EFFECT OF LIGHT ON GLUCOSE ASSIMILATION IN TOLYPOTHRIX TENUIS

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In a previous study (1), it has been revealed that the blue-green alga Tolypothrix tennis can be heterotrophically grown in complete darkness if, besides mineral nutrients, appropriate organic substances are supplied as N- and C- sources. The maximum values for the growth rate and final growth yield obtained under the heterotrophic conditions, however, were found to be far less than those attained in usual autotrophic cultures. The addition of substances known as growth-factors for various microorganisms, such as cyanocobalamin, thiamine, biotin and others, as well as of the filtrate (and also extract) of the algal culture—run either in the light or in darkness—exerted no effect on the algal growth in the dark. It is thus unlikely that the growth under heterotrophic conditions is limited by the low concentration of growth-promoting substances which the alga can form, in situ, in the light but not in the dark, or is suppressed by an accumulation of growth-inhibiting substances produced during the dark incubation. On the other hand, it has been found that the growth rate in the presence of organic substances is enhanced to a considerable extent, to attain a level comparable to, or slightly higher than, that in the autotrophic culture, if culturing is performed under illuminaion with light having so low an intensity (less than 500 lux) as hardly to support autotrophic growth (2). This suggests that the light stimulates the assimilation of carbon source (e.g., glucose). An attempt was made to elucidate how light acts on the metabolism of glucose in Tolypothrix cells.

Tolypothrix tennis, grown for 3 weeks under heterotrophic (in complete darkness) or semi-heterotrophic (illuminated with a dim light of 500 lux) conditions (1), was used as test organism. The harvested cells were washed 3 times with K2SO4 solution (10⁻¹M), suspended in an inorganic medium** and incubated aerobically in the dark for 20 hours at 32° to reduce the level of carbohydrate reserves. The starved cells so obtained were resuspended in M/50 phosphate buffer (pH 7.6) in a concentration of 20 ml packed cells

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** Composition of inorganic medium: per liter, KNO3, 3.0 g; MgSO4·7H2O, 0.5 g; Na2HPO4·12H2O, 0.5 g; CaCl2·2H2O, 0.02 g; FeSO4·7H2O, 0.002 g; Arnon’s A5 solution, 1 ml.
per liter and placed in conical flasks. The flasks were shaken in a thermostat at 32° and illuminated from below with incandescent lamps (6,000 lux). In the dark control, the flasks were covered with aluminium foil to exclude the light. In the anaerobic experiments, Warburg-flasks were used instead of the usual conical flasks and the air in the gas space was replaced with O2-free nitrogen gas. At zero time glucose ($5 \times 10^{-4} M$ in final concentration) was added to the algal suspension. At appropriate intervals (20 minutes), aliquots (5 ml) of algal suspension were removed and frozen immediately. After separating the algal cells by filtration from the reaction mixture, the amounts of glucose in the reaction medium and carbohydrate in the algal cells were estimated colorimetrically by the anthron method (3). The amount of cellular carbohydrates was expressed in terms of glucose equivalent.

The results obtained are presented in Figs. 1 and 2. As will be seen from Fig. 1, the starved cells rapidly consumed the exogenous glucose linearly with time until about 70 per cent of glucose had disappeared from the medium. The rate of glucose consumption was hardly affected by illumination if the incubation was performed in the presence of oxygen. The amount of glucose removed from the reaction medium in the light was recovered almost quantitatively as cellular carbohydrate. On the other hand, little increase in carbohydrate reserves occurred when the cells were kept in the dark. The rate of glucose assimilation in the light remained unaltered even when the air in
gas space was replaced with pure nitrogen (Fig. 2). This was not the case in the dark; anaerobiosis markedly reduced the metabolism of glucose. These findings indicate that (i) the present test organism has, in the light, the capacity for assimilating exogenous glucose as such, and converting it into cellular carbohydrates, and (ii) in the dark, it can utilize glucose as fuel substance but not as building material for constructing cellular substances.

Very instructive in this connection is Bishop's recent finding (4) which has shown that the hydrogen-adapted cells of Ankistrodesmus braunii are capable of incorporating glucose directly into starch by means of phosphorylation, the ATP required for this reaction being furnished from the photochemical process. It appears highly probable that a similar mechanism is operative in the process of light-dependent assimilation of glucose observed in the present organism. In light-aerobic conditions, however, cellular carbohydrate might be partly formed by refixation of the carbon dioxide produced through respiratory combustion of glucose. At any rate, the present experimental results seem to provide a new clue for interpreting the well known fact that heterotrophic culture of cyanophycean algae is generally very difficult.

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REFERENCES


(2) T. Kiyohara: unpublished data.
