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Nitrate reduction and assimilation by a moderately halophilic, halotolerant bacterium Ba_1

Ayala Hochman, Aliza Nissany and Michal Amizur

Department of Biochemistry, George S. Wise Faculty of Life Sciences, Tel-Aviv University, Ramat-Aviv, Tel-Aviv (Israel)

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The moderately halophilic, halotolerant bacterium Ba_1 can utilize either nitrate or ammonium as a nitrogen source, but cannot use nitrate as an electron acceptor for anaerobic respiration. When both nitrate and ammonium are present in the growth medium, nitrate is taken up only after all the ammonium has been exhausted. Nitrate reductase of this bacterium is associated with the respiratory electron transport chain on the cytoplasmic membrane, and is able to accept electrons from NADH, succinate and malate, but not from NADPH. Chlorate is a competitive inhibitor of the enzyme which is also inhibited by antimycin A, cyanide, azide and *p*-chloromercuribenzoate. Cellular activities of the enzyme are not affected by the type of nitrogen source used by the cells. Nitrite reductase is a soluble enzyme, its preferred physiological substrate is ferredoxin, and its synthesis is repressed by ammonium and induced by nitrate. Glutamine synthetase is repressed by ammonium, whereas glutamate dehydrogenase is induced by it; activity levels of glutamate synthase are not affected by the nitrogen source in the growth medium. Glutamine synthetase activity is inhibited by amino acids and by magnesium ions. The ratio of activity $+Mg^{2+}/-Mg^{2+}$ is 0.074 for nitrate-grown cells, and 0.42 for cells grown with ammonium alone or plus nitrate.

Introduction

When nitrate is used as a nitrogen source, it is first reduced to ammonia in two enzymatic steps: nitrate reductase reduces it to nitrite, which is further reduced to ammonia by nitrite reductase. This nitrate assimilatory pathway has been demonstrated in a wide range of organisms, including bacteria, fungi, algae and higher plants [1,2]. In several aerobic bacteria nitrate can also serve as terminal electron acceptor, in an anaerobic respiration [3]. In this dissimilatory pathway, nitrate is also first reduced to nitrite by the enzyme nitrate reductase. Nitrite is frequently the end product of this process, but it may also be reduced to nitric oxide, nitrous oxide or molecular nitrogen [3,4], and in some cases to ammonia [5,6]. It is generally accepted that the dissimilatory nitrate reductase is associated with the respiratory electron transport chain on the cytoplasmic membrane, while the assimilatory enzymes were shown to be either particulate or soluble.

Bacterial regulation of assimilatory nitrate reduction appears to be similar to that of the eukaryotic system. Ammonia was shown to cause its repression, either partial or complete, and sometimes nitrate was required for the induction of the system [7–10]. More is known about the regulation of the dissimilatory nitrate reductase, which was shown to be an inducible enzyme [4], whose synthesis and function occur in cells in-

Correspondence: A. Hochman, Department of Biochemistry, George S. Wise Faculty of Life Sciences, Tel-Aviv University, Tel-Aviv 69978, Israel.

cubated anaerobically or at low oxygen tension. Neither synthesis nor function of the dissimilatory system is affected by nitrogenous compounds, and oxygen has no effect on the assimilatory enzymes.

Ammonia, whether generated intracellularly from nitrate, or taken up from the medium, is incorporated into a carbon skeleton in one of two pathways [11]. It can be assimilated either by the enzyme glutamate dehydrogenase, or by the combined action of glutamine synthetase and glutamate synthase. These two pathways were demonstrated in various bacterial genera (e.g., Refs. 12–16). Ammonia assimilation in bacteria has been studied more intensively than nitrate reduction. When ammonia is available in excess it is usually assimilated by glutamate dehydrogenase [11], while glutamine synthetase and glutamate synthase are operative usually when the intracellular concentration of ammonia is relatively low. The cellular activity of glutamine synthetase was shown to be regulated at four different levels: repression/derepression of its synthesis, modulation of its activity by divalent cations and by other effectors, and covalent modification by adenylylation/deadenylylation [17]. The adenylylation/deadenylylation control of the enzyme seems to be a general phenomenon of Gram-negative bacteria [17,18], but it was demonstrated also in Gram-positive bacteria [19].

There are only scant reports in the literature on the characterization of bacterial assimilatory nitrate reductase (e.g., Refs. 20–22), and there are no published data about nitrate and ammonia assimilation of bacteria capable of growing in high salt concentrations. We have studied the inorganic nitrogen metabolism of a halotolerant, moderately halophilic bacterium, Ba_1. This is a Gram-negative rod, which was isolated from crude salt samples collected at the Dead Sea evaporation ponds [23]. It is not fully characterized yet, but several aspects of its metabolism and enzymatic activities have been investigated (e.g., Refs. 23–28). We describe in this study the enzymatic activities involved in nitrate assimilation in this bacterium: its reduction to ammonia via nitrate reductase and nitrite reductase, and the ammonia-assimilation enzymes. We show that the nitrate reductase which functions in the assimilatory pathway resembles in some aspects the dissimilatory enzyme: it is associated with the respiratory electron transport chain on the cytoplasmic membrane, uses NADH as an electron donor, and is inhibited by chlorate. Furthermore, the synthesis of the enzyme is not affected by nitrate or ammonium. The ammonium-assimilating system is similar to that of other bacteria, and includes the enzymes glutamate dehydrogenase, glutamine synthetase, and glutamate synthase.

Materials and Methods

Methods

Ba_1 was the gift of Professor Y. Avidor, Department of Biology, Technion, Israel. The cells were grown on a minimal medium, containing (g/l): Na_2SO_4, 2.0; K_2HPO_4, 3.0; KH_2PO_4, 1.0; NaCl, 87.5 (1.5 M); KCl, 37.2 (0.5 M); $MgCl_2$, 3 (0.05 M); glucose, 3.09; Na_2MoO_4, 10^{-3}; $CaCl_2$, 10^{-4}; and KNO_3, 0.53 (10 mM) or NH_4Cl, 1.0 (10 mM), or both, as indicated, as nitrogen source. Bacteria were grown at 37 °C in 400 ml batch cultures, in 2 liter Erlenmeyer flasks on a rotary shaker at 300 rpm. Growth was followed by monitoring the absorbance at 660 nm in a Uvicon 810 spectrophotometer. Cells were harvested, at the end of the logarithmic phase, by centrifugation in the cold at $10000 \times g$ for 15 min. The supernatant was decanted and used for measurement of NO_3^- and NH_4^+ in the experiments on substrate utilization.

For enzymatic assays, the cells were resuspended in 20 mM K_2HPO_4, pH 7.5, containing deoxyribonuclease and ruptured by ultrasonic treatment (8×15 s) at 4 °C. The homogenate was centrifuged at $12000 \times g$ for 15 min to remove cell debris and unbroken cells. The supernatant was centrifuged again at $144000 \times g$ for 60 min, and the supernatant from this second centrifugation was designated SupII. The pellet, referred to as membrane preparation, was washed twice with 20 mM phosphate buffer, pH 7.5, and resuspended by homogenization in the same buffer, to a final protein concentration of 25 mg/ml. The membrane preparation could be stored at −20 °C for several weeks without significant loss of activity.

Nitrate reductase activity was measured by following the rate of nitrite formation according to Lorimer et al. [29], in a reaction mixture contain-

ing 30 mM K_2HPO_4, pH 7.5, 10 mM KNO_3, 0.5 mM NADH and enzyme preparation containing 1–2 mg protein, in a total volume of 1 ml. The assay was run for 10 min at 30 °C. Aliquots were taken at different times from the reaction mixture for NO_2^- determination to ensure that the rate was constant. Nitrite reductase activity was determined by following the disappearance of nitrite using dithionite-reduced methyl viologen, as electron donor, according to the method of Losada and Paneque [30]. Glutamine synthetase was assayed by the γ-glutamyltransferase method in whole cells pretreated with cetyltrimethylammonium bromide according to Bender et al. [31]. Glutamate synthase was assayed spectrophotometrically at 30 °C by following the rate of oxidation of NADH or NADPH at 340 nm in a reaction mixture containing 50 mM Tris-HCl, pH 7.6, 5 mM 2-oxoglutarate, 5 mM glutamine, and 0.2 mM NADH or NADPH. Glutamate dehydrogenase activity was measured at 30 °C by monitoring the rate of reduction of NAD^+ or $NADP^+$ at 340 nm in a reaction mixture containing 100 mM Tris-HCl, pH 7.6, 20 mM glutamate, and 0.2 mM NAD^+ or $NADP^+$.

Nitrite was determined by the diazo coupling method according to Nicholas and Nason [32]. Nitrate was estimated by its reduction to nitrite on a cadmium-copper column according to Strickland and Parson [33]. Ammonia was assayed after microdiffusion [34] by the phenol-hypochlorite method according to Chaykin [35]. For protein determination the membrane preparation was boiled for 20 min in 1 M NaOH and centrifuged. Protein was determined in this supernatant and in SupII according to Lowry et al. [36], with bovine serum albumin as standard.

Materials

NADH, NADPH, NAD^+, $NADP^+$, methyl viologen, benzyl viologen and deoxyribonuclease were purchased from Sigma. All other chemicals were of analytical grade except for the NaCl and KCl which were only of the 'pure' grade when used for growth of large quantities of cells.

Results

Ba_1 cells grew aerobically with either NH_4^+ or NO_3^- as the sole nitrogen source, and had the same generation time of 2.8 h with either of the substrates (Fig. 1), or with both in the growth medium. There was no growth under anaerobic conditions in the presence of nitrate and glucose, which means that the cells can neither ferment glucose nor use NO_3^- as a terminal electron acceptor in anaerobic respiration. When nitrate or ammonia was utilized as the sole nitrogen source, their rates of utilization were similar. However, ammonia was used preferentially by the cells, as can be seen in Fig. 1: when both NH_4^+ and NO_3^- were present in the growth medium, NO_3^- was not utilized untill all the NH_4^+ was exhausted. Ba_1 cells had all five enzymes that are generally accepted to participate in the assimilation of inorganic nitrogen: nitrate reductase, nitrite reductase, glutamate dehydrogenase, glutamine synthetase and glutamate synthase (Table I). Nitrate reductase was membrane-bound, while all the other enzymes were found in the soluble fraction of the cells.

TABLE I

INTRACELLULAR LOCALIZATION OF ENZYMES METABOLIZING INORGANIC NITROGEN IN Ba_1

Cells were grown with 20 mM KNO_3 as the nitrogen source. Glutamate dehydrogenase was assayed with NAD^+ and glutamate synthase with NADPH as the electron donors. n.d., not detected.

Enzyme	Specific activities	
	membrane preparation	supernatant (SupII)
Nitrate reductase (nmol NO_2^- · (mg protein)$^{-1}$ · min^{-1})	121	n.d.
Nitrite reductase (nmol NO_2^- · (mg protein)$^{-1}$ · min^{-1})	n.d.	427.0
Glutamate dehydrogenase (μmol NADH · (mg protein)$^{-1}$ · min^{-1})	n.d.	22.3
Glutamine synthetase (nmol hydroxamate · (mg protein)$^{-1}$ · min^{-1})	n.d.	290.0
Glutamate synthase (μmol NADPH · (mg protein)$^{-1}$ · min^{-1})	n.d.	11.8

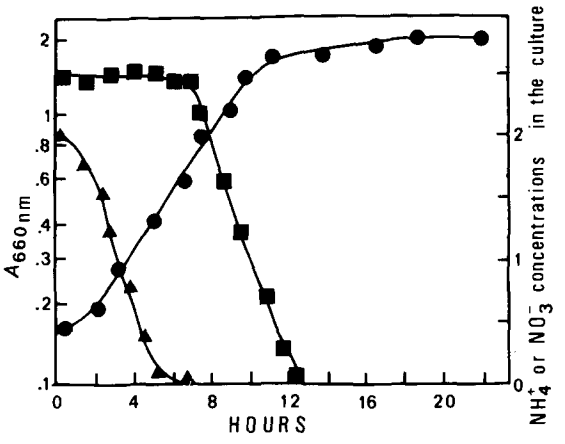

Fig. 1. Time course of growth and ammonia and nitrate utilization by Ba_1. Cells which were precultured on 10 mM KNO_3 were transferred to a medium containing 2 mM NH_4Cl and 2.5 mM KNO_3. ●, $A_{660\,nm}$; ▲, NH_4^+ concentration; and ■, NO_3^- concentration in thr growth medium.

Nitrate reductase

Nitrate reductase activity was associated with the cytoplasmic membrane where the oxidases of NADH, succinate and malate were also localized (not shown). NADH was the best electron donor for nitrate reductase (Table II) while NADPH was a very poor substrate. Nitrate reductases from various sources are known to be stimulated by flavins [30]; in the present case, however, addition of either FAD or FMN did not enhance the activity with either of the nucleotides. With succinate the activity was 53% relative to NADH, but with malate it was less than 10%. Dithionite could also serve as a reductant in the nitrate reductase assay, though with less efficiency, but neither reduced viologen dyes nor flavins enhanced the enzyme rate. The K_m of nitrate reductase for nitrate, with NADH as electron donor, was 66 μM. The pH optimum of the enzyme was 7.3–7.5.

TABLE II

ELECTRON DONORS AND COFACTORS FOR NITRATE REDUCTASE FROM MEMBRANE PREPARATIONS OF Ba_1 CELLS

Electron donor and cofactor	Nitrate reductase activity (nmol NO_2^- formed $\cdot min^{-1} \cdot (mg\ protein)^{-1}$)
None	0
NADH 0.5 mM	121.1
NADH 0.5 mM + FAD (FMN) 2 mM	118.6
NADPH 0.5 mM	5.2
NADPH 0.5 mM + FAD (FMN) 2 mM	5.0
Dithionite 5 mM	17.1
Dithionite 5 mM + methyl viologen 2 mM	20.0
Dithionite 5 mM + benzyl viologen 2 mM	19.5
Dithionine 5 mM + FAD (FMN) 2 mM	18.1
Succinate 20 mM	64.3
Malate 20 mM	10.7

Nitrate reductases from various sources, both of the assimilatory and dissimilatory types, were shown to contain molybdenum in their active site [3,4]. The enzyme from Ba_1 was also dependent on molybdenum, as was shown by tungstate antagonism. A comparison of the effects of tungstate and molybdate on ammonia- and nitrate-grown cells is presented in Fig. 2. Addition of 3 mM tungstate to the medium, instead of molybdate (Fig. 2A), inhibited the growth of Ba_1 cells both on nitrate and on ammonia, probably because tungstate interfered with other molybdenum-containing enzymes. However, while the nitrate-grown culture was almost completely inhibited, the growth of cells on ammonia was only partially influenced. Furthermore, when only 0.75 mM tungstate was added to the medium (Fig. 2B), growth on ammonia was not affected, but growth on nitrate was significantly inhibited.

Nitrate reductase was competitively inhibited by chlorate with a K_i of 1.76 mM. The enzyme was strongly inhibited by *p*-chloromercuribenzoate and azide (Table III) and to a lesser extent by KSCN, KCNO and KCN. $1.5 \cdot 10^{-4}$ M concentrations of the electron transport inhibitor, antimycin A, inhibited 44% of nitrate reduction activity and 50% of NADH oxidase activity (not shown). Neither nitrite, which is the product of nitrate reductase, nor ammonia, which is the end product of the pathway, inhibited nitrate reductase activity. Exclusion of oxygen did not affect the activity (not shown), but extended the time during which the reaction was linear, probably because in the presence of oxygen NADH was consumed also by the NADH oxidase system

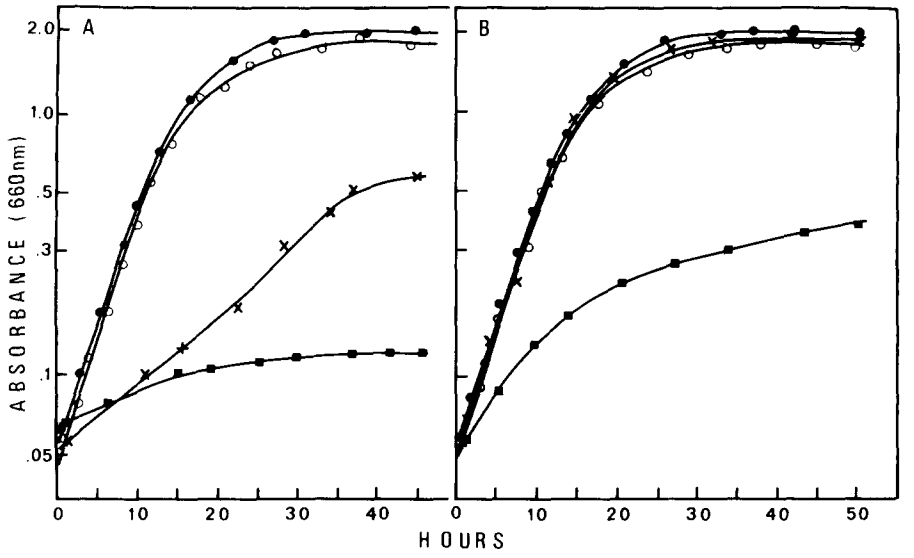

Fig. 2. Effect of tungstate on growth of Ba_1. Cells were grown on standard medium, except that when tungstate was added, molybdate was omitted. (A) Effect of 3 mM tungstate. Cells were grown in the presence of: ○, NH_4^+ and MoO_4^{2-}; ●, NO_3^- and MoO_4^{2-}; ×, NH_4^+ and WO_4^{2-}; ■, NO_3^- and WO_4^{2-}. (B) Effect of 0.75 mM tungstate. Cells were grown in the presence of ○, NH_4^+ and MoO_4^{2-}; ●, NO_3^- and MoO_4^{2-}; ×, NH_4^+ and WO_4^{2-}; ■, NO_3^- and WO_4^{2-}.

associated with the same membrane.

The cellular levels of nitrate reductase were not affected by the type of nitrogen source in the growth medium; specific activities of the enzyme, calculated on the basis of total cell protein, were similar for cells grown on a rich medium or with nitrate, ammonia or glutamate as the sole nitrogen sources.

TABLE III

EFFECT OF DIFFERENT INHIBITORS ON NITRATE REDUCTASE ACTIVITY OF MEMBRANE PREPARATIONS OF Ba_1 CELLS

Inhibitor	Concentration (M)	Nitrate reductase (% of control)
p-Chloromercuri-benzoate	$2.5 \cdot 10^{-5}$	48
KCNO	$1 \cdot 10^{-3}$	38
KSCN	$2 \cdot 10^{-4}$	41
KCN	$1 \cdot 10^{-3}$	41
NaN_3	$3 \cdot 10^{-5}$	43
Antimycin A	$1.5 \cdot 10^{-4}$	44
NH_4Cl	$2 \cdot 10^{-1}$	97
KNO_2	$2 \cdot 10^{-1}$	102

Nitrite reductase

The product of nitrite reductase was NH_4^+, as evidenced by the identical specific activities measured for nitrite utilization and for ammonia formation. Ba_1-ferredoxin, reduced by dithionite, was the best electron donor for nitrite reductase activity and showed a specific activity of 3904 nmol NO^{-2} reduced · (mg protein)$^{-1}$ · min^{-1}. Reduced methyl or benzyl viologens could also serve as electron donors but the rate was only 11% relative to ferredoxin. Activity was detected also with dithionite alone, though the rate was relatively slow. Neither FAD or FMN, nor NADH or NADPH could support NO_2^- reduction. The pH optimum of the enzyme was 7.9. Cellular levels of nitrite reductase were regulated by both nitrate and ammonia. Specific activities of the enzyme, calculated on the basis of total cell protein, were 428 nmol NO^{-2} reduced · (mg protein)$^{-1}$ · h^{-1} for nitrate-grown cells and 230 nmol NO_2^- reduced · (mg protein)$^{-1}$ · h^{-1} for cells grown with both nitrate and ammonia. Ammonia-grown cells had no detectable nitrite reductase activity.

Ammonia assimilation

Glutamine synthetase. As in other Gram-nega-

tive bacteria, magnesium ions inhibited the transferase activity of glutamine synthetase from Ba_1 (Table IV). The inhibition was more significant in NO_3^--grown cells, in which the ratio of activity $+Mg^{2+}/-Mg^{2+}$ was 0.074, while for cells grown on ammonia alone, or on ammonia plus nitrate, it was found to be 0.42–0.43. Glutamine synthetase activity was 10–15-times higher in nitrate-grown cells relative to cells grown with ammonia as the sole nitrogen source (Table IV). When both nitrate and ammonia were present in the growth medium, the activity was similar to that of cells grown on ammonia alone. As in other Gram-negative bacteria [17,18], glutamine synthetase activity of Ba_1 was subject to feedback inhibition by amino acids; 10 mM of alanine, serine, and glycine resulted in 46%, 58% and 29% inhibition, respectively. When all three amino acids were present in the reaction mixture, the inhibition was 69%.

Glutamate synthase. NADPH was a more efficient electron donor to glutamate synthase than NADH. The specific activities of the enzyme were 11.8 and 1.5 $\mu mol \cdot (mg\ protein)^{-1} \cdot min^{-1}$ for NADPH and NADH, respectively. No activity was detected with reduced Ba^1 – ferredoxin. Activity levels assayed with NADH or NADPH were similar in cells grown on either ammonia or nitrate as sole nitrogen sources, or on both.

Glutamate dehydrogenase. Glutamate dehydrogenase activity with NAD^+ as a substrate was 22.3 $\mu mol \cdot (mg\ protein)^{-1} \cdot min^{-1}$, 15–20-times higher than with $NADP^+$. The activity was 2.5–3-times higher for cells grown on ammonia, or on ammonia plus nitrate, than for cells grown on nitrate alone. This finding was similar for both NADH and NADPH as electron donor.

TABLE IV

EFFECT OF NITROGEN SOURCE IN THE GROWTH MEDIUM ON THE LEVELS OF GLUTAMINE SYNTHETASE AND ON THE DEGREE OF INHIBITION OF THE ACTIVITY BY MAGNESIUM IONS IN Ba_1 CELLS

Activity was assayed in whole cells treated with cetyltrimethylammonium bromide for permeabilization and preservation of the adenylylation state of the enzyme.

N-source	Glutamine synthetase activity (μmol hydroxamate · (mg protein)$^{-1} \cdot min^{-1}$)		Ratio: $-Mg^{2+}/+Mg^{2+}$
	$-Mg^{2+}$	$+Mg^{2+}$	
NO_3^-	190.0	14.0	0.074
NH_4^+	14.3	6.1	0.43
$NO_3^- + NH_4^+$	13.9	5.9	0.42

Discussion

Enzymes of the nitrate assimilation pathway in Ba_1 appear to be similar to those of other bacteria, except for the first step, catalyzed by nitrate reductase, which has more in common with the dissimilatory-type enzyme. Like dissimilatory nitrate reductase, the enzyme from Ba_1 was associated with the cytoplasmic membrane, which was also the site of the respiratory electron transport chain to oxygen. While reduced viologen dyes were poor electron donors, nitrate reductase could accept electrons from NADH, succinate and malate, which were also electron donors to the respiratory chain. Furthermore, antimycin A, which is known as a cytochrome *b* inhibitor [37], inhibited nitrate reductase and NADH oxidase activities to the same extent. These results suggest that nitrate reductase of Ba_1 accepts electrons from the respiratory electron transport chain, in a pathway which includes a *b*-type cytochrome, as in the denitrifiers [4]. Further studies of the electron transport chain of Ba_1 are necessary in order to understand why the electron transport to nitrate cannot fulfill the function of anaerobic respiration in these cells. In preliminary experiments we were unable to demonstrate synthesis of ATP coupled to electron transport from NADH to nitrate under anaerobic conditions. We propose, therefore, that this pathway cannot conserve enough energy to support cell growth. A particulate NADH-dependent nitrate reductase has been characterized in *Rhodopseudomonas capsulata* BK5 [38] and *Azotobacter vinelandii* [39]. Like the Ba_1 enzyme, the *R. capsulata* nitrate reductase was not stimulated by flavins and was inhibited by chlorate. However, in contrast to the Ba_1 nitrate reductase, the activity of the *A. vinelandii* enzyme was inhibited by oxygen and stimulated by added FAD and FMN. NADH-driven nitrate reductase was also found in *Acinetobacter calcoaceticum* [9] and *Derxia gummosa* [40], but unlike the Ba_1 enzyme it was found to be a soluble enzyme.

Assimilatory nitrate reduction in several

bacterial strains was shown to be regulated by the nitrogen source in the growth medium, via repression by ammonia and induction by nitrate, but the regulatory patterns of the various strains were somewhat different. In *Klebsiella aerogenes* the enzyme was repressed in cells grown on ammonia [41]. When *A. chroococcum* cells were grown in the presence of both nitrate and ammonia [7], they produced amounts of nitrate reductase intermediate to those present in cultures grown on either of the substrates. A similar situation occurred in the marine pseudomonad PL1, where nitrate reductase synthesis was repressed in cultures growing on excess of ammonia [8], and in *D. gummosa* where NH_4^+ inhibited induction of the enzyme [40]. Contrary to these reports, nitrate reductase of Ba_1 is not regulated by nitrate or ammonia. Nitrate utilization in Ba_1 was regulated at two different levels that will be discussed separately: (1) nitrate uptake, and (2) nitrite reduction to ammonia. (1) When cells that were grown on nitrate were transferred to a medium containing both nitrate and ammonia, only ammonia was taken up, and utilization of nitrate began only after the ammonia had been utilized. Since we have shown that cells had a fully active nitrate reductase under these conditions, it was concluded that the utilization of ammonia in preference to nitrate was due to failure of the cells to take up nitrate. That this is an inhibition, rather than repression, of the nitrate uptake system is concluded from the finding that nitrate uptake started without any lag, after ammonia was exhausted from the medium. Uptake of ammonia in preference to nitrate has been reported for plants, algae and fungi [42], while relatively few studies of this phenomenon in bacteria have been reported. In the marine pseudomonad PL1 [8] the uptake of nitrate was inhibited by ammonia concentrations higher than 1 mM. However, in contrast to Ba_1, both nitrate and ammonia were utilized simultaneously at ammonia concentrations below 1 mM. In *Pseudomonas fluorescens* [43], ammonia was found to regulate nitrate assimilation at two levels: it inhibited nitrate transport as in Ba_1 but, in contrast to Ba_1, it also repressed synthesis of this uptake system. (2) Nitrite reductase of Ba_1 is repressed by ammonia and induced by the presence of nitrate in the growth medium. This was shown by the finding that cells grown on ammonia show no activity of the enzyme, while when they are grown on both nitrate and ammonia, the cellular activity is about one half of that of nitrate-grown cells. It is not clear at this stage whether the inducer is nitrate itself, or its reduction product, nitrite. In *A. chroococcum* both nitrate and nitrite were shown to induce nitrite reductase, but ammonia did not repress its synthesis [22]. The enzyme of *R. capsulata* strain E1F1 [21] was found to be induced by both nitrate and nitrite, and it was suggested that it was actually the nitrite which promoted the synthesis.

Incorporation of nitrate into organic compounds in Ba_1 is regulated also at the level of ammonia assimilation at two points: repression of glutamine synthetase and induction of glutamate dehydrogenase by ammonia. The synthesis and activity of glutamine synthetase in Ba_1, like the enzyme from other sources [16,17,44–46], was regulated by various factors. The enzyme was repressed by ammonia, and its activity was inhibited by amino acids and Mg^{2+}. In various bacterial strains it was shown that 60 mM Mg^{2+} inhibited the transferase activity of the adenylylated form of the enzyme [16,17,31,47]. Therefore, it is suggested that Ba_1 glutamine synthetase is also regulated by adenylylation/deadenylylation.

In conclusion, the assimilation of nitrate in Ba_1 starts by its reduction to ammonia by a nitrate reductase which is associated with the electron transport chain on the cytoplasmic membrane, and a soluble nitrite reductase. This portion of the pathway is regulated by ammonia and nitrate at the level of nitrate uptake and nitrite reduction. Ammonia, generated from nitrate, is assimilated by the glutamine synthetase/glutamate synthase pathway, while ammonia supplied in the growth medium is incorporated into organic compounds by glutamate dehydrogenase.

References

1 Vennesland, B. and Guerrero, M.G. (1978) in Encyclopedia of Plant Physiology, New Series (Gibbs, M. and Latzko, E., eds.), Vol. 6, pp. 425–444, Springer Verlag, Berlin.
2 Hewitt, E.J. and Notton, B.A. (1980) in Molybdenum and Molybdenum Containing Enzymes (Coughlan, M., ed.), pp. 273–325, Pergamon Press, London.
3 Knowless, R. (1982) Microbiol. Rev. 46, 43–70.

4 Payne, W.J. (1973) Bacteriol. Rev. 37, 409–452.
5 McCready, R.G., Gould, W.D. and Barendregt, R.W. (1982) Can. J. Microbiol. 29, 231–234.
6 Schroder, I., Robertson, A.M., Bokranz, M., Unden, G., Bocker, R. and Kroger, A. (1985) Arch. Microbiol. 140, 380–386.
7 Guerrero, M.G., Vega, J.M., Leadbetter, E. and Losada, M. (1973) Arch. Mikrobiol. 91, 287–304.
8 Brown, C.M., MacDonald-Brown, D.C. and Stanley, S.O. (1975) Marine Biol. 31, 7–13.
9 Villalobo, A., Roldan, M.J., Rivas, J. and Cardenas, J. (1977) Arch. Microbiol. 112, 127–132.
10 Cole, J.A. (1978) FEMS Microbiol. Lett 4, 327–329.
11 Tyler, B. (1978) Annu. Rev. Biochem. 47, 1127–1162.
12 Meers, J.L., Tempest, D.W. and Brown, C.M. (1970) J. Gen. Microbiol. 64, 187–194.
13 Brenchley, J.E., Baker, C.A. and Patil, L.G. (1975) J. Bacteriol. 124, 182–189.
14 Nagatani, H., Shimizu, M. and Valentine, R.C. (1971) Arch. Microbiol. 79, 164–175.
15 Brown, C.M. and Herbert, R.A. (1977) FEMS Microbiol. Lett. 1, 39–42.
16 Janssen, D.B., Op den Camp, H.J.M., Leenen, P.J.M. and Van den Drift, C. (1980) Arch. Microbiol. 124, 197–203.
17 Stadtman, E.R. and Ginsburg, A. (1974) in The Enzymes (Boyer, P.D., Ed.), Vol. 10, 3rd Edn., pp. 755–807, Academic Press, New York.
18 Tronick, S.R., Ciardi, J.E. and Stadtman, E.R. (1973) J. Bacteriol. 115, 858–868.
19 Kimura, K., Yagi, K. and Matsuoka, K. (19840 J. Biochem. 95, 1559–1567.
20 Coleman, K.J., Cornish-Bowden, A. and Cole, J.A. (1978) Biochem. J. 175, 483–493.
21 Kerber, N.L., Caballero, F.J. and Cardenas, J. (1981) FEMS Microbiol. Lett. 11, 249–252.
22 Vega, J.M., Guerrero, M.G., Leadbetter, G. and Losada, M. (1973) Biochem. J. 133, 701–708.
23 Rafaeli-Eshkol, D. (1968) Biochem. J. 109, 679–685.
24 Peleg, E., Tietz, A. and Friedberg, I. (1980) Biochim. Biophys. Acta 596, 118–128.
25 Stern, N. and Tietz, A. (1973) Biochim. Biophys. Acta 296, 130–135.
26 Avi-Dor, Y. and Schnaiderman, R. (1981) Stud. Biophys. 84, 43–44.
27 Ken-Dror, S., Preger, R. and Avi-dor, Y. (1986) FEMS Microbiol. Rev. 39, 115–120.
28 Ken-Dror, S., Shnaiderman, R. and Avidor, Y. (1984) Arch. Biochem. Biophys. 229, 640–649.
29 Lorimer, G.H., Gewitz, H.-S., Volker, W., Solomonson, L.P. and Vennesland, B. (1974) J. Biol. Chem. 249, 6074–6079.
30 Losada, M. and Paneque, M. (1971) Methods Enzymol. 23, 481–491.
31 Bender, R.A., Janssen, K.A., Resnick, A.D., Blumberg, M., Foor, F. and Magasanik, B. (1977) J. Bacteriol. 129, 1001–1009.
32 Nicholas, D.J.D. and Nason (1957) Methods Enzymol. 3, 981–982.
33 Strickland, J.D.H. and Parson, T.R. (1968) A Practical Handbook of Sea Water Analysis, pp. 185–192, Roger Duhamel, Ottawa.
34 Burris, R.H. (1972) Methods Enzymol. 24B, 415–431.
35 Chaykin, S. (1969) Anal. Biochem. 31, 375.
36 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
37 Slater, E.C. (1973) Biochim. Biophys. Acta 301, 129–154.
38 Wesch, R. and Klemme, J.H. (1980) FEMS Microbiol. Lett. 8, 37–41.
39 Taniguchi, S. and Ohmachi, K. (1960) J. Biochem. 48, 50–62.
40 Wang, R. and Nicholas, D.J.D. (1986) Arch. Microbiol. 145, 20–26.
41 Van 't Riet, J., Stouthamer, A.H. and Planta, R.J. (1968) J. Bacteriol. 96, 1455–1464.
42 Hewitt, E.J. (1975) Annu. Rev. Plant Physiol. 26, 73–100.
43 Betlach, M.R., Tiedje, J.M. and Firestone, R.B. (1981) Arch. Microbiol. 129, 135–140.
44 Bhandari, B. and Nicholas, D.J.D. (1981) Aust. J. Biol. Sci. 34, 527–539.
45 Deuel, T.F. and Prusiner, S. (1974) J. Biol. Chem. 249, 257–264.
46 Woolfolk, C.A. and Stadtman, E.R. (1967) Arch. Biochem. Biophys. 118, 736–755.
47 Upchurch, P.G. and Elkan, G.H. (1978) Biochim. Biophys. Acta 538, 244–248.