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Inhibition of nitrate reduction by light and oxygen in *Rhodobacter sphaeroides* forma sp. *denitrificans*

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Abstract. Light inhibited each step of the denitrification process in whole cells of *Rhodobacter sphaeroides* forma sp. *denitrificans*. This inhibition by light was prevented in the presence of exogenous electron donors like N,N,N′,N′-tetramethyl-*p*-phenylenediamine (TMPD) plus ascorbate or in the presence of an uncoupler (carbonyl cyanide *m*-chlorophenylhydrazone). Addition of myxothiazol restored the inhibition by light in uncoupled cells. Measurements of light-induced absorbance changes under these conditions showed that this inhibition is due, for the steps of reduction of nitrite to dinitrogen, to the photooxidation of cytochromes c_1 plus c_2 and not due to the photoinduced membrane potential. Moreover, the presence of oxygen inhibited almost all of the reduction of nitrate and nitrous oxide but only 70% of the reduction of nitrite to nitrous oxide. These inhibitions were overcome in the presence of TMPD plus ascorbate. This implies that the inhibition in presence of oxygen was due to a diversion of the reducing power from the denitrifying chain to the respiratory chain. It was concluded from this series of experiments that the reduction of nitrate to nitrite is inhibited when the ubiquinone pool is partly oxidized and that nitrite and nitrous oxide reductions are inhibited when cytochromes c_1 plus c_2 are oxidized by photosynthesis or respiration.

Key words: Photosynthetic bacteria – *Rhodobacter sphaeroides* – Denitrification – Nitrate reductase – Nitrite reductase – Nitrous oxide reductase – Light inhibition – Oxygen inhibition

Denitrification is an anaerobic bacterial respiration in which nitrate (NO_3^-) is reduced to dinitrogen (N_2), via the intermediates nitrite (NO_2^-), nitric oxide (NO) and nitrous oxide (N_2O). Although many of the purple non-sulphur photosynthetic bacteria can assimilate nitrate, only few species (*Rhodobacter sphaeroides* forma sp. *denitrificans* and two strains of *Rhodopseudomonas palustris*) are able to grow with denitrification as the energy conserving process in addition to photosynthesis or oxygen respiration (Satoh et al. 1976; Klemme et al. 1980). Among these species, the most studied is *R. sphaeroides* forma sp. *denitrificans*. Another well characterized species is *R. capsulatus* N22DNAR$^+$, a mutant isolated from strain N22, which reduces nitrate and nitrous oxide under dark and anaerobic conditions (McEwan et al. 1982). However, *R. capsulatus* N22DNAR$^+$ is unable to perform complete denitrification since growth under anaerobic conditions in the dark at the expense of nitrate reduction is not observed.

The nitrate reductase in photosynthetic bacteria is a periplasmic watersoluble enzyme (Sawada and Satoh 1980; McEwan et al. 1984a), in contrast to the membrane-bound nitrate reductase found in most of the non photosynthetic denitrifiers (see however Bell et al. 1990).

The nitrite and nitrous oxide reductases are also periplasmic. They have been purified and partially characterized in a number of species (Sawada et al. 1978; Michalski et al. 1986).

In *R. sphaeroides* forma sp. *denitrificans*, a nitric oxide reductase has been isolated from chromatophores, tightly associated with the cytochrome bc_1 complex, (Itoh et al. 1989a). Recently, Bell et al. (1992) have reported a nitric oxide activity for several strains of *R. capsulatus*. Several reports in the literature have described the nature of the electron donors required for the successive steps of the reduction of nitrate to dinitrogen. In both *R. sphaeroides* forma sp. *denitrificans* and *R. capsulatus* N22DNAR$^+$, the first step, reduction of nitrate to nitrite, induces the oxidation of a *b*-type cytochrome different from that of the bc_1 complex (Bannai and Satoh 1982; Yokota et al. 1984; Richardson et al. 1990). The reduction of nitrite to nitrous oxide probably with nitric

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Abbreviations: R, Rhodobacter; TMPD, N,N,N′,N′-tetramethyl-*p*-phenylenediamine; HOQNO, 2-*n*-heptyl-4-hydroxyquinoline N-oxide; CCCP, carbonyl cyanide m-chlorophenylhydrazone; cytochrome c_t, cytochrome c_2 plus cytochrome c_1

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oxide as intermediate (Urata and Satoh 1985; Goretski and Hollocher 1988) requires the participation of both the cytochrome bc_1 complex and the soluble cytochrome c_2, in the case of *R. sphaeroides* forma sp. *denitrificans* (Urata and Satoh 1985). However, it has been recently suggested that nitric oxide could be reduced by an alternative pathway in *R. capsulatus* (Bell et al. 1992).

The nature of the electron donor for the reduction of nitrous oxide to dinitrogen is still unconfirmed. Based on the observation that myxothiazol inhibits this process in *R. sphaeroides* forma sp. *denitrificans*, Itoh et al. (1989b) have proposed that the cytochrome bc_1 complex and cytochrome c_2 are involved in this last step. Richardson et al. (1989, 1991) proposed a scheme where the nitrous oxide is reduced either by the cytochrome bc_1 complex or an alternative ubiquinol-cyt c_2 oxidoreductase, sensitive to 2-*n*-heptyl-4-hydroxyquinoline *N*-oxide (HOQNO) and not to myxothiazol, via cytochrome c_2 in both cases.

Interactions between the three different energetic processes, photosynthesis, respiration and denitrification, have been documented to some extent.

In *R. sphaeroides* forma sp. *denitrificans* or *R. capsulatus* N22DNAR$^+$, it has been shown that nitrate reduction was inhibited by oxygen (McEwan et al. 1982; McEwan et al. 1984b) but no report can be found for the nitrite or nitrous oxide reduction of these species. Since their nitrate reductase is periplasmic, any control on the transport of nitrate across the membrane as proposed for *Paracoccus denitrificans* (Alefounder et al. 1983) is excluded. In addition, McEwan et al. (1984b) have shown that the inhibition by oxygen was not a consequence of a thermodynamic control on the nitrate reduction chain exerted by the proton motive force related to the respiratory activity.

Light strongly inhibits both respiratory activity (Nakamura 1937; Van Niel 1941) and nitrate reduction (Satoh 1977; McEwan et al. 1982). This inhibition of respiration and nitrate reduction has been explained as the result of thermodynamic control by the light-induced proton motive force on the proton-translocating complexes involved in the electron flow (i.e. dehydrogenases and cytochrome bc_1 complex) (Cotton et al. 1983; McEwan et al. 1984b). In addition, competition between redox components associated with both the respiratory and photosynthetic chains (i.e. ubiquinone, cytochrome bc_1 complex and cytochrome c_2) results in the inhibition of respiratory activity by light (Richaud et al. 1986; Lavorel et al. 1989; see also Rugolo and Zannoni 1983). The participation of ubiquinone, cytochrome bc_1 complex and cytochrome c_2 in the different steps of denitrification led us to suppose similarly, that changes in their oxidation stated during illumination or respiration may also contribute to the light or oxygen induced inhibition of this process. Recently and during completion of this work, Richardson et al. (1991) concluded that cytochrome c_2 photooxidation indeed plays an important role in the light-induced inhibition of nitrous oxide reduction.

In the present article, we have determined which steps of the denitrification process are inhibited by the photosynthetic activity or the respiration process. We have also shown that changes in the redox states of cytochrome c_i and the ubiquinone pool during photosynthesis or respiration, are responsible for the inhibition of the denitrification process. A preliminary account of this work has been presented elsewhere (Sabaty et al. 1991).

Materials and methods

Rhodobacter sphaeroides forma sp. *denitrificans*, generously provided by Prof. T. Satoh (Hiroshima University), was grown in the light (25 μmol photons · $m^{-2} \cdot s^{-1}$) in degassed Hutner medium supplemented with 20 mM KNO_3. All experiments were carried out on cultures grown for 40 h.

The bacterial suspension was placed into a thermostated (25 °C) reaction vessel (1.5 ml). A teflon membrane at the bottom of the reaction vessel allowed the dissolved gases to be directly introduced into a mass spectrometer equiped with three collectors (V.G Instruments MM 14-80). The apparatus has been described previously by Peltier and Thibault (1985). The evolution of nitrous oxide, dinitrogen and oxygen in the bacterial suspension was then followed under dark or continuous illumination. Light was supplied by a projector equipped with a 150 W halogen lamp (300 μmol photons · $m^{-2} \cdot s^{-1}$).

Nitrate and nitrite reduction were directly measured in the presence of atmospheric dinitrogen using $K^{15}NO_3$ (C.E.A., 97.4% ^{15}N) or $Na^{15}NO_2$ (C.E.A., 99%) and following $^{15}N_2$ at m/e = 30. When acetylene, an inhibitor of nitrous oxide reductase was added in these conditions, $^{15}N_2O$ production was followed at m/e = 46. The last step of denitrification has been measured either by the uptake of N_2O or by the production of N_2. As $^{15}N_2O$ was not commercially available, measurements have been made using $^{14}N_2O$. In this case, the cell suspension was carefully purged with helium before starting the experiment to eliminate any atmospheric dinitrogen. $^{14}N_2O$ gives a mass peak in the spectrometer at m/e = 44 like $^{12}CO_2$. To avoid artefacts due to variations in CO_2 concentration, $^{14}N_2O$ concentration has been measured at m/e = 30 (mass peak due to an ionic species formed from N_2O in the ionization chamber). Nitrous oxide was added to the reaction vessel from a stock solution prepared by passing nitrous oxide through 2 ml H_2O contained in a airtight bottle for 5 min. The calculated concentration of dissolved nitrous oxide was taken to be 23 mM at 25 °C. The same procedure was performed for acetylene for which the concentration was taken to be 38 mM at 25 °C.

Aerobic respiration was followed by the uptake of O_2 at m/e = 32.

Spectrophotometric measurements were performed with an apparatus similar to that described by Joliot et al. (1980) and improved according to Joliot and Joliot (1984). Actinic excitation was provided with a 12 Volts Quartz halogen lamp filtered through a Kodak Wratten 89B film (240 μmol photons · $m^{-2} \cdot s^{-1}$).

CCCP, myxothiazol, antimycin A, TMPD and ascorbate were purchased from Sigma, St. Louis, Mo., USA.

Results

Mechanism of the inhibition by light of the different steps of denitrification

Figure 1 illustrates the mass spectrometric measurements we have performed to follow the different steps of the denitrification activity of whole cells of *Rhodobacter sphaeroides* forma sp. *denitrificans*. In part A, the

evolution of the concentrations of O_2 and $^{15}N_2$ are plotted as functions of time in the presence of 2 mM of $^{15}NO_3^-$. This experiment clearly confirms the necessity of strict anaerobiosis for denitrification activity since $^{15}N_2$ production only occurs when all the O_2 has been consumed in the cell suspension. Inhibition by continuous illumination of both the respiration and the denitrification processes are also observed in agreement with previous results (Satoh 1977). The percentage of these inhibitions by light varies from 50 to 100% depending on the batch of bacteria used (compare for example Fig. 1A and 2A for the inhibition of nitrate reduction). This variation may reflect differences in the level of reducing power accumulated by the cells. Although the reduction of nitrite to nitrous oxide probably occurs with nitric oxide as an intermediate (Urata and Satoh 1985; Bell et al. 1992), there is no report for nitric oxide production in whole cells of photosynthetic bacteria. Therefore, only the global reduction of nitrite to nitrous oxide is generally considered. In Fig. 1B, we show that the reduction of nitrite to nitrous oxide, measured in the presence of acetylene to inhibit the last step of denitrification, is also inhibited by light. As already reported (McEwan et al. 1985; Itoh et al. 1989b) continuous illumination inhibits dinitrogen production from nitrous oxide (Fig. 1C). This series of experiments demonstrates that the different steps of the denitrification are inhibited by light in *R. sphaeroides* forma sp. *denitrificans* whole cells. In particular, this is the first report for the inhibition of the overall reduction of nitrite to nitrous oxide by light (Fig. 1B).

To specify the influence of the proton motive force and the redox state of different electron carriers on the processes of inhibition by light, we have performed a series of experiments in the presence of uncouplers and/or inhibitors. The reduction of nitrogen compounds and the light-induced absorbance changes have been followed respectively by mass spectrometry and absorption spectroscopy. The results are shown in Figs. 2 and 3 for nitrate reduction. As already stated, continuous illumination induces an inhibition of the nitrate reduction in a control sample (Fig. 2A). Oxidation of cytochrome c_t and a large membrane potential are observed in the light minus dark difference spectrum (Fig. 3A). On the other hand, in the presence of ascorbate plus TMPD, dinitrogen production is stimulated and continuous illumination only slightly inhibits this activity (Fig. 2B). Under these conditions a membrane potential, identical with that control sample one, is observed with no accumulation of photooxidized cytochromes (Fig. 3B). Several minutes after addition of 20 µM CCCP, the reduction rate of nitrate is decreased (Fig. 2C) with respect to the control experiment. An extreme case is presented in Fig. 2C. This phenomenon has also been observed for respiratory activity (Cotton et al. 1983) and is presumably due to a decrease in the substrate level. Only a small inhibition of nitrate reduction by light is observed in the presence of CCCP (Fig. 2C). No

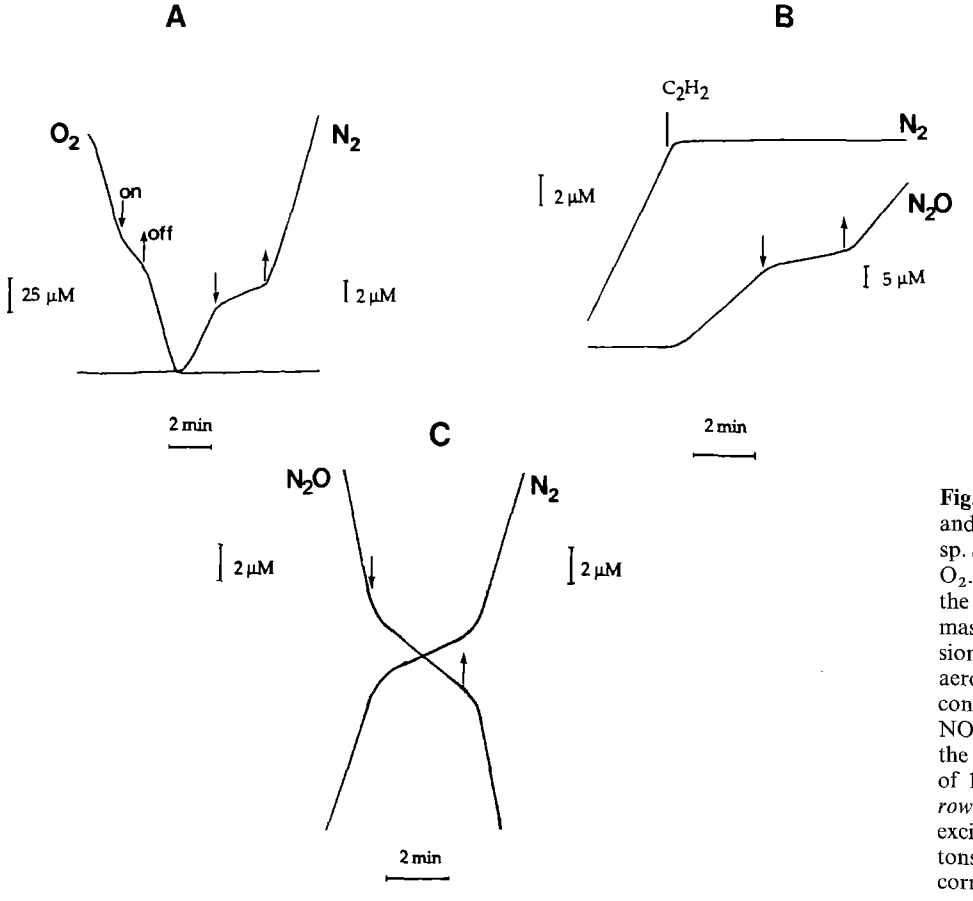

Fig. 1A–C. Reduction of nitrate, nitrite and nitrous oxide by *R. sphaeroides* forma sp. *denitrificans* and inhibition by light and O_2. The evolution of gases dissolved in the bacterial suspension is followed on a mass spectrometer. The bacterial suspension contains **(A)** 2 mM KNO_3 under aerobic conditions, **(B)** and **(C)** anaerobic conditions in the presence of 2 mM $NaNO_2$ and 0.8 mM N_2O respectively. In **(B)** the N_2O reduction is inhibited by addition of 1.3 mM acetylene. The *downwards arrows* indicate the switching on of the exciting continuous light (300 µmol photons · m^{-2} · s^{-1}) while the *upwards arrows* correspond to its cessation

cytochrome c_t oxidation and only a very small membrane potential are photoinduced under this condition (Fig. 3C). On the other hand, subsequent addition of 20 μM myxothiazol in the presence of CCCP, completely restores the inhibition of dinitrogen production by light

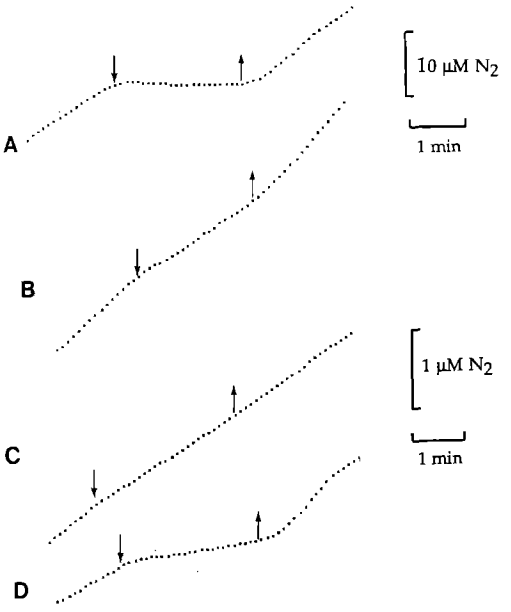

Fig. 2A–D. Effect of light on the nitrate reduction by *R. sphaeroides* forma sp. *denitrificans* in the presence of uncoupler and exogenous electron donors. N_2 production by the bacterial suspension (absorbance at 850 nm of 3.5) in the presence of 2 mM KNO_3 is measured in dark and continuous light, as indicated by *arrows*. **A** No addition, **B** 0.1 mM TMPD + 1 mM ascorbate, **C** 20 μM CCCP, **D** 20 μM CCCP + 20 μM myxothiazol. The *downwards arrows* indicate the switching on of the exciting continuous light (300 μmol photons · m^{-2} · s^{-1}) while the *upwards arrows* correspond to its cessation

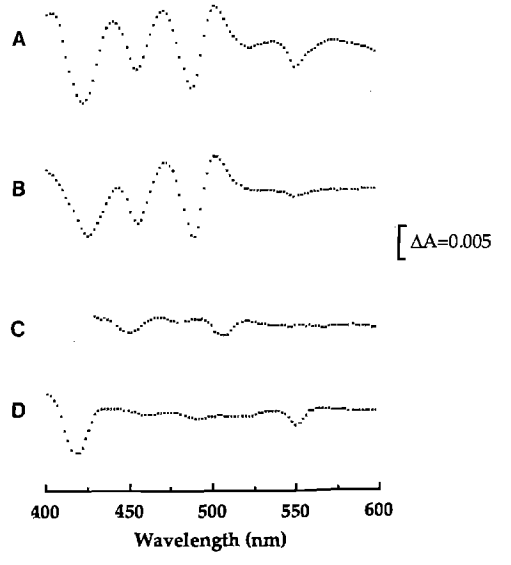

Fig. 3A–D. Light minus dark absorption spectra of whole cells of *R. sphaeroides* forma sp. *denitrificans*. Conditions similar to those of Fig. 2: **(A)** no addition, **(B)** 0.1 mM TMPD + 1 mM ascorbate, **(C)** 20 μM CCCP, **(D)** 20 μM CCCP + 20 μM myxothiazol. In the sample cuvette, bacteria (A_{850} = 1.1) are illuminated with an actinic continuous light (240 μmol photons · m^{-2} · s^{-1})

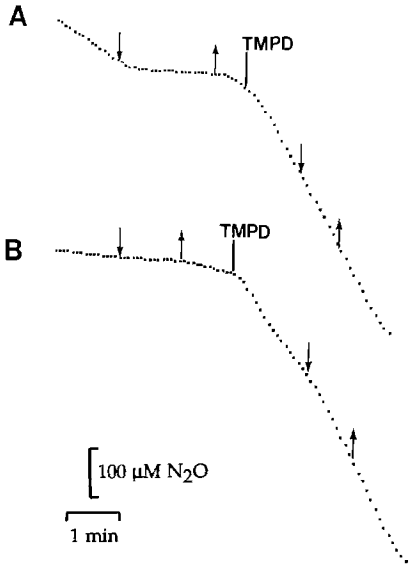

Fig. 4. Overcoming light inhibition of N_2O reduction of *R. sphaeroides* forma sp. *denitrificans* by TMPD, in the presence of myxothiazol and antimycin A. N_2O uptake measured on a bacterial suspension (A_{850} = 3) in the presence of 0.8 mM N_2O: **(A)** control, **(B)** in the presence of 20 μM myxothiazol and 20 μM antimycin A. Addition of 0.1 mM TMPD + 1 mM ascorbate is indicated by TMPD. The *downwards arrows* indicate the switching on of the exciting continuous light (300 μmol photons · m^{-2} · s^{-1}) while the *upwards arrows* correspond to its cessation

(Fig. 2D) in agreement with the results of Richardson et al. (1991). Under the same experimental conditions, cytochrome c_t photooxidation can be observed in the light minus dark difference spectrum (Fig. 3D). This is due to inhibition of the cyclic electron transport by myxothiazol.

Similar results have been obtained for the reduction of nitrous oxide to dinitrogen and nitrite to nitrous oxide. For both processes, inhibition by light is not observed after addition of CCCP but restored in the presence of both CCCP and myxothiazol (data not shown). In the presence of TMPD plus ascorbate, no inhibition by light can be observed for each of these two steps of the denitrification process, even after addition of both myxothiazol and antimycin A. This is illustrated in Fig. 4 for the reduction of nitrous oxide to dinitrogen. This result is in disagreement with the experiment of Richardson et al. (1991) who reported an inhibition by light for *R. capsulatus* N22DNAR$^+$ whole cells, in the presence of TMPD plus ascorbate plus myxothiazol. We have no explanation for this discrepancy. This series of experiments shows that the cytochrome bc_1 complex and the cytochrome c_2 which is reduced by TMPD, are efficient electron donors for the enzymes of the different steps of the reduction of nitrite to dinitrogen.

Moreover, in all the situations where cytochrome c_t are photooxidized (Fig. 3A and D), light inhibits the dinitrogen production (Fig. 2A and D). On the other hand, when no photooxidation of cytochrome c_t are observed, even in the presence of a large membrane potential (Fig. 3B), inhibition of the dinitrogen production by light does not occur (Fig. 2B).

Denitrification in the presence of oxygen

The importance of the oxidation state of cytochrome c_t in the denitrification activity that was demonstrated above suggests that the inhibition observed in the presence of oxygen (Fig. 1A) may be linked to a similar effect. In a preliminary series of experiments, we have determined which steps of denitrification are inhibited by the presence of oxygen. As for the overall reduction of nitrate (Fig. 1A and 5A), the reduction of nitrous oxide to dinitrogen is inhibited (more than 90%) in the presence of oxygen (Fig. 5C). On the other hand, reduction of nitrite to nitrous oxide, measured in the presence of acetylene, is only partially inhibited (70%) by oxygen (Fig. 5E). This difference between the levels of inhibition by oxygen, for the reduction of nitrous oxide to dinitrogen and the reduction of nitrite to nitrous oxide, results in the production of nitrous oxide in whole cells supplied with nitrite even in the absence of acetylene (Fig. 5G). When all of the oxygen has been consumed, the reduction of nitrous oxide is reactivated and concomitant nitrous oxide uptake and dinitrogen production are observed (Fig. 5G). On the other hand, in a similar experiment but with nitrate as the electron acceptor, no nitrous oxide production is observed (Fig. 5A). We conclude from this observation that the first step of denitrification is completely inhibited by oxygen in agreement with the results obtained by McEwan et al. (1982) with *R. capsulatus* N22DNAR$^+$ cells. If this was not the case, production of nitrous oxide should have been observed when nitrate was added to the bacterial suspension, as was the case after addition of nitrite.

To test if the inhibition of some steps of denitrification by oxygen was due to the partial oxidation of cytochrome c_t induced by the respiratory activity or a direct effect of oxygen, we have tried to specifically inhibit the cytochrome oxidase. Under these conditions one expects to maintain all the cytochrome c_t in the reduced state even in the presence of oxygen. We have found, however, that nitrous oxide reductase activity is sensitive to potassium cyanide in a lower concentration range than the one needed to inhibit the cytochrome *c* oxidase (data not shown). Another way to maintain cytochromes in the reduced form in the presence of oxygen is to supply

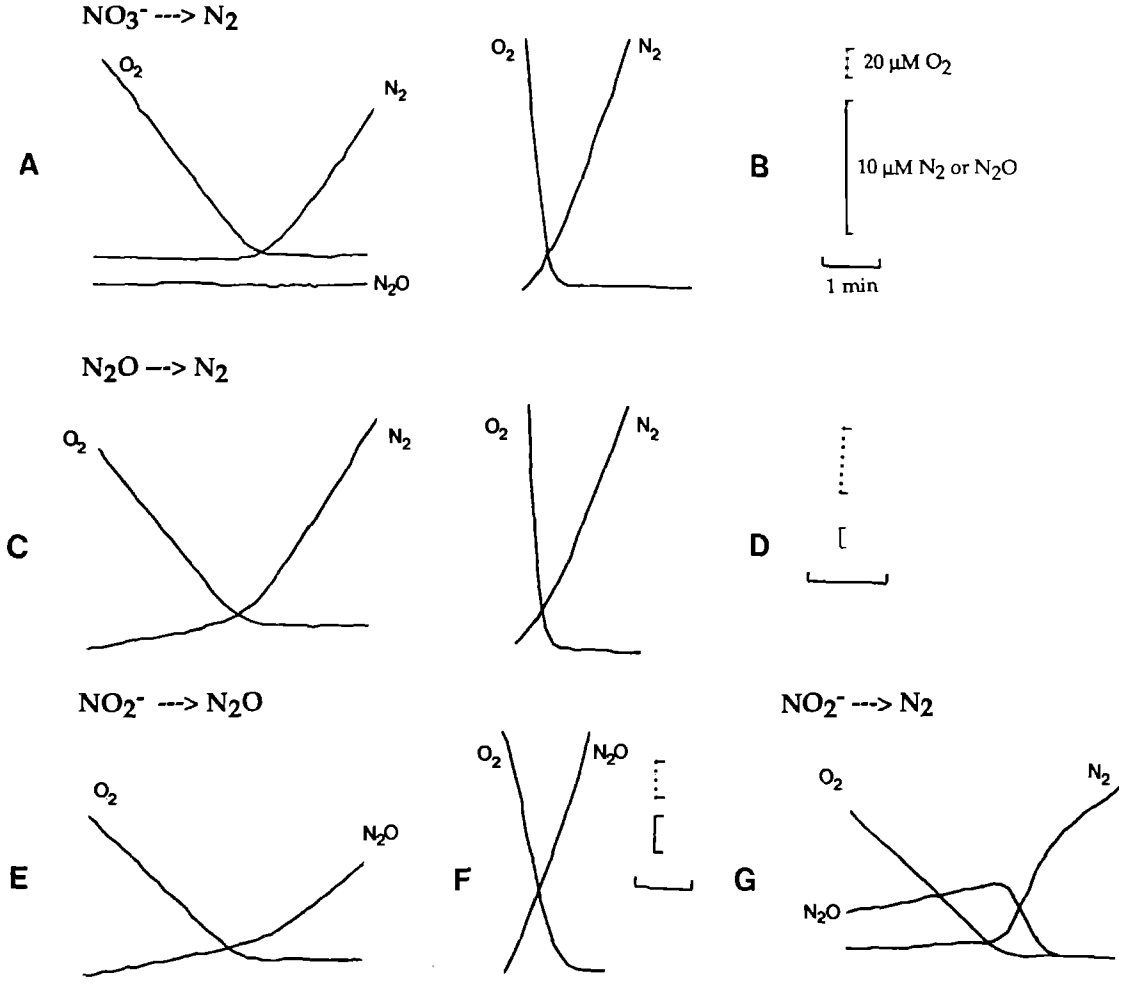

Fig. 5A–G. Effect of oxygen on nitrate, nitrite and nitrous oxide reduction by *R. sphaeroides* forma sp. *denitrificans*. N_2O and N_2 productions and O_2 uptake are measured on a bacterial suspension (A_{850} = 3.5) in the presence of 2 mM KNO_3 (**A**), 0.8 mM N_2O (**C**) or 2 mM $NaNO_2$ (**E** and **G**) under aerobic conditions. **B**, **D** and **F** same as **A**, **C** and **E** respectively but in the presence of 2 mM TMPD and 20 mM ascorbate. In **E** and **F** N_2O reduction is inhibited by addition of 1.3 mM of acetylene. *Dashed* and *full vertical bars* represent 20 µM O_2 and 10 µM N_2 or N_2O, respectively. The *horizontal bar* is equivalent to 1 min

the cells with an excess of electron donors like the couple TMPD plus ascorbate. The minimal concentration of TMPD has been determined spectrophotometrically and found to be 2 mM. In the presence of 2 mM TMPD and 20 mM ascorbate, nitrate, nitrite and nitrous oxide are readily reduced even in the presence of oxygen as shown in Fig. 5B, D, F. Anaerobiosis induces a slight stimulation of the reduction processes. These experiments imply either that none of the enzymes involved in the overall nitrate reduction pathways are sensitive to oxygen or that the denitrification enzymes are protected from the effects of oxygen by a mechanism similar to the one suggested for nitrogenase in *Azotobacter* species (Drozd and Postgate 1970). The first explanation is in line with the results of Richardson et al. (1991) on the nitrous oxide reductase of *R. capsulatus* MTG4/S4 which have shown that the enzyme is not labile to oxygen in periplasmic fractions. These results are also comparable with those reported recently for whole cells of *Thiosphaera pantophora*, an organism able to reduce nitrate under aerobic conditions (Bell et al. 1990; Bell and Ferguson 1991).

Discussion

The observations presented here establish that the steps of the reduction of nitrate to dinitrogen are inhibited by continuous illumination in whole cells of *R. sphaeroides* forma sp. *denitrificans*. This inhibition can be induced simply by the photooxidation of cytochrome c_t and does not require the generation of a photoinduced proton motive force. This is clearly shown using combination of uncoupler, electron transport inhibitors and exogenous electron donors. This conclusion is in agreement with the fact that the cytochrome bc_1 complex and cytochrome c_2 are the electron donors to these reductive steps of denitrification (Urata and Satoh 1984; Itoh et al. 1989b) and with the periplasmic nature of the nitrite and nitrous oxide reductases. These results also confirm the proposal of Richardson et al. (1991), for the mechanism of inhibition of the nitrous oxide reduction by light.

The competition for reducing power at the level of cytochrome c_2 between photosynthetic electron transport chain and denitrification cannot however be invoked for the inhibition of the first step of denitrification, the reduction of nitrate to nitrite. This can be concluded because cytochrome c_2 is not an electron donor to the nitrate reductase (Bannai and Satoh 1982; Richardson et al. 1990). The inhibition by light of the first reduction step in the denitrification process could be explained by a thermodynamic control of the photoinduced membrane potential on the NADH deshydrogenase (McEwan et al. 1984b). However, the reduction of nitrate to nitrite is not inhibited by light in the presence of TMPD plus ascorbate since the complete denitrification process is observed (Fig. 2B). One possible explanation is that in these conditions, light induces a large reduction of the ubiquinone pool at the expense of the artificial electron donors even if the dehydrogenases are largely inhibited by the photoinduced membrane potential. These photoreduced quinones serve as electron donors for the nitrate reduction. Moreover the lack of light inhibition, observed in presence of TMPD plus ascorbate implies that electron transfer between the ubiquinone pool, the cytochrome *b* involved in the nitrate reduction, and the nitrate reductase is not influenced by the photo-induced membrane potential.

We have established that oxygen induces the inhibition of the first and last steps of denitrification. The reduction of nitrite to nitrous oxide is only inhibited by 70% in the presence of oxygen. The complete or partial inhibitions are overcome by addition of exogenous electron donors at concentrations high enough to saturate respiratory activity and keep the cytochrome c_t fully reduced. This behaviour implies that the inhibition by oxygen of the reactions of the denitrification process is due to a diversion of the reducing power from the denitrifying chain to the respiratory chain. In the case of the nitrite and nitrous oxide reductions, this diversion occurs at the level of cytochrome c_2 and cytochrome bc_1 complex. This type of mechanism is in argreement with our proposal stated above for the process of inhibition by light. The complete inhibition of nitrate reduction to nitrite by oxygen (McEwan et al. 1982 and this work) can also be explained by a diversion of electrons from the denitrification chain to the respiratory chain but at the level of the ubiquinone pool. The respiratory activity is limited by the entry of electrons to the ubiquinone pool since addition of TMPD plus ascorbate stimulates the rate of oxygen uptake by a factor of 7 (Fig. 5). This result and the observation of oscillations in the flash-induced modulation of respiratory activity (Richaud et al. 1986) imply that the ubiquinone pool is largely oxidized in the presence of oxygen (see also Zannoni and Moore 1990). The oxidation of the ubiquinone pool induces the inhibition of the reduction of nitrate to nitrite. On the other hand, in the presence of TMPD plus ascorbate, the ubiquinone pool is expected to be fully reduced by the dehydrogenases because respiratory activity is only working at the expense of these exogenous electron donors under these conditions. Reduction of nitrate to nitrite occurs only when the quinone pool is largely reduced (Fig. 5B). Zannoni and Moore (1990) have already observed a marked non-linear relationship between the redox state of the quinone pool and the activity of the alternative oxidase.

In conclusion, we propose a model where the nitrate reductase is only active when the ubiquinone pool is largely reduced. The nitrite reductase and the nitrous oxide reductase require a significant reduction of cytochrome c_t. Inhibition of denitrification in presence of oxygen is therefore due to the oxidation of the ubiquinone pool and cytochrome c_t by respiratory activity. Similarly, the oxidation of cytochrome c_t during photosynthesis is responsible for the inhibition of the reduction of nitrite to nitrous oxide and nitrous oxide to dinitrogen.

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