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Catalytic behavior of a polynuclear Mg—Mo complex and nitrogenase active site (FeMoco) isolated from the enzyme in reactions with C_2H_2, N_2, and CO: a comparative study

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In order to identify common and distinctive features in the catalytic behavior of natural and artificial nitrogen-fixation clusters, the kinetics of the catalytic reduction of C_2H_2 in the presence of Mg—Mo-cluster (**1**) was investigated and compared with the kinetics of acetylene reduction catalyzed by the cluster FeMoco (**2**) isolated from the enzyme nitrogenase we studied previously. The reactions were conducted in the presence of Zn/Hg and Eu/Hg as reducing agents and PhSH and C_6F_5SH as proton donors, *i.e.*, under the same conditions as had been used in the case of **2**. Both polynuclear Mg—Mo-complex and the europium amalgam-reduced FeMoco have multiple interdependent binding sites for substrates and/or inhibitors. Carbon monoxide inhibits the acetylene reduction much less efficiently in systems with cluster **1** than in systems with cluster **2**, although the type of inhibition is mixed in both systems: CO binds to multiple sites of the cluster and affects both C_2H_2 complexation to the reduced cluster and decomposition of the catalyst—substrate complex to give the products. Unlike isolated FeMoco, the Mg—Mo-cluster efficiently catalyzes the reduction of molecular nitrogen. The reaction is greatly inhibited by acetylene, while no inhibiting effect of N_2 is observed in acetylene reduction, as was found earlier for a system with the natural cluster as the catalyst.

Key words: biomimetics, biological nitrogen fixation, metal complex catalysis, metal cluster, active site, structural and functional models, mechanism, nitrogenase, acetylene reduction.

Previously,[1] a nitrogen-reducing system based on polynuclear molybdenum complexes was discovered. Currently, this is the only known non-enzyme system capable of catalytic reduction of N_2 at atmospheric pressure and room temperature at rates comparable with nitrogenase. The reaction is carried out in methanol with a minor water additive, which apparently serves as the proton donor in ammonia and/or hydrazine formation. Sodium amalgam, europium amalgam or a cathode with a specified potential equal to the Na/Hg potential can serve as reducing agents.[2] The molecular structure of the Mg-containing molybdenum anionic cluster $\{[Mg_2Mo_8O_{22}(MeO)_6(MeOH)_4]^{2-}[Mg(MeOH)_6]^{2+}\}\cdot$ $\cdot 6MeOH$ (**1**), which forms the active site of the system upon reduction, has been determined[3] by X-ray diffraction (Fig. 1).

The available data[1—4] indicate that this compound is reduced with retention of the cluster core, although, strictly speaking, the structure of the complex active with respect to N_2 is unknown. The molecular mechanism of N_2 catalytic reduction with participation of this cluster is also unknown.

The active site of the natural nitrogen-fixing enzyme nitrogenase incorporates an octanuclear heterobimetallic cluster FeMoco ($(\mu_6\text{-N})MoFe_7S_9\cdot$homocitrate (**2**)) in which nitrogen is bound and then reduced to ammonia.[5] The chemical mechanism of the multielectron reduction of nitrogenase substrates catalyzed by the cluster is unknown.

In order to elucidate the function of the cofactor and the contribution made by the whole protein matrix and by the amino acids located most closely to FeMoco to the nitrogen reduction under mild conditions, we studied the catalytic reactivity of the FeMoco cluster isolated from the protein toward the reduction of nitrogenase substrates in a nonprotein medium under conditions where electrons, protons, and the substrate were available to it.[6] For FeMoco as a catalyst of one nitrogenase reaction, namely, C_2H_2 reduction without a protein, we found a number of interesting features that are also characteristic of FeMoco functioning in an enzyme system. In particular, the presence of a number of interdependent sites for substrate and inhibitor coordination on the europium amalgam-reduced FeMoco cluster was demonstrated and the type and pa-

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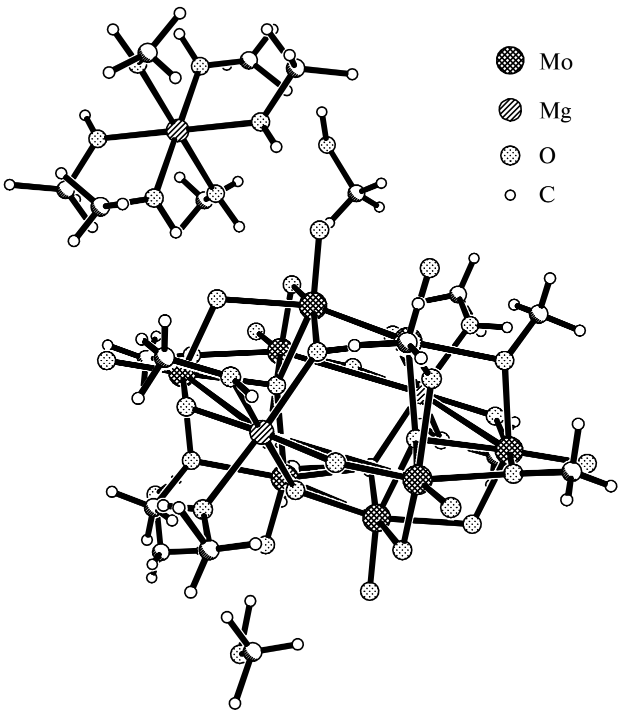


Fig. 1. Molecular structure of $\{[Mg_2Mo_8O_{22}(MeO)_6(MeOH)_4]^{2-}[Mg(MeOH)_6]^{2+}\}\cdot 6MeOH$ (**1**).

rameters for inhibition of acetylene reduction with carbon monoxide and molecular nitrogen were determined. The binding constants of both substrates and inhibitors with the FeMoco cluster reduced by europium amalgam without a protein matrix were close to those found for the nitrogenase M-center (M-center is the FeMoco cluster in the MoFe protein).

Previously,[7] we showed that, apart from N_2 reduction, the polynuclear Mg—Mo-complex can catalyze the reduction of C_2H_2, moreover, by much weaker reducing agents than sodium amalgam, for example, by zinc or europium amalgam, but in the presence of a stronger proton donor, thiophenol.

In this work, we studied the kinetics of C_2H_2 reduction catalyzed by cluster **1** under the same conditions and in the presence of the same reducing agents as have been used previously[6] to study the catalytic reactivity of FeMoco isolated from the enzyme. Thus, it would be possible to compare the mechanistic features of this reaction under the same conditions in the presence of either synthetic polynuclear complex **1** or the natural FeMoco cluster, in order to find out how much they are similar as catalysts.

Experimental

The commercial chemicals: tetra-*N*-butylammonium bromide, thiophenol (Fluka), pentafluorothiophenol (Sigma), phosphatidyl choline (Kharkov, Bacterial Preparation Plant), sodium hydroxide (Chemapol), anhydrous magnesium sulfate (chemically pure grade), molybdenum pentachloride (pure grade), magnesium chloride (chemically pure grade), tributylphosphine, mercury metal 99.9995% (Aldrich), argon (high-purity grade), and nitrogen (special purity grade), were used as received.

Anhydrous methanol (pure grade, Reakhim) purified by refluxing with magnesium methoxide and distilled in an inert gas flow was used as the solvent. Analytical solutions were prepared using triply distilled water.

Acetylene (pure grade) was additionally purified by a known procedure.[6b,c] Dideuterioacetylene was synthesized by the reaction of calcium carbide with heavy water (D_2O, 99.8% deuterium) and purified in a similar way.[6b] Carbon monoxide was synthesized by the reaction of sodium formate (Reakhim) with concentrated sulfuric acid. Sodium, zinc, and europium amalgams were prepared by dissolving the metals in mercury in an inert atmosphere. The metal concentration was determined by a published procedure.[6a] The prepared amalgams were stored under argon. All operations with substances sensitive to oxidation were carried out under strictly anaerobic conditions using Schlenk technique.

Complex **1** was synthesized from $MoCl_5$ and $MgCl_2$ in methanol, as described previously.[4] To prepare the active form of the catalyst (designated in the Schemes as $[Mg_2Mo_8]$), the complex was reduced by sodium amalgam as described previously.[1]

Acetylene reduction. The experiments were carried out in a specially designed thermostated flat-bottom glass vessel with a magnetic stirrer, suitable for working with metal amalgams.[8] The vessel was evacuated and filled with argon, then Eu/Hg (0.5 mL) was introduced under argon into the side tumbler tube and a solution (4—4.3 mL) containing complex **1** ($5\cdot10^{-6}$ mol L^{-1}) and thiophenol (0.01 mol L^{-1}) in MeOH was added into the main vessel. After freezing of the liquid phase, the reaction vessel was connected to a circulation unit and evacuated, then the liquid was unfrozen, and the vessel was filled with a gas mixture. During the experiment, the gas phase was stirred in the circulation unit and the liquid phase was stirred with a magnetic stirrer under conditions that ensured the maximum dispersion of the amalgam. The gas phase composition was varied depending on the task: for acetylene reduction, the vessel was filled with an acetylene—argon mixture in different ratios (to study the reaction stereospecificity, dideuterioacetylene was used); to study the inhibitory effect of CO or N_2, a CO (or N_2)—acetylene—argon mixture was used. In the experiments dealing with the dependence of the reaction rate on the catalyst concentration, the content of the complex in the reaction mixture was varied in a range of $(0.3—3)\cdot10^{-5}$ mol L^{-1} at a constant acetylene pressure of 110 Torr (0.145 atm) and a constant liquid phase volume.

The reaction was monitored by taking gas samples from the reaction vessel at regular intervals for chromatographic analysis (see below). The resulting kinetic curves show the time dependence of the amount of the product formed. Using the initial sections of the kinetic curves, the initial steady-state reaction rate (W), *i.e.*, the amount of the reaction product formed per unit time, was calculated.

Nitrogen reduction with europium amalgam. In the study of nitrogen reduction with europium amalgam in the presence of thiophenol or pentafluorothiophenol, the vessel was filled with the liquid phase similarly to acetylene reduction experiments and then filled with nitrogen up to atmospheric pressure, and the reaction was carried out for 30 min at 20 °C. After completion of the reaction, the solution was analyzed for hydrazine and ammonia (see below).

Nitrogen reduction with sodium amalgam. Inhibition. The reduction of nitrogen was carried out in a vessel similar to that used in acetylene reduction experiments but in this case, a sampling capillary was inserted in the vessel to determine the hydrazine and ammonia contents.[8] The vessel was pre-evacuated and sodium amalgam ($C = 1.07$ mol L^{-1}) (0.7 mL) was introduced in a nitrogen flow into the side tumbler tube, while the main vessel was filled with methanol (8 mL), a solution of NaOMe (4.6 mol L^{-1}) in methanol (0.01 mL), a solution of phosphatidyl choline (0.13 mol L^{-1}) in ethanol (0.02 mL), tributylphosphine (4.013 mol L^{-1}, 0.01 mL), and complex **1** (0.015 mL) with a concentration of $4.9 \cdot 10^{-3}$ mol L^{-1}. After addition of the reactants, the vessel was filled with an N_2—Ar mixture in nitrogen reduction experiments and with an N_2—C_2H_2—Ar mixture when acetylene inhibition of nitrogen reduction was studied.

Experiments on electroreduction of acetylene and determination of the reaction activation energy were carried out in the potentiostatic mode with a zinc amalgam working electrode by a published procedure.[9] All measurements at different temperatures were carried out at the same reactant concentrations and other parameters; therefore, the activation energies were calculated by the Arrhenius equation using reaction rates rather than rate constants.

Analytical procedures. The molybdenum concentration was determined by spectrophotometry in the thiocyanate complex ($\lambda = 460$ nm).[10] The magnesium concentration was determined by titration with Trilon B in an ammonia buffer solution (pH 10) in the presence of Chromogen Black ET-00 as an indicator.[11]

The concentration of hydrazine was determined by spectrophotometry as a colored compound (dihydrazone, $\varepsilon_{max}(458\text{ nm}) = 7 \cdot 10^4$ L mol $^{-1}$ cm^{-1}) *via* the reaction with *p*-dimethylaminobenzaldehyde in an acid medium.[12] The concentration of ammonia was determined by the indophenol method modified as described previously.[13]

The gaseous reaction products, *viz*., ethylene, ethane, and methane, were analyzed by gas chromatography on a Biochrom chromatograph (column filled with activated alumina (0.25—0.5 mm), column temperature 90 °C, argon as the carrier gas, flame ionization detector).

Absorption spectra were recorded on a Hewlett—Packard 8451A Diode Array Spectrophotometer. IR spectra were measured on a Perkin—Elmer Spectrum Bx2 IR spectrometer in a range of 4000—400 cm^{-1} with a 2 cm^{-1} resolution. The spectra were recorded using a sealed KBr cell for gaseous samples. The gaseous products of the catalytic reduction of C_2D_2 were sampled using a Tepler pump from the reaction vessel cooled to –93 °C by an ethanol bath.

Results and Discussion

Dependence on the catalyst concentration. The plot for the ethylene and ethane formation rates *vs.* the volume concentration of catalyst **1** in the reaction solution is shown in Fig. 2. At Mg_2Mo_8 concentration $>5 \cdot 10^{-6}$ mol L^{-1}, an apparent zero order is observed caused by the saturation effect; probably, this concentration corresponds to complete surface coverage of the given amount of amalgam with the reduced cluster at a constant stirring rate. Previously,[6b] it has been shown for a system involving the FeMoco cluster (**2**) that the dependences of the ethylene and ethane formation rates on the volume concentration of the catalyst are described by an equation similar to the adsorption isotherm equation that reflects the change in the catalyst surface concentration upon an increase in its volume concentration. This indicates that the reactions giving the reduction products occur on the amalgam surface without migration of the catalyst—substrate complex to the solution bulk in both systems. This pattern of the process is additionally confirmed by electrochemical experiments, which show (see below) that the electron transfer from the amalgam to the catalytic cluster adsorbed on its surface is the rate-limiting step.

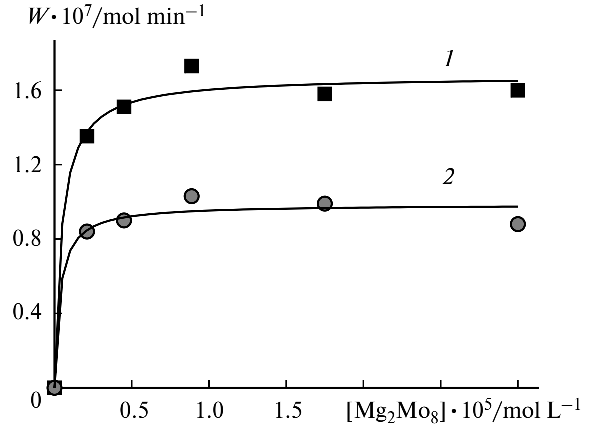


Fig. 2. Initial rates of ethylene (*1*) and ethane (*2*) formation *vs.* volume concentration of the catalyst upon acetylene reduction. Reaction conditions: Eu/Hg (0.35 mol L^{-1}) 0.5 mL; [PhSH] = 0.003 mol L^{-1}; MeOH as the solvent (4.2 mL); $[Mg_2Mo_8]$ as the catalyst; 21 °C; $p_{C_2H_2} = 110$ Torr.

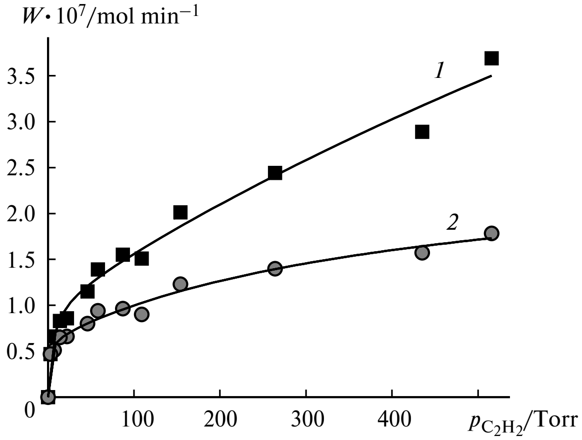
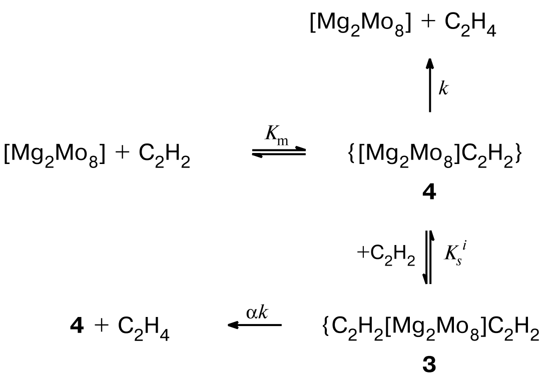


Fig. 3. Rates of ethylene (*1*) and ethane (*2*) formation *vs.* substrate pressure in the reduction of C_2H_2. Reaction conditions: Eu/Hg 0.5 mL; [PhSH] = 0.01 mol L^{-1}; MeOH as the solvent (4.2 mL); [**1**] = $5 \cdot 10^{-6}$ mol L^{-1}; C_2H_2; 21 °C.

Dependence on the substrate concentration. The curves for ethylene and ethane formation rates over a wide range of C_2H_2 pressures in the gas phase above the reaction solution are presented in Fig. 3.

The products of C_2H_2 reduction are formed in parallel. The pattern of the dependence (gradual increase in the reaction rate without smoothing down) suggests a non-Michaelis reaction mechanism,[14] involving activation of the reaction with excess substrate due to the formation of ternary complex **3** (catalyst—substrate—substrate), which differs in reactivity from the catalyst—substrate complex **4** (Scheme 1).

Scheme 1



where K_s^i is equilibrium dissociation constant of ternary complex **3**.

$$K_s^i = \frac{[\{[Mg_2Mo_8]C_2H_2\}][C_2H_2]}{[\{C_2H_2[Mg_2Mo_8]C_2H_2\}]}$$

The reaction rate is described by the equation:

$$W = \frac{V_{max}K_s^iS + \alpha V_{max}S^2}{K_s^iK_m + K_s^iS + S^2}, \tag{1}$$

$\alpha > 1$ and $K^i_s > \alpha K_m$ (see Ref. 15),

where W is the reaction rate, S is the substrate concentration, K_m is the Michaelis constant, V_{max} is the maximum rate attained under conditions of cluster saturation with the substrate, α is the correction factor.

To find the Michaelis constant, which is a measure of the substrate affinity for the catalyst, we made use of the fact that at low concentrations of the substrate, the term containing S^2 in expression (1) can be neglected, and the equation will be simplified to the Michaelis—Menten hyperbola equation.[15a] Figure 4 presents the experimental data for low acetylene pressures (3—22 Torr). The Michaelis constants found for the ethylene and ethane formation reactions in the acetylene concentration range chosen were 0.0048 and 0.0023 atm C_2H_2, respectively. The fact that K_m values for the two products are different may be indicative of the existence of different binding sites on the catalytic cluster, one giving predominantly ethylene and the other, ethane. This difference is also attributable to the fact that under equilibrium conditions, the K_m value is determined by the rate constants for not only binding of the substrate molecule to the cluster but also for all the subsequent steps, resulting in the reduction products (*i.e.*, electron and proton transfer). Meanwhile, the numbers and rates of such steps *en route* to ethylene and ethane are different.

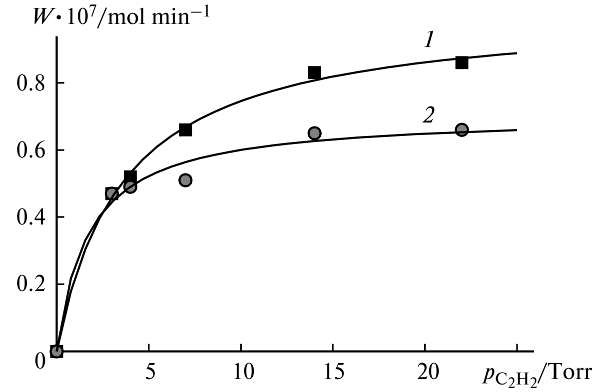


Fig. 4. Rates of ethylene (*1*) и ethane (*2*) formation in the acetylene reduction in the C_2H_2 pressure range of 3—22 Torr in straight coordinates. Reaction conditions: see Fig. 3.

Thus, the obtained data show that the reduced cluster has several substrate-coordinating sites with different acetylene affinities. Apparently, one, the most active site that operates at low acetylene pressures gives equal proportions of ethane and ethylene; indeed, for acetylene pressure of up to 7 Torr, the ethane fraction in the reaction products is ~50%. For a similar reaction in the system with catalyst **2**, we have also observed the existence of several C_2H_2 coordination and reduction sites with different substrate affinities, the K_m values found being close in magnitude in the two systems.

Inhibition of acetylene reduction by carbon monoxide. It is known[16] that CO inhibits the reduction of all nitrogenase substrates except for H^+ in the case of enzyme catalysis. The inhibiting effect is due to the fact that CO, being isoeletronic to N_2 and C_2H_2, is bound to the cluster, which has been reduced during the catalytic cycle, although CO itself is not reduced.

Data on the effect of CO on the rate of C_2H_2 reduction catalyzed by cluster **1** are presented in Fig. 5. It can be seen that the rates of formation of both ethylene and ethane decrease in the presence of CO. Treatment of the kinetic data on the inhibition by CO in the Dixon and Cornish—Bowden coordinates (Figs. 6 and 7) allows one to determine the inhibition type and calculate the process parameters.[15b] Figure 6 shows the dependence of the initial steady-state rate for ethylene and ethane formation on the CO pressure in the system involving cluster **1** in the

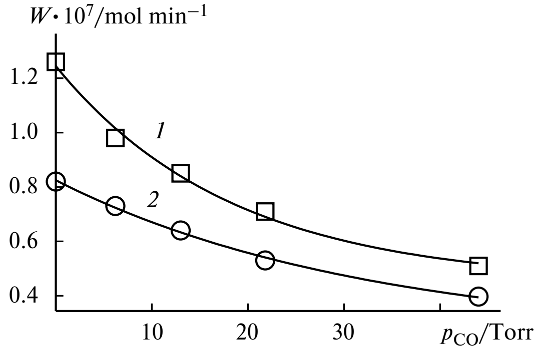


Fig. 5. Rates of ethylene (*1*) и ethane (*2*) formation in the acetylene reduction catalyzed by the Mg—Mo-complex and inhibited by carbon monoxide. Reaction conditions: Eu/Hg 0.5 mL; [PhSH] = 0.01 mol L^{-1}; MeOH as the solvent (4.2 mL); [**1**] = $5\cdot10^{-6}$ mol L^{-1}; $p_{C_2H_2}$ = 107 Torr; CO; 21 °C.

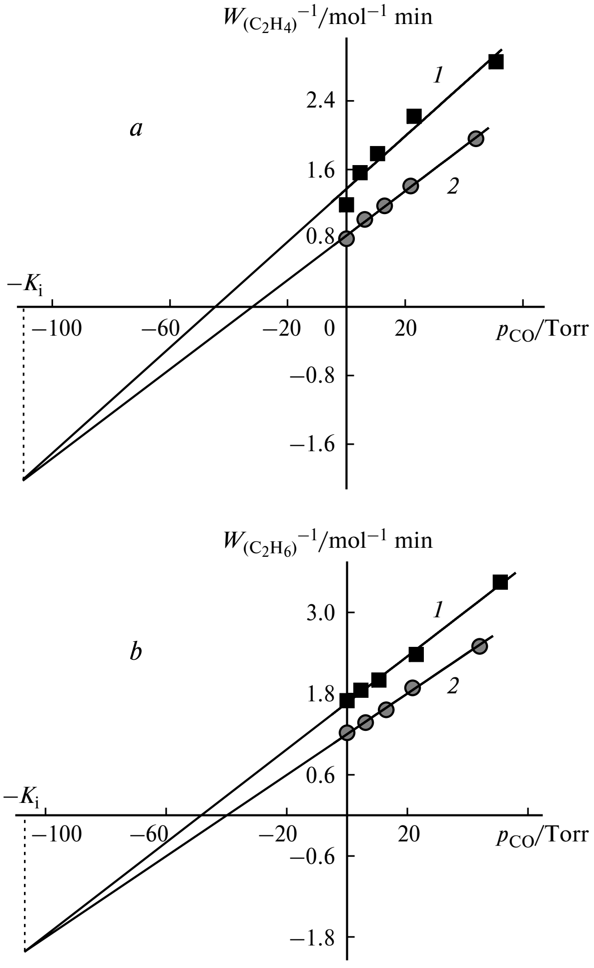


Fig. 6. Carbon monoxide inhibition of C_2H_2 reduction to C_2H_4 (*a*) and C_2H_6 (*b*) with europium amalgam catalyzed by the Mg—Mo-cluster (data in the Dixon coordinates): $p_{C_2H_2}$ = 50 (*1*), 107 Torr (*2*). Reaction conditions: see Fig. 5.

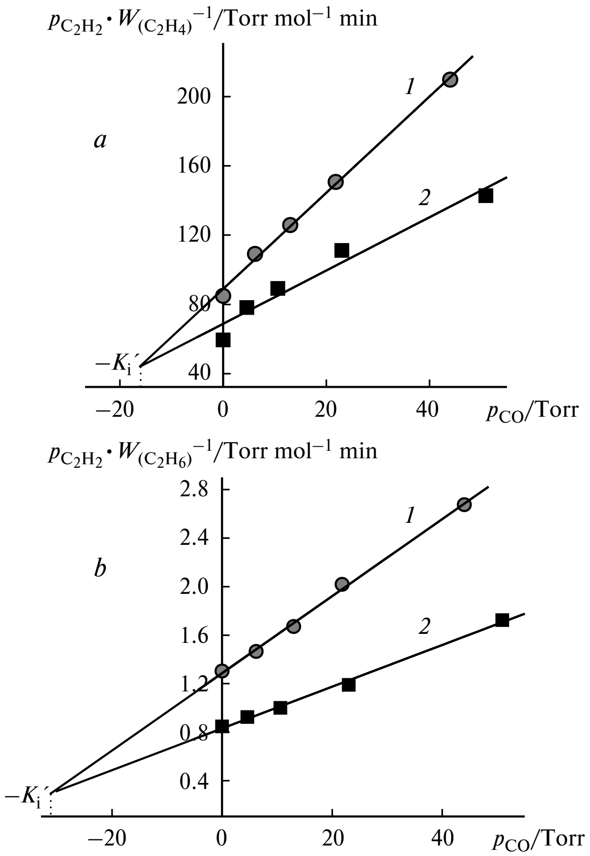


Fig. 7. Carbon monoxide inhibition of C_2H_2 reduction to C_2H_4 (*a*) and C_2H_6 (*b*) (data in the Cornish—Bowden coordinates): $p_{C_2H_2}$ = 50 (*1*), 107 Torr (*2*). Reaction conditions: see Fig. 5.

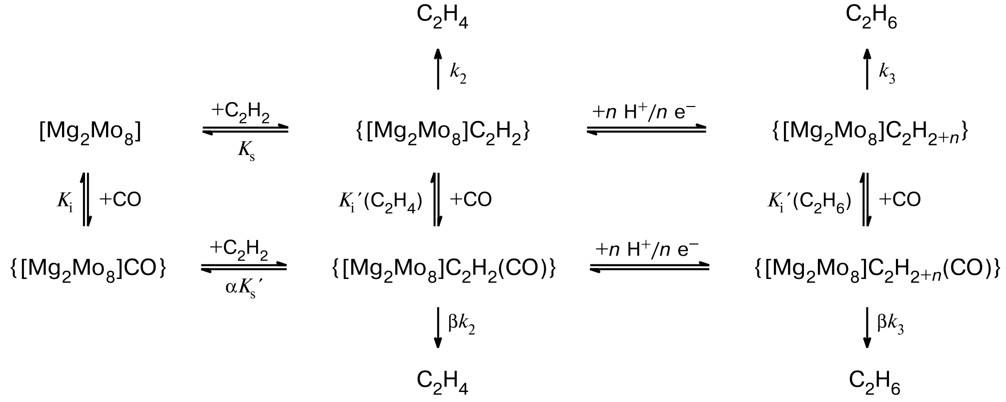
Dixon coordinates. The straight lines related to two different substrate concentrations intersect in the third quadrant. With this form of the plot, one can directly determine the inhibition constant (K_i), which is the reciprocal of the cluster affinity for the inhibitor CO molecule. The K_i values for ethylene and ethane are equal (0.14 atm) and the inhibition is of a mixed type. Since the intersection point of the straight lines lies below the *x* axis showing the inhibitor pressure, the contribution of non-competitive inhibition is greater than that of competitive inhibition, *i.e.*, $K_i > K_i'$, where

$$K_i = \frac{[Mg_2Mo_8][CO]}{[\{[Mg_2Mo_8]CO\}]},$$

$$K_i' = \frac{[\{[Mg_2Mo_8]C_2H_2\}][CO]}{[\{[Mg_2Mo_8]C_2H_2CO\}]}.$$

The constant K_i' for product formation was found using the Cornish—Bowden method. The plots for of the ratio of the substrate pressure to the reduction rate ($p_{C_2H_2}/W$) *vs.* the inhibitor pressure (p_{CO}) are shown in Fig. 7. The resulting constants proved to be different for ethylene and ethane formation; this may imply that CO is attached to the complex that already has a substrate mol-

Scheme 2



$K_i = 0.14$ atm CO, $K_i'(C_2H_4) = 0.02$ atm CO, $K_i'(C_2H_6) = 0.04$ atm CO, $K_s \leq K_i$

ecule in the coordination sphere at different reduction steps.

The catalytic transformation of acetylene into ethylene and ethane in the presence of CO in the gas phase in the system involving **1** is shown in Scheme 2.

A comparison of the data obtained on the carbon monoxide inhibition of the reduction of C_2H_2 for cluster **1** as the catalyst with these data for the FeMoco catalyst[6c] shows qualitatively similar pictures. In both cases, substantial inhibition is observed, which is different for the formation of ethylene and ethane. Quantitatively, the inhibiting effect of carbon monoxide is much higher for the system containing cluster **2**: for $p_{CO} = 0.5p_{C_2H_2}$ the rate of acetylene reduction decreased ninefold; for the same substrate to inhibitor ratio in the system with cluster **1**, the rate of acetylene reduction decreased only twice.

Apparently, a mixed type of inhibition is involved in both cases, in particular, CO is coordinated to several sites of the cluster and affects both C_2H_2 coordination to the reduced cluster and decomposition of the catalyst—substrate complex to give the products. No reduction of CO is observed in either of the cases.

Data on the effect of CO on the reduction of acetylene catalyzed by cluster **1** confirm the conclusion drawn from the study of the variation of the rate *vs.* substrate concentration in this system, namely, there are several interdependent coordination sites for substrates and inhibitors on the reduced catalytic Mg—Mo-cluster. A similar feature is observed for the FeMoco catalyst.[6b,c]

Inhibition of nitrogen reduction by acetylene in the system involving the synthetic Mg—Mo-cluster. While investigating the catalytic reduction of C_2H_2 catalyzed by FeMoco, we found that molecular nitrogen inhibits competitively the reduction of C_2H_2 through the reversible complexation with reduced cluster **2** (see Ref. 6c). In the absence of protein, no subsequent protonation of the resulting nitrogen complex takes place, at least, under conditions where C_2H_2 is efficiently reduced. The Mg—Mo-complex, unlike FeMoco extracted from the protein, efficiently catalyzes the reduction of N_2 to hydrazine and ammonia (see, for example, Ref. 13 and references cited therein). It was of interest to determine the characteristics of the dinitrogen complex formed at the first step of N_2 activation to compare them with the results obtained for the FeMoco catalyst. However, in the presence of the Mg—Mo-complex, we did not find any inhibiting effect of nitrogen toward the C_2H_2 reduction even for very low acetylene concentrations in the gas phase. However, the reduction of N_2 is inhibited by acetylene very efficiently.

The kinetics of the catalytic reduction of nitrogen catalyzed by the polynuclear Mg—Mo-complex has been studied in detail (see, for example, Ref. 7). High rates of N_2H_4 and NH_3 formation are observed in so-called complete system where sodium amalgam is used as the reducing agent and phosphatidylcholine and tributylphosphine are present as the co-catalysts. Under these conditions, acetylene is a very good substrate with K_m of about $3 \cdot 10^{-4}$ atm C_2H_2 (found from inhibition data). The saturation of the reaction rate with respect to nitrogen is observed at N_2 pressures of several tens of atmospheres, while the K_m value for nitrogen calculated from data on acetylene inhibition of nitrogen reduction is 25 atm. It is not surprising that acetylene inhibits efficiently the reduction of nitrogen under these conditions. The plot for the nitrogen reduction rate in the presence of minor amounts of acetylene in the gas phase is presented in Fig. 8. It can be seen that the inhibiting effect is of noncompetitive type (the straight lines intersect in the negative region of the x axis showing the inhibitor pressure) and K_i is equal to 0.009 atm C_2H_2. A comparison with similar data[17] for the nitrogenase system *in vitro* shows that the relationships of the characteristics found for acetylene and nitrogen as substrates in the natural system and in its best functional model are closely similar.

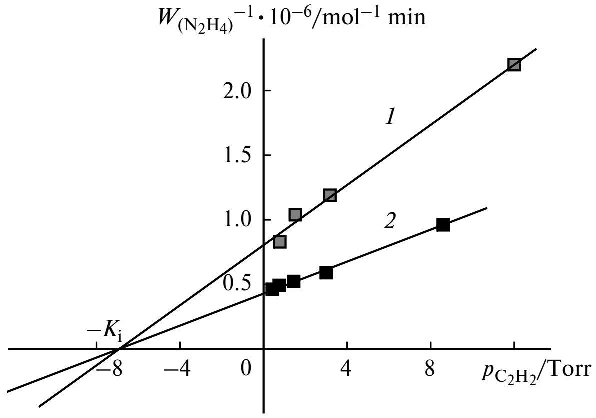


Fig. 8. Acetylene inhibition of N_2 reduction in the complete system at p_{N_2} = 400 (*1*), 760 Torr (*2*) (data in the Dixon coordinates). Reaction conditions: Na/Hg (1.07 mol L^{-1}), $[Bu_3P]$ = $5 \cdot 10^{-3}$ mol L^{-1}, [phosphatidyl choline] = $3 \cdot 10^{-4}$ mol L^{-1}, [NaOMe] = $6 \cdot 10^{-3}$ mol L^{-1}, [**1**]= $8.3 \cdot 10^{-6}$ mol L^{-1}, $[Bu_4NBr]$ = 0.1 mol L^{-1}.

Electrochemical experiments and temperature dependence. Previously, for systems containing the Mg—Mo-cluster[7,13] and FeMoco isolated from a protein[9] as catalysts, the rate of C_2H_2 reduction was found to depend exponentially on the specified potential of a zinc amalgam cathode in the presence of proton donors such as thiophenol or water. This implies that for both systems, electron transfer from the cathode to the catalytic cluster is the rate-limiting step of the process. While investigating the temperature dependences of acetylene reduction catalyzed by cluster **2** with PhSH as the proton donor, we found[9] that the effective activation energies for the formation of ethane and ethylene are different, E_a for the formation of C_2H_6 being ~1.5 times lower than that for C_2H_4 (Table 1). It was concluded that the structure and properties of the natural catalytic cluster are favorable for multielectron processes.

Study of the temperature dependence of C_2H_2 reduction rate with cluster **1** as the catalyst showed that the rates of ethylene and ethane formation increase exponentially with temperature rise in the range from 15 to 35 °C not only in the presence of thiophenol, but also in the presence of a more acidic proton donor, pentafluorothiophenol (Fig. 9). For the system with cluster **2**, an increase in the proton donor acidity resulted in a change in the rate-limiting step, which was manifested as the lack of dependence of the acetylene reduction rate on the cathode potential in the presence of C_6F_5SH. Hence, the system with the Mg—Mo-complex depends to a lesser extent on the acidity of the protonating agent. Having calculated the effective activation energies (E_a) from the Arrhenius equation, we found that with the Mg—Mo-complex as the catalyst, too, E_a for C_2H_6 formation is lower than E_a for C_2H_4 formation (see Table 1). Thus, a common feature of these clusters is that they catalyze multielectron processes. The absolute values of E_a are markedly higher for the reaction in the presence of **2**. Note that the E_a values for **2** were obtained from experiments carried out at a potential of −1.7 V, while for the system with cluster **1**, at a potential of −1.3 V (*vs.* saturated silver chloride electrode $Ag/AgCl/KCl_{sat}$). Since a

Table 1. Activation energies for the formation of ethylene and ethane in the catalytic systems containing clusters **1** and **2** in the presence of thiophenol and pentafluorothiophenol

Catalyst	Reaction conditions		E_a/kcal mol^{-1}	
	Reagent	$-E$/V	C_2H_4	C_2H_6
FeMoco[12]	PhSH	1.7	18.3±1.5	12.8±2
	C_6F_5SH	1.3	32±4	32±4
Mg—Mo-complex	PhSH	1.3	9.4±0.6	5.4±0.6
	C_6F_5SH	1.3	7.1±0.5	3.7±0.9

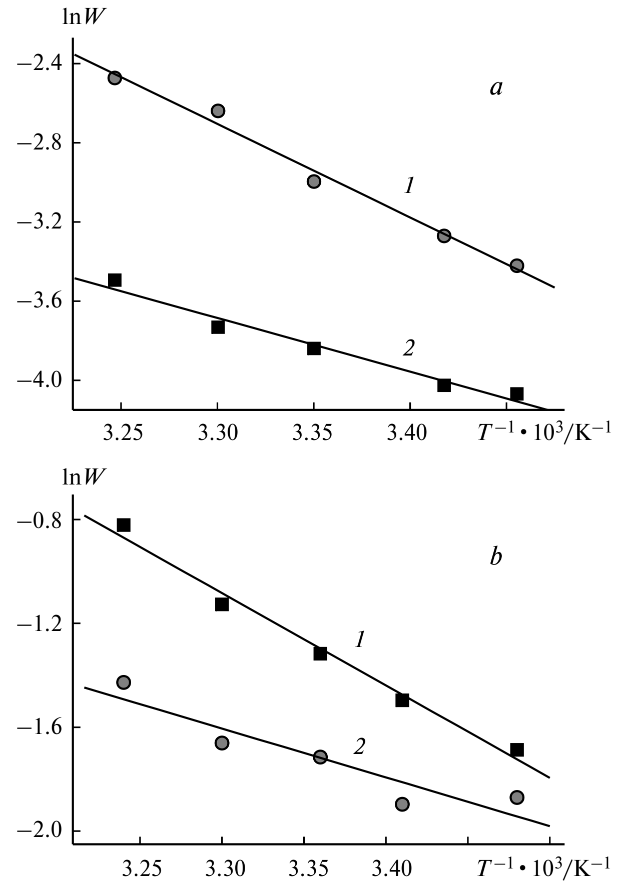


Fig. 9. Temperature dependences of the ethylene (*1*) and ethane (*2*) formation rates during the catalytic reduction of acetylene with the Mg—Mo-complex in the presence of thiophenol (*a*) and pentafluorothiophenol (*b*); Arrhenius coordinates. Reaction conditions: the specified potential of the zinc amalgam cathode *E* = −1.3 V; MeOH as the solvent; the catholyte volume 6.5 mL; the catalyst [**1**] = $1.32 \cdot 10^{-5}$ mol L^{-1}; [PhSH] = $[C_6F_5SH]$ = $1.2 \cdot 10^{-2}$ mol L^{-1}; $p_{C_2H_2}$ = 100 Torr.

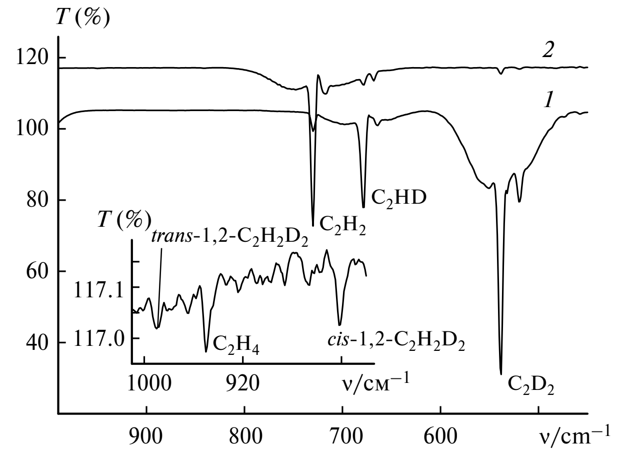


Fig. 10. IR spectra of the initial acetylene C_2D_2 (*1*) and the products of C_2D_2 reduction catalyzed by the Mg—Mo-complex (*2*); the inset shows the enlarged fragment of spectrum *2* corresponding to the reduction products. Reaction conditions: Eu/Hg (0.5 mL); [PhSH] = 0.01 mol L^{-1}; MeOH as the solvent (4.2 mL); [**1**] = $5 \cdot 10^{-6}$ mol L^{-1}; $p_{C_2D_2}$ = 120 Torr; 21 °C.

decrease in the potential, with other factors remaining invariable, entails a decrease in the activation energy for those systems in which electron transfer is the rate-limiting step, the observed difference indicates that a better spatial organization and a stronger contact with the amalgam (reducing agent) are attained in the system with the Mg—Mo-complex. This facilitates the electron transfer and decreases the activation energy.

Reaction stereospecificity. In order to elucidate the type of binding of the C_2H_2 molecule to the cluster and the mechanism of protonation of the coordinated acetylene, we attempted to study the stereospecificity of ethylene formation from C_2D_2 upon protonation with PhSH or MeOH using FT IR spectroscopy. We did not obtain unambiguous information, as under experimental conditions, C_2D_2 underwent fast and almost quantitative H/D exchange on europium amalgam to give a mixture of C_2D_2, C_2HD, and C_2H_2. This reaction occurs on the Eu/Hg surface equally efficiently both in the presence and in the absence of the catalytic cluster in solution, the exchange of C_2D_2 with the PhSH or MeOH protons being faster than the catalytic reduction of C_2D_2. The reduction of a mixture of acetylenes yields a set of all possible ethylene isotopes without predominant formation of either *cis*- or *trans*-product (Fig. 10).

Some characteristics of the reduction of C_2H_2 and nitrogen with europium amalgam in the presence of the Mg—Mo-complex and FeMoco were compared (Table 2). It can be seen that the regular features of these reactions in the systems involving the natural catalytic FeMoco cluster and the synthetic Mg—Mo-complex simulating the function of this cluster are qualitatively similar in many respects.

The most pronounced differences between these systems are related to the reaction with molecular nitrogen. In the presence of cluster **2**, only reversible complexation of nitrogen with the reduced cluster takes place, while with cluster **1**, N_2 is reduced to hydrazine and ammonia. In the latter case, we did not succeed in obtaining any characteristics of the primary complexation of N_2, because no inhibition of the acetylene reduction by nitrogen was observed in this system. Apparently, even under conditions far from saturation with nitrogen, the dinitrogen complex formed is rapidly and irreversibly protonated to give N_2H_4 and NH_3.

Analysis of the data obtained on the comparative behavior of the clusters suggests that it would be possible to create a catalytic system with FeMoco without protein that would perform the reduction of molecular nitrogen. This requires creating a better contact of the catalytic cluster with the surface of the electron donor (amalgam or cathode), as has been done previously in the system with the Mg—Mo-cluster in a lipid film,[8] by selecting appropriate surfactants or using electrodes with special coatings capable of strong adsorption of FeMoco.

Table 2. Comparison of some parameters of the reduction of nitrogenase substrates catalyzed by FeMoco isolated from the protein and by the synthetic Mg—Mo-complex

Catalyst	Substrate							
	Acetylene				CO[a]		Nitrogen	
	$K_m \cdot 10^3$ /atm C_2H_2		p[b] (%)		$K_i \cdot 10^3$ /atm C_2H_2		I[c] K_m	II[d] K_i
	C_2H_4	C_2H_6	C_2H_2	*trans*-$C_2H_2D_2$	C_2H_4	C_2H_6	atm N_2	
FeMoco without protein	6	6	22	11	3	6	—	0.49
Mg—Mo-complex	4.8	2.3	47	—	140	140	~25	—

[a] A mixed inhibition mechanism is involved for both catalysts.

[b] Fraction.

[c] I is the reduction of nitrogen.

[d] II is acetylene inhibition of the reduction of nitrogen.

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References

1. L. P. Didenko, A. B. Gavrilov, A. K. Shilova, V. V. Strelets, N. V. Tsarev, and A. E. Shilov, *Nouv. J. Chim.*, 1986, **10**, 503.
2. L. P. Didenko, A. B. Gavrilov, A. K. Shilova, V. V. Strelets, N. V. Tsarev, A. E. Shilov, V. D. Makhaev, A. K. Banerjee, and L. Pospišhil, *Nouv. J. Chim.*, 1986, **10**, 583.
3. M. Yu. Antipin, L. P. Didenko, L. M. Kachapina, A. E. Shilov, A. K. Shilova, and Yu. T. Struchkov, *J. Chem. Soc., Chem. Commun.*, 1989, 1467.
4. M. Yu. Antipin, Yu. T. Struchkov, A. E. Shilov, and A. K. Shilova, *Gazz. Chim. Ital.*, 1993, **123**, 256.
5. B. K. Burgess and D. J. Lowe, *Chem. Rev.*, 1996, **96**, 2983.
6. (a) T. A. Bazhenova, M. A. Bazhenova, G. N. Petrova, S. A. Mironova, and V. V. Strelets, *Kinet. Katal.*, 2000, **41**, 550 [*Kinet. Catal.*, 2000, **41**, 499 (Engl. Transl.)]; (b) T. A. Bazhenova, M. A. Bazhenova, G. N. Petrova, and S. A. Mironova, *Kinet. Katal.*, 2002, **43**, 382 [*Kinet. Catal.*, 2002, **43**, 351 (Engl. Transl.)]; (c) M. A. Bazhenova, T. A. Bazhenova, G. N. Petrova, and S. A. Mironova, *Kinet. Katal.*, 2002, **43**, 219 [*Kinet. Catal.*, 2002, **43** (Engl. Transl.)].
7. T. A. Bazhenova, M. A. Bazhenova, G. N. Petrova, A. K. Shilova, N. I. Shuvalova, and A. E. Shilov, *Dokl Akad. Nauk*, 1997, **354**, 51 [*Dokl. Chem.*, 1997 (Engl. Transl.)]; T. A. Bazhenova, M. A. Bazhenova, S. A. Mironova, G. N. Petrova, A. K. Shilova, N. I. Shuvalova, and A. E. Shilov, *Inorg. Chim. Acta*, 1998, **270**, 221.
8. L. P. Didenko, O. K. Gavrilina, E. E. Yablonskaya, A. K. Shilova, and A. E. Shilov, *Nouv. J. Chim.*, 1983, **7**, 605.
9. T. A. Bazhenova, N. V. Bardina, G. N. Petrova, and M. A. Borovinskaya, *Izv. Akad. Nauk, Ser. Khim.*, 2004, 1583 [*Russ. Chem. Bull., Int. Ed.*, 2004, **53**, 1646].
10. A. I. Busev, *Analiticheskaya khimiya molibdena* [*Analytical Chemistry of Molybdenum*], Izd-vo Akad. Nauk SSSR, Moscow, 1962, 205 pp. (in Russian).
11. E. V. Alekseevskii, R. K. Gol´ts, and A. P. Musakich, *Kolichestvennyi analiz* [*Quantitative Analysis*], Goskhimizdat, Leningrad, 1957, 391 pp. (in Russian).
12. M. Perez and A. Petit, *Bull. Soc. Chim. Fr.*, 1947, 122.
13. T. A. Bazhenova, M. A. Bazhenova, G. N. Petrova, A. K. Shilova, and A. E. Shilov, *Izv. Akad. Nauk, Ser. Khim.*, 1998, 890 [*Russ. Chem. Bull.*, 1998, **47**, 861 (Engl. Transl.)].
14. S. D. Varfolomeev and K. G. Gurevich, *Biokinetika: prakticheskii kurs* [*Biokinetics. Practical Course*], Fair-Press, Moscow, 1999, 101 (in Russian).
15. (a) M. Dixon and E. C. Webb, *Enzymes*, Longman Group Ltd., London, 1979; (b) *idem*, p. 515.
16. *A Treatise on Dinitrogen Fixation*, Eds R. Hardy, F. Bottomley, and R. Burris, Wiley, New York, 1979.
17. J. M. Rivera-Ortiz and R. H. Burris, *J. Bacteriol.*, 1975, **123**, 537.

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