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Differential Synthesis of Five Primary Electron Transport Dehydrogenases in *Hemophilus parainfluenzae**

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Growth of *Hemophilus parainfluenzae* requires the formation of an electron transport system consisting of six cytochrome components (1). As a compensatory mechanism for changing environment, this bacterium can modify the cytochrome oxidase composition and the proportions of the cytochromes during conditions where there is no cell division (2). The intact bacterium has remarkable permeability to many substrates that reduce the respiratory pigments and produce an oxygen uptake (3). D-Lactate, L-lactate, succinate, reduced diphosphopyridine nucleotide, and formate added to washed suspensions of this bacterium produce rapid rates of oxygen utilization (3). The dehydrogenases for these substrates are part of the membrane-bound electron transport system (3). Rupture of the bacteria with alumina releases the glycolytic enzymes and disrupts the respiratory activity produced by intermediates of the tricarboxylic acid cycle (3). The membrane fragments isolated from bacteria ruptured with alumina contain the substrate-stimulated oxygen uptake properties and the substrate-reducible dehydrogenases and cytochromes found in the intact cells (1, 3).

This report presents evidence indicating that each of these five substrates reduces a distinct membrane-bound dehydrogenase, that these dehydrogenases are very likely flavoproteins, and that each dehydrogenase can be formed differentially during different growth conditions. The great variability in the composition of the cytochrome and flavoprotein components of the electron transport systems during conditions of limited net growth suggests that each respiratory pigment is added individually to the membrane.

EXPERIMENTAL PROCEDURE

Methods

Growth of Bacteria—The strain of *H. parainfluenzae* and the proteose peptone base medium used in this study were described previously (1). However, in the present study Antifoam A was omitted from the medium. Where indicated, metabolites other than glucose were added to concentrations of 40 mM. These bacteria are able to grow in this proteose peptone base medium alone, but the growth rate and final cell yield are increased by adding supplemental metabolites (3). Bacteria were incubated and harvested as described elsewhere (4). Viable counts (2) and turbidity measurements (3) were carried out by described techniques.

Analytical Methods—Oxygen uptake was measured with the Clark oxygen electrode (5). Substrate concentrations were sufficiently large that the initial rate of oxygen utilization was of zero order.

Relative concentrations of cytochromes were estimated from difference spectra (6) measured with a Cary model 15 spectrophotometer between suspensions of cells in the presence and those in the absence of substrates, as described earlier (2, 3, 5). In each case the suspension contained about 10 mg of bacterial protein per ml. Addition of D-lactate, L-lactate, succinate, formate, and DPNH to the suspension in the sample cuvette results in essentially total reduction of the membrane-bound electron transport system. Substrates were added in concentrations that were greater than the concentrations required for maximum oxygen utilization. Estimation of cytochrome b_1 was made from the absorbance difference between 561 mμ and 575 mμ; of cytochrome c_1, from the difference in absorbance between 553 mμ and 575 mμ; and of the flavoprotein dehydrogenase, from the absorbance difference between 409 mμ and 500 mμ. The ferricyanide reductases were assayed from the rate of change in absorbance at 425 mμ, as previously described (7). Assays were carried out at substrate concentrations that were not rate limiting.

Cells were ruptured with the Sonifier (Branson Ultrasonic Corporation, Stamford, Connecticut) at Level 8 in intervals of 30 seconds for a total time up to 4 minutes. The temperature of the bacterial suspension was maintained in an ice-salt bath, and it did not rise above 10° during the treatment.

Protein was determined by a modified biuret reaction (1).

Materials

Thenoyltrifluoroacetone was obtained from the Fisher Chemical Company; sodium secobarbital from the Eli Lilly Company; 2-*n*-heptyl-4-hydroxyquinoline *N*-oxide was a generous gift from Dr. J. W. Lightbown; monofluoroacetate was supplied by the Sigma Chemical Company; and D- and L-lactate were products of the California Corporation for Biochemical Research. Other reagents have been described previously (1–5, 7).

RESULTS

Ferricyanide Assay—Addition of L-lactate, D-lactate, succinate, DPNH, or formate to *H. parainfluenzae* suspended in 50 mM phosphate buffer, pH 7.6, that is also 5 to 7 mM with respect to $K_3Fe(CN)_6$ and 5 mM in KCN produces a change in absorbance at 425 mμ. The effect of the addition of L-lactate is illustrated in Fig. 1. Initially there is a zero order reduction of ferricyanide. As is evident from Fig. 1, the reaction rate diminishes when the

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ferricyanide concentration falls to approximately 2 mM. Addition of L-lactate at this time does not increase the rate of reduction of ferricyanide. At concentrations less than 2 mM, the rate of ferricyanide reduction follows Michaelis-Menten kinetics, as indicated by the linear Lineweaver-Burk (8) plot of the inset graph of Fig. 1. The maximal rate estimated from the intercept of the double reciprocal plot, 4.75 mμmoles per second per 10 mg of protein is identical with the zero order reaction rate at substrate concentrations that are not rate limiting. Therefore in all other measurements the slope of that part of the curve that is characterized by zero order kinetics was taken to represent the maximal rate of reaction of the individual ferricyanide reductases.

The rates of electron transport from various substrates to the artificial electron acceptor, ferricyanide, and to oxygen-using intact *H. parainfluenzae* are given in Table I. The close agreement between these rates indicates that ferricyanide is a satisfactory assay of electron transport except for the case in which formate is used as the substrate.

Because the rate of ferricyanide reduction is unchanged when the cells are ruptured by ultrasonic vibration, it appears that there is no permeability barrier to ferricyanide.

Site (or Sites) of Ferricyanide Reduction—The inhibition of electron transport to ferricyanide and oxygen by various inhibi-

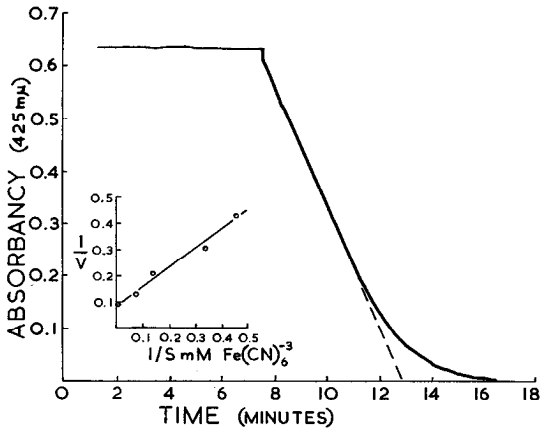

FIG. 1. Rate of ferricyanide reduction at 30° on addition of L-lactate. Concentrations were: L-lactate, 10 mM; ferricyanide, 7 mM; KCN, 5 mM; *H. parainfluenzae*, 12.1 mg of protein per ml. The *inset* shows a Lineweaver-Burk (8) plot of the dependence of reciprocal velocity upon reciprocal ferricyanide concentration at limiting ferricyanide concentrations.

TABLE I

Comparison of rates of reduction of ferricyanide and oxygen by H. parainfluenzae

Substrate	Initial substrate concentration	Ferricyanide reduction	Oxygen uptake × 4*
	mM	*mμmoles/sec/10 mg protein*	
D-Lactate	10	15.8	12.0
L-Lactate	10	15.7	15.0
Succinate	10	17.8	19.8
DPNH	5	71.5	72.0
Formate	10	147.5	690.0

* Because ferricyanide is a 1-electron acceptor and molecular oxygen a 4-electron acceptor, rates of oxygen uptake are multiplied by 4.

TABLE II

Inhibition of reduction of oxygen and of ferricyanide

Substrate	Inhibition*			
	Thenoyltrifluoroacetone	Secobarbital	HOQ-*N*-oxide†	KCN
	% initial rate			
D-Lactate				
Ferricyanide reduction	28	14	90	81
Oxygen uptake	33	18	50	<0.01
L-Lactate				
Ferricyanide reduction	11	7	50	163
Oxygen uptake	19	5.8	32	<0.01
Succinate				
Ferricyanide reduction	63	61	100	145
Oxygen uptake	71	42	48	<0.01
DPNH				
Ferricyanide reduction	34	25	93	250
Oxygen uptake	43	41	29	<0.01
Formate				
Ferricyanide reduction	14	65	46	40
Oxygen uptake	8	46	37	<3

* Expressed as percentage of initial rate without inhibitor. Inhibitor concentrations were: thenoyltrifluoroacetone, 10 mM; secobarbital, 4 mM; 2-*n*-heptyl-4-hydroxyquinoline *N*-oxide, 2 μM; and KCN, 5 mM. Measurements were made at a protein concentration of 7.5 mg per ml at 30°.

† The abbreviation used is: HOQ-*N*-oxide, 2-*n*-heptyl-4-hydroxyquinoline *N*-oxide.

TABLE III

Inhibition of oxygen utilization by 5 mM ferricyanide

Substrate	Oxygen uptake	Oxygen uptake with 5 mM ferricyanide
	mμmoles/sec/10 mg protein	
D-Lactate	2.98	0.32
L-Lactate	3.70	0.66
Succinate	4.98	0.85
DPNH	17.9	2.40
Formate	170.0	143.0

TABLE IV

Effect of 4-minute treatment with ultrasonic vibration on substrate-stimulated ferricyanide reductase and oxygen utilization with H. parainfluenzae

Substrate	Activity remaining in supernatant*	
	Ferricyanide reduction	Oxygen utilization
	mμmoles/sec/10 mg protein	
D-Lactate	75	16.5
L-Lactate	110	10
Succinate	96	1
DPNH	80	20
Formate	25	10

* Expressed as percentage of activity of substrate-stimulated ferricyanide reduction or oxygen utilization contained in the supernatant suspension, which was centrifuged at 105,000 × *g* for 30 minutes, of bacteria ruptured by ultrasonic vibration, compared to the intact bacteria.

tors is shown in Table II. Earlier studies (3) have indicated that thenoyltrifluoroacetone and secobarbital inhibit electron transport between substrates and cytochrome b_1. In this study both oxygen uptake and ferricyanide reduction are inhibited by these agents to nearly the same degree. 2-*n*-Heptyl-4-hydroxyquinoline *N*-oxide inhibits electron transport between cytochrome c_1 and the oxidases in this bacterium (1). The data in Table II indicate that this *N*-oxide has a greater inhibitory effect on oxygen utilization than on ferricyanide reduction. Cyanide forms complexes with the oxidases of *H. parainfluenzae* (1, 3) and completely inhibits oxygen uptake. As has been observed in other systems, ferricyanide reduction is stimulated by cyanide.

If ferricyanide reduction is a true measure of the enzyme activities used in the electron transport system, oxygen utilization should be inhibited by ferricyanide. With the use of intact bacteria, ferricyanide at a concentration of 5 mM inhibits oxygen utilization by 80 to 90% when D-lactate, L-lactate, succinate, and DPNH are added. In the presence of formate, oxygen utilization is decreased by only 16%. These results are documented in Table III. During the period of inhibition, the yellow color of the ferricyanide disappears. After it has disappeared, there is little inhibition of oxygen uptake.

Treatment of the cells with ultrasonic vibration for a total of 4 minutes produces small membrane fragments that remain in the supernatant fluid after centrifugation at 105,000 × *g* for 30 minutes. As is shown in Table IV, these fragments have the ferricyanide reductase activity of the intact bacteria but have lost significant reactivity with oxygen. When the pellet resulting from centrifugation at 105,000 × *g* for 30 minutes is resuspended to its initial volume with 50 mM phosphate buffer, pH 7.6, the suspension shows a loss of ferricyanide reductase activity in the sedimentable membrane fragments that is balanced by the activity recovered in the supernatant suspension. This result is shown in Table V. The ferricyanide reductase activities in the

TABLE V

Ferricyanide reductase activity in bacteria ruptured by treatment with ultrasonic vibration, in pellet, and in supernatant suspensions

Substrate	Broken cell suspension	Pellet	Supernatant suspension
	mμmoles ferricyanide reduced/sec/10 mg protein		
D-Lactate	5.7*	2.3	4.4
L-Lactate	2.1	0.2	2.2
Succinate	6.4	1.2	6.1
DPNH	31.8	7.1	25.4
Formate	5.3	3.2	1.2

* The supernatant suspension was decanted after centrifugation at 105,000 × *g* for 30 minutes. The pellet was resuspended to volume in 50 mM phosphate buffer, pH 7.6.

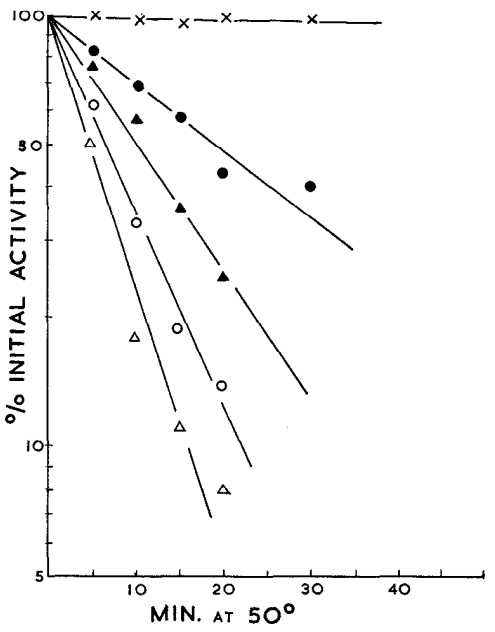

FIG. 2. Thermal inactivation of ferricyanide reductase activity. Ferricyanide reduction in the presence of succinate, ×——×; formate, ●——●; D-lactate, ▲——▲; L-lactate, ○——○; and DPNH, △——△. The reduction was done at 50° in 50 mM phosphate buffer, pH 7.6, with bacteria that had been exposed to ultrasonic energy for 1 minute. Bacteria were grown in glucose and measured at a protein concentration of 4.5 mg per ml. Data are expressed as percentage of initial velocity at zero time.

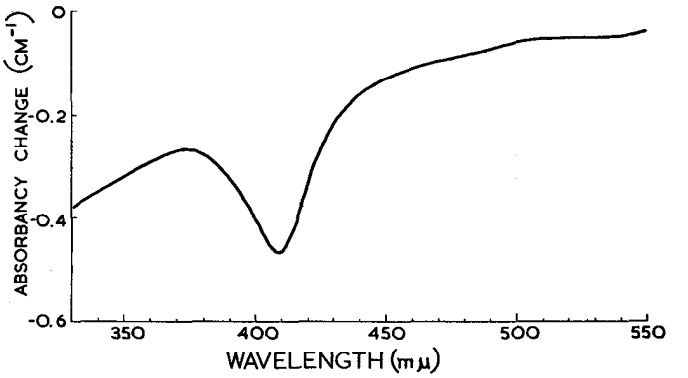

FIG. 3. Difference spectrum between suspensions of small membrane fragments. The sample cuvette contained 10 mM L-lactate and 5 mM KCN. Lactate was omitted from the reference cuvette. Protein concentration was 11.1 mg per ml. The buffer was 50 mM phosphate, pH 7.6. The *ordinate axis* provides the measure for the decrease in absorbance after reduction of the pigment.

TABLE VI

Changes in flavoprotein absorbance resulting from addition of various substrates singly and in combination

Substrate	Absorbance difference* (500 to 409 mμ)	
	Observed	Theoretical
D-Lactate	0.056	
L-Lactate	0.042	
Succinate	0.021	
DPNH	0.035	
Formate	0.065	
D-Lactate + L-lactate	0.095	0.098
D- + L-Lactate + succinate	0.11	0.119
D- + L-Lactate + succinate + formate	0.16	0.184
DPNH + formate	0.091	0.100

* Absorbance difference per 10 mg of protein between 500 and 409 mμ was calculated from difference spectra similar to the one shown in Fig. 3.

TABLE VII

Reduction of ferricyanide, oxygen, and cytochrome resulting from additions of substrates to intact H. parainfluenzae grown under various conditions

The level of reduction of cytochromes b_1 and c_1 was taken from difference spectra of substrate-reduced *versus* oxidized cells at a concentration of 10 mg of protein per ml. Cytochrome b_1 was measured by the absorbance difference between 561 and 575 mμ, and cytochrome c_1 by the absorbance difference between 553 and 575 mμ.

Substrate	$K_3Fe(CN)_6$ reduction	O_2 uptake	Cytochrome b_1 reduced	Cytochrome c_1 reduced
	mμmoles/sec/10 mg protein			
A. Grown with 0.5 M D-lactate and vigorous aeration				
D-Lactate	4.2	1.05	0.007	<0.002
L-Lactate	0.4	0.12	<0.001	<0.001
Succinate	2.84	1.47	0.008	<0.003
DPNH	17.9	5.6	0.009	<0.003
Formate	6.90	8.50	0.009	<0.003
B. Grown with vigorous aeration without supplement				
D-Lactate	<0.02	<0.02	<0.001	<0.001
L-Lactate	<0.02	<0.02	<0.001	<0.001
Succinate	16.2	4.80	0.011	<0.004
DPNH	51.7	14.2	0.041	<0.010
Formate	7.90	9.16	0.042	<0.010
C. Grown with glucose + 0.15 M monofluoroacetate and poor aeration				
D-Lactate	10.1	2.24	<0.002	0.008
L-Lactate	<0.02	<0.02	<0.001	<0.001
Succinate	10.5	2.65	<0.003	0.010
DPNH	38.6	8.72	<0.009	0.029
Formate	17.8	15.5	<0.010	0.034
D. Grown with glucose and poor aeration				
D-Lactate	12.2	2.8	<0.020	0.067
L-Lactate	38.2	9.3	<0.030	0.095
Succinate	22.8	5.8	<0.027	0.083
DPNH	43.8	10.0	<0.045	0.102
Formate	168	150	<0.030	0.091

high speed supernatant have the same sensitivity to thenoyltrifluoroacetone and secobarbital as do the intact bacteria.

These observations are consistent with the conclusion that ferricyanide reductase activity is a true measure of the reactivity of a portion of the electron transport system.

Multiplicity of Ferricyanide Reductases—The ferricyanide reductase activities exhibited by broken cells in the presence of D-lactate, L-lactate, succinate, DPNH, or formate are inactivated at different rates by heat. The results shown in Fig. 2 suggest that each activity arises from a separate enzyme. Similar patterns of inactivation by heat can be demonstrated when oxygen is used as an electron acceptor. Furthermore, the same patterns of inactivation of ferricyanide reductase or respiratory activity can be demonstrated by use of intact cells.

The supernatant suspension used in the experiments reported in Table IV, which contains the ferricyanide reductase activity but has lost a significant proportion of the enzymes necessary for oxygen utilization, can be used to demonstrate the spectral properties of the ferricyanide reductase without significant interference by the cytochromes. D-Lactate, L-lactate, succinate, DPNH, and formate each produce a decrease in absorbance at 409 to 412 mμ. This change is suggestive of reduced flavoproteins. A difference spectrum produced in the presence of 5 mM L-lactate with this preparation is shown in Fig. 3. Similar difference spectra result from the addition of D-lactate, succinate, DPNH, or formate.

If, as is indicated by the differential sensitivity to thermal inactivation, the ferricyanide reductases are five enzymes, the absorbance difference between 409 mμ and 500 mμ with each substrate should increase additively when the various substrates are added in sequence to the same cuvette. The realization of this expectation is shown in Table VI. The slight discrepancies observed may be due to the end absorption of DPNH or to the presence of a small amount of cytochrome c_1, which in the presence of DPNH or formate is slowly reduced and forms a small maximum at 423 mμ.

The finding that the addition of 5 mM DPN to intact or broken cells is without influence on the rate of oxygen uptake or of ferricyanide reduction is consistent with the view that the various ferricyanide reductase systems are flavoproteins. Furthermore, there is no formation of DPNH as measured by absorbance at 340 mμ on addition of D-lactate, L-lactate, succinate, or formate to the high speed supernatant phase in the presence of 5 mM DPN and 5 mM KCN.

Differential Synthesis of Respiratory Dehydrogenases—*H. parainfluenzae* under a variety of growth conditions is capable of greatly modifying the proportions of the five respiratory dehydrogenases, as seen in Table VII. During growth in

D-lactate or 0.15 M monofluoroacetate plus glucose, D-lactate dehydrogenase is formed with little L-lactate dehydrogenase activity. Both lactate dehydrogenases are depressed during growth with vigorous aeration. The ratio of the activity of succinate to that of DPNH dehydrogenase increases 3-fold and the activity of formate dehydrogenase increases 24-fold when bacteria grown in the presence of D-lactate are compared to bacteria grown with glucose and poor aeration. In addition, the proportion of cytochrome b_1 or of cytochrome c_1 reduced by each substrate, which is measured after anaerobiosis is produced in the presence of saturating substrate concentrations, is proportional to the rate of ferricyanide reduction. The changes in dehydrogenase activities measured by the ferricyanide reductase are paralleled by changes in the rates of oxygen uptake. Treatment with ultrasonic vibration for 1 minute does not increase any of the values reported in Table VII. Consequently it is unlikely that the changes in dehydrogenase levels produced by altering the growth conditions are the result of changing the permeability of the bacteria or the affinity of the electron transport system to ferricyanide.

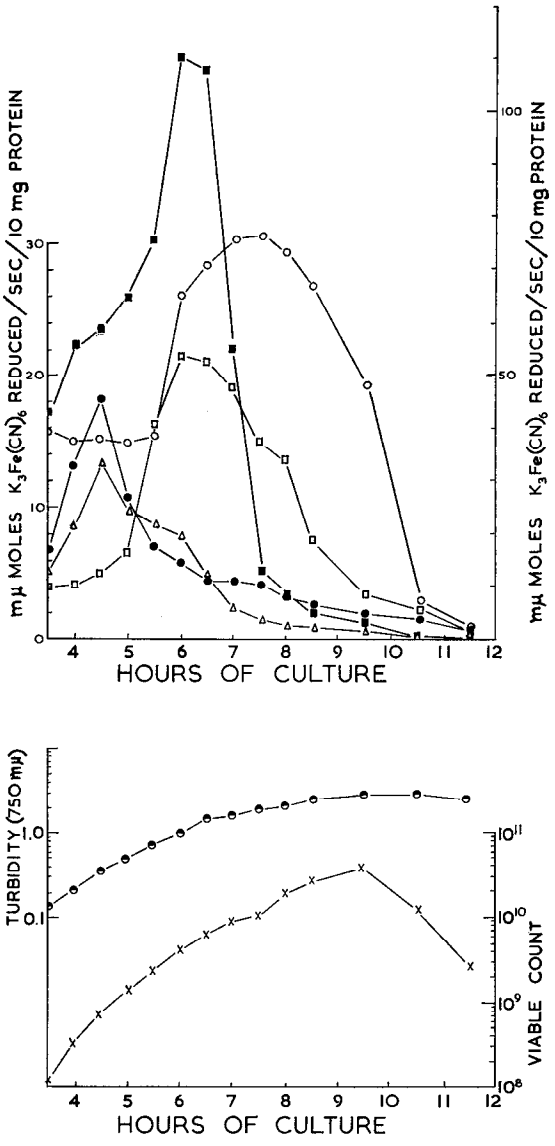

FIG. 4. Differential synthesis of the respiratory dehydrogenases. The synthesis of dehydrogenases occurred during the growth cycle of *H. parainfluenzae* grown with 20 mM glucose with aeration at a rate of 2 liters of air per minute per liter of media. The growth apparatus and methods of sampling have been previously described (2). In *A* (*upper*), ferricyanide reductase activities shown are: D-lactate, ●——●; L-lactate, □——□; succinate, △——△; and formate, ○——○. DPNH-ferricyanide reductase is shown by ■——■. The *right hand axis of ordinates* represents the DPNH activity. These activities were measured with the use of intact bacteria. In *B* (*lower*), turbidity (◓——◑) was measured at 750 mμ, and viable count (×——×), represented on the *right hand axis of ordinates*, was measured as before (2).

Fig. 4 illustrates the differential appearance of these respiratory flavoprotein dehydrogenases during the growth cycle. The largest amounts of D-lactate and succinate dehydrogenase activities appear during the late log phase. Very large amounts of DPNH and L-lactate dehydrogenases are formed at a later time. The greatest amount of formate dehydrogenase is formed during the early stationary phase. Vigorous aeration produces the most striking differential appearance of these activities and effects their rapid loss. The highly aerated environment also causes rapid disruption of the cytochrome system and cell death during the stationary phase (2). If the bacteria used in measuring the ferricyanide reductase activities during the growth cycle are treated with ultrasonic vibration for 1 minute before assay, there is no significant change in the appearance or level of these activities.

DISCUSSION

Use of artificial electron acceptors as measures of respiratory dehydrogenase activities merits careful interpretation (9). Evidence presented in the present study indicates that ferricyanide reduction is an accurate measure of the respiratory dehydrogenases of the electron transport system of *H. parainfluenzae*. Ferricyanide accepts electrons at the same rate as oxygen and is an inhibitor of oxygen uptake. All ferricyanide reductases are similarly inactivated by heat and are inhibited by thenoyltrifluoroacetate and secobarbital in a manner parallel to the oxygen reductases. Rates of both ferricyanide reduction and oxygen utilization correlate to an identical degree with the proportion of cytochrome reduced by each substrate. Large membrane fragments prepared from cells ruptured with alumina contain both the ferricyanide reductases and the oxygen-utilizing components but have lost many other enzymatic activities (3). Disruption of these membrane fragments by treatment with ultrasonic vibration releases an amount of ferricyanide reductase activity into the supernatant phase that is balanced by losses of this activity in the pellet of high speed centrifugation. Ferricyanide reduction assays the activities of the respiratory chain of this bacterium at a point or points closer to the dehydrogenases than oxygen uptake measurements. Oxygen utilization requires the entire electron transport system. Studies with inhibitors show that ferricyanide reduction is less sensitive to 2-*n*-heptyl-4-hydroxyquinoline *N*-oxide and cyanide than oxygen utilization. The greater resistance to treatment with ultrasonic vibration of the ferricyanide reductases indicates that less of the intact electron transport system is required to reduce ferricyanide than is required for oxygen reduction. Also, it has been previously shown (7) that repeated washing of respiratory particle preparations from this bacteria removes cytochrome c_1 progressively and has little effect on the ferricyanide reductase activity. Removal of cytochrome c_1 is attended by loss of the capacity for oxygen uptake.

The rate of ferricyanide reduction in the presence of formate is about 0.25 that expected from the rate of oxygen utilization. The inhibition of oxygen utilization by ferricyanide is also less pronounced when measured in the presence of formate than with the other substrates. In all other criteria mentioned above, the activity produced in the presence of formate behaves similarly to that produced in the presence of the other substrates. With the reservation that ferricyanide reduction measures possibly only 25% of the total activity on addition of formate, this assay is a reasonable assay for formate dehydrogenase.

Consequently ferricyanide reduction is an adequate measure of the respiratory dehydrogenases of the electron transport system of *H. parainfluenzae*. The ferricyanide reductase activity can be liberated in the high speed supernatant phase after treatment with ultrasonic vibration. These preparations in the presence of cyanide show suggestive flavoprotein difference spectra on the addition of D-lactate, L-lactate, succinate, DPNH, and formate. This evidence helps confirm the inferences as to the nature of these dehydrogenases from the earlier inhibitor studies (3). These ferricyanide reductase activities represent the different respiratory dehydrogenases indicated by the differential sensitivity to heating, the differences in the effect of treatment with ultrasonic vibration, and the additivity of the amount of reduction at 409 mμ on sequential substrate addition.

The five membrane-bound respiratory dehydrogenases are under a control system that allows differential synthesis. Formation of D- or L-lactate dehydrogenase, or of both, can be inhibited by growth conditions, and D-lactate dehydrogenase appears earlier in the growth cycle than L-lactate dehydrogenase. The ratio of succinic to DPNH dehydrogenase can change, and maximal succinic dehydrogenase appears before maximal DPNH dehydrogenase in the growth cycle. Formation of formate dehydrogenase is greatly stimulated by low oxygen tension and in the stationary growth phase.

Two insights into the structure of the electron transport system of *H. parainfluenzae* emerge from this study. Since the rate of oxygen uptake closely approximates the activity of the dehydrogenases measured by ferricyanide reduction in the intact bacterium, the rate-limiting step in electron transport must be near the level of the respiratory dehydrogenases. The greater the concentration of the dehydrogenase, measured by the rate of ferricyanide reduction or the absorbance decrease at 409 mμ on reduction, the greater the proportion of the total cytochrome c_1 or b_1 reduced and the more rapid the rate of electron transport.

The second implication of this report concerns the formation of the electron transport system. *H. parainfluenzae* must have electron transport through the oxidases to grow (1), and the bacterium has the capacity to greatly modify the cytochrome composition of its electron transport system to fit various growth conditions (2). It can form primarily cytochrome oxidase *o* and very little cytochrome c_1 during growth with high aeration. During growth with poor aeration, very large amounts of cytochrome oxidase a_2 and cytochrome c_1 are formed relative to the cytochrome b_1—an arrangement that allows the maximum respiratory rate at oxygen concentrations just above the K_m of the system for oxygen (4). Anaerobic growth is nitrate dependent, and during it, no cytochrome a_2 is formed, but the largest amounts of cytochrome oxidase a_1 and cytochrome c_1 relative to the cytochrome b_1 are formed. The present study indicates that there is an additional compensatory mechanism present in these bacteria that alters the proportion of each of the dehydrogenases for particular growth conditions. These respiratory dehydrogenases are membrane bound and, like the cytochromes and cytochrome oxidases, can be changed dramatically. It appears that in this bacterium the formation of the electron transport pigments involves control over the concentration of each respiratory pigment and not the multiplication of a single complete unit containing these pigments.

SUMMARY

Ferricyanide reduction in cyanide measures the activity of five membrane-bound respiratory dehydrogenases that react with D-lactate, L-lactate, succinate, reduced diphosphopyridine nucleotide, and formate and are part of the electron transport system of *Hemophilus parainfluenzae*. Each of these dehydrogenases can be formed differentially during various growth conditions, indicating that they, like the cytochromes, are formed individually in the membrane-multienzyme complex. The primary dehydrogenases appear to be flavoproteins.

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