SOME RESPIRATORY CHARACTERISTICS OF THE BLUE-GREEN ALGA, ANABAENA

GEORGE C. WEBSTER [1] AND ALBERT W. FRENKEL

DEPARTMENT OF BOTANY, UNIVERSITY OF MINNESOTA, MINNEAPOLIS, MINNESOTA

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Introduction

In recent years there has been a considerable increase in our knowledge of the respiratory metabolism in higher plants. The lower plants, however, have received little attention. This is particularly true of the blue-green algae where the amount of available information on their respiration is almost negligible. The only known investigation has been that of EMERSON (**1**) who reported that respiration in a species of Oscillatoria was inhibited about 80% by 10^{-4} M hydrogen cyanide but only 12% by a mixture of 97% carbon monoxide–3% oxygen. The purpose of this investigation has been to determine some of the over-all respiratory characteristics of a blue-green alga, and to compare certain of these characteristics with those generally found in living organisms.

Materials and methods

The alga under investigation was isolated in pure culture by R. Hecker of the University of Illinois, and identified as a species of Anabaena by Dr. E. Pringsheim of Cambridge University (**3**). The alga was grown autotrophically in an inorganic salt solution of the following composition which was modified from the Chroococcus medium of EMERSON and LEWIS (**2**): $MgSO_4 \cdot 7\,H_2O$, 0.25 gm./l.; KH_2PO_4, 0.10 gm./l.; KNO_3, 1.00 gm./l.; $Ca(NO_3)_2$, 0.025 gm./l.; NH_4Cl, 0.05 gm./l.; $FeSO_4(NH_4)_2SO_4 \cdot 6\,H_20$, 0.004 gm./l.; Mn, 2.0 mg./l. (as $MnSO_4$); B, 1.0 mg./l. (as H_3BO_3); Zn, 0.05 mg./l. (as $ZnSO_4$); Cu, 0.01 mg./l. (as $CuSO_4$); Mo, 0.01 mg./l. (as MoO_3). Fifty-ml. aliquots of this nutrient solution were autoclaved in 250-ml. Erlenmeyer flasks, and after cooling the solution, 2.5 ml. of a Seitz filtered solution containing 1.2 gm. $NaHCO_3$ per 50 ml. were added to each flask of nutrient medium. The final pH of the medium was 9.0. The liquid cultures were inoculated from young cultures actively growing on agar slants and then grown under continuous illumination from white fluorescent lamps (at about 50 foot-candles) at a temperature of $24 \pm 1°$ C. In the course of growth the nutrient medium became more alkaline. The pH of a mature culture was about 9.5. Cultures were grown for 15 to 20 days before the cells were used for experiments. The cultures were checked periodically for contamination by bacteria and fungi by growth on glucose-peptone-agar slants.

[1] Present address: Kerckhoff Laboratories of Biology, California Institute of Technology, Pasadena, California.

The alga was maintained on a medium containing 2% agar in nutrient medium to which Seitz filtered $NaHCO_3$ solution was added just before the agar solidified.

Measurements of respiratory activity were carried out using the standard Warburg manometric technique. Both phosphate and carbonate buffers were used during this investigation. Dry weights of aliquots were determined for each experiment. Studies with cyanide were performed in such a way as to maintain a constant concentration of undissociated acid (**6**). Cyanide and azide were added to the test system as their sodium salts, but the concentrations of these inhibitors expressed in this paper refer to final concentrations of the non-dissociated acids, HCN and HN_3 in the Warburg vessels. All experiments with carbon monoxide were corrected for blank uptake of carbon monoxide in the presence of KOH. Appropriate controls using nitrogen-oxygen mixtures of the same percentage composition were employed during all carbon monoxide experiments. Experiments were performed at 25° C.

Fragmented cells were prepared by grinding whole cell masses at 0 to 5° C with equal quantities of 0.05 M phosphate buffer (pH 6.0) and washed Carborundum powder. The unbroken cells and the Carborundum were removed by low speed centrifugation, and the supernatant was kept at 5° C until used. Appropriate controls containing boiled fragmented cells were employed to correct for non-enzymatic oxidations.

Cytochrome c was prepared by the method of KEILIN and HARTREE (**4**).

Results

ENDOGENOUS RESPIRATION

Values for rates of oxygen consumption and for respiratory quotients are given in table I. In the absence of any added organic substrates the algal cells maintained a constant respiratory rate for several hours over a wide range of hydrogen ion concentrations and in two different buffer systems. The course of respiration was examined over an extended period of time and

TABLE I

RATES OF ENDOGENOUS RESPIRATION OF ANABAENA UNDER VARIOUS CONDITIONS OF PH AND SUSPENDING MEDIUM (DURATION OF EXPERIMENT WAS THREE HOURS).

Suspending medium	pH	Respiratory rate	Respiratory quotient
		cmm. O_2 absorbed/ mg. dry wt./h.	*cmm. CO_2 evolved/ cmm. O_2 absorbed*
Culture solution	9.3	4.5	....
0.05 M Bicarbonate-carbonate buffer (Na : K = 2 : 1)	9.1	4.6	....
0.05 M Phosphate buffer	7.9	4.7	0.90
0.05 M Phosphate buffer	7.1	4.2	0.90
0.05 M Phosphate buffer	6.0	4.6	0.90
0.05 M Phosphate buffer	5.3	4.8	0.95

showed that the initial respiratory rate continued for about four hours after the cells were placed in the dark. This was followed by a gradual decrease to a value about 20% of the original rate after 36 to 48 hours. Cells kept in the dark for three to five days became yellow but continued to respire at the low endogenous rate. They became green again after several days exposure to weak light of about 50 foot-candles. Unless otherwise indicated, results in this paper are from experiments which were performed during the first four hours after the cells had been put in the dark.

Effect of respiratory inhibitors

The heavy metal enzyme inhibitors, cyanide, azide, and *o*-phenanthroline strongly inhibited endogenous respiration (table II). It should be noted, however, that a certain portion of the respiration was not cyanide sensitive, indicating the possibility of an alternate respiratory pathway not mediated by heavy metal containing enzymes. All respiratory inhibitions, except those with *o*-phenanthroline, could be completely abolished by washing the cells several times in distilled water.

It was found that endogenous respiration was relatively insensitive to carbon monoxide until very high ratios of CO/O_2 were employed. However, the oxidation of substrate was strongly inhibited by carbon monoxide (table IV). Inhibitions were easily reversed by flushing out the manometer and vessel with air. In figure 1, the results for the inhibition of endogenous

TABLE II

INHIBITION OF OXYGEN CONSUMPTION BY INTACT CELLS OF ANABAENA BY CERTAIN RESPIRATORY INHIBITORS (CELLS IN 0.05 M PHOSPHATE BUFFER AT PH 5.5. DURATION OF EACH EXPERIMENT WAS THREE HOURS).

Inhibitor	Molar concentration	Substrate added	Range of inhibition
			%
Hydrogen cyanide*	0.00001	none	20–30
Hydrogen cyanide	0.0001	none	60–70
Hydrogen cyanide	0.001	none	70–75
Hydrogen cyanide	0.01	none	75–80
Hydrogen cyanide	0.1	none	75–80
Hydrogen cyanide	0.001	0.05 M glucose	80–85
Hydrogen cyanide	0.001	0.05 M ascorbate	85–90
Hydrogen azide	0.0001	none	0–10
Hydrogen azide	0.001	none	20–25
Hydrogen azide	0.01	none	60–70
o-Phenanthroline	0.001	none	30–40
o-Phenanthroline	0.01	none	60–65
Hydroxylamine	0.0001	none	0
Hydroxylamine	0.001	none	5–10
Hydroxylamine	0.01	none	10–20

*Inhibition of endogenous respiration by hydrogen cyanide was the same for 0.1 M to 0.00001 M HCN in carbonate-bicarbonate buffer (pH 9.1) as in phosphate buffer (pH 5.5).

respiration by carbon monoxide were plotted according to the method of LINEWEAVER and BURK (5). From this plot the distribution constant (K_D) was calculated as 29.0 (probable error ± 2.2). This value is considerably higher than the value of 2.25 obtained by WIZLER (8) for yeast respiring on glucose but is smaller than the value of 180 which can be calculated from EMERSON'S data (1) for Oscillatoria. It is apparent, however, that stimulation of oxygen uptake was observed at lower CO/O_2 ratios. It remains to be determined whether this stimulation is due to oxidation of carbon monoxide or due to actual increase in the rate of oxidation of endogenous substrate. Should oxidation of carbon monoxide take place, the distribution constant calculated above would have to be corrected. For glucose, ascorbate, and hydroquinone only one CO/O_2 ratio was tested for each compound and thus

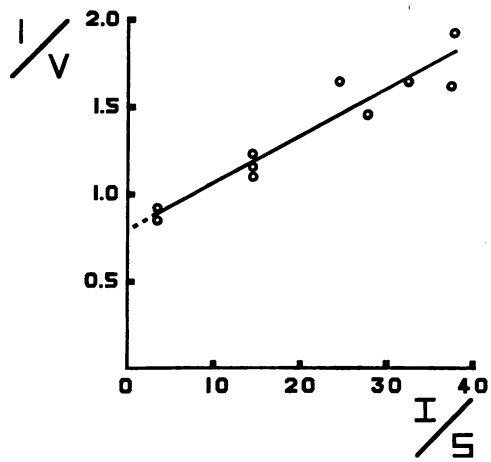

FIG. 1. The effect of different ratios of carbon monoxide to oxygen on the rate of endogenous respiration of Anabaena sp. Abscissa: Ratio of carbon monoxide to oxygen in liquid phase. Ordinate: Reciprocals of the relative rates of endogenous respiration.

only the Warburg distribution constants (7) were calculated for the observed results (table IV). The value thus obtained for glucose is also somewhat higher than those for yeast obtained by WARBURG (7) and WINZLER (8). In contrast, the oxidation of ascorbate by intact cells and of hydroquinone by fragmented cells, fortified with cytochrome c, is considerably more sensitive to carbon monoxide than the oxidation of glucose. The distribution constants in the presence of ascorbate and of hydroquinone are more comparable to those obtained by Warburg for the respiration of yeast in the presence of glucose.

An effort was made to study the effect of light on green cells whose respiration had been inhibited by carbon monoxide. The results were complicated by photosynthetic gas exchange and by an apparent inhibition of respiration in the light. The full details of these experiments will be published in a later paper. In starved cells which had lost their photosynthetic

TABLE III

OXIDATION OF ORGANIC SUBSTRATES BY INTACT AND FRAGMENTED CELLS OF ANABAENA (CELLS IN 0.05 M PHOSPHATE BUFFER AT PH 6.0. DURATION OF EACH EXPERIMENT WAS THREE HOURS. TEMPERATURE WAS 25° C).

Substrate	Oxygen uptake		Per cent. of control	
	Intact cells	Fragmented cells	Intact cells	Fragmented cells
	cmm. O_2 consumed/ mg. dry wt./hr.			
None	1.8	0.08	100	100
Glucose (0.05 M)	10.5	0.10	584	125
Galactose (0.05 M)	2.0	0.08	111	100
Mannose (0.05 M)	2.0	0.08	111	100
Succinate (0.05 M)	2.3	0.15	128	187
Acetate (0.05 M)	2.1	0.09	117	112
Pyruvate (0.05 M)	1.4	....	78	....
Catechol (0.01 M)	1.7	0.08	95	100
Ascorbate (0.05 M)	11.5	1.30	639	1625
Ascorbate + 0.0001 M cytochrome c	....	4.80	....	6000
Hydroquinone (0.01 M)	1.8	1.00	100	1450
Hydroquinone + 0.0001 M cytochrone c	....	3.60	....	4500

pigments after incubation in the dark for several days, the carbon monoxide inhibition of glucose enhanced respiration was completely reversed by light (table IV).

Oxidation of added organic substrates

Algal cells which had been kept in the dark for 24 hours to deplete photosynthetically produced substrates, were able to oxidize several organic materials (table III). In order to eliminate any influence of permeability on the oxidations, fragmented cell preparations were also used. Of interest was the finding that a polyphenol oxidase was absent from these cells since they were unable to oxidize catechol, but a cytochrome oxidase was probably present since oxidation of hydroquinone or ascorbate was enhanced by the presence of cytochrome c. The oxidation stimulated by cytochrome c was inhibited by carbon monoxide (table IV), and thus was probably mediated by a heavy metal enzyme.

Discussion

The results presented here leave little doubt that most of the respiration of Anabaena is mediated by one or more heavy metal-containing enzymes. Of the known heavy metal oxidases which have been postulated as participating in plant respiration (polyphenol oxidase, ascorbic acid oxidase, and cytochrome oxidase), polyphenol oxidase is apparently eliminated from consideration as a terminal oxidase in this organism since neither intact nor fragmented cells possessed any ability to oxidize catechol. The evidence for

TABLE IV

EFFECT OF CARBON MONOXIDE ON OXIDATIONS BY INTACT AND FRAGMENTED CELLS OF ANABAENA (CELLS IN 0.05 M PHOSPHATE BUFFER AT PH 6.0. DURATION OF EACH EXPERIMENT WAS THREE HOURS. TEMPERATURE WAS 25° C).

Substrate	Oxygen uptake		Inhibition	Relative affinity constant
	Intact cells	Fragmented cells		
	cmm. oxygen consumed/ mg. dry wt./hr.		%	K_A
Ascorbate	11.5	....	0	....
Ascorbate + carbon monoxide ($CO/O_2 = 24.6$)*	2.5	....	78	6.9
Hydroquinone + 0.0001 M cytochrome c	....	3.60	0	....
Hydroquinone + 0.0001 M cytochrome c + carbon monoxide ($CO/O_2 = 18.2$)	....	0.90	75	6.1
Glucose (0.05 M)**	13.0	....	0	....
Glucose + carbon monoxide ($CO/O_2 = 14.4$) in the dark**	7.5	....	42	19.9
Glucose + carbon monoxide ($CO/O_2 = 14.4$) in the light**	13.2	....	0	....

*CO/O_2 in the liquid phase.
**Non-photosynthetic cells.

the occurrence of an ascorbic acid oxidase is somewhat uncertain, since the oxidation of ascorbate is stimulated by cytochrome c and would seem, therefore, to be mediated, at least in part, by a cytochrome oxidase.

On the other hand, the possibility of a cytochrome oxidase operating in respiration must be considered. The following facts point to the participation of this enzyme in the respiration of Anabaena: (a) The endogenous respiration is inhibited by cyanide, azide, and carbon monoxide; (b) The oxidation of hydroquinone and ascorbate by fragmented cells is definitely stimulated by the addition of cytochrome c and this stimulated oxidation is carbon monoxide sensitive; (c) The carbon monoxide inhibition of glucose oxidation by non-photosynthetic cells cultured in the dark is reversed by light.

Summary

The endogenous respiration of the blue-green alga, Anabaena, is independent of hydrogen ion concentration between pH 5.3 and 9.3 in phosphate and carbonate-bicarbonate buffers. When the algae are kept in the dark for two days, however, the respiratory rate declines to about 20% of the original rate.

Endogenous respiration is strongly inhibited by cyanide, azide, and *o*-phenanthroline, but is inhibited by carbon monoxide only at high ratios of CO/O_2. The calculated distribution constant is 29.

Oxidation of hydroquinone or ascorbate by fragmented cells is stimulated by cytochrome c, and the stimulated oxidation is strongly inhibited by carbon monoxide. Polyphenol oxidase is not present in Anabaena cells.

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