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MECHANISM OF OXIDATIVE PHOSPHORYLATION IN THE CHEMOAUTOTROPH *NITROBACTER AGILIS*

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SUMMARY

Cytochrome electron transport particles obtained from Nitrobacter, catalyzed nitrite oxidation with concomitant phosphate esterification yielding P/O ratios approaching 1.0. Likewise the enzymatic oxidation of ascorbate or ferrocyanide yielded similar P/O ratios. The oxidation of exogenously added NADH mediated by flavoproteins and cytochrome systems was also coupled to ATP formation and yielded P/O ratios of 2.0.

The phosphorylation coupled to nitrite oxidation was not affected by NADH traps, rotenone (250 μM), amytal (1.5 mM), or antimycin A (10 μg/mg protein). Somewhat higher P/O ratios were obtained in the presence of rotenone or antimycin A due to the inhibition of an energy-dependent reverse electron flow.

A 50 and 70 % inhibition of the NADH-linked phosphorylation was observed in the presence of 2-*n*-heptyl-4-hydroxyquinoline *N*-oxide (HOQNO) (5 μg/mg protein), and rotenone (250 μM), respectively. These inhibitors blocked electron transfer between the flavoproteins and cytochrome systems, and the latter were oxidized when NADH served as an electron donor, thus exhibiting a cross-over point.

Observations based on spectrophotometry and use of uncouplers and inhibitors suggested cytochrome a_1 as the site of entry of nitrite in the Nitrobacter electron transport chain; the reduction of cytochrome *c* involved an energy-dependent reversal of electron transfer from cytochrome a_1.

INTRODUCTION

The obligatory chemosynthetic metabolism of Nitrobacter involves reduction of CO_2 at the expense of ATP and NADH both of which are generated and coupled to the specific oxidation of nitrite[1]. The enzymatic oxidation of nitrite to nitrate is achieved at the expense of oxygen atom from water, a process which involves the dehydrogenation of a hydrated nitrite molecule and subsequent electron transfer to molecular oxygen which is reduced to water[2]. This process is mediated by the cytochrome systems and is coupled to the generation of ATP. The generation of NADH

Abbreviations: HOQNO, 2-*n*-heptyl-4-hydroxyquinoline *N*-oxide; ~ I, ~ II, ~ III, high-energy intermediates generated at coupling sites I, II, and III in the electron transport chain; TMPD, tetramethyl-*p*-phenylenediamine.

involves an energy-dependent reversal of electron transfer from the cytochrome systems[1,3]. The previously reported low P/O ratios of 0.2 with nitrite as the electron donor in this system were due to the prolonged periods of cell disruption by the sonication procedures[4]. By improving the methods of cell disintegration (see MATERIALS AND METHODS) it has been possible to obtain P/O ratios of 1.0 with nitrite, and about 2.0 with NADH as the electron donors.

This paper is concerned with the mechanism of nitrite oxidation and coupled energy conversion in the chemoautotroph, *Nitrobacter agilis* and confirms our previous observations that the enzymatic transfer of electrons from nitrite is effected at the cytochrome a_1 level[5].

MATERIALS AND METHODS

N. agilis (ATCC 9482) was grown in an inorganic liquid medium described previously[6] under forced aeration with 5 % CO_2. An active log phase of the culture was obtained with periodic nitrite additions. When the culture was capable of oxidizing 60 mM nitrite within 24 h for a period of 2 days, the cells were collected at 50000 × *g* by a Sharple's centrifuge at 5°. The intact cells were washed twice with 50 mM Tris–HCl buffer (pH 8.0) by centrifugation at 10000 × *g* for 30 min at 5°.

Preparation of cell-free extracts

A 5-g weight of the cell paste was suspended in 15 ml of the sonication medium containing 50 mM Tris–HCl buffer (pH 8.0), 250 mM sucrose, 1 mM EDTA, and 1 mM glutathione (reduced). The cell suspension was subjected to sonication at 5° for 20-sec intervals for a total period of 3 min using a Bronson cell disintegrator at maximum output. The broken cell suspension was centrifuged at 10000 × *g* for 10 min and the supernatant fraction was further centrifuged at 40000 × *g* for 10 min. The resultant supernatant was subjected to centrifugation at 150000 × *g* for 60 min and the pellet was suspended in 10 ml of the sonication medium described above. This fraction designated as P-150000 was used as the enzyme source in the phosphorylation and electron transport studies.

Experimental procedures

Details of the experimental procedures are given in the legend to the tables and figures. Enzyme fractions containing 2–4 mg protein per ml of reaction mixture, determined according to LOWRY *et al.*[7], were used in the present experiments. The esterification of $^{32}P_i$ into ATP was measured in reaction mixtures deproteinized with 5 % final concentration of trichloroacetic acid as described previously using a scintillation counter[4]. Oxygen uptake was measured polarographically with a Pt electrode in identical reaction mixtures used for phosphate esterification, but contained no $^{32}P_i$. The difference absorption spectra were obtained using a Cary Model-14 recording spectrophotometer equipped with sensitive slide wire; full deflection 0–0.1 O.D.

RESULTS AND INTERPRETATION

Phosphorylation coupled to nitrite oxidation

It may be seen (Table I) that the enzymatic oxidation of one nitrite molecule yields one ATP. The inhibitors which block electron flow at the flavoprotein or cyto-

TABLE I

PHOSPHORYLATION COUPLED TO NITRITE OXIDATION BY NITROBACTER ELECTRON TRANSPORT PARTICLES

A 1.3-ml reaction mixture contained 4.0 mg of enzyme protein, 35 μmoles of Tris–HCl (pH 8.0), 1 mg of hexokinase from yeast (Sigma Chemical Co.,) 17 μmoles of glucose, 7 μmoles of $MgCl_2$, 7 μmoles of NaF, 0.2 μmole of ADP, 4.6 μmoles of potassium phosphate (pH 8.0), $2.5 \cdot 10^5$ counts per min $^{32}P_i$ and additions as indicated. The enzyme was preincubated for 5 min with antimycin A or rotenone before addition of nitrite where indicated. Reaction was stopped with 5 % final concentration of trichloroacetic acid after 2 min.

Additions	*Total $^{32}P_i$ uptake (counts/min)*	*Total ATP formed (μmole)*	*Total O_2 uptake (μatom)*	*P/O*
None	520	0.01	0.00	0.00
NO_2^- (1 μmole)	13 050	0.24	0.30	0.80
NO_2^- + antimycin A (10 μg)	9 783	0.18	0.21	0.86
NO_2^- + pyruvate (2 μmoles) + lactate dehydrogenase (50 μg)	13 674	0.27	0.30	0.90
NO_2^- + rotenone (250 μM)	8 696	0.16	0.18	0.90

chrome *b* level (*i.e.* rotenone or antimycin A), did not affect the P/O ratios. The NADH trapping system (pyruvate–lactate dehydrogenase) did not inhibit the oxidation of nitrite nor the coupled phosphate esterification. These results suggest that the entry of nitrite into the electron transport chain occurs beyond the flavin or cytochrome *b* region towards the oxygen side.

The reduced *minus* oxidized spectra of the enzyme preparations treated with nitrite were not affected, either by added antimycin A or rotenone. However, 3–5 min after addition of nitrite, cytochrome *c* was oxidized (Fig. 1); this process also involved the simultaneous oxidation of cytochrome a_1. The oxidation of the cytochromes was found to be due to the formation of nitrate which acted as electron acceptor from reduced cytochromes *c* and a_1. Subsequent addition of NAD^+ in the presence of 1 mM added CN^- resulted in the rapid reduction of cytochrome *c* and a_1; no such reduction of the cytochrome systems was observed in the presence of an NADH trap. Thus, added NAD^+ may be visualized as being reduced by nitrite. The NADH thus generated is then effective in the observed reduction of the cytochrome systems. However, because of the lower standard redox potential of the NAD^+/NADH couple at pH 7.0 and 25° ($E_0' = -0.32$ V) compared to the standard redox potential of the NO_2^-/NO_3^- couple ($E_0' = +0.43$ V)[8], the observed reduction of NAD^+ by the electrons generated by NO_2^- is clearly against the thermodynamic gradient. Therefore, the energy of nitrite oxidation must be utilized for the coupled generation of reduced pyridine nucleotide. It is also possible that the electron transfer from reduced cytochrome *c* to nitrate catalyzed by the dissimilatory nitrate reductase[10,11] is coupled to the generation of energy which could also be invested in such energy requiring reductive processes.

The site of nitrite entry

Considering the standard free energy change at pH 7.0 ($\Delta F'$) for nitrite oxidation being −17.8 kcal/mole (ref. 8), the standard redox potential for the NO_2^-/NO_3^- couple was calculated to be 0.43 V by using the equation: $\Delta F' = -nF\,\Delta E$ where *n* is

the number of electrons, F is the Faraday (23040 cal) and ΔE is the difference in standard oxidation–reduction potentials of the reacting carriers[12]. Thus, thermodynamically the direct reduction of cytochrome *c* by nitrite is highly unpropitious and an energy equivalence of 8.3 kcal must be spent to achieve this reaction. However, spectrophotometric observations indicated that both cytochrome *c*- and a_1-like components were reduced by nitrite added either to intact cell suspensions or cell-free extracts[9]. Since an energy-linked reversal of electron transfer does occur in Nitrobacter[1,3], it was therefore thought that the reduction of cytochrome *c* by nitrite might be an energy-linked process involving electron reversal from the cytochrome a_1 level.

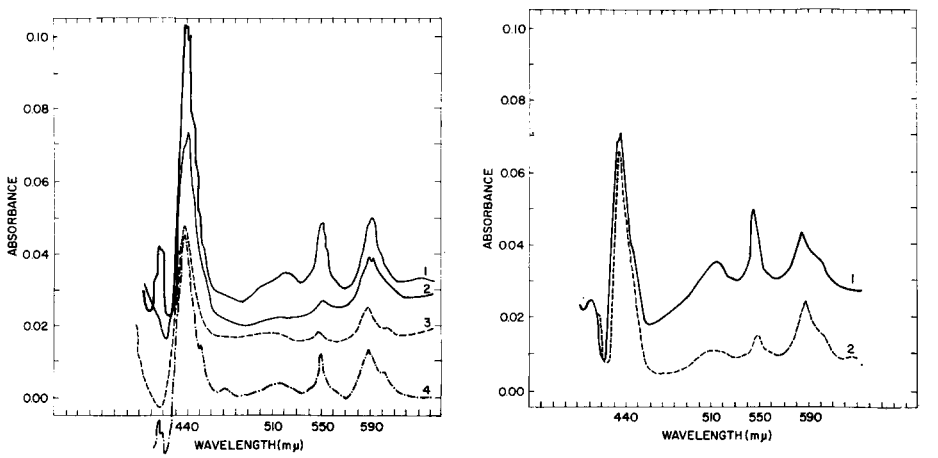


Fig. 1. Reduced *minus* oxidized absorption spectra of Nitrobacter electron transport particles. Reaction mixture in a 2-ml volume contained particulate cell-free fraction, P-150000 × *g*, containing 4 mg protein in 50 mM Tris–HCl buffer (pH 8.0). The treatment cuvette contained in addition, 10 mM KNO_2. Trace (1) shows difference absorption spectra obtained immediately after the addition of NO_2^-, Trace (2) represents absorption after 5 min, Trace (3) was obtained after adding 1 mM KCN to both cuvettes, and Trace (4) was obtained after the addition of 0.5 mM NAD^+ to both cuvettes. See description and details under RESULTS AND INTERPRETATION.

Fig. 2. Effect of 2,4-dibromophenol upon the steady-state reduction of the Nitrobacter cytochromes treated with nitrite. Trace (1) shows the reduced *minus* oxidized absorption spectra of P-150000 × *g* upon addition of 10 mM NO_2^-; Trace (2) was obtained when the particles were preincubated for 2 min with 80 μM 2,4-dibromophenol followed by the addition of nitrite. Experimental conditions were similar as described in Fig. 1.

When a suspension of P-150000 catalyzing oxidative phosphorylation was treated with 10 mM NO_2^-, the steady-state reduced *minus* oxidized absorption spectra revealed α and γ peaks of cytochrome a_3 and a_1, at 603, 445 and 590, 438 mμ, respectively (Fig. 2). Cytochromes *b* and *c* were also reduced as indicated by absorption peaks at 560, 530 and 550, 420 mμ. When this experiment was repeated with the enzyme preparation preincubated for 2 min with 80 μM 2,4-dibromophenol, an uncoupler of oxidative phosphorylation, there was no change in the steady-state reduction of cytochrome $a_1 + a_3$ but the reduction of cytochrome *c* was markedly suppressed, probably due to the hydrolysis of the energy-rich compound generated during NO_2^- oxidation which is effective in the reduction of cytochrome *c* using electrons from cytochrome a_1 reduced by NO_2^-.

This was further confirmed by our observations that nitrite failed to reduce

cytochrome *c* in the presence of 0.5 mM concentration of cyanide; on the other hand the reduction of cytochromes *c* and a_1 and a_3 could easily be seen with ascorbate as the electron donor when the terminal oxidase was blocked by cyanide (Fig. 3). If the

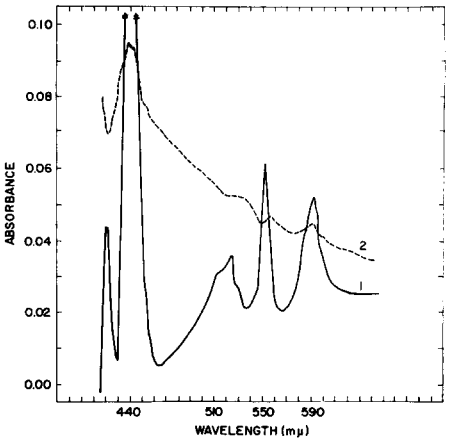


Fig. 3. Effect of cyanide upon the reduction of cytochrome components by ascorbate and nitrite. Trace (1), 10 mM ascorbate + 1 mM CN^-; Trace (2), 10 mM NO_2^- + 1 mM CN^-. Experimental conditions were similar as described in Fig. 1.

TABLE II

PHOSPHORYLATION COUPLED TO THE OXIDATION OF ASCORBATE AND FERROCYANIDE *vs.* NO_2^- BY NITROBACTER PARTICLES

Experimental conditions were similar as described in Table I, except reaction mixture contained 4.9 μmoles of potassium phosphate and $4.3 \cdot 10^5$ counts/min $^{32}P_i$. Reaction was stopped after 3 min.

Additions	*Total $^{32}P_i$ uptake (counts/min)*	*Total ATP formed (μmole)*	*Total O_2 uptake (μatom)*	*P/O*
None	443	0.005	0.00	0.00
NO_2^- (1 μmole)	10 530	0.120	0.20	0.60
Ferrocyanide (3 μmoles)	13 163	0.150	0.19	0.80
Ascorbate (5 μmoles) + TMPD (50 μM)	26 326	0.300	0.54	0.55

nitrite entry takes place at the cytochrome *c* level, then it should yield the same absorption spectra as obtained with ascorbate. These observations indicated not only that nitrite entry in the electron transport chain of Nitrobacter is effected beyond the cytochrome *c* level towards the oxygen side, but also that the phosphorylation observed with nitrite (Table I) involves electron transport carriers between cytochrome a_1 and oxygen. An analogous situation was observed when ferrocyanide served as electron donor. The E_0' of the ferro-ferricyanide couple (+ 0.34 V) being more electropositive[13] compared to the E_0' of the ferri-ferrocytochrome *c* (+ 0.25 V), the oxidation of ferrocyanide by the Nitrobacter electron transport particles was observed to be coupled to ATP formation yielding a P/O ratio of about 1.0 (Table II). Although the entry of ascorbate in the Nitrobacter electron transport chain took place at cytochrome *c*, its oxidation did not result in increased P/O ratios. Thus there appears to

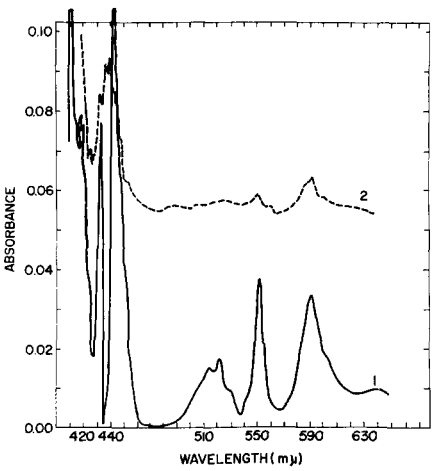
be only one energy-coupling site in the segment of the electron transport chain involving the action of cytochrome *c*: O_2 oxidoreductase.

TABLE III

PHOSPHORYLATION COUPLED TO NADH OXIDATION BY NITROBACTER ELECTRON TRANSPORT PARTICLES

Experimental conditions were similar as described in Table I, except the reaction mixture contained 5.02 μmoles of potassium phosphate (pH 8.0) and $4.7 \cdot 10^5$ counts/min $^{32}P_i$. Additions were made as indicated and the reaction was allowed to proceed for 5 min.

Additions	*Total $^{32}P_i$ uptake (counts/min)*	*Total ATP formed (μmole)*	*Total O_2 uptake (μatom)*	*P/O*
None	415	0.004	0.00	—
+ NADH (1 μmole)	54 300	0.58	0.32	1.81
+ NADH + antimycin A (10 μg)	26 214	0.28	0.24	1.16
+ NADH + HOQNO (10 μg)	20 597	0.20	0.22	0.90
+ NADH + rotenone (250 μM)	5 617	0.06	0.10	0.60
+ NADH + pyruvate (2 μmoles) + lactate dehydrogenase (50 μg)	514	0.005	0.00	0.00



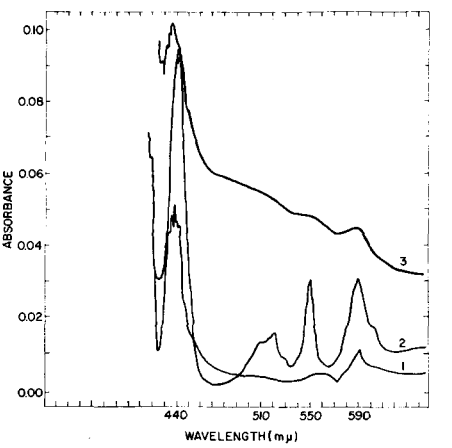


Fig. 4. Effect of antimycin A on the redox state of Nitrobacter cytochromes in the presence of NADH. Trace (1) shows steady-state reduced *minus* oxidized absorption spectra of P-150000 × *g* treated with 10 mM NADH; Trace (2) represents absorption spectra upon the addition of 10 μg antimycin A. Experimental conditions were similar as described in Fig. 1.

Fig. 5. Effect of antimycin A and rotenone on the redox state of respiratory carriers in the presence of NADH. Trace (1) shows difference absorption spectra of Nitrobacter P-150000 × *g* preincubated with 10 μg antimycin A for 2 min followed by the addition of 10 mM NADH. Trace (2) shows the change in absorption spectra after 1 h and Trace (3) was obtained after the addition of 250 μM rotenone. For experimental conditions, see legend to Fig. 1.

Oxidative phosphorylation coupled to the oxidation of NADH

The data in Table III show that the oxidation of 1 NADH molecule yields approx. 2 ATP. It may also be seen that this phosphorylation is sensitive to rotenone as well as to antimycin A or 2-*n*-heptyl-4-hydroxyquinoline *N*-oxide (HOQNO) implicating the participation of the flavoprotein systems and possibly a cytochrome

of *b* type in addition to cytochrome *c* and the cytochrome oxidase (EC 1.9.3.1) components (a_1 and a_3). There was no NADH oxidation or any coupled phosphorylation in the presence of an NADH trapping system (pyruvate–lactate dehydrogenase); as already mentioned this trapping system did not affect the phosphorylation coupled to the oxidation of nitrite. The inhibition of phosphorylation of about 50 and 70 % in the presence of HOQNO and rotenone, respectively, indicates that all the conventional sites of energy conservation were operative when NADH acted as the electron donor. It may be mentioned that in the aged or uncoupled enzyme preparations the phosphorylation coupled to NADH oxidation was normally very low; these preparations were also not very sensitive to antimycin A or HOQNO.

Addition of NADH to the phosphorylating particles resulted in the reduction of the flavoproteins, and cytochromes of *b*, *c*, *a*, and a_3 type (Fig. 4). A subsequent addition of 5 μg of antimycin A resulted in the oxidation of cytochrome *c* and cytochrome oxidase components, indicating a crossover point between cytochrome *b* and *c*. However, when the enzyme preparation was preincubated with antimycin A followed by the addition of NADH, the difference absorption spectra did not reveal the reduction of cytochrome *c* and a_1 components until after about 1 h (Fig. 5). Upon addition of 100 μM rotenone, all the cytochrome components were oxidized and the absorption spectra did not change upon a further incubation period of 1 h. It is of interest to note that rotenone caused the oxidation of the flavoprotein systems as well (Fig. 5). The Nitrobacter electron transport chain thus appears to be analogous to the mitochondrial respiratory chain reported by CHANCE *et al.*[14].

$$\text{NADH} \longrightarrow \underset{\text{Site I}}{\underset{\text{Rotenone}}{F_{P_{D_1}}}} \longrightarrow \underset{\text{Site II}}{\underset{\text{Antimycin A}}{\underset{\text{HOQNO}}{F_{P_{D_2}}}}} \longrightarrow (\text{Cyt. } b, \text{ Q}) \longrightarrow \text{Cyt. } c \longrightarrow \underset{\text{Site III}}{\underset{CN^-}{\text{Cyt. } a_1, a_3}} \longrightarrow O_2$$

Our unpublished observations have indicated the presence of ubiquinones in Nitrobacter preparations but wether or not the quinones are associated with the electron transport and coupled energy conservation is subject to further work.

DISCUSSION

The results reported in this paper have clearly demonstrated that the cytochrome electron transport particles obtained from *N. agilis* yield 1 mole of ATP concomitant to the oxidation of 1 mole of nitrite. The high efficiency of energy conservation by the Nitrobacter particles was found to be due to two important factors: (1) the cultural conditions of the organism and (2) the procedures involved in the disruption of intact cells. Actively growing Nitrobacter cultures must oxidize three additions of 20 mM nitrite within a period of 24 h for at least 2 days. When the growing cultures do not meet these specifications the P/O ratios are normally very low. Likewise cell disruption by prolonged sonication periods of 30–45 min results in the destruction of structural integrity of the phosphorylating particles although the electron transport ability is not markedly affected. We have circumvented the denaturation of the phosphorylating particles by disrupting cell suspensions in isotonic sucrose–EDTA–GSH solutions for not more than 3 min maintaining a temperature of 5–8°.

Efficiency of phosphorylation by the nitrite oxidase particles

Assuming $\Delta F'$ for nitrite oxidation = −17.8 kcal (ref. 8) and $\Delta F'$ for ATP hydrolysis = −7 kcal/mole, the efficiency of energy conservation by the isolated Nitrobacter particles is about 40 % and is comparable to the efficiency of the mammalian mitochondria. If, however, we take into account of the standard free energy change as a result of electron transport from the site of nitrite entry, *e.g.* cytochrome a_1, to molecular O_2 ($\Delta F'$ = −14.6 kcal), the efficiency of the cytochrome oxidase system in Nitrobacter is about 48 %. This high efficiency of chemosynthetic energy conservation is in striking contrast to the low free energy efficiency of the organism. It is conceivable, however, that during cell disintegration and subsequent fractionation the link between the energy generating and energy consuming components can be easily broken. Investigations to elucidate such phenomena are in progress. The low P/O ratios of 0.1–0.2 observed by FISHER AND LAUDELOUT[15] in *Nitrobacter Winogradskyi* have been explained on the basis of its low molar growth yield. However, these observations might be due to the variation in the strain of the organism in addition to the cultural and cell disintegration procedures used by these investigators.

The failure of the electron transport particles to reduce cytochrome *c* with nitrite in the presence of dibromophenol without appreciably affecting the reduction of cytochrome $a_1 + a_3$ indicates that the observed reduction of cytochrome *c* by nitrite in the absence of an uncoupling agent is an energy-dependent phenomenon. This was further supported by our observations that no reduction of the electron carriers was observed in the presence of added cyanide; however, the reduction of cytochrome *c* and cytochrome oxidase components ($a_1 + a_3$) by electron donors, such as ascorbate, capable of entering at the cytochrome *c* level, was not affected in the presence of cyanide. Thus the entry of nitrite in the electron transport chain of Nitrobacter may be visualized to take place at the level of cytochrome a_1. The complete inhibition of nitrite-dependent electron transport by cyanide supports our previous observations* that cyanide-inactivated nitrite oxidase could be reactivated by Fe^{2+} which is likely to be involved in the electron transport and coupled energy generation. In view of our recent work[2,5], the electron transport system of Nitrobacter reported previously[9] has been modified (Fig. 6).

Of particular interest is the ability of the nitrite oxidase particles to involve the complete electron transport sequence with NADH, the oxidation of which is mediated by flavoproteins, and cytochromes of *b*, *c*, a_1 and a_3-like components. This oxidation is sensitive to rotenone, antimycin A, HOQNO and is completely blocked by 0.5 mM concentration of cyanide. The electron transport from NADH to molecular oxygen is coupled to ATP formation and thus the chemoautotroph Nitrobacter does catalyze the process of oxidative phosphorylation analogous to the mitochondrial systems. Thus far there is no information in the present literature that such a process occurs in other chemosynthetic bacteria.

Attempts to demonstrate phosphorylation coupled to the oxidation of NADH by the chemoautotroph, *Thiobacillus neapolitanus* have so far been unsuccessful; and one of the reasons for autotrophy was based on the inability of chemosynthetic bacteria to catalyze oxidative phosphorylation with NADH as the electron donor[16]. According to recent investigations by SMITH, LONDON AND STANIER[17] the obligately chemoautotrophic thiobacilli and certain of the blue green algae lack α-ketoglutarate

* M. I. H. ALEEM AND A. NASON, unpublished experiments.

dehydrogenase (EC 1.1.99.4) and NADH oxidase. If Nitrobacter is an obligate chemoautotroph, then the occurrence of oxidative phosphorylation with NADH in this system appears to be a unique phenomenon. However, SMITH AND HOARE[18] have been able to demonstrate in Nitrobacter cell-free extracts the presence of all the enzymes necessary for the activation and complete oxidation of acetate *via* the tricarboxylic acid cycle. These workers have also been able to grow Nitrobacter heterotrophically in the complete absence of nitrite. Moreover, the DNA base ratios in the autotrophically and heterotrophically grown Nitrobacter cells were identical. SMITH AND HOARE have therefore concluded that *N. agilis* is not an obligate autotroph but rather a facultative autotroph.

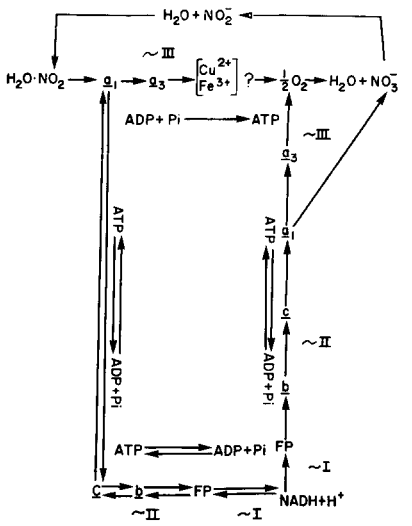


Fig. 6. Electron transport and coupled energy conversion pathways in *N. agilis*. According to this scheme the nitrite molecule is hydrated prior to electron removal. The subsequent electron transport is mediated by cytochromes $a_1 + a_3$ for the reduction of molecular O_2 to water; and nitrite is oxidized by the oxygen atom from water. The electron transport from nitrite to O_2 is coupled to the conservation of energy in the terminal site denoted as ~ III which is the precursor of ATP. NAD^+ is reduced by reverse electron flow from cytochrome a_1; this process is driven by ATP and is mediated by cytochromes *b*, *c*, and flavoprotein systems. The electron transport from NADH to molecular O_2 involves complete electron transport chain. This process is also coupled with the energy conservation steps indicated by ~ I, ~ II and ~ III. Nitrate can also accept electrons from NADH and this pathway of electron transport is also mediated by the same carriers upto cytochrome a_1 (ref. 10) and the process is coupled to energy generation[11].

In view of the presence of a potent NADH oxidase in Nitrobacter an important question arises as to how the reducing power is conserved in this sytem when the organism grows autotrophically. It is our feeling that since the nitrite oxidase is localized in the cell membranes and the NADH-consuming enzymes catalyzing CO_2 reduction reside in the soluble cytoplasmic fractions[1], there appears to be a compartmentation between the oxidative and reductive processes. Thus it would appear that nitrite oxidation consuming all the available oxygen in the vicinity of cellular membranes could render the cytoplasmic compartment virtually anaerobic where the reducing power may be visualized to be conserved and eventually used to drive the carbon reduction cycle.

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