Biophysical Chemistry 29 (1988) 155–159
Elsevier

BPC 01224

Signal perception and amplification in photoresponses of cyanobacteria

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Accepted 15 October 1987

Cyanobacteria; Electric gradient; Gating channel; Phobic response; Phototaxis; Proton-motive force; (*Phormidium uncinatum*)

In addition to a number of other external stimuli, the gliding cyanobacterium, *Phormidium uncinatum*, uses light as a clue to control movement in order to find and stay in a microhabitat with optimal conditions for growth and survival of the population. Of the three photoresponses developed in cyanobacteria, phototaxis, photokinesis and photophobic responses, the latter are the most prominent for this task. Step-down phobic responses are mediated by the photosynthetic pigments and are linked to the linear electron-transport chain. A change in the fluence rate is reflected by a change in the proton-motive force which is amplified by a massive Ca^{2+} influx after a phobic stimulation. This event reverses the electric gradient between front and rear end of the filament which controls the direction of movement. Step-down phobic responses prevent the organisms from moving into dark areas, while step-up responses elicited by an increase in the fluence rate, found recently, allow the organisms to avoid excessively bright areas in which their photosynthetic pigments would be photobleached within a short time. The action spectra of the two responses differ drastically. The antagonism of the two phobic responses allows a population to find a suitable niche in their environment by a rather delicate balance and fine-tuned adjustments.

1. Introduction

Like many other motile microorganisms, gliding cyanobacteria respond to a number of external stimuli, such as light, chemical and thermal gradients as well as mechanical clues, in order to select a suitable niche in their environment with optimal conditions for survival and growth of the population [1]. Light certainly plays a major role not only for photosynthetic but also for other organisms.

Both prokaryotic and eukaryotic microorganisms have developed three basically different strategies to respond to the various physical properties of light [2]: Phototaxis, which is defined as a movement with respect to the direction of the light, may conceptually be the easiest of these, however, it poses a formidable task for a microorganism. Humans utilize an extensive amount of optics and neurophysiology to determine the brightest spot in an environment; obviously this is not a trivial problem for an aneural organism. In fact, while some cyanobacteria, such as *Anabaena*, have developed a true phototactic steering mechanism [3], others, including the filamentous *Phormidium*, operate on a trial-and-error basis [4].

Photokinesis describes a dependence of the linear velocity of an organism on the incident fluence rate (independent of the direction of light). In all photosynthetic prokaryotes as well as some eukaryotes this phenomenon can be explained by the extra photosynthetic energy available to the motor apparatus [2]. In cyanobacteria this may be either ATP from cyclic and/or noncyclic photophosphorylation or the proton-motive force itself generated in the thylakoids. The third and, probably in many cyanobacteria, ecologically most important mechanism is the photophobic response which is a transient motor response triggered by a sudden change in the fluence rate (dI/dt).

In *P. uncinatum* a phobic response can be induced by a temporal change in the fluence rate

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or by a spatial variation, e.g., when an organism crosses a light/dark boundary [5]. Both a step-up and a step-down in the fluence rate may cause a photophobic reaction. A typical response is a delayed stop followed by a resting period of up to several seconds and a final reversal of movement [6].

2. Photoperception and modulation of the thylakoid proton-motive force

The photoreceptor molecules responsible for step-down phobic responses are the photosynthetic pigments. (It should be mentioned in passing that the action spectra for phototaxis and photokinesis are different from that for photophobic responses in *Phormidium*). It has been shown in a number of studies using uncouplers that the organisms do not detect a change in fluence rate in terms of a modulation of the energization of the cells (ATP) [7]. Rather, the primary signal is linked to the linear electron-transport chain [8]. The first step in the transduction chain is thought to be a modulation of the proton gradient across the thylakoid membrane [9]. In light, the plastoquinone shuttle transports protons from the cytoplasm into the thylakoid vesicles which may or may not have a connection to the cytoplasmic membrane. When the front end of a filament moves into a dark area the proton-motive force breaks down rather rapidly. The resulting electric potential change is sensed by the organism and initiates a sequence of events finally resulting in the reversal of movement.

In fact, by using very fine microglass electrodes (200 nm tip diameter) light-dependent electric potential changes could be detected intracellularly [10]. If light-induced potential changes are actually involved in sensory transduction it should be possible to bypass the photoreceptor and initiate a reversal of movement through pH-jump experiments. In fact, when the pH is suddenly dropped from the growth pH into the range between 4.9 and 5.5, reversal of movement can be induced in the dark (or better still in infrared light of wavelength > 780 nm, which serves as the monitoring light for the infrared-sensitive CCD camera, but does not affect any visible response in the organisms) [9]. Inhibition of the membrane potential change by application of the lipophilic cation triphenylmethylphosphonium ($TPMP^+$), which penetrates membranes and thus breaks down existing electric gradients, strongly inhibits photophobic responses [11].

3. Signal amplification by gated cation currents

However, the threshold of step-down photophobic responses is rather low so that the resulting change in proton-motive force cannot account for the measured potential change. Thus, we have to assume an amplification process which could be an enzyme cascade, as has been found in, e.g., vision [12] or gating of ionic currents [13]. Enzymatic reactions and cyclic nucleotides have been postulated to occur but have not yet been established beyond doubt in phobic responses of *Phormidium* [14]. Instead, the main mechanism seems to involve voltage-dependent channels.

Ionophores specific for monovalent cations such as valinomycin were found to be ineffective in impairing phobic responses [15]. The Ca^{2+} ionophore calcimycin (A23187), however, specifically inhibits phobic responses [15–17]. Likewise, Ca^{2+} blockers, such as lanthanum and ruthenium red, as well as organic Ca^{2+} antagonists, such as gallopamil hydrochloride (D600) and nitrendipine, block phobic response specifically [15]. *Phormidium* survives the complete removal of cations from the medium and is even motile after being washed in distilled water with some EGTA added for 24 h. After this treatment the filaments were unable to respond photophobically at a light/dark boundary [18]. Subsequent addition of 1 mM Ca^{2+}, which is about the concentration found in pond water, completely restores the photophobic response.

From the experimental results detailed above a model was derived (fig. 1) which assumes that the initial change in the fluence rate detected by photosynthetic pigments (C-phycoerythrin, C-phycocyanin, chlorophyll *a* and possibly some carotenoids) causes a change in the proton-motive force across the thylakoid membranes. The con-

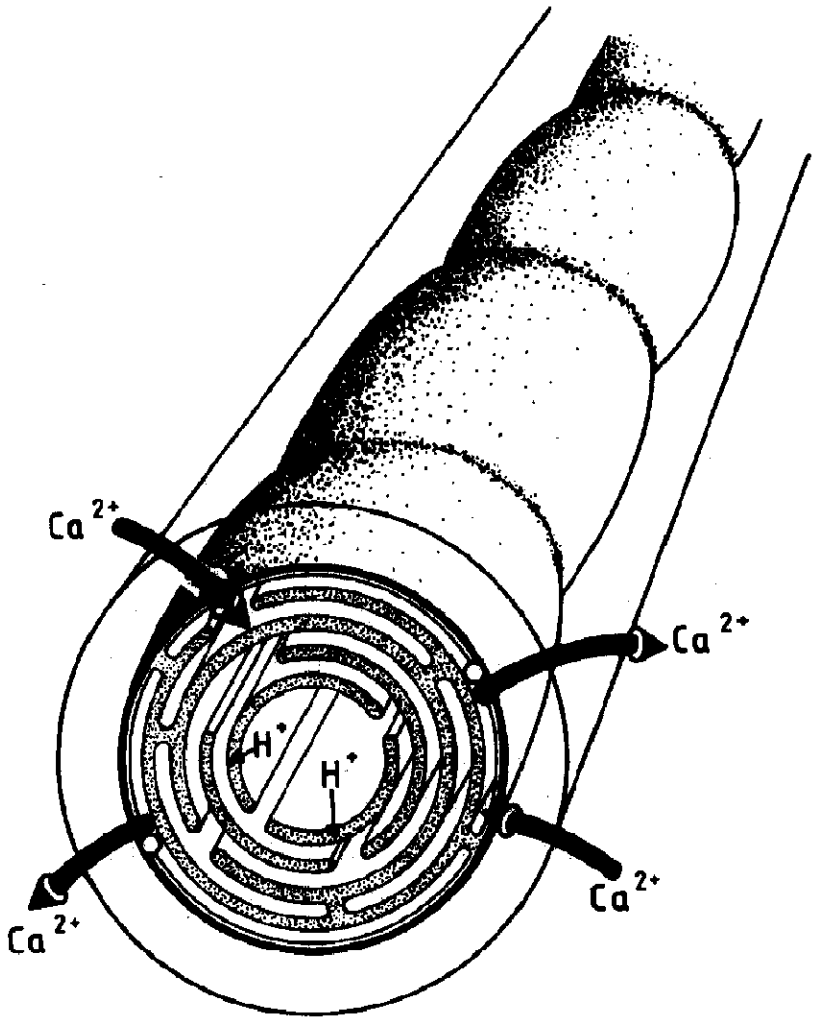

Fig. 1. Model of the sensory transduction chain of photophobic responses in *Phormidium uncinatum* (from ref. 1).

comitant electric potential change triggers the opening of voltage-dependent, Ca^{2+}-selective channels in the cytoplasmic membrane which allows a temporary massive influx of Ca^{2+} into the cells along a previously established gradient. Two factors remain to be established: the direction of the ionic current has to be demonstrated and the existence of Ca^{2+} pumps must be confirmed.

The direction of the cation current during a photophobic response can be analyzed using radioactively labelled Ca^{2+}. Dark-adapted filaments bind a certain amount of $^{45}Ca^{2+}$, probably in their slime sheath when incubated for 5 s [18]. After transfer into moderate light, which does not induce a photophobic response, almost the same amount of label is incorporated. However, upon darkening of the light-adapted cells (which causes a phobic response) $^{45}Ca^{2+}$ is taken up in massive quantities by the cells. The kinetics corresponds well with that of a photophobic reaction. Subsequently, the $^{45}Ca^{2+}$ is again extruded by the action of the pumps. The existence of a Ca^{2+}-dependent ATPase can be demonstrated by the use of the inhibitor poly(L-lysine) which decreases the phobic response; however, it has only limited solubility in water and thus the effect is not total.

4. Step-up photophobic responses

Recently, a second, step-up photophobic response was found using bright laser beams in a microscope preparation of *P. uncinatum* (unpublished data). The fluence rate-response curve indicates that this response is used to avoid bright areas in the environment. Upon entering a field of bright light a reversal of movement is initiated. The action spectrum is different from that of step-down photophobic responses, showing a three-peaked maximum in the blue region which resembles the activity of a blue light receptor [19] and also having a second maximum in the absorption range of C-phycoerythrin; however, it shows no involvement of chlorophyll (fig. 2).

5. Control of the motor apparatus

Both step-up and step-down photophobic responses have been simulated by a computer model [20,21] which assumes that each cell has a negative electric potential with respect to the outside which is modulated by light, ionic currents and active pumps. The direction of movement is determined by a gradient between the two ends (morphologically not different from each other) of a trichome: the temporarily leading front has a higher potential than that of the rear. In fact, such potential differences have been measured using extracellular electrodes [10,22,23]. The light-dependent potential change was found to exceed 10 mV between the two ends and the action spectrum resembles that of step-down photophobic responses [24].

Upon entering a dark area, the electric potential in the front end breaks down due to the massive Ca^{2+} influx which eventually causes a reversal of the front-of-rear gradient resulting in reversal of movement. Since such potentials are probably based on ionic currents inside the cells moving through the length of the filament, coun-

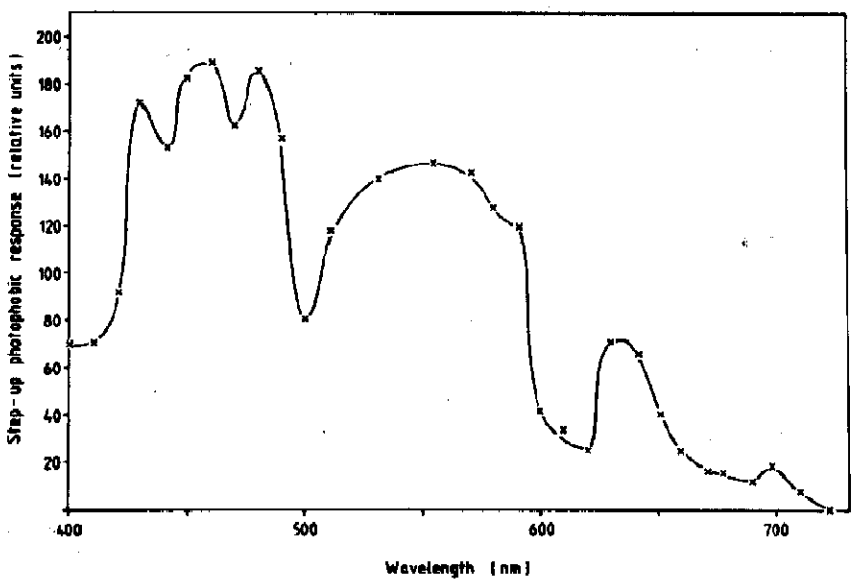

Fig. 2. Action spectrum of step-up photophobic responses in *P. uncinatum*.

tercurrents were sought in the vicinity of the trichome using the vibrating platinum electrode technique [25]. Howéver, no currents could be detected in the medium adjacent to the *Oscillatoria* species involved in this test. Thus, it could be speculated that the countercurrent is restricted to the slime sheath which is constantly produced by all cells and in which the filament moves.

The next step to be revealed concerns the mechanism of movement which is still enigmatic in cyanobacteria and the control of the motor apparatus by the temporary electric gradient between the front and rear ends. Basically, two mechanisms of movement are being considered currently [5,26]: one is based on the extrusion of slime which is sometimes produced in large quantities and is constantly shed by the organisms. However, it is difficult to imagine a reversal of movement as being effected by this mechanism; either two sets of pores need to be present or the direction of ejection must be controlled. The alternative explanation is based on the existence of undulating elements in the outer cell layers. In this case, the sheath is thought to be a passive tube and the motive force is generated at the interface between the tube and the cell wall. This has the advantage that the cells move constantly in the same milieu, generate the mechanical force over the whole circumference and that the sheath adheres to all kinds of substrata.

While no travelling waves have yet been demonstrated on the surface of cyanobacterial filaments, Halfen and Castenholz [27,28] have observed fibrils in electron micrographs of the outer layers of an *Oscillatoria* species, which might be contractile. It is interesting to note that the pitch with respect to the long axis of the filament agrees with the pitch of rotation during forward movement. Addition of 1 mM lysozyme almost instantaneously arrests movement (Häder, unpublished data). Production of the slime sheath can be suppressed by adding bacitracin which results in a reduced velocity. It can be speculated that after removal of the sheath the cells are left with a small contact zone with the substratum which decreases the motive force, since when the filaments are incorporated into a block of 1% agar after removal of the sheath the velocity increases to almost the normal value. In this experiment the organisms produce visible tunnels in the agar block and propel themselves by moving against the agar instead of the slime sheath.

6. Ecological importance of the photobehavior in cyanobacteria

Both photophobic responses serve important ecological functions. The step-down photophobic response prevents the filaments from moving into a dark area, such as the sediment in their natural habitat, simply by inducing a reversal of movement upon a sudden decrease in the fluence rate detected by the front end. By means of this simple but effective mechanism the filaments remain in irradiated areas which is a prerequisite for growth of these photosynthetic organisms [29,30]. However, most cyanobacteria are known to be typical shade organisms which thrive at fluence rates of a few hundred to a few thousand lux but are photobleached and irreversibly damaged when exposed to high fluence rates exceeding several thousand lux [31,32].

The step-up phobic response is an effective mechanism to prevent movement into and exposure to bright light. By these two antagonistic responses the organisms select a suitable habitat with an optimal fluence rate and effectively adjust to the constant changes in the light climate. This

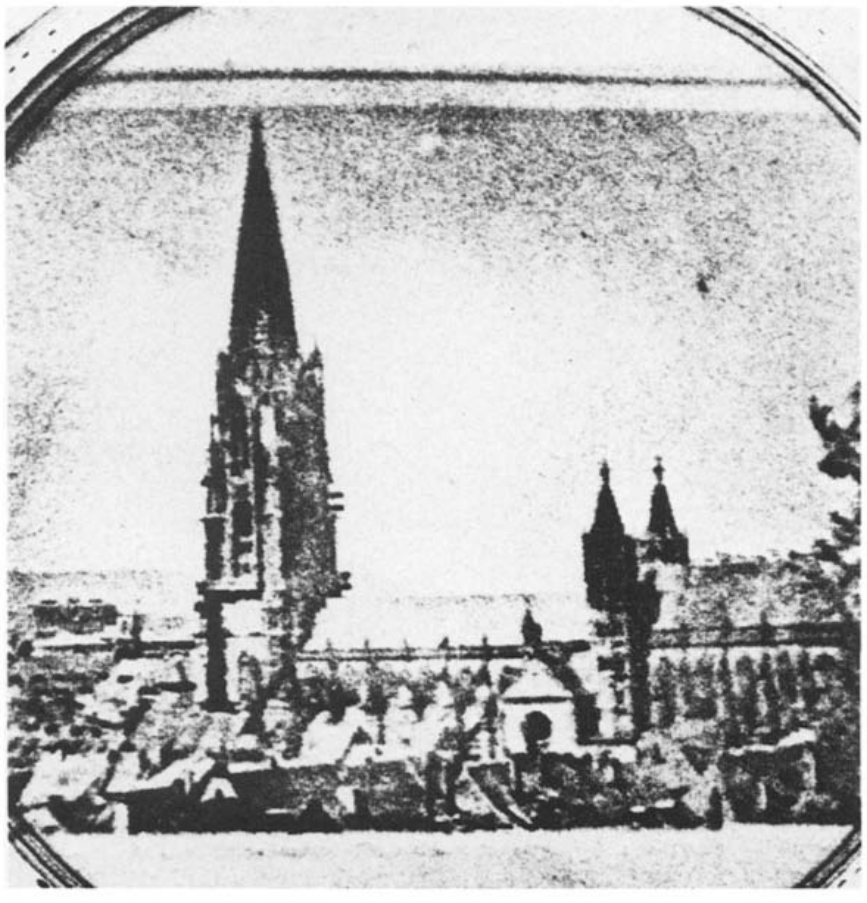

Fig. 3. Freiburger Münster. A positive was formed by photoaccumulating *P. uncinatum* when a photographic negative was projected into a preparation of the filaments (from ref. 33).

can be demonstrated with an easy and instructive experiment: when a photographic negative is projected into a homogeneous suspension of *Phormidium* filaments, the organisms avoid both excessively bright and dark areas and populate those of moderate fluence rates [33]. When the organisms are dried and fixed in their position during exposure they produce a photographic positive of the original negative (fig. 3), which shows an astonishing spatial resolution and faithfully reflects the shades of grey.

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