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PHOTORESPIRATION IN THE PROTONEMATA OF FUNARIA HYGROMETRICA HEDW.

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SUMMARY

The protonemata of *Funaria hygrometrica* Hedw. were shown to photorespire, first by demonstrating that immediately after a period of illumination oxygen was consumed at a rate greater than that characteristic of long-term dark respiration, and secondly by observing a greater release of $^{14}CO_2$ from 1-^{14}C glycolate in light than in darkness. The glycolate-oxidizing enzyme had the ability to decompose L(−)lactate but not D(−)lactate, and must therefore be considered to be a glycolate oxidase and not a glycolate dehydrogenase such as operative in some algae. Some implications of these findings are discussed.

1. INTRODUCTION

In the past few years, a great deal of attention has been given to photorespiration, i.e., the production of CO_2 in the light from recently formed photosynthate (CHOLLET & OGREN 1975, GOLDSWORTHY 1969, JACKSON & VOLK 1970, ZELITCH 1971). If the process is only wasteful, as claimed by some, greater crop-yields could conceivably be obtained by inhibiting it (LAWYER & ZELITCH 1978, SERVAITES & OGREN 1977, ZELITCH 1966, 1978, ZELITCH & DAY 1968). It is also imperative to study the interaction between photorespiration and other biochemical processes, especially photosynthesis (BULLY et al. 1969, OSMOND & BJÖRKMAN 1972, PAUL et al. 1975, RASTORFER & HIGGINBOTHAM 1968). Unfortunately, understanding photorespiration in a particular plant species does not mean that the process is fully understood in all green plants. Different plant materials not only have different rates of photorespiration, they often use different enzymes or may not photorespire at all. Thus far, most of the work concerned with photorespiration has been done with terrestrial angiosperms and with algae. Several differences have been found in the manner in which different plant species handle the important photorespiratory intermediate glycolate. Algae may either oxidize this compound by using glycolate oxidase, or they may employ glycolate dehydrogenase (COLLINS & MERRETT 1975, FLOYD & SALISBURY 1977, FREDERICK et al. 1976, FREDERICK et al. 1973, HOUGH & WETZEL 1972, MERRETT & LORD 1973, PAUL et al. 1975); they may also excrete the glycolate (TOLBERT & ZILL 1956, TOLBERT 1963). Terrestrial angiosperms, on the other hand, can only oxidize glycolate by means of glycolate oxidase.

Very little is known about the photorespiration of Bryophytes, although a survey of their gametophytes by DILKS (1976) has shown that a great number of species do photorespire, probably through a mechanism similar to that of algae.

The present investigation is concerned with the question of whether the protonemata of *Funaria hygrometrica* photorespire and, if so, what mechanism they use to handle glycolate.

2. MATERIALS AND METHODS

Unopened capsules of *Funaria hygrometrica* were collected at Discovery Park in Seattle, Washington. They were brought to the laboratory, washed, and sterilized in undiluted chlorox for 5 minutes. This was followed by rinsing, drying, and storage under sterile conditions. To start a culture, several capsules were opened in sterile water, and aliquots were transferred to sterile Kofler's medium B (KOFLER 1959) with a sterile pipette. Cultures were grown in a lighted incubator at 5 °C for 2–3 months. The low growth temperature was adopted to discourage the formation of buds followed by differentiation into gametophytes (BOPP 1959). Prior to each experiment, cultures were checked under the microscope for contamination and for signs of differentiation.

Measurements of O_2-production by photosynthesis or consumption by respiration were done in a YSI Model 53 oxygen-monitor, equipped with a YSI 5301 stirred bath and a Lauda K2 thermoregulator set to run at 25 °C. Light (when needed) was provided by a focused 100 watt GE spot bulb and was passed through a layer of circulating water 7 cm thick. The intensity was held constant at 500 $W.m^2$ ($= 5 \times 10^5$ $ergs.cm^{-2}.sec^{-1}$) by adjusting a diaphragm. The solution in which dissolved O_2 was measured consisted of 3 ml of a 0.3 M mannitol-solution with 0.5 mM $NaHCO_3$ and 10 mg (fresh wt.) protonemata, at pH 7.2. When 100% airsaturated, this solution contains approximately 240 mM O_2 (CHEVALIER & DOUCE 1976).

Release of $^{14}CO_2$ from 1-^{14}C glycolate was followed in the light and the dark by capturing the released $^{14}CO_2$ on a filter paper wetted with ethanolamine in the center well of Warburg vessels. The protonematal filaments, in phosphate buffer, pH 5.6, were kept in the main compartment. Light was supplied by several flood lamps, and was passed through a layer of circulating water 10 cm thick. The temperature was held constant at 27 °C. After the incubation period, the filter papers were immediately immersed in a scintillation fluid composed of toluene – omnifluor – trition and shaken for 1 hour at room temperature. Radioactivity was determined by using a Packard 3000 liquid scintillation counter. In some experiments the protonemata were first fragmentized into short sections composed of from 3 to 10 cells by a short burst of high-intensity sonication. This prevented clumping of the filaments and thus promoted glycolate uptake. Filaments thus treated were separated from the cell sap released from broken cells by several low-speed centrifugations. The amount of radioactivity absorbed and retained by the cells was determined by rapidly filtering and washing the protonemata, digesting in toluene, and counting a 0.1 ml sample in a toluene-omnifluor. Several parameters were changed, one at a time, in a series of these experiments, in an effort to demonstrate the difference between the light- and the dark-release of $^{14}CO_2$. Experiment 4 (see *fig. 2*) was carried out with 15 mg protonemata, 2 ml buffer, and

0.1 ml 3 M ethanolamine. Following a 1-hr equilibration period, 50 μl (6.25 μCi) 1-^{14}C glycolate was added and the vessels were sealed off from the atmosphere. Incubation time was 2 hours. Experiment 5 was identical except that equilibration time was reduced to 30 minutes. Experiment 8 was carried out with 13 mg fragmentized protonemata, 1 ml buffer, and 0.05 ml ethanolamine. Following 30 min. of equilibration, 25 μl (3.12 μCi) 1-^{14}C glycolate was added. Incubation time was shortened to 1 hour. Experiment 14 was identical to experiment 8 except that the equilibration was carried out in the absence of ethanolamine instead of in the absence of glycolate. For the determination of the type of glycolate-oxidizing enzyme present in an extract of *Funaria hygrometrica* protonemata, the O_2-monitor was used to determine the ability of the extract to oxidize L(−) or D(−) lactate, as well as glycolate. The extract was prepared by grinding 160 mg of protonemata in 10 ml phosphate buffer pH 7.0 with the aid of a glass-glass homogenizer. The resultant slurry was centrifuged at 500 × g for 10 minutes and the pellet was discarded. These steps were done at 5 °C. To 1 ml of this extract was added 0.02 ml FMN to make the solution 0.1 mM, and the mixture was introduced into an O_2-monitoring cell. The apparatus was darkened and the O_2-monitoring probe sealed over the cell. A waiting-period of a few minutes was allowed to obtain a basal rate of O_2-consumption; then, 0.1 ml of either 0.2 M glycolate, 0.5 M D(−) lactate or 0.5 M L(−) lactate, or water (control) was injected, and the rate of O_2-consumption was determined.

3. RESULTS

3.1. O_2-consumption after illumination

The production of O_2 by photosynthesis is a result of the photolysis of water, a process which has an absolute requirement for light-energy. Photorespiration also requires light; however, unlike the photolysis of water, photorespiration is not an energetically unfavorable process, therefore the requirement for light is something of a different complexion and may even be indirect (see the discussion-section for a consideration of the role of light in photorespiration). When a plant is actively photosynthesizing in the light and the light is suddenly turned off, the photosynthetic production of O_2 is immediately stopped. Photorespiration, however, which is also proceeding in the light, continues for a short time, because its requirement for light is not as absolute. Plants which immediately after a period of active photosynthesis demonstrate a rate of O_2-consumption in the dark which is considerably greater than that of long-range dark-respiration, can be said to photorespire.

In *fig. 1*, the rate of O_2-consumption immediately after a period of illumination is approximately 2 1/2 times the eventual rate of O_2-consumption which reflects dark-respiration. This indicates that the protonemata of *F. hygrometrica* are photorespiring in the light, at a rate considerably greater than that of dark-respiration.

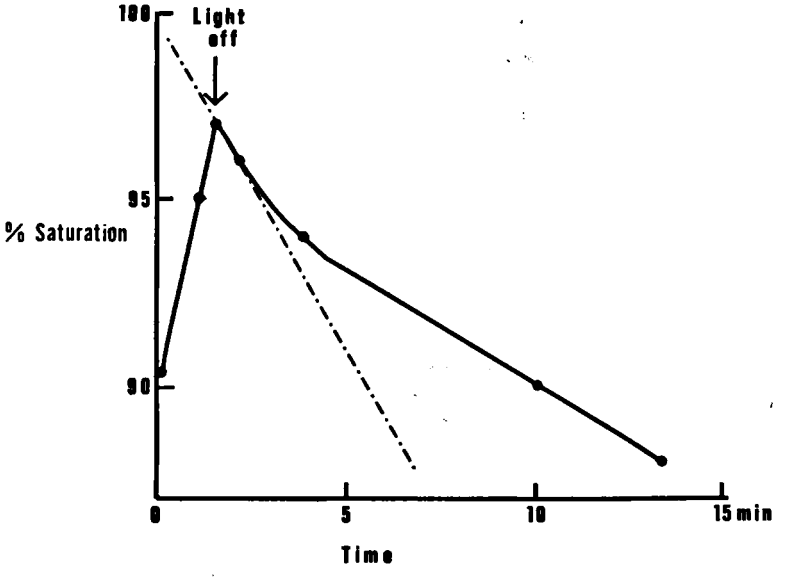

Fig. 1. Rate of O_2-production/O_2-consumption in light and in darkness. The dotted line represents the rate of O_2-consumption immediately after a period of photosynthesis.

Expt. No.	$\bar{X}_L$	n	$\bar{X}_D$	n	$\bar{X}_L/\bar{X}_D$
4	239 $\frac{cpm}{mg \cdot hr}$	3	185 $\frac{cpm}{mg \cdot hr}$	3	1.29
5	160	3	115	3	1.39
8	176	3	106	3	1.66
14	376	3	342	2	1.10
	$\bar{\bar{X}}_L = 238$		$\bar{\bar{X}}_D = 173$		$\bar{\bar{X}}_L/\bar{\bar{X}}_D = 1.37$

$H_o: \bar{\bar{X}}_L = \bar{\bar{X}}_D$ can be rejected at $\alpha = .01$ using 2 sample t-test with paired data

Fig. 2. Rates of $^{14}CO_2$ release in light (X_L) and in darkness (X_D) from protonemata fed with 1-^{14}C glycolate under various conditions (see text for these).

3.2. Comparison of ^{14}-CO_2 release from 1-^{14}C glycolate in light and in dark

Further evidence for the existence of photorespiration in *Funaria hygrometrica* protonemata comes from the comparison of the light- and dark release of $^{14}CO_2$ when protonemata are fed 1-^{14}C glycolate. The glycolate is rapidly absorbed and metabolized to CO_2 in both the light and the dark. Although the rates of absorption of 1-^{14}C glycolate are the same, *fig. 2* shows that the protonemata in the light release significantly more $^{14}CO_2$ than the protonemata in the dark. The statistical test chosen was a paired t-test because the parameters of each experiment were different, causing the magnitude of the response to be different in both the light and the dark (see Materials and Methods section).

3.3 Demonstration of the presence of glycolate oxidase

All angiosperms investigated have shown the presence of the enzyme glycolate oxidase. This flavo-protein oxidizes glycolate to glyoxylate, using O_2 as an electron acceptor and generating H_2O_2 in the process which according to some investigators plays a further role by oxidizing glyoxylate non-enzymically (GLIDEWELL & RAVEN 1976, HALLIWELL & BUTT 1974, KENTEN & MANN 1952). Glycolate oxidase can be identified by its ability to oxidize, in addition to glycolate, L(−) lactate but not D(−) lactate. Many green algae utilize a different enzyme for the oxidation of glycolate called glycolate dehydrogenase. This enzyme does not use O_2 as an immediate electron acceptor and thus does not generate H_2O_2. Glycolate dehydrogenase can be identified by its ability to oxidize D(−) lactate but not L(−) lactate in addition to glycolate.

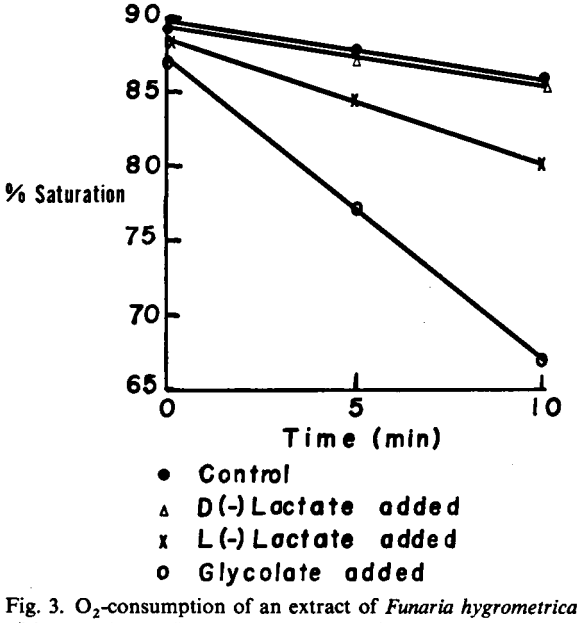

Fig. 3. O_2-consumption of an extract of *Funaria hygrometrica* protonemata upon confrontation with water (control), glycolate, and D(−) or L(−) lactate.

Fig. 3 shows that when an extract of *Funaria hygrometrica* protonemata is confronted with glycolate or L (–) lactate, its O_2-consumption rate is increased, presumably due to the activity of an oxidizing enzyme; however, when confronted with D (–) lactate there is no response. This indicates that glycolate oxidase and not glycolate dehydrogenase is responsible for the oxidation of glycolate in *Funaria hygrometrica* protonemata.

4. DISCUSSION

Moss protonemata constitute an interesting biological material to study because of their position in the evolutionary hierarchy. Many (perhaps most) algae are microscopic aquatic organisms which are often filamentous. Angiosperms are macroscopic and terrestrial, usually possessing roots, stems, and leaves with stomata. Mosses develop from an organism very much resembling an alga (the protomema) to an organism very similar to a land plant (the gametophyte, which has the same ploidy level as the protonema). Thus, there is some reason to think of the development of a moss plant as a recapitulation of what happened during evolution.

We have here investigated the alga-like protonema and found that it does photorespire, while with respect to its glycolate-oxidizing enzyme its photorespiration resembles that of a land plant. One must remember, however, that not all algae use glycolate dehydrogenase to oxidize glycolate, in fact it is only the unicellular algae which employ this enzyme (DILKS 1976, FLOYD & SALISBURY 1977, FREDERICK et al. 1976). The presence of glycolate-dehydrogenase might then be considered a primitive characteristic which moss protonemata have progressed above or beyond.

Photorespiration in the protonemata of *Funaria hygrometrica* appears to be a rapid process when one considers the results of our O_2-monitoring experiments; however, it is barely detectable in experiments dealing with the release of $^{14}CO_2$ from $1-^{14}C$ glycolate. How can we reconcile the two cases? To answer this question, we must consider what the role of light is in photorespiration. The simplest hypothesis is that in the light, due to active photosynthesis, larger pools of glycolate are created than those available in the dark; the increased rate of photorespiration would then be due to an increase in substrate concentration. Support has come from the work of BULLY et al. (1969) in which it was shown that in radish plants the action-spectrum for photosynthesis is identical with that for photorespiration. This indicates that chlorophyll is the acceptor-pigment for photorespiration (as would be expected if the rate of photorespiration were indeed controlled by the glycolate pool-size). However, our data indicate that in the protonemata of *Funaria hygrometrica* this is not the case; under the hypothesis, one would expect the same amount of ^{14}C to be released from introduced $1-^{14}C$ glycolate in the light and in the dark. (Actually, one might even expect less $^{14}CO_2$ to be released in the light, since photosynthetically produced glycolate would be unlabeled and might compete with the labeled glycolate for active sites on the glycolate-oxidase.) It thus appears likely that light plays a dual role in governing

the rate of photorespiration: that of controlling the pool-size of glycolate, and some other role as yet not understood. Experiments in which 1-^{14}C glycolate is fed to protonemata prior to a sojourn in light or in darkness ignore the effect of pool-size, in spite of the latter's potential importance in controlling the rate of photorespiration

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