NADH OXIDASE IN BLUE-GREEN ALGAE

A. A. Horton

Department of Biochemistry, University of Birmingham, Great Britain

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Although particulate fractions prepared from two colorless members of the Cyanophyta were shown by Webster and Hackett (1966) to possess NADH oxidase, it was recently reported that no NADH oxidase activity could be detected in particles isolated from some pigmented members of the Cyanophyta (Smith et al., 1967). This communication reports the results of studies on two pigmented and one colorless member of the Cyanophyta. Particles prepared from all three organisms possessed NADH oxidase activity, but lacked other respiratory activities e.g. cytochrome c oxidase.

Materials and Methods

Pure cultures of Anacystis nidulans, a gift from M. B. Allen (University of Alaska, Fairbanks, U.S.A.) and Anabaena variabilis, a gift from N. G. Carr (University of Liverpool, U. K.) were grown in a mineral salts medium supplemented with sodium acetate (0.01 M) and yeast extract (0.1 %). Leucothrix sp., a gift from R. Y. Stanier (University of California, Berkeley) was grown as described by Webster and Hackett (1966).

Inoculations were made by the transfer of a dense suspension of late log phase cells. Leucothrix sp. was incubated in still culture (25°) and the pigmented organisms in an illuminated rotary shaker at 35°. Cells of all organisms were harvested in the

late log phase, washed with buffer (0.1 M sodium phosphate pH 7.4; 0.001 M EDTA) and resuspended in a small volume of mannitol-medium to yield a 20 % cell suspension. The mannitol medium was a modification of that of Wiskich and Bonner (1963) and contained sucrose 85.50 g; mannitol, 67.00 g; $Na_2 \cdot EDTA \cdot 2H_2O$, 1.86 g; cysteine, 0.50 g all dissolved in 0.01 M sodium phosphate pH 7.0 (1000 ml). Cells of Anacystis and Anabaena were disintegrated in the cold (2^{o}) by the addition of an equal volume of glass beads (0.025 mm. diameter) and treatment for 10 min. in a Mickle shaker. In the case of Leucothrix, it was necessary to first subject the cell suspensions to treatment in a Potter-Elvehjem type homogenizer before disintegration in the Mickle shaker. Cells and cell fragments were decanted from the beads and centrifuged at 8,500 x g for 10 min. to remove whole cells and the cell-free supernatant recentrifuged at 35,000 x g for 30 min. to sediment the respiratory particles which were washed again in the same buffer.

Spectrophotometric assays of the following respiratory activities, cytochrome c oxidase (Polakis et al., 1964), succin-oxidase, NADH oxidase and NADH-cytochrome c reductase (Green and Ziegler, 1963) were performed in mannitol medium as indicated. Assays were started by addition of substrate either directly, or from the side arm of a Thunberg tube. For anaerobic experiments, the Thunberg cuvettes were alternately evacuated and gassed with O_2-free argon three times.

Antimycin A, amytal, HOQNO (2-Heptyl-4-hydroxyl-quinoline-N-oxide) and rotenone were dissolved in ethanol and the appropriate controls were included in assay systems. Dry weight determinations were made by centrifuging and washing the cells or particles with water in preweighed tubes followed by drying to

constant weight under vacuum. To check for heterotrophic contamination, samples from cultures used in experiments were streaked on 0.1 % yeast agar supplemented with 0.01 M sodium acetate.

Results and Discussion

Cytologically, the blue-green algae are quite distinct from the higher algae but resemble the bacteria inasmuch as they are procaryotic and lacking mitochondria. The cytochrome system in bacteria is found in association with the cell wall (Smith, 1961) and the results of experiments with Anacystis nidulans, Anabaena variabilis and Leucothrix sp. suggest that the respiratory system of these organisms is associated with the cell wall.

Cell-free particulate preparations of these three organisms, prepared as described, were assayed for respiratory activity. NADH oxidase was found to be present in all three organisms in contrast to a previous report (Smith et al., 1967). It is possible that these apparently conflicting results may be explained on the basis of the different procedures used e.g. the preparation of the particulate fractions, and the conditions employed for culture of the microorganisms. Assays for cytochrome c oxidase, succinoxidase and NADH-cytochrome c reductase showed no activity. The observed rates of activity of NADH oxidase in particles from Anacystis and Anabaena were lower than in particles prepared from Leucothrix (Table I) where the rate exceeded that reported for some colorless members of the Cyanophyta examined in earlier studies (Webster and Hackett, 1966). The higher rate is probably due in part to the previously noted stimulatory effect of EDTA which was included in the assay medium. The variations in

TABLE I. NADH Oxidase Activity in Particles Isolated from Three Organisms

Organism	NADH Oxidase Activity*
Anacystis nidulans	10 (±4)
Anabaena variabilis	9 (±3)
Leucothrix sp.	100 (±23)

*mμ moles/mg dry wt./min.
Particles isolated as in text.

specific activity of NADH oxidase observed are reported as standard deviations (Table I).

In order to confirm that the NADH oxidase was generally associated with the cytoplasmic membrane and not confined to one size of particle, the disintegrated cells were centrifuged at a series of successively higher speeds and the sedimented particles washed in mannitol medium and assayed for NADH oxidase activity. The results show (Table II) that the enzymic activity is generally distributed among particles of different size and is mainly confined to the particulate fraction despite the fact that the highest centrifugal force used was only 40,000 x g. Some NADH dehydrogenase activity seen in the supernatant after high speed centrifugation is also often observed in similar preparations in higher plants.

TABLE II. Localization of NADH Oxidase Activity in Particles of Different Size from Anacystis nidulans.

Fraction	NADH Oxidase Activity*
10,000 x g sediment	9
20,000 x g sediment	10
40,000 x g sediment	8
40,000 x g supernatant	1

*mμ moles/mg dry wt./min. Mean of two experimental results. Particulate preparation was first centrifuged at 8,500 x g for 10 min. to remove whole cells.
Time for each sedimentation = 30 min.

The effects of different respiratory inhibitors on the particulate NADH oxidase activities of the three organisms studied revealed differences in response from the effects observed on the respiratory systems of higher organisms. (Table III). The results quoted represent the mean of five determinations and include the standard deviations. The inhibitory effect of azide on the particulate preparations tested compared with its action on eucaryotic organisms was extremely slight and provides

TABLE III. Effects of Inhibitors on the NADH Oxidase of Three Organisms.

Inhibitor	Final Concentration (Molarity)	% Inhibition		
		Anabaena variabilis	Anacystis nidulans	Leucothrix sp.
Anaerobic	...	100	100	100
Boiled Particles	...	100	100	100
KCN	1.0×10^{-3}	82 (±3)	65 (±8)	92 (±8)
NaN_3	1.0×10^{-3}	7 (±5)	4 (±3)	4 (±3)
Antimycin A	2.0×10^{-6}	11 (±2)	8 (±3)	7 (±3)
HOQNO	7.6×10^{-6}	45 (±7)	41 (±3)	49 (±3)
Rotenone	6.0×10^{-5}	59 (±6)	53 (±6)	53 (±6)
Amytal	1.0×10^{-3}	47 (±5)	47 (±6)	42 (±8)

Control rates as in Table I.

further support for the view that this terminal oxidase in these organisms is different to that found in higher plants and algae. The effects of other inhibitors (e.g. antimycin and HOQNO) resembled the response typical of certain bacteria. Complete inhibition of changes in optical density at 340 mμ under anaerobic conditions provides strong evidence for concluding that the enzyme systems studied are true oxidases.

The results reported here clearly show the existence of an NADH oxidase in particulate preparations isolated from Anacystis and Anabaena. Studies on the respiratory systems of these organisms are continuing.

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