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ELECTRON-TRANSPORT SYSTEM OF *VIBRIO SUCCINOGENES*

II. INHIBITION OF ELECTRON TRANSPORT BY 2-HEPTYL-4-HYDROXYQUINOLINE *N*-OXIDE

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SUMMARY

The effect of the inhibitor 2-heptyl-4-hydroxyquinoline *N*-oxide on the reduction by formate of fumarate, O_2 and cytochromes was studied in extracts of *Vibrio succinogenes*. The inhibitor causes 90% inhibition of the couple between formate and fumarate but less than 10% inhibition of the formic dehydrogenase or fumarate reductase activity. Spectrophotometric studies showed that 2-heptyl-4-hydroxyquinoline *N*-oxide causes an appreciable delay in the reduction of both cytochromes *b* and *c* by formate. No effect of the inhibitor on the oxidation of reduced cytochrome *b* by fumarate could be detected. With succinate as substrate, the inhibitor causes a delay in the reduction of cytochrome *c* but no delay in the reduction of cytochrome *b* was detected. The effect of other inhibitory agents on the reduction of fumarate by formate and H_2 was also studied.

Oxygen consumption with formate as substrate is not inhibited by 2-heptyl-4-hydroxyquinoline *N*-oxide, but a marked increase in H_2O_2 formation occurs in the presence of this inhibitor. The formate peroxidase system of this extract is partially inhibited by 2-heptyl-4-hydroxyquinoline *N*-oxide.

The implications of these findings with regard to the role of cytochrome *b* as an electron carrier between the formic dehydrogenase and the fumarate reductase, and the role of the cytochromes in the oxidation of formate by O_2 are discussed. It is suggested that the presence of the H_2O_2-producing formate oxidase system and the formate peroxidase system explains the microaerophilic nature of this organism when grown with O_2 as an electron acceptor.

INTRODUCTION

In the preceding paper[1], enzyme systems which couple the oxidation of either H_2 or formate to the reduction of fumarate were demonstrated in extracts of *Vibrio succinogenes*. Data were also presented which were consistent with the hypothesis that the cytochrome *b* of this vibrio is an intermediate electron carrier in these

Abbreviation: QO, 2-heptyl-4-hydroxyquinoline *N*-oxide.

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coupled systems since this cytochrome is reduced by H_2 or formate and is oxidized by fumarate.

This present paper, through the use of the inhibitor QO, presents further evidence for the role of the cytochrome *b* of the vibrio. This evidence is based on the observation that this inhibitor inhibits both the reduction of the cytochromes and fumarate by formate. The ability of certain other inhibitory treatments to inhibit the couple between formate and fumarate is also described.

The inhibitory action of QO has also been used to characterize the mechanism of formate oxidation with O_2 as an electron acceptor. The results suggest that an unusual mechanism for O_2 consumption, which includes a formate oxidase system which forms H_2O_2 and a peroxidase system which destroys H_2O_2, exists in this organism. Evidence regarding the role of the cytochromes in O_2 consumption is also presented. Finally, an hypothesis is presented which suggests that it is the existence of this unusual mechanism for O_2 consumption which explains the microaerophilic type of growth which this organism manifests with O_2 as an electron acceptor. Experimental data are presented to support this latter hypothesis.

MATERIALS AND METHODS

QO was kindly supplied by Dr. J. W. LIGHTBOWN. It was dissolved in 0.01 *N* KOH. When it was necessary to compare results with and without this inhibitor, an equal volume of 0.01 *N* KOH was added to the reaction mixture without inhibitor. H_2O_2 was determined as described in the preceding paper[1].

Spectrophotometric studies of cytochrome reduction were conducted as previously described[1] in anaerobic cuvettes with a Cary automatic recording spectrophotometer. It was possible to record the spectrum between 600 and 500 mμ within 1 min from the time of addition of substrate. Following this recording, the instrument was reset at 600 mμ and another spectrum was recorded. This procedure was repeated in order to follow the extent of cytochrome reduction at various time intervals after substrate addition.

Cell-free extracts of *V. succinogenes* were prepared as described in the preceding paper[1] from cells grown on formate and fumarate, and stored under H_2 at 0° until used. Acetone-dried cells were prepared according to the method of GUNSALUS[2]. In order to prepare cell-free extracts of these acetone-dried cells, 50 mg of cells were suspended in 5 ml of 0.03 *M* K_2HPO_4 (pH 7.1) containing 0.5 mg β-mercaptoethanol. The suspension was sonicated for 15 min and centrifuged at 4300 × *g* for 20 min to remove large debris.

Cell-free extracts of *Clostridium pasteurianum* were used as the source of hydrogenase. Cells were prepared as described by WILSON *et al.*[3] and disrupted by sonic oscillation for 45 min. The extract was centrifuged at 14000 × *g* for 10 min to remove remaining whole cells and large debris.

RESULTS

Inhibition of the couple between formate and fumarate by QO

Table I shows that QO has little effect on formic dehydrogenase activity, measured with methylene blue or benzyl viologen, whereas the reaction between

the formic dehydrogenase and the fumarate reductase is almost completely inhibited. Although inhibition of formic dehydrogenase activity was always less than 10% in extracts assayed within 2 or 3 days after preparation, occasionally a more marked inhibition (20–40%) of the formic dehydrogenase activity was observed in extracts which had been aged for several days.

The ability of QO to inhibit the fumarate reductase activity of the extracts was next investigated. The fumarate reductase was assayed by a modification of the method of PECK *et al.*[4] which measures the uptake of H_2 in the presence of fumarate, *C. pasteurianum* hydrogenase and benzyl viologen. Since the vibrio extract itself is capable of reducing fumarate with H_2, and since this reaction is inhibited by QO, it was necessary to use very small amounts of vibrio extract in the presence of excess *C. pasteurianum* hydrogenase. Under these conditions, approx. 90% of the rate of hydrogen uptake was dependent upon the presence of *C. pasteurianum* extract and benzyl viologen. The results shown in Table II demonstrate that QO causes considerably less than 12% inhibition of the fumarate reductase, especially since about 10% of the measured H_2 consumption can be attributed to the activity which was due to the H_2–fumarate couple of the vibrio extract itself and which would be expected

TABLE I

EFFECT OF QO ON FORMATE OXIDATION WITH VARIOUS ELECTRON ACCEPTORS

The formate oxidation rate is expressed as μl CO_2 evolved/min/mg protein at 37° with N_2 as gas phase calculated from CO_2 evolved between 5 and 10 min after addition of electron acceptor. Warburg cups contained 111 m*M* K_2HPO_4 (pH 6.2), 0.15 mg β-mercaptoethanol, either 0.1 ml of 0.01 *N* NaOH or 3.2 μg of QO and H_2O to 3.15 ml. 10 μmoles of sodium formate and the electron acceptor were added from the sidearm to start the reaction. 20 μmoles of methylene blue, 20 μmoles of sodium fumarate or 40 μmoles of benzyl viologen were added.

	Electron acceptor					
	Methylene blue		*Fumarate*		*Benzyl viologen*	
	−QO	*+QO*	*−QO*	*+QO*	*−QO*	*+QO*
Formate oxidation rate	2.90	2.70	1.50	0.08	1.54	1.54
Inhibition by QO (%)	—	7	—	95	—	0

TABLE II

EFFECT OF QO ON FUMARATE REDUCTASE

The rate of fumarate reduction is expressed as μl H_2 uptake/min/mg protein at 37° with H_2 as gas phase. Warburg-cup contents as in Table I except that 5 μmoles of benzyl viologen and 3.2 μg of QO were added. 0.5 ml of *C. pasteurianum* extract and 50 μg of *V. succinogenes* extract protein were used. Fumarate was present in the sidearm and was tipped after 30 min equilibration to start reaction.

Additions	*Rate of fumarate reduction*
V. succinogenes and C. pasteurianum extracts, benzyl viologen	32.8
V. succinogenes and C. pasteurianum extracts, benzyl viologen, QO	28.2
V. succinogenes and C. pasteurianum extract	3.9
V. succinogenes extract, benzyl viologen	1.9
V. succinogenes extract	2.3

to be inhibited. From the results presented in Tables I and II, it can be concluded that QO causes little inhibition of formic dehydrogenase or fumarate reductase but causes marked inhibition of some intermediate site coupling the two enzymes.

Inhibition of cytochrome reduction and oxidation by QO

In order to examine the relationship of the cytochromes of the vibrio to the inhibited site, we investigated the ability of QO to inhibit the reactions of the cytochromes. These studies were conducted within 2 or 3 days from the time the extracts were prepared.

Fig. 1 shows a typical recording of several time intervals following reduction with formate. After 25 min, the extent of reduction of both cytochromes *b* and *c* with formate is about 80% of that which can be obtained by addition of hydrosulfite. It will also be noted that cytochrome *b* approaches 80% reduction more slowly than cytochrome *c*. 1 μg per ml of QO causes a definite delay in the appearance of both the cytochrome *b* and *c* peaks. This marked inhibition of cytochrome reduction cannot be due to inhibition of the formic dehydrogenase since it has been shown that the dehydrogenase is inhibited less than 10% by similar concentrations of QO.

Fig. 2 illustrates the oxidation of the reduced cytochromes by fumarate in the presence and absence of QO. There is no doubt that fumarate causes the oxidation of reduced cytochrome *b*, since only the peak due to cytochrome *c* remains after the addition of fumarate. Due to the overlapping of the *b* and *c* peaks, it is difficult to estimate if any of the cytochrome *c* has been oxidized by fumarate. Since QO, at a concentration three times greater than that used in the experiment of Fig. 1 has no visible effect on cytochrome oxidation, it can be concluded that inhibition by QO of cytochrome *b* oxidation by fumarate was not detected by this method. It is of interest to note that in *Escherichia coli* no effect of this inhibitor on the reduction of cytochrome b_1 by formate was detected, although the oxidation of reduced cytochrome b_1 by NO_3^- was inhibited[5].

Fig. 3 shows a typical recording at various time intervals following addition of succinate. On comparing this reduction with the total reduction obtained with hydrosulfite, it would appear that a little more than 1/2 of the cytochrome *c* is reduced by succinate and about 1/3 of the cytochrome *b* is reduced. The effect of QO on the course of cytochrome reduction by succinate is also illustrated. Although the reduction of cytochrome *b* is again only partial, no obvious difference in the extent or rate of reduction of cytchrome *b* can be detected whether or not the inhibitor is present. On the other hand, the inhibition of cytochrome *c* reduction is obvious. The technique used has the disadvantage that the rate of cytochrome reduction cannot be followed since the cytochrome, in the absence of inhibitor, is usually more than 50% reduced before the first spectrum can be taken. Therefore, these data should only be interpreted to indicate that the inhibition by QO of cytochrome *b* reduction by succinate cannot be detected under conditions where QO inhibition of both cytochrome *b* and *c* reduction by formate and of cytochrome *c* reduction by succinate can be readily detected. This criticism also applies to the experiment illustrated in Fig. 2 where no inhibition of cytochrome *b* oxidation by fumarate was detected. If QO is used at a concentration 9 times higher than shown in Fig. 3, there is still no detectable inhibition of cytochrome *b* reduction by succinate.

It was necessary to evaluate the effect of QO on the succinic dehydrogenase itself.

Nitroblue tetrazolium was used to assay succinic dehydrogenase activity since it is one of the few electron acceptors which is reduced by succinate with these extracts[1]. The succinic dehydrogenase was not found to be inhibited by QO since there was no inhibition of nitroblue tetrazolium reduction by succinate in the presence of QO.

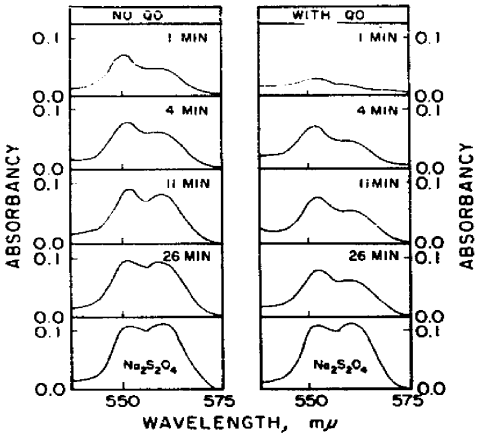

Fig. 1. Reduction of cytochromes by formate. Anaerobic cuvettes (prepared as in MATERIALS AND METHODS) contained 110 m*M* K_2HPO_4 (pH 6.2), 0.15 mg β-mercaptoethanol, 20 mg extract protein and H_2O to 3.15 ml total volume. The inhibited series contained 3.3 μg of QO. 10 μmoles of formate were added from sidearm. The time intervals indicate the time elapsed from addition of substrate to completion of spectrum. The lines were traced directly from the recorded spectrum. Aerated reaction mixtures without formate were used as blanks.

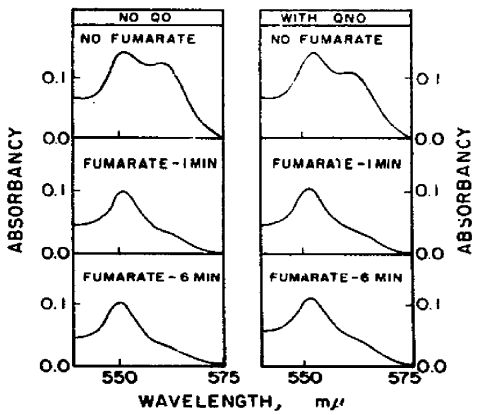

Fig. 2. Oxidation of reduced cytochromes by fumarate. Anaerobic cuvettes as in Fig. 1 with 19 mg extract protein. 2 μmoles of formate were in the siderarm. After evacuation, the formate was tipped in order to reduce the cytochromes. The sidearms were replaced with new sidearms, containing either 0.1 ml of 0.01 *N* NaOH or 0.1 ml of a solution of QO (100 μg/ml), with a minimum of aeration. The cuvettes were again evacuated and the sidearms tipped. The sidearms were then replaced with sidearms containing 0.1 ml of 0.2 *M* fumarate and again evacuated. The spectra shown at the top of the figure were recorded before the addition of fumarate.

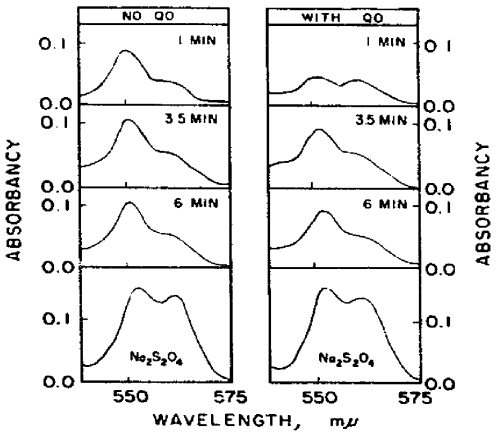

Fig. 3. Reduction of cytochromes by succinate. Conditions as in Fig. 1 with 25 mg extract protein and 0.2 ml of 0.4 *M* succinate added to start the reaction.

Effect of iron chelators on the couple between formate and fumarate

In an attempt to reveal the nature of the intermediate site which is inhibited by QO, other reagents were tested for their ability to inhibit the couple between

formate and fumarate. Iron-chelating agents were investigated since QO is known to form a coordination compound when reacted with ferric chloride[6], and since LASCELLES AND STILL[7] reported that the reduction of fumarate by H_2 in *Escherichia coli* is much more inhibited by *o*-phenanthroline at a concentration of $2 \cdot 10^{-3}$ *M* than is the reduction of methylene blue by H_2. We therefore tested the ability of an iron chelator such as α,α′-dipyridyl to inhibit the couple. We have also tested other iron chelators such as *o*-phenanthroline and tiron (disodium-1,2-dihydroxybenzene-3,5-disulfonate monohydrate) on the formic dehydrogenase and on the formate–fumarate couple. The results were essentially the same as they were with α,α′-dipyridyl. Table III compares the inhibition patterns of QO and α,α′-dipyridyl.

TABLE III

INHIBITION OF FORMATE OXIDATION BY QO AND α,α′-DIPYRIDYL

Electron acceptor	*Inhibitor*	*Inhibitor concentration (M)*	*Inhibition* (%)*
Methylene blue	QO	$4 \cdot 10^{-6}$	7
Fumarate	QO	$4 \cdot 10^{-6}$	95
Methylene blue	α,α′ dipyridyl**	$1.1 \cdot 10^{-3}$	20–30
Fumarate	α,α′-dipyridyl**	$1.1 \cdot 10^{-3}$	40

* Gas evolution measured as in Table I.

** α,α′-Dipyridyl was dissolved in 1.25% ethanol. An equal amount of 1.25% ethanol was added to the cups without α,α′-dipyridyl.

The results demonstrate that α,α′-dipyridyl does not exhibit the same pattern of inhibition that QO does. QO is a relatively specific inhibitor of the formate–fumarate couple and a relatively poor inhibitor of the formic dehydrogenase at the concentrations used. On the other hand, when compared with QO, the ordinary iron-chelating agent is a much poorer inhibitor of the couple. In fact, it is difficult to decide whether or not the ordinary iron-chelating agent has any specific effect on the couple since the per cent inhibition with fumarate as acceptor is not a great deal higher than the per cent inhibition with methylene blue. $FeSO_4$ (10^{-4} *M*) does not reverse the inhibition of the couple by QO, while it does cause some reversal of the inhibition of the formic dehydrogenase by QO and of both the formic dehydrogenase and the couple by α,α′-dipyridyl. Preincubation of enzyme with formate under N_2 prior to the addition of QO does not relieve the inhibition of the couple by QO, although it does prevent the small amount of inhibition of the formic dehydrogenase caused by QO. The inhibition of the formic dehydrogenase of *E. coli* by iron-chelating agents has been discussed by PECK AND GEST[8].

Effect of QO on the couple between H_2 and fumarate

In order to determine whether or not the QO-inhibited site which coupled formate to fumarate is similar to the one coupling H_2 to fumarate, the effect of QO on the reaction between H_2 and fumarate was studied. This reaction can be studied with much lower enzyme concentrations because the hydrogenase and the fumarate reductase are in much higher concentration in extracts than the formic dehydrogenase. QO at $1.2 \cdot 10^{-5}$ *M* caused no inhibition of the hydrogenase as measured by methylene blue reduction. At the same concentration, it caused 87% inhibition of fumarate

reduction by H_2. These experiments were conducted under approximately the same conditions as shown in Table I of the preceding paper[1]. 1 μg per ml of Antimycin A or 1 μmole per ml of amytal did not inhibit the reduction of fumarate by hydrogen.

We have also investigated one other procedure which will preferentially uncouple the hydrogenase from the fumarate reductase. If the cells are first made into an acetone-powder (cells dried in cold acetone and ether) before being disrupted by sonic oscillation, the cell-free extracts are unable to couple the hydrogenase to the fumarate reductase. Methylene blue can still be reduced by H_2, and the couple can be restored with catalytic quantities of benzyl viologen. Neither the hydrogenase nor the fumarate reductase is markedly inactivated in these extracts. It would appear that the solvent treatment has preferentially inactivated some intermediate site(s) necessary for the couple between the two enzymes.

Effect of QO on the formate oxidase system

It was shown that these extracts oxidized formate with O_2 (see ref. 1). In order to examine the role of the cytochromes in this reaction, the ability of QO to inhibit this formate oxidase system was tested. The results are shown in Fig. 4. It will be noted that QO has little or no ability to inhibit the initial rate of O_2 consumption, but QO does cause a more rapid decline in the rate of O_2 uptake. This decline in the rate of O_2 consumption suggested the possibility that QO caused a more rapid accumulation of an inhibitory end product such as H_2O_2. When the Warburg-cup contents were assayed for H_2O_2 it was found that in the absence of QO, about 0.5 μmole of H_2O_2 was formed during the consumption of 8.4 μmoles of O_2 whereas in the presence of QO about 3.3 μmoles of H_2O_2 were formed during the consumption of 6.0 μmoles of O_2. These values for the amount of H_2O_2 are probably slightly low due to the presence of reducing substances, such as β-mercaptoethanol, in the enzyme preparation which could react with H_2O_2. There was also some variation in the ratio of H_2O_2 formed to O_2 consumed depending upon the batch of extract which was used.

The fact that more H_2O_2 accumulated in the presence of QO suggested that QO was at least partially inhibiting a reaction which could dispose of the H_2O_2 which was formed by the formate oxidase system. The obvious mechanism for H_2O_2 disposal in this catalase-negative organism was the formate peroxidase system which was described in the preceding paper[1]. It was found that this formate peroxidase system, as assayed under the conditions shown in Table III of the preceding paper, was about 50 % inhibited by 1 μg per ml of QO.

Since these data and the data presented in the previous paper suggested that an important mechanism for O_2 consumption in these extracts involved a combination of a formate oxidase system which formed H_2O_2 and a formate peroxidase system which destroyed H_2O_2, the possibility was considered that this unusual mechanism for O_2 consumption explained the observation that this organism exhibits a microaerophilic type of growth when grown with O_2 as an electron acceptor. Previous studies have shown that this organism will utilize O_2 as an electron acceptor for growth only if the O_2 concentration is below 4 %.

From the observation that H_2O_2 is formed even in the absence of QO under the conditions shown in Fig. 4, it was concluded that the peroxidase was not active enough under those conditions to destroy all of the H_2O_2 formed by the oxidase system. If conditions could somehow be altered to reduce the activity of the oxidase,

but not the peroxidase, the result should be that less H_2O_2 should be produced per mole of O_2 consumed. The most obvious method for achieving this result would be to lower the O_2 concentration of the atmosphere.

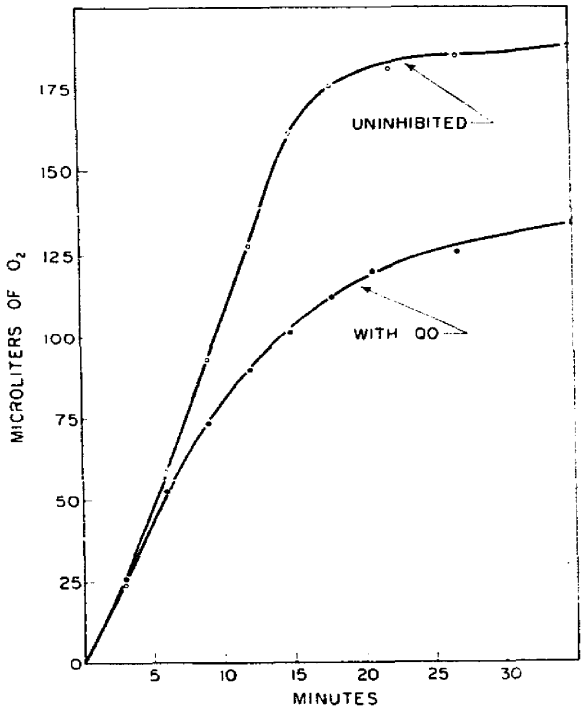

Fig. 4. Effect of QO on O_2 uptake in cell-free extracts. Each cup contained 110 m*M* K_2HPO_4 (pH 6.2), 11 mg extract protein, 0.15 ml of 20% KOH in the center well, H_2O to 3.0 ml total volume and 3 μg QO where indicated. After equilibration under N_2 gas for 10 min, the cups were flushed with air for 8 min before tipping 15 μmoles of sodium formate from the sidearm. A reaction mixture without formate was used as an endogenous blank both for correcting O_2 uptake and H_2O_2 values. After 35 min, aliquots were removed and diluted to 4.0 ml with 5% trichloroacetic acid. Precipitated protein was removed by centrifugation. The supernatant solutions were analyzed for H_2O_2 by the procedure in MATERIALS AND METHODS. Standard curves for H_2O_2 were determined with the same procedure using 30% H_2O_2 (Fischer Certified Reagent) as a standard.

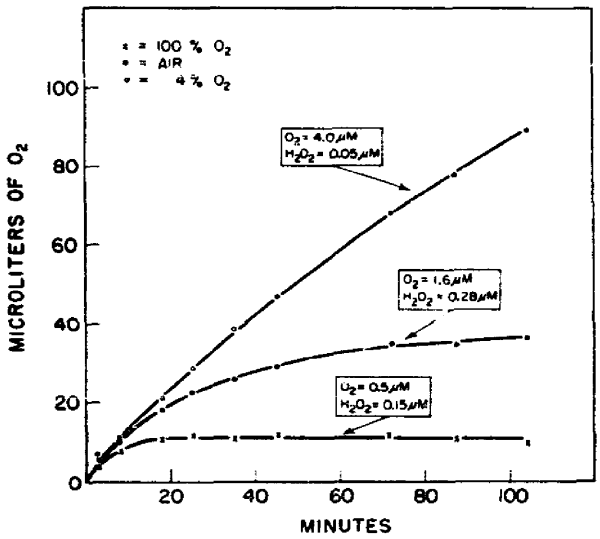

Fig. 5. Formate oxidase activity by whole cells at various O_2 concentrations. Cup contents as in Fig. 4 with 0.1 ml of a washed cell suspension containing 0.31 mg cells (dry weight). The cup used to measure endogenous gas uptake and the cup under air were allowed to equilibrate in air. The cups under 100% O_2 and 20% air–80% N_2 were equilibrated for 20 min before tipping 15 μmoles formate from the sidearm. After 165 min, the cup contents were analyzed for H_2O_2 as in Fig. 4. The endogenous cup was used as blank for calculation of O_2 consumption and H_2O_2 production. The total μmoles of O_2 consumed and H_2O_2 formed per cup are shown on the figure.

Fig. 5 illustrates an experiment in which O_2 consumption and H_2O_2 production with formate as substrate were measured under atmospheres of varying O_2 concentration. This experiment was conducted with dilute suspensions of whole cells in order to duplicate as closely as possible the conditions under which this organism

grows. The results show that as the O_2 concentration of the atmosphere is increased, the ratio of H_2O_2 produced to O_2 consumed also increases. This increased amount of H_2O_2 is probably responsible for the more rapid decline in the rate of O_2 consumption at higher O_2 concentrations.

DISCUSSION

The fact that QO inhibits both the reduction of cytochrome *b* by formate and the reduction of fumarate by formate, lends further support to the suggestion that cytochrome *b* functions as a quantitatively important electron carrier between the formic dehydrogenase and the fumarate reductase in this organism.

It should be emphasized that the results obtained with this inhibitor do not prove that cytochrome *b* is a required intermediate in this reaction since they only establish that the inhibited site is necessary for both cytochrome *b* and fumarate reduction by formate. Nevertheless, for purposes of discussion we have constructed

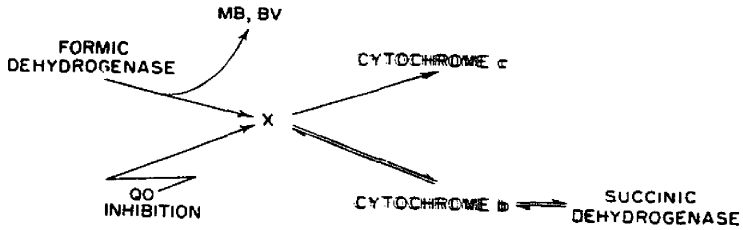

Fig. 6. Path of electrons from formate or hydrogen to cytochromes and fumarate.

an electron transport scheme (Fig. 6) which suggests a possible pathway of electron flow which is consistent with the data presented. In this scheme, X represents the site which QO inhibits. The scheme shows that, with formate as substrate, QO would inhibit the reduction of cytochrome *b*, of cytochrome *c*, and of fumarate. With succinate as substrate, QO would inhibit the reduction of cytochrome *c*, but not the reduction of cytochrome *b*. It is of interest to point out that, with the exception of formic dehydrogenase, the scheme resembles that which has been postulated for mammalian mitochondria. Both LIGHTBOWN AND JACKSON[9] and CHANCE[10] have shown in mammalian mitochondria, with succinate as substrate, the reduction of cytochrome *c* but not of cytochrome *b* is inhibited by QO. The scheme presented must only be regarded as a tentative one since several facts have not been experimentally established which would be required before the scheme could be accepted in its entirety. For instance, although we were unable to detect any inhibition of the reduction and oxidation of cytochrome *b* by succinate and fumarate by the methods available, these reactions should be followed kinetically in order to insure that QO does not inhibit them to any degree. Also, the scheme assumes without any experimental verification that the dehydrogenases and the cytochromes are all interconnected and that only one site of action of QO exists in this organism.

The fact that QO is an effective inhibitor for both the couple between formate and fumarate and the couple between H_2 and fumarate suggests that the pathway of electron transport between formate and fumarate is similar to the pathway between H_2 and fumarate. The inability of Antimycin A to substitute for QO as an inhibitor is similar to effects observed with other microbial systems[11].

The implications of the effect of QO on the enzyme systems responsible for oxidation of formate by O_2 will now be discussed. The results which have been presented indicate that in extracts QO does not inhibit O_2 uptake with formate but that QO does cause an increase in H_2O_2 production. It has also been shown that QO causes a partial inhibition of the formate peroxidase system. The most obvious interpretation of these data is that a mechanism which involves a combination of Eqns. 1 and 2 is a quantitatively important mechanism for the oxidation of formate by O_2

$$HCOOH + O_2 \longrightarrow CO_2 + H_2O_2 \quad (1)$$

$$HCOOH + H_2O_2 \longrightarrow CO_2 + 2H_2O \quad (2)$$

This interpretation is based on the fact that the formate peroxidase system in the extracts is inhibited by QO and on the observation that an increased amount of H_2O_2 is formed during O_2 consumption in the presence of QO. The existence of another formate oxidase system which does not form H_2O_2 and which is not inhibited by QO cannot be ruled out. However, the fact that at least 3.3 μmoles of H_2O_2 were formed during the uptake of 6.0 μmoles of O_2 in the presence of QO suggests that the mechanism illustrated by Eqns. 1 and 2 is responsible for at least half of the total O_2 consumption.

The fact that QO does not inhibit the initial rate of O_2 consumption on formate indicates that neither cytochrome *b* nor cytochrome *c* participates in the formate oxidase system because QO inhibits the reduction of cytochrome *b* and cytochrome *c* by formate. Since this formate oxidase system does not involve cytochromes, and since it forms appreciable amounts of H_2O_2, it may be similar to the one proposed by HAUGE[12] to be operative in *Aspergillus niger*.

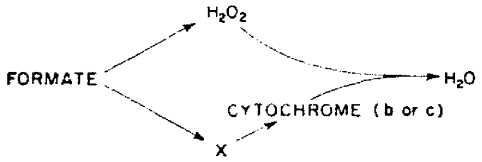

Fig. 7. Pathway for oxygen utilization.

Although the formate oxidase system apparently does not involve cytochrome *b* or *c*, the formate peroxidase system may involve cytochromes *b* or *c* since this system is partially inhibited by QO. A possible scheme of electron transport during formate oxidation by O_2 is shown in Fig. 7. In this scheme, X is the site which is inhibited by QO. The mechanism proposed by this scheme would predict that one molecule of formate would be oxidized by O_2 without the mediation of cytochromes *b* or *c* to yield a molecule of H_2O_2. A second molecule of formate would reduce cytochromes *b* or *c*. The reduced cytochrome could then react with the molecule of H_2O_2 to form H_2O through the mediation of a cytochrome peroxidase. A cytochrome peroxidase system has been demonstrated in *P. fluorescens* (see ref. 13). The fact that QO inhibits the formate peroxidase should not be interpreted as final proof that the cytochromes are involved in this system for the same reasons which have already been discussed with regard to the role of cytochromes in fumarate reduction.

It seems reasonable to conclude that this unusual oxidase--peroxidase system is responsible for the restricted use of O_2 as an electron acceptor for growth at low partial pressures of O_2. The amount of the toxic product, H_2O_2, which accumulates during the oxidation of formate is dependent upon the ratio of the velocities of Eqns. 1 and 2. The experiment illustrated in Fig. 5 demonstrates that an increase in O_2 concentration results in increased H_2O_2 production presumably because the velocity of Eqn. 1 is increased without a corresponding increase in the velocity of Eqn. 2. Growth of the vibrio would, therefore, be inhibited at higher O_2 concentrations due to the accumulation of toxic H_2O_2. Since this experiment was conducted with a dilute suspension of whole cells, it is felt that the result can be used to explain the extreme sensitivity to O_2 which this organism exhibits during growth.

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