JOURNAL OF BACTERIOLOGY, Mar. 1971, p. 1083-1089
Vol. 105, No. 3

Respiratory Mechanisms in the *Flexibacteriaceae*: Terminal Oxidase Systems of *Saprospira grandis* and *Vitreoscilla* Species

WILLIAM E. DIETRICH, JR.,[1] AND JOHN BIGGINS[2]

Department of Biology, University of Pennsylvania, Philadelphia, Pennsylvania 19104

Received for publication 30 December 1970

Particles from both *Saprospira grandis* and *Vitreoscilla* species, obtained by high-pressure extrusion and sonic treatment, respectively, actively catalyze the oxidation of reduced nicotinamide adenine dinucleotide (NADH) and succinate with O_2. These activities are inhibited by cyanide but not by antimycin; *Saprospira* is also amytal- and rotenone-insensitive. *Vitreoscilla* preparations were unable to oxidize mammalian ferrocytochrome *c* and reduced tetramethyl-*p*-phenylenediamine, whereas the *Saprospira* preparations did so actively. Low-temperature (77 K) difference spectroscopy of *Vitreoscilla* cells and particles indicates the presence of three maxima in the cytochrome alpha-region at 554, 558, and 562 nm. All three cytochromes are active in NADH and succinate oxidation, but none is ascorbate reducible. Cytochrome *o* is the only CO-binding pigment present and is probably the terminal oxidase; it has properties similar to the cytochrome *o* isolated in solubilized form from this organism. *Saprospira* cells and membranes exhibit four cytochrome absorption bands whose maxima are at 550, 554, 558, and 603 nm at 77 K. The latter component has not been noted previously. NADH and succinate reduce all four cytochromes, but ascorbate reduces only the 550- and 603-nm pigments. CO spectra indicate the presence of cytochrome a,a_3 which is probably the oxidase. A second CO-binding pigment is present which is not a peroxidase but may be a cytochrome.

Saprospira grandis and *Vitreoscilla* species are both considered to be members of the *Flexibacteriaceae*, a group which shares several properties with the blue-green algae and is often considered to be homologous with them (17, 21). Both of these procaryotes are filamentous, exhibit gliding motility, and are obligate aerobes (15, 19). In a survey of respiration in colorless algae, Webster and Hackett (23) obtained difference spectra of the cytochromes of three flexibacter species, *S. grandis*, *Vitreoscilla* sp., and *Leucothrix mucor*. Carbon monoxide spectra showed that all three possessed cytochrome *o*. Our previous report was a detailed study of the oxidase system of *L. mucor* (2). Membrane preparations exhibited reduced nicotinamide adenine denucleotide (NADH) and succinate oxidases which were inhibited by antimycin, 2-leptyl-4-hydroxyquinoline-*N*-oxide, and cyanide. The cytochrome chain was shown to be composed of cytochromes b_{562}, $c_{552(548)}$, and b_{558} which was shown to be cytochrome *o* and also the oxidase.

Virtually nothing is known of the carbon metabolism of *Vitreoscilla* and *Saprospira;* however, it has been suggested that *S. thermalis* does have an operative Krebs cycle (16). The present report presents data on the nature of the respiratory electron transport chain of these two flexibacteria as derived from measurements of low-temperature difference spectra and of oxidative activities and inhibitor interactions. In light of the postulated homology of flexibacters and blue-green algae, this study may be of additional value in understanding respiration in the latter organisms.

MATERIALS AND METHODS

Growth of organisms. Both *S. grandis* (no. 23116) and *Vitreoscilla* sp. (no. 13981) were obtained from the American Type Culture Collection (ATCC).

Vitreoscilla was maintained on slants of the medium suggested by the ATCC. Cells for study were grown in 10-liter batches in a fermentor (MF 14, New Brunswick

[1] Present address: Biology Department, Indiana University of Pennsylvania, Indiana, 15701. This paper was submitted in partial fulfillment of the requirements for the Ph.D. degree in the Biology Graduate Group, University of Pennsylvania.

[2] Present address: Division of Biological and Medical Sciences, Brown University, Providence, R.I. 02912.

Scientific Co., New Brunswick, N.J.) at 25 C, stirred at 300 rev/min, and sparged with sterile air at 7.5 liters per min. Each liter of the medium contained: 4.0 g of yeast extract, 4.0 g of tryptone, 2.0 g of beef extract, 2.0 g of sodium glycerophosphate, 1.0 ml of HoLe trace metals solution (2), and 50 μliters of antifoam SAG 5441 (Union Carbide Corp., New York, N.Y.) per liter.

S. grandis was cultured at 30 C in 2-liter flasks containing 500 ml of medium on a rotary shaker (200 rev/min). The growth medium was that of Webster and Hackett (23) with the exception that artificial sea water (Triton Marine Salts or Neptune Salts) was used. The organism was maintained on slants or semisolid plates of the same medium.

Cells of both organisms were harvested during the late log phase by centrifugation, washed with deionized water, and stored as a pellet at −15 C. For in vivo studies, freshly harvested cells were resuspended in sterile growth medium and aerated.

Cell free extracts. *Vitreoscilla* cells, suspended in 50 mM potassium phosphate (*p*H 7.0)–0.4 M sucrose, were broken either by sonic treatment (10 kc) or by grinding with sand. Membrane fragments were sedimented, after a preliminary centrifugation at 6,800 × *g* for 10 min, by successive centrifugations at 27,000 × *g* for 10 min and 48,000 × *g* for 30 min. In each case a gray pellet and a milky supernatant fluid resulted. The latter was clarified by centrifugation at 144,000 × *g* for 2 hr, which gave a pale-yellow supernatant and a pinkish pellet. The combined 27,000 × *g* and 48,000 × *g* pellets were used in the experiments described.

Cell homogenates of *Saprospira*, suspended in 50 mM tris(hydroxymethyl)aminopropane sulfonic acid TAPS (*p*H 8.5)–0.4 M sucrose-1 mM ethylenediaminetetraacetic acid, were prepared by extrusion in a French pressure cell (20,000 psi). After preliminary centrifugation at 12,000 × *g* for 10 min., the homogenate was further fractionated by centrifugation as described for *Vitreoscilla*.

Biochemical assays. NADH oxidase, succinate oxidase, and ascorbate-tetramethyl-*p*-phenylenediamine (TMPD) oxidase were measured by using a covered Clarke oxygen electrode (Yellow Springs Instrument Co., Yellow Springs, Ohio). The oxidation of both NADH and ferrocytochrome *c* as well as succinate dehydrogenase (EC 1.3.99.1) were measured spectrophotometrically as previously described (2). Peroxidase activity was measured by the method of Gregory (10) with ascorbate as substrate. Any increase in the rate of absorbance change, at 550 nm upon the addition of 18 μM (final concentration) H_2O_2 to membranes which were already oxidizing ferrocytochrome *c*, was assumed to be a measure of cytochrome *c* peroxidase activity (29).

Equine cytochrome *c* and NADH were obtained from the Boehringer Mannheim Corp., New York, N.Y.; 2,6-dichlorophenol-indophenol, antimycin A, and rotenone were from Sigma Chemical Co., St. Louis, Mo.; and TMPD was purchased from Eastman Organic Chemicals, Rochester, N.Y. Amytal was a gift from B. Chance of this University.

Protein was assayed by a modified biuret procedure (13).

Difference spectroscopy. Both room and liquid nitrogen (77 K) temperature difference spectra were carried out by using the modernized version of the split-beam spectrophotometer which was used in our previous study (2). The samples for low-temperature spectra were injected into precooled plexiglass cuvettes (2-mm light path); the temperature was maintained close to 77 K in an unsilvered glass Dewar. Pyrex cuvettes of 1.0-cm path length were used for room temperature spectra.

The "trapped" steady-state technique was utilized to assess the role of the cytochromes in the respiration of these two flexibacters. This technique has been shown faithfully to represent the room-temperature steady state (7). Particles in isolation medium or whole cells in growth medium were kept aerobic before use by bubbling with air; anaerobiosis was attained by incubation for several minutes under a layer of mineral oil.

Carbon monoxide spectra were obtained by bubbling CO gas into a dithionite-reduced sample for a few minutes. When respiratory substrate was used as reductant, the reference sample was bubbled with nitrogen.

All spectra shown are uncorrected for the baseline which was flat from 650 to 500 nm; however, it curved upwards below 500 nm.

RESULTS

Oxidative activities. NADH oxidation catalyzed by particles from *Vitreoscilla* had a *p*H optimum of 7.0. When measured immediately after the addition of the preparation to buffers of various *p*H values, *S. grandis* particles exhibited a very high *p*H optimum (9.5) for the same activity. Incubation of these particles for 30 min at room or ice temperatures before assaying caused the optimal *p*H to drop to 8.5. NADH oxidase rates were very much lower when the assays were run in tris(hydroxymethyl)aminomethane buffer. Subsequent work with *Saprospira* preparations was, therefore, carried out with TAPS buffer at *p*H 8.5.

The distribution of NADH oxidase activity of these two organisms (Table 1) shows the same general pattern for both. The final, 144,000 × *g*, supernatants did not scatter light and, in *Saprospira*, possessed no detectable carotenoids; this indicates that these fractions were essentially free of membrane fragments. Rates of NADH oxidation by *Vitreoscilla* homogenates decreased sharply in fractions sedimented at higher forces, indicating that the activity is either very labile, requiring a high degree of membrane integrity, or requires a loosely bound component.

Table 2 lists electron transport reactions carried out by these membranes. *Vitreoscilla* particles are much more active in NADH oxidation than those of *Saprospira*, whereas respiration rates involving succinate are similar. *Vitreoscilla* exhibits a fundamental difference between it and other flexibacters (2) in that mammalian ferrocytochrome *c* or the ascorbate-TMPD couple will not act as electron donors.

High cyanide concentrations were needed to

TABLE 1. *Distribution of NADH oxidase in cell-free homogenates of Saprospira and Vitreoscilla*

Fraction	Rate[a]	
	Saprospira	*Vitreoscilla*
27,000 × *g*, 10-min pellet (P_2)	37	386
48,000 × *g*, 30-min pellet (P_3)	31	160
144,000 × *g*, 120-min pellet (P_4)	26	15
144,000 × *g*, 120-min supernatant (S_4)	5	3

[a] Expressed as nanomoles of NADH oxidized per minute per milligram of protein.

TABLE 2. *Oxidative activities of Saprospira and Vitreoscilla particles*

Electron donor	Electron acceptor	Rate[a]	
		Saprospira	*Vitreoscilla*
NADH[b]	O_2	81	278
Succinate	PMS[c]/DPIP[b]	36	64
Succinate	O_2[b]	30	25
Ascorbate/TMPD	O_2[b]	39	2
Ferrocytochrome c[b]	O_2	24	2
NADH	Ferricytochrome c[b]	733	
Succinate	Ferricytochrome c[b]	14	

[a] Expressed as nanomoles per minute per milligram of protein.

[b] Indicates that which was measured.

[c] PMS, phenazine methosulfate.

inhibit electron transport to oxygen; 1.2 mM cyanide inhibited respiration of *S. grandis* and *Vitreoscilla* particles 73% and 88%, respectively. Diaphorase activities were stimulated and oxygen uptake was completely abolished by 10 mM cyanide. Antimycin was noninhibitory in both organisms at concentrations and inhibitor-protein ratios higher than needed to elicit complete respiratory inhibition of *Leucothrix* (2). Additionally, *Saprospira* NADH oxidase was not inhibited by either amytal or rotenone; insensitivity to rotenone has been shown in *Vitreoscilla* particles (23).

Difference spectroscopy. A low-temperature reduced-minus-oxidized difference spectrum of *Vitreoscilla* cells is shown in Fig. 1. Three cytochrome alpha bands are resolved at 554, 558, and 562 nm. At room temperature, these bands overlap, making a single peak at 557 nm with a shoulder at 563 to 565 nm. In the blue region of the spectrum, a cytochrome Soret band at 421 to 422 nm and a broad trough centered at 450 nm due to reduced flavines was observed. Thus, the psoitions of the alpha-maxima indicate the presence of both *b*- and *c*-type cytochromes.

The low-temperature difference spectrum of *S. grandis* (Fig. 2b) showed four absorption peaks in the wavelength span characteristic of the alpha absorption bands of cytochromes, at 550, 554,

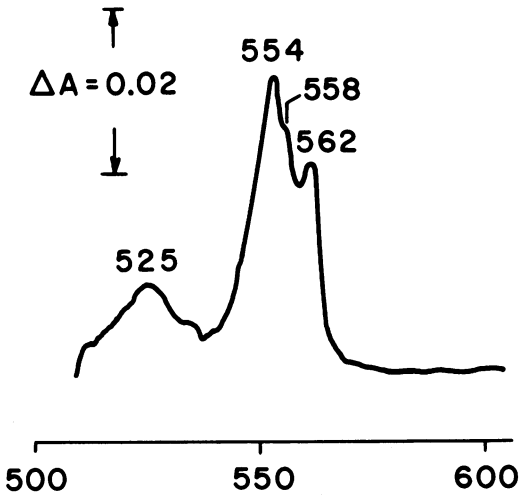

FIG. 1. *Dithionite-reduced minus ferricyanide-oxidized difference spectrum (77 K) of Vitreoscilla cells in growth medium, 13.9 mg dry weight/ml. The numbers on the abscissa are wavelength in manometers.*

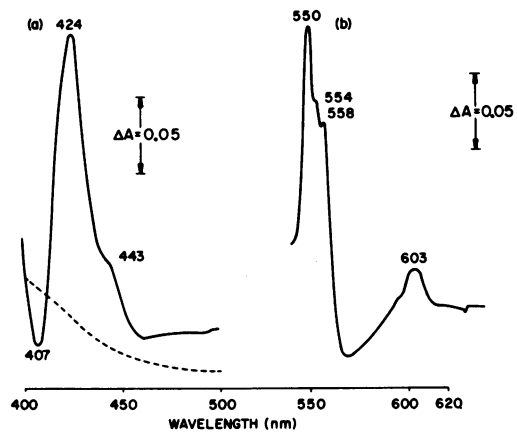

FIG. 2. *Difference spectra of S. grandis particles. a, Dithionite-reduced minus untreated, room temperature, 3.2 mg of protein/ml. Broken line indicates baseline. b, Dithionite-reduced minus ferricyanide-oxidized (77 K), 4.3 mg of protein/ml.*

558, and 603 nm. As Webster and Hackett found (23), the high carotenoid content of this organism made measurement in the blue region difficult; however, it was less so when the spectra were recorded at room temperature. At this temperature (Fig. 2a), a Soret maximum at 424 nm is observed which has a prominent shoulder at 443 to 445 nm. *Saprospira* thus seems to possess not only cytochromes *b* and *c* but also type *a*.

All of the cytochromes of both organisms are firmly bound to membrane since none could be detected in the 144,000 × *g* supernatant, nor were the relative proportions of the cytochromes in the

various membrane fractions significantly different from those of whole cells. Table 3 gives the cytochrome concentrations in particles of both organisms. They were calculated from the absorbance difference of reduced-minus-oxidized spectra by using a millimolar extinction of 20 cm^{-1} for each pigment and a low-temperature absorbance-intensification factor which was empirically determined; concentrations are uncorrected for the contributions of band overlap. The concentrations for both organisms are in the range of values found for other bacterial preparations and for mitochondrial fragments (20).

Our observations differ from those of Webster and Hackett (23) not only in the detection of an *a*-type cytochrome in *Saprospira*, but also in the positions of some of the alpha-maxima in both organisms. These disparities may have arisen from differences in strains of experimental organisms and in the growth conditions employed, especially the rate of culture aeration. Our preliminary observations on both these flexibacters indicate, however, no cytochrome variations between log- and stationary-phase cells and between cells grown in flasks and those grown in a fermentor with high aeration. This contrasts with *L. mucor* which showed a high dependence of cytochrome concentration and ratios on aeration (2).

Table 4 presents steady-state data on *S. grandis* particles. All four absorbance entities are due to separate cytochromes, all of which are reduced by NADH and succinate. However, only cytochromes 550 and 604 are reduced by ascorbate, indicating high redox potentials. With succinate as electron donor, the 558-nm pigment is the most reduced in the aerobic steady state, whereas the 603-nm pigment is the most oxidized. In the presence of NADH, however, the 550-nm pigment also becomes very much reduced. The high level of reduction of the 603-nm pigment may be indicative of both the presence of a nonautoxidizable component and the low concentration of this cytochrome relative to the rest of the chain.

TABLE 3. *Cytochrome composition of Vitreoscilla and Saprospira particles*

Organism	Cytochrome composition[a]				
	550 nm[b]	554 nm[b]	558 nm[b]	562 nm[b]	603 nm[b]
Saprospira	0.60	0.47	0.41		0.12
Vitreoscilla		0.26	0.21	0.17	

[a] Expressed as nonomoles per milligram of protein.
[b] Alpha-region wavelength maxima.

When cyanide was added to a sample of *Saprospira* particles, an additional absorbance peak at 590 to 592 nm appeared. This resembles closely the CN complex of cytochrome oxidase from mitochondria (28).

Steady-state data obtained with *Vitreoscilla* cells and particles is shown in Table 5. About 80% of all three cytochromes was reduced by NADH and succinate but none was reduced by either the ascorbate-TMPD couple or ascorbate alone. The reduction levels of all the cytochromes are high and lack a clear correlation with their position in the chain. In comparison with *Saprospira*, the cytochrome concentration is low, yet the rates of substrate oxidation are the same or higher, indicating a more rapid turnover of the whole respiratory chain.

A room-temperature CO difference spectrum of *Vitreoscilla* cells (Fig. 3) shows maxima at 566, 533, and 414 to 418 nm and troughs at 550 and 430 nm. This spectrum is similar to that previously published for *Vitreoscilla* cells (23) and for the two CO-binding pigments isolated from

TABLE 4. *Aerobic steady-state levels of reduction of Saprospira respiratory pigments*

Treatment	Alpha-region wavelength maxima			
	550 nm	554 nm	558 nm	603 nm
	Per cent reduction relative to anaerobic-minus-oxidized sample[a]			
NADH: aerobic	60	59	56	81
Ascorbate-TMPD: aerobic	78	0	0	100
	Per cent reduction relative to substrate: cyanide sample[a]			
NADH: aerobic minus oxidized	88	103	123	56
NADH: aerobic minus untreated	92	72	84	72
Succinate: aerobic minus oxidized	37	42	80	22
Succinate: aerobic minus untreated	13	37	80	0
Ascorbate-TMPD: aerobic minus oxidized	79	0	nd	60
Ascorbate-TMPD: aerobic minus untreated	93	0	nd	72

[a] Data, calculated to the nearest per cent, are the averaged results of several independent experiments.

TABLE 5. *Aerobic steady-state levels of reduction of Vitreoscilla respiratory pigments*

Aerobic steady-state	Alpha region wavelength maxima		
	554 nm	558 nm	562 nm
	Per cent reduction relative to substrate: anaerobic sample[a]		
NADH	33	37	42
Succinate	14	17	28
	Per cent reduction relative to anaerobic (whole cells) sample[a]		
Aerobic	56	43	64
	Per cent reduction relative to substrate: cyanide sample[a]		
NADH	59	56	117
Succinate	20	25	48

[a] Data, calculated to the nearest per cent, are the averaged results from several independent experiments.

this organism (24) and is due to cytochrome *o* (6).

Figure 4 shows the Soret region of the CO difference spectrum of *S. grandis* particles at room temperature; no consistent spectrum could be obtained in the alpha-region because of interference from the high concentrations of carotenoids absorbing in the same region. A main peak at 414 to 420 nm with a shoulder at 433 nm and a trough at 445 nm was observed when either dithionite or respiratory substrate was used as reductant. Such a spectrum indicates the presence of the CO complex of cytochrome a_3 (433 nm peak and 445 nm trough; reference 28) and either cytochrome *o* or another hemoprotein (6). However, when either ascorbate or ferrocytochrome *c* was used as donor, no peroxidase activity was observed.

DISCUSSION

Our data on activity and cytochrome distribution indicate that the respiratory chains of both *Saprospira* and *Vitreoscilla* are membrane-bound. Since no internal lamellae were observed in micrographs of these flexibacters (15, 25), the cell membrane is the probable source of the particles.

The ability of both flexibacters to catalyze the oxidation of NADH and succinate may indicate a conventional carbon metabolism. An active tricarboxylic acid cycle has been postulated for *S. thermalis* (16). It is not known at this time whether the lack of an ascorbate oxidase in *Vitreoscilla* is reflected in the overall pattern of substrate metabolism.

Like many other procaryotic respiratory systems (9), *Saprospira* and *Vitreoscilla* both are insensitive to antimycin and require high cyanide concentrations for complete inhibition. Another flexibacter, *L. mucor* (2), was shown to be antimycin sensitive, however. The inhibitors amytal and rotenone failed to elicit an inhibitory response in *Saprospira* as was also the case in *L. mucor*. *Vitreoscilla*, however, is known to be sensitive to these two compounds (23).

Three distinct cytochrome components are active in the respiration of *Vitreoscilla*. In agreement with Webster and Hackett (23), no *a*-type cytochrome was seen and cytochrome *o* is implicated as the terminal oxidase. It is not known

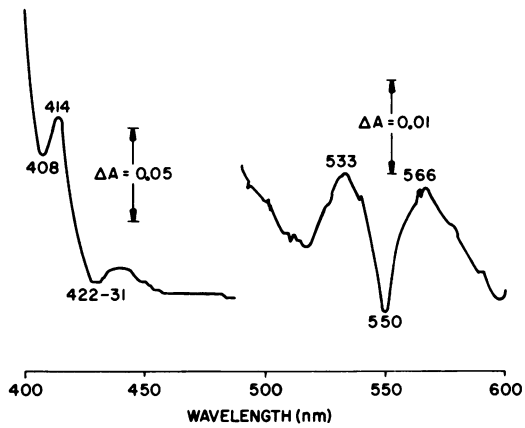

FIG. 3. *Room-temperature CO spectrum of Vitreoscilla cells in growth medium. Dithionite-reduced: CO minus dithionite-reduced, 2.9 mg dry weight/ml.*

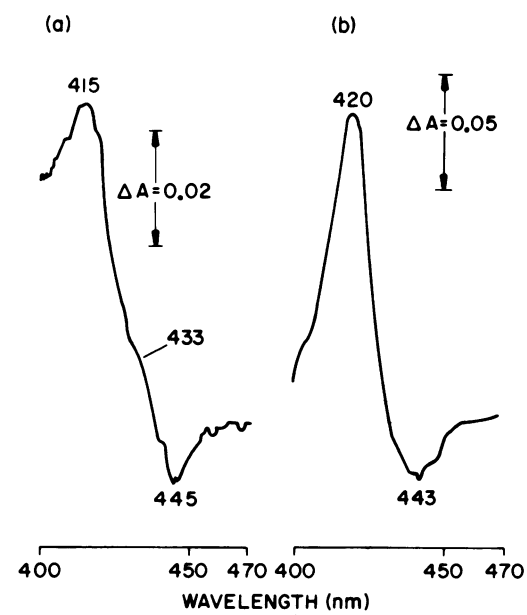

FIG. 4. *Room-temperature CO spectra of S. grandis particles. a, Succinate (66 mM): CO minus succinate: N_2; b, dithionite-reduced: CO minus dithionite-reduced.*

whether the 558-nm pigment is, as in *L. mucor*, cytochrome *o*.

Two CO-binding protohematin-containing proteins have been isolated from *Vitreoscilla* (24). Both have CO complexes whose spectra resemble cytochrome *o*, but only one is autoxidizable and not reducible by ascorbate. The presence of a low-potential oxidase would mean not only that the respiratory chain of this flexibacter spans a narrow range of redox potential as our data indicates, but also that the chain may contain a low-potential cytochrome *c*, a feature not often observed in strictly heterotrophic aerobes (9). Additionally, the low-temperature spectra of the reduced forms of the two *Vitreoscilla* components are not entirely incompatible with a respiratory chain made up entirely of *b*-type cytochromes.

Our data on the respiratory system of *S. grandis* show that it is made up of four functional cytochromes, one of which is an *a*-type cytochrome, which disagrees with Webster and Hackett's observations (23). From the cyanide and carbon monoxide spectra, this latter component is similar to the cytochrome a,a_3 of mitochondria and several bacterial sources (6, 28). In the aerobic steady state, this cytochrome was usually the most oxidized and thus may function as a terminal oxidase.

The CO spectra of *Saprospira* particles also show a Soret band which resembles those exhibited by the proteins isolated by Webster and Hackett from *Vitreoscilla*. The presence of a second oxidase cannot be eliminated by our data on aerobic steady states. We have been unable to demonstrate the presence of a peroxidase in these preparations. It is conceivable, though, that a portion of the 558-nm pigment may be able to combine with CO and be autoxidizable in the manner described for cytochrome b_{558} from *Acetobacter suboxydans* by Iwasaki (12).

Our conclusions concerning the nature of the prosthetic group of the cytochromes await heme analysis. Furthermore, our data on the aerobic steady states in cells and particles of these flexibacters need confirmation by kinetic experiments, since the "trapped steady state" technique is unable to resolve kinetically distinct but spectrally similar cytochromes.

Little is known about the respiration of the other procaryotes which are considered to be flexibacters. *Beggiatoa* is known not to possess cytochromes (4) but oxidizes H_2S as an energy source for the fixation of carbon dioxide. Some of the aerobic flexibacters which have been studied have been shown to possess the Embden-Meyerhof pathway and other enzymes for the generation of reduced pyridine nucleotide (22).

Flexibacters are often thought of as apochlorotic descendants of the blue-green algae (17, 21), a contention which is supported by some biochemical data. In a study of the deoxyribonucleic acid base composition of several flexibacters and blue-green algae, it was shown that base composition of deoxyribonucleic acid isolated from members of the *Oscillatoriales* (filamentous) were very similar to the filamentous gliding bacteria (8). Willard and Gibbs (27) showed that the properties of aldolases isolated from *Anacystis nidulans* and *S. thermalis* were similar; both were metal-dependent enzymes similar to those found in other procaryotes.

The respiratory chain of the blue-green algae is membrane bound (3). Particle preparations from *Anabaena* species have been shown to carry out not only the oxidation of pyridine nucleotides but also of ascorbate and hydroquinone; these oxidations are inhibited by CO and cyanide (14, 26). Biggins (1) demonstrated cyanide-sensitive oxidation of succinate, malate, and ferrocytochrome *c* by particles from *Phormidium luridum*. In a direct comparison of the NADH oxidases of photoheterotrophically grown *Anacystis nidulans* and *Anabaena variabilis* with that of *L. mucor*, Horton (11) found that all were inhibited by cyanide, 2-heptyl-4-hydroxyquinoline-*N*-oxide, amytal, and rotenone but not by antimycin. In all cases the respiratory rates of the algae were found to be much lower than those of the flexibacters; this is most likely due to the autotrophic habit of the algae. Although no cytochromes have been directly implicated in blue-green algal respiration, the cyanide and CO sensitivities do suggest a heme-containing oxidase.

ACKNOWLEDGMENT

This investigation was supported by Public Health Service grant 5T01GM00517 from the National Institute of General Medical Sciences and by grant GB-6922 from the National Science Foundation.

LITERATURE CITED

1. Biggins, J. 1969. Respiration in blue-green algae. J. Bacteriol. **99**:570-75.
2. Biggins, J., and W. E. Dietrich, Jr. 1968. Respiratory mechanisms in the *Flexibacteriaceae*. I. Studies on the terminal oxidase system of *Leucothrix mucor*. Arch. Biochem. Biophys. **128**:40-50.
3. Bisalputra, T., D. L. Brown, and T. E. Weier. 1969. Possible respiratory sites in a blue-green alga *Nostoc sphaericum* as demonstrated by potassium tellurite and tetranitro-blue tetrazolium reduction. J. Ultrastruct. Res. **27**: 182-197.
4. Burton, S. D., R. Y. Morita, and W. Miller. 1966. Utilization of acetate by *Beggiatoa*. J. Bacteriol. **91**:1192-1200.
5. Carr, N. G., and M. Hallaway. 1965. Reduction of phenolindo-2,6-dichlorophenol by the blue-green alga, *Anabaena variabilis*. J. Gen. Microbiol. **39**:335-344.
6. Castor, L. N., and B. Chance. 1959. Photochemical determinations of the oxidases of bacteria. J. Biol. Chem. **234**: 1587-1592.
7. Chance, B., and E. L. Spencer. 1959. Stabilization of

"steady states" of cytochromes at liquid nitrogen temperature. Discuss. Faraday Soc. **27**:200-205.

8. Edelman, M., D. Swinton, J. A. Schiff, H. T. Epstein, and B. Zeldin. 1967. Deoxyribonucleic acid of the blue-green algae (*Cyanophyta*). Bacteriol. Rev. **31**:315-31.
9. Gel'man, N. S., M. A. Lukoyanova, and D. N. Ostrovskii. 1967. Respiration and phosphorylation of bacteria. Plenum Press, New York.
10. Gregory, R. P. F. 1966. A rapid assay for peroxidase activity. Biochem. J. **101**:582-3.
11. Horton, A. A. 1968. NADH oxidase in blue-green algae. Biochem. Biophys. Res. Commun. **32**:839-845.
12. Iwasaki, H. 1966. Lactate oxidation system in *Acetobacter suboxydans* with special reference to the carbon monoxide-binding pigment. Plant Cell Physiol. **7**:199-216.
13. Jacobs, E. E., M. Jacob, D. R. Sanadi, and L. R. Bradley. 1956. Uncoupling of oxidative phosphorylation by cadmium ion. J. Biol. Chem. **233**:147-56.
14. Leach, C. K., and N. G. Carr. 1969. Oxidative phosphorylation in an extract of *Anabaena variabilis*. Biochem. J. **112**:125-26.
15. Lewin, R. A. 1962. *Saprospira grandis Gross* and suggestions for reclassifying helical, apochlorotic, gliding organisms. Can. J. Microbiol. **8**:555-563.
16. Lewin, R. A. 1965. Isolation and some physiological features of *Saprospira thermalis*. Can. J. Microbiol. **11**:77-86.
17. Lewin, R. A. 1969. A classification of Flexibacteria. J. Gen. Microbiol. **58**:189-206.
18. Mandel, M., and R. A. Lewin. 1969. Deoxyribonucleic acid base composition of Flexibacteria. J. Gen. Microbiol. **58**: 17-178.
19. Pringsheim, E. G. 1951. The Vitroscillaceae: a family of colorless, gliding, filamentous organisms. J. Gen. Microbiol. **5**:124-149.
20. Smith, L. 1968. The respiratory chain system of bacteria, p. 55-122. *In* T. P. Singer (ed.), Biological oxidations. Interscience Publishers, New York.
21. Soriano, S., and R. A. Lewin. 1965. Gliding microbes: Some taxonomic reconsiderations. Antonie van Leeuwenhoek J. Microbiol. Serol. **31**:66-80.
22. Starr, T. J., and H. P. Klein. 1954. Enzymes involved in the utilization of carbohydrates by two strains of myxobacteria. Arch. Mikrobiol. **30**:235-42.
23. Webster, D. A., and D. P. Hackett. 1966. Respiratory chain of colorless algae. II. Cyanophyta. Plant Physiol. **41**:599-605.
24. Webster, D. A., and D. P. Hackett. 1966. The purification and properties of cytochrome o from *Vitreoscilla*. J. Biol. Chem. **241**:3308-3315.
25. Webster, D. A., and D. P. Hackett, and R. B. Park. 1967. Respiratory chain of colorless algae. III. Electron microscopy. J. Ultrastruct. Res. **21**:514-523.
26. Webster, G. C., and A. W. Frenkel. 1952. Some respiratory characteristics of the blue-green alga, *Anabaena*. Plant Physiol. **28**:63-69.
27. Willard, J. M., and M. Gibbs. 1968. Purification and characterization of the fructose diphosphate aldolases from *Anacystis nidulans* and *Saprospira thermalis*. Biochim. Biophys. Acta **151**:438-48.
28. Yonetani, T. 1960. Studies on cytochrome oxidase. I. Absolute and difference absorption spectra. J. Biol. Chem. **235**:845-852.
29. Yonetani, T. 1967. Cytochrome *c* peroxidase (baker's yeast), p. 336-339. *In* R. W. Estabrook and M. E. Pullman (ed.), Methods in enzymology, vol. 10. Academic Press Inc., New York.