Chemical Evolution of a Nitrogenase Model. VIII. Ferredoxin Model Compounds as Electron Transfer Catalysts and Reducing Agents in the Simulation of Nitrogenase and Hydrogenase Reactions

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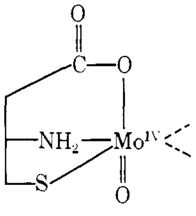
Abstract: Ferredoxin model compounds, i.e., anions of the type $[Fe_4S_4(SR)_4]^{z-}$ (z = 2-4) significantly accelerate the transfer of electrons from reductants such as $S_2O_4^{2-}$ or BH_4^- to molybdothiol catalysts, whose nitrogenase-like chemical behavior is now well established. New experimental data are presented which demonstrate that the reduced ferredoxin-like cluster complexes can be employed as stoichiometric reducing agents for the molybdothiol catalyzed reduction of C_2H_2 to C_2H_4. In the absence of reducible substrate, H_2 is evolved under these conditions, both by a molybdenum- and an iron-dependent pathway. The iron-dependent H_2 evolution is not associated with species $[Fe_4S_4(SR)_4]^{z-}$. Other as yet unidentified mercaptoiron sulfides appear to be the active components in these systems. The most active H_2-evolving systems contain Fe^{2+}, S^{2-}, and RS^- in the molar ratios of 1:2:6.

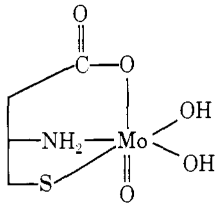
It is now well established that complexes of oxomolybdate ions with thiol ligands simulate the molybdenum active site of nitrogenase (N_2-ase), and that anions of ferredoxin model compounds of the type $[Fe_4S_4(SR)_4]^{z-}$ (z = 2-4) are efficient electron transfer catalysts for the conversion of the molybdothiol complexes into the active reduced form.[1] In the last paper of this series[2] we have demonstrated, for example, that systems containing Mo, Fe, S^{2-}, and RS^- in proportions similar to those observed in N_2-ase holoenzyme may be used for model studies of the reduction of typical N_2-ase substrates. In the present paper we report further details on the reactions of these systems with acetylene as the substrate. Functional N_2-ase is known to reduce the protons of the medium to yield H_2 in the absence of added reducible substrates.[3] This reaction has thus far not been duplicated in the model systems. We will show that H_2 evolution can be demonstrated and that H_2 is formed by both molybdenum- and iron-dependent mechanisms. The latter observation prompted us to conduct experiments with the aim of duplicating some of the reactions of bacterial hydrogenases as well. The known hydrogenase enzymes have been shown to contain Fe, S^{2-}, and protein-S^- groups,[4] but to date no satisfactory model systems for these important enzymes have been described. Evidence will be presented for H_2 evolution in systems containing Fe, S^{2-}, and RS^- as the sole active components. Although these systems did not yet function very efficiently, the present study demonstrates the existence of unstable mercaptoiron sulfide complexes capable of reducing protons to hydrogen. Our work thus might lead to the eventual development of functional hydrogenase systems, once the nature of the species catalyzing H_2 evolution is elucidated. Both tasks may prove to be very difficult, however, in view of the multitude of possible reactions and equilibria in Fe-S^{2-}-RS^- systems.

Model Systems of N_2-ase Employed. The molybdenum catalyst employed throughout this study consisted of the binuclear oxo-bridged Mo^{5+} complex of L(+)-cysteine (complex I). The ferredoxin model compounds possess an idealized "cubane" structure[5-7] as shown for the dianion in Figure 1. The alkyl-substituent R in the anions $[Fe_4S_4(SR)_4]^{z-}$ was chosen to be the n-propyl group, primarily to prevent possible interference in the assay for C_2H_4 during C_2H_2 reduction experiments. The Fe_4-cluster complexes undergo partial decomposition with hydrocarbon formation under reducing conditions; if R = n-C_3H_7, the products of decomposition are propylene (C_3H_6) and propane (C_3H_8), whose gas-chromatographic retention times are sufficiently different from those of C_2 hydrocarbons. As in the past, we shall refer to the active reduced form of the catalyst as Mo^{red} and to the oxidized derivatives thereof as Mo^{ox}. All experiments were performed in aqueous methanolic solutions containing pH 9.6 borate buffer. The use of this solvent system was preferred over water in view of the instability of the ferredoxin model compounds in an aqueous medium. Since ATP decomposes the mercaptoiron sulfides as well, all experiments were run in its absence. The omission of ATP is of no consequence for the aims of this study.

The catalytically active species Mo^{ox} and Mo^{red} are generated from complex I under the reaction conditions chosen. We have shown previously[1] that both are mononuclear oxomolybdate-cysteine complexes of possible structure **1** and **2**.



1 ("Mo^{red}")

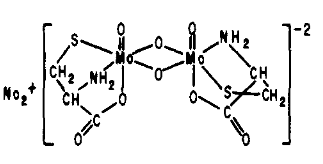


2 ("Mo^{ox}")

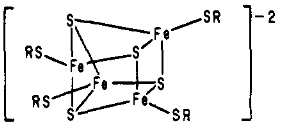
Mo^{red} is most probably a complex of cysteine with the highly reactive Mo^{4+}. Mo^{ox} contains molybdenum either in the +5 or +6 state of oxidation. The stationary concentrations of Mo^{red} and Mo^{ox} are low due to the tendency of mononuclear oxomolybdate ions to form catalytically inactive oxo-bridged dimers.[1]

Results

Acetylene Reduction. The addition of salts of the anions $[Fe_4S_4(SR)_4]^{2-}$ to solutions containing complex I and excess $S_2O_4^{2-}$ leads to a substantial increase in the rate of reduction of Mo^{ox} to Mo^{red}, as evidenced by the up to 3000-fold enhancement of the rate of C_2H_2 reduction. Since the mercaptoiron sulfide clusters are unstable under the reaction conditions, slowly decomposing into colloidal iron sulfides or iron mercaptides, it was necessary to determine whether the accelerating effects observed were due to the



"COMPLEX I"
(SOURCE OF THE CATALYTICALLY ACTIVE SPECIES "Mo^{ox}" AND "Mo^{red}")



FERREDOXIN MODEL COMPOUND AS ELECTRON TRANSFER CATALYST

Figure 1. Model systems employed.

added intact cluster anions or their decomposition products. A "minimum component" study, whose results are summarized in Table I, indicates that ferrous ion and iron mercaptides are essentially inactive; colloidal iron sulfide showed some activity, but the most active system resulted only if all components were present and the solutions were homogeneous. Such systems remain functional for about 40 hr. They are initially homogeneous, but after 40 min of reaction deposit increasing amounts of iron sulfides, which, however, do not affect the course of the molybdenum dependent C_2H_2 reduction to C_2H_4. Nevertheless, unless specified, all yields quoted in the figures and tables refer to homogeneous or essentially homogeneous reaction systems during the first 40-60 min of reaction. Table I also shows that the yields of C_2H_4 in the complete system (no. 17) with chemically synthesized $[Fe_4S_4(SR)_4]^{2-}$ instead of the equivalent amount of the same species generated in situ (no. 16) is about the same.

Table I. Reduction of C_2H_2 by Components of the Nitrogenase Model Systems[a]

		Yields of C_2H_4 after 60 min reaction	
No.	System	Absolute (μmol)	Relative
1	$LiS\text{-}n\text{-}C_3H_7$, $S_2O_4^{2-}$	0	0
2	1+ complex I	Trace	Trace
3	$Li_2S + S_2O_4^{2-}$	0	0
4	3 + complex I	0.02	0.6
5	$FeCl_3$, $S_2O_4^{2-}$ *	Trace	Trace
6	5 + complex I	Trace	Trace
7	$LiS\text{-}n\text{-}C_3H_7$, $S_2O_4^{2-}$, Li_2S	Trace	Trace
8	7 + complex I	0.06	2.1
9	1 + $FeCl_3$	Trace	Trace
10	9 + complex I	0.07	2.4
11	Li_2S, $FeCl_3 + S_2O_4^{2-}$	0.04	1.2
12	11 + complex I	0.90	30.5
13	$LiS\text{-}n\text{-}C_3H_7$, $FeCl_3 + S_2O_4^{2-}$	Trace	Trace
14	13 + complex I	0.07	2.4
15	$LiS\text{-}n\text{-}C_3H_7$, Li_2S, $FeCl_3$, $S_2O_4^{2-}$	0.07	2.4
16	15 + complex I ("complete system")	3.4	100.0
17	16, but with equivalent amounts of authentic $[N(C_4H_9)_4^+]_2[Fe_4S_4(SR)_4]^{2-}$	3.2	94.1

[a] Where indicated, reaction solutions contained 0.48 mmol of $LiS\text{-}n\text{-}C_3H_7$, 0.16 mmol of Li_2S, and 0.16 mmol of $FeCl_3$, with or without complex I (0.008 mmol), in a total reaction volume of 2 ml: solvent, water–CH_3OH (1:1, by volume); buffer, 0.2 *F* pH 9.6 borate; reaction temperature, 27°.

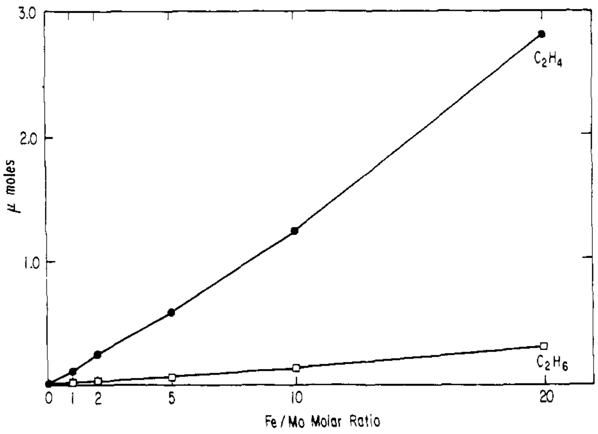


Figure 2. Dependence of the rate of C_2H_2 reduction by complex I on the concentration of $[Fe_4S_4(SR)_4]^{2-}$, generated in situ. Reaction solutions contained, in a total volume of 4 ml: complex I, 0.008 mmol; $S_2O_4^{2-}$, 0.3 mmol; and the amounts of mercaptoiron sulfide as indicated by the Fe/Mo ratios. Solvent was a 1:1 mixture of CH_3OH with 0.2 *F* pH 9.6 aqueous borate buffer. The initial $p_{C_2H_2}$ was 1 atm; the C_2H_4 yields were determined after 20 min of reaction at 27°.

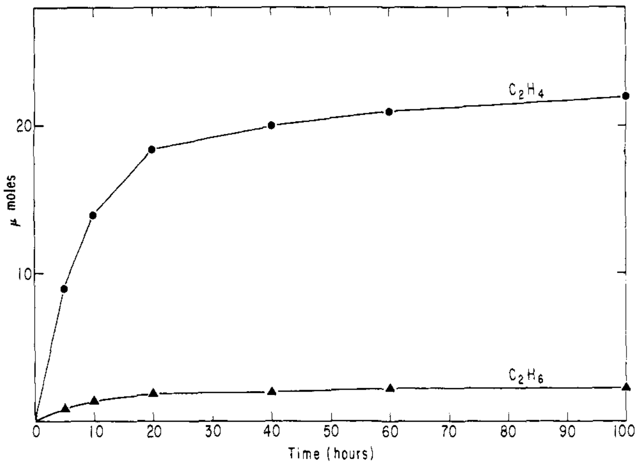


Figure 3. Time dependence of the yield of C_2H_4 from C_2H_2 in a system containing complex I (0.024 mmol), $[Fe_4S_4(SR)_4]^{2-}$ (0.048 mmol, generated in situ), and $S_2O_4^{2-}$ (initial concentration, 0.50 mmol), in a total reaction volume of 2 ml (solvent: CH_3OH borate buffer, 1:1), pH 9.6. The reaction temperature was 27°; the yields were determined after 18 hr of reaction.

The rate of C_2H_2 reduction increases with increasing concentration of added $[Fe_4S_4(SR)_4]^{2-}$ almost linearly (Figure 2), affording C_2H_4 as the main product with only traces of C_2H_6. A time-yield plot from a typical experiment is reproduced in Figure 3.

Ferredoxin Model Compounds as Stoichiometric Reducing Agents. The anions $[Fe_4S_4(SR)_4]^{z-}$ can be generated in solution in their different oxidation states by combining stoichiometric amounts of iron salt (e.g., mixtures of $FeCl_2$ and $FeCl_3$) with LiSR and Li_2S in anhydrous methanol. If a mixture of $FeCl_2$ and $FeCl_3$ is employed in the molar ratio of 1:1, the anions with $z = 2$ result according to eq 1.

$$2Fe^{2+} + 2Fe^{3+} + 4RS^- + 4S^{2-} \rightarrow [Fe_4S_4(SR)_4]^{2-} \quad (1)$$

Crystalline salts of these anions can be isolated by the addition of, e.g., $[(n\text{-}C_4H_9)_4N]^+I^-$. Solutions of the anions with $z = 3$ are obtained similarly, except that the proportion of Fe^{2+} to Fe^{3+} is increased to 3:1. The fully reduced anions with $z = 4$ may be expected to form if the Fe is added exclusively in the ferrous state. Formally, at least, even the cluster anions with $z = 1$ or 0 should be accessible in this

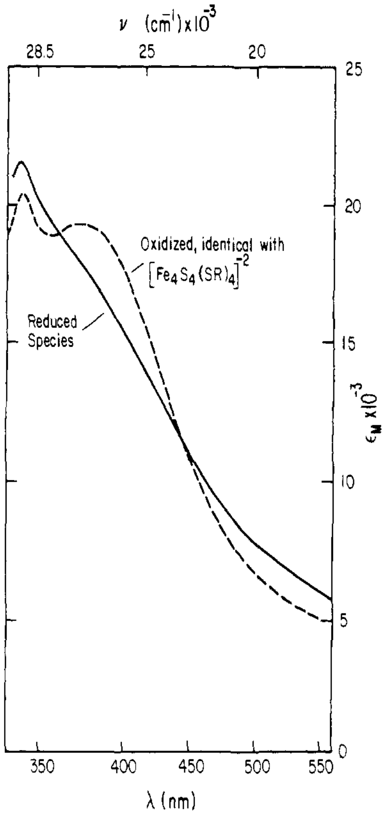


Figure 4. Absorption spectrum of a solution of reduced $[Fe_4S_4(SR)_4]^{z-}$ (z = 4, R = n-C_3H_7) or related species obtained by the combination of equivalent amounts of Fe^{2+}, S^{2-}, and RS^- in CH_3OH (—). Exposure to 1 μl of O_2 changes the spectrum to that of authentic $[Fe_4S_4(SR)_4]^{2-}$ (- - -) within 30 min at 27°.

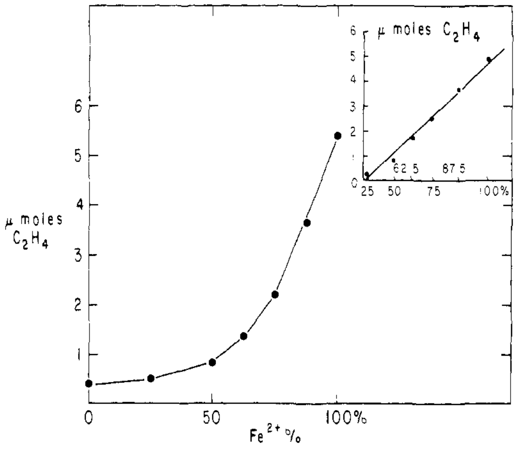


Figure 5. Reduction of C_2H_2 to C_2H_4 with complex I and stoichiometric amounts of anions $[Fe_4S_4(SR)_4]^{z-}$ or equivalent species present in solutions containing Fe, S^{2-}, and RS^- at the molar ratios of 1:1:1, plotted as a function of the per cent Fe^{2+} at t = 0. Reaction solutions contained, in a total volume of 2 ml: complex I, 0.006 mmol; Fe, S^{2-}, RS^-, each 0.12 mmol. The solvent was a 1:1 mixture of CH_3OH and 0.2 F pH 9.6 borate buffer in H_2O. The C_2H_2 pressure at t = 0 was 1 atm, the reaction temperature, 27°. Yields were determined after 18 hr of reaction. Insert shows C_2H_4 yield plotted against the square of the percentage of Fe^{2+}.

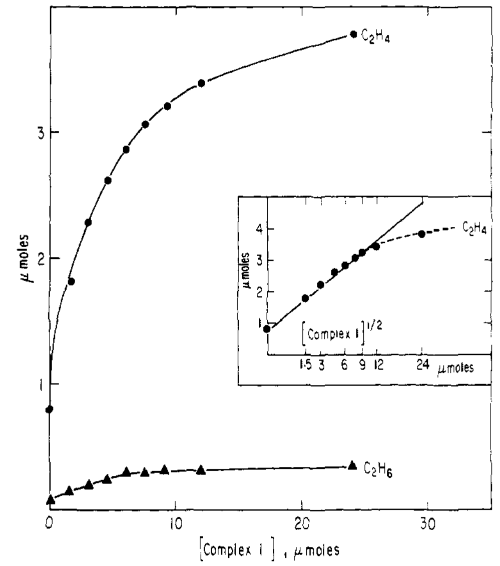


Figure 6. Dependence of the yield of C_2H_4 on the concentration of complex I in the presence of $[Fe_4S_4(SR)_4]^{4-}$ or equivalent species containing Fe^{2+}, S^{2-}, and RS^- at the molar ratios of 1:1:1. Conditions as given in legend to Figure 4.

fashion by increasing the proportion of Fe^{3+} to Fe^{2+}. However, the Fe^{3+} may cause oxidation of RS^- to R_2S_2, giving rise to the formation of the cluster anion with z = 2 instead. Investigating the reducing power of mercaptoiron sulfides with respect to the Mo^{red}-catalyzed reduction of C_2H_2 to C_2H_4, we employed solutions of RS^-, S^{2-}, and Fe salt containing 0–100% Fe^{2+} regardless of complications such as mentioned above. Salts of the anions with z = 3 or 4 proved difficult to isolate due to their high solubility and oxygen sensitivity. In the presence of added $[(n\text{-}C_4H_9)_4N]^+I^-$, the salts of the anions with z = 2 precipitate from the solutions of the reduced cluster anions upon exposure to limiting amounts of O_2. The oxidation was also followed spectrophotometrically (Figure 4). The results in Figure 5 demonstrate the presence of powerful reducing species in solutions containing more than 50% Fe^{2+}, which is ascribed to the formation of Fe_4-cluster anions with z = 3 and 4. The yield of C_2H_4 from C_2H_2 in the presence of molybdenum catalyst increases nonlinearly with the concentration of Fe^{2+} under otherwise identical conditions. Plotted as a function of $[Fe^{2+}]^2$, a linear relationship is observed (see insert in Figure 5). This result is consistent with the reaction stoichiometry in eq 2.

$$C_2H_2 + 2H^+ + 2\,[e^-] \rightarrow C_2H_4 \qquad (2)$$

The anions $[Fe_4S_4(SR)_4]^{3-,4-}$ presumably function as one-electron reducing agents, although the possibility of electron transfer steps involving two electrons also exists. The ion with z = 2, however, is not a reductant in the system of our study.

In Figure 6, the yield of C_2H_4 from C_2H_2 is shown to depend linearly on [complex I]$^{1/2}$. This demonstrates that the active reduced species Mo^{red} is a mononuclear rather than dimeric species, in accord with our previous studies with the N_2-ase model systems. It is furthermore apparent that the reactions of Mo^{red} with C_2H_2 are basically the same as in the absence of mercaptoiron sulfides, the latter obviously do not participate directly in the reduction of the substrate.

Hydrogen Evolution. Only traces of H_2 are formed in systems composed of complex I and $S_2O_4^{2-}$ in pH 9.6 buffered aqueous solution. The addition of catalytic amounts of $[Fe_4S_4(SR)_4]^{2-}$ does not stimulate H_2 evolution under these conditions, a fact which is primarily attributed to the instability of the ferredoxin model compounds in aqueous alkaline media. However, in the experiments with the reduced ferredoxin model compounds in methanol solution, some H_2 was detectable even in the presence of C_2H_2 as the substrate. Subsequent experiments revealed that systems containing Fe^{2+}, S^{2-}, and RS^- in the ratios of 1:2:6 in

Table II. Hydrogen Evolution in Systems Containing Fe^{2+}, S^{2-}, and RS^-, With and Without Complex I[a]

No.	Components	Hydrogen yields[b] in systems (μmol) I	II
1	LiSR, Li_2S, $FeCl_2$ (1:1:1)	0.26	0.031
2	1 + 0.06 mmol of complex I	0.30	0.063
3	1 + 0.12 mmol of complex I	0.36	0.065
4	1 + 0.18 mmol of complex I	0.42	0.060
5	1 + 0.24 mmol of complex I	0.47	0.054
6	5 at 1 atm of C_2H_2	0.28	0.018
7	5 at 1 atm of CO	0.46	0.050
8	5 at 1 atm of N_2	0.37	0.045

[a] Reaction solutions in column I contained $Fe:S^{2-}:RS^-$ in the molar ratios of 1:2:6, in a total volume of 2 ml: 0.72 mmol of LiSR (R = n-C_3H_7), 0.24 mmol of Li_2S, and 0.12 mmol of $FeCl_2$, and varying amounts of complex I. Reaction solutions in column II contained $Fe:S^{2-}:RS^-$ in the ratios of 1:1:1 under otherwise identical conditions: solvents, H_2O–CH_3OH, 1:1; reaction temperature, 27°. [b] After 1 hr of reaction.

methanol produce increasing amounts of H_2 as more complex I is added. A small stimulation of H_2 evolution in analogous systems containing Fe^{2+}, S^{2-}, and RS^- in the ratios of 1:1:1 was also observed. Some H_2 is even formed in the absence of complex I (Table II), suggesting the existence of both iron- and molybdenum-dependent pathways of H_2 evolution. No inhibition of H_2 production by CO was observed, but C_2H_2 as well as N_2 caused partial inhibition if present at 1 atm of pressure.

A systematic study of H_2 evolution in Mo-free systems containing only Fe^{2+}, S^{2-}, and RS^- revealed that maximum yields of H_2 are formed if the three components are in the molar ratio of 1:2:6 (Figure 7). Such systems are homogeneous and evolve H_2 continuously for about 12 hr (Figure 8). (The total yield of H_2 approaches 20% relative to the amount of Fe^{2+} present, after 24 hr of reaction at 27°.) Gas chromatographic analysis of the reaction solutions indicated that R_2S_2 is formed as a by-product, suggesting that RS^- functions as the source of electrons for H^+ reduction. The overall reaction catalyzed by the active iron complex thus may be formulated according to eq 3.

$$2H^+ + 2RS^- \xrightarrow[\text{[Fe}^{2+}\text{, S}^{2-}\text{, RS}^-\text{ complex]}]{} H_2 + R_2S_2 \quad (3)$$

We have been unable thus far to obtain H_2-evolving systems of similar or higher efficiency using $S_2O_4^{2-}$ as the reducing agent in aqueous or aqueous-methanolic solutions; the iron complex(es) responsible for H_2 production are sensitive both to water and alkali. However, work is currently in progress to modify the systems and to enhance their resistance to water. Our aim is to find active hydrogenase model systems operating under more nearly biological conditions.

Discussion

Ferredoxin Model Compounds as Electron Transfer Catalysts. Although the structure of the non-heme iron components in N_2-ase has not yet been elucidated, it appears highly probable that individual units of $[Fe_4S_4(S\text{-protein})_4]$ clusters are present which serve primarily as storage compartments of electrons for the reduction of the molybdenum active site. The experiments with N_2-ase model systems in the presence of Holm's ferredoxin model compounds demonstrate that the reduced cluster anions $[Fe_4S_4(SR)_4]^{z-}$ (z = 3 or 4), or equivalent species, convert the oxidized forms of the molybdothiol catalyst efficiently into the active reduced form Mo^{red}.

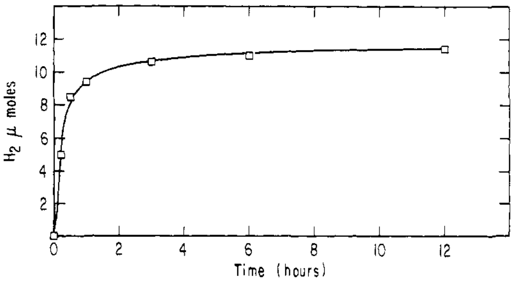


Figure 7. Yields of H_2 from systems containing Fe^{2+} (0.12 mmol) and LiSR (0.72 mmol) as a function of $[S^{2-}]$, as expressed by the Fe^{2+}/S^{2-} ratios. Total reaction volume was 2 ml. Solvent was anhydrous CH_3OH. Yields of H_2 were measured after 30 min of reaction at 27°.

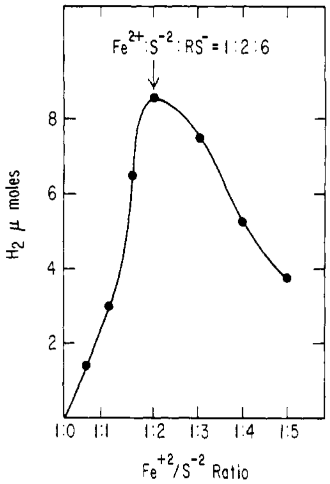
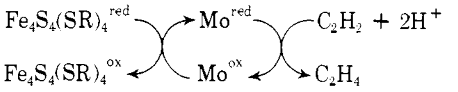


Figure 8. Dependence of the yield of H_2 as function of time. Reaction solutions contained Fe^{2+} (0.12 mmol), RS^- (0.72 mmol), and S^{2-} (0.24 mmol), respectively, in a total volume of 2.0 ml of anhydrous CH_3OH.

The available evidence suggests that the conversion of Mo^{ox} to Mo^{red} requires two electrons which are either transferred in successive one-electron steps or perhaps simultaneously. The overall reaction is schematically represented in eq 4, for C_2H_2 as the substrate. The structure of

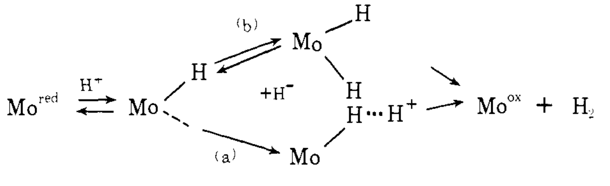


$$\begin{matrix} Fe_4S_4(SR)_4^{\ red} & \searrow\!\!\nearrow & Mo^{red} & \searrow\!\!\nearrow & C_2H_2 + 2H^+ \\ Fe_4S_4(SR)_4^{\ ox} & \swarrow\!\!\nwarrow & Mo^{ox} & \swarrow\!\!\nwarrow & C_2H_4 \end{matrix} \quad (4)$$

the non-heme iron cluster units in N_2-ase remains to be established until a more detailed mechanism of electron transfer can be formulated.

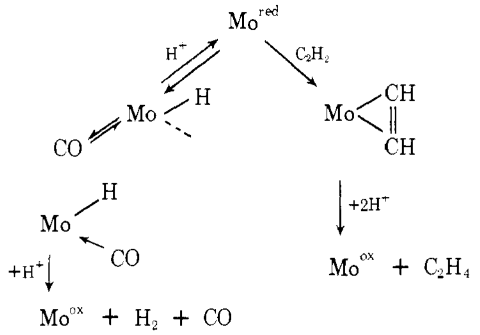
Molybdenum Dependent H_2 Evolution. The observed evolution of H_2 in systems containing stoichiometric amounts of reduced ferredoxin model compound and complex I as the catalyst shows that Mo^{red} is capable of discharging protons of the medium. Two mechanisms by which this can occur are shown in Scheme I. The species Mo^{red} possesses

Scheme I



the properties of a metallonucleophile and thus could form an unstable hydride or dihydride on protonation. The formation of a monohydride and the generation of H_2 via path (a) in Scheme I could explain why CO is not inhibitory.

Scheme II



However, with C_2H_2, inhibition of H_2 evolution is observed since this substrate is bound in the side-on-fashion (Scheme II). Hydrogen evolution according to path b in Scheme I cannot be entirely excluded, however.

Iron-Dependent H_2 Evolution. The only bacterial hydrogenase which thus far has been obtained in reasonably pure form is that of *Clostridium pasteurianum*. It is an iron-sulfur protein of molecular weight 60,500 containing Fe^{2+}, S^{2-}, and Cys-SH in the approximate ratios of 12:~12:~12.[4] The enzyme as isolated is paramagnetic and exhibits a "1.94"-type EPR signal which disappears on oxygenation and reappears on exposure to H_2. Neither the mechanism of H_2 uptake or H_2 formation is as yet understood. It appears likely that the H-H bond undergoes heterolytic, rather than homolytic cleavage, and evidence has been presented for equilibration of the bound hydrogen with the protons of the solvent. The nature of the active site is unknown; current mechanistic postulates assume a reaction of H_2 with centers containing either one or two iron-sulfur atoms. The present work shows that ferredoxin-type Fe_4 cluster anions do not provide a model for hydrogenase. Instead, systems containing Fe^{2+}, S^{2-}, and RS^- in the molar ratios of 1:2:6 were found to release H_2 most efficiently. It thus appears that H_2 evolution is associated with the presence of transient, unidentified mercaptoiron sulfide complexes. Work is in progress to characterize these species with the aim to develop functional models of hydrogenase and to establish their mechanism of action.

Experimental Section

Reagents and Starting Materials. All commercially available reagents and chemicals were "Reagent" or "Analytical" grade and were used without further purification. The Mo^{5+} complex of L(+)-cysteine (complex I) was prepared according to published methods and was purified by recrystallization from water-methanol. The tetra-*n*-butylammonium salt of $[Fe_4S_4(SR)_4]^{2-}$ (R = *n*-C_3H_7) was synthesized according to ref 2. Preparation of the Fe_4-cluster anions in situ is outlined below.

Assays. The reduction of C_2H_2 to C_2H_4 was followed by gas-liquid chromatography using a Hewlitt-Packard Series 700 laboratory gas chromatograph equipped with a phenylisocyanate-porasil, 80-100 mesh column. Hydrogen was detected by means of a column of 6 ft length, filled with molecular sieve (5A). Assays for $C_2H_4(C_2H_2)$ as well as those for H_2, were performed at the operating temperature of 27°.

Typical Experimental Procedures. In the following sections typical examples for all essential experiments are outlined. Further details on reaction conditions, quantities of reactants, etc., are given in the figures and tables.

(a) Acetylene Reduction with $S_2O_4^{2-}$ as the Reductant. Reaction vials of 25 ml volume were equipped with rubber septum caps and filled with water-washed C_2H_2 at 1 atm of pressure. Into these vials were injected: an aqueous solution of complex I in 0.2 *F*, pH 9.6 borate buffer (1.5 ml); a methanol solution containing the ion $[Fe_4S_4(SR)_4]^{2-}$ at concentrations specified (1.4 ml); and at $t = 0$, a freshly prepared 1.2 *M* solution of $Na_2S_2O_4$ in 0.2 *M* pH 9.6 borate buffer (0.5 ml). For analysis of gas-liquid chromatography, 0.2-ml gas samples were withdrawn at convenient time intervals, initially every 10 min.

(b) Acetylene Reduction with Stoichiometric Amounts of Fe_4 Cluster Complexes. Stock solutions of the required components were prepared as follows: solution I, anhydrous $FeCl_3$ (4.9 g, 30 mmol) dissolved in 50 ml of anhydrous methanol; solution II, anhydrous $FeCl_3$ (4.9 g) in 50 ml of anhydrous methanol, subsequently reduced to $FeCl_2$ by the addition of 3 g of Fe powder; solution III, anhydrous lithium sulfide, Li_2S (Research Organic/Inorganic Chemical Corporation, fresh bottle), 1.38 g (30 mmol), in 50 ml of anhydrous CH_3OH; solution IV, lithium methoxide (3.42 g, 90 mmol), dissolved in 38 ml of anhydrous methanol, followed by injection of 6.85 g (90 mmol) of pure, distilled *n*-C_3H_7SH. All stock solutions were stored under argon.

To prepare solutions containing the ion $[Fe_4S_4(SR)_4]^{2-}$, 3.0 and 3.0 ml of solutions I and IV, respectively, were injected into a vial, followed by 3 ml of solution III. Solutions containing the ion $[Fe_4S_4(SR)_4]^{3-}$ were generated similarly except that mixtures of solutions I and II, containing 75% $FeCl_2$ and 25% $FeCl_3$, were employed. The fully reduced Fe_4 clusters $[Fe_4S_4(SR)_4]^{4-}$, or equivalent mercaptoiron sulfides of Fe^{2+}, were generated as described above, using Fe^{2+} salt only (solution II).

Iron Dependent Hydrogen Evolution. An iron-dependent evolution of hydrogen can be observed in methanolic solutions containing Fe^{2+}, S^{2-}, and RS^- in various ratios, optimally at the ratios of 1:2:6 (see Figures 6 and 7). The most active systems result if the calculated amount of Fe^{2+} in anhydrous methanol (e.g., solution II, see above) is injected into a methanolic solution of S^{2-} and RS^- containing both components in the ratio of 2:6 relative to Fe^{2+}. The systems are sensitive to water and alkali. It is essential, furthermore, to use freshly prepared solutions of S^{2-} for these experiments and not to change the order of reagent addition given above.

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