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Competition between nitrate and nitrite uptake in the cyanobacterium *Anacystis nidulans*

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Nitrate and nitrite reciprocally inhibited the utilization of each other in the unicellular cyanobacterium *Anacystis nidulans*. Nitrate hampered active nitrite uptake both in wild-type cells lacking active nitrate reductase and in a nitrate reductase-defective mutant. Nitrate uptake in a nitrite reductase-defective mutant was inhibited by nitrite. The competitive interaction between nitrate and nitrite utilization in *A. nidulans* seems to take place at the level of substrate transport into the cell.

Cyanobacteria can use nitrate or nitrite as the source of nitrogen for growth [1]. The assimilation of both nitrogenous compounds in this group of organisms has been studied mainly in the unicellular species *Anacystis nidulans* (= *Synechococcus elongatus*, see Ref. 2). The systems involved in nitrate and nitrite uptake are both operative in nitrate-grown cells. Nitrate uptake appears to be mediated by an active transport system with a high affinity for nitrate [3–5]. Nitrite entrance into the cell seemingly takes place both by diffusion (probably in the form of nitrous acid) and via active transport of the nitrite anion [4,6]; the relative contribution of each component depends on the pH of the medium, that of the active system being prevalent at high pH values [6]. Nitrate uptake and the active component of nitrite uptake share common properties in *A. nidulans*, as they exhibit analogous pH-dependence profiles and are subject to regulation by ammonium and carbon dioxide in a similar fashion [6]. In view of the above similarities it seems plausible that a common transport system participates in both nitrate and nitrite uptake. The results presented in this report actually show that there is a marked competitive interaction between nitrate and nitrite uptake in *A. nidulans*.

Anacystis nidulans (strain L 1402-1 from Göttingen University's Algal Culture Collection, also known as *Synechococcus leopoliensis*) cells incubated in the presence of both nitrate and nitrite took up both substrates simultaneously, but at rates lower than those observed in control cell suspensions supplemented with only one nitrogenous compound (Table I). This was also the case for cells treated with L-methionine-D,L-sulfoximine (Table I), a glutamate analog that inactivates glutamine synthetase [7], the L-methionine-D,L-sulfoximine-treated cells quantitatively releasing to the outer medium the ammonium resulting from reduction of the nitrate or nitrite taken up [4]. Thus, the competition between nitrate and nitrite utilization takes place at the early stages of the processes, prior to the assimilation, via glutamine synthetase, of the ammonium resulting from

Abbreviations: Mops, 4-morpholinepropanesulfonic acid; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine.

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TABLE I

COMPETITION BETWEEN NITRATE AND NITRITE UPTAKE IN *ANACYSTIS NIDULANS*

Nitrate-grown cells [10] were harvested and resuspended (20 μg chlorophyll/ml) in 25 mM Tricine-NaOH buffer (pH 8.3) with or without 1 mM L-methionine-D,L-sulfoximine (MSX). After 15 min of incubation at 40°C in the light (100 W/m^2), the assays were started by the addition of KNO_3 or/and KNO_2 at a concentration of 0.32 mM each (Expt. 1) or 0.4 mM KNO_3 or/and 0.6 mM KNO_2 (Expt. 2). Samples were withdrawn at different times, filtered (Millipore HA 0.45 μm), and the remaining substrates determined in the filtrates [13,14].

Expt.	Addition	Substrate taken up (nmol/μg chlorophyll per 25 min)	
		NO_3^-	NO_2^-
1	KNO_3	12.1	–
	KNO_2	–	10.7
	KNO_3, KNO_2	3.7	6.4
2	MSX, KNO_3	16.0	–
	MSX, KNO_2	–	21.0
	MSX, KNO_3, KNO_2	12.5	15.6

reduction of the substrates.

Because in *A. nidulans* both nitrate reductase and nitrite reductase use reduced ferredoxin as the physiological electron donor [8], a competition between the enzymes for reductant could account for the observed competitive interaction above described. Alternatively, competition might take place at the level of substrate transport into the cell. To decide between these two possibilities, cells devoid of nitrate reductase or nitrite reductase have been used in studies of the effect of nitrate on nitrite uptake and vice versa.

Cells of *Anacystis nidulans* L 1402-1 essentially devoid of active nitrate reductase but with full activity of other components of the nitrate assimilating system were obtained by treatment with tungstate as described in Ref. 9. Nitrate reductase activity [10] in these cells was only 0.02 mU/μg chlorophyll (chlorophyll measured as in Ref. 11), which represents about 0.5% of the activity level of normal untreated cells. The tungstate-treated cells exhibited high rates of nitrite uptake and reduction, whereas they did not show any detectable nitrate utilization activity. Nitrite uptake by these cells was effectively inhibited by nitrate, however (Table II), suggesting an effect at the level of nitrite transport, since nitrate could not be reduced, which excludes a competition at the reduction stage. The extent of the inhibition of nitrite uptake caused by nitrate depended on the pH of the outer medium, increasing with the alkalinization of the medium (Table II). This pattern of inhibition resembles the activity profile with respect to pH of the active nitrite transport system [6], supporting the contention of the active transport of nitrite as the likely target of the nitrate effect on nitrite uptake. Increasing the nitrate concentration in the medium resulted in enhanced inhibition of nitrite uptake. Under conditions in which nitrite uptake takes place almost exclusively via the active transport system (L-methionine-D,L-sulfoximine-treated cells, pH 9.6; see Ref. 6), nitrate at 20 mM concentration caused 95% inhibition of the utilization of 0.2 mM nitrite (data not shown).

The effect of nitrate on nitrite uptake has also been studied in a nitrate reductase-defective mutant of *A. nidulans*. This mutant (strain FM10), which will be described in detail elsewhere, is a derivative of *Anacystis nidulans* R2 (a strain closely related to *A. nidulans* L 1402-1; Ref. 2) obtained by transposon mutagenesis by the method described by Kuhlemeier et al. [12]. Nitrate reductase level in nitrite-grown cells of strain FM10

TABLE II

NITRATE INHIBITION OF NITRITE UPTAKE IN TUNGSTATE-TREATED *A. NIDULANS* CELLS

Ammonium-grown cells were treated with 1 mM sodium tungstate and the nitrate utilization system induced in the presence of nitrate and tungstate as described in Ref. 9. The cells were harvested and resuspended (11 μg chlorophyll/ml) in 25 mM Mops-NaOH buffer (pH 7.2), 25 mM Tricine-NaOH buffer (pH 8.1), or 25 mM glycine-NaOH buffer (pH 9.6). The assays were started by the addition of 0.2 mM KNO_2 and carried out at 40°C in the light (100 W/m^2), in the absence or in the presence of 2 mM KNO_3. Samples were withdrawn at different times (0–15 min) and the remaining nitrite determined [13].

pH	Nitrite uptake (nmol/μg chlorophyll per min)		Inhibition by nitrate (%)
	$-NO_3^-$	$+NO_3^-$	
7.2	1.66	1.17	30
8.1	1.28	0.56	56
9.6	1.29	0.31	76

was negligible, below 0.02 mU/μg chlorophyll. Nitrate also inhibited nitrite utilization by cells of this strain, but no negative effect of nitrate up to 50 mM on nitrite reductase activity could be observed (nitrite reductase activity was about 0.8 mU/μg chlorophyll as estimated in situ in cells made permeable with mixed alkyltrimethylammonium bromide [9]). These results again suggest that competition between nitrate and nitrite uptake does not take place at the reduction stage but rather at the level of transport of the anions into the cell.

Results from kinetic studies of the effect of nitrate on nitrite uptake by L-methionine-D,L-sulfoximine-treated FM10 cells at different nitrite concentrations showed that nitrate actually behaves as a competitive inhibitor of nitrite uptake (Fig. 1). L-Methionine-D,L-sulfoximine-treated cells were used in this experiment in order to avoid regulatory interferences resulting from the assimilation via glutamine synthetase of the ammonium resulting from nitrite reduction (see Refs. 3 and 6). The calculated K_i value for nitrate was 24 μM, quite close to the K_m value for nitrite of nitrite uptake, which was 22 μM (Fig. 1).

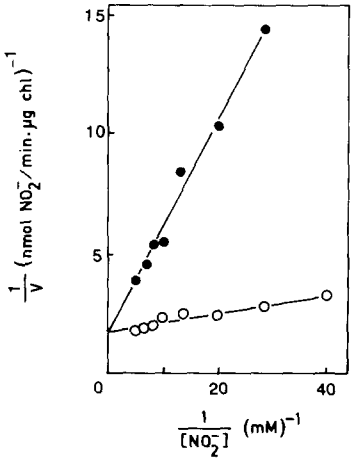

Fig. 1. Lineweaver-Burk plot of the effect of nitrite concentration on the rate of nitrite uptake by *A. nidulans* strain FM10 (defective in nitrate reductase) in the absence (O) or in the presence (●) of 0.25 mM KNO_3. Nitrite-grown cells (9 μg chlorophyll per ml of 50 mM Tricine-NaOH buffer, pH 8.1) were treated with 1 mM L-methionine-D,L-sulfoximine for 15 min in the light at 40 °C. After harvesting, the cells were resuspended in 25 mM glycine-NaOH buffer (pH 9.6), and the nitrite uptake assays were carried out as described in Table II.

The effect of nitrite on nitrate uptake has also been investigated. For this study, another transposon-induced mutant of *A. nidulans* R2 (strain FM2) has been used. Strain FM2 is a nitrite reductase-defective mutant exhibiting undetectable nitrite reductase level. (Nitrite reductase activity of strain R2 estimated as described in Ref. 9 was about 1 mU/μg chlorophyll.) The effect of nitrite on nitrate uptake was tested in cell suspensions of strain FM2 at pH 9.6, conditions under which nitrite uptake takes place almost exclusively via the active transport system. Nitrite effectively inhibited nitrate uptake in FM2 cells, 1 mM nitrite inhibiting the utilization of 0.1 mM nitrate by 75% (Table III). Because nitrite at moderately high concentrations is toxic for cyanobacteria, the effect of nitrite on [^{14}C]CO_2 fixation by FM2 cells has been tested. No inhibition was detected, the CO_2-fixation rate being 5.8 nmol/μg chlorophyll per min in the absence or in the presence of KNO_2 at concentrations up to 1 mM. These experiments show that nitrite inhibition of nitrate utilization in *Anacystis* does not result from a competition for reducing equivalents or from non-specific inhibition of photosynthetic electron flow which is required for nitrate utilization [3,4].

The data presented in this paper indicate a reciprocal competition between the active trans-

TABLE III

NITRITE INHIBITION OF NITRATE UPTAKE BY NITRITE REDUCTASE-DEFECTIVE *A. NIDULANS* STRAIN FM2

Ammonium-grown cells were incubated for 3 h in culture medium lacking any added nitrogen source, in order to induce the nitrate-assimilating system. The assays were carried out as described in Table II and were started by addition of 0.1 mM KNO_3 to cells suspended in 25 mM glycine-NaOH buffer (pH 9.6), with or without KNO_2. At different time intervals samples were withdrawn and filtered (Millipore HA-0.45 μm filters), the remaining nitrate being measured in the filtrates [14].

Nitrite added (mM)	Nitrate uptake (nmol/μg chlorophyll per min)
0	0.34
0.2	0.25
0.5	0.13
1.0	0.09

port of nitrate and that of nitrite in the cyanobacterium *Anacystis nidulans*. These results support the contention that in *A. nidulans* nitrate and nitrite might share a common permease for their transport into the cell. Our results with *Anacystis* are in contrast to those reported for *Neurospora*, where nitrite behaves as a non-competitive inhibitor of nitrate uptake [15] and nitrate does not inhibit nitrite uptake [16]. However, for the diatom *Phaeodactylum*, the similarities between nitrate and nitrite uptake have led to the suggestion that both anions may be taken up by the same mechanism [17].

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