Plant Physiol. (1966) 41, 599–605

Respiratory Chain of Colorless Algae
II. Cyanophyta

D. A. Webster and D. P. Hackett[1]
Department of Biochemistry, University of California, Berkeley, California

Received September 16, 1965.

Summary. Whole cell difference spectra of the blue-green algae, *Saprospira grandis*, *Leucothrix mucor*, and *Vitreoscilla sp.* have one, or at the most 2, broad α-bands near 560 mμ. At $-190°$ these bands split to give 4 peaks in the α-region for b and c-type cytochromes, but no α-band for a-type cytochromes is visible. The NADH oxidase activity of these organisms was shown to be associated with particulate fractions of cell homogenates. The response of this activity to inhibitors differed from the responses of the NADH oxidase activities of particulate preparations from the green algae and higher plants to the same inhibitors, but is more typical of certain bacteria. No cytochrome oxidase activity was present in these preparations. The respiration of *Saprospira* and *Vitreoscilla* can be light-reversibly inhibited by CO, and all 3 organisms have a CO-binding pigment whose CO complex absorbs near 570, 535, and 417 mμ. The action spectrum for the light reversal of CO-inhibited *Vitreoscilla* respiration shows maxima at 568, 534, and 416 mμ. The results suggest that the terminal oxidase in these blue-greens is an o-type cytochrome.

As very little is known of the respiratory chain of algae and virtually nothing is known of their terminal oxidase (16), we have studied algae from 3 divisions with regard to respiratory characteristics, cytochrome content, and the nature of the terminal oxidase that is present. To avoid the presence of interfering photosynthetic pigments, colorless algae have been used in this study. The first paper of this series gave the results for the *Chlorophyta* and the *Euglenophyta* (30). This paper reports the results obtained for the *Cyanophta*. Preliminary reports of this work have already been published (28, 29).

Materials and Methods

Saprospira grandis was kindly sent to us by Ralph Lewin of Scripps Institution of Oceanography. It was grown in Fernbach flasks at 30° with shaking. The medium used (R. A. Lewin, personal communication) was made up as follows: potassium nitrate, 0.5 g; sodium glycerophosphate, 0.1 g; Tris buffer, 1.0 g; tryptone, 5.0 g; and yeast extract, 5.0 g; all in 1000 ml filtered sea water. The medium was autoclaved for 5 minutes, the precipitate removed by filtering through Reeve-Angel No. 835 filter paper and then reautoclaved for 15 minutes. Cells were harvested in late log phase by centrifugation at 2500 $\times$ *g* in a Servall refrigerated centrifuge.

Vitreoscilla species and *Leucothrix mucor* were gifts of R. Y. Stanier of this University. *Leucothrix mucor* was grown on medium made up as follows (R. Y. Stanier, personal communication): 1.0 g yeast extract, 1.0 g tryptone, 0.2 g beef extract, 1000 ml synthetic sea water (20). The pH was adjusted to 8.0 to 8.3. *Vitreoscilla* species were grown on medium of the following composition (R. Y. Stanier, personal communication): 5.0 g yeast extract, 5.0 g peptone, and 0.1 g sodium acetate in 1000 ml tap water; the final pH was adjusted to approximately 7.5. Both *Leucothrix* and *Vitreoscilla* were grown in Fernbach flasks, each containing 500 ml, at room temperature and were harvested by low speed centrifugation as for *Saprospira*. When larger amounts of *Vitreoscilla* were required for the disintegration experiments, it was grown in 9 liter bottles as previously described for *Polytoma* and *Astasia* (30).

Saprospira was disintegrated using the method which was successful for the higher algae (30), but with a few modifications. Instead of mannitol medium, potassium phosphate buffer (0.1 M, pH 7.0) was used as the disintegration and suspending medium (24). After blending for 5 minutes at 30 % maximum speed, the extract was poured through a pre-cooled fritted glass filter into a vacuum flask to remove the glass beads. The extract was then centrifuged for 15 minutes at 2000 to 3000 $\times$ *g* and then at 30,000 to 35,000 $\times$ *g*. The 2 precipitates were washed and suspended in phosphate buffer. Both suspensions were orange while the supernatant fluid was practically colorless. All 3 fractions were as-

[1] Died January 21, 1965.

sayed for NADH oxidase activity and protein. All the above operations were performed at 0 to 4°, except the assays, which were done at room temperature.

Particulate preparations could be obtained from *Vitreoscilla* by either blending a suspension of cells in 0.1 M potassium phosphate buffer, pH 7.5, with glass beads or by treating the cells with lysozyme + EDTA (3, 10). The former method yielded particles with a higher NADH oxidase activity than the latter method, but even after centrifugation at 80,000 × *g* for 20 minutes there were still some cytochromes present in the supernatant. The latter method produced an extract which on centrifugation at 35,000 × *g* for 20 minutes had all of the cytochromes in the precipitate.

NADH oxidase assays on fractionated *Saprospira* and *Vitreoscilla* cells were performed using 0.1 M potassium phosphate, pH 7.0 and 7.5, respectively, as the assay medium. Otherwise, the procedure for this assay, as well as the cytochrome c oxidase assay, dry weight determinations, and protein determinations, was the same as described in the first paper of this report (30). Bovine serum albumin (Sigma) was added as the standard for the protein determinations. The spectral methods and the methods employed for the CO inhibition and light reversal experiments were also described in this first paper.

The action spectrum for the light reversal of CO inhibited *Vitreoscilla* respiration was performed with the action spectrophotometer similar to that described by French and Myers (15) and the electrode assembly and circulatory system described by Fork (13). The circulatory system contained 60 ml of sterilized medium, and the air space was flushed with about 1000 ml of the 19:1 CO:O_2 gas mixture. After this initial flushing, the remaining gas in the mixing bottle was circulated with an aquarium pump.

Results

Saprospira grandis Gross. Difference Spectra of Whole Cells and Cell Fragments. The cultivation of *S. grandis* is somewhat more difficult than that of the other algae studied because of the rapid death of the cultures. Maximum growth is achieved in 2 days at 30°, but by the fourth day, the characteristic silky, nacreous appearance of healthy cultures has disappeared. A suspension of healthy cells has a bright orange color. Unlike *Polytoma*, however, there are multiple bands of carotenoid absorption in *Saprospira;* these bands caused endless difficulty in obtaining difference spectra. An absolute spectrum (versus filter paper) shows absorption maxima at 633, 576 (shoulder), 514, 481, 455, and 411 mμ (cf. 14).

Whole cell difference spectra reveal bands at 560 (shoulder) and 553 mμ and a Soret at 425 mμ (cf. fig 1, a difference spectrum of particles prepared from *S. grandis*). There was no α-band visible in the 600 mμ region. CO difference spectra show bands at about 570 and 540 mμ, a Soret at 418 mμ, and a trough at about 430 mμ. The same type of CO difference spectrum has been observed for *Leucothrix* (fig 4) and *Vitreoscilla* (fig 6).

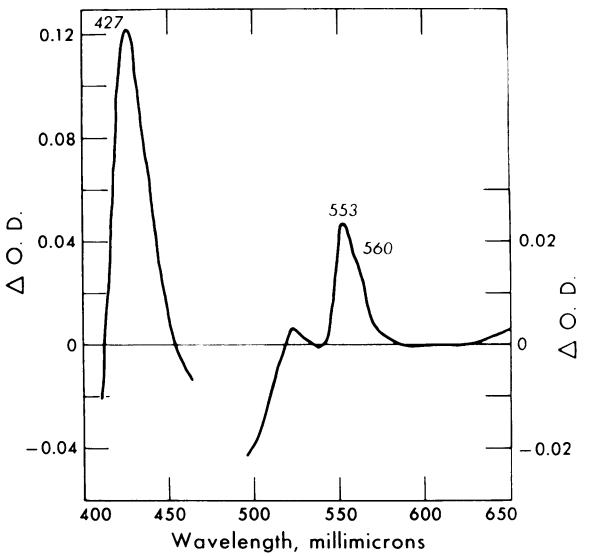

FIG. 1. Difference spectrum of 10,000 × *g* particles from *S. grandis* (dithionite reduced)−(oxidized). Protein concentration was 6.4 mg/ml in 0.067 M phosphate, pH 7.0 and 33 % glycerol.

Disintegration and Sedimentation Experiments. The blue-green algae are cytologically quite unlike the higher algae but resemble the bacteria in that they are procaryotic and possess no mitochondria. In the bacteria the cytochrome system has always been found to be associated with the particulate fractions of extracts (24). To see if the respiratory activity of *Saprospira* is indeed particulate, cells were disintegrated and assayed as described (table I). Note that the specific activities of the 2 washed particulate fractions are identical while the specific activity of the supernatant is much lower. Table I shows that over 80 % of the total respiratory activity is particulate even when the extract is sedimented at relatively low velocity (30,000 × *g*). The effects

Table I. *Localization of NADH Oxidase Activity in* S. grandis *Homogenates*

Fraction	Total protein (mg)	*NADH oxidase activity	**Ratio of activities	% of Total activity
2000 × *g* precipitate	38	58	0.98	59
30,000 × *g* precipitate	16	59	1.00	25
30,000 × *g* supernatant	65	0	0.15	16

* mμmoles per minute per mg protein.

** Activity of 30,000 × *g* precipitate arbitrarily defined as 1.00.

Table II. *Effects of Inhibitors on the NADH Oxidase Activity of* Saprospira grandis *Particles*

The average control rate was 76 mμmoles/minute per mg protein.

Inhibitor	Conc (M)	% Inhibition
Anaerobic	...	100
KCN	1.0×10^{-3}	57
HOQNO	7.7×10^{-6}	56
Antimycin A	2.2×10^{-6}	0
Rotenone	6.0×10^{-5}	0

of various inhibitors on the NADH oxidase activity of the 2 particulate fractions were the same; the average of 6 determinations of the effects of inhibitors on the NADH oxidase activity of *Saprospira* particles is given in table II. Diphenylamine gave an anomalous result: a concentration of 3.3×10^{-4} M caused about a 60 % stimulation in the rate of NADH oxidation by these particles (cf. 1). EDTA (2.0×10^{-4} M) also gave a stimulation (42 %). There was no cytochrome c oxidase activity present in any of the fractions.

A difference spectrum of a particulate preparation is given in figure 1, which shows the same absorption maxima that are present in whole cell difference spectra. The supernatant contained a relatively small amount of cytochromes.

CO Inhibition of Respiration. The best test for a heme protein terminal oxidase is light reversible CO inhibition (*27*). That the terminal oxidase of *S. grandis* clearly meets this specification is demonstrated in figure 2. In this experiment the lights were turned on and off successively at the end of each hour for 4 hours. It is clear that light reverses the CO inhibition and that light has no effect on the nitrogen control. From many such experiments an average value of 48 % inhibition is obtained for CO which contains 10 % O_2.

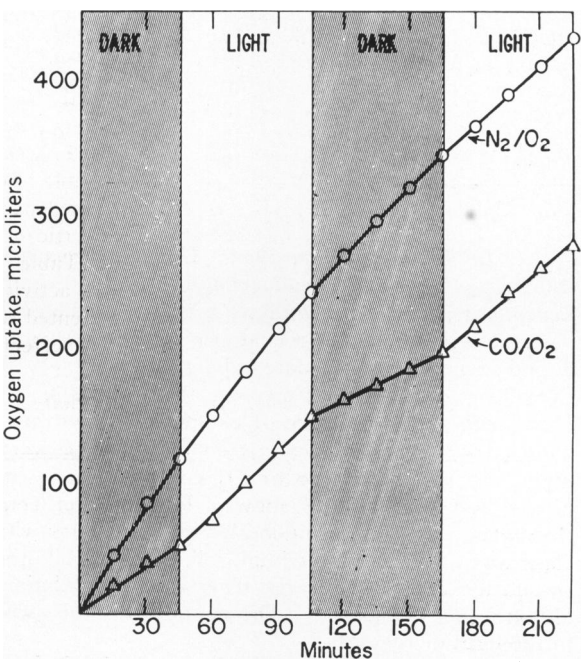

FIG. 2. CO inhibition of *S. grandis* respiration. Each flask contained 4.8 mg dry weight of cells in 3.0 ml of boiled sea water + 0.01 M phosphate, pH 7.0. CO/O_2 = 9/1. The control flasks contained N_2 instead of CO. The temperature of the water bath was 30°.

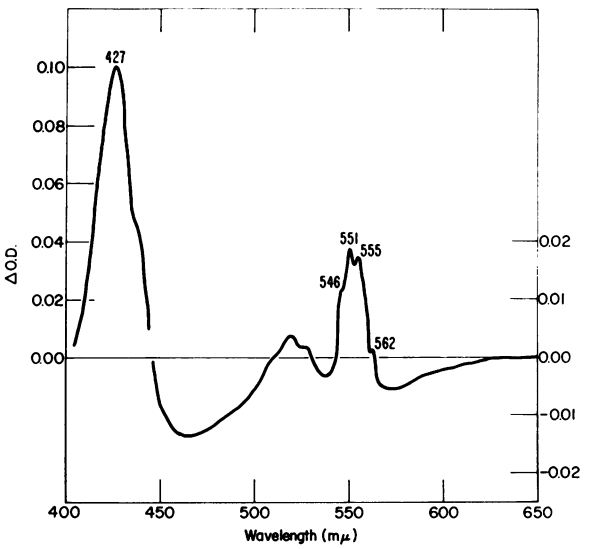

FIG. 3. Difference spectrum of *L. mucor* at −190° (dithionite reduced)−(oxidized). Cell concentration was 18.6 mg dry weight/ml of 0.1 M phosphate, pH 7.5.

Leucothrix mucor. Difference Spectra of Whole Cells. Room temperature difference spectra were quite similar to those of *Saprospira*, with bands at 553 and 560 mμ and a Soret at 426 mμ. With the hope of unmasking an a-type cytochrome and to resolve the 550 to 560 mμ region of the spectrum, liquid nitrogen difference spectra were tried with excellent results (fig 3). Four bands are now visible in the α-regions for b- and c-type cytochromes, but still no band is visible in the α-region for a-type cytochromes. The shoulder on the Soret peak is 10 mμ too low to be the Soret band for cytochrome oxidase.

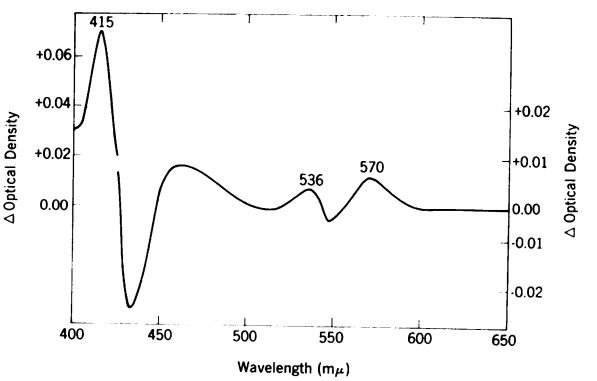

FIG. 4. CO difference spectrum of *L. mucor* (dithionite + CO)−(dithionite). Cell concentration same as figure 3.

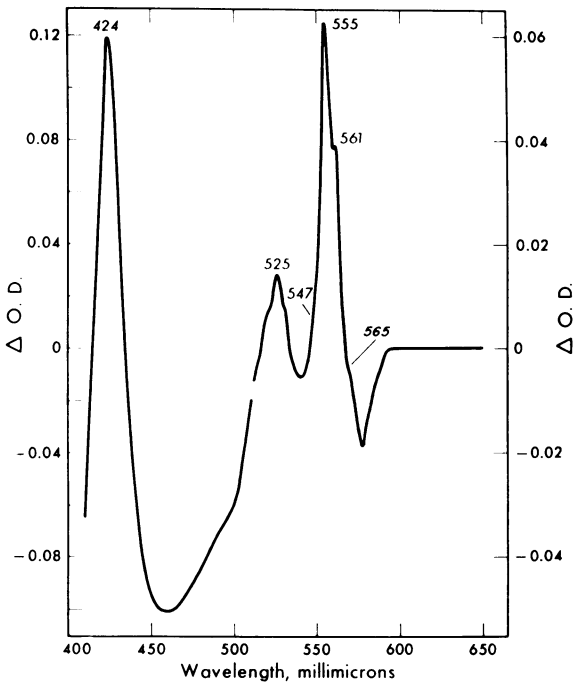

FIG. 5. Difference spectrum of *Vitreoscilla sp.* at −190° (dithionite reduced)−(oxidized). Cell concentration was 21.3 mg dry weight/ml of 0.1 M phosphate, pH 7.5.

A CO difference spectrum is shown in figure 4, and, as in *Saprospira*, the spectrum is quite unlike the CO difference of cytochrome oxidase.

Vitreoscilla species. Difference Spectra of Whole Cells. As for *Leucothrix* it was necessary to use low temperature spectrophotometry to resolve the α-region of the spectrum because room temperature difference spectra of *Vitreoscilla* revealed only a broad α-band at 560 mμ and a Soret band at 427 mμ. Four α-bands are visible in low temperature difference spectra of this organism, but there is no evidence for an a-type cytochrome (fig 5). *Vitreoscilla* has the same type of CO binding pigment that is present in *Saprospira* and *Leucothrix*, but this pigment is apparently present in larger amounts in *Vitreoscilla* than in the other 2 organisms (fig 6). CO difference spectra of *Vitreoscilla*, such as figure 6, are characterized by 2 symmetrical α-bands of the same intensity.

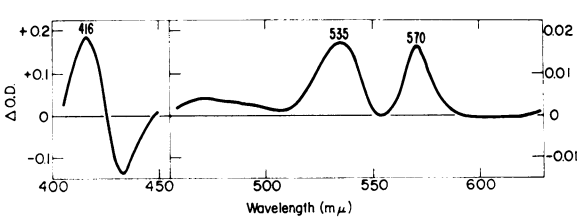

FIG. 6. CO difference spectrum of *Vitreoscilla sp.* (dithionite + CO)−(dithionite). Cell concentration same as figure 5.

Disintegration and Sedimentation Experiments. As for *Saprospira*, the respiratory activity of *Vitreoscilla* was found to be associated with the particulate fractions of cell homogenates. It was observed that the untreated cells were capable of oxidizing NADH (cf. 31). If the cells were treated with EDTA alone (4×10^{-4} M in 0.02 M phosphate, pH 7.5) for approximately 1 hour at room temperature, no protein was solubilized, but there was a large increase (2–8-fold) in the NADH oxidase activity of the cells. Furthermore, EDTA at a concentration of 10^{-3} to 10^{-4} M stimulated the NADH oxidase activity of cell particles prepared from cells disintegrated in the omni-mixer for 15 minutes at 30 % maximum speed or of cells treated with EDTA-phosphate buffer and then washed with phosphate buffer. The effects of inhibitors on the NADH oxidase activity of both of these 2 systems was treated and the results are given in table III. The results obtained for EDTA-treated *Vitreoscilla* cells are similar to those obtained with particulate preparations from *Vitreoscilla*, but are somewhat different from those observed with *Saprospira* (*cf.* table II).

Table III. *Effects of Inhibitors on the NADH Oxidase Activity of* Vitreoscilla *Particles*

The control rate was 58 mμmoles/minute per mg protein.

Inhibitor	Conc (M)	% Inhibition
Anaerobic	...	100
KCN	1.0×10^{-3}	48
HOQNO	7.7×10^{-6}	49
Antimycin A	2.2×10^{-6}	10
Rotenone	6.0×10^{-5}	68
Diphenylamine	3.3×10^{-4}	27
NaN_3	1.0×10^{-3}	0
Amytal	1.0×10^{-3}	53

CO Inhibition of Respiration. Unlike *Saprospira*, which has a very high endogenous respiration, both *Leucothrix* and *Vitreoscilla* have a very low endogenous respiration (ca. 2 μl O_2/hr per mg dry wt), and in both genera it is stimulated about 80 % by a 19 : 1 CO : O_2 gas mixture. However, the respiration of *Vitreoscilla* in the presence of exogenous growth medium (0.5 % yeast extract + 0.5 % peptone) is very high, and this high rate of O_2 consumption is inhibited by CO as figure 7 shows. In this experiment the lights were on continuously, and the dark CO flask was wrapped in aluminum foil. CO inhibits the respiration and light clearly reverses this inhibition, although not completely. The control was the same in the light or the dark.

Action Spectrum for Light Reversal of CO Inhibition of Vitreoscilla Respiration. Is the CO-binding pigment, whose CO difference spectrum is shown in figure 6, responsible for the light reversible inhibition of respiration by CO (fig 7)? This question can

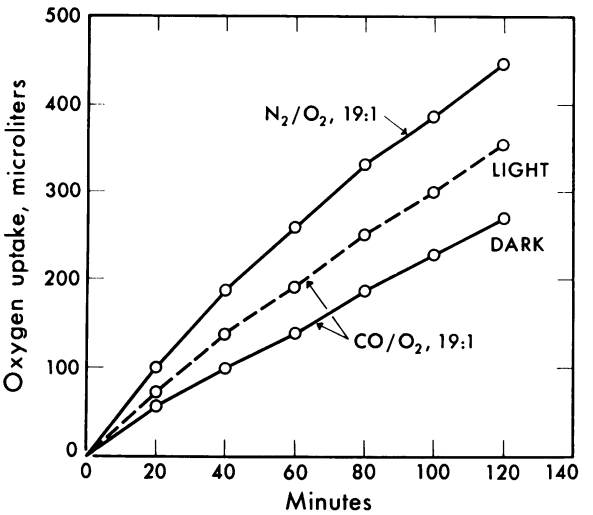

FIG. 7. Light reversal of CO inhibition of *Vitreoscilla* respiration. Each flask contained 1.24 mg dry weight of cells in 3.0 ml *Vitreoscilla* medium. A 19:1 $CO:O_2$ gas mixture was employed. The dark CO flasks were wrapped in aluminum foil. The control flasks contained a 19:1 $N_2:O_2$ gas mixture. The water bath was at 25°.

be answered by performing an action spectrum for the light reversal of the CO inhibition.

The reversals of the CO inhibition obtained with monochromatic light are shown in figure 8. The maxima of figure 8a were found at 534 and 568 mμ with light from the monochromator. The O_2 uptake in relative units, corrected to equal incident quanta, is plotted on the ordinate and the wavelength on the abscissa. As the monochromator does not function below 430 mμ, owing to short-wavelength absorbing materials in the optical system, the action spectrum in the Soret region was measured using interference filters (fig 8b). Light at 416 mμ is most effective for reversal of the CO inhibition; the band is somewhat broad, probably because of the rather wide half-width (average about 15 mμ) of the filters. Parts a and b of figure 8 are plotted in different relative units and are not directly comparable.

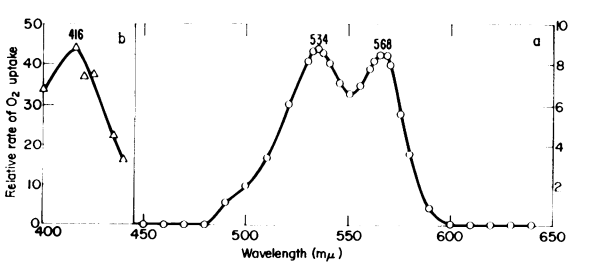

FIG. 8. Action spectrum for the light reversal of CO inhibition of respiration in *Vitreoscilla* cells. A 19:1 $CO:O_2$ gas mixture was employed. The experiment was performed at room temperature. Experimental details given in text.

Control experiments showed that the respiration of cells exposed to air (without CO) was not affected by the light emerging from the 565 and 416 mμ filters. The light energy impinging on the electrode assembly after passing through these filters was an order of magnitude greater than light emerging from the monochromator.

The similarity of the CO difference spectrum of the CO binding pigment (fig 6) and the action spectrum (fig 8) supports the view that this pigment is indeed functioning as a respiratory enzyme.

Discussion

The difference spectra of the 3 organisms of this division studied are all alike in that there is no evidence for any a-type cytochrome. The respiratory chain of these organisms thus appears quite unlike the respiratory chain of higher plants, animals, and the higher algae studied in the first report of this series. This supposition is supported by the responses of the particulate NADH oxidase activity to inhibitors (tables II, III). Although particles from *Saprospira* and *Vitreoscilla* do not show identical responses to inhibitors, both are inhibited by low concentrations of 2-heptyl-4-hydroxyl quinoline-*N*-oxide while antimycin A has little or no effect; this response is quite typical of certain bacteria (19). Also, the relatively low inhibition of the NADH oxidase activity by cyanide is quite unlike the responses of the NADH oxidase activity of mitochondria from eucaryotic organisms to this inhibitor. Rotenone, an effective inhibitor of electron transfer in mammalian mitochondria (9, 12) and algal mitochondria (30) rather surprisingly elicits different responses from particulate preparations of the 2 *Cyanophyta* studied. It is an effective inhibitor of *Vitreoscilla* particles but rather ineffective on *Saprospira* particles. Also, diphenylamine, which stimulated the oxidation of NADH by *Saprospira* particles, proved to be an inhibitor of NADH oxidation by *Vitreoscilla* particles.

The specific NADH oxidase activity of particles isolated from both *Saprospira* and *Vitreoscilla* was quite variable. For example, the specific NADH oxidase activity of particles isolated from *Saprospira* varied from 27 to 190 mμmoles/minute per mg protein and the inhibition of this activity by HOQNO varied from 35 to 76%. This variation in specific NADH oxidase activity and the variation in response of this activity to inhibitors could be due to lack of careful enough control in the growth or harvesting of the cells or to lack of control in the preparation of the particles. Lack of control in the preparation of the particles might also be responsible for the low inhibitions by cyanide and HOQNO. Certainly not enough is known about the necessary conditions and controls needed for the isolation of respiratory particles from procaryotic organisms.

Neither the endogenous respiration of intact cells of *Saprospira* nor the yeast extract and peptone medi-

ated respiration of intact cells of *Vitreoscilla* was tested for sensitivity to cyanide. If the respiration of these intact cells were also inhibited only about 50 % by cyanide and HOQNO it would give validity to the integrity of the particles isolated.

The fact that the low endogenous respiration of both *Leucothrix* and *Vitreoscilla* is stimulated by CO may be explained in the same way that the similar effect of cyanide on the endogenous respiration of *Prototheca* was explained (30): at low rates of substrate oxidation the CO-inhibited terminal oxidase may not be rate-limiting. The slight uncoupling effect of CO may thus increase the respiration.

Although there are no a-type cytochromes visible in difference spectra of the 3 *Cyanophyta* studied, all 3 organisms possess what appears to be the same type of CO-binding pigment. That this pigment is functioning as the terminal oxidase, at least in *Vitreoscilla*, is supported by the action spectrum (fig 8). Both the CO difference spectra and the action spectrum presented here are similar to those which have been observed in certain bacteria by Chance (6, 7), Castor and Chance (4), and Chance, Smith, and Castor (8). The pigment responsible for these spectra has been termed cytochrome o (5). This cytochrome appears to have a wide distribution in the bacteria, both as one of several terminal oxidases, as in *Escherichia coli*, or as the only terminal oxidase, as in *Acetobacter suboxydans* (24). It has been found in hydrogen oxidizing bacteria (23), in a fruiting myxobacterium (11), in *Hemophilus parainfluenzae* (31), and in the nitrogen-fixing *Rhizobium* (26).

Bartsch and Kamen (2) have purified a highly auto-oxidizable, CO-binding heme protein, which they have called RHP (*Rhodospirillum* Heme Protein), from the facultative photoheterotroph, *Rhodospirillum rubrum*. This pigment, which appears to be localized in the chromatophores (22), has a CO difference spectrum which resembles that of cytochrome o. The question of the identity of cytochrome o and RHP has been considered by Kamen, who at first thought them to be the same pigment (2, 21). In support of this, Horio and Yamashita (17) performed an action spectrum for the relief of the CO-inhibited respiration of dark grown cells of *R. rubrum* and found it to be of the same type as had been found for cytochrome o (4). Later, however, Kamen began questioning the role of RHP as an oxidase (18), and he has recently published strong evidence for the non-identity of the 2 CO-binding pigments (25).

Acknowledgments

The authors are indebted to Dr. C. Stacy French for the use of his action spectrophotometer and to Dr. William Vidaver for invaluable assistance in performing the action spectrum. This work was supported by a grant from the National Science Foundation (GB-1751). D. A. Webster is a Graduate Fellow of the NSF.

Literature Cited

1. Baker, J. E. 1961. Effects of diphenylamine on electron transport in plant mitochondria. Plant Physiol. 36: xxxii.
2. Bartsch, R. G. and M. D. Kamen. 1958. On the new heme protein of facultative photoheterotrophs. J. Biol. Chem. 230: 41–63.
3. Brown, W. C., W. E. Sandine, and P. R. Elliker. 1962. Lysis of lactic acid bacteria by lysozyme and EDTA. J. Bacteriol. 83: 697–98.
4. Castor, L. N. and B. Chance. 1955. Photochemical action spectra of carbon monoxide-inhibited respiration. J. Biol. Chem. 217: 453–65.
5. Castor, L. N. 1961. As cited by L. Smith in The Bacteria, Vol. II. I. C. Gunsalus and R. Y. Stanier, eds. Academic Press, New York. p. 374.
6. Chance, B. 1953. The carbon monoxide compounds of the cytochrome oxidases. I. Difference spectra. J. Biol. Chem. 202: 383–96.
7. Chance, B. 1953. The carbon monoxide compounds of the cytochrome oxidases. II. Photodissociation spectra. J. Biol. Chem. 202: 397–406.
8. Chance, B., L. Smith, and L. Castor. 1953. New methods for the study of the carbon monoxide compounds of respiratory enzymes. Biochem. Biophys. Acta 12: 289–98.
9. Chance, B. and G. Hollunger. 1963. Inhibition of electron and energy transfer to mitochondria. I. Effects of amytal, thiopental, rotenone, progesterone, and methylene glycol. J. Biol. Chem. 238: 418–31.
10. Crespi, H. L., S. E. Manderville, and J. J. Katz. 1962. The action of lysozyme on several blue-green algae. Biochem. Biophys. Res. Commun. 9: 569–73.
11. Dworkin, M. and D. J. Niederpruem. 1964. Electron transport system in vegetative cells and microcysts of *Myxococcus xanthus*. J. Bacteriol. 87: 316–22.
12. Ernster, L., G. Dallner, and G. E. Azzone. 1963. Differential effects of rotenone and amytal on mitochondrial electron and energy transfer. J. Biol. Chem. 238: 1124–31.
13. Fork, D. C. 1963. Action spectra for O_2 evolution by chloroplasts with and without added substrate, for regeneration of O_2 evolving ability by far-red, and for O_2 uptake. Plant Physiol. 38: 323–32.
14. Fox, D. L. and R. A. Lewin. 1963. A preliminary study of the carotenoids of some flexibacteria. Can. J. Microbiol. 9: 753–68.
15. French, C. S. and J. Meyers. 1959. Recording action spectra of photosynthesis automatically. Carnegie Inst. Wash. Yearbook 58: 323–27.
16. Gibbs, M. 1962. In: Physiology and Biochemistry of Algae. R. A. Lewin, ed. Academic Press, New York. p 61–90.
17. Horio, T. and J. Yamashita. 1963. In: Bacterial Photosynthesis. H. Gest, A. San Pietro, and C. P. Vernon, eds. Antioch Press, Yellow Springs, Ohio. p 275–306.
18. Kamen, M. D. 1963. In: Bacterial Photosynthesis. H. Gest, A. San Pietro, and C. P. Vernon, eds. Antioch Press, Yellow Springs, Ohio. p 61–70.
19. Lightbown, J. W. and F. L. Jackson. 1956. Inhibition of cytochrome systems of heart muscle and

certain bacteria by the antagonists of dihydrostreptomycin: 2-alkyl-4-hydroxquinoline *N*-oxides. Biochem. J. 63: 130–37.

20. LYMAN, J. AND R. H. FLEMING. 1940. Composition of sea water. J. Marine Res. 3: 134–46.
21. NEWTON, J. W. AND M. D. KAMEN. 1961. In: The Bacteria, Vol. II. I. C. Gunsalus and R. Y. Stanier, eds. Academic Press, New York. p 397–423.
22. ORLANDO, J. A., L. LEVINE, AND M. D. KAMEN. 1961. Immunochemical studies on *Rhodospirillum* heme protein. Biochim. Biophys. Acta 46: 126–33.
23. PACKER, L. 1958. Respiratory carriers involved in the oxidation of hydrogen and lactate in a facultative autotroph. Arch. Biochem. Biophys. 78: 54–65.
24. SMITH, L. 1961. In: The Bacteria, Volume II. I. C. Gunsalus and R. Y. Stanier, eds. Academic Press, New York. p 365–96.
25. TANIGUCHI, S. AND M. D. KAMEN. 1965. The oxidase system of heterotrophically-grown *Rhodospirillum rubrum*. Biochim. Biophys. Acta 96: 395–428.
26. TAZIMURA, K. AND I. WATANABE. 1964. Electron transport systems of *Rhizobium* grown in nodules and in laboratory medium. Plant Cell Physiol. Tokyo 5: 157–70.
27. WARBURG, O. 1949. Heavy Metal Prosthetic Groups and Enzyme Action. Oxford University Press, London.
28. WEBSTER, D. A. AND D. P. HACKETT. 1964. The respiratory chain in colorless algae. Plant Physiol. 39: lix.
29. WEBSTER, D. A. AND D. P. HACKETT. 1964. Action spectrum for light reversal of carbon monoxide inhibition of *Vitreoscilla* respiration. Carnegie Inst. Wash. Yearbook 63: 483–85.
30. WEBSTER, D. A. AND D. P. HACKETT. 1965. The respiratory chain of colorless algae. I. *Chlorophyta* and *Euglenophyta*. Plant Physiol. 40: 1091–1100.
31. WHITE, D. C. AND L. SMITH. 1962. Hematin enzymes of *Hemophilus parainfluenza*. J. Biol. Chem. 237: 1332–36.