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Influence of different forms of nitrogen on uptake of ammonium, glutamate and glutamine in the cyanobacterium *Nostoc muscorum*

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Effect of various types of nitrogen nutrition was studied on the uptake of ammonium, glutamate and glutamine by *Nostoc muscorum* and its Het^-Nif^- mutant. Ammonium nitrogen acted as a potent inhibitor/repressor of ammonium, glutamate and glutamine transport. Nitrate nitrogen was found to be a strong inhibitor/repressor of ammonium transport, a partial inhibitor/repressor of glutamate transport but, caused a partial stimulation of glutamine transport.

Heterocystous cyanobacteria can assimilate N_2, nitrate, ammonium or glutamine as nitrogen source. While repression-derepression mechanism of nitrogen control governs their diazotrophic nutrition and induction/repression-derepression mechanisms of nitrogen control their nitrate nutriton, molecular details of such nitrogen controls are virtually unknown[1-4]. In heterotrophic enteric bacteria, a well defined *ntr* system has been shown to control assimilation of various inorganic and organic nitrogen compounds as nitrogen source[5]. No comparable *ntr* system for control of nitrogen assimilation in any cyanobacteria has hitherto been documented. It is well established that cellular control of nitrogen nutrition normally operates at the level of transport and/or assimilation[6]. In recent years, considerable attention has been paid to nitrogen control of ammonium transport system in heterocystous forms in view of its potential role in generation of ammonia excreting strains[7]. In comparison, very few studies have been conducted to understand the nature and kinds of cyanobacterial amino acid transport systems[8]. However, existence of a common transport system for glutamate and glutamine in *Anabaena variabilis* and *Anabaena* PCC 7120 has been demonstrated[9-11]. A *Nostoc* cyanobiont isolated from *Geosiphon pyriforme* has been found to have a highly functional transport system common for glutamate and aspartate[12]. Nitrogen regulation of amino acid transport and metabolism has not been systematically studied in any cyanobacteria. In the present study, we provide evidence for the first time that ammonium nitrogen is a general and strong inhibitor/repressor of ammonium transport, glutamate transport and glutamine transport; that nitrate nitrogen, like ammonium nitrogen, is a strong inhibitor/repressor of glutamate transport; and that nitrate and glutamine nitrogen sources both stimulate glutamine transport.

Materials and Methods

Growth of organism and culture conditions— *Nostoc muscorum* parent and its Het^-Nif^- mutant[13] were grown in axenic batch cultures in 5 m*M* KNO_3 supplemented BG-11_0 medium[14] at 25°C and photon fluence rate of 50 µmole $m^{-2}s^{-1}$. When required, NH_4Cl (1 m*M*) or glutamine (1 m*M*) was added to the medium and buffered with 10 m*M* HEPES†-NaOH buffer (*p*H 7.5).

Chlorophyll estimation — Chlorophyll a concentration was measured according to Mackinney[15].

Measurement of ammonium, glutamate and glutamine uptake— Ammonium, glutamate and glutamine uptakes were measured using $^{14}CH_3NH_3^+$, [*U*-^{14}C]glutamic acid and [*U*-^{14}C]glutamine, respectively. Exponentially growing cells were harvested by centrifugation (10,000 *g* for 10 min), washed and resuspended in 10 m*M* HEPES-NaOH bufer (*p*H 7) and equilibrated for 30 min at 25°C and 50 µmole $m^{-2}s^{-1}$ photon fluence rate. Respective ^{14}C-labelled compounds were then added to a final concentration of 50 µ*M* (specific activity 185 kBq $\mu mole^{-1}$). Samples were

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† *Abbreviations used*—GS, glutamine synthetase; GOGAT, glutamate synthase; HEPES, 4-(2-hydroxyethyl)-1-piperazine ethane sulphonic acid.

withdrawn at time intervals and cells separated from the medium by silicon oil microcentrifugation technique[16]. ^{14}C-label in the cells was estimated by liquid scintillation spectrometer (Beckman, LS 1801). Nonspecific binding of ^{14}C label was determined by measuring its incorporation into toluene-treated cells as described earlier[7].

Chemicals — $^{14}CH_3NH_2Cl$ was purchased from Amersham International Plc., Amersham, UK. [^{14}C]glutamate and [^{14}C]glutamine were obtained from BARC, Bombay, India. Silicon oil DC 550 and dinonyl phthalate were purchased from Fluka AG, Buchs, Switzerland. All other chemicals were obtained from Sigma Chemical Company, St. Louis, USA.

Results

The status of ammonium transport was studied in ammonium-grown, nitrate-grown and N_2-grown cultures of *Nostoc muscorum* parent strain (Het^+Nif^+) and its Het^-Nif^- mutant strain. As shown in Fig.1 (a and b), N_2-fixing/N-starved cultures showed a characteristic biphasic pattern of ammonium transport, while their corresponding nitrate- or ammonium-grown cultures almost completely lacked both phases of the transport. Thus, nitrate nitrogen and the ammonium nitrogen, strongly inhibit ammonium transport.

Glutamate (1 m*M*) was found to be strongly toxic for growth of diazotrophic cultures but not of nitrate assimilating or ammonium assimilating cultures (data not shown). Therefore, the pattern of glutamate uptake as a function of nitrogen source in the growth medium was examined.

As shown in Fig.2 (a and b), both parent and its mutant exhibited maximal glutamate uptake under nitrogen-limited (N_2-fixing/N-starved) growth conditions. However, in nitrate-grown cultures of the two strains a significant reduction (nearly 50%) in glutamate uptake was observed, which diminished further in cultures grown with ammonium as nitrogen source. Apparently, nitrate functions as partial, and ammonium as a complete inhibitor/repressor of glutamate uptake.

The mutant and parent strains could grow with glutamine as nitrogen source. Therefore, uptake of glutamine in cultures grown on N_2, nitrate, ammonium or glutamine as nitrogen source was also studied (Fig.3). Glutamine uptake was very low in ammonium-grown cultures and maximum in

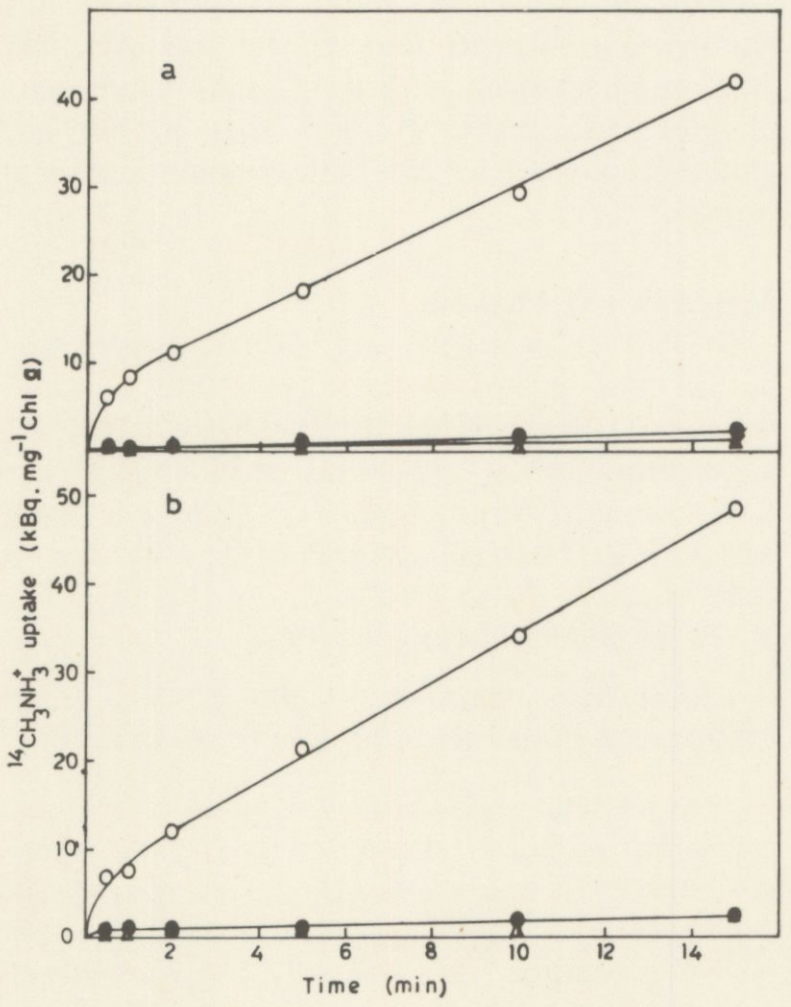

Fig. 1—$^{14}CH_3NH_3^+$ uptake in *Nostoc muscorum* (a) and its Het^-Nif^- mutant strain (b) [○, N_2-grown cells; ●, nitrate (5 m*M*)-grown cells; △, ammonium (1 m*M*)-grown cells. In Figs 1b and 2b, the uptake rates in "N_2-grown cells" refer to the nitrate-grown cells which were subjected to nitrogen starvation for 24 hr before measuring uptake; this is because the mutant does not grow in N_2-medium]

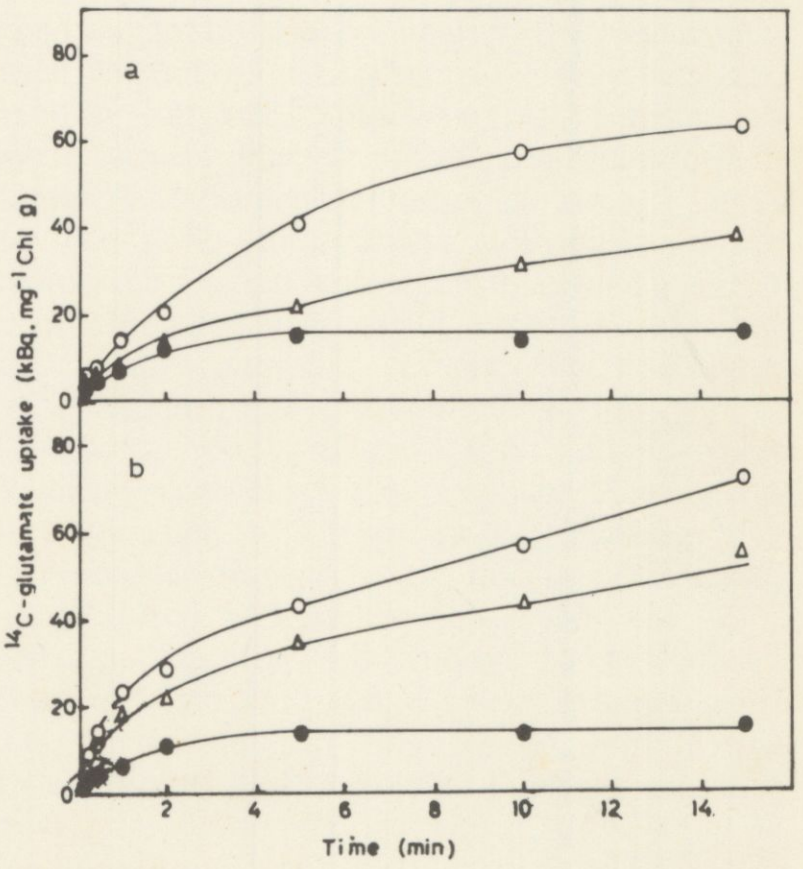

Fig. 2—[^{14}C]Glutamate uptake in *Nostoc muscorum* (a) and its Het^-Nif^- mutant strain (b) [○, N_2-grown cells; △, nitrate (5 m*M*)-grown cells; ●, ammonium (1 m*M*)-grown cells]

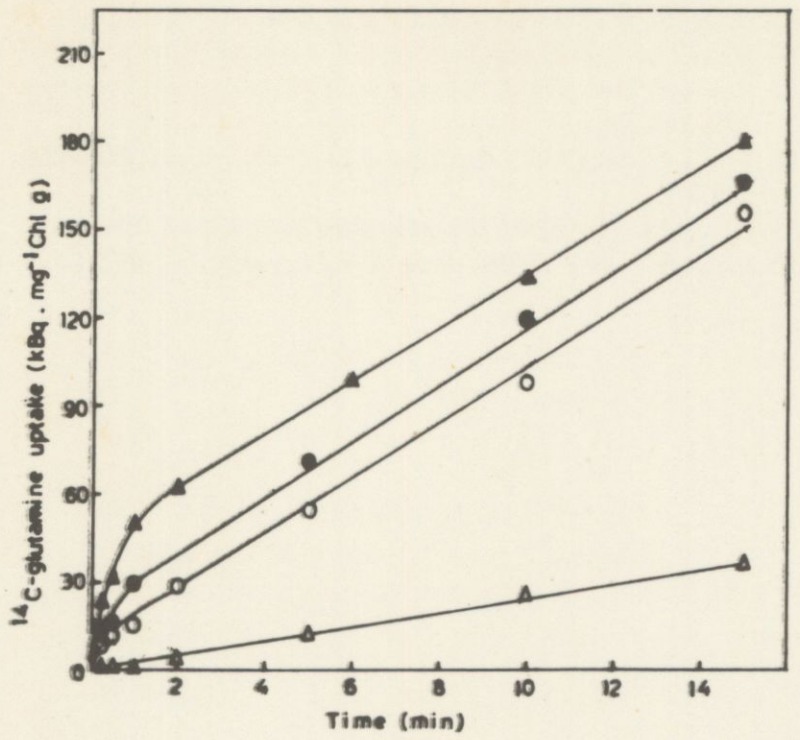

Fig. 3—[^{14}C]Glutamine uptake in N_2-grown (○), 5 m*M* nitrate-grown (●), 1 m*M* ammonium-grown (△) and 1 m*M* glutamine-grown (▲) *Nostoc muscorum* cells

glutamine-grown cultures. Even nitrate-grown cultures showed slightly higher level of uptake than its corresponding N_2-grown cultures. Ammonium nitrogen certainly appears to inhibit the process of glutamine uptake as it does for glutamate and ammonium uptake. A similar pattern was found in the case of the Het$^-$Nif$^-$ mutant (data not shown).

Discussion

Ammonium is a well-known repressor of its own transport system in diazotrophic cyanobacteria[17,18]. A possible role of the ammonium transport system was proposed by Kleiner[19], according to which, the ammonium transport system enables retention of ammonia generated intracellularly.Nitrate,like N_2, is reduced to ammonia before its assimilation into organic nitrogen through the GS-GOGAT pathway[3,20]. While the ammonium transport system remains fully functional during diazotrophic growth, extracellular ammonia is never detected. This lack of extracellular ammonia in diazotrophic cultures has been attributed to the functioning of ammonium transport system. Accordingly, nitrate- assimilating cultures, deficient in ammonium transport system, should be expected to liberate extracellular ammonia during their growth. No such liberation of ammonia was found in the nitrate assimilating cultures of the parent or its Het$^-$Nif$^-$ mutant strain (data not shown). These findings question the proposed role of ammonium transport system as a physiological mechanism of ammonia conservation during growth with N_2 or nitrate as nitrogen source in this cyanobacterium.

Glutamate transport system is also inhibited by fixed inorganic nitrogen sources like nitrate and ammonium. The degree of inhibition is partial in nitrate-grown cultures and almost complete in ammonium-grown cultures. Such control of glutamate uptake by fixed inorganic nitrogen may explain why glutamate is toxic to diazotrophic cultures but not to nitrate assimilating or ammonia assimilating cultures of *Nostoc muscorum*. Glutamate toxicity to growth has also been reported for N_2-fixing cultures of *Anabaena variabilis*[9].

Glutamine uptake process remains relatively more active in nitrate- and glutamine-grown cultures than in N_2-grown cultures. However, ammonium-grown cultures remain uniformly deficient in glutamine, ammonium and glutamate uptake. Thus, the response of glutamine transport system to N_2 and ammonium as nitrogen source is also similar to those observed for ammonium transport system and glutamate transport system under comparable conditions. However, this transport differs from ammonium and glutamate transport in respect of its response to nitrate which stimulated its activity.

To conclude, ammonium, in addition to being a repressor for ammonium transport system (data in the present study, and ref.18) and nitrate transport[3], was found to be an inhibitor/repressor of glutamate transport and glutamine transport in *Nostoc muscorum*. Nitrate, while functioning like ammonium as an inhibitor/repressor of ammonium transport, causes partial inhibition/repression of glutamate uptake but stimulates uptake of glutamine.

Acknowledgements

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