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STIMULATION OF NITROGENASE BY ACETYLENE : FRESH SYNTHESIS OR CONFORMATIONAL CHANGE ?

K. A. V. David, S. K. Apte and Joseph Thomas

Biology and Agriculture Division
Bhabha Atomic Research Centre
Trombay, Bombay 400 085, INDIA.

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ABSTRACT : Alternate exposure of the nitrogen-fixing blue-green alga Anabaena L-31 to acetylene in air and ambient atmosphere resulted in substantial enhancement in the rate of acetylene reduction activity. The stimulation occurred even when protein synthesis was inhibited in the dark or in the presence of rifampicin and chloramphenicol. Residual nitrogenase of ammonium chloride treated cultures also showed the stimulation effect. The results indicate that the stimulation is due to a substrate (acetylene) mediated alteration of the enzyme molecule and not due to fresh synthesis.

INTRODUCTION : Nitrogenase from different organisms has very similar enzymatic properties (1). In addition to dinitrogen (N_2), it reduces a variety of substrates like azide, nitrous oxide, acetylene, cyanides and isocyanides. The reduction of acetylene to ethylene (2, 3) has been routinely used in assays for nitrogenase activity (4) and long periods of incubation with acetylene have been employed to compensate for the low rates of acetylene reduction in certain field studies (5) and in free-living rhizobial cultures (6, 7). It was reported earlier (8) that continuous or alternate incubation under acetylene resulted in multifold enhancement of the rate of acetylene reduction activity in a variety of nitrogen-fixing organisms tested. Two reasons for such enhancement were proposed : (a) derepression of nitrogenase and fresh synthesis of the enzyme and (b) a conformational change on the enzyme mole-

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cule. It was pointed out (8) that assessments based on long term incubation with acetylene may result in gross overestimation of N_2 fixed. Here we report the details of the enhancement of acetylene reduction in the blue-green alga Anabaena L-31 and show that it is due to a direct effect of acetylene on the enzyme molecule.

MATERIALS AND METHODS : Anabaena L-31 was grown in cyanophycean medium (9) with, or without combined nitrogen (2.5mM NH_4Cl) as described previously (10). When grown in combined nitrogen the medium was buffered to pH 7.0 with 4mM Tris (hydroxymethyl) methylamine/HCl. All experiments were performed with log phase (48 h old) cultures. Nitrogenase activity (acetylene reduction) was measured as described previously (8). Prior to normal incubation for 30 min to perform the acetylene reduction assay, algal suspensions were alternately exposed to a gas phase of 0.1 atm acetylene in air and to ambient atmosphere respectively, each period lasting for 30 min. Control samples were incubated under air without acetylene for the same period prior to the normal 30 min acetylene reduction assay. Chlorophyll a was measured after Mackinney (11). Chloramphenicol and rifampicin were supplied by Sigma Chemical Co., London. Other chemicals were used at the highest purity available from the British Drug Houses Ltd., Poole, England.

RESULTS AND DISCUSSION

Effect of preincubation with acetylene. Alternate exposure of Anabaena L-31 cultures to 0.1 atm acetylene (in air) and ambient atmosphere respectively, resulted in enhancement of acetylene reduction activity. A 2.2-fold increase was observed after 1 h of preincubation (30 min under 0.1 atm C_2H_2 + 30 min in air) in light (Fig. 1a). Prolonged preincubation however resulted in a decline in the rate of C_2H_2 reduction. This was possibly due to rapid consumption of ATP and reduction in a physiologically non-productive reaction. A similar treatment with C_2H_4 did not produce any enhancement of acetylene reduction ruling out the possibility of a product ($C_2H_2 \longrightarrow C_2H_4$) catalyzed stimulation. Fig. 1b shows that enhancement occurs even in

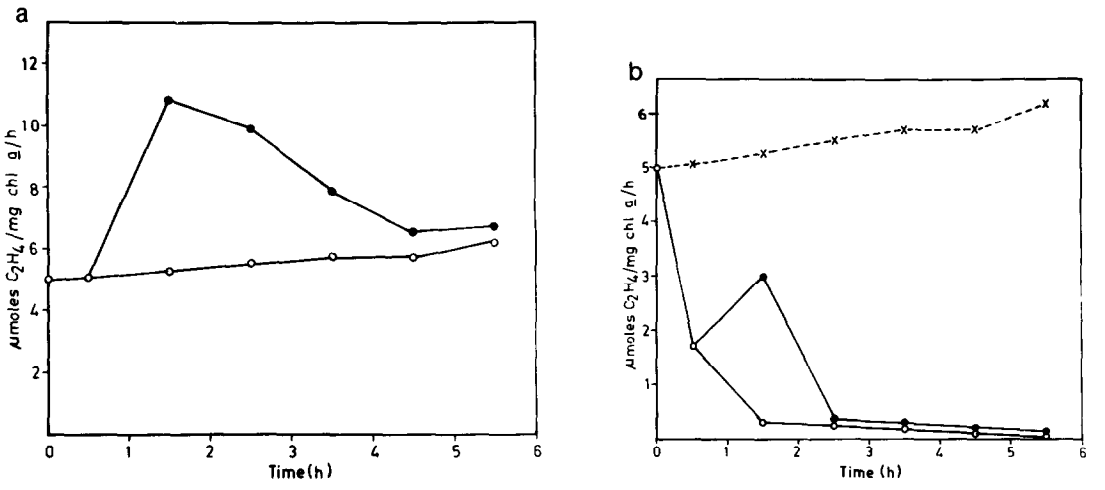

Fig. 1. Effect of preincubation under acetylene (0.1 atm. in air)on ethylene production by N_2-fixing cultures of Anabaena L-31 in (a) light and (b) dark. Alternate incubation under acetylene and ambient atmosphere respectively (●) is compared with untreated control (O). In Fig. 1b control (light) rates (X) are provided for comparision.

dark. In the dark, control activity declines rapidly due to nonavailability of reductant and ATP (12). However a 60 min preincubation produces an almost 10-fold increase in C_2H_2 reduction activity. Since protein synthesis does not occur in dark (13) this suggests a conformational change on the enzyme molecule.

Effect of inhibitors. C_2H_2 reduction activity is inhibited rapidly in the presence of inhibitors of protein synthesis (Fig. 2a and b). With rifampicin (2.5μM) - an inhibitor of transcription - the decline in enzyme activity is rapid while in presence of chloramphenicol (0.3mM) - an inhibitor of translation - it is comparatively slow. In either case, however, preincubation resulted in 2-3-fold increase in the enzyme activity. The treated cultures revived on transfer to inhibitor-free medium indicating that the concentrations of the inhibitors used were non-toxic.

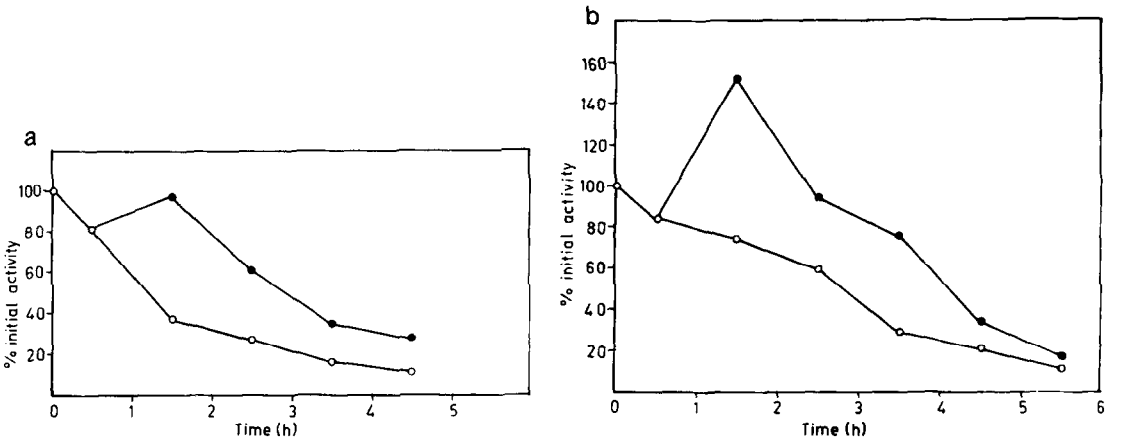

Fig. 2. Ethylene production by N_2-fixing cultures of Anabaena L-31 in the presence of (a) 2.5 μM rifampicin and (b) 0.3 mM chloramphenicol added at zero time. Alternate incubation under acetylene and ambient atmosphere respectively (●) is compared with control (○). Activities are expressed as % of initial activity.

The above results obtained from experiments in the dark and using inhibitors of protein synthesis suggest that the stimulation in acetylene reduction in Anabaena L-31 may be due to a conformational change rather than new synthesis of the enzyme. However, an alternative interpretation is possible. Incubation under acetylene prevents ammonium synthesis resulting in the depletion of nitrogenous metabolites in the cell. A product of ammonium metabolism is the putative repressor of nitrogenase (14, 15) and as pointed out earlier by David and Fay (8) nitrogenase may remain derepressed under the conditions cited above. Thus, a fresh synthesis of the enzyme may account for the observed enhancement. To establish that this is not the case, it is essential to demonstrate the enhancement effect after fresh enzyme synthesis is repressed completely and also by using physiological inhibitors specific to nitrogenase synthesis.

Using NH_4Cl (2.5 mM) - a physiological repressor of nitrogenase

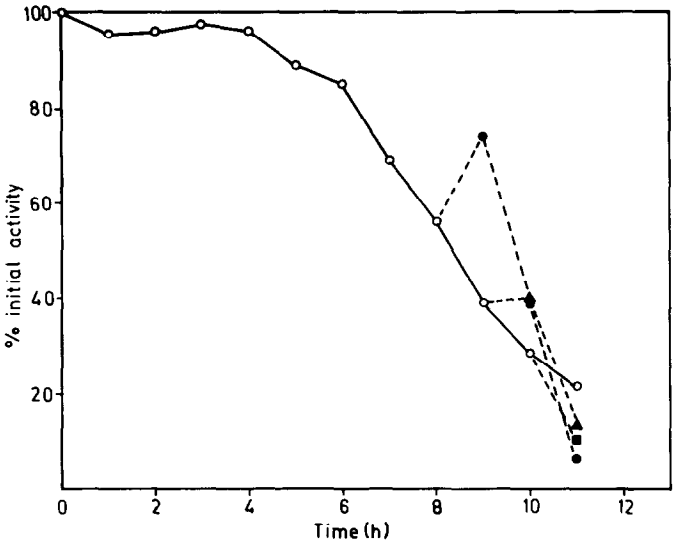

Fig. 3. The effect of NH_4Cl (2.5 mM) added at zero time on ethylene production by N_2-fixing cultures of Anabaena L-31. Alternate incubation under acetylene and ambient atmosphere repectively at 8 h (●), 9 h (▲) and 10 h (■) compared with control (○). Activities are expressed as % of initial activity.

synthesis (16, 17) - it was shown (Fig. 3) that when C_2H_2 reduction was lowered by over 50% of initial activity, nearly 2-fold increase in activity occurred in response to preincubation. This shows that stimulation is not due to fresh synthesis. This was further confirmed using rifampicin (Fig. 4). It can be seen that when the activity was reduced to 30% of the initial level (i. e. fresh syntheis was completely stopped) preincubation produced an almost 7-fold enhancement. Even at 10% of initial activity preincubation resulted in 2-fold stimulation, in contrast with experiments using NH_4Cl (Fig. 3) where no enhancement occurred after the initial activity had been reduced to 30%. This short-lived nature of the stimulation in NH_4Cl may be due to an additional inactivation of existing nitrogenase by ammonia as has also been reported previously (18).

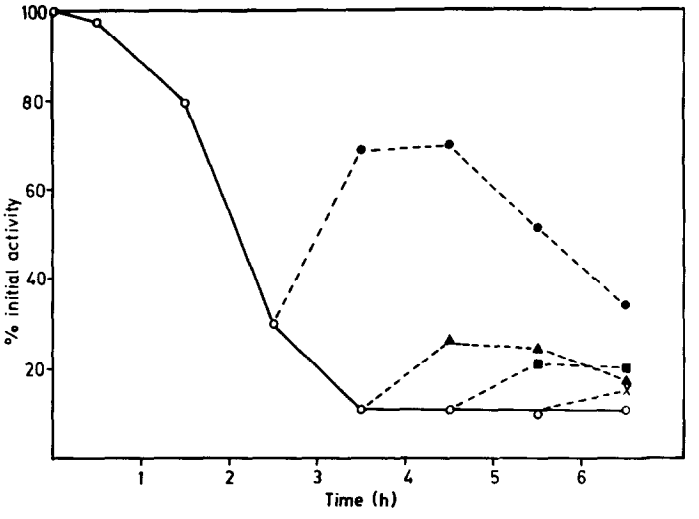

Fig. 4. The effect of rifampicin (2.5 μM) added at zero time on ethylene production by N_2-fixing cultures of Anabaena L-31. Alternate incubation under acetylene and ambient atmosphere respectively at 2.5 h (●), 3.5 h (▲), 4.5 h (■) and 5.5 h (X) compared with control (O). Activities are expressed as % of initial activity.

In blue-green algae nitrogen-starvation leads to the utilization of usually ample cellular reserves of organic nitrogen and it takes several hours before any appreciable increase of nitrogenase activity is detectable (19). It is therefore unlikely that enzyme derepression and synthesis would result owing to nitrogen-starvation caused by incubation under acetylene. A direct involvement of C_2H_2 in fresh synthesis of enzyme is also ruled out by our results (Fig. 3, 4). Thus, the observed enhancement of C_2H_2 reduction is likely to be due to a substrate (C_2H_2) mediated modification of enzyme molecule and not due to synthesis. There is increasing evidence for the presence of multiple sites for substrates on nitrogenase (20, 21) and it is possible that such preincubation may make more sites amenable to C_2H_2 binding.

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