THIOSULFATE AS AN ELECTRON DONOR IN SEVERAL STRAINS OF BLUE-GREEN ALGAE

by

Lisa A. Harvill

Biology

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C.O. Patterson

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SUMMARY

Thiosulfate was tested as a possible electron donor for anoxygenic photosynthesis in the blue-green algae Anacystis nidulans, Nostoc muscorum, Anabaena variabilis, and Anabaena flos-aquae. Thiosulfate did not appear to be appreciably toxic to cell growth in any of the species examined, but it also did not seem to stimulate growth. No electron donation by thiosulfate was demonstrated for any of the organisms, using 3(3,4-dichlorophenyl)-1,1-dimethyl urea (DCMU), an inhibitor of photosystem II of the photosynthetic apparatus, as a check.

INTRODUCTION

Blue-green algae are unique organisms among those groups capable of photosynthetic growth. They possess a prokaryotic cellular organization, like that found in photosynthetic bacteria, but unlike the bacteria, the blue-greens have the capability of performing non-cyclic (oxygenic) photosynthesis, like that found in eukaryotic phototrophs including higher plants. This seemingly intermediary position of the blue-green algae between the relatively primitive bacteria and the more highly developed green plants has led to the suggestion that these organisms indeed represent an evolutionary link between the two rather widely separated groups (7).

The finding that some species of blue-green algae are capable of bacterial-type (anoxygenic) cyclic photosynthesis as well with the aid of an electron-donating compound (1,2,3,4,5,6,10,11) adds evidence to the theory that they are intermediates between

bacteria and green plants in the evolutionary tree. A number of the organisms tested were found to perform anoxygenic photosynthesis using sulfide as an electron donor (3,4,5,6). Cohen et.al. (5) found that the blue-green alga Oscillatoria limnetica seems to photo-oxidize sulfide quantitatively to elemental sulfur, which is then excreted from the cells. This use of sulfide is similar to that noted in many phototrophic sulfur bacteria and was verified by the use of 3(3,4-dichlorophenyl)-1,1-dimethyl urea (DCMU), an inhibitor of photosystem II (found only in blue-green algae and in eukaryotic phototrophs) of the photosynthetic apparatus. The fact that O. limnetica can also carry out oxygenic photosynthesis makes it ideally suited for growth in its natural habitat, one which fluctuates between aerobic and anaerobic conditions.

Molecular hydrogen and thiosulfate have also been found to act as electron donors for a few species of blue-green algae (1, 2,10,11). O. limnetica and Aphanothece halophytica have been shown to use molecular hydrogen efficiently in anoxygenic metabolism of CO_2 (1). Utkilen (11) has reported that the species Anacystis nidulans appears to utilize thiosulfate as an electron donor to the cyclic photosynthetic condition created by the use of DCMU. This organism seems to completely oxidize thiosulfate to sulfate.

This work is an attempt to further investigate the possible use of thiosulfate as an electron donor for anoxygenic photosynthesis by several species of blue-green algae. Anacystis nidulans,

Nostoc muscorum, Anabaena variabilis, and Anabaena flos-aquae were first grown in the presence of thiosulfate to test for its possible toxicity to the cells. Then DCMU was used to inhibit photosystem II in medium containing thiosulfate to allow demonstration of any actual use of the thiosulfate as an electron donor by the organisms.

MATERIALS AND METHODS

Organisms. Stock cultures of Anacystis nidulans, Nostoc muscorum, and Anabaena variabilis were obtained from Professor Jack Myers of the University of Texas. Anabaena flos-aquae came from Professor S. E. Stevens of Pennsylvania State University. All stock cultures were maintained at room temperature on 1% agar slants containing the standard growth medium (refer to Cell Culture) and were illuminated by one 60 watt incandescent bulb placed 6 inches away from a rack of tubes. Caps of all tubes were loosened so as to allow gas exchange.

Cell Culture. The organisms were maintained in liquid culture using the standard medium for blue-green algae described by Stevens, Patterson, and Myers (8), Referred to also as medium C. The medium was adjusted to pH 8.2 before sterilization by autoclaving at 121°C under steam pressure for 20 minutes. Tubes were incubated in a water bath at 32°C, and illumination was provided by 1-3 60 watt incandescent bulbs placed at a distance of 3 inches from the water bath. The number of bulbs used was determined by the number of tubes in the bath. The major source of carbon was either 1% CO_2 (Bailey Oxygen Company) in air, which

was mixed in the laboratory and bubbled into tubes via Pasteur pipettes, or 1mM $NaHCO_3$ added to the medium using sealed growth tubes. The tubes with $NaHCO_3$ were used to avoid possible oxidation of thiosulfate by molecular oxygen to sulfate which is not known to be an electron-donating compound.

Reagents. $Na_2S_2O_3 \cdot 5H_2O$ was used at a concentration of 1mM and was added to medium C, with $MgCl_2 \cdot 6H_2O$ substituted for the usual $MgSO_4 \cdot 7H_2O$ of medium C without thiosulfate. 3(3,4-dichlorophenyl)-1,1-dimethyl urea (Sigma Chemical Company), or DCMU, was made up in 95% ethanol and used at a concentration of 1μM. All chemicals used for media preparation were reagent grade.

Growth Experiments. Inoculums were taken from pre-existing liquid cultures in medium C and transferred into tubes containing medium C or medium C with thiosulfate added. After 24 hours, the log rhythmically-growing cells were used to inoculate fresh tubes of the respective media. These growth tubes were observed, and spectrophotometer readings were taken of them at 640nm to determine turbidity at intervals of several hours over a 60-hour period. Uninoculated tubes of each medium were used as blanks.

Electron Donation Experiments. Cultures containing medium C or medium C with thiosulfate added were again initiated from medium C liquid cultures. Following approximately 24 hours, fresh tubes were inoculated as in the Growth Experiments. In addition to these tubes, other fresh tubes of medium C or medium C with thiosulfate added were modified by adding DCMU, and then inoculations were made. Observation and turbidity readings were carried

out as before.

RESULTS

Growth Determinations. Growth rate comparisons for the organisms grown in either medium C or in medium C plus thiosulfate showed that thiosulfate was not appreciably toxic to growth (refer to Figures 1 and 2). In fact, the Anabaena species and Nostoc showed almost identical growth curves with or without thiosulfate. Growth curves for Anacystis showed essentially the same growth rate, as evidenced by the similar slopes of the curves, but with a slightly longer lag phase with thiosulfate present. Observation of the tubes over the growth period showed no appreciable differences in the appearances of the cells grown with or without thiosulfate.

Electron Donation. All cultures in which DCMU was present showed no growth over the incubation period. Those cells without DCMU and with or without thiosulfate showed normal growth.

DISCUSSION

The data presented in this study seem to indicate that while thiosulfate is not toxic to growth of the blue-green algae tested, it cannot be used as an electron donor under the conditions maintained here. If electrons had been obtained from thiosulfate by the organisms, growth would have occurred in cultures with DCMU and thiosulfate present, while those without thiosulfate but with DCMU would have no source of electrons for photosystem I and therefore could not carry out normal metabolic functions. Instead, no growth was observed in any of the cultures in

electron donation experiments.

This finding for Anacystis nidulans is contrary to results reported by Utkilen (11), who found stimulation of growth in the presence of thiosulfate and actual electron donation using DCMU for verification. Slight differences in culture techniques, involving temperature and/or pH, may have played some part in causing the conflicting results. A comparison of the DCMU concentrations used shows that of Utkilen to be 10 times greater than the concentration noted in this report, which would seem to mean that the latter concentration would even be preferable, since it appears to successfully inhibit photosystem II while being less likely to damage the cells in other ways. It is also possible that there is some undetermined factor that may play an important role in enabling A. nidulans to use thiosulfate as an electron source, such as an organic growth factor.

While Nostoc muscorum, Anabaena variabilis, and Anabaena flos-aquae have not been tested with thiosulfate in other laboratories, A. flos-aquae has been tested using sulfide as a possible electron donor by Stewart and Pearson (9). They found no growth with DCMU and sulfide present, indicating no donation of electrons when photosystem II was completely inhibited. However, when photosystem II was only partially inhibited by salicylaldoxime, they noted greater metabolic activity when sulfide was present than when it was absent. This would seem to indicate that some functioning of photosystem II is essential for growth of

A. flos-aquae in the presence of sulfide but that sulfide may play some sort of auxiliary role in electron donation when partial inhibition of photosystem II leaves the cell with an insufficient supply of electrons for metabolic reactions.

Further experimentation must be done with the various possible electron donors for the species of blue-green algae used in this study. Other possible donors include ascorbate and sulfite. Experiments with thiosulfate should be repeated, with further modification of culture techniques, and testing of the effect of salicylaldoxime on electron donation might prove useful. It may be that Nostoc muscorum and the Anabaena species would show some increased growth with thiosulfate as A. flos-aquae did in the Stewart and Pearson study (9) when photosystem II was only partially inhibited. In addition, better controls should be devised to make certain that the thiosulfate is not being oxidized by some force other than by the cells and that conditions are indeed favorable for thiosulfate uptake by the cells.

Figure 1. Growth Curves for Nostoc muscorum and Anabaena variabilis (1% CO_2 in air, 32°C).

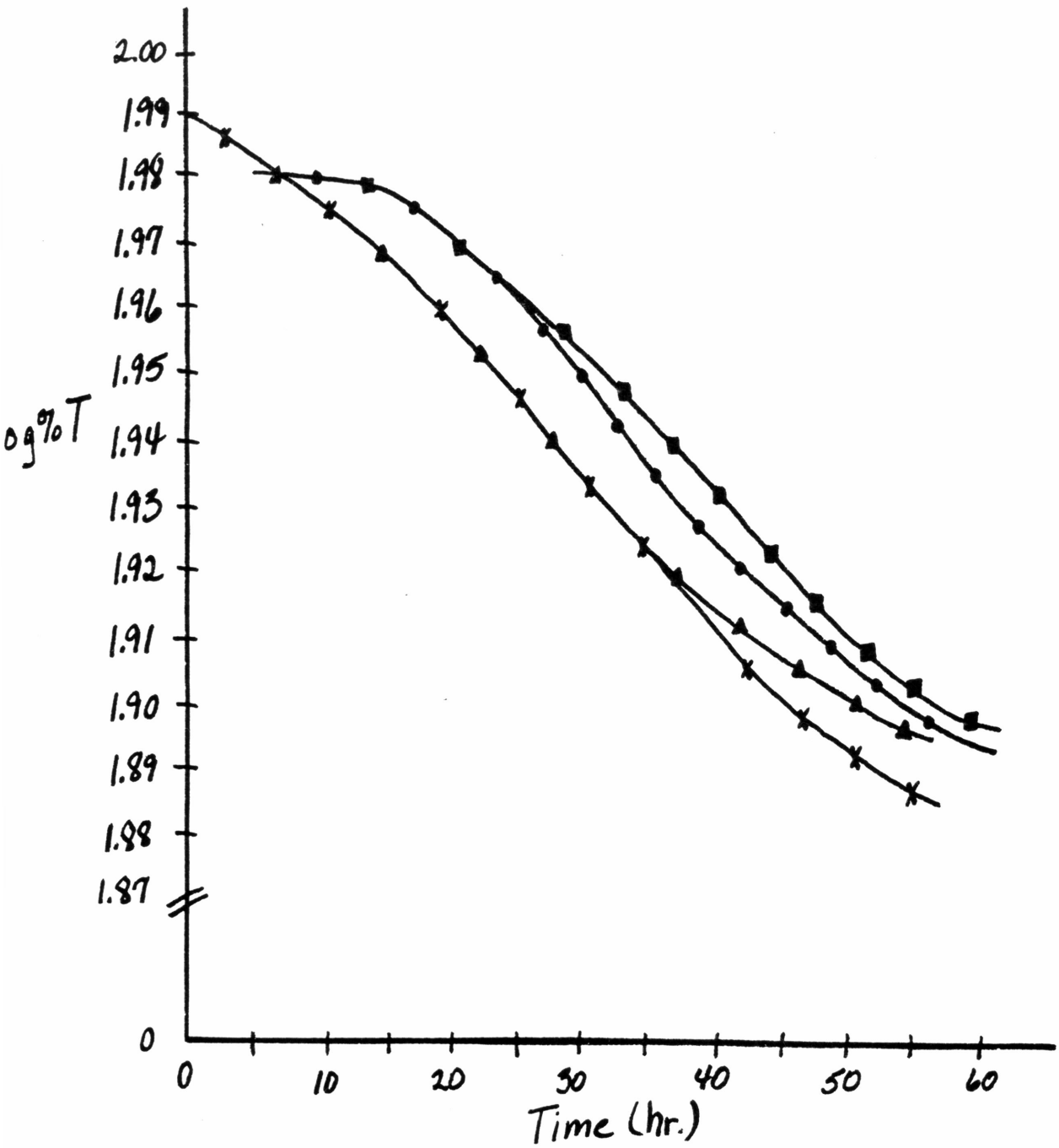
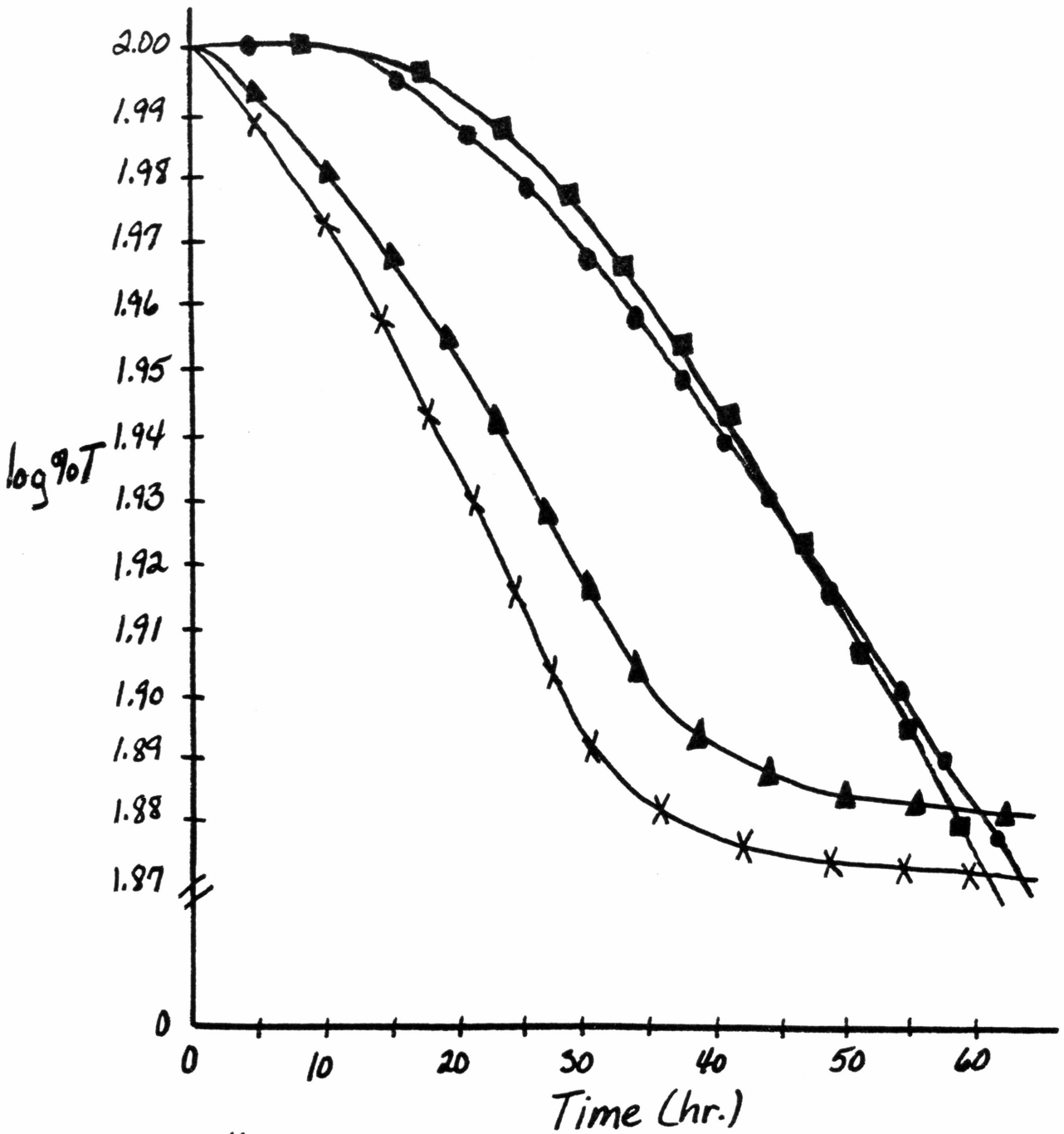


Figure 2. Growth Curves for Anacystis nidulans and Anabaena variabilis (1% CO_2 in air, 32°C).



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