Interactions Among Substrates and Inhibitors of Nitrogenase

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Examination of interactions among various substrates and inhibitors reacting with a partially purified nitrogenase from Azotobacter vinelandii has shown that: nitrous oxide is competitive with N₂; carbon monoxide and acetylene are noncompetitive with N₂; carbon monoxide, cyanide, and nitrous oxide are noncompetitive with acetylene, whereas N₂ is competitive with acetylene; carbon monoxide is noncompetitive with cyanide, whereas azide is competitive with cyanide; acetylene and nitrous oxide increase the rate of reduction of cyanide. The results are understandable if nitrogenase serves as an electron sink and substrates and inhibitors bind at multiple modified sites on reduced nitrogenase. It is suggested that substrates such as acetylene may be reduced by a less completely reduced electron sink than is required for the six-electron transfer necessary to reduce N₂.

Nitrogenase is a versatile enzyme which is capable of reducing N₂, nitrous oxide, acetylene, azide, cyanide, methyl isocyanide, protons, and analogs of some of these compounds. Nitrogenase consists of two proteins, a molybdenum-iron protein (MoFe protein) and an iron protein (Fe protein); neither has been demonstrated to have catalytic activity by itself. Under physiological conditions the Fe protein is reduced by ferredoxin or a flavoprotein. The Fe protein specifically binds magnesium adenosine 5′-triphosphate (MgATP) (3, 29); upon binding the MgATP the potential of the Fe protein becomes substantially more negative (7, 27, 31), and it acquires the unique ability to reduce the MoFe protein. The reduced MoFe protein serves as an electron sink capable of reducing all of the substrates of nitrogenase; all substrates compete for electrons from the reduced MoFe protein. Although reduction of substrates always has required both proteins in experimental systems, it is quite possible that reduced MoFe protein if separated from the Fe protein would be capable of reducing substrates by itself. The present paper extends earlier work (2, 20, 23) and explores the interactions among substrates and inhibitors of nitrogenase in an attempt to learn more about the substrate and inhibitor binding sites and the mode of action of nitrogenase. The information discussed has been recorded in more detail by Rivera-Ortiz (M. S. thesis, Univ. of Wisconsin, Madison, 1973).

MATERIALS AND METHODS

Cylinder gases (Matheson Gas Products) were passed over BASF catalyst R3-11 (Chemical Dynam-
separated component proteins, removes the necessity for recombination of nitrogenase components in the proper proportions and catalyzes all the reactions reported for nitrogenase.

The mixture for measurement of nitrogenase activity contained in 1 ml: 40 µmol of creatine phosphate, 0.1 to 0.25 mg of creatine kinase (2.7.3.2, Sigma Chemical Co., 50 to 100 U/mg of protein), 6 µmol of ATP, 5 µmol of MgCl₂, 50 µmol of tris(hydroxymethyl)aminomethane at pH 7.3, 15 µmol of Na₂S₂O₄, enzyme preparation with 0.5 to 2.0 µg of protein (protein determined by the method of Gornall et al. [14]). The components (minus Na₂S₂O₄ and the enzyme preparation) were mixed in a 21-ml serum bottle closed with a rubber serum stopper; the bottle was evacuated and refilled four times with argon through a hypodermic needle. Substrate (often gaseous) was added, and the pressure was brought to 1 atm with argon. The bottle was shaken for 5 min in a 30°C water bath before the Na₂S₂O₄ and enzyme preparation in anaerobic solution were injected to start the reaction. After 15 min, the reaction was terminated by injecting 1 ml of 1.25 M trichloroacetic acid (when following reduction of C₂H₂ or CN⁻) or 1 ml of saturated K₂CO₃ (when measuring N₂ fixation).

Fixation of N₂ was evaluated by measuring NH₃ formed; after microdiffusion of NH₃ (6), it was determined with the indophenol method of Chaykin (10). C₂H₂ and CH₄ produced (13, 15, 17, 26) from C₂H₂ and CN⁻, respectively, were measured by gas chromatography with a flame ionization detector (150 cm long, 2 mm ID column of Porapak R, 50 C; N₂ carrier gas: 0.5 ml gas samples). H₂ evolution was determined in Gilson constant pressure all glass volumeters or by the appearance of mass 2 as analyzed in an isotope ratio mass spectrometer.

Data used to calculate Michaelis constants were treated by the computer program described by Cleland (11, 12). Inhibition patterns were obtained initially with double reciprocal plots (1/velocity versus 1/substrate concentration); these patterns defined the appropriate rate equations conforming to fortran programs of Cleland (11, 12). Analysis of these programs yielded values for inhibition constants (Kᵢ, Kᵢᵣ relative to intercept; Kᵢᵣ relative to slope) maximum velocities (V), Michaelis constants (Kₘ), and standard error of the estimates. The computer plots included all data and assumed equal variances for all velocities; lower velocities carry a greater intrinsic error. Data showing competitive inhibition were fitted to the equation: $v = \frac{VA}{[K(1 + I/Kᵢᵣ) + A]}$ in which $A$ = substrate concentration, $I$ = inhibitor concentration, and $v$ = velocity. Data showing noncompetitive inhibition were fitted to the equation: $v = \frac{VA}{[K(1 + I/Kᵢᵣ) + A(1 + I/Kᵢ)]}$.

**RESULTS AND DISCUSSION**

Measurements of the time course of NH₃ formation at 0.05 and 0.40 atm N₂ indicated no initial lag and linear production of NH₃ with time for 25 min. Likewise, production of C₂H₂ from C₂H₂ at 0.005 and 0.125 atm showed no lag and was linear for 25 min. Production of CH₄ from 1.04 and 2.96 mM CN⁻ was linear for about 20 min and showed no lag. H₂ evolution was linear without lag for 35 min. A 15-min reaction period was adopted for all experiments to avoid any nonlinear responses with time.

**Apparent Michaelis constants.** Measurements of the $Kₘ$ for N₂ gave a value of 0.136 ± 0.003 atm (7.75 × 10⁻⁵ M); this is in good agreement with other reported values (18, 19, 28). The $Kₘ$ for C₂H₂ was 0.012 ± 0.002 atm (4.33 × 10⁻⁴ M); the value did not differ greatly from most others in the literature (13, 15, 19, 21, 26), but was quite different from the value of 0.001 atm reported by Hardy and Knight (18). Hardy et al. (16) later revised their estimates upward to 0.002 to 0.009 atm for A. vinelandii.

Determining the $Kₘ$ for CN⁻ poses special problems, because above 4 mM concentration CN⁻ inhibits its own reduction markedly (see Fig. 5). By assuming that self-inhibition by CN⁻ is negligible at low concentrations and by using data up to 4 mM CN⁻, one arrives at an apparent $Kₘ$ of 1.23 ± 0.22 mM for CN⁻. Hwang and Burris (19) reported 1.28 mM, but Biggins and Kelly (1) reported 0.19 mM for nitrogenase of Klebsiella pneumoniae. Hardy and Knight (18) found that CN⁻ inhibited ATP hydrolysis and they pointed out that their apparent $Kₘ$ of 4 mM for an extract from A. vinelandii, "is susceptible to error because of substrate inhibition which occurs above 5 mM." Later Hardy et al. (16) revised the $Kₘ$ downward to 0.4 to 1.0 mM.

**Evolution of H₂.** Reduced nitrogenase plus MgATP reduces H⁺ to H₂; the reaction is termed ATP-dependent H₂ evolution. Because this reaction draws electrons from the common electron sink of nitrogenase, evolution of H₂ is inhibited by the presence of other nitrogenase substrates competing for electrons from the sink. As indicated by Hardy et al. (16), C₂H₂ is particularly effective in suppressing evolution of H₂.

Figure 1 indicates that H₂ evolution continues at about 35% of its maximal rate even in the presence of 1 atm of N₂. If the distance from line A to the curve is plotted versus pN₂, a curve is obtained corresponding to the mirror image of the inverted curve shown (appearance is like a substrate concentration versus rate curve). Analysis of this derived curve by a 1/u versus 1/pN₂ plot (insert curve is calculated with the Hypoer program of Cleland [11] which gives apparent maximum velocity and the standard error of the estimate) defines the maximum velocity (y intercept) in relative terms at infinite pN₂. Values for V from three experiments
were 0.749 ± 0.018, 0.808 ± 0.025 and 0.760 ± 0.016; average 0.77 (23% residual H₂ evolution). If one uses measured velocities of H₂ evolution and produces a double reciprocal plot by visual inspection, the velocity of H₂ evolution at infinite pN₂ appears to be 13 to 15% of its velocity in the absence of N₂. The results with either analysis indicate that the pN₂ cannot be raised to a point where it will block evolution of H₂ completely; at infinite pN₂ the evolution of H₂ still continues at a rate 13% to 23% its maximum value.

Figure 2 indicates the influence of the partial pressure of C₂H₂ on evolution of H₂. Treating the data as in Fig. 1 yielded the following values for V (Maximum velocity from Hypero computer program of Cleland, 11): 0.95 ± 0.01, 1.01 ± 0.07 and 1.04 ± 0.02; average 1.00. In contrast to the response to N₂, evolution of H₂ would be suppressed completely by infinite pC₂H₂. Evolution of H₂ responds in a qualitatively different fashion to N₂ and to C₂H₂. The effect of CN⁻ on evolution of H₂ is similar to the effect of C₂H₂ in the sense that an infinite concentration of cyanide would suppress evolution of H₂ completely. However, CN⁻ in addition exhibits powerful substrate inhibition.

N₂ is unable to block evolution of H₂ completely, whereas C₂H₂ or CN⁻ at infinite concentration is able. These observations emphasize that there are differences in the reduction of N₂ and its alternative substrates, hence observations on reduction of C₂H₂ or CN⁻ should be related to N₂ reduction with caution.

**Inhibition of reduction of N₂.** Carbon monoxide. Nitrogenase was incubated for 15 min in the presence of 0.01 or 0.02 atm CO. When the CO was pumped off, the activity was restored completely; this confirms that the inhibitory effect of CO at these levels is completely reversible. In contrast, inhibition by NO was only partially reversible, and its inhibitory action was not studied further.

CO generally is accepted as a noncompetitive inhibitor of N₂ fixation, but there have been ambiguities in some experiments (2, 8, 23). The data obtained with the nitrogenase preparations from *A. vinelandii* showed unequivocal noncompetitive inhibition of N₂ reduction by CO. The $K_{1a} = 0.0004 ± 0.00008$ atm and the $K_{1b} = 0.0015 ± 0.0002$ atm CO.

**Acetylene.** Reports have differed on the nature of C₂H₂ inhibition of N₂ reduction (20, 26). Figure 3 shows clearly that the inhibition is noncompetitive in agreement with Hwang et al. (20). The $K_{Ia} = 0.0052 ± 0.0019$ atm and the $K_{II} = 0.0064 ± 0.0021$ atm C₂H₂.

**Nitrous oxide.** There have been few studies of N₂O as an inhibitor of N₂ fixation, but it has been concluded that N₂O is competitive with N₂ (25). The present studies yielded the data summarized in Fig. 4; the inhibition is competitive in these preparations. The inhibitor constant was $K_I = 0.108 ± 0.015$ atm N₂O. Although it is evident that N₂ and N₂O bind to the same enzyme site, it should be pointed out that
In preliminary experiments there was a deviation from linearity for points corresponding to a low pN$_2$ in the presence of a higher pN$_2$O. Nevertheless, analysis confirmed the competitive nature of the inhibition.

**Inhibition of reduction of cyanide.** The use of cyanide as a substrate poses problems, because it is inhibitory to its own reduction (Fig. 5), it is volatile under acid conditions, and the relative effectiveness of HCN and CN$^-$ as substrate species is not established. Ljones (22) has shown that CN$^-$ inhibits electron transfer in the nitrogenase system. All our experiments were performed with concentrations of CN$^-$ less than 4 mM to minimize any inhibition by the CN$^-$. Rates of reduction of CN$^-$ by nitrogenase are lower than its rates of reduction of most other substrates (16, 30).

**Carbon monoxide.** CO inhibits all reductions by nitrogenase except the reduction of protons to H$_2$. CO proved to be a noncompetitive inhibitor of CN$^-$ reduction (Fig. 6). A somewhat higher level of CO is required to give 50% inhibition of CN$^-$ than of N$_2$ reduction. The

![Figure 3](http://jb.asm.org/)

**FIG. 3.** Inhibition of N$_2$ reduction by C$_2$H$_2$. The reaction mixture was described in Fig. 1. All lines were derived by computer analysis. Velocity $v = \text{micromol of NH}_4^+$ produced in 15 min. The pC$_2$H$_2$ in atm was as follows: (▲) 0.0, (□) 0.002, (△) 0.004, (○) 0.006.

![Figure 4](http://jb.asm.org/)

**FIG. 4.** Inhibition of N$_2$ reduction by N$_2$O. The reaction mixture was described in Fig. 1. All lines were derived by computer analysis. The pN$_2$O in atm was as follows: (▲) 0.0, (□) 0.02, (△) 0.04, (○) 0.08.

![Figure 5](http://jb.asm.org/)

**FIG. 5.** Effect of cyanide on methane formation from cyanide. The reaction mixture was described in Fig. 1. The reaction was run at 30 C for 15 min under argon. Velocity $v = \text{micromoles of CH}_4$ produced in 15 min. The insert is a Lineweaver-Burk plot of the first six points of the main curve.
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Fig. 6. Inhibition of cyanide reduction by carbon monoxide. The reaction mixture was described in Fig. 1. Reactions were run under argon at 30°C, and the cyanide concentration was varied as indicated. Velocity, \( v = \text{micromoles CH}_4 \text{ produced in 15 min.} \) The pCO in atm was as follows: (\( \triangledown \)) 0.0, (\( \square \)) 0.001, (\( \triangle \)) 0.003, (\( \bigcirc \)) 0.005. Computer analysis indicated that \( K_H = 0.0026 \pm 0.0008 \text{ atm and } K_{is} = 0.0028 \pm 0.0010 \text{ atm CO.} \)

Azide. Azide reduction is competitively inhibited by CN\(^-\) (20). As shown in Fig. 7, azide also is a competitive inhibitor of CN\(^-\) reduction. Apparently azide, CN\(^-\) and methyl isocyanide all bind at the same site, as they are mutually competitive. The \( K_i \) for azide as an inhibitor of CN\(^-\) reduction was found to be 2.38 \pm 0.28 mM. The \( K_m \) for azide has been reported as 1.15 mM (19) and 1.0 mM (16).

Acetylene. Biggins and Kelly (1) reported that \( \text{C}_2\text{H}_2 \) enhanced formation of \( \text{CH}_4 \) from CN\(^-\) by nitrogenase. We also observed that \( \text{C}_2\text{H}_2 \) enhanced \( \text{CH}_4 \) formation from CN\(^-\); the concentration of \( \text{C}_2\text{H}_2 \) giving greatest enhancement varied with the level of CN\(^-\) present. \( \text{C}_2\text{H}_2 \) at a level of 0.2 atm enhanced production of \( \text{CH}_4 \) from 0.5 mM CN\(^-\) by about 40%. When 0.05 atm \( \text{C}_2\text{H}_2 \) was present, CN\(^-\) inhibition of CN\(^-\) reduction to \( \text{CH}_4 \) (up to 6 mM CN\(^-\)) was suppressed as shown in Fig. 8 (compare with Fig. 5). Apparently CN\(^-\) inhibits its own reduc-

![Fig. 6](http://jb.asm.org/)

![Fig. 7](http://jb.asm.org/)

![Fig. 8](http://jb.asm.org/)
suggested that CN⁻ was bound specifically by the MoFe protein. Hardy and Knight (18) found that when the reduction of CN⁻ is blocked by CO, CN⁻ still inhibits evolution of H₂ (CO does not inhibit evolution of H₂).

Nitrous oxide. N₂O like C₂H₂ enhances the rate of CN⁻ reduction (Fig. 9). Enhancement was seen when 0.05, 0.1 and 0.2 atm N₂O was added to CN⁻ at concentrations of 0.30, 0.50, 0.99, and 1.28 mM. The enhancement increased with increasing pN₂O up to 1.0 atm. The highest concentration of N₂O used (Fig. 9), and the curve still was rising at this point. It is interesting that C₂H₂ and N₂O have similar effects on reduction of CN⁻, because N₂O is a noncompetitive inhibitor of the C₂H₂ reduction, and hence they do not appear to bind at the same site for their reduction.

Dinitrogen. N₂ had little effect on reduction of CN⁻. When the concentration of CN⁻ was 1.96 mM, 1.0 atm of N₂ decreased CN⁻ reduction to CH₄ by 14%. When concentrations of CN⁻ as low as 0.1 mM were used, the highest inhibition by N₂ was 22%. Under these conditions, the pattern of N₂ inhibition could not be determined.

It would be interesting to determine the production of H₂ simultaneously with production of CH₄ to obtain a more complete picture of reductive processes when CN⁻ is furnished as substrate and alternative substrates also are present. It is evident that CN⁻ must be used as a nitrogenase substrate with caution because of its self-inhibitory action and the enhancement of its reduction by C₂H₂ or N₂O.

Inhibition of reduction of acetylene. Reduction of C₂H₂ has acquired importance because of the extensive use of C₂H₂ reduction as an index of N₂ fixation. The assumption often is made that N₂ and C₂H₂ are strictly equivalent as substrates for nitrogenase. However, there are demonstrable differences between them, other than the requirement of six electrons for reduction of N₂ to 2 NH₃ and 2 electrons for reduction of C₂H₂ to C₂H₄. Hwang et al. (20) reported that azide and CO are noncompetitive inhibitors of C₂H₂ reduction, and that H₂ does not inhibit the reduction.

Carbon monoxide. When 0.1 atm of C₂H₂ was present, 0.005 atm CO gave about 50% inhibition. The inhibition was noncompetitive as reported by Hwang et al. (20).

Nitrous oxide. C₂H₂ was furnished as substrate at 0.1 atm, and its reduction was inhibited about 50% by 0.3 atm N₂O. The inhibition is noncompetitive (Fig. 10). The observations that H₂ competitively inhibits N₂ fixation but has no effect on reduction of C₂H₂ and that N₂O is a competitive inhibitor of N₂ fixation but a noncompetitive inhibitor of reduction of C₂H₂ give further support to the suggestion that the binding sites for N₂ and C₂H₂ are not equivalent. The fact that C₂H₂ is a noncompetitive inhibitor of N₂ reduction reinforces the concept of distinguishable sites.

Cyaniide. CN⁻ strongly inhibits reduction of C₂H₂; in the presence of 0.05 atm C₂H₂, 0.5 mM CN⁻ effects about 50% inhibition. The inhibition pattern (Fig. 11) is noncompetitive. CN⁻ has been reported to be noncompetitive toward N₂ and competitive toward azide reduction (20). Whereas CN⁻ is a powerful inhibitor of C₂H₂ reduction, C₂H₂ enhances reduction of CN⁻.

Dinitrogen. Reduction of C₂H₂ is inhibited by N₂ (Fig. 12); when 0.02 atm C₂H₂ was present, 0.6 atm N₂ caused about 33% inhibition. With 0.10 atm C₂H₂ no measurable inhibition was demonstrable with 0.80 atm N₂. The inhibition is competitive, and the Kᵢ values determined in five experiments were: 0.389 ± 0.059, 0.395 ± 0.060, 0.572 ± 0.118, 0.381 ± 0.025 and 0.262 ± 0.023 atm N₂; the average was 0.40 atm N₂. The competitive nature of this inhibition is not reciprocal in the sense that C₂H₂ is a noncompetitive inhibitor of N₂ reduction.

Conclusion. Nitrogenase is a versatile enzyme system which can reduce a variety of substrates. It is clear from the current observations and the earlier work of others (16, 18–20, 23) that the interactions are complex among these substrates and among inhibitors which are not reduced by nitrogenase. Table 1 summarizes observations which have been made to date on substrate and inhibitor interactions. Although some blanks remain in the
chart, notably N₂O and CH₂NC as substrates and NO as inhibitor, a number of conclusions can be drawn from the available data.

In interpreting the interactions among substrates and inhibitors, we will assume that the MoFe protein binds substrates and that the reduced MoFe protein of nitrogenase serves as the electron sink which reduces all nitrogenase substrates. Any blockage of electron transport before the electrons reach the MoFe protein would block all reductions by nitrogenase, although a partial inhibition in electron flow to the MoFe protein could affect reduction of substrates differentially.

The fact that the presence of one substrate decreases the reduction rate of other substrates supports the concept that all are bound to the reduced MoFe protein. However, there is a nonequivalence of the binding sites. As pointed out by Hwang et al. (20), the responses of the substrates and inhibitors suggest modified sites to accommodate:

(i) N₂, H₂, N₂O (H₂ and N₂O competitive inhibitors of N₂);
(ii) CN⁻, N₂O⁻, CH₂NC (mutually competitive but noncompetitive with N₂);
(iii) C₂H₂ (noncompetitive with the N₂ and N₂O; C₂H₂ enhances CN⁻ reduction; C₂H₂ can block H₂ evolution completely; N₂ is competitive with C₂H₂);
(iv) CO (noncompetitive with substrates and does not inhibit H⁺ reduction);
(v) H₂ evolution (not identical to N₂ site as H₂ does not inhibit; unique in escaping CO inhibition).

New elements which have been added by the present work are the demonstration that N₂
Table 1. Nature of inhibition by some electron acceptors and inhibitors of nitrogenase

<table>
<thead>
<tr>
<th>Substrate</th>
<th>( N_2 )</th>
<th>( N_2^{*} )</th>
<th>( C_2H_2 )</th>
<th>HCN</th>
<th>( N_2O )</th>
<th>( H_2 )</th>
<th>CO</th>
<th>( CH_3NC )</th>
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<tr>
<td>( N_2 )</td>
<td>NC</td>
<td>NC</td>
<td>NC(^*)</td>
<td>C</td>
<td>C</td>
<td>NC</td>
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<td>C</td>
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<tr>
<td>( N_2^{*} )</td>
<td>NC(^*)</td>
<td>C</td>
<td>NC(^*)</td>
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<tr>
<td>( C_2H_2 )</td>
<td>C</td>
<td>NC(^*)</td>
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<td>C</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>NC</td>
<td>NC(^*)</td>
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<tr>
<td>( H^+ )</td>
<td>I</td>
<td>I(^*)</td>
<td>I</td>
<td>I</td>
<td>I(^*)</td>
<td>I</td>
<td>NI</td>
<td>NI(^*)</td>
</tr>
</tbody>
</table>

\(^a\) Symbols: C, competitive inhibition; NC, noncompetitive inhibition; NI, does not inhibit; I, inhibits; E, enhances.

\(^b\) Reference 20.

\(^c\) Reference 5.

Competitively inhibits reduction of \( C_2H_2 \) (it had been known that \( C_2H_2 \) is noncompetitive with \( N_2 \)); that \( CN^-, N_2O \) and CO are noncompetitive with \( C_2H_2 \); that \( N_2O \) enhances reduction of \( CN^- \); and that \( N_2^+ \) is a competitive and CO a noncompetitive inhibitor of \( CN^- \) reduction.

Assimilating most of the data into the earlier scheme of Hwang et al. (16) presents no difficulty. However, the escape of \( H_2 \) evolution from CO, which inhibits all other reductions, and explaining the nonreciprocal responses of \( N_2 \) and \( C_2H_2 \) pose problems.

The escape of \( H_2 \) evolution, which requires electrons from reduced MoFe protein, from CO inhibition indicates that CO cannot block electron transfer before the electrons reach the MoFe protein; rather, CO blocks electron transfer from the MoFe protein to the various substrates. The site for reduction of \( H^+ \) is unique, for steric or other reasons, in escaping the CO block (note that other substrates decrease the rate of \( H^+ \) reduction, because all draw from the same electron sink).

The nonreciprocal response of \( N_2 \) and \( C_2H_2 \) poses a different problem. The competitive inhibition of \( C_2H_2 \) reduction by \( N_2 \) provides evidence for suggesting that \( C_2H_2 \) and \( N_2 \) bind at the same site, and a model for reduction of these substrates can be based on the concept of a single site. However, the noncompetitive inhibition of \( N_2 \) reduction by \( C_2H_2 \) indicates that these substrates bind at separate sites, and the competitive inhibition of \( C_2H_2 \) reduction by \( N_2 \) shows that \( C_2H_2 \) at high concentrations can completely overcome the inhibitory effect of \( N_2 \). We will postulate a model possessing separate sites for \( N_2 \) and \( C_2H_2 \). A model which predicts nonreciprocal inhibitory behavior between \( C_2H_2 \) and \( N_2 \) calls for the electron sink of the MoFe protein to contain six electrons in order to reduce \( N_2 \), but only two electrons to reduce \( C_2H_2 \), and for the flow of electrons from the Fe protein to be rate limiting for reduction of good substrates such as \( C_2H_2 \) and \( N_2 \). \( C_2H_2 \) and \( N_2 \) each has its own binding site, and their mutual inhibition arises from the fact that they are tapping a common sink of electrons. Whereas high \( C_2H_2 \) will keep this sink depleted, so that it never will contain more than two electrons (and thus will be incapable of reducing \( N_2 \)), high \( N_2 \) never can prevent access to the sink by \( C_2H_2 \), with the result that \( C_2H_2 \) is noncompetitive versus \( N_2 \). If it is assumed that electrons leak from the sink when it contains two or more electrons, this model also explains why \( H_2 \) evolution is completely suppressed \( C_2H_2 \), but not by \( N_2 \). By keeping storage in the MoFe protein less than two electrons, a high \( pC_2H_2 \) prevents \( H_2 \) evolution, whereas the residual 23% of \( H_2 \) evolution that cannot be suppressed by \( N_2 \) results from leakage from the electron sink when it contains at least two but fewer than six electrons. Chatt et al. (9) have presented evidence that reduction of \( N_2 \) to 2 \( NH_3 \) occurs with a six-electron transfer in model compounds containing molybdenum or tungsten, but they express doubts that this occurs in nitrogenase.

This model also suggests an explanation for the failure to observe intermediates before \( NH_3 \) in \( N_2 \) reduction. If the electron sink must contain six electrons to start the reduction of \( N_2 \), it is likely that the first electron transfer will be the slowest step, and subsequent electron transfers will be much faster; thus only the final end product of reduction, \( NH_3 \), will be observed. This absolute requirement for all the electrons for reduction to be in the sink before the process can start may not apply to other substrates; alternate sets of reaction products are observed for some substrates, e.g., \( CN^- \) may be reduced with six electrons to \( CH_4 + NH_3 \) or alternatively may be reduced with 4 electrons to \( CH_3NH_2 \).

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LITERATURE CITED


