Effect of the potential of an external electron donor on C_2H_2 reduction catalyzed by the nitrogenase active center (FeMoco) isolated from the enzyme

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The effect of potential value and chemical properties of an external electron donor on C_2H_2 reduction catalyzed by nitrogenase active center (cluster $[(\mu_6\text{-N})Fe_7MoS_9\cdot$homocitrate] FeMoco isolated from the enzyme) has been investigated in the presence of proton donors of different acidity. The temperature—reaction rate dependences of these reactions have been studied. It has been shown that the rate-limiting steps of the reactions differ depending on the proton donor used. When thiophenol or water are used as proton donors, and electrochemical step — the electron transfer from cathode to adsorbed catalytic cluster — has been found to be a rate-limiting one. The effective activation energy of ethane formation as a product of four-electron C_2H_2 reduction is found to be 1.5 times lower than that of ethylene, namely, ~13 kcal mol^{-1}. When stronger acid, pentafluorothiophenol, is used as a proton donor, the chemical step of intramolecular rearrangement of the catalyst—substrate complex taking place in solution becomes a rate-limiting one. The effective activation energies of both ethylene and ethane become equal to 32 kcal mol^{-1}.

Key words: iron-molybdenum cofactor, nitrogenase, acetylene reduction, metallocomplex electrocatalysis.

The main function of the enzyme nitrogenase is the catalysis of the mild reduction of atmospheric nitrogen to ammonia which is the first step in the global "nitrogen" cycle. The enzyme has already been studied for a long time, and the last decade was the most productive in terms of obtaining information on the enzyme structure. The three-dimensional X-ray crystal structures of both protein components of nitrogenase, Fe protein and MoFe protein, were determined for several nitrogenases from different bacteria.[1—3] The structures of the metal clusters included in the protein components of nitrogenases were established. The Fe protein contains a [4Fe—4S] cluster, while the MoFe protein contains two types of metal clusters unique in composition and structure: the so-called P-cluster [8Fe—7S],[2] and M-center: iron-molybdenum cofactor $[(\mu_6\text{-N})Fe_7MoS_9\cdot$homocitrate] or FeMoco.[2,3] Generally, the functions of both protein components of the enzyme and all three types of metal clusters are clear. From the experimental data available up to date, it is commonly accepted that FeMoco acts as catalytic center of enzyme.[4] Although the composition and molecular structure of the cluster are known, the detailed mechanism of catalysis of N_2 reduction involving FeMoco is to be elucidated. Where and how a substrate binds to the reduced cofactor? What is the mechanism of nitrogen protonation to form ammonia? What intermediate states are formed during this process?

Unlike for P-cluster, it is possible to probe the reactivity of FeMoco not only as a part of enzyme but also as an isolated cluster.[5] FeMoco as the M-center of the MoFe protein and the cofactor isolated from the protein are not identical species but very similar.[6,7] We study the reactivity of FeMoco extracted from the protein as a catalyst of reactions of nitrogenase substrate reduction when electrons, protons, and the substrate are provided to the cluster.[8—10] A comparison of the catalytic behavior of the FeMoco as a part of the protein and that of isolated one makes it possible to clarify the role of the cluster itself and the contribution of the protein matrix to the catalysis of reduction of such a "difficult" substrate as molecular nitrogen.

We found that FeMoco cluster, extracted from the MoFe protein of nitrogenase, catalyzes actively the reduction of acetylene, one of the most well studied substrates of nitrogenase which stands as a model for molecular nitrogen.[8,9] The reaction proceeds in aprotic sol-

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 8, pp. 1583—1591, August, 2004.

1066-5285/04/5308-1646

vents (DMF, *N*-methylformamide, THF) where FeMoco is stable in the absence of oxygen for a long time, in the presence of electron donors, for instance, zinc amalgam (Zn/Hg; $E_0 = -0.84$ V against the standard hydrogen electrode (SHE)) or europium amalgam (Eu/Hg; $E_0 = -1.4$ V against SHE)[8] and proton donors, *e.g.*, thiophenol.

To compare the mechanisms of the cofactor-catalyzed reduction of acetylene in enzymatic and non-enzymatic variants, we studied the kinetics of this reaction under non-protein conditions. The system involving Eu/Hg as a reducing agent and thiophenol (PhSH) as a proton donor was studied in most detail. The dependences of the rates of C_2H_4 and C_2H_6 formation on concentrations of the catalyst, substrate, proton donor, and amalgam (Eu/Hg) were studied. The stereospecificity of the reaction was studied by FTIR spectroscopy. The inhibition effect of CO and N_2 on acetylene reduction was found and studied in detail. The kinetic parameters of both C_2H_2 reduction and inhibition of this process by CO and N_2 were shown to be very similar to those observed for enzyme-catalyzed processes. In particular, the inhibition of both C_2H_4 and C_2H_6 formation by molecular nitrogen was shown to be competitive (inhibition constant $K_i = 0.5$ atm of N_2 for both products[10,11]). This implies that, as for enzymatic catalysis, N_2 and C_2H_2 as ligands compete for binding at the same coordination site on the FeMoco cluster reduced by amalgam, with quantitative parameters characteristic of nitrogenase enzyme *in vitro,* both wild type[12,13] and mutant.[13,14]

Our data[8–11] show that in many aspects FeMoco isolated from the enzyme catalyzes C_2H_2 reduction quite similarly to M-center of nitrogenase. Therefore, it can be concluded that the FeMoco cluster is an obligatory and, to some extent, sufficient active center of the enzyme: it is responsible for the formation of a substrate—enzyme complex and specificity of the nitrogenase action as a catalyst.

In enzymatic systems, the coordination of a nitrogen molecule to the reduced FeMoco cluster is followed by its protonation forming ammonia. In model non-protein systems involving the FeMoco isolated from the enzyme, most likely, no protonation of the complex $\{FeMoco_{red}N_2\}$ happens: we have not found products of N_2 reduction under the conditions when C_2H_2 is actively reduced. To reveal whether this is related to the fact that dinitrogen in the $\{FeMoco_{red}N_2\}$ complex is not sufficiently reduced by europium amalgam for subsequent protonation, we studied the effect of potential of the external electron donor on the catalytic reduction of C_2H_2 to C_2H_4 and C_2H_6 in the presence of thiophenol ($pK_a^{H_2O} = 6.43$) and pentafluorothiophenol ($pK_a^{H_2O} = 2.68$)[15] as proton donors. Here we present the data we obtained in this study showing that the kinetics of the process mentioned above differs depending on the acidity of proton donor used. It should be noted that acetylene is a very convenient substrate for studying the kinetics and mechanism of the cofactor-catalyzed electrocatalytic reaction, because it is easy to analyze the hydrocarbons formed. In addition, the relative amount of ethylene/ethane formed is sensitive to the reaction mechanism, namely, to the structure of catalytically active intermediate and the way of its reduction and protonation.

Experimental

The following reagents were used without additional purification: tris(hydroxymethyl)aminomethane (TRIS), benzylviologen (Serva); sodium creatine phosphate, tetra-*N*-butylammonium bromide, sodium dithionite, thiophenol (Fluka); pentafluorothiophenol (Sigma); diethylaminoethylsepharose (DEAE-Sepharose) CL-6B, creatinekinase (Sigma); magnesium chloride, trichloroacetic acid, mercury 99.9995% (Aldrich); tetra-*N*-butylammonium hexafluorophosphate, adenosinetriphosphoric acid (Aldrich); 2,2′-bipyridine (analytically pure grade, Reanal); argon (specially pure grade).

Molecular sieves 4 Å (Fluka) were activated by evacuation with heating and then stored in argon of atmosphere.

Dimethylformamide (pure grade, Reakhim) was used as a solvent. It was dried and distilled *in vacuo* (15 Torr) above molecular sieves 4 Å and then degassed by evacuation at reduced temperature; after evacuation, DMF was stored under argon before use.

Acetylene (pure grade) was additionally purified as follows: it was frozen with liquid nitrogen and then evacuated from an ethanol bath ($T = -100$ °C) to remove oxygen traces to a residual pressure of $5 \cdot 10^{-3}$ Torr. Then C_2H_2 was evaporated into a glass cylinder by increasing the temperature of the bath to −50 °C.

Tetrabutylammonium dithionite was synthesized using a known procedure.[5b]

Preparations of the Fe and MoFe proteins of nitrogenase from *Azotobacter vinelandii* bacteria were obtained according to a published procedure.[16] The FeMoco-deficient MoFe protein from the mutant strain *Klebsiella pneumoniae Kp* 5058 was kindly provided by Prof. B. E. Smith (Nitrogen Fixation Laboratory, John Innes Centre, United Kingdom).

Samples of the FeMoco in various solvents and solutions of the Fe and MoFe proteins were stored in the frozen state in liquid nitrogen.

Zinc and europium amalgams were synthesized as described earlier.[8] Prepared amalgams were stored under argon.

All manipulations with substances sensitive to oxidation (including chromatographic procedures) were carried out under strictly anaerobic conditions using Schlenk technique. All aqueous buffer solutions contained sodium dithionite ($5 \cdot 10^{-3}$ mol L^{-1}), and all organic solvents contained tetrabutylammonium dithionite ($(2—5) \cdot 10^{-3}$ mol L^{-1}). The presence of dithionite was monitored with the use of benzylviologen indicator.

Preparation and analysis of FeMoco. The iron-molybdenum cofactor was isolated from the MoFe protein of nitrogenase from *Azotobacter vinelandii* bacteria (concentration of the protein solution was 40—70 mg mL^{-1} in 0.25 *M* NaCl/25 m*M* TRIS · HCl) using protocol described in papers[5b,c] with some slight modifications. The cofactor was extracted from the MoFe

protein, pre-bound to an anion-exchanger DEAE-Sepharose, and then denaturated by DMF solution of Bu_4NBr.

Based analysis of the molybdenum and iron content in FeMoco samples (see below), we found the yield of FeMoco to be 70 to 85%. The [Fe]/[Mo] molar ratio ranged in an interval of 7—10. The quality of the FeMoco after isolation (retention of the cluster assay and the presence of homocitrate bound) was checked by the biological activity of FeMoco, *i.e.*, by its ability to recover the catalytic activity of the FeMoco-deficient MoFe protein from *Klebsiella pneumoniae Kp* 5058 towards C_2H_2 reduction. The assay was carried out according to a published protocol.[17] The molybdenum content in the FeMoco samples was determined by atomic absorption analysis on a Carl Zeiss AAS1 spectrometer with a Perkin Elmer HGA 74 graphite furnace. The iron content was determined spectrophotometrically as an Fe^{3+} complex with the CNS^- ion, measuring the absorbance of solutions in 95% EtOH at 500 nm. Iron in the cofactor samples was oxidized to the Fe^{3+} state by heating with dilute nitric acid (1 : 10). Absorption spectra were recorded with Hewlett Packard 8451A Diode Array Spectrophotometer.

Experiments on the electrochemical reduction of acetylene were carried out in a three-electrode glass cell with temperature control. The volume of the catholyte was 6.5 mL, zinc amalgam or pure mercury served as a cathode (surface area $S = 3.1$ cm^2), and the anode was a glass filter-top stick containing platinum wire placed inside the cell. Electrolysis was carried out at a controlled potential of the working electrode using a P-5827M potentiostat. The reference electrode was a silver chloride electrode (Ag/AgCl/KCl(sat.)) connected to reacting solution by electrolytic bridge. Argon was pumped through a cell by power circulation,[18] and a reaction solution containing FeMoco and thiophenol or pentafluorothiophenol in DMF was introduced into the cell in an argon flow. The cell and the circulation system were filled with a gas mixture of C_2H_2 (100 Torr) and argon. The overall pressure in the working system was kept at the atmospheric level. The gas mixture was circulated through the catholyte solution by means of an electromagnetic pump. The reaction was monitored by taking sample for analysis directly from the circulating gas mixture into an evacuated loop of calibrated volume. Then the sample was transferred from the loop to a chromatograph by carrier gas. Gaseous reaction products (C_2H_4, C_2H_6, and CH_4) were analyzed by gas chromatography on a Biochrom chromatograph supplied with flame-ionization detector. The column was packed with activated alumina (Al_2O_3, 0.25—0.5 mm), the temperature of the column was 90 °C, and argon was used as a carrier gas.

As a result, we obtained kinetic curves which were time dependences of the amount of product formed. Then the reaction rate was calculated from the initial regions of these curves and expressed in the number of moles of the product (C_2H_4 or C_2H_6) formed in 1 min. Each value of the reaction rate was averaged of two—three parallel experiments, with usual divergence of the rates in compared experiments not exceeding 10%.

Results and Discussion

Electrochemical reduction of C_2H_2 catalyzed by FeMoco. In the absence of FeMoco, no direct electroreduction of acetylene occurs by any cathode and at any value of the electrode potential. The cofactor catalyzes the reduction of C_2H_2, since FeMoco itself can be reduced by cathode to a substrate-binding state, and then it performs metallocomplex catalysis. In this case, C_2H_4 and C_2H_6 formation occurs in parallel. To determine the limiting step in the sequence of events leading to product formation, we studied the dependence of the rate of C_2H_4 and C_2H_6 accumulation on specified cathode potential.

Thiophenol or water as protonating agents. The experimentally obtained plot of the rate of C_2H_2 reduction to C_2H_4 *vs.* potential of the cathode (Zn/Hg) is presented in Fig. 1. It is seen that the rate of C_2H_4 formation increases sharply with an increase in the negative potential of the working electrode; the reaction rate reveals an exponential dependence on the cathode potential in a wide range of potential values. The plot becomes linear in the semilogarithmic coordinates (see Fig. 1, insert), *i.e.*, obeys the Tafel equation which relates an electrochemical reaction rate an the electrode potential E

$$E = a + b\log W_{C_2H_4}, \qquad (1)$$

where a and b are constant, and $W_{C_2H_4}$ is the rate of C_2H_4 accumulation at a given potential of the working electrode.

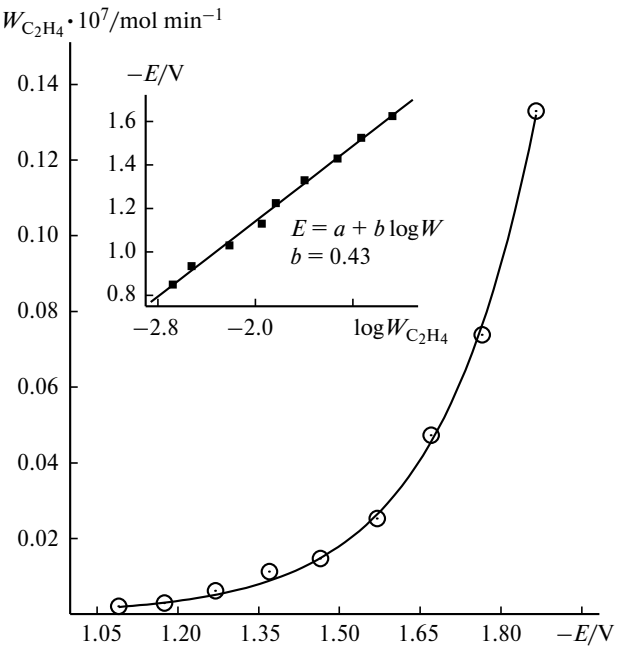


Fig. 1. Plot of the rate of C_2H_2 reduction (rate of C_2H_4 accumulation ($W_{C_2H_4}$)) *vs.* specified potential of the working electrode (E) (against Ag/AgCl/KCl(sat.)); the Tafel dependence for the reduction of C_2H_2 to C_2H_4 is given in the insert. Reaction conditions: solvent DMF; catalyst [FeMoco] = $1.2 \cdot 10^{-5}$ mol L^{-1}; proton donor thiophenol; [PhSH] = $1.2 \cdot 10^{-2}$ mol L^{-1}; electrode zinc amalgam (2 wt.%), 2 mL; cathode surface area 3.1 cm^2; catholyte volume 6.5 mL; 20 °C; $P_{C_2H_2}$ = 0.13 atm.

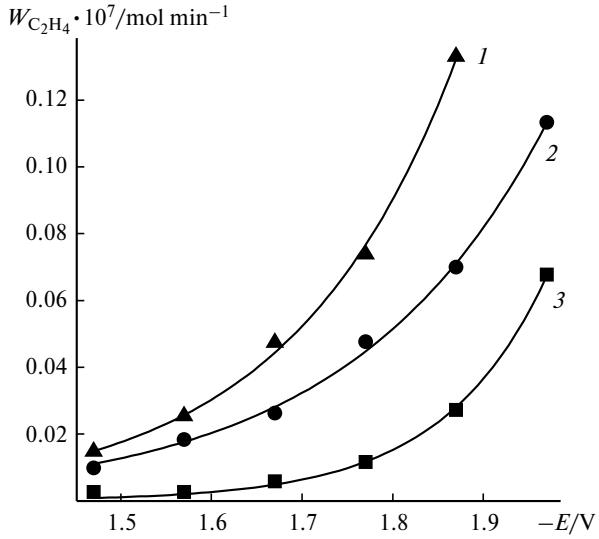


Fig. 2. Plots of the rate of C_2H_2 reduction (the rate of C_2H_4 accumulation ($W_{C_2H_4}$)) *vs.* specified potential (E) on different working electrodes (against Ag/AgCl/KCl(sat.)): Zn/Hg (2 wt.%; 2 mL), [PhSH] = $1.2 \cdot 10^{-2}$ mol L^{-1} (*1*); mercury (2 mL), [PhSH] = $1.2 \cdot 10^{-2}$ mol L^{-1} (*2*); mercury (2 mL), [H_2O] = $1.2 \cdot 10^{-2}$ mol L^{-1} (*3*). For other conditions, see Fig. 1.

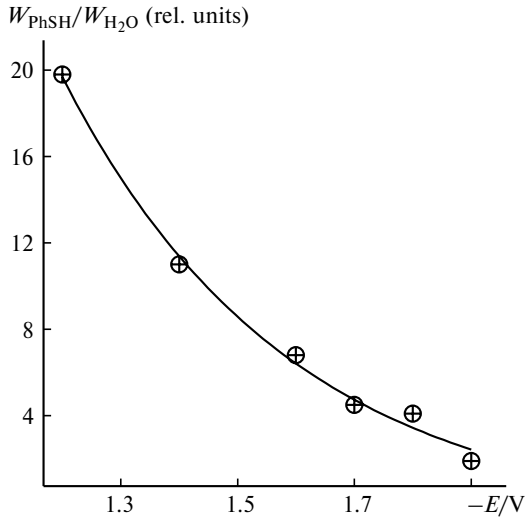


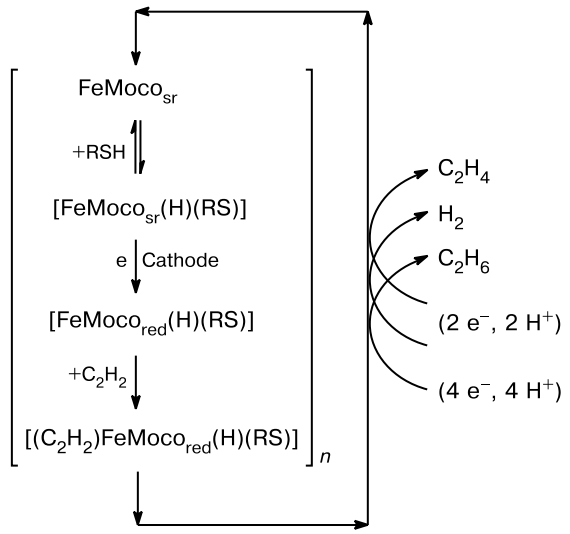
Fig. 3. Influence of the acidity of the protonating agent on the rate of C_2H_2 reduction at different values of the specified potential (E) of the mercury cathode (against Ag/AgCl/KCl(sat.)). W_{PhSH} and W_{H_2O} are the rates of C_2H_2 reduction in the presence of thiophenol and water, respectively. Reaction conditions: solvent DMF; catalyst [FeMoco] = $2.1 \cdot 10^{-5}$ mol L^{-1}; [PhSH] = $1.2 \cdot 10^{-2}$ mol L^{-1}; [H_2O] = $2 \cdot 10^{-2}$ mol L^{-1}; electrode mercury, 2 mL; 20 °C.

Such a dependence of the reaction rate on the cathode potential indicates that the electrochemical step, *i.e.*, electron transfer through the interface between the cathode and adsorbed FeMoco cluster, is the slowest in the reaction sequence. Similar exponential dependences of the rate of C_2H_4 formation on the working electrode potential were also obtained for polarized pure mercury as a cathode in the presence of both thiophenol and H_2O (Fig. 2). The corresponding plots for the range of potentials from -1.4 to -2.0 V (*vs.* silver chloride electrode) are presented in Fig. 2. In the absence of thiophenol* the detectable reduction of C_2H_2 starts only at $E \approx -1.5$ V. In this case, no C_2H_6 is formed at any values of the cathodic potential of mercury electrode.

A comparison of the reaction rates with pure mercury as a cathode at different values of specified potential with thiophenol or water as proton donors reveals that the weaker the reducing agent, the more weight is given to the acidity of proton donor (see Fig. 3). For the mercury cathodic potential of $E = -1.2$ V, the reaction rates in the presence of different proton donors differ by ~20 times. When $E = -1.9$ V, the difference in rates for thiophenol and water is two times only. In addition to a cathodic potential, the electrode composition also causes great influence on the reaction rate and mechanism. For the cathodes made of different materials (pure mercury, Zn/Hg, or Eu/Hg) at the same specified potential value equal, *e.g.*, to the potential of europium amalgam (-1.4 V against SHE) and the same protonating agent (thiophenol), the absolute rates of C_2H_4 accumulation on europium amalgam are ~20 times higher (and for C_2H_6 formation even 30 times higher) than those at the pure mercury cathode, and tenfold higher than those for the zinc amalgam cathode for the same specified potential.

For reaction products to form, the catalytic system has to pass through several steps (Scheme 1).

Scheme 1



R = Ph, C_6F_5

* When water serves as proton donor, the H_2O content in DMF used was found to be $5 \cdot 10^{-2}$ mol L^{-1} or higher.

The first step is chemical binding a thiophenol molecule to the $FeMoco_{sr}$ cluster in the semireduced state.[19,20] This step is followed by electrochemical step, which is the reduction of [FeMoco·PhSH] complex bound to the electrode surface up to the substrate-binding state $FeMoco_{red}$.[7,8] Then a number of chemical steps occur, namely, coordination of acetylene, intramolecular electron transfer, and C_2H_2 protonation to form C_2H_4 and C_2H_6 as products.

In theory, any of these steps can limit the process as a whole: both the preceding chemical step, an electrochemical one, or subsequent chemical steps. It is known that the rate constant of electrochemical reaction depends on electrode potential, unlike that for chemical reaction. If the process is limited by one of the chemical steps, the overall rate of the process would not depend on the electrode potential, at least starting from the potential value when a catalytically active intermediate forms.

The kinetic data on the electrocatalytic reduction of C_2H_2 in the presence of thiophenol indicate that for this process an electrochemical reaction appears to be the rate-limiting one. The protonation of the $[FeMoco]^{n-}$ anion by thiophenol decreases the negative charge of the anion, which should facilitate the subsequent step of reduction of the [FeMoco·PhSH] complex when the complex is either adsorbed to the electrode surface or locates nearby the surface in a thin near-electrode layer. Quite often it happens that a particle adsorbs prior to it undergoes electrochemical transformation. In this case, the reaction rate depends on the electrode material. In the case when no adsorption happens, the reaction rate at a given potential is independent of the electrode composition. In experiments with thiophenol or water, the absolute values of reaction rates do depend on the cathode material for the same specified potential. It happens due to the difference in potential of zero-charge values for mercury and the amalgams which determine the binding energy of an adsorbed particle to the cathode surface. The potentials of zero charge of the amalgam surfaces are close to the equilibrium potentials. In this case, the surface charge favors the adsorption of a negatively charged particle, such as the $[FeMoco]^{n-}$ anion, which leads to a high reaction rate. Unlikely, the value of the zero-charge potential of the mercury cathode (−0.19 V *vs.* SHE[21]) does not favor the adsorption of the $[FeMoco]^{n-}$ anion: a negatively charged particle will have a hard time approaching the surface of negative charge, namely, mercury with the applied negative potential. When water is used as a proton donor instead of thiophenol, the conditions for FeMoco adsorption at the mercury cathode are even worse: first, water as weaker proton donor protonates poorly the cluster; second, the cofactor with thiophenol bound possesses better surfactant properties. That is why, the reaction rates are low in the presence of water (see Fig. 2). Note that when the electrochemical step involving adsorbed particles is limiting, the dependence of the reaction rate on concentration of the catalyst in volume should have the same shape as a classical adsorption plot: proportionality at low concentrations and saturation at high concentrations. It is exactly what we observe as a dependence of C_2H_2 reduction on FeMoco concentration in solution for europium amalgam electrode in the presence of PhSH.[9]

The rate of C_2H_6 formation which accumulates in parallel to C_2H_4, also depends on the specified cathode potential as an exponential function. This implies that for both products the reaction rate is limited by electron transfer from an external reducing agent to the catalyst. In the case of C_2H_6, the rate dependence is more complicated than that for C_2H_4: two Tafel regions with different sets of *a* and *b* parameters can easily be seen (Fig. 4) with a break in the plot at potential of −1.67 V. From the plot of C_2H_6 accumulation rate (which is the product of four-electron reduction of C_2H_2) *vs.* potential of the external reducing agent one can see that for FeMoco as a catalyst (with PhSH as a proton donor) an increase in the cathodic potential decreases the rate of the multielectron process compared to that of the two-electron process (C_2H_4 or H_2).

These are several examples of efficient electrocatalytic processes when small molecules, in particular, C_2H_2, are reduced with participation of transition metal clusters.[22,23] We are especially interested in systems involving eight- and sixteen-nuclear Mg—Mo clusters in MeOH and Ti—Mo-pyrocatechol clusters in water, which are also, presumably, eight-nuclear.[24] In these systems, metal (Zn, Na, Eu) amalgams or mercury cathode were used as reducing agents. They are of special interest, because, unlike the system involving FeMoco, they catalyze the reduction of molecular nitrogen in addition to C_2H_2 reduction. For example, the system with Mg—Mo clusters as catalysts and Na/Hg as electron donor reduces actively N_2 to N_2H_4 and NH_3, and even a weaker reducing agent

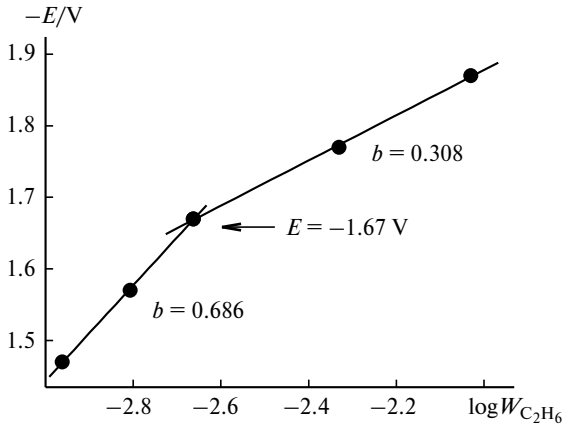


Fig. 4. Tafel dependence for the reduction of C_2H_2 to C_2H_6 at the zinc amalgam electrode (against Ag/AgCl/KCl(sat.)), catalyst FeMoco. For conditions, see Fig. 1.

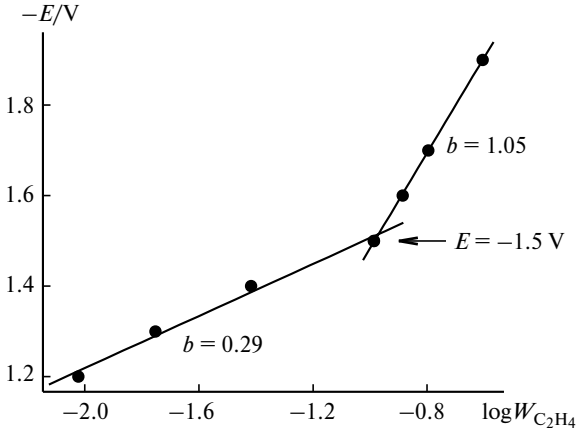


Fig. 5. Tafel dependence for C_2H_2 reduction at the zinc amalgam electrode (against Ag/AgCl/KCl(sat.)) using the polynuclear molybdenum complex as the catalyst. Reaction conditions: solvent MeOH; catalyst $[Mg_2Mo_8O_{22}(MeO)_6(MeOH)_4]^{2-} \cdot [Mg(MeOH_6)]^{2+} \cdot 6MeOH$; [Mo] = $5.1 \cdot 10^{-5}$ mol L^{-1}; proton donor thiophenol; [PhSH] = $4.7 \cdot 10^{-3}$ mol L^{-1}; electrode zinc amalgam (2 wt.%), 2 mL; cathode surface area 2.5 cm^2; catholyte volume 6 mL; 20 °C; $P_{C_2H_2} = 0.13$ atm.

(Eu/Hg) can be used for this reaction in the presence of a more acidic proton source (thiophenol).[25,26] For this system, in particular, it has been studied the dependence of the rate of catalytic C_2H_2 reduction on the specified potential of the zinc amalgam cathode. The rate showed an exponential dependence plotted on the electrode potential.[26,27] This dependence plotted in semilogarithmic coordinates is presented in Fig. 5. Comparing the similar plots for systems involving FeMoco and Mg—Mo cluster one can see that the overall situations are similar for those systems except the possibility of multielectron processes under high reduction potential of the external electron donor. An increase in the cathodic potential for the system with the Mg—Mo cluster increases the rates of C_2H_4 and C_2H_6 accumulation, whereas at $E = -1.5$ V the Tafel plot contains a break, and the second branch of the Tafel region has the parameter $b_2 = 1.05$, which is much higher than $b_1 = 0.29$ for the first region. Starting at $E = -1.5$ V, the rates of accumulation of products of four- and even six-electron reduction increase significantly, in particular, a noticeable amount of methane (to 20%) is formed among the products.

Aiming at finding the conditions for catalysis of dinitrogen reduction by FeMoco isolated from the protein, it looks like an increase in the reduction potential of the external electron donor is not reasonable since it facilitates the competitive two-electron process of H_2 formation comparing to the multielectron reduction of N_2.

The temperature dependence of the rate of C_2H_2 reduction was recorded in the potentiostatic regime with the specified potential of the working electrode (Zn/Hg) equal to −1.7 V. We found that the rates of both C_2H_4 and

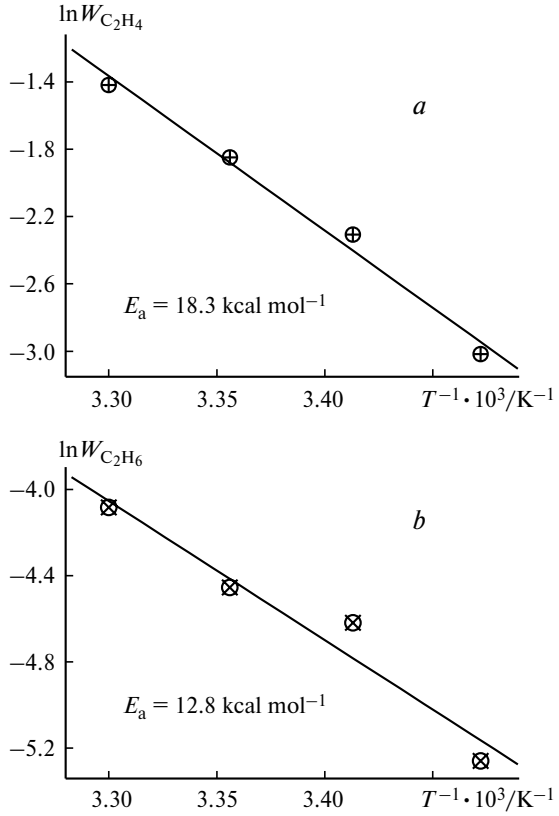


Fig. 6. Temperature plots of the rates of C_2H_4 (*a*) and C_2H_6 (*b*) accumulation by the catalytic reduction of C_2H_2 in the Arrhenius coordinates. Reaction conditions: specified potential of the zinc amalgam −1.7 V; solvent DMF; catalyst [FeMoco] = $1.2 \cdot 10^{-5}$ mol L^{-1}; [PhSH] = $1.2 \cdot 10^{-2}$ mol L^{-1}; $P_{C_2H_2} = 0.13$ atm.

C_2H_6 formation increase exponentially with the temperature increase in the interval from 13 to 30 °C. We did not raise the temperature further because it has previously been found that FeMoco possesses a restricted thermal stability and decomposes, to a great extent, at 40 °C.[8] Since all measurements for different temperatures were carried out at the same concentrations of all reactants and other reaction parameters being the same, the rate values were used for the calculation of activation energies instead of the rate constants. The apparent activation energy of acetylene reduction with C_2H_4 formation was calculated using the Arrhenius equation and was found to be equal to 18.3±1.5 kcal mol^{-1}, and that of the reduction to C_2H_6 was approximately 1.5-fold lower (12.8±2 kcal mol^{-1}) (Fig. 6). Therefore, the relative yield of C_2H_6 decreases remarkably with the temperature increase (Fig. 7).

The fact that the rate of C_2H_2 reduction *vs.* temperature dependence obeys Arrhenius equation indicates that no strong chemosorption of FeMoco occurs on the cathode surface, because the temperature dependence would be quite different in this case: the reaction rate would

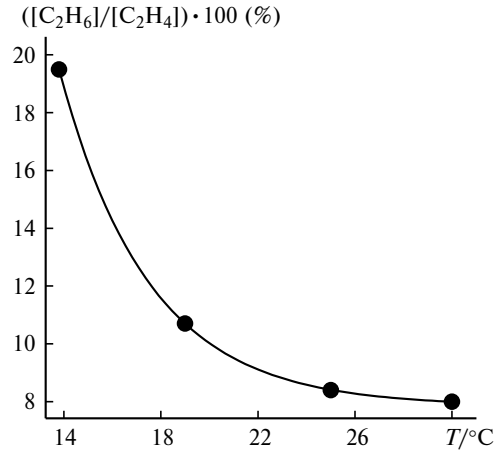


Fig. 7. Decrease in the relative fraction of C_2H_6 in the products of C_2H_2 reduction with the temperature increase. Reaction conditions: cathode zinc amalgam; $E = -1.7$ V; for other conditions, see Fig. 1.

decrease with the temperature increase due to the temperature increase makes chemosorption worse.

The apparent activation energy of C_2H_6 formation, which is the product of four-electron reduction of C_2H_2, is 1.5-fold lower than the activation energy of C_2H_4 formation. This means, most likely, that the reaction of ethane formation occurs on the electrode surface having a constant contact with the electron donor. Usually, the more ordered an electron donor—catalyst—substrate structure and the higher the adsorption component of the reaction rate, the lower the energy barrier for the electron transfer to the coordinated substrate molecule. The experimental data observed show that the formation of C_2H_6 requires better ordered electron donor—catalyst—substrate structure and a stronger contact with the cathode indicated by a relatively low activation energy found. The temperature increase makes the adsorption conditions worse, *i.e.*, the contact time of the catalyst with the electron donor shortens and, therefore, the relative contribution of the reaction leading to C_2H_6 and its relative yield decrease with the temperature increase (see Fig. 7). It is most likely that a decrease in the relative yield of C_2H_6 among the products with an increase in the cathodic potential of the zinc amalgam or mercury electrodes (see Fig. 4) is also related to deteriorating of conditions for the adsorption of the catalytic cluster on the negatively charged cathode surface. The two-electron process of C_2H_4 formation is less sensitive to the spatial organization of the system and can occur in solution even with no contact between the catalytic cluster and the electron donor, which is indicated by a much higher activation energy of C_2H_4 formation.

It can be concluded that for the multielectron process catalyzed by FeMoco cluster and involving thiophenol as a proton donor to occur, it is obligatory to have a constant contact of FeMoco with an electron donor.

Pentafluorothiophenol as a protonating agent. When pentafluorothiophenol is used instead of thiophenol, the reaction rate of C_2H_6 formation increases by five times and that of C_2H_4 by three times (these products are formed in parallel, as in the case of thiophenol) and the overall kinetics of the process also changes. First, when the cathodic potential increases from -1.07 V (the potential of zinc amalgam) to -1.2 V, the rate increases with the potential increase. Then, beginning from -1.2 V and to $E = -1.75$ V, the reaction rate is independent of the potential. After the current is switched off, the stationary potential of the reaction solution is -1.15 V. This is likely to be the potential of the $FeMoco_{sr}/FeMoco_{red}$ redox couple. At this potential, the catalyst is reduced to the substrate-binding state. When air is introduced into the system, *i.e.*, when FeMoco is irreversibly oxidized, the potential of the solution decreases to -1.07 V, which is the potential of zinc amalgam. It is of interest that, in this case, the accumulation rates of both C_2H_6 and C_2H_4 are virtually independent of the cathode material: the polarization of pure mercury and zinc amalgam gives almost the same rates at the same potential values. The fact that the reduction rate is independent of the cathodic potential and electrode material implies that the electron transfer from the cathode is not the limiting step in this case. The reaction of C_2H_2 reduction in the presence of pentafluorothiophenol is ceased more rapidly (Fig. 8) due to the fact that C_6F_5SH is much more actively consumed than thiophenol in the reaction of hydrogen evolution on

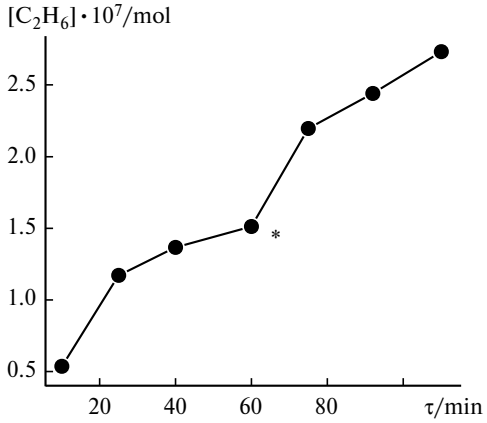


Fig. 8. Kinetic curves for C_2H_6 accumulation by the electrocatalytic reduction of C_2H_2. Reaction conditions: solvent DMF; catalyst [FeMoco] = $2.1 \cdot 10^{-5}$ mol L^{-1}; proton donor pentafluorothiophenol; $[C_6F_5SH] = 1.2 \cdot 10^{-2}$ mol L^{-1}; $P_{C_2H_2} = 0.17$ atm; specified potential of the mercury cathode -1.36 V *vs.* SHE; cathode surface area 3.1 cm^2; catholyte volume 6.5 mL; 25 °C; $P_{C_2H_2} = 0.13$ atm.
* After 60 min, C_6F_5SH ($8 \cdot 10^{-5}$ mol) was added to the system, and the C_6F_5SH concentration became equal to the initial value.

the cathode parallel to the catalytic reduction of C_2H_2. In the presence of pentafluorothiophenol, the FeMoco is stable and does not decompose for a long time as confirmed by the repeated addition of C_6F_5SH to the reaction mixture (when C_2H_2 reduction has almost stopped due to the consumption of the proton donor) results in C_2H_2 reduction with the rate almost the same as the initial one (see Fig. 8). The reaction rate increases with the temperature increase from 15 to 30 °C. The temperature dependence obeys the Arrhenius equation. The apparent activation energy for this system measured at a specified cathodic potential of −1.3 V is 32±4 kcal mol^{-1} for both products.

Pentafluorothiophenol, being a stronger acid, can perform, most likely, the multiple protonation of the FeMoco anion.[19] This would decrease the anion charge to a greater extent than in the case of thiophenol and would facilitate the way of the catalytic cluster approaches the cathode. In addition, the complex (C_6F_5S—FeMoco—H) formed contains the electron-withdrawing substituent which also facilitates its reduction. All these factors result in such an increase in the rate of the electrochemical step that one of the subsequent chemical steps in the coordination sphere of the metal cluster will limit the whole process. This step is, most likely, the intramolecular proton transfer to the substrate in the $[(C_6F_5S^-H^+)FeMoco_{red}\cdot(C_2H_2)]$ complex, leading to the formation of products from coordinated acetylene. The same apparent activation energy for the formation of C_2H_4 and C_2H_6 much higher than that for thiophenol indicates the presence of the limiting step common in parallel reactions, *via* which these products are formed, accompanied by considerable structural changes.

The data presented above show that, depending on the acidity of the proton donor used, the FeMoco-catalyzed reduction of the triple bond can be limited by both the electron transfer from the external donor and intramolecular rearrangements in the coordination sphere of the metal complex with the substrate and hydrogen donor.

In the presence of such protonating agents as PhSH or H_2O, the C_2H_2 reduction reactions occur in the adsorption layer on the cathode surface without intermediate reduction products release into solution. The kinetics of these reactions can be described by the surface concentration of the catalyst rather than the volume concentration; the reaction is heterogeneous and limited by the electron transfer.

For pentafluorothiophenol as a proton source (FeMoco, DMF), the reaction rate is independent of the cathodic potential and electrode material starting from $E = -1.2$ V. This indicates that some step of intramolecular protonation of coordinated C_2H_2 in the associate (catalyst—substrate—thiol) followed by formation of products and subsequent electrochemical regeneration of the catalyst limits the whole reaction rather than the electron transfer.

The authors thank A. E. Shilov and A. F. Shestakov for helpful discussion of the work and M. V. Rudakova for help in determination of the water content in DMF.

This work was financially supported by the Russian Foundation for Basic Research (Project Nos 01-03-33278 and 04-03-32635) and the President of the Russian Federation (Program of Support for Leading Scientific Schools, Grant NSh-2065.2003.3).

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Received November 4, 2003;
in revised form February 17, 2004