Table I. The Rotational Strength Contributions (When the Sugar-Base Conformation is Syn or Anti, As Indicated) of a Transition (2.3 D in Magnitude) Localized on the Bond Indicated. Calculations Are Made with Eq 4 of Text

	Syn			Anti						
Bond[a]	140°	130°	150°	0°	−10°	−20°	−30°	−40°	−50°	−60°
1–2	1.94	1.26	2.29	0.94	1.00	0.91	0.71	0.46	0.21	0.03
2–3	2.60	2.98	2.30	−1.32	−1.36	−1.42	−1.45	−1.44	−1.40	−1.33
3–4	−1.67	−1.31	−2.35	10.16	9.60	8.58	7.28	5.79	4.20	2.26
4–5	6.11	5.24	3.26	−3.25	−2.89	−2.61	−2.37	−2.16	−1.96	−1.73
5–6	−0.25	−0.26	−0.52	2.27	1.98	1.62	1.20	0.73	0.22	−0.30
6–1	−0.23	−0.20	−0.30	2.60	2.70	2.61	2.37	2.00	1.54	1.01
2–11	−1.83	−0.44	−2.40	1.62	1.54	1.45	1.37	1.17	0.97	0.71
6–10	1.18	0.70	1.62	−1.08	−1.00	−0.92	−0.92	−0.74	−0.64	−0.50
5–7	1.12	−0.09	2.19	2.91	3.26	3.25	2.90	2.23	1.33	0.28
7–8	−2.88	−2.74	−2.91	5.78	5.81	5.47	4.74	3.65	2.25	0.65
8–9	11.58	8.94	12.76	−12.05	−10.77	−9.41	−8.11	−6.96	−5.98	−5.13
9–4	−4.29	−3.11	−5.39	12.28	10.60	8.54	6.18	3.64	1.05	−1.59

[a] The official IUPAC system for numbering the purine ring is used in this paper.

and shows the B_{2u} transition to remain localized in the vicinity of the C_2–N_3 bond (see Figure 9). From a purely geometrical point of view, the C_2–N_3 bond is to the anti conformer of **15** as the C_6–N_1 bond is to the anti conformer of **1a**. Referring to Table I, we find that the rotational strength contribution of the C_6–N_1 bond is positive in the anti range. Referring to Figure 6, which gives the CD curve of **15**, we note that the predicted and experiment results are in agreement.

Acknowledgment. We wish to thank Dr. Dov Elad for samples of **7a** and **7b**. The excellent technical assistance of Clark Wyatt Miles and Ali Ghandehari is acknowledged. This research was supported by the Petroleum Research Fund, administered by the American Chemical Society, Grant No. GM 12862-06 from the National Institutes of Health, and Grant No. CA-08109-06 from the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service.

Chemical Evolution of a Nitrogenase Model. II. Molybdate–Cysteine and Related Catalysts in the Reduction of Acetylene to Olefins and Alkanes

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Abstract: The catalytic reduction of acetylene to ethylene by molybdenum complexes with sulfur-containing ligands (*e.g.*, cysteine or thioglycerol) and $NaBH_4$ or $Na_2S_2O_4$ as the reducing agent strikingly resembles the enzymatic reduction of acetylene by nitrogen-fixing enzymes. Under these conditions, molybdenum exhibits the highest catalytic activity of all metals. The catalytically active species are presumably Mo(IV)–thiol complexes in which two cis positions are available for the binding of acetylene in the side-on fashion. Under certain conditions acetylene is also reduced to ethane. In this case the reaction apparently involves binuclear catalytically active species. As in the enzymatic reaction the stereochemical course of the reduction is predominantly cis and is significantly stimulated by biological phosphorylating agents such as ATP. Higher alkynes, *e.g.*, propyne, propargyl alcohol, and 2-butyne are also reduced by the model systems. In the absence of substrate the molybdenum–thiol complexes catalyze hydrogen evolution which is stimulated by ATP, unaffected by CO, and inhibited by acetylene. On the basis of these analogies, we conclude that the reactions of acetylene with nitrogenase occur at a molybdenum-containing binding site of the Mo–Fe protein. Iron, the more abundant metal in nitrogenase, does not seem to participate directly in the binding and the reduction of acetylenic substrates, but may function as an electron-transfer catalyst.

The molybdenum–iron protein of *Azotobacter vinelandii* nitrogenase (N_2-ase) has recently been isolated in crystalline form[1] and was shown to consist of a protein of molecular weight between 270.000 and 300.000, containing molybdenum, iron, cysteine, and "labile sulfide" in the ratio of 1:20:20:15.

The protein, which was also isolated from various other sources, is essential for typical N_2-ase reactions, in combination with an additional iron protein which presumably serves as an electron-transfer system.[2–6]

(1) R. C. Burns, R. D. Holsten, and R. W. F. Hardy, *Biochem. Biophys. Res. Commun.*, **39**, 90 (1970).

One of the salient features of N_2-ase is its ability to reduce many substrates other than nitrogen, *e.g.*, acetylene, 1-alkynes, cyanide, isocyanides, saturated and unsaturated nitriles, and nitrous oxide.[7,8] The reaction with acetylene is of particular interest because it is widely employed for the assay of N_2-ase activity, and because acetylene is isoelectronic with molecular nitrogen. In the reduction of acetylene, N_2-ase functions as a two-electron reducing agent, whereas six electrons are required for the reduction of nitrogen to ammonia. The reduction of isocyanides, which has been shown to yield C_1–C_4 hydrocarbons, occurs by multi-electron reduction steps. Reactions of this type have hitherto been completely unknown and have added fascination to the as yet unresolved problem of biological nitrogen fixation.

The high iron and low molybdenum content of N_2-ase suggest that thioprotein complexes of these two metals are present at the active site, but does not permit a distinction as to whether the chemical reactions of the substrates take place at sites containing molybdenum, iron, or both. For all reactions of N_2-ase, stoichiometric amounts of ATP are required, for reasons which until now have been unexplained. ATP as a source of energy is not needed, since all N_2-ase catalyzed reductions are exothermic. The possibility therefore exists that the ATP consumption during the reactions is linked to processes other than the chemical conversion of the substrates. A hypothesis connecting the ATP requirement to the induction of an essential conformational change of the enzyme protein was formulated in 1965[9] but has been criticized by Hardy and Knight.[7] Most recently,[10,11] it has been suggested that ATP may function by phosphorylating the oxidized metalloenzyme of N_2-ase to generate a better leaving group for the displacement of a metal-bound hydroxyl group.

In order to obtain initial insights into the nature of the catalytic processes involved in nitrogen fixation, we have investigated the reactions catalyzed by N_2-ase using low molecular weight model systems consisting of inorganic molybdenum salts, a thiol or related sulfur ligand, and a reducing agent in buffered aqueous solution. These initial studies indicated that the models duplicate many reactions of N_2-ase to an amazing extent.[12]

In this and in forthcoming papers we will outline parallels and differences between the reactions of substrates of N_2-ase and our models. Although we will restrict ourselves in the present paper to acetylenic substrates, we will also show how the ATP requirement in the N_2-ase reactions can be rationalized on the basis of model experiments.

Results

Development of Nitrogenase Models. Ideally, N_2-ase models should be based on the metals present in the enzyme and should operate under approximately physiological conditions. To be acceptable, the model should be capable of duplicating a maximum number of the reactions of the enzyme with a minimum number of essential constituents in homogeneous solution.

To develop such models in a stepwise and logically consistent fashion, we have utilized the following approach.

Rather than using nitrogen fixation itself as a criterion for "emerging N_2-ase-type behavior" of our model systems, we chose the reduction of acetylene to ethylene because of its conceptual simplicity. The studies were begun[12] with two-component systems consisting of solutions of simple salts of various metals in the presence of various reducing agents. Neither molybdenum nor iron exhibited significant catalytic activity under these conditions. Salts of certain heavy metals, *e.g.*, of Ni, Fe, and Pd, reduced acetylene to mixtures of ethylene and ethane with $NaBH_4$ as the reducing agent. However, these reactions were soon recognized to be catalyzed by the finely divided metals in heterogeneous systems and were consequently eliminated from further consideration.[12a] The lack of catalytic activity of the two-component systems suggested that a third component was essential for activity. In view of the high cysteine content of the Mo–Fe protein of N_2-ase, thiols appeared to be logical cofactors. Solutions of Na_2MoO_4 in the pH range between 7 and 12 to which various thiols were added were subsequently found to possess high catalytic activity for acetylene reduction to ethylene, using $Na_2S_2O_4$ as the reducing agent.[12] Similar experiments with $NaBH_4$ in place of $Na_2S_2O_4$ again demonstrated that for this reaction molybdenum possesses the highest catalytic activity of all eligible metals (Table I). Some of the first and most active catalysts were obtained from aqueous solutions of Na_2MoO_4 to which cysteine (Cys) or 1-thioglycerol (Tg) was added, or with the well-characterized[13,14] binuclear molybdenum–cysteine complex **1**. Experimental results to be described in this paper were obtained mainly with these catalyst systems. For other reactive combinations of molybdenum salts and reducing agents, see ref 12. Most experiments to be reported here were conducted with $NaBH_4$ as reducing agent.

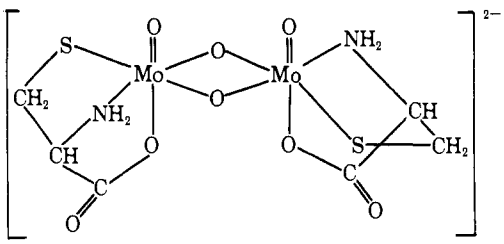
However, since N_2-ase also contains labile sulfide, completely inorganic catalyst systems composed of, *e.g.*, MoO_3 and NaSH at various molar proportions, both in the presence and absence of thiols as cofactors, were tested with various reducing agents. Mixtures of Na_2MoS_4 and Na_2MoO_4 were also evaluated as catalysts. The catalytic activity of these systems was modest compared to the molybdate–thiol mixtures described above. In addition, all tended to decompose by forming insoluble MoS_2, which itself showed little if any catalytic activity.

(2) W. A. Bulen and J. R. LeComte, *Proc. Nat. Acad. Sci. U. S.*, **56**, 979 (1966).
(3) L. E. Mortenson, J. A. Morris, and D. Y. Jeng, *Biochim. Biophys. Acta*, **141**, 516 (1967).
(4) R. W. Detroy, D. F. Witz, R. A. Parejko, and P. W. Wilson, *Proc. Nat. Acad. Sci. U. S.*, **61**, 537 (1968).
(5) R. V. Klucas, B. Koch, and H. J. Evans, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, **27**, 593 (1968).
(6) J. P. Vandecasteele and R. H. Burris, *J. Bacteriol.*, 101, 794 (1970).
(7) R. W. F. Hardy and E. Knight, Jr., *Progr. Phytochem.*, **1**, 407 (1968).
(8) R. W. F. Hardy, and R. C. Burns, *Ann. Rev. Biochem.*, **37**, 331 (1968).
(9) W. A. Bulen, J. R. LeComte, R. C. Burns, and J. Hinkson in "Non-heme Iron Proteins: Role in Energy Conversion," A. San Pietro, Ed., Antioch Press, Yellow Springs, Ohio, 1965, p 261.
(10) R. W. F. Hardy and E. Knight, Jr., *Bacteriol. Proc.*, 112 (1967).
(11) G. W. Parshall, *J. Amer. Chem. Soc.*, **89**, 1822 (1967).
(12) G. N. Schrauzer and G. Schlesinger, *ibid.*, **92**, 1808 (1970).
(12a) NOTE ADDED IN PROOF. The nitrogenase model systems recently proposed by Newton, *et al.*, must be rejected for similar reasons [W. E. Newton, J. L. Corbin, P. W. Schneider, and W. A. Bulen, *ibid.*, **93**, 268 (1971)].
(13) A. Kay and P. C. H. Mitchell, *Nature (London)*, **219**, 267 (1967).
(14) J. R. Knox and C. K. Prout, *Chem. Commun.*, 1227 (1968).

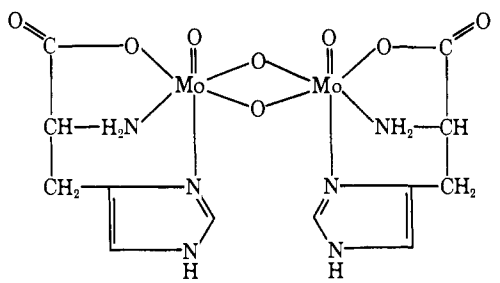
Table I. Relative Activities of Transition Metals in the Reduction of Acetylene to Ethylene at 27°. Cofactors and Reducing Agents are Cysteine and $NaBH_4$ (System I) and 1-Thioglycerol and $Na_2S_2O_4$ (System II)[a,b]

Metal	Relative activity System I	Relative activity System II	Metal	Relative activity System I	Relative activity System II	Metal	Relative activity System I	Relative activity System II
Ti	0	0	Y	0	0	Hf	*d*	*d*
V	0	0	Zr	0	0	Ta	*d*	0.1
Cr	0	0	Nb	0	0.1	W	700	0
Mn	0	0	Mo	10.000	100	Re	200	0.1
Fe	20	0	Tc	*d*	*d*	Os	0	0.1
Co	*c*	0	Ru	150	2.9	Ir	0	15.5
Ni	*c*	0	Rh	270	0.4	Pt	*c*	0.1
Cu	0	0	Pd	*c*	1.3	Au	0	0
Zn	0	0	Ag	0	0	Hg	0	0

[a] For reaction conditions, see Experimental Section. [b] Relative rates based on Mo = 10.000 in system I. Data for system II are from ref 12. Activities of metals other than Mo are uncorrected for possible contamination by Mo. [c] Reduction of acetylene to ethylene and ethane occurs due to formation of finely divided metal. [d] Not determined.



1



2

In systems free of sulfur-containing ligands, the catalytic activity was usually much less than in any of the above-mentioned combinations with thiols. Traces of C_2H_4 are formed in the reaction of acetylene with mixtures of Na_2MoO_4 and bipyridyl. The Mo(V) complex of histidine[15] of probable structure **2** reduces acetylene with $NaBH_4$ as the reducing agent to a 1:1 mixture of ethylene and ethane at a rate corresponding to only about 5% of the rate of the reaction with complex **1** as the catalyst. Little if any catalytic activity resulted when the thiol component was replaced by thioethers, indicating that the presence of a thiol or mercaptide group is essential for catalytic activity.

Accordingly, mixtures of molybdenum salts with thiols as cofactors represent the most active, minimum-component functional systems for the catalytic reduction of acetylene to ethylene.

The following experiments were carried out to establish the optimal conditions of catalytic activity in the system MoO_4^{2-}–Cys. However, measurements were also performed with MoO_4^{2-}–Tg, MoO_4^{2-}–glutathione, and complexes **1** and **2** as catalysts.

Acetylene Reduction in the MoO_4^{2-}–Cysteine System. Complex **1** catalyzes the reduction of acetylene in aqueous solutions optimally at pH values between 9 and 10 with $NaBH_4$ as the reducing agent. With $Na_2S_2O_4$ in place of $NaBH_4$ best results are obtained at pH 12, but the rates are only about 1% of those achieved with $NaBH_4$.

On standing at room temperature, ethylene is formed together with ethane, the latter in yields of between 0.1 and 50% relative to ethylene, depending on the reaction conditions employed. If freshly prepared solutions of **1** are used the rate of ethylene production initially increases exponentially, presumably due to the conversion of **1** into a catalytically active species. The first stage of this process probably consists in the solvent-assisted cleavage of **1** into reactive Mo(V) monomers, a well-known reaction which has recently been studied by Huang and Haight.[16] The participation of the solvent in this process is indicated by the large kinetic H/D effect of 8 at the beginning of the reaction in D_2O as the solvent, which continuously diminishes and eventually disappears. The disturbing influence of the conversion of **1** into the active species can be largely eliminated by preparing the reaction solutions at least 30 min before starting the experiment. On prolonged standing of the solutions of **1** the catalytic activity diminishes somewhat due to other deactivating effects.

The changes of the concentrations of C_2H_2, C_2H_4, and of C_2H_6 in a typical experiment are shown in Figure 1.

The rate of C_2H_2 uptake is first order in acetylene and, at concentrations of **1** between 0.02 and 0.20 *M*, proportional to the total concentration of molybdenum complex. The rate of acetylene uptake in this concentration range may thus be represented by

$$-d[C_2H_2]/dt = k_2[C_2H_2][Mo_{total}] \quad (1)$$

At higher concentrations of molybdenum complex the rate of acetylene uptake diminishes continuously, in part as a result of the diminished solubility of C_2H_2 with increasing electrolyte concentration in the reaction solution and the diminution of the catalytic activity of complex **1**.

Values for k_2, defined according to eq 1, initial rates of C_2H_4 and C_2H_6 production, as well as the final C_2H_4:C_2H_6 ratios under various conditions are summarized in Table II. The relative amounts of C_2H_6 produced are as a rule smaller at the beginning of the reaction, but later approach a constant value which has been recorded in Table II.

(15) L. R. Melby, *Inorg. Chem.*, **8**, 1539 (1969).

(16) T. J. Huang and G. P. Haight, Jr., *J. Amer. Chem. Soc.*, **92**, 2336 (1970).

Table II. Rate Constants of Acetylene Uptake (k_2), Initial Rates of C_2H_4 and C_2H_6 Production, and Final C_2H_4/C_2H_6 Ratios in the Reduction of C_2H_2 with Molybdenum–Cysteine Catalysts at 27°

Reaction conditions	Total catalyst concn, mmol[a]	k_2,[b] min^{-1} $mmol^{-1}$	Initial rates, mmol/min: C_2H_4	C_2H_6	$C_2H_4:C_2H_6$
Complex **1** as catalyst, fresh solution in H_2O, unbuffered	0.070	0.22	1.66×10^{-3}	0.22×10^{-3}	7.8
	0.141	0.14	2.81×10^{-3}	0.63×10^{-3}	3.7
	0.220	0.11	3.15×10^{-3}	1.54×10^{-3}	3.6
	0.282	0.10	3.14×10^{-3}	1.05×10^{-3}	2.9
	0.355	0.10	3.29×10^{-3}	1.34×10^{-3}	3.1
	0.420	0.11	3.39×10^{-3}	1.90×10^{-3}	1.3
Complex **1** as catalyst, preincubated for 5 hr in pH 9.6 buffer	0.070	0.32	3.36×10^{-3}	0.19×10^{-3}	18
	0.141	0.21	3.43×10^{-3}	0.29×10^{-3}	11
	0.220	9.080	3.45×10^{-3}	0.34×10^{-3}	10
	0.282	0.061	3.18×10^{-3}	0.32×10^{-3}	10
	0.355	0.060	2.82×10^{-3}	0.28×10^{-3}	10
	0.42	0.060	2.78×10^{-3}	0.36×10^{-3}	7.9
	1.50	0.0043	1.54×10^{-3}	0.62×10^{-3}	2.5
MoO_4^{2-}:Cys[c]					
9:1	2.69	0.0038	1.93×10^{-3}	0.29×10^{-3}	6.5
2.3:1	2.10	0.0072	2.54×10^{-3}	0.33×10^{-3}	7.8
2:1	1.98	0.020	4.01×10^{-3}	1.29×10^{-3}	3.1
1:1	1.50	0.015	1.54×10^{-3}	0.62×10^{-3}	2.5
1:1.5	1.20	0.0002	0.10×10^{-3}	0.05×10^{-3}	2.1
1:1.9	0.98	0.0002	0.10×10^{-3}	0.05×10^{-3}	0.5

[a] All measurements were performed in a total solution reaction volume of 3.5 ml. [b] Average error limits in k_2, ±5%. [c] Molar ratio.

The observed kinetic behavior is consistent with the reaction of acetylene with a catalytically active reduced molybdenum complex designated $[Mo^*_{red}]$, which is continuously regenerated in the catalytic process.

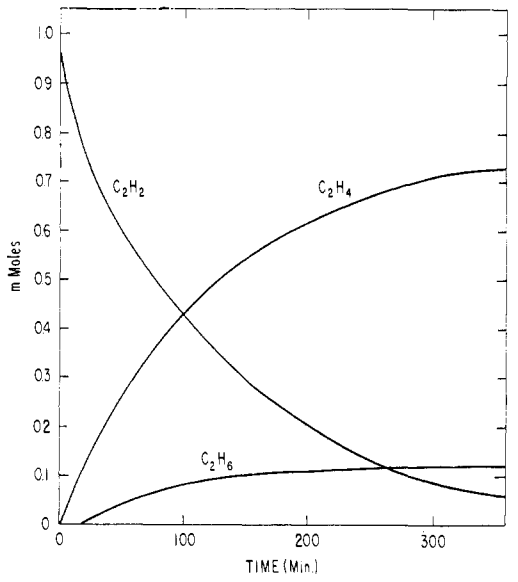


Figure 1. Changes of the gas-phase concentrations of C_2H_2, C_2H_4, and C_2H_6 in a typical acetylene reduction experiment with complex **1** as catalyst and $NaBH_4$ as reducing agent. The reaction solution contained, in a final volume of 3.5 ml of doubly distilled deionized water, complex **1**, 0.048 mmol, and $NaBH_4$, 0.67 mmol (added as 1.33 *M* solution in 0.2 *M* borate buffer of pH 9.6). The initial concentration of acetylene in the gas phase was 0.82 mmol. The gas-phase concentrations are corrected for solubility in the liquid phase.

Disregarding the initial conversion of **1** into $[Mo^*_{red}]$, as well as monomer–dimer equilibria between Mo(V) and other complex intermediates, the overall reaction can be represented by eq 2–4, in which $[Mo_{ox}]$ is the oxidized form of the catalyst.

$$[Mo^*_{red}] + C_2H_2 \rightleftharpoons [Mo \cdot C_2H_2] \quad (2)$$

$$[Mo \cdot C_2H_2] + H_2O \longrightarrow [Mo_{ox}] + C_2H_4 \quad (3)$$

$$[Mo_{ox}] + 2e \longrightarrow [Mo^*_{red}] \quad (4)$$

Plotting x/t *vs.* $(1/t)$ ln $[(A_0 - x)/A_0]$ according to Henri[17] affords a straight line from which a "K_m" value of 0.33 mmol is calculated (Figure 2). The energy of activation between 27 and 50° is 13 kcal.[18]

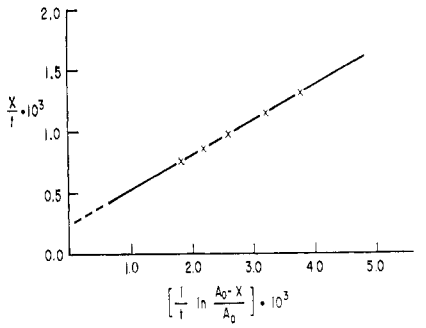


Figure 2. Henri plot of an acetylene reduction experiment with complex **1** as the catalyst: total volume of reaction solution, 3.5 ml; amount of catalyst, 0.15 mmol; initial $NaBH_4$ concentration, 0.20 *M* in 0.1 *M* pH 9.6 borate buffer; A_0 = initial concentration of substrate C_2H_2; x = concentration of substrate reacted to form product at time t. K_m is calculated from the slope of the line to be 0.33 mmol. Reaction temperature 27°.

The rate of ethylene production is very slow at 0°, however, suggesting a break in the Arrhenius plot between 0 and 27°. The turnover numbers are 0.01–0.05 mol of C_2H_4 produced/(min mol equiv of complex **1**) at 27°, depending on the concentration of the reaction solution. Turnover numbers calculated from the rates of acetylene uptake are somewhat larger due to

(17) V. Henri, "Lois Genérales de l'Action des Diastases," Hermann, Paris, 1903; see also J. Westley in "Enzymic Catalysis," Harper and Row, New York, N. Y., 1969, p 32.

(18) E_a was calculated from the rate of C_2H_2 uptake at 23, 35, and 45°, in experiments with complex 1 as the catalyst and $NaBH_4$ as reducing agent.

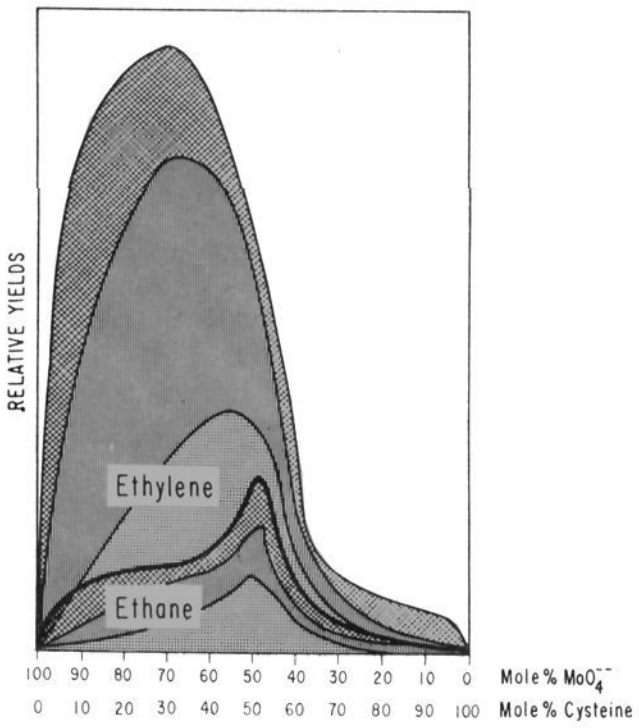


Figure 3. Catalytic activity in the system MoO_4^{2-}–cysteine (reducing agent $NaBH_4$) with respect to reduction of acetylene to ethylene and ethane. The diagram shows the relative yields of ethylene and ethane after 10, 90, and 229 min. The sum of the molar concentrations of Na_2MoO_4 and of cysteine is 2.0 mmol at each molar ratio. Initial $NaBH_4$ concentration was 0.22 *M* in a total volume of 3.0 ml. Initial C_2H_2 concentration was 0.84 mmol (1 atm, 27°).

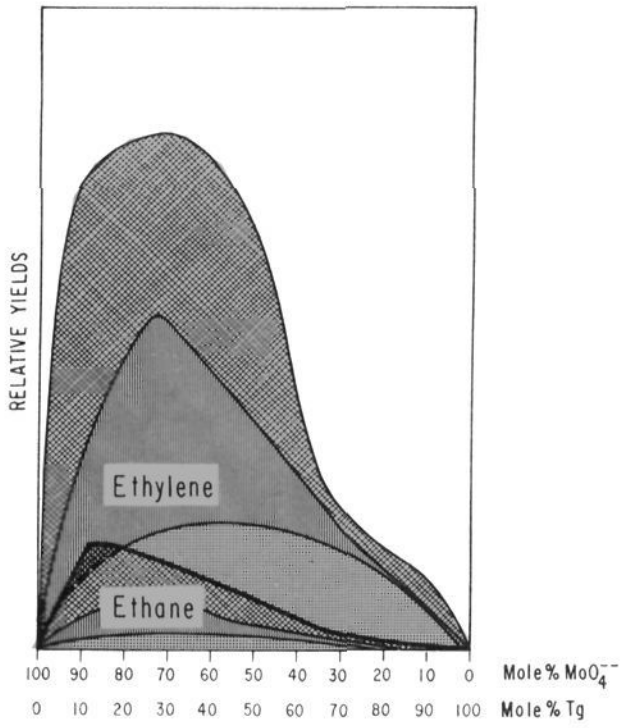


Figure 4. Catalytic activity in the system MoO_4^{2-}–thioglycerol (reducing agent $NaBH_4$) with respect to reduction of acetylene to ethylene and ethane after 10 min, 110 min, and 24 hr of reaction. The sum of the molar concentrations is 2.0 mmol of Na_2MoO_4 and thioglycerol at each molar ratio. Initial Na_2BH_4 concentration was 0.22 *M* in a total volume of 3.0 ml. Initial C_2H_2 concentration in the gas phase was 0.84 mmol (1 atm, 27°).

the reaction independent saturation of the solvent with C_2H_2.

In the system MoO_4^{2-}–Cys (Figure 3), a maximum activity for C_2H_4 production is observed at a 1:1 ratio initially, and at MoO_4^{2-}–Cys ratios of between 3:1 and 2:1 at later stages of the reaction. The Mo–Cys catalysts are active only if, and as long as, excess reducing agent is present. The addition of fresh $NaBH_4$ partially restores catalytic activity after all of the reducing agent is consumed. However, after 2 days of continuous reaction, catalyst inactivation becomes significant and is indicated by the formation of increasing amounts of molybdenum blue in the reaction solution. At this point the addition of fresh $NaBH_4$ causes only a marginal regeneration of catalytic activity.

Ethane is formed as a by-product of acetylene reduction in yields depending on the MoO_4^{2-}–Cys ratio, the catalyst concentration, pH, and age of the reaction solution. Except for the initial reaction periods, the C_2H_4/C_2H_6 ratio remains approximately constant during the experiment.

The relative amounts of ethane produced increase with increasing catalyst concentration (Table II). Maximum amounts of C_2H_6 are formed at the MoO_4^{2-}–Cys ratio of 1:1 (Figure 3). More C_2H_6 is furthermore generated in freshly prepared solutions of **1** than after a 5-hr incubation period (Table II).

Acetylene Reduction in the System MoO_4^{2-}–Thioglycerol. Mixtures of Na_2MoO_4 and Tg produce ethylene with maximum rates initially at 1:1, later at ratios between 2:1 and 3:1 (Figure 4), just like in the MoO_4^{2-}–Cys system. In contrast to the latter system the maximum amount of ethane is formed at the composition of catalyst which also yields the largest amounts of ethylene (Figure 4). The overall catalytic activity of the system is less than that of Mo–Cys [calculated turnover numbers are between 0.001 and 0.002 mol of C_2H_4 produced/(min mol of catalyst)]. Although catalyst life is appreciably longer in the MoO_4^{2-}–Cys systems, some irreversible catalyst deactivation is nevertheless noticeable. Under the conditions of reaction Tg slowly decomposes into allyl alcohol. With mercaptoethanol, ethylene is formed by the analogous degradation reaction and for this reason cannot be used as a replacement for Tg in acetylene reduction experiments.

Mixtures of glutathione with MoO_4^{2-} reduce acetylene to ethylene and ethane without offering noticeable advantages over MoO_4^{2-}–Cys or MoO_4^{2-}–Tg catalyst systems.

Stereochemistry of Reduction and Substrate Selectivity. The MoO_4^{2-}–Cys or –Tg catalysts reduce acetylene, propyne, and 2-butyne to ethylene, propylene, and 2-butene at relative rates of 1:0.8:0.2, with $NaBH_4$ as the reducing agent. Reduction of 2-butyne with $Na_2S_2O_4$ and the catalysts is too slow to be observed.[12] In the reduction of propyne, 1–5% propane is formed. In a typical experiment with 2-butyne (catalyst, complex **1**; reducing agent, $NaBH_4$), the reaction products after 5 hr consisted of 7.1% *cis*-2-butene, 0.05% *trans*-2-butene, and 1.9% *n*-butane, based on the initial amount of 2-butyne. The Mo(V)–histidine complex **2** under otherwise identical conditions afforded 0.3% *cis*-2-butene and 0.05% *n*-butane. These results suggest that the stereochemical course of the reduction is predominantly cis. This was confirmed by conducting the reduction of acetylene in D_2O as the solvent. With complex **1** as well as MoO_4^{2-}–Tg as the catalysts, the 1,2-dideuterioethylene produced was found to consist of 95%+ of the cis isomer. In qualitative experiments it was established that phenylacetylene is slowly reduced to styrene and propargyl alcohol is reduced to allyl alcohol with complex **1** as the catalyst. No measurable amounts of deuterium were introduced into the ethylene if the reduction catalyzed by complex **1** was carried out in H_2O solvent with $NaBD_4$ as the reducing

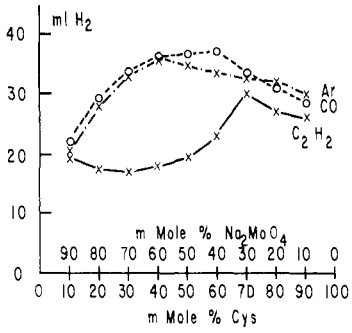


Figure 5. Hydrogen evolution in the system MoO_4^{2-}–cysteine in the presence of excess $NaBH_4$ under argon, CO, and C_2H_2 (all at 1 atm, 27°). Solution concentrations as given in the legend to Figure 3.

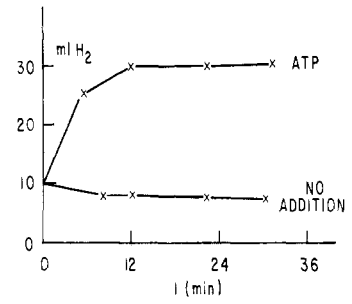


Figure 6. Effect of ATP on the evolution of H_2 in a C_2H_2 reduction experiment with $NaBH_4$ as the reducing agent and complex **1** as the catalyst. At t = 0, 3.0 ml of a solution containing 0.09 mmol of complex **1** and 0.128 mmol of $NaBH_4$ in 0.2 M pH 9.6 buffer was injected into a test tube containing 100 mg of solid ATP.

agent. This eliminates the possibility that hydride ions are transferred directly to the substrate.

Catalytic Hydrogen Evolution. In the absence of acetylene, pH-9.6 buffered solutions of complex **1** evolve hydrogen upon the addition of $NaBH_4$. This hydrogen evolution is clearly related to the catalytic activity of the MoO_4^{2-}–Cys systems in acetylene reduction. Thus, the maximum amount of hydrogen is evolved in MoO_4^{2-}–Cys mixtures at molar ratios of 3:1–2:1 (Figure 5). The hydrogen evolution is inhibited by acetylene, but not affected by CO.

Effects of ATP and of Related Phosphorylating Agents. Using solutions of **1** as the catalyst and $NaBH_4$ as the reducing agent, the effects of various phosphorylating or potential phosphorylating agents on the rate of C_2H_4, C_2H_6, and H_2 production in C_2H_2 reduction was studied. Under the conditions employed (see Experimental Section), ATP and GTP were found to stimulate all reactions significantly and reproducibly. ADP was less active, $Na_5P_3O_{10}$ inactive, and AMP actually inhibitory if present in substrate amounts (Figures 6–8). It was also noted that ATP and ADP stimulate the formation of C_2H_6 more than that of C_2H_4 (Table III).

Inhibition of Acetylene Reduction. The production of C_2H_4 from C_2H_2 is inhibited by various agents in systems containing MoO_4^{2-}–Tg catalyst and alkaline $Na_2S_2O_4$ as the reducing agent. The observed sequence of decreasing inhibitory effect, *e.g.*, R–NC > O_2 > $P(C_4H_9)_3$ > CN^- > CO, under these conditions suggests that these compounds compete with the substrate for the metal binding site.

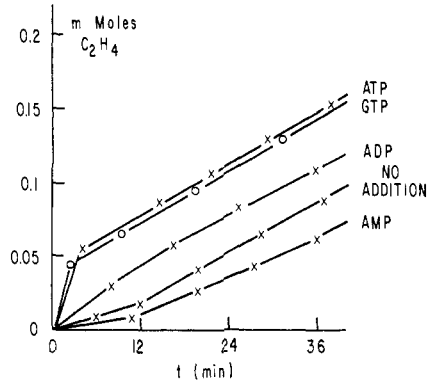


Figure 7. Effects of ATP, GTP, ADP, and AMP on ethylene production in a reaction catalyzed by complex **1**. Reaction conditions are identical with those given in the legend to Figure 6.

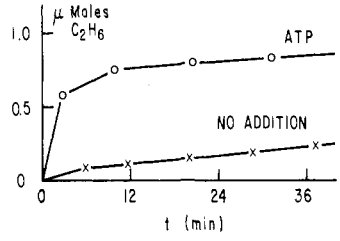


Figure 8. Effect of ATP on the production of ethane. Reaction conditions are given in the legend to Figure 6.

However, the same compounds have little if any effect in systems containing complex **1** as the catalyst and $NaBH_4$ as the reducing agent. Carbon monoxide may be slightly inhibitory in early stages of the reaction, but later actually appears to stimulate C_2H_4 production. The various effects are presently being studied in greater detail.

Table III. Effect of ATP and of Other Phosphates on Yields and Ratios of Ethylene and Ethane[a]

Phosphate added (0.2 mmol)	C_2H_4, %	C_2H_6, %	C_2H_4:C_2H_6
None	3	0.2	15
$Na_5P_3O_{10}$	2.7	0.1	27
AMP	3	3	1.0
ADP	13	5	2.6
ATP	20	9	2.2

[a] Catalyst, complex **1** (3 ml of 0.15 M solution in pH 9.6 buffer); reducing agent, $NaBH_4$ (0.67 mmol); 27°; 30 min after the addition of the phosphates.

Metals as Cocatalysts and Inhibitors. Considerable importance must be attached to the low catalytic activity of iron–Cys or Tg complexes in acetylene reduction. Since it is unlikely that the reactivities of molybdenum and iron should be reversed in the enzyme, we conclude that iron cannot be directly involved in the acetylene reduction reaction of N_2-ase.

Iron could have a cocatalyst effect, however, by facilitating the transfer of electrons to the molybdenum active site. This function is difficult to simulate in a simple model, since the iron in the Mo–Fe protein is present as a ferredoxin-type complex. However, the addition of catalytic amounts of $FeSO_4$ to reacting solutions of Na_2MoO_4–Tg catalysts with $NaBH_4$

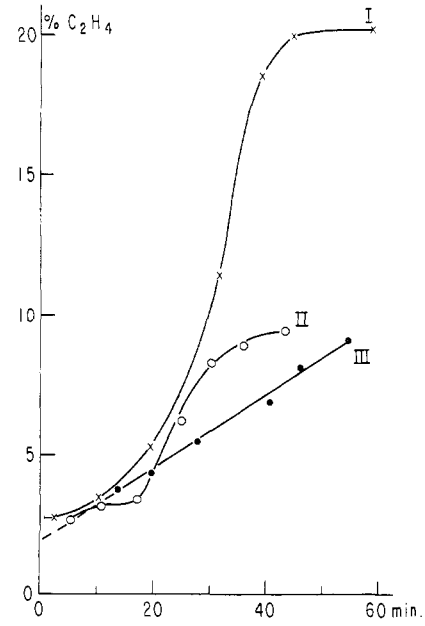


Figure 9. Effect of added iron salt on the production of ethylene from acetylene by MoO_4^{2-}–thioglycerol catalysts: curve I, $FeSO_4$ (12 mol %) added immediately before the experiment; curve II, no iron salt added; curve III, $FeSO_4$ (12 mol %) added 12 hr prior to the experiment. Each reaction tube contained 0.15 mmol of Na_2MoO_4, 0.15 mmol of thioglycerol, and 0.67 mmol of $NaBH_4$ (initial concentration) in a final solution volume of 3.5 ml. The initial acetylene concentration in the gas phase was 0.82 mmol (1 atm, 27°).

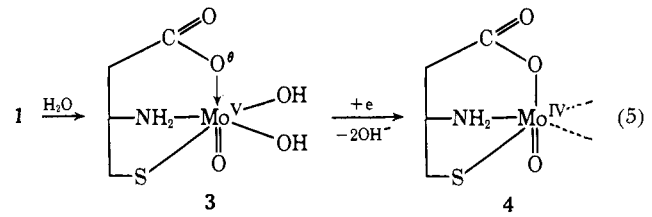
stimulated both hydrogen evolution and ethylene production (Figure 9). It may be significant that only salts of iron, rhodium, and palladium stimulated ethylene production, while most other metal salts acted inhibitory. Particularly potent inhibitors are salts of Hg^{2+}, Zn^{2+}, Cu^{2+}, Co^{2+}, Os^{3+}, VO_4^{3-}, and WO_4^{2-}. These metals presumably displace molybdenum by forming complexes with Tg. This even seems to be the case with iron, whose salts were found to act as cocatalysts only if added to an already reacting MoO_4^{2-}–Tg system. Iron salts proved inhibitory if added to solutions of **1** 12 hr prior to the experiment, an effect which is attributed to the slow formation of an iron–Tg complex *via* a ligand-displacement reaction.

Discussion

The reduction of acetylene to ethylene by our catalysts involves reduced Mo–Cys or Mo–Tg complexes as the catalytically active species $[Mo^*_{red}]$ whose reaction with acetylene and subsequent rapid solvolysis affords ethylene and the oxidized form of the catalyst, $[Mo_{ox}]$, without permitting the isolation or detection of the presumed intermediate $[Mo \cdot C_2H_2]$. The observed rate law of acetylene uptake (eq 1) suggests that the formation of this catalyst–substrate complex is rate determining. It has also not been possible to either isolate or detect $[Mo^*_{red}]$ because of its short lifetime, even in the absence of substrate. On the other hand, $[Mo_{ox}]$ appears to be a Mo(VI) derivative, either MoO_4^{2-} or a labile complex of Mo(VI) with Cys or Tg, since Mo(VI) accumulates in the reaction solutions after all of the reducing agent is consumed (*viz.*, molybdenum blue formation). Accordingly, $[Mo^*_{red}]$ could be a Mo(IV)–Cys or –Tg complex, which could readily be envisaged to function as a two-electron reducing agent.

The cis stereochemistry of the acetylene reduction, as well as the fact that 2-butyne is reduced to *cis*-2-butene, establishes that the alkyne molecules are attached to the catalyst in the side-on fashion; two cis positions must therefore be available in $[Mo^*_{red}]$ for the coordination of the substrates.

Prior to its reduction to $[Mo^*_{red}]$, complex **1** must first dissociate into monomeric Mo(V) species. Evidence for the slow dissociation of **1** into paramagnetic monomers has recently been obtained by Huang and Haight.[16] The reaction is presumably initiated by the nucleophilic attack of OH^-, followed by the cleavage of the μ-oxo bridges. A plausible structure of monomeric Mo(V)–Cys is therefore **3**.[16] In the subsequent reduction of **3** to $[Mo^*_{red}]$, metal-bound OH groups must be displaced to generate a binding site for the acetylenic ligands. Assuming that Cys remains tridentate, $[Mo^*_{red}]$ could therefore be thought to have structure **4**.



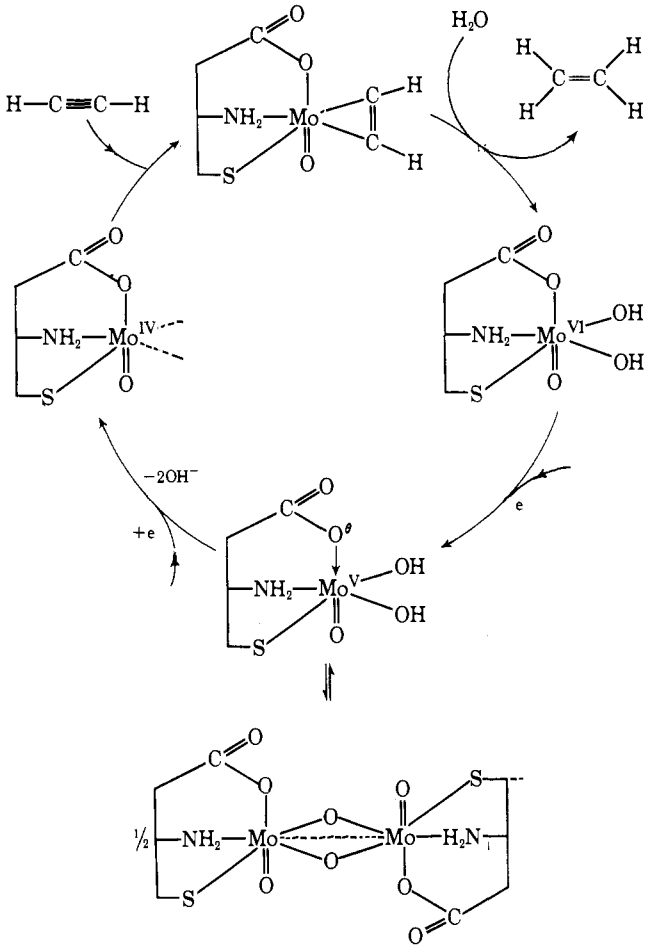
The mechanism of acetylene reduction to ethylene by Mo–Cys catalysts may thus be represented by Scheme I.

Although the exact structure of Mo–Tg complexes is unknown, the mechanism of acetylene reduction by MoO_4^{2-}–Tg catalysts must be similar to that shown in Scheme I. We furthermore mention that the catalytic activity of MoO_4^{2-}–Tg systems is subject to aging, particularly at MoO_4^{2-}–Tg ratios of 1:1, which is attributed to the slow formation of inactive dimeric species in the reaction solution.

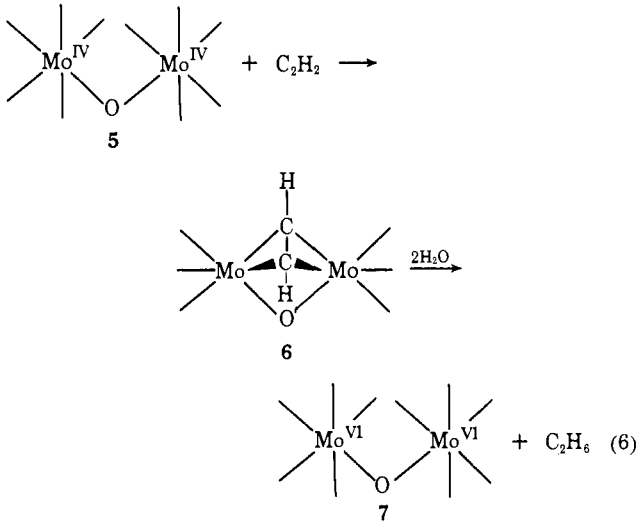
The Mechanism of Ethane Production. The formation of ethane as a by-product of acetylene reduction requires the transfer of four electrons from the catalyst to the substrate. Since ethylene is not reduced by our catalysts, the ethane is certainly not formed by the reduction of product ethylene. This possibility is also eliminated by the observed near constancy of the ethylene:ethane ratio during the reaction. Although the catalyst could function as a four-electron reducing agent if it were reduced to a low valence state, say, to Mo(II), no evidence is available to substantiate this suggestion.

A reduction of acetylene by two independent two-electron transfer steps could be achieved if two molecules of the active Mo(IV) complex or a binuclear catalytic species were involved. The remarkable dependence of the ethylene:ethane ratio on the age of the solutions of complex **1**, as well as the increased ethane production at high catalyst concentration in the MoO_4^{2-}–Cys system, indicates that the ethane formation is linked to time-dependent processes which are probably associated with monomer–dimer equilibria. We therefore postulate that ethane is formed by the reaction of one molecule of acetylene with a binuclear active reduced catalyst species $[Mo^*_{red}]_2$. Plausible

Scheme I. Suggested Mechanism of Ethylene Formation



structures for such a species and of its reaction product with acetylene are shown in formulas **5** and **6** (eq 6).

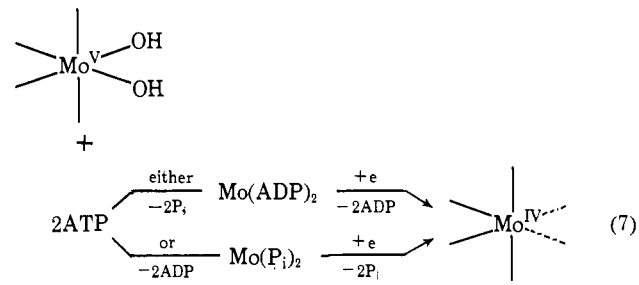


The postulated interaction of acetylene with two metallic centers is not without precedent. What appears to be unusual about the complexes with the molybdenum catalysts is their great reactivity, however. We accordingly expect the Mo–C bonds in, *e.g.*, **6** to have a high degree of σ character.

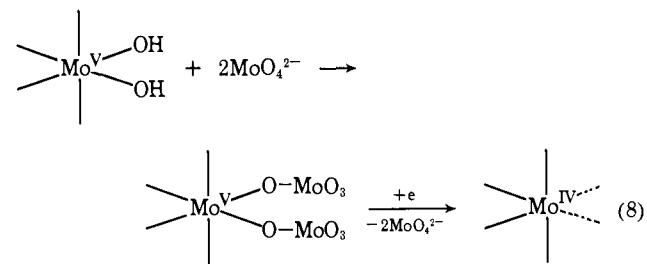
Since the extent of dissociation of Mo(V) dimers is known to depend on the nature of the ligands,[16] it is possible to rationalize the reactivity differences between complexes **1** and **2**. In the latter, the μ-oxygen bridges are more stable than in the former, causing a diminished tendency of **2** to dissociate into monomers. This is consistent with the observed low catalytic activity and the large relative yields of ethane with **2** as the catalyst. Sulfur ligands are known[16] to labilize the μ-oxygen bridges, causing **1** to be a much better catalyst. These considerations provide an explanation for the requirement of sulfur-ligand cofactors in our catalyst systems and possibly also for nitrogenase.

The Effect of ATP. The observed rate-enhancing effect of ATP and of related biological phosphorylating agents is of utmost importance and provides a clue to the understanding of the ATP requirement of the nitrogen-fixing enzymes.

We have previously mentioned that the conversion of the monomeric Mo(V)–thio ligand complexes to the catalytically active species [Mo^*_{red}] requires the removal of two molybdenum-bound OH groups. The ATP could phosphorylate the OH groups and thus improve their leaving-group properties. This reaction could occur by the two alternative mechanisms shown in eq 7. The maximum catalytic activity of MoO_4^{2-}–



Cys or –Tg systems at high MoO_4^{2-}–thiol levels similarly could be due to the enhancement of the rate of OH-group removal during the formation of the active complex, *i.e.*, according to



It is of interest that ATP stimulates the production of ethane more than that of ethylene. This suggests that ATP activates binuclear catalyst precursors whose conversion into catalytically active species would normally be slow.

The Catalytic Evolution of Hydrogen. The hydrogen evolution in MoO_4^{2-}–Cys–$NaBH_4$ mixtures must occur at the same active site as the acetylene reduction, as it is inhibited by acetylene and stimulated by ATP. Presumably, the $NaBH_4$ reduces the catalytically active Mo–Cys complex to a labile hydride or dihydride which decomposes with H_2 evolution. This proposition is supported by the observed activity profile in the system MoO_4^{2-}–Cys (Figure 5). The maximum rates of hydrogen evolution and acetylene reduction are both observed at the same MoO_4^{2-}–Cys ratios of 2:1–3:1.

Comparison with Nitrogenase Reactions. Our work establishes strong and important parallels between the

Table IV. Characteristics of the Reactions of Nitrogenase and of the Model Systems with Acetylene

Characteristic	N_2-ase	Model systems
Products	C_2H_4	C_2H_4 (C_2H_6)
Electrons per molecule	Two	Two, (four)
Proposed intermediates	None	None
Proposed bonding to site	Side on	Side on
Estd activation energy, kcal/mol	14	13
Estd K_m	0.4–1 mmol	0.33 mmol
Requirements	ATP, $Na_2S_2O_4$, N_2-ase	(ATP, GTP) $NaBH_4$, $Na_2S_2O_4$, MoO_4^{2-}, cysteine or other thio ligands
% of electrons transferred to added substrate[a]	98	52
Effect of CO	Inhibits	Inhibits weakly[b]
Effect of H_2	None	None
Turnover number[c]	150–200	0.01–0.05
Reaction in the absence of substrate	H_2 evolution (ATP dependent)	H_2 evolution (ATP stimulated)
Ref	7	This work, 12

[a] Balance of electrons transferred to H_3O^+. [b] May stimulate C_2H_4 production under certain conditions. [c] Moles of C_2H_2 reduced per mole of enzyme or catalyst per minute.

mechanism of acetylene reduction of N_2-ase and the corresponding reactions of model systems. The far-reaching analogies have become strikingly evident by the observed significant stimulating effect of ATP in the nonenzymatic model systems. To our knowledge, this is the first example of a nonenzymatic reaction in which ATP functions in direct correspondence to an enzymatic process. This observation opens the way for the systematic investigation of other ATP-requiring enzymatic reactions by means of suitable functional model systems.

Nitrogenase reduces acetylene with a specific activity of 1500 nmol (mg of protein)$^{-1}$ min^{-1},[7] corresponding to a turnover number of approximately 150 mol of C_2H_2 reduced per minute per mole of enzyme per number of active centers. The best of our model systems operate at about 0.03% of the enzymatic rate in the absence of ATP at about 0.3% in the presence of ATP. This activity can therefore probably still be increased. It is perhaps even more important that our models are less active than the enzyme, and that they produce ethane as a by-product of acetylene reduction to ethylene. Ethane is not formed in the corresponding reaction of N_2-ase.[19] The diminished selectivity and reactivity of the models has been attributed to the presence of binuclear catalyst species at equilibrium with the reactive monomers. It may be significant, therefore, that complex **1** has been independently shown to dissociate into paramagnetic species only to the extent of about 2%.[16] The high activity and selectivity of N_2-ase in acetylene reduction to ethylene therefore suggests that the active enzyme has a diminished tendency to dimerize, and that dimers, if formed, are unreactive with acetylene. A necessary condition for the dimers to yield ethane from acetylene is the availability of coordination sites at the molybdenum atoms. Such sites are evidently accessible in the models, but this may not be the case in the hypothetical N_2-ase dimers. The fact that 2-butyne is reduced slowly by the models, while it is not a substrate of the enzyme, may mean that the enzymic binding site is slightly sterically hindered, although other interpretations of this effect are possible. A reinvestigation of 2-butyne as substrate of N_2-ase would seem to be advisable in this context.

Table IV compares characteristic features of N_2-ase, as tabulated by Hardy and Knight,[7] with the properties of our model systems. The close correspondence between the enzyme and the models permits us to draw several pertinent conclusions concerning the "Two-Site-Hypothesis" of N_2-ase action by Hardy, Knight, and D'Eustachio.[20] It was suggested by these authors that N_2-ase consists of an H_2- and CO-insensitive "electron activating site," designated X_{ox}, whose reduction to the active reduced form X^*_{red} was assumed[7,10,11] to be ATP dependent in analogy to the mechanism of ATP action postulated for our model systems in eq 7.

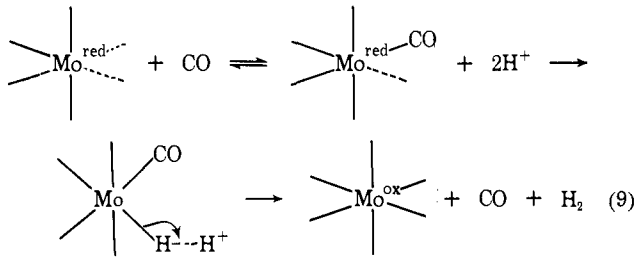
In addition, a CO-sensitive substrate complexing site "Y" was assumed to exist either on a separate metal or at the same metal containing site X. In the absence of substrate X^*_{red} reacts with protons of the medium to evolve molecular hydrogen. The identity of X and Y has not been established. In particular, it has not been possible to assign specific functions to the Mo–Fe and the Fe proteins.

Our work suggests that X_{ox} and X^*_{red} must be molybdenum atoms associated with the Mo–Fe protein. Furthermore, the molybdenum is likely to be bound to one or more cysteine residues of the apoprotein.

Our studies show that the reactions of the acetylenic substrates can be accommodated without the need of a separate binding site Y, and that X and Y are either identical or at the same molybdenum atom. The greater sensitivity of N_2-ase to CO, which is not a substrate, as compared to model systems, and the dependence of the inhibitory action of various compounds on the nature of the reducing agent in our systems suggest that the inhibitors may interfere with the transfer of electrons to the active site. On the other hand, the ATP-dependent H_2 evolution of N_2-ase is essentially unaffected by CO. We conclude, therefore, that the CO interacts weakly with the active site X^*_{red} without preventing H_2 evolution, perhaps in the fashion indicated in eq 9. The lack of inhibition by CO in the model systems could accordingly be due to the

(19) The amount of C_2H_6 formed in the reduction of C_2H_2 by N_2-ase of *Azotobacter vinelandii* is at best 0.01% relative to ethylene: R. W. F. Hardy, personal communication.

(20) R. W. F. Hardy, E. Knight, Jr., and A. J. D'Eustachio, *Biochem. Biophys. Res. Commun.*, **20**, 539 (1965).



availability of an additional coordination site on molybdenum, which is not accessible in the active site of the enzyme. We hope to obtain support for this hypothesis by studying the effect of CO on catalyst systems containing molybdenum complexes with quadridentate ligands.

Since all essential reactions of N_2-ase with acetylenic substrates are duplicated by iron-free systems, we conclude that the iron present in the enzyme in part of the intramolecular electron-transport system. Although the complex organization of the presumably ferredoxin-like iron protein is more difficult to mimic with simple models, the observed cocatalyst effect of iron salts indicates that such a role or iron in the enzyme is possible in principle.

The reduction of acetylene to ethylene by N_2-ase and the models supports the contention that the reduction of molecular nitrogen produces *cis*-diimide initially, which is subsequently reduced to ammonia.

Experimental Section

Reagents and Chemicals. Sodium borohydride (Ventron Corp.), sodium molybdate (Baker Analyzed reagent), cysteine hydrochloride, histidine hydrochloride (both from Matheson Coleman and Bell), and 1-propyne, 2-butyne, phenylacetylene, and propargyl alcohol (all from Farchan Research Laboratories) were used without further purification. Standard sodium borate buffer solutions were prepared from analytical grade chemicals in doubly distilled, deionized water.

Cylinder acetylene (Matheson] was passed through two gas-wash flasks filled with H_2O. 1-Thioglycerol, obtained from various commercial sources, was purified by boiling with 50 ml of 20% NaOH in methanol per 200 g of thioglycerol. After neutralization with concentrated HCl, the solution was vacuum distilled twice. The main fraction boiled between 122 and 124° (3 mm) and was collected.

The Mo(V) complexes of cysteine (complex **1**) and histidine (complex **3**) were prepared according to ref 13 and 15 and were recrystallized three times from water–methanol.

Standard Gas Chromatographic Technique. Hydrocarbons were determined by glpc using an F & M Model 700 chromatograph equipped with dual Porapak-N 6 ft × 0.25 in. chromatography columns, both with thermal conductivity and flame ionization detectors. The C_2–C_4 hydrocarbons may be adequately separated at ambient operating temperature.

Relative Activity of Metals in Catalytic Acetylene Reduction (see Table I). The data summarized in Table I were obtained by the following technique. Solutions of the metal salts (0.1 *M*) were prepared prior to the experiment. Into argon-filled, 4-in. Pyrex test tubes closed with rubber serum caps were injected 2.0 ml of the metal salt solution and 0.3 ml of a freshly prepared 1 *M* cysteine solution in 0.2 *M* borate buffer of pH 9.6. The test tubes were subsequently purged with washed acetylene gas for 10 min. At t = 0, 0.5 ml of a freshly prepared 1.33 *M* solution of $NaBH_4$ in pH-9.6 borate buffer was injected. After 18 hr of reaction at 27°, the gas phase was analyzed by glpc for C_2H_2, C_2H_4, and C_2H_6. The sample tubes were gently shaken during the experiments.

Catalytic C_2H_2 Reduction in the Molybdenum–Cysteine System (see Table II and Figures 1 and 2). **(a) Experiments with Complex 1,** $Na_2Mo_2O_4(Cys)_2 \cdot 5H_2O$. Stock solutions of complex **1** were prepared by dissolving 4.5 g of crystalline complex **1** in 50 ml of doubly distilled deionized H_2O. The solutions were kept in rubber-serum-capped flasks under pure argon. Varying amounts of this solution, which is 0.142 *M*, were injected into 4-in. Pyrex test tubes. The solutions were diluted with H_2O to a total volume of 3 ml in each case. Into each tube 20 cm^3 of acetylene gas was injected through the serum caps. At t = 0, 0.5 ml of freshly prepared 1.33 *M* $NaBH_4$ solution in pH-9.6, 0.2 *M* borate buffer was injected. The reaction was followed by glpc by periodically withdrawing 0.1-cm^3 gas samples.

(b) The System MoO_4^{2-}–Cys. Stock solutions of 1 *M* Na_2MoO_4 and 1 *M* cysteine in pH-9.6 borate buffer (0.2 *M*) were prepared and stored in serum-capped flask under pure argon. Reaction tubes consisting of 4-in. Pyrex test tubes equipped with rubber serum caps were first filled with argon. Subsequently, varying known amounts of Na_2MoO_4 and Cys solutions were added, maintaining the sum of the volumes of the two solutions in each tube at 3.0 ml. These MoO_4^{2-}–Cys mixtures were purged with argon for 15 min. After this, 0.2 ml of 1.33 *M* $NaBH_4$ solution in pH-9.6 borate buffer was injected into each tube and the solutions were allowed to stand overnight. The addition of the borohydride at this point serves the purpose of pre-equilibrating the reaction solutions. The next morning the reaction tubes were purged with acetylene. At t = 0, 0.3 ml of fresh 1.33 *M* $NaBH_4$ solutions was injected. The course of reduction was followed at 27° in most measurements by the periodic withdrawal of 0.1-cm^3 gas samples. Typical time intervals were 5, 25, 50, 80, 150 min, etc. The concentration of the C_2 hydrocarbons was determined from the peak areas. Since hydrogen is evolved during the reaction it proved preferable to equalize the pressure in the reaction tubes. This was achieved in a simple but effective way by allowing the gases in the tubes to expand into empty syringes of 20-ml capacity. The amount of hydrogen evolved could also be determined in this way. After sample withdrawal the gas in the syringe was injected back into the reaction tubes.

Catalytic C_2H_2 Reduction in the System MoO_4^{2-}–Thioglycerol (see Figure 3). Stock solutions of 1 *M* Na_2MoO_4 and 1 *M* Tg were prepared by dissolving 24.2 g (0.1 mol) of $Na_2MoO_4 \cdot 2H_2O$ and 8.1 ml (0.1 mol) of Tg each in 100 ml of 0.2 *M* pH-9.6 borate buffer solution. These solutions were kept in serum-capped flasks and were made anaerobic by flushing for 20 min with argon. For the system study, aliquots of the stock solutions were injected into argon-filled reaction tubes, *e.g.*, 1 ml of Na_2MoO_4 solution plus 2 ml of Tg solution. The total volume of both solutions injected was maintained at 3 ml in each tube. The tubes were then purged with acetylene. At t = 0, 0.5 ml of fresh 1.33 *M* $NaBH_4$ solution in pH-9.6 buffer was injected. The course of the reaction was followed as described above under (a).

H/D Effect. The kinetic H/D effect of acetylene reduction with complex **1** as the catalyst was determined by preparing 0.15 *M* solutions of complex **1** in H_2O and D_2O pH-9.6 borate buffer. Reaction tubes were filled with 3 ml each of the solutions of complex **1** in H_2O and D_2O. After purging with acetylene, the reactions were started by injecting 0.5 ml of 1.33 *M* $NaBH_4$ solutions in H_2O and D_2O, respectively. The results are summarized in Table V.

Table V. Kinetic H/D Effect on the Rate of C_2H_4 and C_2H_6 Production

	—% C_2H_4—		—% C_2H_6—		—H/D—	
Time, min	H_2O	D_2O	H_2O	D_2O	C_2H_4	C_2H_6
4	0.16	0.02			8.0	
46	12.9	1.8	0.39	0.01	7.8	4
123	44.5	12.3	1.46	0.80	3.62	1.8
1044	93.1	81.7	2.10	5.60	1.14	0.37

Catalytic Reduction of 2-Butyne. Three 15-ml vials were fitted with rubber serum caps and filled with argon. Subsequently, 0.35 mmol each of complex **1** and of complex **2**, dissolved in 5 ml of water, were injected into the first two reaction tubes. Into the third tube 5 ml of a solution containing 5 mmol of Na_2MoO_4 and 4 mmol of 1-thioglycerol was added similarly. Into each tube 1 ml of 2-butyne was injected, followed by 1 ml of 1.33 *M* $NaBH_4$ in pH-9.6 borate buffer. After 5 hr of reaction 0.2-cm^3 gas samples were withdrawn for analysis. The identification of the products was achieved by glpc, using a Duropak-N–octane/Porasil-C 120/150 mesh, 6 ft × 0.25 in. copper tube column at 40°, and helium as the carrier gas. The retention times of the C_4 hydrocarbons were as follows: *n*-butane, 84 sec; *trans*-2-butene, 108 sec; *cis*-2-butene, 120 sec; 2-butyne, 9.5 min. The reduction products consisted in all three cases predominantly of *cis*-2-butene (see Results section).

Table VI. Relative Yields of C_2H_4 with MoO_4^{2-}–Tg Catalyst in the Presence of Catalytic Amounts of Metal Salts (Reducing Agent, $NaBH_4$)[a]

Salt	Rel yield	Salt	Rel yield
$FeSO_4$	3.1	$Mn(OAc)_2$	0.7
$RhCl_3$	3.0	$CrCl_3$	0.7
$PdCl_2$	1.64	$CuSO_4$	0.6
$TiOSO_4$	1.4	$NiCl_2$	0.5
$ReCl_5$	1.2	Na_3VO_4	0.4
$RuCl_3$	1.0	$CoCl_2$	0.3
K_2PtCl_4	0.9	Na_2WO_4	0.3
$IrCl_3$	0.7	$OsCl_3$	0.3

[a] Hydrated forms of the salts not indicated.

Metal Salts as Cocatalysts and Inhibitors. Reaction tubes containing 0.15 mmol each of Na_2MoO_4 and Tg in a total volume of 3 ml of H_2O were filled with 1 atm of C_2H_2. Aliquots of solutions of metal salts (mostly chlorides) corresponding to 0.02 mmol were injected. At $t = 0$, immediately after the addition of the metal salts, 0.5 ml of 1.33 *M* $NaBH_4$ solution in pH-9.6 buffer was injected into the reaction tubes. After 30 min of reaction the relative yields of ethylene (no metal salt added:1.00) were as shown in Table VI.

Miscellaneous Inhibitors. The inhibition of the acetylene reduction by CO was first studied with Na_2MoO_4–Tg catalyst and $Na_2S_2O_4$ as the reducing agent. Solutions containing MoO_4^{2-}–Tg in the molar ratio of 1:1 (1.5 mmol in 3 ml of H_2O) were injected into acetylene-filled test tubes as described above. The inhibitors in amounts corresponding to 0.5 mmol were injected in concentrated aqueous or alcoholic solutions or, in the case of O_2 and CO, in the gaseous state, by means of syringes. At $t = 0$, 0.5 ml of a freshly prepared 1 *M* solution of $Na_2S_2O_4$ in 1 *M* NaOH was injected. The relative yields of C_2H_4 were determined after 30 min of reaction at 27° and are summarized in Table VII.

Table VII. Relative Yields of Ethylene Formed in the Presence of Substrate Amounts of Various Inhibitors

$C_6H_{11}NC$	O_2	$P(C_4H_9)_3$	KCN	$HgCl_2$	$CuSO_4$	$ZnCl_2$	CO	None	System[a]
0.068	0.20	0.29	0.31	0.55	0.55	0.56	0.82	1.00	I
1.10	1.15	1.2	1.50	nd[b]	0.65	0.70	1.10	1.00	II

[a] System I: MoO_4^{2-}–Tg, 1:1; reducing agent, alkaline $Na_2S_2O_4$. System II: Complex I, pH-9.6 buffer, $NaBH_4$. [b] nd = not determined.

Identification of *cis*-1,2-Dideuterioethylene. In order to determine the stereochemistry of the acetylene reduction, 2.42 g of $Na_2MoO_4 \cdot 2H_2O$ and 0.77 ml of 1-thioglycerol were dissolved in 10 ml of D_2O. Of this solution, 3.3 ml was transferred into a 50-ml glass bottle, which was sealed with a rubber serum cap. The air in the bottle was displaced with gaseous acetylene. Subsequently, a solution containing 0.75 g of $NaBH_4$ in 10 ml of D_2O was injected. After 3 days of standing at room temperature, approximately one-half of the gas phase in the reaction flask was transferred into an evacuated ir gas cell of 10-cm path length. The presence of *cis*-1,2-dideuterioethylene was established by ir analysis (presence of the characteristic band at 842.1 cm^{-1} for *cis*-1,2-dideuterioethylene[21]).

Stimulation of Acetylene Reduction by Phosphorylating Agents (Figures 6–8, Table IV). A stock solution containing 30 ml of a 0.03 *M* solution of complex **1** in 0.2 *M* pH-9.6 buffer was prepared first and stored under argon. Subsequently, 100 mg each of ATP, ADP, and AMP were placed into 3-in. test tubes. After sealing the tubes with rubber serum caps, the air was displaced by 1 atm of acetylene. In the meantime, 5 ml of a 1.33 *M* buffered $NaBH_4$ solution was added to the stock solution of complex **1**. At $t = 0$, 3 ml of this solution was injected into the acetylene-filled test tubes containing the phosphates, as well as into test tubes containing just acetylene. The reaction solutions immediately darkened and showed increased H_2 evolution as compared to the controls. The course of the reactions was followed both by measuring the H_2 pressure and by periodically withdrawing samples for glpc.

The effect of CO in the MoO_4^{2-}–Cys system was investigated at molar ratios of MoO_4^{2-}:Cys of 3:1, 1:1, and 1:2. The reaction solutions were prepared by mixing the appropriate amounts of 1 *M* Na_2MoO_4 and 1 *M* Cys stock solutions in 0.2 *M* pH-9.6 buffer, maintaining a total volume of 3.0 ml. After transferring the solutions into the test tubes, the air was displaced by a gas mixture containing C_2H_2 and CO in a ratio of 3:1. After the injection of 0.5 ml of 1.33 *M* $NaBH_4$ the reaction tubes were allowed to stand at 27° for 20 min. Analysis of the gas phase indicated 54 and 35% inhibition of ethylene formation at the MoO_4^{2-}–Cys ratios of 1:2 and 1:1 (relative to samples containing the equal amount of Ar in place of CO), respectively. At the ratio of 3:1, however, a 31% stimulation of ethylene production was observed relative to the control. After 45 min of reaction the inhibiting effect of CO largely disappears.

With complex **1** as the catalyst and $NaBH_4$ as reducing agent no measurable inhibition of acetylene reduction was observed after 45 min in the presence of substrate amounts of $C_6H_{11}NC$, KCN, $P(C_4H_9)_3$, CO, or air (Table VII) at a catalyst concentration of 0.03 *M*.

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