Bioenergetics (1970) **1**, 139–145.

Phototaxis in *Euglena*. VI. Correlations Between ATP Production by Light and Phototactic Rates*

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Abstract

Using DCMU and CCP to specifically affect the photosynthetic system, the kinetics of inhibition of photophosphorylation and phototaxis in *Euglena* are shown to be the same. In the case of DCMU, measurements of oxygen evolution demonstrate that the delay observed between exposure to inhibitor and maximum effect is not due to the time required for the DCMU to reach its site of action, thus suggesting the presence of an endogenous pool of electron donors which can bypass photosystem II. Placing a culture in darkness causes a cessation of growth and an increase in ATP content per cell, during the same time period in which phototactic activity remains constant. The results are interpreted in terms of the utilization of photosynthetically generated ATP as an energy source for phototactic orientation and a control system which acts to maintain phototactic energy reserves during an extended period of little or no photosynthetic activity.

Introduction

Phototaxis in *Euglena* involves a form of sensory perception, utilizing a well-defined photosensory organelle (stigma or eyespot plus paraflagellar swelling), in which the organism orients its swimming motion with respect to the direction of a beam of visible light.[1] As is the case with the visual response in higher organisms, it has been shown that the stimulating light serves to trigger the release of previously stored metabolic energy.[2] Experiments utilizing metabolic inhibitors have suggested that the photophosphorylation system of the photosynthetic apparatus in *Euglena* is specifically involved in providing this energy pool.[3] This work must be considered tentative, inasmuch as direct comparisons between inhibition of phototaxis and of photosynthetic responses were not made. In the present investigation we have examined the kinetics of inhibition by DCMU† and CCP of both phototaxis and photophosphorylation in a single *Euglena* culture. As will be shown, the results provide further support for the contention that photosynthetically produced high-energy compounds are involved in mediating the phototactic response.

Materials and Methods

Euglena gracilis, Z strain, was grown at 28° in a previously described[2] medium to which had been added 1 mg/l of vitamin B_1. A 10 h light, 14 h dark cycle was used. Cultures

* A portion of this work was presented at the "Conference on Phototaxis and Photokinesis in Flagellated Cells," University of California, Santa Barbara, August 21–22, 1969.

† Abbreviations used are as follows: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethyl urea; CCP, carbonylcyanide *p*-trifluoromethoxyphenyl hydrazone.

were assayed after approximately one week of growth. The general procedure was to divide a culture into four aliquots, taking care to obtain representative samples. Into two of these was pipetted an aqueous solution of inhibitor (usually 1–3 ml/10 ml of culture), and into the remaining two an equivalent amount of distilled water. The cultures were placed in the incubator at 28° until assays of activity were performed. Measurements of phototaxis and photophosphorylation were then carried out on these samples over a period of several hours.

Phototactic response was determined using the phototaxigraph.[4] The maximum slope of the accumulation curves was used as a measure of activity. White light provided the stimulus for phototactic accumulation. Photophosphorylation was measured using the firefly luciferin-luciferase assay[5] to determine ATP levels in the intact organisms. The general procedure was as follows. A 1 ml aliquot of *Euglena* suspension was removed from the culture into a 2·5 ml hypodermic syringe. This was then illuminated with red light from a 650 W tungsten-iodine lamp (Corning CS 2-62 filter plus water-cooled infra-red filter) for a given length of time (5 min in most experiments) and quickly injected into a small volume (0·5 ml) of boiling distilled water. The injection was done while the light was still on in order to minimize effects of endogenous ATPase activity. Heating of the sample was continued for 1 min, after which the sample was stored in an ice bath until the ATP assay was performed. As a control, an unilluminated sample was treated in an identical manner. The actual ATP assay was performed using either a Turner Model 111 fluorometer (without exciting light) coupled to a Heath model EUW-20A recorder or a specially built luminescence measuring device consisting of a temperature-controlled cell

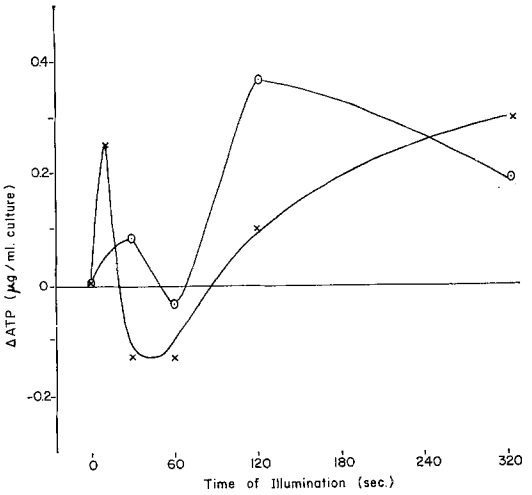

Figure 1. Effect of time of illumination with red light on ATP levels in *Euglena*. Ordinate represents difference in ATP between illuminated and unilluminated aliquots. Two separate experiments are shown.

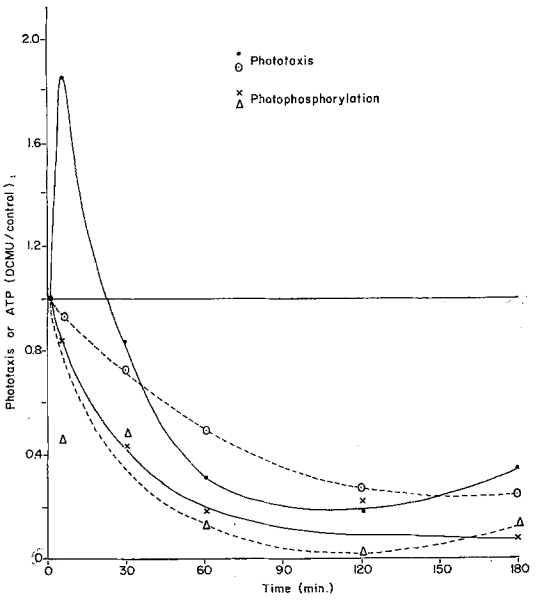

Figure 2. Time course of effect of DCMU (2×10^{-6} M) on phototaxis and photophosphorylation in *Euglena*. Ordinate represents ratio of responses of DCMU-treated culture to control culture. The results of two separate experiments are shown; comparisons should be made between solid curves and between dashed curves.

compartment (25° ± 0·1°), a Dumont 6292 photomultiplier, a Keithley Model 240 power supply, a Keithley Model 150A microvoltmeter and a Leeds and Northrup recorder. The firefly lantern extract was placed in a test tube mounted close to the face of the photomultiplier tube. The boiled *Euglena* extract was quickly injected into the tube using a hypodermic syringe with a needle which extended below the surface of the firefly enzyme solution. This allowed sufficient mixing to take place quickly. The luminescence pulse was recorded and the peak signal was used as a measure of ATP concentration. The use of the peak value avoided complications arising from the myokinase activity of the firefly extract, inasmuch as the ADP-induced luminescence burst had significantly slower kinetics than that produced by ATP. An ATP standard solution was used to calibrate the apparatus. Photophosphorylation values were calculated from the difference in response between the illuminated and unilluminated aliquots. All samples were run in duplicate. Overall reproducibility was better than 10%. The ATP levels in the illuminated samples were generally from 25 to 200% larger than in the dark controls.

There are many possible sources of error in these determinations. Probably the most significant is obtaining reproducibly representative samples of the *Euglena* suspension. This is made difficult by the settling out of the cells and by the tendency of some cultures to develop clumps of dead (or immotile) cells. The latter problem was alleviated by filtering the culture through glass wool before dividing it, and the former by thorough mixing of the suspension before removing an aliquot.

Photosynthetic oxygen evolution was measured using a Yellow Springs Instrument Co. Model 55 oxygen monitor. A Leeds and Northrup recorder was used to follow the time course of oxygen production by light.

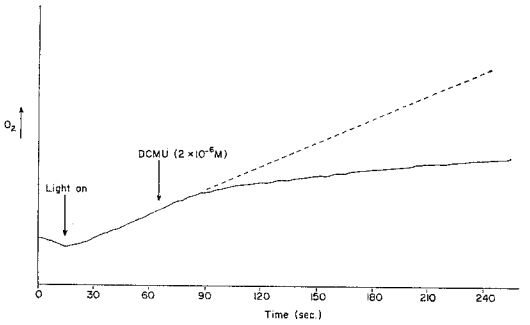

Figure 3. Effect of DCMU addition on photosynthetic oxygen evolution in *Euglena*.

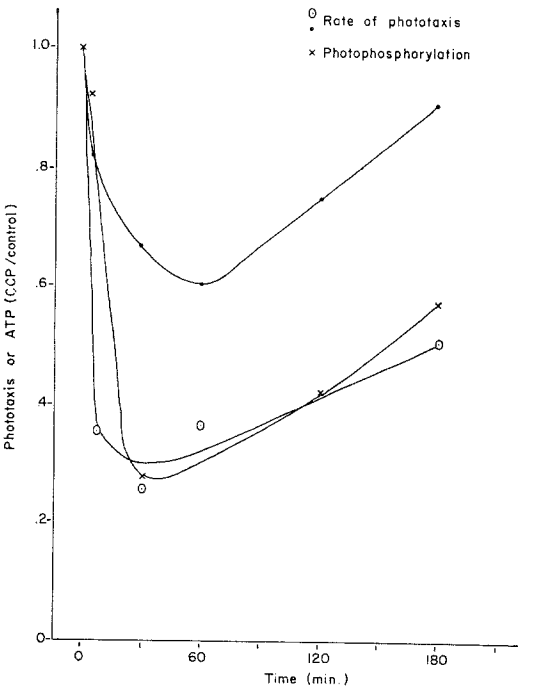

Figure 4. Time course of effect of CCP (5×10^{-6} M) on phototaxis and photophosphorylation in *Euglena*. Ordinate represents ratio of responses of CCP-treated culture to control culture. The results of two separate phototaxis measurements are shown.

Results and Discussion

The effect of varying times of red light illumination on the ATP content of a normal culture of *Euglena* is shown in Fig. 1. Transients, similar to those observed with O_2 evolution[6] and fluorescence[7] are generally found to occur during the first minute of illumination. By the end of 5 min, the ATP level in the illuminated sample is invariably greater than that in the dark control. Longer periods of illumination generally did not further increase the ATP level in the organism. We routinely used a 5 min illumination period in the inhibitor experiments. Several experiments were carried out by first concentrating the *Euglena* suspension approximately five-fold by centrifugation and using white light to elicit the response. Quite similar results were obtained with this protocol, although the light signals were considerably larger.

The effect of DCMU on phototaxis and photophosphorylation in *Euglena* is shown in Fig. 2. As was shown in our earlier work,[3] approximately 2 h of exposure to this inhibitor is required to achieve the maximum effect on phototaxis. Note that a similar time course is observed for photophosphorylation. It is pertinent to ask whether this delayed response is due to the time required for the inhibitor to penetrate the cell (or the chloroplast). In order to test this, we measured the time course of the DCMU inhibition of oxygen evolution. The results are shown in Fig. 3. The initial downward slope of the curve represents respiratory oxygen uptake. As soon as the light is turned on, oxygen begins to be evolved. The arrow marks the time at which an aliquot of DCMU solution was quickly injected into the culture. As is evident, inhibition of oxygen production*

* Note that, at the DCMU levels used in these experiments, oxygen evolution is not completely inhibited.

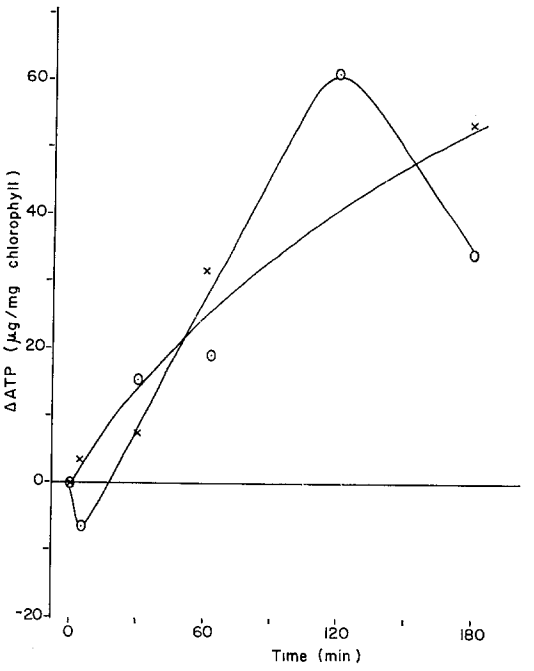

Figure 5. Time course of effect of DCMU (2×10^{-6} M) on ATP levels in *Euglena* prior to illumination. Ordinate represents difference in ATP between inhibited and uninhibited cultures. The results of two separate experiments are shown.

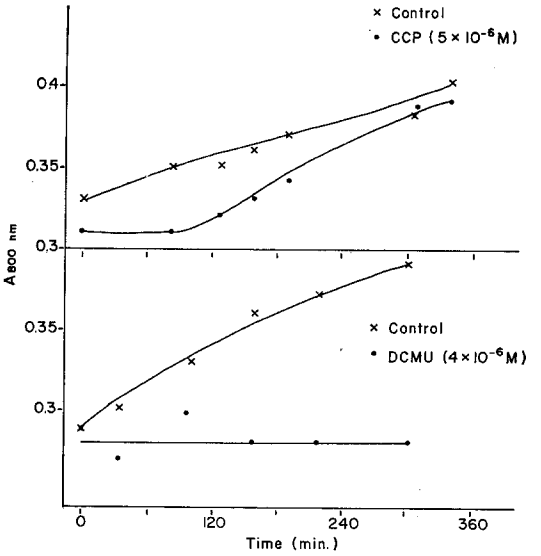

Figure 6. Effect of DCMU and CCP on growth of *Euglena* cultures as measured by absorbance at 800 nm.

is manifested within 30 sec. The results shown in Figs. 2 and 3 can be explained in terms of an endogenous pool of electron donors in *Euglena*, which allow photosystem II of the photosynthetic pathway to be bypassed and thus permit ATP production to occur in the presence of DCMU until the pool is exhausted.

In Fig. 4 is shown the results of phototaxis and photophosphorylation measurements in the presence of CCP. Although the time course of the CCP effects are somewhat different than those observed with DCMU, we again observe similar patterns in the two types of responses.

It is of interest to look at the ATP content of the *Euglena* cells in the presence of the inhibitors but prior to illumination. Figure 5 shows these levels as a function of time after addition of DCMU. Inasmuch as this inhibitor causes cessation of cell growth (Fig. 6), we have expressed the results in terms of chlorophyll content. It is apparent that rather large increases in ATP concentration occur shortly after exposure to DCMU. On a relative scale, the maximum increase is about five-fold. In contrast to this, CCP causes a decrease in ATP levels (Fig. 7) and does not appreciably affect cell growth (Fig. 6). Thus, it would appear that the ATP reserves of the *Euglena* are depleted by cell growth processes in the CCP-inhibited cultures, whereas this does not occur with DCMU.

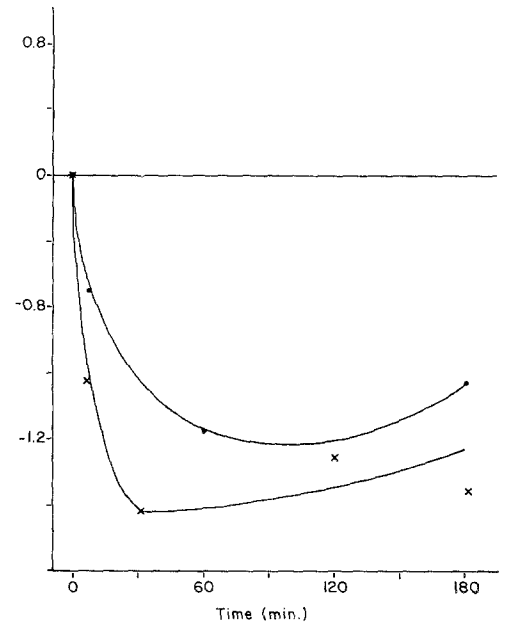

Figure 7. Time course of effect of CCP (5×10^{-6} M) on ATP levels in *Euglena* prior to illumination. Ordinate represents difference in ATP between inhibited and uninhibited cultures. The results of two separate experiments are shown.

In our previous work,[3] we showed that placing a culture of *Euglena* in the dark (and thus stopping photosynthetic ATP production) did not cause a rapid loss of phototactic ability; in fact, phototaxis was maintained at approximately the normal level for 24 h. We therefore thought it might be instructive to monitor ATP levels under these conditions. In Table I are the results of experiments in which *Euglena* were placed in the dark during a normal light period. Two cultures were used, one relatively young and still growing and the other an older culture which had reached saturation. Two things may be seen in these experiments. First, placing the organisms in darkness inhibits growth (as measured by the absorbance at 800 nm). Secondly, ATP levels per cell in the dark-adapted culture actually increase over a 24 h period.* These results, considered in relation to our earlier observations,[3] suggest the presence of a control system which acts to maintain phototactic energy reserves during an extended period of little or no photosynthetic activity, perhaps at the expense of cell growth and division.

* We have carried some experiments out to longer times and find that the ATP levels eventually decrease to quite low values (after two to three days).

TABLE I. Effect of darkness on ATP levels in *Euglena* cultures

(*a*) *6-day-old culture*

	Normal		Dark	
Time (h)	A_{800nm}	ATP (μg/ml)	A_{800nm}	ATP (μg/ml)
0	0·26	0·35	0·26	0·30
2	0·27	0·27	0·27	0·36
4	0·33	0·30	0·28	0·40
6	0·37	0·30	0·28	0·38
24	0·43	0·53	0·30	0·54
26	0·45	0·75	0·30	0·55
28	0·50	0·81	0·30	0·61
30	0·53	0·82	0·30	0·64

(*b*) *11-day-old culture*

Time (h)	Normal ATP (μg/ml)	Dark ATP (μg/ml)
0	0·66	0·75
2	0·62	0·84
4	0·57	0·90
6	0·60	0·99
23	0·69	0·81
25	0·75	0·89
27	0·99	1·04
29	1·00	1·09

Conclusions

The correlations between phototaxis and photophosphorylation observed in these experiments provide further support for our contention[3] that the photosynthetic phosphorylation system supplies the energy required by *Euglena* for phototactic orientation. Inasmuch as the organelles which mediate these two phenomena are physically separated in this organism, a transport system and storage reservoir for ATP (or some other energy source derived from the ATP-producing system in the chloroplast) is implicated, with the primary photoprocess triggering the release and/or utilization of the material thus stored. It is worthy of note that the euglenoid flagellates are the only algae in which the eyespot is separated from the chloroplast.[1] In all other species, these two organelles are in intimate contact. If the eyespot is indeed a shading device for a photoreceptor organelle in all species, as present evidence suggests,[1] then it may be that *direct* photocontrol of photophosphorylation occurs in non-euglenoid species (although a transport system from chloroplast to phototactic apparatus would still be required).

Acknowledgements

This work was supported in part by the U.S. Atomic Energy Commission (Contract AT(11-1)908). The technical assistance of Elinor S. Hostetter is gratefully acknowledged.

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