Journal of Photoscience (2000), Vol. 7(1), pp. 1 – 4

Effect of Electron Acceptors on Step-up Photophobic Responses of *Blepharisma japonicum*

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The photosensory ciliates *Blepharisma japonicum and Stentor coeruleus* use the hypericin-derived pigments blepharismin and stentorin, respectively, as photoreceptor chromophores. Fluorescence quenching studies have shown that the first excited singlet state of hypericin and the purified chromophores blepharismin and stentorin can be deactivated by electron transfer to an acceptor molecule with a suitable reducing potential [1,2]. This paper reports the result of a series of photobehavioral experiments performed with the aim to ascertain if the same electron acceptors which quench the photoreceptor pigment fluorescence in vitro may also compete with the native acceptor molecule in its natural physiological environment. Individual cell trajectories were examined before and after light stimulation, in the presence and in the absence of potential "in vivo" electron acceptors, with a microvideo-recording apparatus. Our data, on *Blepharisma* cells, showed that as the negative reduction potential of the electron acceptor increases, a pronounced decrease in cell photoresponsiveness was detected. A dramatic effect on cell photoresponsiveness was noticed in the presence of 1,4-benzoquinone that has the lowest negative reduction potential. Such an effect on the percentage of photoreacting cells was moderate in the case of 1,4-naphthoquinone, with a relatively higher negative reduction potential. In the presence of benzophenone, which has the highest negative reduction potential, no significant effect on photoreacting cells was noticed. Our results can support the hypothesis that in the pigment granules such a light-induced charge transfer from excited blepharismin to a suitable electron acceptor triggers sensory transduction processes in *B. japonicum*.

key words: Electron acceptor, Photophobic response, *Blepharisma japonicum*

INTRODUCTION

At present it is ascertained that blepharismin and stentorin, the endogenous pigments of the heterotrichous ciliates *Blepharisma japonicum* and *Stentor coeruleus* respectively, are the photoreceptor molecules which transduce the light stimulus into an intracellular signal ultimately yielding a motile response in these cells (step-up photophobic response and negative phototaxis) [3-5]. The pigment containing granules, which are regularly spread all over the cell just beneath the plasmatic membrane [6-8], were proposed to be the cellular structures where the photosensory process initiates [5,8]. These photomotile reactions allow the cells to avoid brightly illuminated regions, where they can be killed by photodynamic reactions sensitized by blepharismin [7,9,10].

Recently, blepharismin and stentorin have been characterized and found to have a molecular structure similar to the natural photosensitizer hypericin [11,12,13]. It remains to ascertain the molecular mechanism through which these chromophores can transduce photic stimuli.

Fluorescence quenching studies performed on purified chromophores (blepharismin and stentorin) have shown that the first excited singlet state can be deactivated by electron transfer to an acceptor molecule with a sufficient reducing potential [1,2]. This light-driven charge transfer, which plays a major role in the fast deactivation of the excited pigment, has been suggested to be a possible mechanism for the primary photoprocess for photomotile responses in *B. japonicum* and *S. coeruleus* [1,2]. The same electron acceptors that quench the photoreceptor pigment fluorescence could act as electron acceptor competitors with the native acceptor molecule/amino-acid residue in its natural physiological environment and could affect the normal path of the photosensory transduction chain.

This piece of work was devoted to study the effects of some electron acceptors on the photomotile responses of *Blepharisma* red cells. The fact that the photo-responsiveness of the cells was significantly inhibited by efficient electron acceptors, such as 1,4-benzoquinone, can support the hypothesis that photoinduced electron transfer from the excited state

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Received 15 February 2000; accepted 30 March 2000

may be the signal-initiating event which the primary transduction processes originate from.

MATERIALS AND METHODS

Cell culture: *Blepharisma japonicum* was grown as previously described [14]. The cells were collected by gentle centrifugation at about 100/g, resuspended in a saline resting medium (SMB, Saline Medium for *Blepharisma*, [15]) and kept in the dark overnight to get them fully adapted to the new medium.

Chemicals: Electron acceptors (1,4-benzoquinone, 1,4-naphthoquinone and benzophenone) were purchased from Sigma-Aldrich. Mother solutions of each acceptor in SMB were prepared and kept as a stock.

Sample preparation: Cell samples were transferred in the dark into the observation chamber. The effects of different concentrations of the above electron acceptors on the photophobic response were studied by adding microliters of the acceptor stock solution to the medium and cell photoresponsiveness was examined immediately. Control measurements have also been performed in the absence of the acceptor, under the same experimental conditions. The cell samples had the same concentration and all measurements were performed at 25°C.

Photobehavioral analysis: Step-up photophobic reactions of individual cells were analyzed by means of a previously described microvideorecording apparatus [4]. A quartz-iodine lamp (50 Watt) was used as stimulating light source. An anti-heat filter was provided in front of the lamp to eliminate the extra-heat that may disturb the cells. An interference filter at 580 nm (bandwidth at half-height 10-12 nm) was also used to select the narrow band of light stimulus. All cell samples were exposed to equal irradiances of about 0.2 W m^{-2}. A shutter placed between the lamp and the cell sample controlled the actinic light source for photic stimulation. As photoresponsiveness index the percentage of cells modifying their motile pattern within 5 seconds after stimulus onset minus the percentage of unstimulated cells modifying their trajectory in the dark within 5 seconds was taken.

RESULTS AND DISCUSSION

Our data indicated that cell photoresponsiveness was clearly inhibited with decreasing the negativity of the reduction potentials of the electron acceptors. Acceptor of high negative reduction potential, on the contrary, did not show any significant effect either in dark or light.

In the presence of 1,4-benzoquinone, which has a relatively low negative reduction potential (E°(V)=-0.134), a pronounced inhibition in cell photoresponsiveness was detected (Fig. 1). As the concentration of this electron acceptor

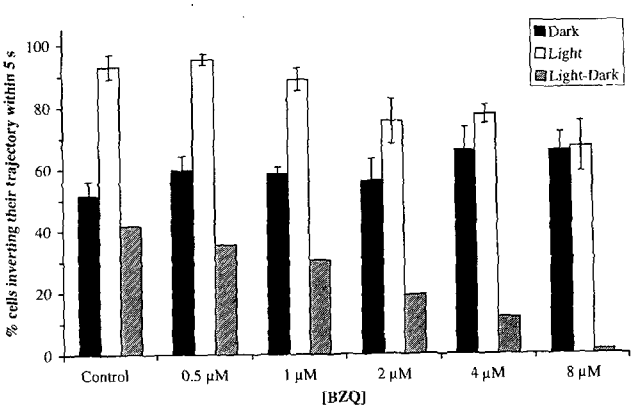

Figure 1. Histograms of the percentage of cells inverting their trajectories (step-up) versus the concentration of 1,4-benzoquinone in the dark and following light stimulation. The difference between the percentage of photoresponding cells after light stimulation and in the dark is indicative of the cell photoresponsiveness.

increases, a gradual decrease in the number of cells inverting their trajectories following light stimulation was observed (Fig. 1). In the dark too, however, cells were found to invert their trajectories at a much higher rate than in the absence of the electron acceptor. Cell photoresponsiveness was significantly reduced to zero at 8 (M concentration (Fig. 1).

As shown in Fig. 2, also in the case of 1,4-naphthoquinone, an electron acceptor with a relatively high negative reduction potential (E°(V)=-0.309), similar results were obtained. Cell photoresponsiveness was slightly decreased and again an increase of the percentage of cells modifying their trajectories in the dark was observed. The effect on photoresponsiveness was found to be moderate compared to the case of 1,4-benzoquinone in the same concentration range (Fig. 2).

No significant effects, either in dark or light (Fig. 3), were

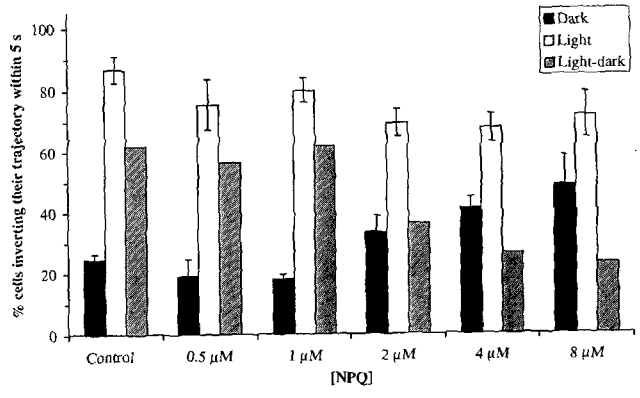

Figure 2. Histograms of the percentage of cells inverting their trajectories (step-up) versus the concentration of 1,4-naphthoquinone in the dark and following light stimulation. The difference between the percentage of photoresponding cells after light stimulation and in the dark is indicative of the cell photoresponsiveness.

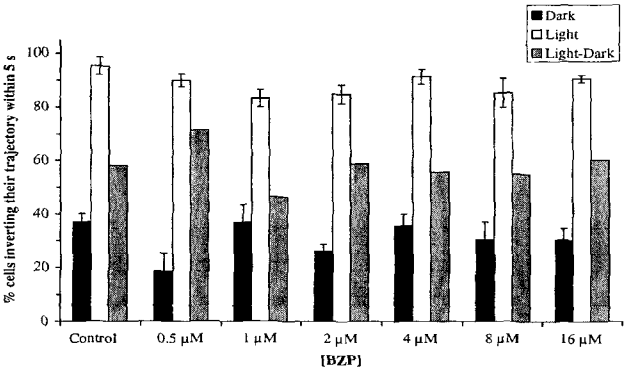

Figure 3. Histograms of the percentage of cells inverting their trajectories (step-up) versus the concentration of benzophenone in the dark and following light stimulation. The difference between the percentage of photoresponding cells after light stimulation and in the dark is indicative of the cell photoresponsiveness.

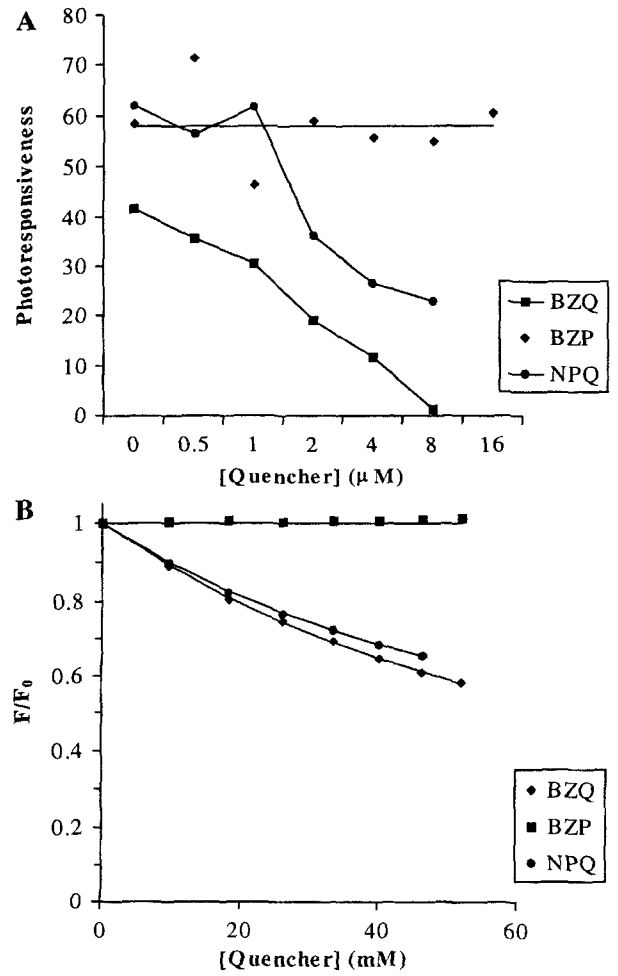

Figure 4. (A) Summary of the effects on cell photoresponsiveness of different electron acceptors (1,4-benzoquinone, 1,4-naphthoquinone and benzophenone in function of their concentration.
(B) Stern-Volmer plot of oxyblepharismin fluorescence quenching by 1,4-benzoquinone, 1,4-naphthoquinone and benzophenone (from N. Angelini et al. 1998).

observed on the cells up to 16 μM in the case of benzophenone, which has the highest negative reduction potential among the used electron acceptors (E°(V)=-1.448).

Fig. (4A) summarizes the effects of the different electron acceptors on cell photoresponsiveness as a function of their concentrations. It is clear that as the acceptor negative reduction potential decreases, a pronounced decrease in cell photoresponsiveness is detected. A dramatic effect on cell photoresponsiveness was noticed with the highest reduction potential acceptor, 1,4-benzoquinone. A moderate effect was detected with the relatively lower reduction potential 1,4-naphthoquinone. Benzophenone, with the lowest reduction potential, did not show any significant effects on the cells.

These results suggest that the same electron acceptors, which quench the photoreceptor pigment fluorescence, can be responsible for the inhibition of the photomotile responses of *Blepharisma*.

When added to cell suspension, these electron acceptors may affect the photo-transduction chain by scavenging an electron from the excited pigment, thus playing the role of a competitor of the physiological electron acceptor.

The fact that electron acceptors that inhibit the step-up photophobic response also significantly alter the unstimulated behavior of cells clearly indicates that these substances affect some metabolic pathways related to cell motility regulation. Consequently, more experiments are needed to draw unambiguous conclusions. Since now, however, the close parallelism between the effect of these electron acceptors on the photoresponsiveness of the cells (Fig. 4A) and on the fluorescence yield of the isolated photoreceptor pigment allow to suggest a photoinduced electron transfer can be an early step of the photosensory perception and transduction chain in B. japonicum.

Acknowlegements–Authors gratefully acknowledge financial support from CNR in the framework of the Strategic Project "Biological Sensors and Biosensors". Tareq Youssef is indebted to "ICTP Programme for Training and Research in Italian Laboratories, Trieste, Italy" for supporting his work in Istituto di Biofisica, CNR, Pisa. This work was accomplished within the framewark of the CNR-asrt agreement.

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