# Reduction of $N_3^-$ by $[Mo_2Fe_6S_8(SPh)_9]^{3-}$ Modified Glassy Carbon Electrode—Model Study of Nitrogenase

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(Received October 6, 1988)

The reduction of $N_3^-$ by a $(Bu_4N)_3[Mo_2Fe_6S_8(SPh)_9]$ modified glassy carbon electrode has been conducted in a pH region 5 to 12 in water. In neutral and acidic conditions, $N_3^-$ undergoes two-and six-electron reductions to afford not only $N_2$ and $NH_3$ but also $N_2H_4$, and the total amounts of $N_2$ and $N_2H_4$ produced were essentially consistent with that of $NH_3$. On the other hand, no $N_2H_4$ formation was observed in the pH region higher than 10, where an equal amount of $N_2$ and $NH_3$ was formed. The rate of $N_2H_4$ formation was linearly proportional to a concentration of $N_3H$, which exists as an equilibrium mixture with $N_3^-$ in the aqueous phase, while that of $N_2$ evolution was controlled by the concentration of free $N_3^-$ in $H_2O$. These observations indicate that $N_2H_4$ and $N_2$ result from the coordination of $N_3H$ and $N_3^-$ to the molybdenum-iron sulfur cluster, respectively.

Nitrogenase is composed of iron proteins and molybdenum-iron proteins and reduces not only $N_2$ but also various unsaturated small molecules such as $C_2H_2$, $N_3^-$, RCN, RNC, and $N_2O$.[1] Among those nitrogenase substrates, $N_3^-$ is the only anionic substrate which is reduced with inequivalent numbers of electrons and protons. In the initial study on the reduction of $N_3^-$ by crude nitrogenase in the presence of MgATP, $N_3^-$ was reduced with two electrons to give an equal amount of $N_2$ and $NH_3$.[2] Later, Hardy et al. have reported that the reduction of $N_3^-$ afforded one $N_2$ molecule and two $NH_3$ molecules at low concentration of $N_3^-$, and the excess $NH_3$ has been attributed to result from the further reduction of the $N_2$ formed.[3] Furthermore, the formation of $N_2H_4$ in addition to $N_2$ and $NH_3$ was observed in the reduction of $N_3^-$ by purified nitrogenase.[4] It has been argued whether $N_3^-$ or $N_3H$ is the actual substrate of nitrogenase[2-5] since both species exist as an equilibrium mixture around pH 7. Furthermore, $N_3^-$ has been shown to disturb the electron flow in enzymatic reaction by inhibiting the rate of MgATP hydrolysis by nitrogenase.[3,6] Despite these difficulties, $N_3H$ has been suggested to be a potent substrate for the formation of $N_2H_4$ based on the reduction of $N_3^-$ conducted in a restricted pH range 6.5—7.3.[7]

It has been demonstrated that various nitrogenase substrates such as $C_2H_2$,[8] $CH_3CN$,[9] $CH_3NC$,[9] and $N_2O$[10] are reduced by the reduced species of $[Fe_4S_4(SPh)_4]^{2-}$ and $[Mo_2Fe_6S_8(SPh)_9]^{3-}$. One of the principal advantage of those model studies is that the reduction of nitrogenase substrates can be conducted in wide pH range, where proteins are readily denatured. The reduction of $N_3^-$ by a model study, therefore, may give a clue to elucidate whether $N_3^-$ or $N_3H$ is the actual substrate of nitrogenase. The construction of a system which can not only rapidly but also continuously transport electrons to a catalyst having ability of multi-electron reduction of $N_3^-$ is required in order to simulate the enzymatic reaction since $N_3^-$ undergoes two-, six-, and eight-electron reductions by nitrogenase.[3,4] This paper describes the reduction of $N_3^-$ on a $(Bu_4N)_3[Mo_2Fe_6S_8(SPh)_9]$ modified glassy carbon electrode in $H_2O$.

## Experimental

Commercially available guaranteed reagent grade of $NaN_3$, $H_3PO_4$, and NaOH were used without further purification. Water insoluble and soluble MoFeS clusters, $(Bu_4N)_3[Mo_2Fe_6S_8(SPh)_9]$[11] ($(Bu_4N)_3$[Mo–Fe]) and $(Et_4N)_3[Mo_2Fe_6S_8(SCH_2CH_2OH)_9]$,[12] and $(Et_4N)N_3$[13] were prepared according to the literature. Solvents used for electrochemical measurements were purified by distillation over appropriate desiccants; DMF from $CaH_2$ under reduced pressure and $CH_3CN$ from $P_2O_5$, and stored under $N_2$ atmosphere. Immediately before the electrochemical reduction of $N_3^-$, those solvents were bubbled with He at least for 1 h to remove nitrogen dissolved in the solvents. A $(Bu_4N)_3$[Mo–Fe] modified glassy carbon electrode was prepared by dropping a $CH_3CN$ solution of $(Bu_4N)_3$[Mo–Fe] (1.0 mmol $dm^{-3}$) on a glassy carbon plates with areas 3.0 and 1.0 $cm^2$ (Tokai Carbon Co., Ltd., SC-2), and dried under $N_2$ atmosphere. The details of the cluster modified glassy carbon electrode was described in previous papers.[10,14]

**Physical Measurements.** Electronic absorption spectra were measured with a Union SM-401 spectrophotometer. Spectroelectrochemical experiments were carried out by the use of an optically transparent thin layer electrode.[15] Cyclic voltammetry was carried out by using a potentiostat (Hokuto Denko HA-301), a function generator (Hokuto Denko HB-107A), and an X-Y recorder (Yokogawa Electric Inc., 3077).

**Reduction of $N_3^-$ by the [Mo-Fe]/GC Electrode.** The reduction of $N_3^-$ by the [Mo–Fe]/GC electrode in water were carried out under the controlled potential electrolysis conditions using an electrolysis cell consisting of three components; a working, a counter, and an SCE reference electrode compartments.[8] A cation exchange membrane (Nafion film) was used as a separator between the working electrode and the counter electrode compartments. After passing He through the electrolysis cell for 1 h to remove air, an aqueous buffer solution ($H_3PO_4$–NaOH) of $NaN_3$ was introduced into the working electrode compartment by syringe techniques. Then, the electrolysis cell was placed in a thermostat at 30 °C and the reduction of $N_3^-$ was started by

applying a fixed potential to the [Mo–Fe]/GC electrode with a potentiostat. The charge consumed in the course of reduction was measured with a Hokuto Denko HF-201 coulometer. Analyses of $H_2$ and $N_2$ evolved in the reduction were conducted on a Shimadzu GC-3BT gas chromatograph with a 2.0 m column filled with Molecular Sieves 13X and on a Shimadzu GC-7A gas chromatograph with Porapack Q, respectively. Ammonia and $N_2H_4$ formed in solutions were determined by Shimadzu GC-6A gas chromatograph with Chromosorb 103 and by colorimetric titration,[16] respectively.

## Results and Discussion

**Interaction of [Mo-Fe]$^{3-}$ with $N_3^-$.** The electronic absorption spectrum of $(n\text{-}Bu_4N)_3$[Mo–Fe] in DMF shows two absorption bands at 360 and 460 nm (a broken line in Fig. 1), which did not change at all by addition of $(Et_4N)N_3$ to the solution. The electrochemical reduction of [Mo–Fe]$^{3-}$ to [Mo–Fe]$^{4-}$ at −1.10 V vs. SCE[17] in DMF resulted in a decrease of the absorptivity at the 460 nm band (a dotted broken line in Fig. 1), while the electrochemical reduction of the cluster conducted in the presence of $(Et_4N)N_3$ under otherwise the same conditions leads to an appearance a new band at 306 nm (a solid line of Fig. 1). The 306 nm band can be assigned to $PhS^-$[14,18] dissociated from [Mo–Fe]$^{4-}$, and the intensity of this band corresponds to one molecule of $PhS^-$ per one molecule of the cluster. On the other hand, this band gradually disappeared by reoxidation of [Mo–Fe]$^{4-}$ at −0.60 V vs. SCE, and the final spectrum became almost same as that of original [Mo–Fe]$^{3-}$ in DMF. These results indicate that the oxidized cluster [Mo–Fe]$^{3-}$ has no interaction with $N_3^-$, while a $PhS^-$ ligand of [Mo–Fe]$^{4-}$ is substituted by $N_3^-$ to afford the 1:1 adduct. Such a substitution reaction may take place on the Fe atom of [Mo–Fe]$^{4-}$ rather than the Mo atom, since a terminal $RS^-$ is subject to a substitution reaction, while the bridging $RS^-$ ligands of the cluster are inert for substitutions.[19]

**Cyclic Voltammograms of the [Mo-Fe]/GC Electrode.** The cyclic voltammogram of the [Mo–Fe]/GC electrode in $H_2O$ (pH 8) shows a cathodic wave around −1.25 V vs. SCE, followed by an irreversible cathodic current due to $H_2$ evolution catalyzed by the reduced species of the cluster, and an anodic wave around at −0.75 V in the reverse scan (a dotted line in Fig. 2). Although the charge consumed in the cathodic process cannot be determined exactly due to the following irreversible cathodic current, that of the anodic one is just two electrons per a cluster modified on the glassy carbon plate. Thus, all the clusters modified on a glassy carbon plate undergo simultaneous two-electron reduction in water. The [Mo–Fe]$^{3-/5-}$ redox cycle stably works on the glassy carbon plate since the cyclic voltammogram was essentially unchanged by successive potential scan at least for 2 h.[14] The cathodic current density at more negative potentials than −1.1 V fairly increases by addition of $NaN_3$ (0.2 mol $dm^{-3}$) to the aqueous buffer solution at the same pH value (a solid line of Fig. 2). The threshold potential of the cathodic current in the presence of $NaN_3$ is essentially consistent with the reduction wave from [Mo–Fe]$^{3-}$ to [Mo–Fe]$^{5-}$ in the absence of $NaN_3$, suggesting that the reduced forms of the cluster can effectively reduce $N_3^-$ in water. The current density at more negative potentials than −1.1 V increases with decreasing the pH value (dotted broken and broken lines in Fig. 2).

**Reduction of $N_3^-$ by the [Mo-Fe]/GC Electrode.** As expected from the cyclic voltammograms of the

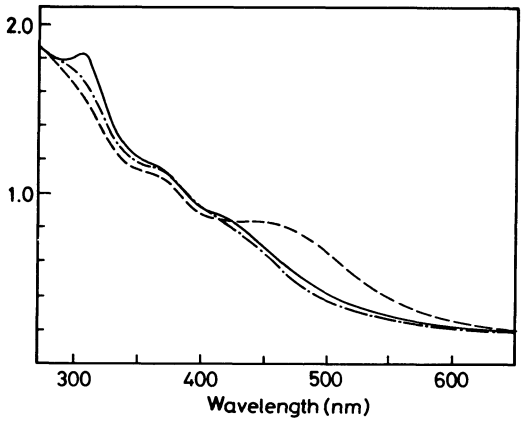


Fig. 1. Electronic absorption spectra of $(Bu_4N)_3$-$[Mo_2Fe_6S_8(SPh)_9]$ ($4.0\times10^{-4}$ mol $dm^{-3}$) (-----) and the reduced species produced at −1.10 V vs. SCE in the absence (—·—) and the presence (------) of $(Bu_4N)N_3$ ($5.0\times10^{-2}$ mol $dm^{-3}$) in DMF.

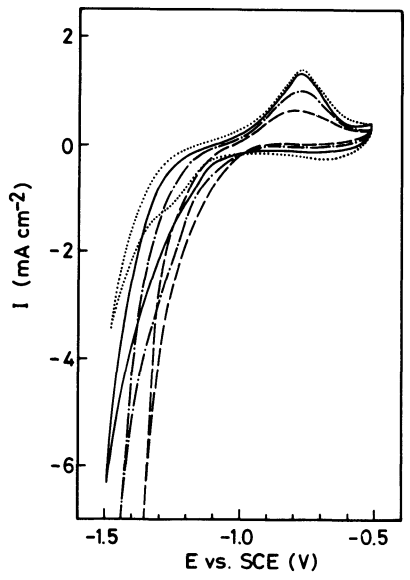


Fig. 2. Cyclic voltammograms of the [Mo–Fe]/GC electrode in $H_2O$ (pH 8.0) in the absence (·····) and the presence of $NaN_3$ (0.2 mol $dm^{-3}$) in $H_2O$ at pH 8.0 (——), 7.0 (—·—), and 6.0 (----) : $dE/dt$= 100 mV $s^{-1}$.

[Mo-Fe]/GC in the presence of $NaN_3$ at various pH, the reduction of $N_3^-$ by the [Mo-Fe]/GC electrode at pH 6.0 is much faster than that at pH 12.0 under the controlled potential electrolysis at −1.25 V vs. SCE (Fig. 3). In addition, the reduction products are also altered by the change of pH; $N_3^-$ is reduced to not only $N_2$ and $NH_3$ but also $N_2H_4$ at pH 6.0 (Fig. 3b), while only $N_2$ and $NH_3$ are formed in the same reduction at pH 12.0 (Fig. 3a). Thus, in acidic solutions both two- and six-electron reductions of $N_3^-$ (Eqs. 1, 2) take place, and two-electron reduction of $N_3^-$ (Eq. 1) occurs predominantly in

$$N_3^- + 3H^+ + 2e^- \longrightarrow N_2 + NH_3 \quad (1)$$

$$N_3^- + 7H^+ + 6e^- \longrightarrow N_2H_4 + NH_3 \quad (2)$$

alkaline solutions. In order to elucidate such a pH dependency of the $N_3^-$ reduction, reduction of $N_3^-$ was conducted under various reaction conditions, and the results are summarized in Table 1.

The formation of $N_2H_4$ (Eq. 2) was observed in the pH less than 10 (Entries 1—7 in Table 1). The sum of the amounts of $N_2$ and $N_2H_4$ formed in that pH region is essentially consistent with the amount of $NH_3$, suggesting that $N_2H_4$ is not the precursor of $NH_3$. In fact, $N_2H_4$ was not reduced by the [Mo-Fe]/GC electrode in $H_2O$ under the present experimental conditions. Thus, the [Mo-Fe]/GC electrode can catalyze two- (Eq. 1) and six-electron (Eq. 2) reductions of $N_3^-$ but has no ability to catalyze the eight-electron reduction of $N_3^-$ (Eq. 3). The amounts of not only the

$$N_3^- + 9H^+ + 8e^- \longrightarrow 3NH_3 \quad (3)$$

reduction products of $N_3^-$ but also $H_2$ increase with decreasing the pH value of the aqueous phase. It should, however, be noticed that the amount of $H_2$ evolved are controlled by the potential of the [Mo-Fe]/GC electrode under a constant pH, while the amounts of $N_2$, $NH_3$, and $N_2H_4$ are not largely dependent on the electrode potential (Entries 2—4 in Table 1). The result indicates that $H_2$ evolution is controlled principally by the electron transfer from the GC plate to the cluster, and that the rate of the $N_3^-$ reduction is governed mainly by the chemical reaction. Thus, the cluster modified on the glassy carbon plate effectively functions as a catalyst for the reduction of $N_3^-$. Holm et al. have reported that the reduced species of the present molybdenum-iron sulfur cluster has an

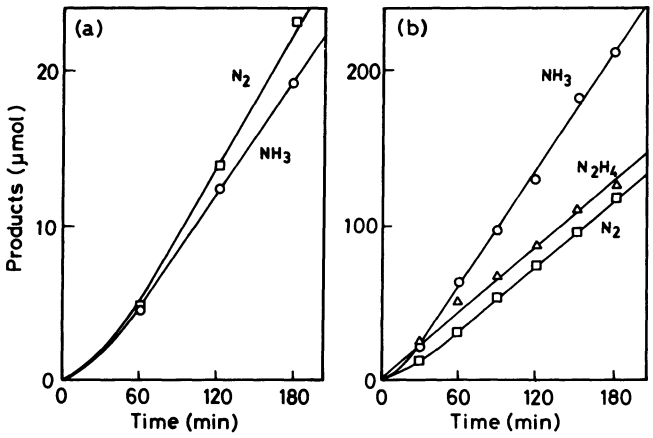


Fig. 3. Reduction of $NaN_3$ catalyzed by the [Mo-Fe]/GC under the controlled potential electrolysis at −1.25 V vs. SCE in $H_2O$ at pH 12.0 (a) and 6.0 (b). The concentrations of $NaN_3$ are 0.5 mol dm$^{-3}$ (a) and 0.05 mol dm$^{-3}$ (b).

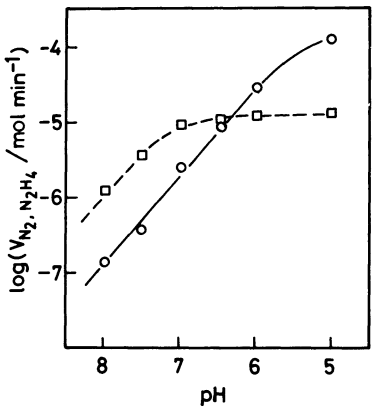


Fig. 4. Rates of the $N_2H_4$ (○) and $N_2$ (□) formation against pH of the electrolyte solutions in the reduction of $NaN_3$ (0.05 mol dm$^{-3}$) by the [Mo-Fe]/GC electrode at −1.25 V vs. SCE.

Table 1. The Reduction of $N_3^-$ Catalyzed by the [Mo-Fe]/GC Electrode in $H_2O$ for 150 min

| Entry | $NaN_3$ | pH | $E$ | Products[a] | | | |
|---|---|---|---|---|---|---|---|
| | mmol dm$^{-3}$ | | V vs. SCE | $NH_3$ | $N_2$ | $N_2H_4$ | $H_2$ |
| 1 | 50 | 5 | −1.25 | 254 | 96 | 165 | 453 |
| 2 | 50 | 6 | −1.10 | 179 | 92 | 89 | 16 |
| 3 | 50 | 6 | −1.25 | 183 | 86 | 103 | 234 |
| 4 | 50 | 6 | −1.40 | 188 | 89 | 95 | 1235 |
| 5 | 25 | 7 | −1.25 | 49 | 23 | 28 | 77 |
| 6 | 50 | 7 | −1.25 | 83 | 45 | 34 | 58 |
| 7 | 50 | 8 | −1.25 | 3.6 | 3.2 | 0.3 | 51 |
| 8 | 50 | 10 | −1.25 | 2.8 | 2.5 | 0 | 18.2 |
| 9 | 50 | 12 | −1.25 | 1.3 | 1.3 | 0 | 1.1 |

a) μmol.

ability to reduce protons and metal-hydride species has been proposed as the precursor for the $H_2$ evolution.[20] The difference of the rate determining steps in the reductions of protons and $N_3^-$ by the [Mo-Fe]/GC electrode suggests that the reaction mechanisms of those two reactions are different from each other. The reduction of $N_3^-$ may, therefore, be initiated by the coordination of $N_3^-$ to the molybdenum-iron sulfur cluster modified on the GC plate since $N_3^-$ is not reduced at all by a glassy carbon electrode at −1.25 V vs. SCE in $H_2O$.

As described above, the rates of $N_2$ and $N_2H_4$ formation[21] increased with decreasing pH values. The former, however, reaches a constant value below around 7 (a broken line in Fig. 4), and the latter also show the saturation behavior around 5 (a solid line in Fig. 4). Such a distinct difference in the formation rates between $N_2H_4$ and $N_2$ toward the pH change suggests that the reductions of Eqs. 1 and 2 take place independently. It is well-known that $N_3^-$ exists as an equilibrium mixture with $N_3H$ in $H_2O$, and the $pK_a$ of $N_3H$ is 4.6 at 30 °C (Eq. 4).[22] The presence of the turning point in the plot of $\log V(N_2H_4)$ vs. pH ($V(N_2H_4)$: the rate of $N_2H_4$ formation) around pH 5 may, therefore, be associated with the $pK_a$. The concentrations of $N_3H$ and $N_3^-$ are expressed by Eqs. 5 and 6, where $[N_3^-]_0$ is the initial concentration of $NaN_3$. Equations 5 and 6 also can be converted into Eqs. 7 and 8 in terms of $pK_a$ and pH in place of

$$N_3H \rightleftharpoons N_3^- + H^+ \quad (4)$$

$$[N_3H] = [N_3^-]_0[H^+]/(K_a + [H^+]) \quad (5)$$

$$[N_3^-] = K_a[N_3^-]_0/(K_a + [H]^+) \quad (6)$$

$$[N_3H] = [N_3^-]_0/(10^{(pH-pK_a)} + 1) \quad (7)$$

$$[N_3^-] = 10^{(pH-pK_a)}[N_3^-]_0/(10^{(pH-pK_a)} + 1) \quad (8)$$

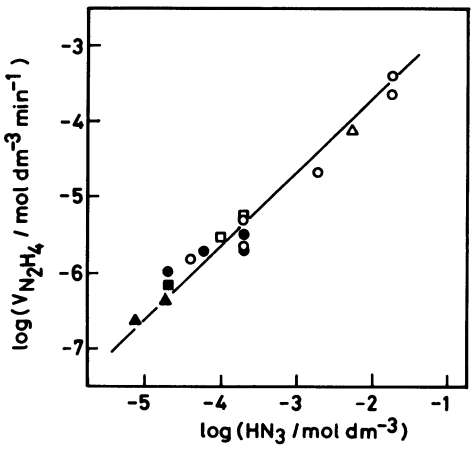


Fig. 5. Relationship between the rate of $N_2H_2$ formation and the $N_3H$ concentration in the reduction of $NaN_3$ by the [Mo-Fe]/GC electrode at pH 6.0 (○), 6.5 (△), 7.0 (□), 8.0 (■), and 10.0 (▲).

$K_a$ and $[H^+]$, respectively. Although any linear relationship was not observed between $\log V(N_2H_4)$ and $\log[N_3^-]$ calculated from Eq. 8, a plot of $\log V(N_2H_4)$ vs. $\log[N_3H]$ obtained from Eq. 7 gives a straight line, as shown in Fig. 5. A linear dependence of $V(N_2H_4)$ upon the $N_3H$ concentration in the aqueous phase suggests that $N_3H$ is the actual substrate for the formation of $N_2H_4$. This conclusion may be supported by the fact that $N_2H_4$ is not produced above pH 10, where $N_3H$ essentially does not exist as an equilibrium mixture of $N_3^-$. Equation 9 rather than Eq. 2 may,

$$N_3H + 6H^+ + 6e^- \longrightarrow N_2H_4 + NH_3 \quad (9)$$

therefore, be appropriate for the formation of $N_2H_4$ in the present study.

The saturation behavior of $\log V(N_2)$ around 7 (Fig. 4) also may be explained by a protonation equilibrium of the reaction intermediate. The reduction of $N_3H$ formed in the aqueous phase may be initiated by the coordination of $N_3H$ to the cluster on the [Mo-Fe]/GC, followed by the electron transfer to the $N_3H$ from the cluster. It should be noticed that $N_3^-$ also can coordinate to the reduced cluster but the rate of the electron transfer to the $N_3^-$ from the cluster may be fairly slower than that to the $N_3H$ ligated on the cluster since $N_3^-$ is apparently much more basic than $N_3H$. Accordingly, smooth electron transfer from the cluster to the $N_3^-$ coordinated may take place when the $N_3^-$ undergoes a reversible protonation, as expressed by Eq. 10,

$$[[Mo\text{-}Fe]\text{-}N_3^-]_{sur} + H^+ \rightleftharpoons [[Mo\text{-}Fe]\text{-}HN_3]_{sur} \quad (10)$$

where $[[Mo\text{-}Fe]\text{-}N_3^-]_{sur}$ and $[[Mo\text{-}Fe]\text{-}HN_3]_{sur}$ are the cluster-$N_3^-$ adduct and its protonated species, respectively, on the [Mo-Fe]/GC. The protonated cluster-$N_3^-$ adduct ($[[Mo\text{-}Fe]\text{-}HN_3]_{sur}$) formed by the protonation (Eq. 10) is not necessarily the same species as the reaction intermediate involved in the reduction of $N_3H$ affording $N_2H_4$ and $NH_3$ (Eq. 9) (vide infra). The proton dissociation constant $pK_a'$ of $[[Mo\text{-}Fe]\text{-}HN_3]_{sur}$ (Eq. 10) may be fairly different from the $pK_a$ of free $HN_3$ (Eq. 4; $pK_a$=4.6). As similar to Eq. 5, the surface concentration of $[[Mo\text{-}Fe]\text{-}HN_3]_{sur}$ of Eq. 10 can be expressed by Eq. 11,

$$[[Mo\text{-}Fe]\text{-}HN_3]_{sur} = [[Mo\text{-}Fe]\text{-}N_3^-]_0[H^+]/(K_a' + [H^+]) \quad (11)$$

where $[[Mo\text{-}Fe]\text{-}N_3^-]_0$ is the total surface concentration of the cluster-$N_3^-$ adduct ($[[Mo\text{-}Fe]\text{-}N_3^-]_{sur}$) and its protonated species ($[[Mo\text{-}Fe]\text{-}HN_3]_{sur}$). As discussed in a previous section, the formation of $H_2$, $N_2$, and $N_2H_4$ takes place independently on the [Mo-Fe]/GC. To avoid an extreme difficulty in the determination of each surface concentrations of the active species in those reductions, the surface concentration of [[Mo-

Fe]-$N_3^-$]$_0$ is assumed to be proportional to the bulk concentration of $N_3^-$ calculated from Eq. 8. The surface concentration of the cluster-$N_3^-$ adduct ([[Mo-Fe]-$N_3^-$]$_{sur}$ and its protonated species ([[Mo-Fe]-$HN_3$]$_{sur}$), therefore, can be expressed in terms of [$N_3^-$], $K_a'$, and [H+] (Eqs. 12, 13). On the basis of the fact

$$[[Mo\text{-}Fe]\text{-}N_3^-]_{sur} \propto K_a'[N_3^-]/(K_a' + [H']) \quad (12)$$

$$[[Mo\text{-}Fe]\text{-}HN_3]_{sur} \propto [N_3^-][H^+]/(K_a' + [H^+]) \quad (13)$$

that $V(N_2)$ is saturated around pH 7, a simulation fitting was conducted in the plots of log $V(N_2)$ vs. log [[Mo-Fe]-$N_3^-$]$_{sur}$ and log [[Mo-Fe]-$HN_3$]$_{sur}$ by changing $K_a'$ value around $10^{-7}$. The best result was obtained at $K_a'$ is $10^{-6.8}$ in the plot of log $V(N_2)$ vs. log [[Mo-Fe]-$HN_3$]$_{sur}$, where the rate of $N_2$ formation in the reduction of $N_3^-$ under various conditions shows a linear dependence on the calculated value $[N_3^-][H^+]/(K_a'+[H^+])$, as shown in Fig. 6, suggesting that [[Mo-Fe]-$HN_3$]$_{sur}$ selectively affords $N_2$ and $NH_3$ (Eq. 1). The large p$K_a'$ value (6.8) of [[Mo-Fe]-$HN_3$]$_{sur}$ compared with the p$K_a$ value (4.6) of $N_3H$ in water can be explained by an increase in the electron density of $N_3^-$ moiety upon the coordination to the cluster. Such an enhancement of the basicity of $N_3^-$ may be caused by electron transfer through the terminal nitrogen atom bonded to the cluster, since $N_3^-$ usually coordinates to metal complexes with a terminal nitrogen.[23] The initial protonation of $N_3^-$ ligated on the cluster may, therefore, take place on the nitrogen atom bonded to the cluster to produce [Mo-Fe]-HN-N=N (**1**) rather than [Mo-Fe]-N=N=NH (**2**). The structure of **1** seems to explain reasonably the dissociation of the terminal $N_2$ moiety upon further protonation coupled with electron transfer from the cluster to HN-N=N moiety. In accordance with this, protonation of M-$N_3^-$ complexes (M=Ru,[24] Co,[25]

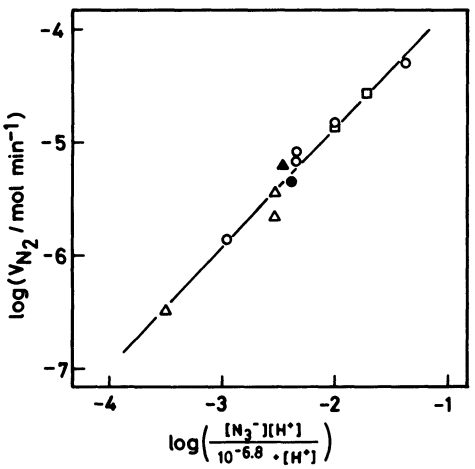


Fig. 6. Relationship between the rate of $N_2$ formation and log ($[N_3^-][H^+]/(10^{-6.8}+[H^+])$) in the reduction of $NaN_3$ by the [Mo-Fe]/GC electrode at pH 6.0 (○), 6.5 (△), 7.0 (□), and 10.0 (▲).

$$\bar{N}=\overset{+}{N}=\bar{N} + H^+ \rightleftharpoons \bar{N}=\overset{+}{N}=NH$$

Scheme 1.

Ir[26]) also occurs at the nitrogen atom bonded to the metal, followed by the dissociation of terminal $N_2$.

The protonation of $N_3^-$ in a solution has been shown to occur at one of a terminal nitrogen atom of $N_3^-$[27] (Scheme 1). The linear dependence of the rate of $N_2H_4$ formation upon the concentration of $N_3H$ in the aqueous phase (Eq. 4) indicates the appropriateness of Eq. 9. In contrast to the protonation of [Mo-Fe]-$\bar{N}=\overset{+}{N}=\bar{N}$ affording [Mo- Fe]-HN-$\overset{+}{N}=\bar{N}$ (**1**), $N_3H$ may bind to the cluster with the nonprotonated terminal nitrogen atom affording [Mo-Fe]-$\bar{N}=\overset{+}{N}$=NH (**2**) rather than **1**. The intramolecular electron transfer from the cluster to the $N_3H$ moiety of **2** may cause also the increase of the basicity of the nitrogen atom bonded to the cluster, resulting in further protonation to form [Mo-Fe]-NH-N=NH (**3**). The following electron transfer coupled with protonation of **3** may lead the dissociation of $N_2H_4$. It should be noticed that such the six-electron reduction of $N_3^-$ (Eq. 2) hardly took place under the controlled potential electrolysis of an aqueous solution (pH 7.0, 12 cm$^3$) containing $(Et_4N)_3$-$[Mo_2Fe_6S_8(SCH_2CH_2OH)_9]$ (0.37 mmol dm$^{-3}$) and $NaN_3$ (0.37 mol dm$^{-3}$) at −1.25 V vs. SCE, where the two-electron reduction of $N_3^-$ (Eq. 1) predominantly occurred with concomitant $H_2$ evolution. The mole ratios of $N_2$ and $H_2$ to $NH_2NH_2$ formed in this homogeneous system were 25 and 194, respectively, and the turnover number for the formation of $N_2H_4$ was as low as 0.385/h based on the amount of the cluster. On the other hand, the [Mo-Fe]/GC catalyzes the six-electron reduction of $N_3^-$ affording $N_2H_4$ with the turnover number 235/h under the same electrolysis potential at pH 7 (Entry 6 in Table 1). Despite that both $[Mo_2Fe_6S_8(SPh)_9]^{3-}$ and $[Mo_2Fe_6S_8(SCH_2CH_2OH)_9]^{3-}$ similarly undergo two successive one-electron reduction at more positive potentials than −1.25 V in solutions, the outstanding ability of the former modified glassy carbon electrode for the six-electron reduction of $N_3^-$ may be explained by the view that the cluster of the [Mo-Fe]/GC exists as the reduced form even after two-electron reduction of $N_3^-$ due to fast and continuous electron transport to $[Mo_2Fe_6S_8(SPh)_9]^{3-}$ from the glassy carbon plate. This assumption is supported by the fact that the cyclic voltammogram of the [Mo-Fe]/GC clearly exhibits the anodic wave of the cluster even in the presence of $N_3^-$ (Fig. 2). Thus, the system which is provided with rapid and continuous electron transport to a redox catalyst may be one of the essential factor to catalyze a multi-electron reduction involving unstable reaction intermediates.

The reduction of $N_3^-$ by nitrogenase affords also $N_2$, $NH_3$, and $N_2H_4$ around pH 7, and the amount of $NH_3$ has been shown to be larger than the total amount of

$N_2$ and $N_2H_4$. This has been ascribed to further six-electron reduction of $N_2$ formed in the two-electron reduction of $N_3^-$. The [Mo–Fe]/GC electrode has no ability of catalyzing the eight-electron reduction of $N_3^-$ though the [Mo–Fe]/GC reasonably explains the difference in the two- and six-electron reduction of $N_3^-$ in a wide pH range.

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