were exposed to light for 30 and 60 min. Each sample was irradiated only once and examined immediately after irradiation, dividing the cell suspension in two parts: one for microscopic observations and behavioural analysis and the other one for spectroscopic measurements. Control measurements have also been performed on dark-kept samples.

Photoresponsiveness and velocity of individual cells were measured by means of a microvideorecording apparatus. The percentage of cells responding to light stimulation was calculated by analysing the videorecorded paths. To induce step-up photophobic responses, a non-saturating actinic light (580 nm, 0.2 W m^{-2} [22]) was used. Cell velocity was measured by means of an on-line computerised image-analysis system controlled by a previously described software [26].

Absorption and fluorescence spectra (measured on cell suspensions, rather than on extracts, in order not to alter the physiological molecular environment of the pigment systems) were recorded with a JASCO 7850 spectrophotometer, equipped with a JASCO TIS-417 integrating sphere, and a JASCO FP 770 spectrofluorimeter, respectively. All the absorption and fluorescence measurements have been performed by diluting 1/1 (v/v) the cell suspensions in a 0.2% agar suspension. In such a viscous medium cells do not undergo sedimentation and, at the same time, keep their shape and remain alive for many hours.

3. Experimental results

3.1. Behavioural measurements

UV-B irradiation, in our experimental conditions, markedly altered the shape and the movement pattern of *B. japonicum*: cells appeared more and more rounded as a function of irradiation time, an increasing number of them becoming completely spherical (about 20% and 40% after irradiation for 30 and 60 min, respectively). A gradual rise in the number of cells that swim following circular trajectories, with a short curvature radius, was also observed. Furthermore, the number of avoiding reactions to mechanical stimuli (swimming direction reversal upon collision with an obstacle) decreased after 30 min and was reduced to zero after 60 min of UV-B irradiation. Visible-irradiated cells, on the contrary, did not show any of the above-described morphological modifications and appeared to swim like dark-kept control cells.

However, both UV-B and visible irradiation caused a decrease in the cell population speed, even though with a quite different time scale. As shown in Fig. 1, in fact, after 30 and 60 min of UV-B irradiation, the mean velocity of the cells was reduced from 118 μm s^{-1} (measured in dark-kept control samples) to 59 μm s^{-1} and 7 μm s^{-1}, respectively, whereas, with visible irradiation, it did not significantly change after 30 min (111 μm s^{-1} and still had a value of 63 μm s^{-1} after 60 min.

A specific and dramatic effect of UV-B irradiation on *B. japonicum* step-up photophobic responses could be ascertained measuring the percentage of motile cells which still responded to light stimuli. From Fig. 2 it is clear that, already after 30 min, UV-B exposure made about 50% of motile cells unable to react to photic stimuli, whereas visible irradiation was substantially ineffective in suppressing the response. Sixty minutes of UV-B irradiation were, finally, enough to fully cancel photoresponsiveness of *B. japonicum*. Despite the decrease in the mean velocity of the cells, also after 60 min of UV-B irradiation the number of motile

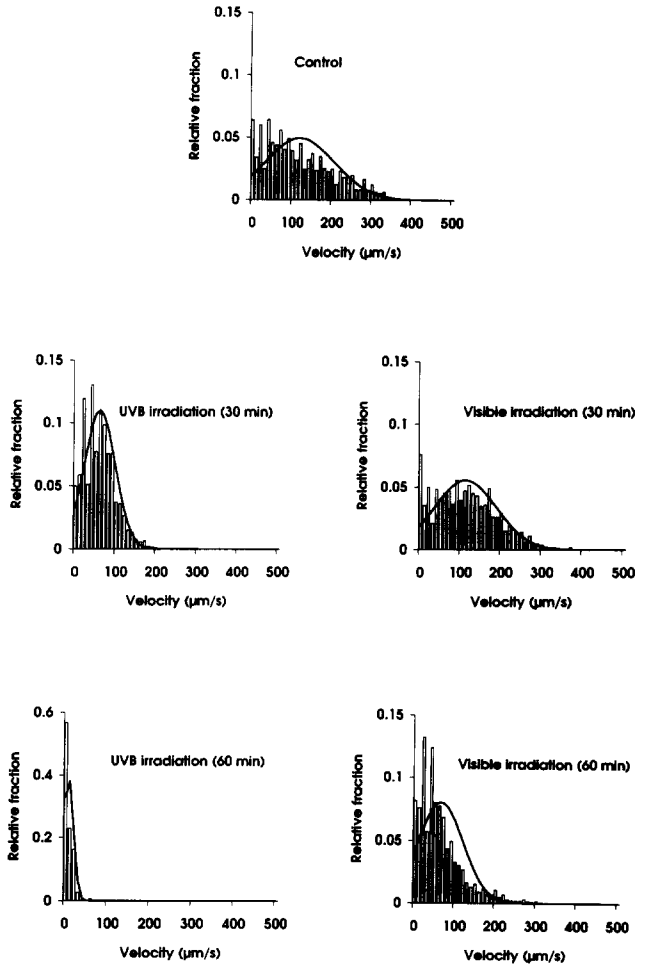

Fig. 1. Histograms of the velocity distribution of *B. japonicum* cells before (control) and after 30 and 60 min irradiation with UV-B and visible light ($\lambda \geq 280$ nm and $\lambda \geq 340$ nm respectively). Continuous lines represent the corresponding gaussian distributions, calculated from mean velocities and standard deviations of the sample distributions. Each histogram is the result of about 3500 measurements, except in the case of 60 min of UV-B irradiation, where the number of measurements was about 1500.

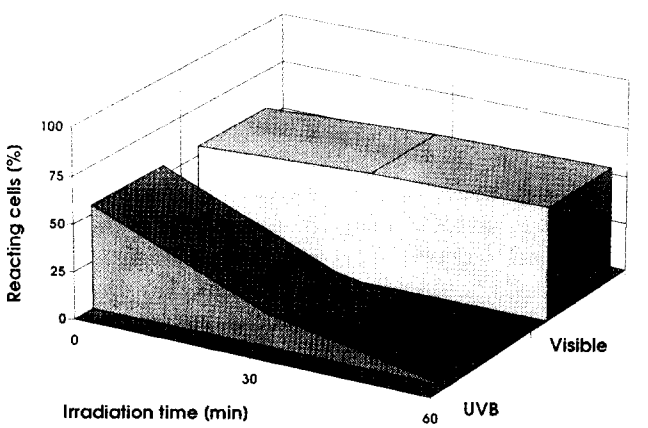

Fig. 2. Effect of irradiation time with UV-B and visible light ($\lambda \geq 280$ nm and $\lambda \geq 340$ nm respectively) on the percentage of motile *B. japonicum* cells showing step-up photophobic responses.

cells in each sample was still high enough to allow reliable photobehavioural measurements.

3.2. Spectroscopic measurements

Absorbance measurements were performed in the range 400–700 nm, where the characteristic structure of blepharismin absorption spectrum [20] can better give information about specific UV-B-induced fading and/or molecular changes of the pigment.

As shown in Fig. 3(A), for irradiation times up to 30 min, no significant difference was observed between the optical absorption spectra of UV-B- and visible-irradiated cells. Both of them, however, slightly differed from the absorption spectrum of the control sample because of a partial intracellular photo-oxidation of blepharismin. This process always occurs when *B. japonicum* cells are irradiated with dim light in the presence of oxygen [23,27]. When photo-oxidation attains saturation, the cells appear blue–grey and, while maintaining their photosensing capability [21,22], do not undergo photokilling, even if exposed to high light intensities [28]. After 60 min of irradiation (Fig. 3(B)),

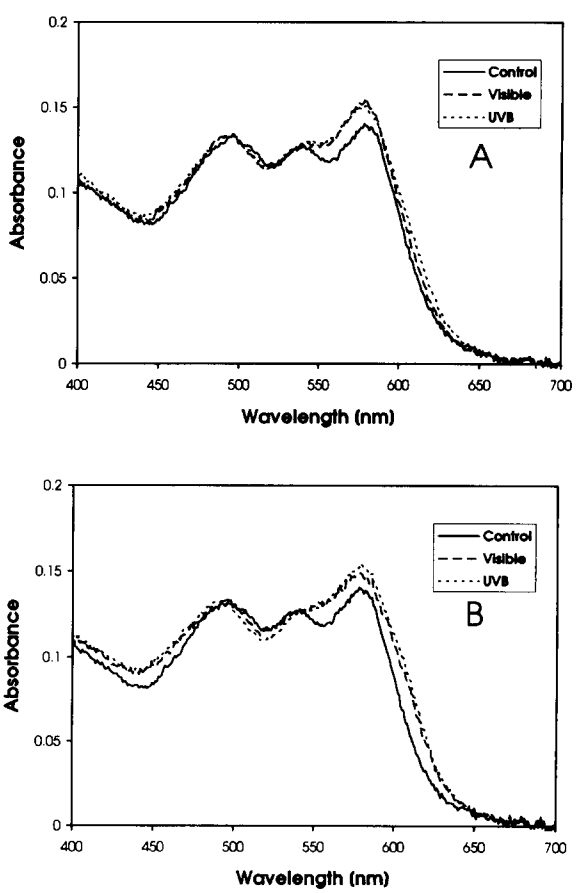

Fig. 3. Absorption spectra of agar suspensions of *B. japonicum* cells, before (control) and after irradiation for 30 min (A) and 60 minutes (B) with UV-B and visible light ($\lambda \geq 280$ nm and $\lambda \geq 340$ nm respectively).

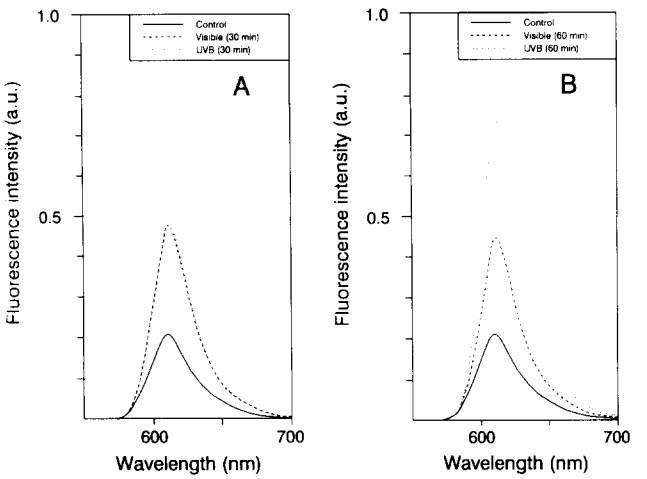

Fig. 4. Fluorescence emission spectra (excitation wavelength = 364 nm) of agar suspensions of *B. japonicum* cells, before (control) and after irradiation for 30 min (A) and 60 min (B) with UV-B and visible light ($\lambda \geqslant 280$ nm and $\lambda \geqslant 340$ nm respectively).

a larger amount of blepharismin was intracellularly photo-oxidized, but still no difference was observed between the optical absorption spectra of the two irradiated samples.

Fluorescence spectra of cell suspensions were contaminated by scattered light and the blepharismin emission peaked at about 600 nm largely overlapped the long-wavelength tail of the "natural" blue–green fluorescence emitted by the cell body [29]. Therefore, a cut-off filter ($T < 0.1\%$ for $\lambda \leqslant 575$ nm, $T = 50\%$ at 592 nm and $T \geqslant 85\%$ for $\lambda \geqslant 615$ nm) was put on the emission beam in order to select blepharismin fluorescence.

After 30 min of irradiation, fluorescence emission increased of the same amount, in comparison with the control, both in UV-B- and visible-irradiated samples (Fig. 4(A)). After 60 min, whereas the emission from the latter did not significantly change, that from the former showed another marked increase (Fig. 4(B)). The slight bathochromic shift observed in the fluorescence spectra can be attributed to the above mentioned partial intracellular photo-oxidation of blepharismin to oxyblepharismin. However, as suggested by one of the referees, a minor change in the molecular environment of the chromophore due to photodynamic action cannot be definitely ruled out.

4. Discussion

In the case example of the flagellate *Euglena gracilis* (see Table 2), the specific UV-B-induced suppression of photomotile responses has been attributed to a direct effect of high energy radiation on the photodetector system, responsible also for a loss in the efficiency of the energy transfer process from pterins to flavins [12,13].

In the case of *B. japonicum*, neither photoinduced fading in the optical absorption spectra nor spectral distribution variations in both absorption and fluorescence spectra were observed (Fig. 3 and Fig. 4). This indicates that UV-B irradiation did not cause specific molecular transformation of blepharismin, the photo-oxidation to oxyblepharismin having also been observed after visible irradiation.

The observed apparent increase of fluorescence quantum yield (Fig. 4) might be due to a partial photoinduced disruption of the functional structure of the photoreceptor apparatus, as revealed in single cells by means of a fluorescence imaging technique [29]. However, as previously reported [23], *B. japonicum*, even in the presence of dim light, tends to extrude pigment, in order to avoid the hurtful consequences of blepharismin-sensitized photodynamic reactions. Taking into account that our cells have been grown and kept in the dark before irradiation, the increase in fluorescence intensity observed in the visible-irradiated samples can be attributed to the beginning of this process, with a detachment of a certain percentage of blepharismin from the photoreceptor apparatus. In the case of UV-B-irradiated cells, this effect appeared to be much more relevant, probably indicating a severe damage of the molecular environment of blepharismin.

Conversely, already after 30 min of irradiation, even though the fluorescence increase was identical to that of visible-treated cells, only about 17% of the UV-B-irradiated cells still responded to light stimulation, in comparison to 59% and 55%, in the case of the visible-treated and the control cells respectively. After 60 min of irradiation, photoresponsiveness was totally suppressed with UV-B and not substantially altered with visible light.

On the basis of these experimental results, the UV-B-induced inhibition of photoresponsiveness in *B. japonicum* can be explained by assuming that UV-B causes a damage to the photosensory transduction chain, rather than a failure in the light detector system. This hypothesis is supported also by recent time-resolved fluorescence measurements on blepharismin, showing that the fluorescence lifetimes and the relative amplitudes of the different species emitting at 600 nm are not affected by UV-B irradiation [30].

Some hypotheses can be put forward concerning the nature and the localisation of these UV-B targets in the sensory chain, such as, for instance, electron/proton translocating macromolecules and membrane channels. A support to the latter suggestion can be provided by the observation that mechanical avoiding reactions too were suppressed by UV-B irradiation. Such an inhibition too, in fact, could be due to a damage at the membrane level. A contribution to answer some of the open

questions can certainly come from the determination of the action spectrum for this photoresponse suppression, and work is in progress to clarify this point.

As mentioned in the introduction, a problem deserving particular attention in coloured microorganisms is the possible relevance of photosensitized reactions mediated by UV-B absorbing endogenous pigments. At this time, however, it is possible to suggest that in *B. japonicum* the lethal effects of UV-B are not mediated by a blepharismin sensitized photodynamic reaction. Preliminary experiments, in fact, showed that UV-B damages nearly at the same extent both red and blue *B. japonicum* cells, the latter having been shown not to undergo sensitized photokilling [28].

From this work it is possible to conclude that UV-B radiation, besides causing foreseeable global cellular damages, the consequences of which are for example the altered cell shape and motile behavior, can specifically impair the photosensory capability of *B. japonicum* in relatively shorter times, thus "trapping" the cell in a noxious light environment.

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