Light Dependent Ammonium Inhibition of Nitrate Assimilation in *Rhodopseudomonas capsulata* AD2

Kassem Alef

Lehrstuhl für Mikrobiologie der Universität D-8580 Bayreuth, Bundesrepublik Deutschland

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Nitrate assimilation by *Rhodopseudomonas capsulata* AD2 was completely inhibited by ammonium only in young well illuminated cultures. At higher densities (A_{660} about 0.5) the addition of ammonium had no inhibitory effect, but the nitrate was only reduced to the level of nitrite, which appeared in the medium. Under both conditions the cellular level of nitrate reductase activity remained unaffected. In marked contrast to other *R. capsulata* strains both ammonium sensitive and insensitive cells could reduce nitrate in the light and in the darkness. In the light up to 90% of the reduced nitrate was assimilated, but in the dark the reduced nitrate was stoichiometrically excreted as nitrite. This behaviour was only shown by *R. capsulata* AD2 and BK5, while in other strains the nitrate assimilation was always completely inhibited by ammonium.

The role of photosynthesis and respiration by the regulation of nitrate reduction is discussed.

Introduction

Ammonium, the end product of assimilatory nitrate reduction, behaves as co-repressor of nitrate assimilation in practically all types of organisms.

The ability of the Rhodospirillaceae to assimilate nitrate is restricted to some strains of *Rhodopseudomonas capsulata* and *Rhodopseudomonas sphaeroides* [1, 2]. Some strains of *R. sphaeroides* and *Rhodopseudomonas palustris* are capable of growth by nitrate dissimilation [3, 4]. This process of nitrate reduction and its regulation have been poorly studied in the Rhodospirillaceae and the few data available are often incomplete and contradictory. In one strain *R. sphaeroides* DSM 158 for example, nitrate was stoichiometrically transformed into nitrite, although the cells were unable to grow with nitrate as their sole of nitrogen source or electron acceptor [5]. *R. capsulata* E_1F_1 can grow with nitrate as N-source but in ammonium containing medium, added nitrate was not reduced aerobically or anaerobically in the dark nor in the light. This strain contained a nitrate reductase, which was not repressed by ammonium ions and governed by the partial pressure of oxygen in the gas phase [6, 7]. Jackson *et al.* [8] described the assimilatory uptake of nitrate by *R. capsulata* N22, which was light-dependent and reversibly inhibited by ammonium. In marked contrast to *R. capsulata* N22, the nitrate reductase of the mutant *R. capsulata* N22DNR$^+$ was active in the dark, produced nitrite stoichiometrically from nitrate and was strongly inhibited by light, but not by ammonium [9].

The present work reports a new type of regulation of nitrate assimilation in *R. capsulata* AD2 and shows that the relative roles of photosynthesis and respiration in controling energy production is the central factor controling nitrate reduction in this strain.

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Methods

Rhodopseudomonas capsulata AD2, BK5 and DSM155 were grown photoheterotrophically in the medium of Alef *et al.* [10], using nitrate, thiamine as nitrogen and vitamin sources. Illumination if not otherwise stated was 100 µ Einstein $m^{-2}s^{-1}$. The cultures were grown in 250 ml screw-cap Roux bottles (18 × 6.5 × 3.5 cm), filled completely with the medium or under argon atmosphere. Nitrate reductase assays in whole cells (toluene treated) and with cell free extracts were carried out as described by Alef and Klemme [11]. Nitrite was measured according to [12], nitrate with salicylic acid according to [13], ammonium was determined by the method of Fawcett and Scott [14].

Results

Effect of ammonium on the assimilatory nitrate reduction

Fig. 1 A shows that the addition of ammonium to a culture grown in the light with nitrate (A_{660} of

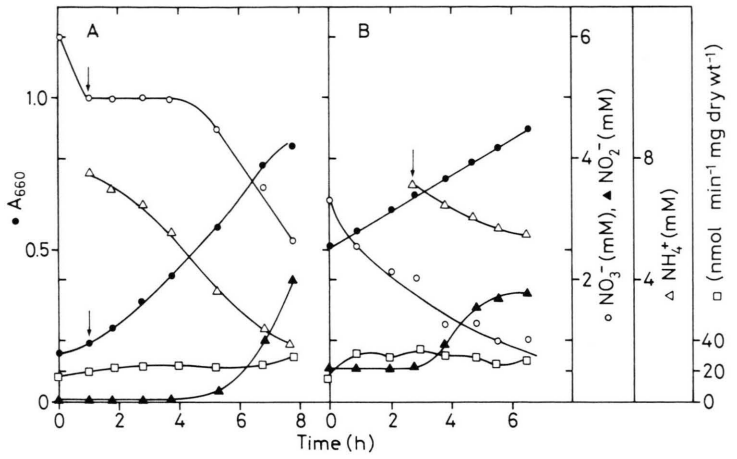

Fig. 1. The effect of ammonium on nitrate assimilation in *Rhodopseudomonas capsulata* AD2. The cells were grown photoheterotrophically under illumination of 136 μ Einstein $m^{-2} s^{-1}$. A) At A_{660} of 0.195 (arrow) ammonium was added. B) Ammonium was added at A_{660} of 0.67. (□) Nitrate reductase activity was measured in whole cells.

0.195) of *Rhodopseudomonas capsulata* AD2 inhibits the nitrate assimilation. This inhibition, however, lasts only to a density of A_{660} about 0.5 (illumination of 136 μ Einstein $m^{-2} s^{-1}$) then nitrate reduction occurs again, but only to nitrite. Fig. 1 B shows that the addition of ammonium to culture with A_{660} of about 0.6 under the same illumination, had no inhibitory effect, but again the reduced nitrate appeared stoichiometrically as nitrite in the medium. Moreover, Fig. 1 A, B shows that this phenomenon was completely independent of the cellular level of nitrate reductase activity in the cells, since the enzyme was insensitive to ammonium. Our attempts to follow the nitrite-reductase activity in this strain were unsuccessful.

Similar results were also obtained, when the closest structural analogue of ammonium, methylammonium was used. This ion inhibits the nitrate assimilation and also the growth of young (A_{660} of 0.205) well illuminated cultures (136 μ Einstein $m^{-2} s^{-1}$) with nitrate as the only N-source, but when ammonium was added the cells grew again, and at A_{660} of about 0.6 they started to reduce nitrate stoichiometrically to nitrite. Similar experiments were carried out, where instead of adding ammonium, the light was switched off (Fig. 2). As expected no growth was observed but the cells reduced nitrate stoichiometrically to nitrite with an initial rate of 20 nmol min^{-1} mg dry wt^{-1}. When the light was switched on again, slow growth due to nitrite assimilation was observed. Under all conditions the cellular level of nitrate reductase activity (30 nmol min^{-1} mg dry wt^{-1}) remained unaffected. This dependence of the inhibition on the density strongly suggests a role of the light in this process and that at high densities, self-shading reduced the light availability. Other experiments also emphasize the role of the light intensity in the regulation of this process. Fig. 3A shows that *R. capsulata* AD2 cells grown in malate and ammonium nitrate under insufficient illumination (45 μ Einstein $m^{-2} s^{-1}$) started to reduce nitrate and excrete nitrite at low density (A_{660} of 0.08), while at higher illumination (136 μ Einstein $m^{-2} s^{-1}$) nitrate reduction started at A_{660} of about 0.5 (Fig. 3B). Similar results were also obtained in *R. capsulata* BK 5. In *R. capsulata* DSM 155, however, nitrate assimilation was completely blocked by ammonium. This inhibition was

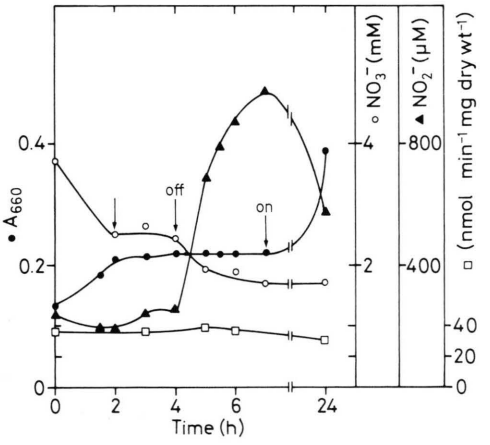

Fig. 2. Reduction of nitrate by *R. capsulata* AD2 in the dark and inhibition by methylammonium. The cells were grown with NO_3^- as in Fig. 1. At A_{660} of 0.205, methylammonium (6 mM) was added (first arrow). The light was switched off and on where shown. (□) Nitrate reductase activity was measured in whole cells.

independent of the light intensity and the growth phase of the culture.

Fig. 3C shows the prevention by ammonium of the assimilatory nitrate reduction under phototrophic conditions in *R. capsulata* AD2 at low densities. When the cells entered the stationary phase, ammonium, nitrate and nitrite were completely assimilated. The assimilation of nitrite started 2 h after the depletion of ammonium from the medium. These results demonstrate the assimilatory character of the nitrite reductase in this strain. Furthermore when *R. capsulata* AD2 was grown photoheterotrophically with nitrate up to A_{660} of 0.7 and nitrate reduction became ammonium insensitive, then diluted to A_{660} of 0.2, further growth without a lag phase was observed, but the nitrate assimilation became ammonium sensitive. This indicates the presence of the assimilatory nitrate reductase in the cells of cultures with high densities. Jackson *et al.* [8] found that fresh cell suspension of the nitrate assimilating strain *R. capsulata* N22 did not take up nitrate in the dark, but in the light. This uptake was inhibited by ammonium. In contrast to this strain *R. capsulata* AD2 cultures with low or high cell densities reduced nitrate in the dark (Fig. 4). The initial rate of nitrate reduction was 18

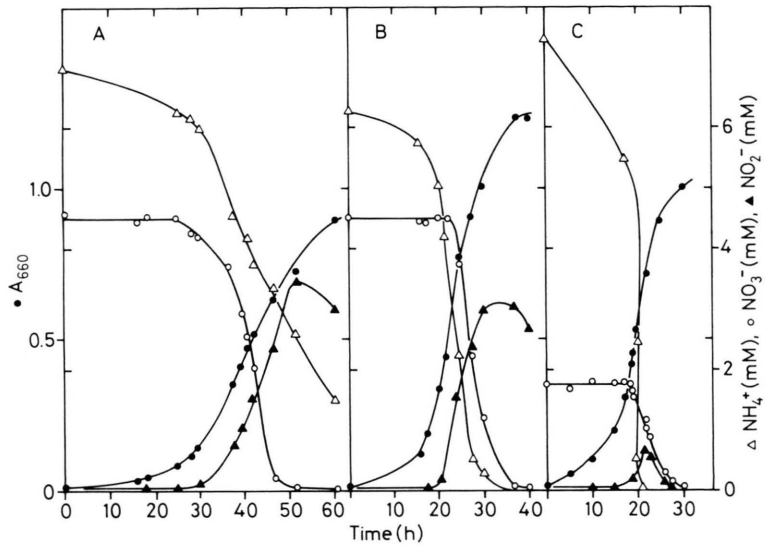

Fig. 3. Nitrate assimilation in *R. capsulata* AD2 in the presence of ammonium. A) The cells were grown photoheterotrophically under illumination of 45 μ Einstein $m^{-2} s^{-1}$. B) Cultures were grown under illumination of 136 μ Einstein $m^{-2} s^{-1}$. C) The illumination was 100 μ Einstein $m^{-2} s^{-1}$.

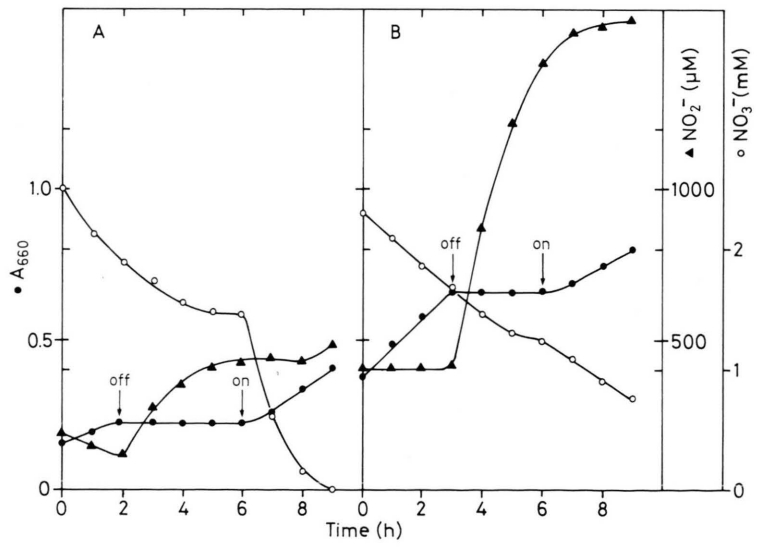

Fig. 4. The effect of light and darkness on nitrate assimilation by *R. capsulata* AD2 culture with low or high cell densities. The cells were grown with NO_3^- as N-source. The light was switched off and on where shown at A_{660} of 0.23 (A) or A_{660} 0.64 (B).

and 21 nmol min^{-1} mg dry wt^{-1} respectively. In both cases nitrite was produced stoichiometrically with nitrate disappearance, and showed a hyperbolic time course. Nevertheless *R. capsulata* AD2 and BK5 were not able to grow with nitrate as electron acceptor. Both cultures with low or high cell densities were able also to reduce nitrate in the light (Fig. 4), but in contrast to the dark reduction only 5–10% of the reduced nitrate appeared as nitrite in the medium. In order to show that nitrate reductase investigated was the same enzyme under all conditions, some of its properties were compared with respect to the effect of different electron donors and some inhibitors on this activity. The results (data not shown) indicate that the enzyme in all crude extracts was active with reduced methylviologen, reduced flavins and NADH. The NADPH-dependent activity was hardly detectable. NAD(P)H-dependent activities were more stimulated by FMN than FAD.

Azide (1 mM), cyanide (1 mM), chlorate (10 mM) and perchlorate (10 mM) as possible inhibitors of the nitrate reductase had practically no inhibitory effect on the reduced methylviologen-dependent activities. All these results provided strong evidence, that *R. capsulata* AD2 possesses only one type of nitrate reductase.

Discussion

The data indicate that regulation of nitrate assimilation by *Rhodopseudomonas capsulata* AD2 and BK5 differs from that in other Rhodospirillaceae [2, 7–9]. In *R. capsulata* AD2 and BK5 ammonium caused a complete blockade of nitrate assimilation under anaerobic growth conditions only, when the cultures were well illuminated. This inhibition decreased, when the cultures were grown under insufficient illumination. I conclude that at low cell densities photosynthesis is the sole energy source whereas at high cell densities there will be an additional kind of energy generation (a kind of nitrate respiration) due to self-shading effects. Since the cultures are grown anaerobically they will require a suitable electron sink to accept electrons produced from malate assimilation. Under these conditions (in the presence of ammonium) nitrate functions as a suitable electron acceptor and there is a stoichiometric excretion of nitrite into the growth medium. This explanation would account for the fact that at low cell densities nitrate reduction is ammonium sensitive whereas at high cells densities there is no inhibition. Further evidence to support this view is provided by the experiments when the light was switched off. Under these conditions nitrate was reduced and excreted as nitrite in the growth medium. Nevertheless in the absence of ammonium *R. capsulata* AD2 cultures with both low and high cell densities were able anaerobically to reduce nitrate in darkness and in the light. Our experiments (Fig. 3C) show the assimilatory character of the nitrite reductase. Furthermore the nitrate reductase obtained under all conditions had similar properties. This reinforces our working hypothesis that *R. capsulata* AD2 possesses only an assimilatory nitrate reductase. Its activity (determined *in vitro*) is insensitive to ammonium and possesses a kind of respiratory function anaerobically under insufficient illumination.

Nitrate reduction should be considered in connection with other reactions of nitrate metabolism. But our attempts to follow the nitrite reductase activity in *R. capsulata* AD2 were unsuccessful. So the excretion of nitrite under insufficient illumination or in the presence of ammonium could be due to the inhibition of nitrite reductase, since nitrite was assimilated only in the light and about 2 h after the complete depletion of ammonium.

Since the inhibitory effect of ammonium and methylammonium was light dependent, ammonium may act also on a putative nitrate transport system. The effect of methylammonium supports this view, because no metabolism of methylammonium in the cells could be observed [2, 8, 15, 16].

Further studies are needed to provide insights into the mechanism, whether nitrate under insufficient illumination functions as electron acceptor for electron transport driven phosphorylation, since *R. capsulata* AD2 and BK5 were not able to grow with nitrate as electron acceptor. Contrary to the situation described in other Rhodospirillaceae we wish to emphasize that this is the first demonstration of an assimilatory nitrate reductase in these bacteria with respiratory function.

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