CONTROL OF CHLOROPLAST FORMATION IN EUGLENA GRACILIS: DEPENDENCE OF RATE OF CHLOROPHYLL SYNTHESIS ON PREVIOUS NUTRITIONAL HISTORY OF CELLS

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Received August 7, 1967

It is well established that chloroplast formation in certain algae is repressed by the addition of utilizable carbon sources to the growth medium. This has been shown for various Chlorella species (Beijerinck, 1904; Kufferath, 1913; Fuller and Gibbs, 1959; Shihira-Ishikawa and Hase, 1964) and also for Euglena gracilis (Smillie and Krotkov, 1960; Buetow, 1967; Ternetz, 1912). In the case of E. gracilis chloroplast development by etiolated cells grown in the dark and then illuminated under non-multiplying conditions, is also partially inhibited by the presence of utilizable carbon sources during the incubation in the light (App and Jagendorf, 1963; Harris and Kirk, unpublished). In the present paper we report that chlorophyll synthesis by etiolated cells of E. gracilis suspended in phosphate buffer in the light is strongly inhibited if the cells are supplied with high concentrations of ethanol as a carbon source during their previous growth in the dark. Thus, chlorophyll formation by these etiolated cells is controlled not only by nutrients present during the greening process but also by the nutritional history of the cells.

METHODS

The basal defined growth medium used in these experiments contained

the following components, per litre - KH_2PO_4, 0.766g.; K_2HPO_4, 0.136g.; NH_4NO_3, 0.3g.; Na_3 citrate.$2H_2O$, 0.5g.; $FeCl_3.6H_2O$, 0.01g.; $MgSO_4.7H_2O$, 0.3g.; $Ca(NO_3)_2$, 0.06g.; $CH_3COONa.2H_2O$, 2.0g.; thiamine, 1.0mg.; cyanocobalamin, 10µg.; trace element solution (Sager and Granick, 1954), 10 ml. The basal medium was distributed in 20 ml. quantities, in 50 ml. Erlenmeyer flasks. The appropriate amounts of ethanol were added to the flasks after autoclaving. Each flask was inoculated with 6 drops of a stationary-phase light-grown culture of Euglena gracilis, strain Z. The flasks were then incubated in the dark at 30° in a New Brunswick rotary shaker for 3 or 4 days. The cells from each flask were harvested by centrifugation, and washed by resuspension and centrifugation three times with 30 ml. quantities of 0.15M NaCl. Each batch of washed cells was resuspended in 11 ml. of 0.04M KH_2PO_4/Na_2HPO_4, pH 7.0, containing 1.0mM $MgSO_4$, in a 50 ml. Erlenmeyer flask. 1.0 ml. was then removed from each flask, 4.0 ml. of acetone were added, the cells removed by centrifugation, and the extinctions at 663 and 645mµ measured on a Hitachi-Perkin Elmer 139 spectrophotometer, for determination of total chlorophyll content (Arnon, 1949). Another 0.5 ml. was removed from each flask for determination of cell number with the Coulter Counter. The flasks were then incubated for 24 hours at 30° in the New Brunswick Psychrotherm rotary shaker, at a light intensity ("daylight" fluorescent tubes) of 250 foot-candles. At the end of the incubation samples were once again taken for chlorophyll and cell number determination.

RESULTS AND DISCUSSION

Growth of the cells in the dark was very much better when ethanol was added to the basal medium (in addition to the acetate already present). 0.2% ethanol gave a three-fold increase in final yield of cells, and 0.4-1.5% ethanol gave a four-fold increase (Table 1). Cell

numbers generally remained the same or increased slightly during the incubation in phosphate buffer in the light, except for cells grown in the absence of ethanol, which sometimes approximately doubled their numbers during the light incubation. In the particular experiment described in Table 1, cell numbers did not increase by more than 15% in any of the flasks during 24 hours illumination.

Although Euglena gracilis grew very well on high concentrations of ethanol, the cells so grown synthesized chlorophyll during a subsequent incubation in the light much more slowly than cells grown on lower levels of ethanol. At ethanol levels during growth of more than 0.2% there was a very marked decrease in the rate of subsequent chlorophyll synthesis per 10^6 cells as the ethanol concentration increased (Table 1): cells grown on 1.0 or 1.5% ethanol formed little or no chlorophyll during 24 hours in the light. Although cells grown on 1.0 or 1.5% ethanol formed no significant amount of chlorophyll during the light incubation, they had not permanently lost the ability to form chloroplasts - when cells from these flasks were inoculated into a complex medium containing peptone and yeast extract (Kirk, 1962) they yielded vigorous green cultures.

It is clear from our results that there is a distinct difference in

TABLE 1: Rate of chlorophyll synthesis in the light by cells previously grown in the dark in the presence of different concentrations of ethanol.
Conditions as in text.

Ethanol conc. (v/v) in growth medium (%)	0	0.2	0.4	0.7	1.0	1.5
Cells x 10^{-6}/ml. in growth medium after 4 days in dark	0.31	0.98	1.22	1.34	1.30	1.38
Cells x 10^{-6}/ml. in buffer at start of light incubation	0.57	1.79	2.22	2.44	2.36	2.50
µg. chlorophyll synthesized per flask in 24 hours	70.8	195.0	134.5	41.3	4.7	0.0
µg. chlorophyll synthesized per 10^6 cells in 24 hours	13.1	11.5	6.4	1.8	0.2	0.0

the properties of cells grown in the dark on low or high concentrations of ethanol, with particular respect to their ability to form chloroplasts during a subsequent incubation in the absence of a carbon source in the light. The cells grown on high levels of ethanol either lack something which is required for chlorophyll formation, or possess something which prevents chlorophyll formation: i.e. chlorophyll formation is prevented either by a nutrient deficiency, or by a type of repression. We are currently studying the nature of the metabolic block in cells grown on high concentrations of ethanol.

These results have some immediate practical significance. They indicate that in order to obtain optimal rates of chloroplast formation by etiolated cells of E. gracilis in the light, close attention should be paid to the concentration of oxidizable carbon source on which the cells are grown in the dark. It should be noted that the concentration of carbon source which gives the highest rate of synthesis per cell is not necessarily the concentration which gives the highest rate for a given volume of culture. For instance, in the present system, the highest rate of synthesis per cell was obtained with cells grown in the absence of ethanol: however, the highest rate of synthesis per 20 ml. of original culture was given by cells grown on 0.2% ethanol (Table 1). This results from the fact that although cells grown on 0.2% ethanol synthesized chlorophyll at a somewhat lower rate per cell, there were more than three times as many cells produced in the presence of 0.2% ethanol than in its absence. Thus, in practice, it would often be more convenient to use cells grown with a certain amount of ethanol in addition to the acetate already present.

ACKNOWLEDGMENT

We are indebted to the Science Research Council for financial support.

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