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Effect of Light on Glucose Utilization by *Euglena gracilis*

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ABSTRACT

The effect of light on glucose consumption by wild-type *Euglena gracilis* Z. and mutant cells has been studied. When dark- or light-grown wild-type cells are transferred from a medium containing sodium butyrate as the only carbon source to a glucose-containing medium, glucose consumption is blocked for 6 to 7 days when cultures are incubated under a light intensity of at least 600 lux. During this time cells multiply at the same rate as controls kept on media devoid of any utilizable organic carbon source. This light-induced inhibition of glucose consumption and of growth on glucose-containing medium is not related to photosynthesis since: (*a*) glucose consumption is inhibited by light intensities much lower than those required for high phototrophic growth; (*b*) the inhibition of photosynthesis by 3-(3,4-dichlorophenyl)-1,1-dimethylurea does not overcome the inhibition of glucose consumption; and (*c*) nonphototrophic-growing mutants also show light-induced inhibition of glucose consumption and of growth on glucose-containing medium. This inhibition of growth by light might be explained by modification in the permeability of the cellular membrane.

Euglena gracilis is a unicellular alga which can utilize numerous carbon sources in the dark or in the light over a wide range of pH values (10). For example, the wild-type strains *bacillaris* (14) and Z. (12) grow on medium containing glucose as the only utilizable carbon source. However, *Euglena* cells grown phototrophically require an adaptation period in order to grow organotrophically on acetate (9) or glucose (3).

The main effect of light on dark-grown *Euglena* cells is to induce chloroplast development (25). Other effects of light include inhibition of the activity of glyoxylic enzymes (11, 15), decrease of the growth rate of mutants (13, 24), increase in paramylum consumption (16, 19, 28, 31), increase in the transcription of cytoplasmic rRNAs (7) and synthesis of some cytoplasmic-localized enzymes (18, 29, 30). In addition carbon sources may inhibit chloroplast development and Chl synthesis (1, 6, 32, 33).

To get a better understanding of the relationship between an exogenous carbon source, light, and the activity of the chloroplast we have studied the effect of light on the growth of *Euglena* cells on glucose-containing medium. The results indicate that light interferes with glucose consumption and slows growth on glucose-containing medium; these effects do not seem to be related to photosynthesis activity.

MATERIALS AND METHODS

Euglena gracilis Z, and the mutants W_3BUL (17) and $W_{34}ZUD$ (28) were grown at 28 C under constant shaking on NC-type media (19) as given in Table I. NCbut and NCglu contain, respectively, sodium butyrate and glucose; phototrophic medium, NCo, lacks an organic carbon source. A pH of 7.0 is used for NCbut since butyrate is toxic below pH 6.5; a pH of 4.5 is used for NCglu because it allows a better uptake of glucose than does pH 7.0 (5, 12). In the text media will be abbreviated as, for example, NCglu-4.5 which refers to NC medium with glucose at pH 4.5. Cells were kept in the light or in the dark. Light was from a battery of fluorescent white tubes which gives an intensity of 3,000 or 6,000 lux. In a few cases light intensity was decreased by intercalating gray neutral Kodak Wratten filters.

Cells were counted with a Coulter counter. Chl was extracted with 80% acetone saturated with Mg-hydroxycarbonate and quantified using Arnon's formula (2). Results are expressed in nmol Chl/cell using a mol wt of 900.

Glucose uptake was measured as follows: 1 μCi [^{14}C]glucose (obtained from the Centre à l'énergie atomique, 50.5 Ci/mol) was added to the cultures. After incubation, aliquots of the cultures were taken, cells were collected by centrifugation and washed three times with 0.9% NaCl. Radioactivity was estimated in both the supernatant and the washed pellet in 10 ml of a solution containing 0.5 liter of Triton X-100, 5.5 g PPO, and 0.1 g (POPOP) per liter toluene, with a Packard 3375 Tri-Carb scintillation counter.

RESULTS

GROWTH OF EUGLENA ON GLUCOSE-CONTAINING MEDIA

We have studied the effect of the composition of the growth medium and of the incubation conditions on the growth of wild-type or mutant *Euglena*. Cells were grown in one of the media described in Table I, in the dark or in the light, and the cell density was measured every day. Values in Table II represent (A), the cell density after 7 days of incubation, this gives an idea of the growth rate during the first week, and (B), the cell density at the plateau indicating the maximum cell density obtained for one given condition. Results obtained using different experimental conditions can be summarized as follows.

Transfer of cells from NCbut-7.0 Medium to NCglu-4.5 or NCbut-7.0 Medium. Growth observed is different depending on whether incubation is in the dark or in the light. (*a*) NCglu-4.5 cultures incubated in the dark show a growth similar to that of NCbut-7.0 cultures (Table II, lines 1 and 3). The length of the lag phase is different from one experiment to another but the maximum cell density is always close to 5–6 $\times$ 10^6 cells/ml. (*b*) When the same two types of cultures are incubated in the light, NCbut-7.0 (Table II, lines 2) develop in the same way as those kept in the dark whereas NCglu-4.5 cultures (Table II, line 4) behave differently. At the beginning they grow as cells deprived of an organic carbon source (Table II, lines 4 and 5), then, after 1 week of incubation they show a rate of division higher than that of phototrophic cultures. However, they never reach the cell density of cultures incubated in the dark.

Transfer of Cells from NCbut-7.0 Medium to NCglu-7.0 Medium. The growth inhibition described above is not due to a shift in pH since similar observations were made with NCglu-7.0 medium (Table II, lines 6–8). The inhibition seems even higher. The lag phase is longer in the dark compared to that of NCglu-4.5

cells. In the light, cultures stay for a longer time at the cell density of the NCo-7.0 cultures.

Transfer from NCglu-4.5 Medium to NCglu-4.5 Medium. Cells adapted for more than 50 generations on NCglu-4.5 medium in the dark and transferred to fresh NCglu-4.5 medium have a less pronounced light-induced inhibition; however, the plateau level is still lower for the cultures incubated in the light compared to those incubated in the dark (Table II, lines 9–11). This inhibition seems specific to glucose since it is not found when cells adapted to NCbut-7.0 medium are transferred to fresh NCbut-7.0 medium (Table II, lines 1 and 2).

The effect described here is highest with wild-type cells. It is also present with mutant cells, $W_{34}ZUD$ showing more light-induced inhibition of growth than W_3BUL (Table II, lines 1–4, 9, and 10).

PROPERTIES OF CELLS GROWN ON GLUCOSE-CONTAINING MEDIA

Table III gives Chl content of wild-type cells grown under various conditions. As expected from previous observations (1, 6, 32, 33), under most conditions tested the addition of an organic carbon source, glucose, or sodium butyrate inhibits Chl formation (Table III, lines 1–2, 5–6, and 7–8). However, when the cultures were not previously adapted to the use of glucose (Table III, lines 3–4) this carbon source has no effect on the Chl content. This is probably due to the fact that under this condition glucose is not used by the cells (Fig. 1) and thus cannot have an inhibitory effect.

To follow uptake and metabolism of glucose under the various experimental conditions, we added [^{14}C]glucose at the time of the transfer of the cells to a fresh medium, and followed its disappearance from the medium. We then noticed that after 10 days of growth in the dark, cells used 55% of the glucose contained in the medium whereas cells incubated in the light have metabolized only 5%. It is worth noting that of the glucose metabolized by dark-incubated cells, 30% were found in the cell pellet whereas 70% were lost, probably released as $^{14}CO_2$. A more precise analysis of the light-induced inhibition of glucose uptake indicates that this uptake is blocked for 7 days when cultures are incubated under a light intensity higher than 600 lux (Fig. 1A). As for the inhibition of growth, light-induced inhibition of glucose uptake is less pronounced in cells preadapted to glucose (Table IV and Fig. 1B). As observed before for growth inhibition the effect on glucose uptake is lower in mutants than in wild-type, and in W_3BUL compared to $W_{34}ZUD$ (Table IV).

Table I. *Composition of Media*

Media were prepared as described (19). Identification of media follows nomenclature already described (19). The NCbut and NBglu media are initially deficient in nitrogen, then carbon; the carbon source is, respectively, sodium butyrate or glucose.

Components	NCbut-7.0[a]	NCglu-7.0[a]	NCglu-4.5[a]	NCo-7.0	NCo-4.5
			g/l		
Sodium butyrate	7.8	0	0	0	0
Glucose	0	8.5	8.5	0	0
K_2HPO_4	0.27				
NH_4Cl	0.72				
Vitamin B_1	12×10^{-3}				
Vitamin B_{12}	12×10^{-6}				
Meb[b]	10 ml concentrated solution				
pH[c]	7.0	7.0	4.5	7.0	4.5

[a] Carbon content of these media was 3.4 g/l.
[b] Meb represents the mineral fraction described previously (19).
[c] After mixing of the organic and mineral fractions.

Table III. *Effect of the Nature of the Previous Growth Conditions and of the Actual Growth Conditions on Chl Content of Wild-type Euglena*

Cells grown on either NCbut-7.0 or NCglu-4.5 medium were inoculated into different media and incubated under a light intensity of 3,000 lux. Chl content was determined after three days of growth.

Previous Growth Conditions		Actual Growth Conditions during Experiment	Chl Content
			nmol/10^6 cells
NCbut-7.0, light	1	NCbut-7.0	6
	2	NCo-7.0	15
NCbut-7.0, light	3	NCglu-4.5	16
	4	NCo-4.5	15
NCglu-4.5, dark	5	NCglu-4.5	8
	6	NCo-4.5	18
NCglu-4.5, light	7	NCglu-4.5	8.2
	8	NCo-4.5	16.5

Table II. *Effect of Previous Growth Conditions of the Culture and Actual Condition of Incubation on Growth of Euglena*

Cells grown on NCbut-7.0 or NCglu-4.5 media were transferred to several growth media and incubated in the dark or under a light intensity of 3,000 lux. Results represent the cell density of the different cultures after 7 days of incubation (A) and the value obtained at the plateau (B).

Previous Growth Conditions		Growth Conditions during Experiment	Cell Density					
			Wild-type		$W_{34}ZUD$		W_3BUL	
			A	B	A	B	A	B
					10^6 cells/ml			
NCbut-7.0, dark	1	NCbut-7.0, dark	5.6	5.6	4.6	4.6	2.4	2.4
	2	NCbut-7.0, light	5.9	5.9	4.9	4.9	3.0	3.0
	3	NCglu-4.5, dark	5.5	5.5	4.3	4.3	3.7	3.7
	4	NCglu-4.5, light	1.1	3.6[a]	0.2	2.1	0.8	4.0
	5	NCo-4.5, light	0.9	1.9[a]				
	6	NCglu-7.0, dark	0.1	6.8				
	7	NCglu-7.0, light	0.9	4.6[a]				
	8	NCo-7.0, light	0.9	1.8[a]				
NCglu-4.5, dark	9	NCglu-4.5, dark	6.9	6.9	4.5	4.5	3.8	4.0
	10	NCglu-4.5, light	4.4	4.4	1.2	2.5	2.6	3.8
	11	NCo-4.5, light	0.6	1.5[a]				

[a] These cultures were not at the plateau after 2 weeks of growth. This is probably due to the fact that these cultures are still able to use CO_2 from the air since the medium is not entirely deprived of the other nutrients as is the case in cultures at the plateau.

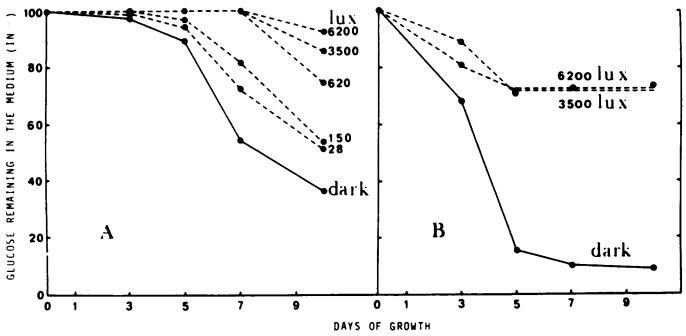

FIG. 1. Effect of light intensity on glucose consumption of wild-type *Euglena*. Cells grown in the dark on NCbut-7.0 (A) or NCglu-4.5 (B) medium were incubated into flasks containing NCglu-4.5 medium. [^{14}C]glucose (20 nCi/ml) was added and the flasks incubated in darkness (——) or under different light intensities (— — —). The amount of glucose remaining in the medium was measured every day. Results were expressed in percent of the radioactivity added to the medium.

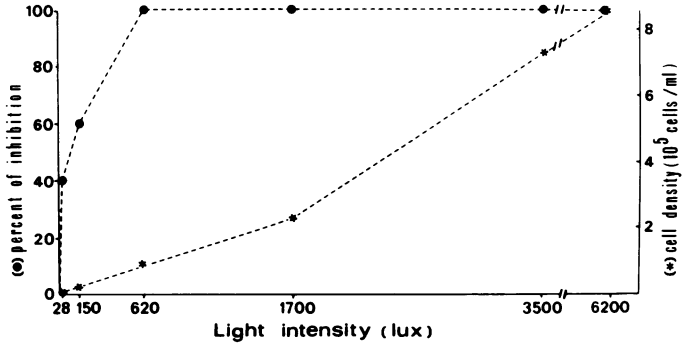

FIG. 2. Effect of light intensity on glucose uptake and on autotrophic growth of wild-type *Euglena*. Cells grown in the dark on NCbut-7.0 medium were inoculated into flasks of NCglu-4.5 medium containing [^{14}C]glucose (20 nCi/ml) and into flasks of NCo-4.5 medium at an initial cell intensity of 5×10^4 cells/ml. Flasks were incubated in darkness or under different light intensities. After 7 days of growth we determined the increase of cell density of NCo-4.5 cultures (*) and the amount of radioactivity present in the supernatant of NCglu-4.5 cultures (●). This last parameter was expressed as the percentage of the light-induced inhibition of glucose uptake.

Table IV. *Light-induced Inhibition of Cell Division and Glucose Uptake in Wild-type and Mutant Cells of Euglena*

Wild-type or mutant cells grown in the dark on NCbut-7.0 or for at least six generations on NCglu-4.5 were inoculated into NCglu-4.5 medium containing [^{14}C]glucose (20 nCi/ml). The cultures were incubated in the dark or in the light. Cell density and radioactivity remaining in the medium were determined after 7 days of incubation.

Origin of Cells	Wild-type	W$_{34}$ZUD		W$_3$BUL	
	Glucose[b]	Cell Density[a]	Glucose[b]	Cell Density[a]	Glucose[b]
	% inhibition				
NCbut-7.0	100	97	92	80	90
NCglu-4.5	70	80	77	30	35

[a] Represents the per cent inhibition on the increase of cell density in the cultures incubated in the light compared to that of cultures incubated in the dark (only for mutant strains where no phototrophic growth is possible).

[b] Represents the per cent inhibition of the glucose consumption in the cultures incubated in the light compared to that of cultures incubated in the dark.

Table V. *Effect of DCMU on Glucose Uptake of Wild-type Euglena Cultures*

Cells grown in the dark on NCbut-7.0 were inoculated into NCglu-4.5 medium containing [^{14}C]glucose (20 nCi/ml). The cultures were incubated in the light or in the dark, with or without 10 μM DCMU. The glucose remaining in the medium was measured after 7, 10, and 14 days of growth; glucose lost from the medium was assumed to have been consumed. Results are expressed in per cent of the glucose added to the cultures.

Conditions of Incubation	Glucose Metabolized		
	after 7 days	after 10 days	after 14 days
		%	
Dark			
No DCMU	47	65	74
10 μM DCMU	19	30	53
3,000 lux			
No DCMU	0	6	20
10 μM DCMU	0	6	25

A comparison of the effect of light intensity on glucose uptake and on phototrophic growth (Fig. 2) indicates that glucose uptake is completely inhibited with light intensities much lower than those needed for maximal phototrophic growth. To test further the possible relationship between photosynthesis and inhibition of glucose uptake we incubated the cultures with 10 μM DCMU which blocks photosynthesis and phototrophic growth. Results indicate that DCMU reduces glucose uptake (Table V) and growth in the dark probably due to the effect of DCMU on mitochondria (8). They also indicate that inhibition of photosynthesis does not modify the inhibition of glucose uptake by light unless the effect of DCMU on glucose uptake observed in the dark compensates exactly to a possible activation of glucose uptake after inhibition of photosynthesis by DCMU.

DISCUSSION

Results presented here show that glucose uptake by *Euglena* and its growth on glucose-containing medium are inhibited by light. The extent of inhibition is a function of light intensity, previous and present growth conditions and strains, wild-type or mutants. Nonadapted wild-type cells grown in the light on glucose-containing medium multiply phototrophically and have a behavior similar to that of cells grown in the absence of organic carbon source which indicates that, under these conditions, glucose is not toxic. An adaptation phase is necessary when *Euglena* cells are transferred from a butyrate to a glucose-containing medium; the length of this phase is increased by light. Even after cell adaptation to glucose by growth in the dark on glucose-containing medium for more than 50 generations, the use of this substrate remains more light-sensitive than the use of butyrate. In addition, Chl synthesis is lower only when *Euglena* cells preadapted to glucose are used, in agreement with observations showing the repression of chloroplast development by certain carbon substrates (1, 6, 32, 33). The lack of effect on Chl synthesis with nonadapted cells confirms the light effect on glucose uptake. Finally, results with mutant cells suggest that light regulation of glucose uptake is controlled by extrachloroplast photoreceptors. Inasmuch as these mutants still contain rudimentary plastidial structures and some chloroplast-localized components (20, 22, 27, 28) the influence of such structures and components cannot be ruled out.

Our results suggest that the inhibition observed is probably not due to an interaction between photosynthesis and glucose metabolism because: (*a*) glucose consumption is completely blocked by

light intensities too low to allow efficient phototrophic growth; and (*b*) inhibition is still present when photosynthesis is blocked chemically with DCMU or by mutation.

The effects described here could be a consequence of a defect either in the metabolism of glucose or in its transport; hexokinase is necessary since strains lacking this enzyme are unable to grow on glucose (26). *Euglena* hexokinase activity is formed of a glucokinase specific for the glucose and a fructokinase specific for the fructose (23). The glucokinase and the enzymes for paramylum synthesis are located outside the chloroplast (34). However, Graves (21) showed that glucokinase activity is not the only factor limiting glucose uptake, an activation of a specific transport system may be required. In some strains of *Astasia longa,* a permanently white species close to *Euglena,* the lack of glucose consumption is due to a defect in permeability (4). In addition, Boehler and Danfort (5) suggested that glucose consumption in *Euglena* cells could be regulated by permeability of the cell membrane.

It is possible that there exists a competition between the development of a system allowing the use of glucose and the formation of chloroplasts. This competition would likely be less pronounced when the plastidial structure has been modified by mutation. Among several possible controls the following is proposed: light may increase permeability of the chloroplast for cytoplasmic-localized components in competition with similar effect on the permeability of the cellular membrane. Such would be the case if some components are identical in the two systems of permeability, such as peptides or energetic factors.

Our results show that light interferes with growth on glucose-containing medium and with glucose uptake. However, specific experiments are needed to determine whether the effect observed is related to a defect in the metabolism of glucose or in its transport as proposed above and further to correlate this effect with observations indicating that glucose inhibits chloroplast development in *Euglena* (32).

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