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ACETYLENE REDUCTION BY THE IRON-MOLYBDENUM COFACTOR FROM NITROGENASE

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SUMMARY

The iron-molybdenum cofactor isolated from component I of nitrogenase catalyzes the reduction of acetylene to ethylene in the presence of sodium borohydride. Like nitrogenase, this activity is strongly inhibited by carbon monoxide. From the initial rates of the reaction, the specific activity of the iron-molybdenum cofactor is 34 nmoles of ethylene formed per minute per nmole of Mo in the cofactor. This activity is about 8% of the activity of nitrogenase with an equivalent amount of Mo. ATP has no stimulatory effect on the cofactor activity.

INTRODUCTION

One of the interesting properties of nitrogenase is that the enzyme catalyzes the reduction of a variety of substrates other than N_2 (1). One of these substrates is acetylene, which is reduced to ethylene (2). In fact, this reaction is the basis of the acetylene-reduction assay for N_2 fixation--a reaction that has greatly simplified studies of N_2 fixation at the basic and applied levels. Nitrogenase is composed of two proteins, components I and II (3). Component I seems to be the protein to which the substrate binds and subsequently is reduced. Component II donates electrons to component I (4). Nitrogen reduction to NH_4^+ and acetylene reduction to ethylene are catalyzed by these two proteins in vitro in the presence of a suitable reducing agent and ATP (5). Component I contains 2 Mo, 33 Fe, and 27 acid-labile sulfides per molecular weight of 250,000 (6).

Several mutant strains of N_2-fixing bacteria (7, 8) produce an inactive component I which can be activated in vitro upon addition of FeMo-co isolated

Abbreviation : FeMo-co, iron-molybdenum cofactor

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from pure component I (6). The FeMo-co contains Mo, Fe, and $S^{=}$ in the ratio of 1: 8 : 6. In this paper we show that FeMo-co, by itself, catalyzes acetylene reduction.

MATERIALS AND METHODS

The FeMo-co was isolated from crystalline component I (9) of Azotobacter vinelandii by the method of Shah and Brill (6). Sodium borohydride and the Mo standard solution were obtained from Fisher Scientific Co., Fairlawn, N.J. Other chemicals used were of analytical grade available commercially. Acetylene was passed through concentrated H_2SO_4 before use.

Acetylene reduction was carried out in a 3 ml reaction volume in 25 ml serum vials under a H_2 atmosphere in a water-bath shaker at 24°, unless otherwise specified. All reagents were evacuated and flushed with H_2 gas. The complete test system (3 ml) contained 0.2 mmoles of borate-NaOH buffer (pH 9.6), 1.2 mmoles of $NaBH_4$, 2.5 µmoles of $Na_2S_2O_4$, 1 to 10 nmoles of Mo as FeMo-co, and 0.25 atm of C_2H_2. Control vials contained equivalent amounts of N-methylformamide because FeMo-co was in a N-methylformamide solution. Ethylene formed was measured by gas chromatography (10). Molybdenum content was determined by the method of Clark and Axley (11).

RESULTS AND DISCUSSION

The dependence of acetylene reduction on the concentration of FeMo-co is shown in Fig. 1. From the initial reaction rates, the specific activity was 34 nmoles ethylene formed per min per nmole Mo in the cofactor. On the basis of Mo contents, the apparent turnover of FeMo-co is 8% that of the turnover of nitrogenase (6) in the acetylene-reduction assay. Under the assay conditions described for acetylene reduction, we have been unable to reduce N_2 to NH_4^+.

Like nitrogenase (12), FeMo-co dependent acetylene reduction is strongly inhibited by CO (Fig. 1). In contrast, acetylene reduction by synthetic Mo-complexes (13-15) is only weakly inhibited by CO. Under comparable conditions, the turnover number of FeMo-co in the acetylene reduction system is about 600 times more than that of the Mo-glutathione (13) and Mo-cysteine systems (14, 15).

In nitrogenase-catalyzed acetylene reduction, ethylene is the only product (2). In contrast, during acetylene reduction by the Mo-glutathione and Mo-cysteine systems, high percentages of ethane and/or butadiene also are formed along with ethylene (13-16). In FeMo-co catalyzed acetylene reduction the major product is ethylene. Within experimental error, no ethane or butadiene were detected.

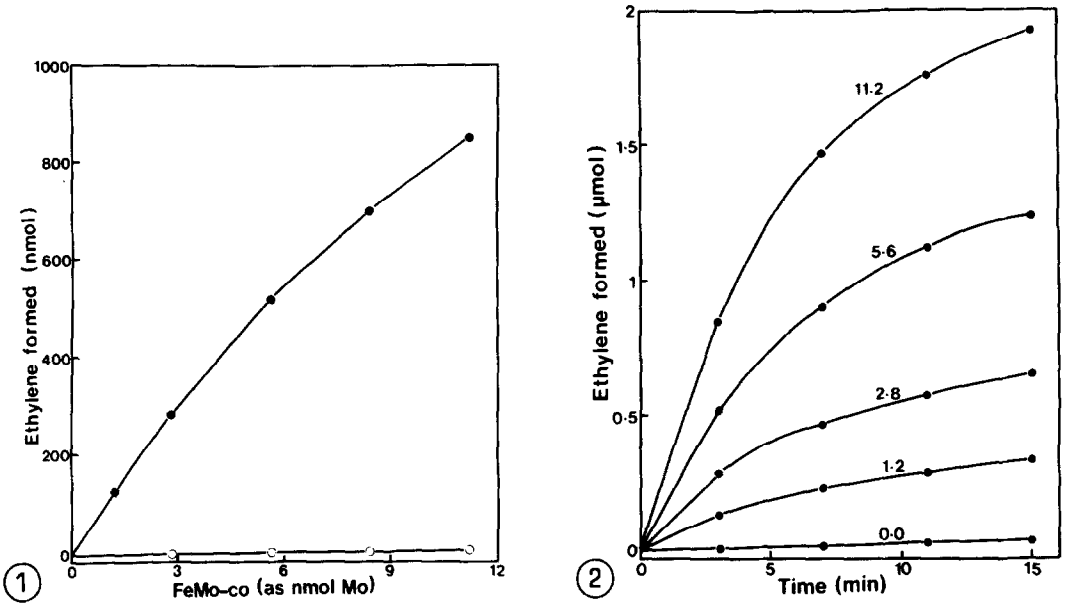

Fig. 1. Effect of FeMo-co concentration on acetylene reduction. • —— • 0.75 atm H_2 + 0.25 atm C_2H_2, o —— o 0.75 atm CO + 0.25 atm C_2H_2. The incubation time is 3 min.

Fig. 2. Effect of time of incubation on acetylene reduction. Values on the figure represent FeMo-co concentrations (as nmoles Mo) added to the assay.

The effect of incubation time on acetylene reduction with varying concentrations of FeMo-co is shown in Fig. 2. The rate is high initially and then drops progressively with time. At lower concentrations of FeMo-co, ethylene formed is proportional to the concentration of FeMo-co. At higher concentrations of FeMo-co, more ethylene is formed but the specific activity decreases substantially. One atmosphere of acetylene or 0.75 atm of helium (instead of H_2) plus 0.25 atm acetylene had no effect on the rate of acetylene reduction. Increasing the reaction temperature to 30° or addition of ATP did not stimulate the rate of acetylene reduction. When FeMo-co was exposed to air for 18 h at 4°, it still retained 84% of the original acetylene-reducing activity. This is in contrast to the extreme O_2-sensitivity of FeMo-co, when tested by in vitro activation of a mutant strain (6). Pure

component I did not reduce acetylene under the conditions used for FeMo-co catalysis.

Physiological and genetic studies of nitrogenase (7, 17) showed that an electron paramagnetic resonance signal with g= 3.65 was caused by an active site on component I. FeMo-co is responsible for this signal (18). The data presented here further support the idea that FeMo-co is an active site of nitrogenase and probably is the site to which N_2 binds and is subsequently reduced. An important question that remains to be answered is whether this activity is catalyzed by the same site on FeMo-co as acetylene reduction in nitrogenase. If the mechanisms are the same, then insight into the mechanism of acetylene reduction by FeMo-co should yield important clues into the mechanism of N_2 fixation by nitrogenase. This should be helpful for designing new catalysts for industrial N_2 fixation.

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