Thiosulphate as Electron Donor in the Blue-green Alga *Anacystis nidulans*

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INTRODUCTION

Blue-green algae are frequently observed in anaerobic environments (Brock, 1973; Castenholtz, 1973), and there have been several reports of stimulation of growth in the laboratory under reducing conditions (Setlike, 1957; Allen, 1959; Ferguson Wood, 1965; Knoblock, 1966; Stewart & Pearson, 1970). Such results pose the question of whether an oxidizable inorganic substrate can act as an electron donor in photosynthesis, or whether such compounds merely create a reducing environment. It is known from laboratory studies that blue-green algae do not thrive under high oxygen tension (Sirenko *et al.*, 1968; Stewart, 1969; Stewart & Pearson, 1970). Stewart & Pearson (1970) suggested that the stimulating effect of hydrogen sulphide on growth of *Anabaena flos-aquae* was due to the removal of oxygen, but they also considered the possibility that hydrogen sulphide could act as an electron donor. Recently a blue-green alga has been isolated that can adopt either bacteria-like photosynthetic growth at the expense of hydrogen sulphide, or the usual oxygen-evolving photosynthesis, depending on environmental conditions (Cohen *et al.*, 1975).

This paper describes the effect of thiosulphate on the growth of *Anacystis nidulans* under light-limiting conditions. Thiosulphate is a potential electron donor that does not directly affect the oxygen tension.

METHODS

Organism. Axenic cultures of *Anacystis nidulans* 14011 (Algal Culture Collection, Institute of Plant Physiology, University of Göttingen, West Germany), purified by single-colony isolation, were used, except in growth experiments where non-axenic cultures were used. In these initial experiments, some bacteria were present in the cultures, but in view of the small numbers observed (0.1% of the total number of cells), it was considered that their presence did not invalidate the results.

Growth conditions. Cultures were grown in glass cylinders (4 cm diam.), with conical bottoms, containing 250 ml medium, and incubated in a water bath at 42 °C. Continuous illumination was provided by three Warm de Luxe Philips TL40 W/32 and two Daylight TL40 WI/55 fluorescent tubes. The medium was that described by Lorenzen & Venkaraman (1969), except that, where indicated, 1 mM-MgSO₄ was replaced by 1 mM-Na₂S₂O₃ and 1 mM-MgCl₂. Phosphate was autoclaved separately to avoid precipitation. Cultures were gassed and agitated with a mixture of air/CO₂ (98.5:1.5, v/v). The light intensity was varied by changing the distance between the water bath and the light source. Unless otherwise stated, the algae were grown in a medium for about 24 h, and were transferred to a similar medium at the start of each experiment.

Growth measurements. Numbers of cells were determined in a suitable dilution of the

Fig. 1. The effect of DBMIB and DCMU on oxidation of thiosulphate by *A. nidulans*. (a) ○, 10 μ M-DBMIB; ●, no inhibitor. (b) □, 10 μ M-DCMU; ■, no inhibitor.

culture using a Celloscope model 302 (Lars Ljungberg, Stockholm, Sweden), tube aperture 30 μ m.

Estimation of light intensity. The photocell (Luxmeter 370-393, Joffe, Italy) was submerged in the water bath beside the culture vessel, thereby allowing determination of the incident light intensity at the surface of the vessel.

Estimation of CO_2 uptake. CO_2 fixation was measured in volumetric respirometers (Scholander & Iversen, 1958) using 20 to 25 ml flasks, under the same conditions as for growth, except that a row of five 25 W incandescent lamps was used for the experiments at 1 klx. The gas phase was air. Algae were grown in thiosulphate medium, harvested during exponential growth, washed twice with 1 mM- MgCl_2 and resuspended in 1 mM- MgCl_2 . Portions (1 ml) of washed cell suspension were transferred to respirometer flasks containing 1 ml thiosulphate or sulphate medium and 15 μ mol NaHCO_3 . CO_2 uptake was stopped by adding 0.05 ml 50% (w/v) citric acid through the rubber injection stopper. The acid served both to inactivate the cells and to liberate from the medium the remaining bound CO_2 which could then be measured. In each experiment one respirometer was used to measure the initial amount of bound CO_2 , and fixation was then calculated.

Estimation of thiosulphate oxidation. Thiosulphate was determined iodometrically, and sulphate turbidimetrically by precipitation with BaCl_2 (Gleen & Quastel, 1953).

Inhibitors. 2,5-Dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (DBMIB) was a gift from Professor W. A. Trebst. 3-(3,4-Dichlorophenyl)-1,1-dimethylurea (DCMU) was obtained from Kebo Grave Labsenter, Spånga, Sweden, and recrystallized from ethanol.

RESULTS AND DISCUSSION

With the experimental conditions used, optimal light intensity for growth of *A. nidulans* was about 14 klx (Utkilen, 1973). Growth of *A. nidulans* at this and suboptimal (8.8 klx) light intensity showed that lowering the light intensity had no effect on the doubling time in cultures with thiosulphate, whereas the doubling time increased from 2.1 to 2.8 h in cultures with sulphate.

Increasing the sulphate or thiosulphate concentration from 1 to 100 μ M caused the final

cell yield to increase from 2.3×10^8 to 6.9×10^8 cells ml^{-1} in cultures containing sulphate and from 3.8×10^8 to 1.0×10^9 cells ml^{-1} in those containing thiosulphate, at optimal light intensities. While further increase in sulphate concentration had no effect, raising the concentration of thiosulphate to 1 mM resulted in a still higher cell yield (1.8×10^9 cells ml^{-1}). The cell density at the start of the experiments was about 10^6 cells ml^{-1} .

These results show that in light-limited conditions, *A. nidulans* can grow faster in the presence of an oxidizable inorganic substrate (thiosulphate) than in its absence. They also indicate that the algae can maintain growth in the presence of thiosulphate at light intensities that would not permit growth in its absence, since the lack of an increase in cell yield when sulphate concentrations were raised above $100 \mu\text{M}$ must be due to light limitation.

The rate of CO_2 fixation at different light intensities (0 to 20 klx) was examined. A different algal suspension was used for experiments at each light intensity and the number of cells was not determined. There was no CO_2 fixation with sulphate or thiosulphate in the dark. At 1 klx, the algae incubated with thiosulphate fixed $0.27 \mu\text{mol CO}_2 \text{ ml}^{-1} \text{ h}^{-1}$ whereas there was no CO_2 fixation in the algae incubated with sulphate. At 8 klx the algae incubated with thiosulphate fixed $0.85 \mu\text{mol CO}_2 \text{ ml}^{-1} \text{ h}^{-1}$ and the algae incubated with sulphate $0.45 \mu\text{mol CO}_2 \text{ ml}^{-1} \text{ h}^{-1}$. At 20 klx the algae fixed $0.70 \mu\text{mol CO}_2 \text{ ml}^{-1} \text{ h}^{-1}$ in both cases. These results are in accord with those of the growth experiments.

The oxidation of thiosulphate appeared to be dependent on enzyme induction, as shown by the following experiment. Two cultures were grown at 8 klx, one with thiosulphate and the other with sulphate. Both were harvested during exponential growth and resuspended in thiosulphate medium to a density of about 10^8 cells ml^{-1} . The disappearance of thiosulphate and appearance of sulphate were followed. The thiosulphate-grown cells oxidized thiosulphate rapidly after a lag period of about 30 min. The sulphate-grown cells required 2 h contact with thiosulphate before oxidation began, and then this was at a much slower, though increasing rate.

It is also evident that thiosulphate was completely oxidized to sulphate since $1.0 \mu\text{mol}$ thiosulphate gave about $2 \mu\text{mol}$ sulphate (for which thiosulphate was the sulphur source); whereas Cohen *et al.* (1975) found that *Oscillatoria limnetica* oxidized hydrogen sulphide only as far as elemental sulphur.

To show that oxidation of thiosulphate was directly associated with photosynthesis, the effect of two inhibitors was examined. Cells growing in thiosulphate medium at 8 klx were harvested during exponential growth and resuspended in the same medium to a density of about 10^8 cells ml^{-1} . DCMU, which is known to block photosystem II in blue-green algae (Krogmann, 1973), had no effect on thiosulphate oxidation by *A. nidulans* at a concentration of $10 \mu\text{M}$, for at least 8 h (Fig. 1*b*). DBMIB has been reported (Trebst & Harth, 1970) to inhibit electron transport between photosystems I and II close to, or at, the functional site of plastoquinone. When this halogenated quinone was added ($10 \mu\text{M}$), it inhibited the oxidation of thiosulphate by about 70 % (Fig. 1*a*). The effect of thiosulphate on oxygen evolution has not been determined. It would appear that *A. nidulans* can utilize thiosulphate as an electron donor, in a manner analogous to that in bacterial photosynthesis, when the supply of ATP or of electrons from water is curtailed by light limitation.

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