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Effect of Nitrate, Fumarate, and Oxygen on the Formation of the Membrane-Bound Electron Transport System of *Haemophilus parainfluenzae*

PETER R. SINCLAIR AND DAVID C. WHITE

Department of Biochemistry, University of Kentucky Medical Center, Lexington, Kentucky 40506

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The composition of the membrane-bound electron transport system of *Haemophilus parainfluenzae* underwent modification in response to the terminal electron acceptor in the growth medium. *H. parainfluenzae* was able to grow with O_2, nitrate, fumarate, pyruvate, and substrate amounts of nicotinamide adenine dinucleotide (NAD) as electron acceptors. When O_2 served as the electron acceptor and its concentration was lowered below 20 μM, the bacteria formed more cytochromes *b*, *c*, a_1, a_2, and *o* than were present in the cells grown at 150 to 200 μM O_2. Nitrate and nitrite reductase activities also appeared during growth at the low O_2 concentrations in the absence of added nitrate. Cytochrome levels in cells grown anaerobically with fumarate, pyruvate, or NAD as terminal acceptors were similar to those formed in cells grown at low O_2 concentrations. Cells grown with nitrate had higher levels of cytochromes *c*, *b*, and *o*, and of nitrate and nitrite reductases, than did cells grown with the other acceptors. The formation of cytochrome oxidase a_2 was repressed by the presence of nitrate in the growth medium. The critical O_2 concentration (the O_2 concentration at which the rate of O_2 uptake becomes demonstrably dependent on the O_2 concentration) was about 100 μM in cells grown with nitrate and about 15 μM in cells grown with the other acceptors. A mutant of *H. parainfluenzae* was found to make about 10% as much cytochrome *c* as the wild type, and its formation of cytochrome a_2 was not repressed by nitrate. The critical O_2 concentration of the mutant was high when it was grown with nitrate, suggesting that the high levels of cytochrome *c* and the absence of cytochrome a_2 from the wild type are not responsible for the high critical O_2 concentration. The modifications of the respiratory system induced by changing the terminal electron acceptor were inhibited by the presence of chloramphenicol, which suggests that protein synthesis is involved.

Facultative aerobes can grow with a variety of terminal electron acceptors. These terminal electron acceptors can induce changes in the composition of the electron transport system. *Haemophilus parainfluenzae* requires a functional membrane-bound electron transport system for the oxidation of reduced pyridine nucleotide (24). This membrane-bound system has been found in cells during all conditions of growth tested. The formation of the membrane-bound electron transport system is controlled in two ways. The activities of the primary membrane-bound dehydrogenases and of the soluble tricarboxylic acid cycle enzymes are markedly affected by the presence of glucose and the other catabolites in the medium (22). These catabolites do not affect the concentration of the respiratory pigments in the membrane (25). In addition, the terminal electron acceptors regulate the cytochrome composition of the cells (20, 25).

In this study, we examined the effects of high and low levels of O_2, fumarate, pyruvate, nicotinamide adenine dinucleotide (NAD), and nitrate in the growth medium on the formation and function of the respiratory system. From the results, some conclusions were drawn about the mechanisms which control the formation of the respiratory system in *H. parainfluenzae*.

MATERIALS AND METHODS

Growth of bacteria. The strains of *H. parainfluenzae*, the medium, and the procedures for determination of the purity of the cultures were described previously (19). Cultures were inoculated with 10 ml of a 12-hr culture grown without nitrate. Cells were grown in 1.7 liters of medium in 2.5-liter low-form Erlenmeyer

flasks which were shaken at high speed on a gyratory shaker (26). In experiments in which the entire content of the flask was harvested, chloramphenicol was added 10 min before the cultures were harvested. Chloramphenicol prevented any increase of the bacterial density and of the cytochromes. In some experiments, samples (500 ml) were removed at intervals, made to 0.30 mM with chloramphenicol (Calbiochem, Los Angeles, Calif.), cooled rapidly in ice, and centrifuged as described. As indicated in the text, 20 mM KNO_3, 20 mM potassium fumarate, or 20 mM KNO_3 plus 0.15 mM chloramphenicol was added aseptically. In some experiments, the agitation was stopped after the additions. Cells were cultured anaerobically as described (24). Bacterial density was determined as the absorbance at 750 nm in round test tubes and was related to dry weight (24).

Cytochromes. Cytochromes were estimated from the difference spectra measured by comparing a suspension of bacteria in which the respiratory pigments were reduced in the presence of 10 mM formate with a part of the same suspension in which the respiratory pigments were oxidized by shaking in air. The measurements were performed in a Cary 14-CM spectrophotometer (19). Cytochrome a_2 was measured as the absorbancy increment between the maximum at 635 nm and a line connecting points at 610 and 660 nm; cytochrome *c* was measured as the absorbancy increment between the maximum at 553 nm and a line connecting points at 540 and 580 nm; cytochrome *b* was measured as the absorbancy increment between its maximum at 560 nm and the line used for cytochrome *c*. Cytochrome oxidase *o* was measured as the absorbancy increment between the maximum at 416 nm and the minimum between 430 and 440 nm in difference spectra obtained with bacteria in which pigments were reduced and saturated with carbon monoxide versus bacteria in which pigments were reduced.

The α peaks of reduced cytochromes *c* and *b* overlap. Two equations were derived for the absorption increments of each cytochrome: cytochrome *c* absorption increment (ΔA) = 1.37 A_{552} − 0.62 A_{560} and cytochrome *b* absorption increment (ΔA) = 1.07 A_{560} − 0.15 A_{552}. These equations were derived from the absorption increment of each cytochrome at the α peak of the other. Protoheme was extracted from cells with 20 volumes of acetone-HCl (10). (A second extraction with acetone-HCl removed a negligible amount of protoheme.) The pyridine hemochromes of the extracted protoheme and of the heme *c* of the residue were prepared, and the reduced minus oxidized difference spectra were measured (8). Figure 1 shows that the equations gave ΔA values for each cytochrome that are proportional to the amount of each heme over the range of ratios of heme *c* to heme *b* from 20:1 and from 1:20. Equations previously derived did not give accurate values of cytochrome *c* to *b* when the ratios were either high or low (26).

The new equations may give an overestimation of cytochrome *b* if cytochrome *o* is present at similar concentrations. Cytochrome *o* has protoheme as the prosthetic group in many organisms (2). The concentration of cytochrome *o* was calculated from the Soret

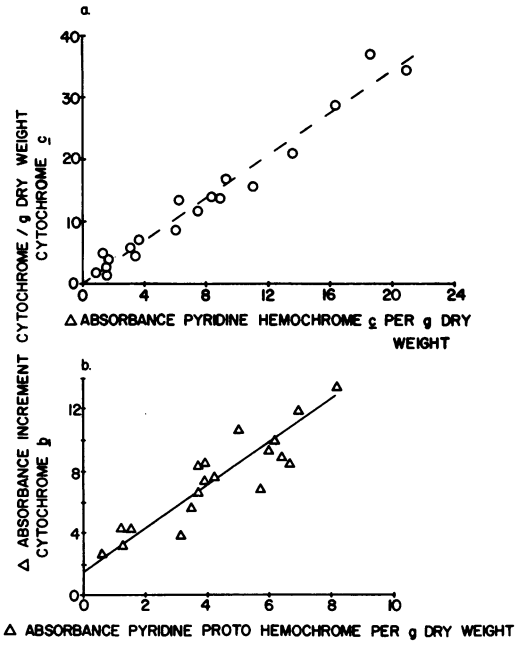

FIG. 1. *Correspondence between total cytochrome c and pyridine hemochrome c (a) and between cytochrome b and the pyridine protohemochrome (b). The absorbances of cytochrome c and b were calculated with the following expressions:* ΔA *cytochrome c* = 1.37A_{552} − 0.62A_{560} *and* ΔA *cytochrome b* = 1.07A_{560} − 0.15A_{552}, *in which* ΔA_{552} = *the absorbance increment between 552 nm and a line connecting 539 and 575 nm and* ΔA_{560} = *the absorbance increment between 560 nm and the same line in the reduced minus oxidized difference spectrum. Protoheme was extracted from the cells with acetone-HCl and the pyridine hemochromes of protoheme and heme c in the residue were measured as the absorbance increment between the maxima and the minima between the* α *and* β *maxima in the reduced minus oxidized difference spectrum.*

peak to trough absorption difference in the CO minus reduced difference spectrum [ϵ = 80 × 10^3 (4)]. The concentration of cytochrome *b* was calculated from the absorption difference between the α peak and the minimum between the α and β peaks in the reduced minus oxidized difference spectrum [ϵ = 20.7 × 10^3 (8)]. In cells in which the concentration of cytochrome *b* was twice that of cytochrome *c*, up to 30% of the absorbance increment of cytochrome *b* was due to cytochrome oxidase *o*. In cells which contained twice as much cytochrome *c* as cytochrome *b*, cytochrome oxidase *o* accounted for 20% of the absorbance at 560 nm. The values for cytochrome *b* reported thus represent at most a 30% overestimation.

Nitrate reductase. Whole cells were incubated in evacuated Thunberg tubes for 3 min at 37 C in 0.9 ml of 50 mM phosphate buffer (*p*H 7.5) containing 20 μmoles of sodium formate. After the incubation, 10 μmoles of KNO_3 was tipped in from the side arm, and the mixture was incubated for 10 min. The reaction

was stopped by opening the tubes and placing them in ice. Nitrite was then measured colorimetrically (11). Nitrite formation was proportional to the dry weight of cells present and to the time of incubation for 20 min. Nitrite could not be detected by the nitrate reductase assay with particulate preparations after the cells were ruptured either by sonic treatment (24) or with a French pressure cell (24). Consequently, whole cells were used for the assay. Nitrite reductase was assayed as above except that the nitrate was replaced with 2 μmoles of KNO_2.

Demethyl vitamin K_2 was assayed spectrophotometrically after extraction with isopropanol-isooctane (23).

Respiratory activity. O_2 utilization was measured polarographically with a Clark electrode in an open vessel in the presence of 17 mM sodium formate. Formate used as substrate gave the most rapid rate of oxygen utilization (26) and reduced the cytochromes to the greatest extent (24). The critical O_2 concentration was measured in triplicate (21) in washed cells with initial rates of O_2 utilization of about 0.54 μmole/min.

RESULTS

Effect of nitrate. When *H. parainfluenzae* was grown to high cell densities with aeration and then the aeration was suddenly stopped, the O_2 tension in the medium dropped rapidly (25). When the O_2 tension was low, the cells grew slowly if no alternative terminal electron acceptor was provided or if protein synthesis was inhibited in the presence of an alternative electron acceptor (upper curves, Fig. 2). In the presence of nitrate, growth continued at the same rate as in aerated cultures. Nitrite appeared in the medium during growth in the presence of nitrate (lower curves, Fig. 2).

In the period of growth after the aeration was stopped, there was a marked change in the composition of the electron transport system (Fig. 3). The concentrations per gram (dry weight) of cytochrome *c* and cytochrome *b* increased 7-fold and 4-fold, respectively; the initial rate of O_2 utilization (with formate as substrate) increased 7-fold; nitrite reductase activity increased at least 20-fold, and nitrate reductase activity increased 16-fold (Fig. 3A–F). Cytochrome oxidase *o* increased during the adaptation to growth with nitrate. The spectral determination of cytochrome *o* was complicated because some of the cytochrome *c* combined with CO. The concentrations of cytochrome a_1 and a_2 remained relatively constant.

The critical O_2 concentration is the O_2 concentration at which the rate of O_2 utilization becomes dependent on the O_2 concentration present in the reaction mixture. Nitrate induced a striking increase in the critical O_2 concentration (Fig. 3F).

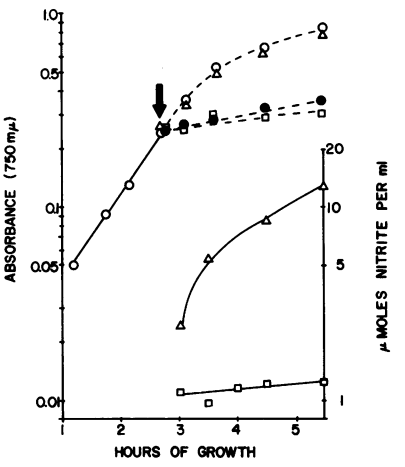

FIG. 2. *Growth rate and nitrite appearance in cultures of Haemophilus parainfluenzae. Cultures were grown with agitation; the agitation was stopped and the following additions were made at* ↓ : △, *20 mM* KNO_3; ●, *20 mM KCl;* □, *20 mM* KNO_3 *and 0.15 mM chloramphenicol;* ○ *indicates a culture agitated continuously without additions. The absorbance at 750 nm measured in samples withdrawn at intervals indicates the bacterial density. The lower curves indicate the nitrite in the medium in cultures grown with* KNO_3 (△) *and with* KNO_3 *and chloramphenicol* (□).

One flask was shaken continuously during the experiment (Fig. 2). In this culture, the concentration of each component of the respiratory system was less than in the cells grown with nitrate in stationary culture (Fig. 3). The critical O_2 concentration was low in the aerated culture. The molar ratio of the demethyl vitamin K_2 to cytochrome *b* was 16:1 in these two cultures, and the concentration of demethyl vitamin K_2 was at about the same concentration in each. Thus, it is unlikely that the high critical O_2 concentration in cells grown with nitrate was related to the high concentration of the demethyl vitamin K_2.

The rate of growth in the presence of 0.15 mM chloramphenicol was 11% of that in the absence of inhibitor. Chloramphenicol prevented the changes in the electron transport system that occurred when the O_2 tension of the medium decreased and nitrate became the terminal electron acceptor. The electron transport system evidently does not change in the absence of protein synthesis. This suggests that preexisting proteins are not activated during the induction.

The cells grown with KCl instead of KNO_3 grew, because O_2 diffused from the surface of the medium. If the air above the medium was

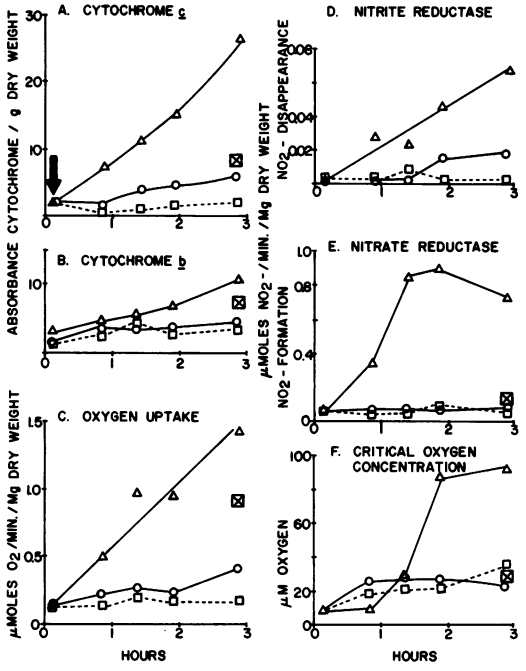

FIG. 3. *Effect of nitrate on the formation of the electron transport system of Haemophilus parainfluenzae. The cultures used in Fig. 2 were vigorously agitated, and the agitation was stopped as indicated by the arrow in A. At this time, additions of 20 mM KNO_3 (△), 20 mM KCl (○), and 20 mM KNO_3 plus 0.15 mM chloramphenicol (□) were made. Analyses from cultures agitated continuously are indicated (⊠). Cytochromes c (A) and b (B) were corrected for overlap as in Fig. 1. Measurements of cytochromes were taken from difference spectra measured at cell densities of about 2.5 mg (dry weight) per ml. O_2 utilization (C) with 17 mM formate as substrate was measured polarographically in an open vessel (21). Nitrite reductase activity (D) is the rate of disappearance of nitrite from an initial concentration of 2 mM. Nitrate reductase activity (E) is the rate of nitrite appearance from an initial concentration of 10 mM nitrate. The critical O_2 concentration (F) is the concentration of O_2 at which the rate of O_2 utilization becomes dependent on the O_2 concentration.*

replaced with nitrogen, growth and cytochrome formation stopped.

Effect of low O_2 concentration. In the experiment shown in Fig. 3, the cells did not grow significantly in the absence of added nitrate. When sufficient O_2 was supplied by agitating the culture, the cells grew at a rate equal to the rate in the presence of nitrate (Fig. 4A). The level of the cytochrome *c*, of the cytochrome *b*, and of the activities of nitrate and nitrite reductases in cells grown without nitrate were about half those found in cells grown with nitrate (Fig. 4).

The rate of O_2 utilization was the same regardless of the presence of nitrate, but the critical O_2 concentration increased in the presence of nitrate.

Effect of fumarate. Fumarate can act as a terminal electron acceptor for the anaerobic growth of *H. parainfluenzae* (24). The growth rates in the presence of fumarate or of nitrate were equal (Fig. 5A). Fumarate did not induce as great an increase of either total cytochrome *c* or of fumarate-reducible cytochrome *c* as did nitrate (Fig. 5C and D). More cytochrome a_2 was formed in the presence of fumarate than in the presence of nitrate (Fig. 5E). The concentrations of cytochromes *b* and a_1 as well as the rates of O_2 utilization were the same in cultures grown with either fumarate or nitrate. The critical O_2 concentration did not increase during growth in the presence of fumarate. Cells grown anaerobically in the presence of fumarate contained

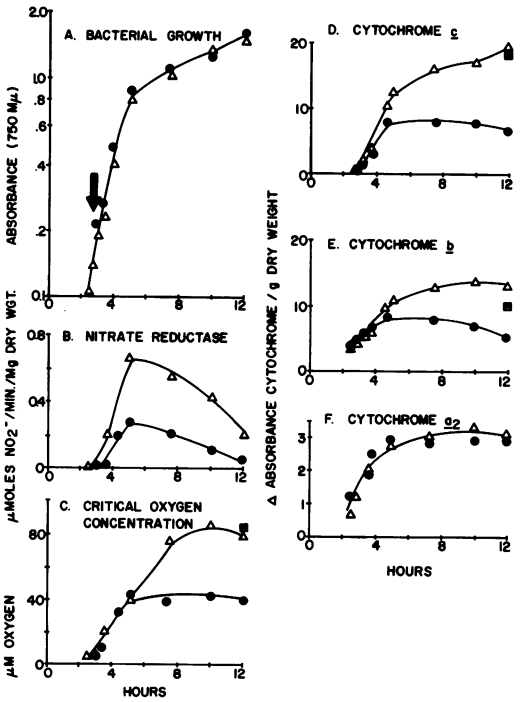

FIG. 4. *Effect of nitrate on the formation of the electron transport system of Haemophilus parainfluenzae during aerobic growth. Symbols: △, 20 mM nitrate added at the arrow; ●, levels in cells grown with 20 mM KCl; ⊠, levels in cells grown anaerobically with nitrate as the terminal electron acceptor. Growth (A), nitrate reductase activity (B), critical O_2 concentration (C), cytochrome c (D), and cytochrome b (E) were measured as in Fig. 3. Cytochrome oxidase a_2 was measured from the absorbance maximum at 635 nm and a line connecting 610 and 650 nm in the reduced minus oxidized difference spectrum.*

concentrations of cytochromes *b*, *c*, a_1, and a_2 equal to those in cells grown aerobically to high cell densities in the absence of additional acceptors. The electron transport system of cells grown anaerobically with both nitrate and fumarate had the same concentrations of respiratory pigments as did cells grown on nitrate alone. Nitrate reductase and nitrite reductase were formed during anaerobic growth with fumarate in the absence of added nitrate or nitrite. The activities of these two enzymes were about half those found in cells grown anaerobically with nitrate.

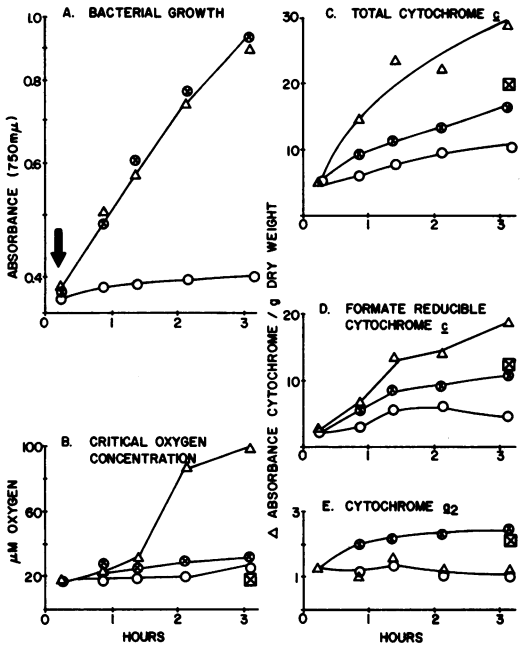

FIG. 5. *Comparison of the effects of fumarate and nitrate on the formation of the electron transport system of Haemophilus parainfluenzae. Cultures were grown with aeration until ↓; then the following were added: 20 mM KNO_3 (△), 20 mM KCl (○), 20 mM sodium fumaratei (⊗). The symbol ⊠ indicates cultures grown anaerobecally with fumarate. Growth (A) and critical O_2 concentration (B) were measured as in Fig. 3. Total cytochr me c (C) indicates the absorbance increment after reduction with dithionite and as corrected as in Fig. 1. Cytochrome oxidase a_2 was measured as in Fig. 4.*

Under the growth conditions illustrated in Fig. 3–5, about half of the total cytochrome *c* was reduced in the presence of formate. As described previously (18), the portion of cytochrome *c* that was not enzymatically reducible was not bound to the membrane and could be recovered quantitatively after rupture of the cells.

Other terminal acceptors. Pyruvate and NAD are also alternative terminal electron acceptors for *H. parainfluenzae* (24). A comparison of the cytochrome composition of cells grown anaerobically in the presence of 20 mM acceptor (0.65 mM for NAD) is given in Table 1. Total cytochrome *c* was highest in cells grown with nitrate as terminal electron acceptor. The cytochrome oxidase *o* levels paralleled the cytochrome *c* levels except in the cells grown with NAD. The level of cytochrome a_2 was lowest in cells grown with nitrate, was intermediate with cells grown with NAD and pyruvate, and was highest in cells grown with fumarate. The critical O_2 concentration was high only in cells grown with nitrate. Cells grew poorly with NAD as the terminal acceptor.

The ability of a chemical to act as a terminal electron acceptor may depend on its oxidation-reduction potential. Two acceptors, tetramethyl phenylenediamine [$E_0' = 0.26$ v (13)] and sodium

TABLE 1. *Comparison of the respiratory pigments of H. parainfluenzae grown anaerobically with different terminal electron acceptors*[a]

Acceptor	Cytochrome absorbance/g (dry wt)						O_2 uptake (μmoles per min per mg, dry wt)	Critical O_2 concn (μM)
	a_2	a_1	*c*	Total *c*	*b*	*o*		
Nitrate.....	0.5	1.2	24.8	39.0	10.5	31.0	0.7	110
Fumarate	3.3	1.0	17.5	25.7	8.8	18.7	0.92	18
Pyruvate.....	1.6	1.1	23.0	27.4	4.9	36.0	0.36	22
NAD.....	1.1	1.4	15.5	26.0	6.5	7.1	0.32	11
Oxygen.....	2.8	1.0	7.5	10.0	8.0	12.5	0.8	20

[a] Cultures were grown anaerobically to early stationary phase with a 20 mM concentration of each acceptor (0.65 mM NAD). For the aerobic culture, cells were grown with agitation in air to early stationary phase. Cytochromes were determined from difference spectra as described in Materials and Methods. Total cytochrome *c* represents the cytochrome *c* reduced by addition of sodium dithionite. The other cytochromes were reduced in the presence of 17 mM formate. O_2 uptake was measured polarographically in an open vessel in the presence of 17 mM formate. The bacterial density at harvest measured as absorbance at 750 nm was between 0.68 and 0.8, except for pyruvate (0.31) and NAD (0.06).

silicomolybdate [E_0' = 0.45 v (12)], which have been used in the assay of cytochrome oxidase, did not support growth of *H. parainfluenzae* when used at concentrations between 0.10 and 10 mM. Aerobic or anaerobic growth with nitrate was not inhibited at these concentrations.

Mutant strain. The results with the parent strain suggested that the high critical oxygen concentration was related to high cytochrome *c*. However, this was not confirmed by the behavior of a mutant strain which makes 10% of the cytochrome *c* in the parent (26).

The mutant makes higher levels of cytochrome *b* than the parent. Table 2 shows that the synthesis of cytochrome a_2 was not repressed in the mutant by nitrate. High concentrations of nitrite were found in the medium after growth with nitrate, which shows that nitrate reductase activity was present in these cells. The other respiratory pigments were at similar concentrations whether the mutant was grown with nitrate or fumarate. However, the critical O_2 concentration of the mutant was affected by the presence of nitrate in the same way as in the parental type. The critical O_2 concentration of the cells grown with nitrate was high, whereas in cells grown with fumarate the critical O_2 concentration was low.

DISCUSSION

Different terminal electron acceptors affect the composition of the respiratory system of *H. parainfluenzae* in three major ways. (i) Growth with high O_2 concentrations suppresses the effects on the formation of the cytochromes by the other terminal electron acceptors. High O_2 concentrations are maintained in shaken cultures at bacterial densities less than 0.2 mg (dry weight) per ml (26). (ii) Growth with limiting O_2 concentrations or with the alternative terminal electron acceptors fumarate, pyruvate, or NAD results in increased synthesis of cytochromes and of nitrate and nitrite reductases. (iii) Growth in the presence of nitrate either anaerobically or at limiting O_2 concentrations stimulates the synthesis of a respiratory system different from that formed under the other growth conditions.

Control of the synthesis of cytochrome a_2 differs from that of the other cytochromes, as cytochrome a_2 synthesis is repressed when nitrate is the terminal electron acceptor. Although low oxygen levels stimulate cytochrome a_2 synthesis in *H. parainfluenzae* (Fig. 4) and in other bacteria (14, 15), the presence of nitrate in anaerobically growing cells inhibits the synthesis of cytochrome a_2 (5, 28). However, nitrate apparently does not repress the synthesis of cytochrome a_2 in the mutant of *H. parainfluenzae* or in *Achromobacter* (1). When fumarate is the terminal electron acceptor for the wild type, the addition of nitrate represses cytochrome a_2 synthesis. This inhibition is removed in the presence of oxygen. For inhibition of cytochrome a_2 synthesis in the wild type, both a high level of nitrate reductase and the presence of nitrate are necessary. Oxygen represses nitrate reductase synthesis, and although cells grown with fumarate contain half the maximal level of nitrate reductase, no repression is seen unless nitrate is present. This suggests that a product of nitrate reduction produces the repression.

The initial rates of O_2 utilization measure the activity of the primary dehydrogenases, as the dehydrogenase reaction is rate-limiting for the whole electron transport chain (22). In *H. parainfluenzae*, high levels of dehydrogenase activity are found in anaerobically grown cells. In *Escherichia coli*, low initial rates of O_2 utilization are found in anaerobically grown cells (3).

The critical O_2 concentration is the O_2 concentration at which the rate of O_2 utilization becomes dependent on the O_2 concentration and is a measure of the affinity of the entire electron transport system for O_2 (21). The critical O_2 concentration is high when cells are grown with nitrate but not with any of the other acceptors tested. The high critical O_2 concentration found in cells grown with nitrate is not dependent on the absence of cytochrome a_2 or on the presence of high levels of cytochrome *c*, as the mutant has a high critical O_2 concentration when grown with nitrate. The parental type grown anaerobically with both fumarate and nitrate has a high critical

TABLE 2. *Respiratory pigment concentrations in the low cytochrome c mutant of Haemophilus parainfluenzae grown anaerobically with nitrate or fumarate as terminal electron acceptors*[a]

Acceptor	Cytochrome absorbance/g (dry wt)						O_2 uptake (μmoles per min per g, dry wt)	Critical O_2 concn (μM)
	a_2	a_1	*c*	Total *c*	*b*	*o*		
Fumarate....	4.7	1.5	6.0	9.7	12.8	21.5	0.72	26
Nitrate.....	3.7	1.0	3.5	7.8	17.0	25.0	1.62	110

[a] Bacteria were grown and cytochromes were measured as in Table 1.

O_2 concentration, which shows that this effect is due to the presence of nitrate in the medium during growth.

The induction of nitrate reductase by nitrate and repression by high O_2 concentrations has been found with *E. coli* (28) and *A. aerogenes* (16). The nitrate reductase activity in cells grown without nitrate at low O_2 concentrations in *H. parainfluenzae* may have resulted from inducers present in the complex medium. Nitrate reductase activity was found in *E. coli* and *A. aerogenes* grown on complex medium without nitrate and was decreased considerably by growth without nitrate in a simple salts medium (16, 28). *H. parainfluenzae* will not grow in a simple salts medium.

Recent theories for the control of the formation of the respiratory system of bacteria grown under different conditions include that of a repressor sensitive to the intracellular redox potential of the cell. Wimpenny and Cole found that, in continuous culture of *E. coli*, changes in either the concentrations of nitrate during anaerobic growth or of O_2 produced similar changes in the concentration of the components of the respiratory system (7). They postulated that the redox potential rather than the terminal electron acceptor determines the composition of the respiratory system. Nitrate, however, induced high levels of nitrate reductase activity and repressed the formation of fumarate reductase. Wimpenny has measured the redox potential of continuous cultures of *E. coli* and found that the cytochrome *b* level is related to the redox potential whether nitrate or O_2 was the terminal electron acceptor (27). Nitrate repressed cytochrome a_2 formation over the range of redox potential at which cytochrome a_2 formation was maximal with O_2 as terminal electron acceptor.

Showe and DeMoss, from their studies of nitrate reductase in *E. coli*, suggested both a redox-sensitive and a nitrate-sensitive repressor (17). Our results are in some agreement with this suggestion. It should be noted that the respiratory system of *H. parainfluenzae* was similar, with alternative terminal electron acceptors of quite different mid-point potentials (fumarate, NAD, pyruvate, and low oxygen). However, the intracellular redox potentials under these conditions are not known.

In *Spirillum itersonii*, the activity of δ-aminolevulinic acid synthase was elevated under conditions of low aeration and maximal cytochrome synthesis (6). Thus, the total heme synthesis may be controlled at the first step by the intracellular redox potential. In *H. parainfluenzae* the control must be more complicated, as there are four different cytochrome prosthetic groups: protoheme, heme *c*, heme *a*, and the iron chlorin a_2.

Other theories for the control of cytochrome formation include controls dependent on the ratio of NAD to reduced NAD (27) or on the adenosine triphosphate levels (9).

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