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CONFORMATIONAL CHANGES IN THE NITROGENASE COMPLEX *IN VIVO* BY PREINCUBATION UNDER ACETYLENE

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ABSTRACT: Preincubation of *Anabaena* L-31 under acetylene results in conformational changes in the nitrogenase enzyme-complex. In particular, the site common to both nitrogen and acetylene is modified such that the affinity towards acetylene increases considerably and there is a corresponding decrease in the affinity towards nitrogen. The site for azide remains unaltered. The binding site for carbon-monoxide (CO) also undergoes change as revealed by the remarkable decrease in the affinity for CO. The results explain the enhancement in acetylene reduction upon preincubation under acetylene.

INTRODUCTION: Continuous preincubation under acetylene or alternate preincubation under acetylene and air results in multifold enhancement of acetylene reduction *in vivo* in a variety of nitrogen-fixing microorganisms (1). We reported earlier that such enhancement does not involve derepression and fresh synthesis of nitrogenase(2). The possibility of substrate (acetylene) mediated structural modification of enzyme molecule was therefore suggested. Data on the interactions among substrates and inhibitors of nitrogenase (3,4) have indicated that the enzyme has either multiple sites for binding of different substrates and/or the sites are modified in the presence of specific substrates. In the present communication we provide data favouring the latter possibility and show that *in vivo* preincubation of the blue-green alga *Anabaena* L-31 under acetylene results in conformational changes in the nitrogenase enzyme-complex, thereby increasing its affinity towards acetylene.

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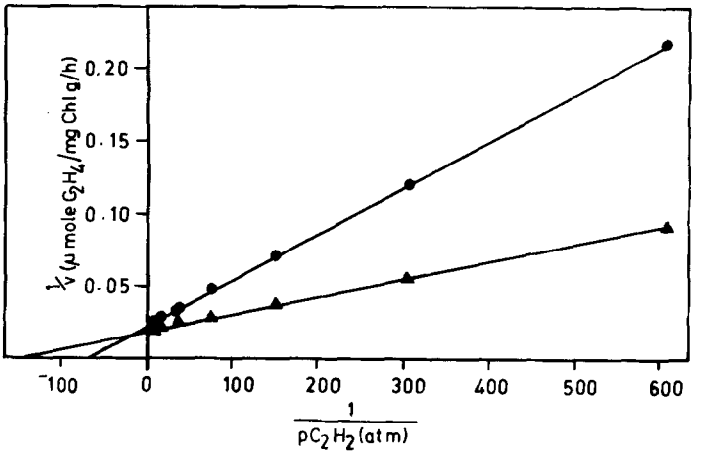

Fig. 1. The effect of acetylene concentration on acetylene reduction by Anabaena L-31. Preincubated algae (▲) are compared with untreated control (●).

MATERIALS AND METHODS: Anabaena L-31 was grown as described previously (6). Prior to assay, algal suspensions were sparged with argon for 90 minutes to remove the dissolved nitrogen (and other gases) completely. Procedures for preincubation (alternate exposure to acetylene in argon and argon respectively, each period lasting for 30 min) and acetylene reduction were described earlier (1,2). All assays were carried out in anaerobic (argon) atmosphere. Inhibitors (substrates) were added to control or preincubated algae prior to assay. All the data were treated by the standard linear regression analysis (7). Chlorophyll a was measured after Mackinney (8). Gases used were high purity products from Indian Oxygen Ltd., Bombay, India. All the chemicals were obtained from British Drug Houses, Poole, England.

RESULTS AND DISCUSSION: It has been established (3,4,5) that multiple reactions of nitrogenase are not catalysed at a single unmodified active site. However, it has not been clear whether there are multiple sites or the properties of one or more sites are modified in the presence of specific substrates. The data in Fig. 1 show normal Michaelis-Menten kinetics for the effect of acetylene concentration on acetylene reduction activity of Anabaena L-31 in vivo. The calculated values of Michaelis constants (K_m) for control and preincubated algae are 0.015 atm

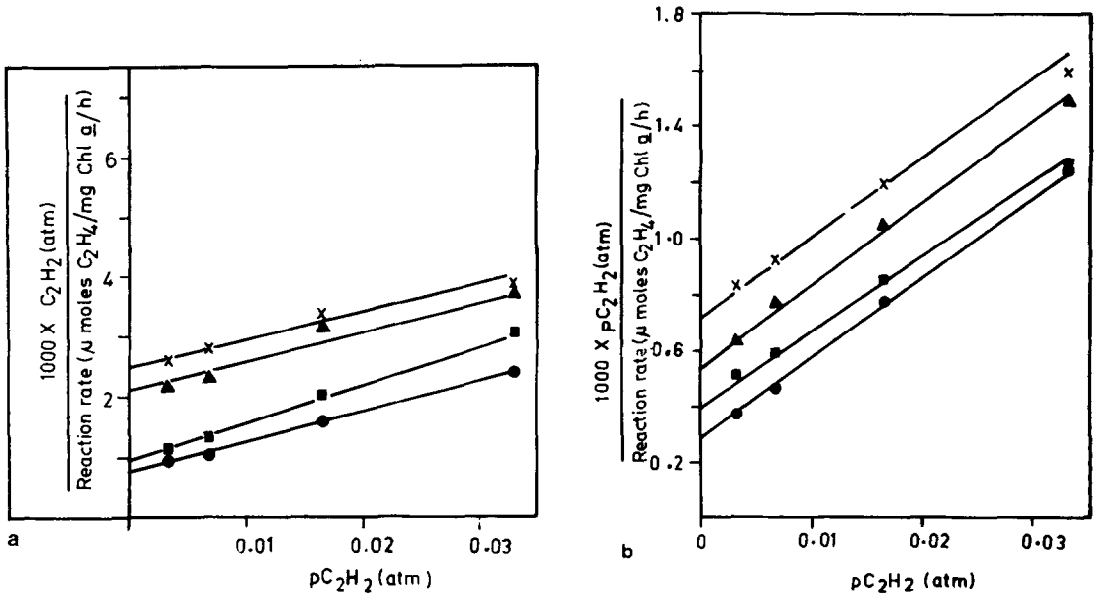

Fig. 2. Effect of nitrogen on acetylene reduction by (a) control and (b) preincubated Anabaena L-31 The pN_2 (atm) was as follows: (●) 0.0, (■) 0.2, (▲) 0.5, (✖) 1.0.

and 0.006 atm respectively. This clearly shows that upon preincubation under acetylene a conformational change occurs at the site of acetylene binding, such that its affinity for acetylene increases by nearly 2.5 fold.

Nitrogen has been shown to inhibit acetylene reduction competitively (4) while azide and carbon-monoxide (CO) are non-competitive inhibitors of acetylene reduction (3,4). Figs. 2-4 show the effect of these inhibitors on the normal and modified (after preincubation) site for acetylene.

Nitrogen and acetylene appear to share the same site on the nitrogenase complex as is shown by competitive inhibition of acetylene reduction by nitrogen (Fig. 2). The calculated

Table 1

Changes in affinity for substrates and inhibitors of acetylene reduction after preincubation

Substrate or inhibitor	Nature of inhibition	Calculated K_m/K_i*	
		Control	Preincubated algae
1. C_2H_2	-	0.015 atm	0.006 atm
2. N_2	Competitive	0.37 atm	0.80 atm
3. CO	Non-competitive	0.0005 atm	0.0013 atm
4. NaN_3	Non-competitive	2.2 mM	2.2 mM

*K_i values were calculated using secondary plots (intercepts versus inhibitor concentrations) of Figs. 2-4.

values of inhibition constants (K_i) for control and preincubated algae are 0.37 atm and 0.80 atm respectively (Table 1). Thus the sensitivity to nitrogen decreases by about 2.2 fold (in agreement with the increase in affinity for acetylene by 2.5 fold). This again demonstrates that a structural modification at the nitrogen/acetylene site does occur after preincubation.

Azide inhibits acetylene reduction non-competitively (Fig. 3) and calculated (K_i) value (2.2 mM) does not change after preincubation (Table 1). This is as would be expected since earlier work indicates that azide and acetylene do not share the same site (3,4).

Carbon-monoxide also inhibits acetylene reduction non-competitively (Fig.4). Surprisingly however K_i value for CO (0.0005 atm) increases to 0.0013 atm after preincubation. This perhaps means that yet another structural alteration

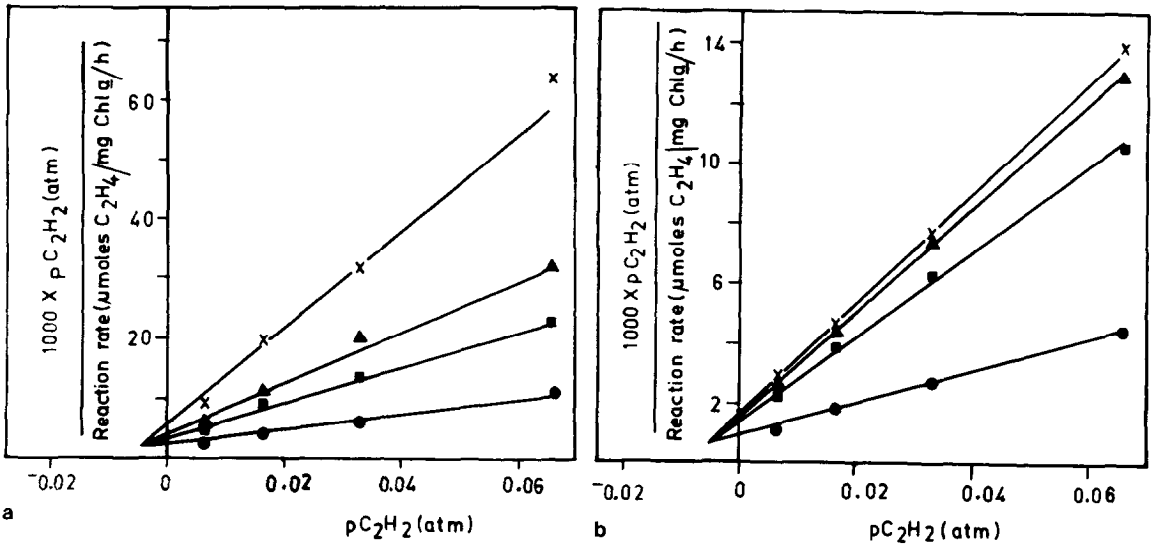

Fig. 3. Effect of azide on acetylene reduction by (a) control and (b) preincubated Anabaena L-31. The concentration of azide in mM was as follows: (●)0.0, (■)1.0, (▲)2.0, (×)5.0.

occurs at the site for CO (in addition to the one that occurs at the site for C_2H_2) upon preincubation under acetylene.

Table 1 summarizes all the results. It is evident that preincubation under acetylene results in certain conformational changes in the enzyme-complex especially at the site shared by N_2 and C_2H_2. Based on the findings that N_2 is competitive with C_2H_2 while C_2H_2 is non-competitive with N_2 (3,4) it was suggested that N_2 and C_2H_2 have different binding sites but "tap a common sink of electrons" (4). However this does not seem to be the case since acetylene preincubation equally alters both the K_m and K_i for C_2H_2 and N_2 respectively. It seems more likely therefore that C_2H_2 and N_2 share a common site. The possibility that preincubation may make more sites amenable to acetylene binding is ruled out by the present finding (Fig. 1) that

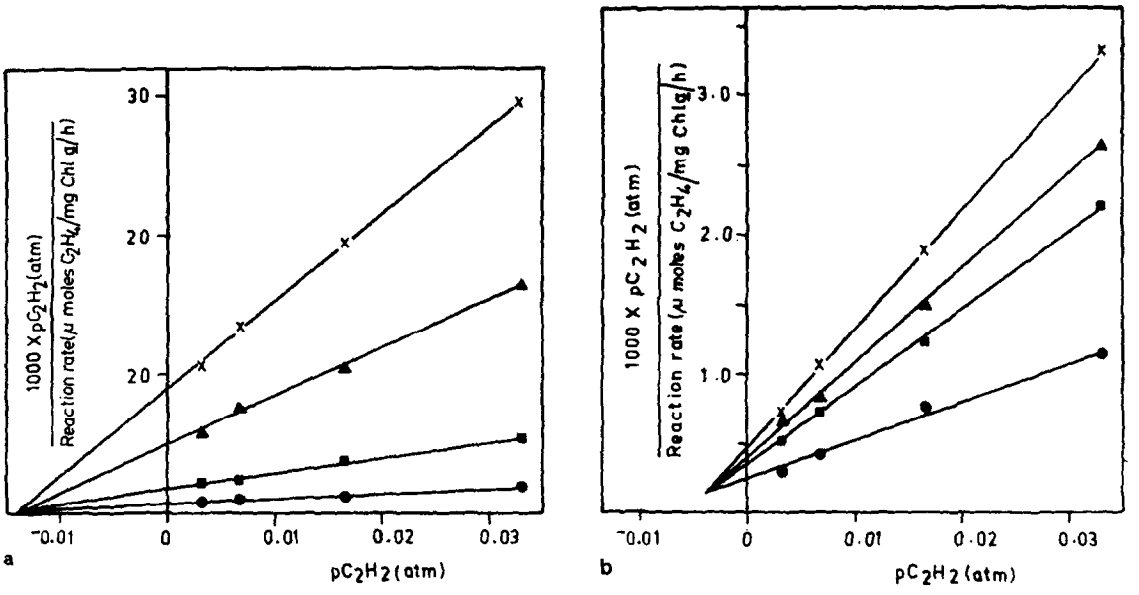

Fig. 4. Effect of carbon-monoxide on acetylene reduction by (a) control and (b) preincubated Anabaena L-31. The pCO (atm) was as follows: (●)0.0, (■)0.0006, (▲)0.0012, (✖)0.002.

acetylene reduction shows a linear Michaelis-Menten kinetics even after preincubation. Further, the competitive or non-competitive nature of inhibition by different inhibitors does not change after preincubation, as would be expected if: (i) new sites were coming into play or (ii) sites for other substrates were also binding acetylene after preincubation. The structure and arrangement of the various sites on nitrogenase molecule are not known at present and the nature of these conformational changes still remains unclear.

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