Carbon Metabolism in *Microcystis* under Low Light Intensities

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The rate of total $^{14}\text{CO}_2$ incorporation in the bloom-forming cyanobacterium *Microcystis* varied with light intensity, being minimal at 10 lux and increasing at both higher and lower intensities. The total labelling of compounds arising from phosphoenolpyruvate carboxylation (aspartate, glutamate and citrate) showed a minimum just below the light compensation point. At lower light intensities a marked increase in the percentage labelling of these compounds from $^{14}\text{CO}_2$ occurred, together with a decrease in that of sugar phosphates and phosphoglycerate. The operation of the reductive pentose phosphate pathway was retarded at low light intensities; with increasing intensities the synthesis of sugars was favoured in relation to that of amino acids. This regulation of the route of CO_2 fixation is discussed in relation to the survival of cyanobacteria under unfavourable environmental conditions.

INTRODUCTION

The reductive pentose phosphate pathway is the main route of cyanobacterial CO_2 fixation in the light (Norris *et al.*, 1955; Pelroy & Bassham, 1972; Ihlenfeldt & Gibson, 1975; Weathers & Allen, 1978). In the dark, an alternative CO_2 fixation reaction leads to the labelling of aspartate and glutamate (Moses *et al.*, 1959). However, the light intensities which induce a change in the rates of these reactions are not known. Jansz & Maclean (1973) reported that the type of incubation vessel used had a marked influence on the mechanism of CO_2 fixation in *Anacystis nidulans*, owing to differences in the quantity of light received by the cells. In vessels with a long light-path radioactivity from $^{14}\text{CO}_2$ was incorporated mostly into aspartate and to a smaller extent into glutamate; in those with a short light-path sugar phosphates and phosphoglycerate were the major radioactive products. Contradictory results were obtained in subsequent experiments performed under low and high light intensities, the ratio of sugar phosphates plus phosphoglycerate to aspartate being 8:1 under low intensities and 2.5:1 under high intensities. The interpretation of these results is complicated because relatively light and dark zones exist in the dense suspensions employed and consequently individual cells were subjected to varying conditions. Unequivocal information on the effect of different light intensities can only be obtained if mutual shading of the cells is restricted to a minimum. In this communication we describe the patterns of $^{14}\text{CO}_2$ incorporation in dilute suspensions of *Microcystis* incubated at different light intensities in vessels with a short light-path which produced an approximately unicellular layer.

METHODS

Organism and culture. *Microcystis* 7005 (Stanier *et al.*, 1971) was obtained from Professor R. Y. Stanier, Institut Pasteur, Paris, France, and cultivated as described by Jüttner (1977). The cultures were proven to be axenic by plating on DEV-agar (Merck no. 11471).

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Fig. 1

Fig. 1. Total ¹⁴CO₂ incorporation in *Microcystis* at different light intensities.

Fig. 2

Fig. 2. Incorporation of ¹⁴CO₂ label in soluble aspartate (○), glutamate (●) and citrate/isocitrate (□) at different light intensities.

Incorporation of ¹⁴CO₂. Several *Microcystis* cultures (containing 2.6 to 5.5 µg chlorophyll *a* ml⁻¹) were mixed, divided into 50 ml portions, and incubated under low light intensity in an incubator shaker for 1 to 4 h. To measure ¹⁴CO₂ incorporation at each light intensity, a 50 ml culture was centrifuged, resuspended in 50 ml of 10-fold diluted medium (pH 7.7) and placed in a cuvette of Plexiglas (total volume 70 ml, 2.5 mm light-path) which was aerated from below [0.05 % (v/v) CO₂ in air]. The illumination was supplied by 1 to 3 fluorescent tubes (Osram Interna 39/20 W) which were either partly shaded or placed at different distances from the cuvette. The light intensity (1 lux ≈ 5 erg cm⁻² s⁻¹) was determined with a standard lux meter (Lange, Berlin). After adaptation for 15 min to the light intensity of a particular treatment, 100 µCi of NaH¹⁴CO₃ solution [100 µCi ml⁻¹, 59.3 mCi mmol⁻¹ (2.19 GBq mmol⁻¹)] was added. After incubation for 5 min, the reaction was stopped by draining the cyanobacterial suspension into 50 ml boiling ethanol and the suspension was boiled for 2 min.

Determination of total CO₂ incorporation. The volume of the ethanolic suspension was adjusted to 100 µl, glacial acetic acid (20 µl) was added to a 500 µl sample and the sample was dried in a stream of N₂ at 50 °C. The residue was dissolved in 500 µl water and counted by liquid scintillation after adding 10 ml Aquasol.

Chromatographic determination of the soluble compounds. Acetic acid (0.5 ml) was added to the ethanolic suspension to give a pH below 4.0, and the sample was bubbled with N₂ for 5 min. The suspension was centrifuged and the supernatant was dried at 30 °C in a rotary evaporator, redissolved in 300 µl water and stored at -18 °C. A 5 µl sample was applied to a thin-layer plate and separated by electrophoresis and chromatography, essentially as described by Schürmann (1969).

Determination of total sugars and amino acids. A 5 ml sample of the ethanolic suspension was brought to dryness. The residue was hydrolysed in 1 ml 8 M-HCl at 110 °C for 24 h. The sample was again dried, redissolved in 2 ml 0.1 M-HCl and separated on an ion-exchange resin (Dowex 50 in H⁺ form; 5 cm × 1.0 cm i.d.). The sugars were eluted with 10 ml distilled water and subsequently the amino acids with 5 × 2 ml 3 M-NH₄OH and 25 ml distilled water. The fractions were counted in a liquid scintillation counter after concentration.

Chlorophyll determination. Chlorophyll was extracted from 3 ml cyanobacterial suspension with 3 ml methanol and its concentration was determined from the absorbance at 666 nm (Ogawa & Vernon, 1971).

RESULTS AND DISCUSSION

Total CO₂ incorporation in *Microcystis* followed a linear relationship with light intensity over a wide range of intensities. However, CO₂ fixation was higher in the dark than at low light intensities, a minimum being observed at 10 lux, well below the light compensation point (Fig. 1).

Chromatographic separation of the water-soluble metabolites from ¹⁴CO₂ fixation over 5 min demonstrated that the ¹⁴C label of aspartate, glutamate and citrate/isocitrate (the

Fig. 3

Fig. 4

Fig. 3. Incorporation of $^{14}\text{CO}_2$ label in sugar phosphates (○) and phosphoglycerate (●) at different light intensities.

Fig. 4. Percentage distribution of ^{14}C label in soluble metabolites, sugar phosphates (□), phosphoglycerate (■), aspartate (●) and citrate/isocitrate (○), at different light intensities.

Fig. 5. Incorporation of $^{14}\text{CO}_2$ label in the sugar (●) and amino acid (○) fractions at different light intensities.

latter were not separated) also showed a light-dependent minimum which, however, was shifted to a higher light intensity (Fig. 2). This value corresponded with the light compensation point (close to 200 lux) determined for a similar *Microcystis* strain (Jüttner, 1977). Labelled sugar phosphates and phosphoglycerate could not be detected at light intensities below 100 lux. Above this value, the label in these compounds increased rapidly and then constituted the main fraction of the labelled metabolites (Fig. 3). The percentage distribution of the label in sugar phosphates and phosphoglycerate increased rapidly at a light intensity of 200 lux (Fig. 4) with a corresponding decrease in the label of citrate, aspartate and glutamate (not shown).

These fixation patterns indicate a severely restricted function of the reductive pentose phosphate cycle under low light intensities. However, the labelling of aspartate, glutamate

and citrate, which are typical compounds of phosphoenolpyruvate carboxylation, was much less affected. Phosphoenolpyruvate carboxylation is of importance for the regeneration of intermediates of the citrate cycle utilized in the synthesis of amino acids. There is no other pathway for organic acid pool regeneration, due to the low activity of the glyoxylate pathway (Pearce & Carr, 1967) and the lack of 2-oxoglutarate decarboxylase (Pearce *et al.*, 1969). These results explain the different $^{14}\text{CO}_2$ fixation patterns reported for *Anacystis* by Richter (1961) and Kandler (1961). If dense suspensions and long light-paths are used the labelling of aspartate and glutamate is favoured. However, the fixation pattern of Calvin cycle products is obtained under high light intensities and if vessels with short light-paths and dilute suspensions are used. Increased light intensities stimulate the formation of sugars which form the storage product glycogen; the synthesis of amino acids is comparatively less affected (Fig. 5). This control system is of importance for the survival of cyanobacteria under varying environmental conditions: if sufficient light energy is available reserve material is formed whereas under limited light conditions the synthesis of essential compounds necessary for the maintenance of cell activity is favoured.

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