RESPONSE OF CYANOBACTERIAL NITROGENASE EXPRESSION TO EXOGENOUS NO_3^- AND NH_4^+

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THE synthesis of nitrogenase in all nitrogen-fixing organisms is repressed on confrontation with NH_4^+. The only reported exception is the marine cyanobacterium, *Anabaena* CA[1], which partially expresses nitrogenase even in excess NH_4^+, whereas the enzyme is not expressed in the presence of NO_3^-. This preliminary report deals with the response of nitrogenase expression in five cyanobacteria, *Anabaena variabilis*, *Calothrix marchica*, *Nostoc rivulare*, *Hapalosiphon intricatus* and *Scytonema schmidlei* to nitrate- and ammonium-nitrogen. Details of the regulatory mechanisms will form a separate communication.

All the cyanobacterial strains, except *Anabaena variabilis*, were grown in Fogg's nitrogen-free medium[2] at 29 ± 1°C under 2500 lux. *A. variabilis* was grown in BG 11 nitrogen-free medium[3]. Nitrogenase activity was measured in terms of acetylene reduction activity (ARA) with a Neucon Model GLC 5500[4]. In experiments to determine the immediate effects of exogenous NO_3^- or NH_4^+, 1 mM KNO_3 or NH_4Cl was added to suitably buffered dinitrogen-growing cultures and ARA was monitored over a 24 hr period. Subsequently, KNO_3 and NH_4Cl were removed from the medium by centrifugal washing with nitrogen-free medium. The washed cultures were resuspended in nitrogen-free medium and ARA was again measured over a 24 hr period in the nitrogen-free medium.

Table 1 shows considerable response-variations not only among the strains but also between NO_3^- and NH_4^+. When 1 mM KNO_3 was added to dinitrogen-growing culture of *A. variabilis*, there was no inhibition of nitrogenase activity upto 8 hr, after which the activity decreased to 82% of that obtained with N_2-grown cells. The trend was similar in *N. rivulare*, where again there was no inhibition upto 4 hr. However, the activity decreased to 28% after 24 hr. In *C. marchica*, there was no change in nitrogenase activity upto 1 hr after which the activity decreased. Unlike other forms, addition of KNO_3 brought about an immediate inhibition in the nitrogenase activity of *H. intricatus*. The time required for a 50% reduction of the nitrogenase activity in presence of NO_3 showed a wide range of variations among the strains. While it is around 4 to 8 hr in *C. marchica*, *N. rivulare* and *H. intricatus*, it is over 24 hr in *A. variabilis* (table 1).

On the other hand, there was an immediate decline in the nitrogenase activity on exposure to NH_4^+, until only 6–23% activity could be detected at 24 hr.

Of particular interest is *S. schmidlei* which was found to express appreciable nitrogenase activity (91–93%) in presence of both NO_3^- and NH_4^+ over a 24 hr period. The reason for this curious behaviour is under study.

When NO_3^- and NH_4^+ were removed from the medium, nitrogenase activity in all the strains returned to its original level within a 24 hr period. The effects of NO_3^- and NH_4^+ were quite different in that the inhibitory effect of NO_3^--N was much less than that of NH_4^+-N.

Table 1 *Relative nitrogenase activity (acetylene reduction) in presence and absence of 1 mM KNO_3 and NH_4Cl.*

	Anabaena variabilis		*Calothrix marchica*		*Nostoc rivulare*		*Hapalosiphon intricatus*		*Scytonema schmidlei*	
Treatment	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+
Control (N_2)	100	100	100	100	100	100	100	100	100	100
Addition (hr)										
1	100	84	100	79	100	69	83	65	100	100
3	100	71	83	61	100	24	75	53	100	100
4	100	67	60	27	100	16	61	38	100	100
8	100	36	48	23	64	15	52	16	100	100
24	82	23	42	20	28	12	50	6	93	91
Removal (hr)										
4	100	100	82	51	100	45	93	39	100	100
8	—	—	100	81	100	100	100	82	100	100
24	100	100	100	100	100	100	100	100	100	100

Genetic studies on nitrogen fixation in *Klebsiella pneumoniae*[5,6] have shown that NH_4^+ regulation of nitrogenase synthesis is mediated through glutamine synthetase (GS) and inhibitors of GS allow *K. pneumoniae*, *Azotobacter vinelandii*[7], *Anabaena cylindrica*[8] and *Rhodopseudomonas spheroides*[9] to produce nitrogenase even in the presence of excess NH_4^+, showing that a product of NH_4^+ assimilation is the repressor of nitrogenase. In *Anabaena variabilis*, nitrogenase activity was found to be severely reduced in 2 mM NH_4Cl even in the presence of methionine-sulphoximine (MSX), an inhibitor of GS (figure 1), indicating that GS may not act as a positive regulator of nitrogenase in cyanobacteria[10].

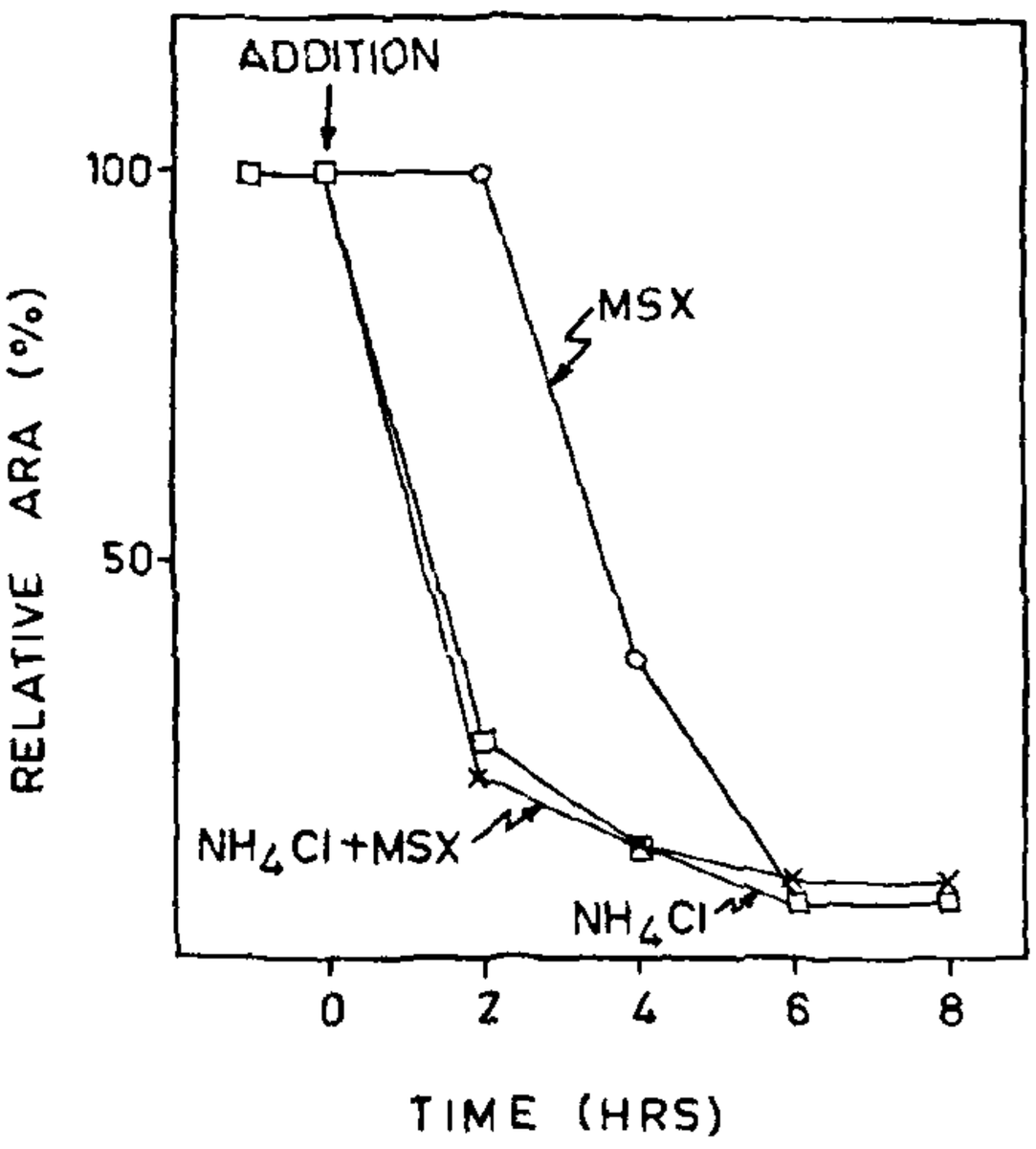

Figure 1. Effect of added NH_4Cl (2 mM), MSX (2 mM) and a mixture of NH_4Cl and MSX to dinitrogen-growing cultures of *Anabaena variabilis* on the activity of nitrogenase (acetylene reduction) as a function of time after the additions. Control rate of acetylene reduction was 6.2 μmol $C_2H_4 \cdot mg\ Chl^{-1} \cdot hr^{-1}$.

Besides repression, NH_4^+ may also inactivate the existing nitrogenase[11-14]. In *Klebsiella*, NH_4^+ inactivation needs protein synthesis, since in the presence of a mixture of NH_4^+ and chloramphenicol half-life of nitrogenase increases as compared to NH_4^+ alone[15]. Our studies with *Anabaena variabilis* (unpublished) suggest that NH_4^+ inactivation may not require fresh protein synthesis as in *Anabaena* L-31[15]. It is interesting that as in photo-synthetic bacteria[12,13], NH_4^+ causes a rapid inhibition of cyanobacterial nitrogenase which is reversible. The mechanism for this reversible inhibition is not clear.

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1. Bottomley, P. J., Grillo, J. F., Baalen, C. V. and Tabita, R. F., *J. Bacteriol.*, 1979, **140,** 938.
2. Fogg, G. E., *Ann. Bot.*, 1949, **13,** 241.
3. Stanier, R. Y., Kunizawa, R., Mandel, M. and Cohen-Bazire, G., *Bacteriol. Rev.*, 1971, **35,** 171.
4. Kaushik, B. D. and Venkataraman, G. S., *Curr. Sci.*, 1983, **52,** 321.
5. Streicher, S. L., Shanmugham, K. T., Ausubel, F., Morandi, C. and Goldberg, R. B., *J. Bacteriol.*, 1974, **120,** 815.
6. Tubb, R. S., *Nature* (*London*), 1974, **251,** 481.
7. Gordon, J. K. and Brill, W. J., *Biochem. Biophys. Res. Commun.*, 1974, **59,** 967.
8. Stewart, W. D. P. and Rowell, P., *Biochem. Biophys. Res. Commun.*, 1975, **65,** 846.
9. Jones, B. L. and Monty, K. J., *J. Bacteriol.*, 1979, **139,** 1007.
10. Tuli, R. and Thomas, J., *Biochem. Biophys. Acta*, 1980, **613,** 526.
11. Shah, V. K., Davis, L. C. and Brill, W. J., *Biochem. Biophys. Acta*, 1972, **256,** 498.
12. Neilson, A. H. and Nordlund, S., *J. Gen. Microbiol.*, 1975, **91,** 53.
13. Zumft, W. G. and Castillo, F., *Arch. Microbiol.*, 1978, **117,** 53.
14. Tubb, R. S. and Postgate, J. R., *J. Gen. Microbiol.*, 1973, **79,** 103.
15. Tuli, R. and Thomas, J., *Arch. Biochem. Biophys.*, 1981, **206,** 181.