Simulation of the nitrogen cycle in microbial systems

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Abstract – The electrochemical reduction of $HOC_2H_4N_3$ with a $(\underline{n}\text{-}Bu_4N)_3[Mo_2\text{-}Fe_6S_8(SPh)_9]$ modified glassy carbon electrode([Mo-Fe]/GCE) in H_2O at -1.25 V $\underline{vs}$. SCE produces NH_3 (and $HOC_2H_4NH_2$) as an eight-electron reduction product $\underline{via}$ N_2, N_2H_2, and N_2H_4 successively. Similarly, $\underline{n}\text{-}C_5H_{11}N_3$ is reduced by $[MoFe_3S_4(SC_6H_4\text{-}\underline{p}\text{-}\underline{n}\text{-}C_8H_{17})_3(O_2C_6Cl_4)(solvent)]^{2-}$ in an aqueous micellar solution containing $Na_2S_2O_4$ and methyl viologen. The reduction of NO_3^- under the electrolysis conditions is catalyzed by [Mo-Fe]/GCE to yield NH_3 as an assimilation product $\underline{via}$ NO_2^-, NO^-, and NH_2OH, while the electrochemical reduction of NO_2^- at -1.10 V $\underline{vs}$. SCE results in the formation of N_2O as a dissimilation product.

INTRODUCTION

Inorganic nitrogen compounds, N_2, NH_3, NO_3^-, and so on, in the natural world are regulated in amount by the nitrogen cycle (Scheme 1). Among those inorganic nitrogen molecules only NH_3 can be converted into organic nitrogen molecules in the metabolism. Most of the higher plants and micro-orgasms that are not provided with the ability of N_2 fixation reduce NO_3^- and NO_2^- to produce NH_3 (assimilation). On the other hand, various denitrification bacteria reduce NO_3^- and NO_2^- to N_2O, which is further reduced to N_2 (dissimilation). These enzymatic reductions require multi-electrons. Such multi-electron reductions of those substrates can be performed by using an Fe-S or Mo-Fe-S cluster as catalysts under the controlled potential electrolysis conditions.
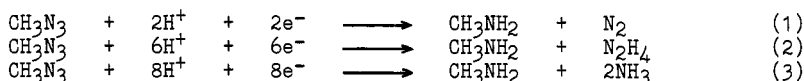
Scheme 1
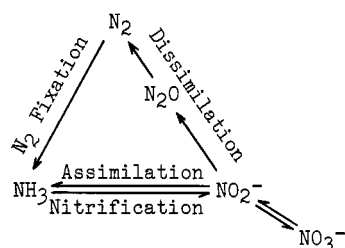
MULTI-ELECTRON REDUCTION OF ALKYLAZIDE (AND DINITROGEN) TO AMMONIA (ref. 1)

Under the electrolysis conditions at -1.25 V vs. SCE with an Hg working electrode in MeOH-THF (1:1 v/v, 20 cm^3) containing $[Mo_2Fe_6S_8(SPh)_9]^{3-}$ (1: 8.0×10^{-4} mol dm^{-3}), CH_3N_3 (6.6×10^{-2} mol dm^{-3}), and LiCl (0.24 mol dm^{-3}) as a supporting electrolyte under He atmosphere, the reduction of CH_3N_3 produces equal amounts of CH_3NH_2 and N_2 with a current efficiency nearly 100%, suggesting that almost all electrons transferred from the electrode
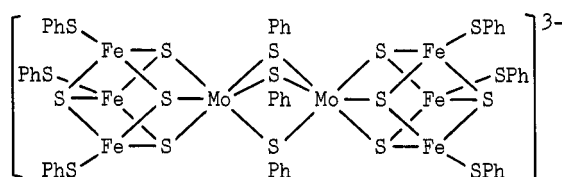
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to the clusters are consumed in the two-electron reduction of CH_3N_3 (eq. 1). On the other hand, when the initial concentration of CH_3N_3 is decreased to 8.7×10^{-3} mol dm^{-3}, the six- and eight-electron reductions of CH_3N_3 take place to afford N_2H_4 (eq. 2) and NH_3 (eq. 3), respectively, though the amounts are small. Thus, in a homogeneous system the electron

$$CH_3N_3 + 2H^+ + 2e^- \longrightarrow CH_3NH_2 + N_2 \qquad (1)$$
$$CH_3N_3 + 6H^+ + 6e^- \longrightarrow CH_3NH_2 + N_2H_4 \qquad (2)$$
$$CH_3N_3 + 8H^+ + 8e^- \longrightarrow CH_3NH_2 + 2NH_3 \qquad (3)$$

transfer from the electrode to the cluster may not be so effective for the multi-electron reduction of CH_3N_3, since only the clusters on the electrode surface can accept additional electrons from the electrode to prompt the multi-electron reductions.

The reduction of $HOC_2H_4N_3$ by an $\underline{n}$-Bu_4N salt of **1** modified glassy carbon electrode ([Mo-Fe]/GCE) under the controlled potential eletrolysis at -1.25 V $\underline{vs}$. SCE, therefore, was conducted in an aqueous solution (pH = 10) containing $HOC_2H_4N_3$ and H_3PO_4-NaOH buffer as a supporting electrolyte. The reduction produces not only NH_3 but also H_2; the turnover number for the formation of NH_3, based on the amount of modified clusters, attains more than 1×10^4 in 2 h (Fig. 1 and Table 1). The reaction ceases within 2 h irrespective of the initial concentration of $HOC_2H_4N_3$ and the amount of NH_3 formed is proportional to the concentration. Thus, the [Mo-Fe]/GCE can efficiently be used for multi-electron reductions of N_2ase substrates.

It has been proposed that N_2ase reduces N_2 to yield NH_3 $\underline{via}$ N_2H_2 and N_2H_4 as intermediates. Although the existence of neither free nor enzyme-bound N_2H_2 has so far been identified in the reduction of N_2 by N_2ase. In the present study, the reduction of $HOC_2H_4N_3$ by [Mo-Fe]/GCE in H_2O in the presence of allyl alcohol under electrolysis at -1.25 V $\underline{vs}$. SCE produces N_2 and HOC_2H_4-NH_2 together with H_2; neither NH_3 nor N_2H_4 has been formed at all, suggesting that N_2H_2 is involved as a reaction intermediate for the formation of N_2H_4 and NH_3 in the reduction of $HOC_2H_4N_3$ by [Mo-Fe]/GCE.
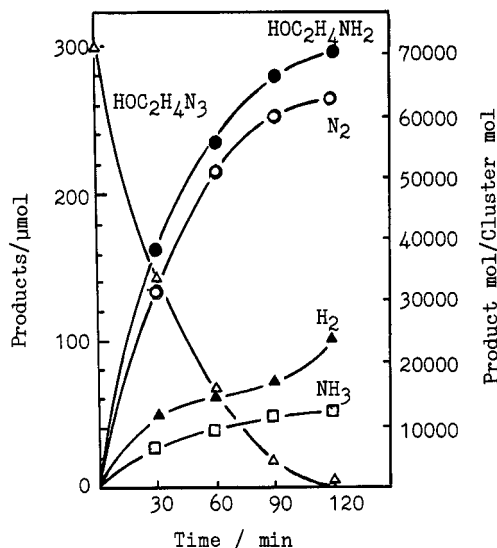
Fig. 1. The reduction of $HOC_2H_4N_3$ by [Mo-Fe]/GCE (4.2×10^{-9} mol/3.1 cm^2) at -1.25 V $\underline{vs}$. SCE in H_3PO_4-NaOH buffer (0.2 mol dm^{-3}, 20 cm^3). The amount of N_2H_4 formed is omitted.

Table 1. Amounts of the products in the reduction of $HOC_2H_4N_3$ catalyzed by [Mo-Fe]/GCE (4.2×10^{-9} mol/3.1 cm^2) at -1.25 V $\underline{vs}$. SCE

$HOC_2H_4N_3$[a]	Time	Product mol / Cluster mol				
mol	min	H_2	N_2	$HOC_2H_4NH_2$	NH_3	N_2H_4
50	120	3.8×10^3	1.0×10^4	1.2×10^4	2.8×10^3	0.9×10^2
100	120	7.3×10^3	2.1×10^4	2.4×10^4	4.4×10^3	1.6×10^2
300	120	2.0×10^4	6.9×10^4	7.1×10^4	1.1×10^4	5.1×10^2
160[b]	240	1.8	9.8	10.8[c]	1.8	0.13

[a]In a 0.2 mol dm^{-3} H_3PO_4-NaOH buffer (20 cm^3). [b]Reduction of CH_3N_3 catalyzed by $[Mo_2Fe_6S_8(SPh)_9]^{3-}$ in MeOH-THF (20 cm^3). [c]CH_3NH_2.

REDUCTION OF n-$C_5H_5N_3$ BY $Na_2S_2O_4$, CATALYZED BY Mo-Fe-S AND Fe-S SINGLE CUBANE CLUSTERS IN AQUEOUS MICELLAR SOLUTIONS (ref. 2)

The comparison of the catalytic activities between Mo and Fe atoms toward multi-electron reductions of N_2ase or pseudo-N_2ase substrates may be important in connection with the fact that the reduction of N_2 by N_2ase takes place with eight-electrons to give two moles of NH_3 and one mole of H_2. Thus, the reductions of $\underline{n}$-$C_5H_{11}N_3$ catalyzed by $[MoFe_3S_4(SR)_3(O_2C_6Cl_4)$-(L)]$^{2-}$ (**2**: R = C_6H_4-$\underline{p}$-$\underline{n}$-C_8H_{17}, L = Me_2CO or DMF) and $[Fe_4S_4(SR)_4]^{2-}$ (**3**) solubilized in aqueous Triton X-100 micellar solutions containing $Na_2S_2O_4$ and methylviologen dibromide ($MVBr_2$).

The cyclic voltammograms of clusters **2** (L = DMF) and **3** both exhibit the (2-/3-) redox couple at $E_{1/2}$ = -0.64 V $\underline{vs}$. SCE in an aqueous Triton X-100 micellar solution at pH 7.0. Thus, both clusters can be reduced by $Na_2S_2O_4$ (E° = -0.90 V $\underline{vs}$. SCE at pH 9.2) in aqueous micellar solutions. In addition, the reduced species produced in the reduction of cluster **2** or **3** with $Na_2S_2O_4$ reduces $\underline{n}$-$C_5H_{11}N_3$ catalytically to afford almost equal amounts of $\underline{n}$-$C_5H_{11}NH_2$ and N_2 in an aqueous micellar solution at pH 6.0 (Table 2). When MV^{2+} was added to the reaction mixture, the electron flow from $S_2O_4^{2-}$ in water to clusters **2** or **3** in micelles was accelerated to increase the rates of reaction by 20 and 50 times, respectively, compared
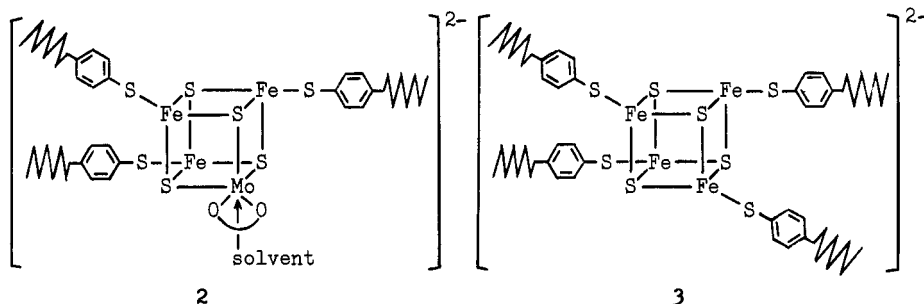
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Table 2. Reduction of $\underline{n}$-$C_5H_{11}N_3$ (2.0×10^{-2} mol dm^{-3}) by $Na_2S_2O_4$ (8.0×10^{-2} mol dm^{-3}) in aqueous micellar solutions (pH 6.0) of **2** or **3** (2.0×10^{-4} mol dm^{-3}) in the absence and presence of MVBr$_2$ at 30°C for 1 h

Cluster	MV^{2+} mol dm^{-3}	Solvent for solubilization	Product mol / Cluster mol			
			N_2	$\underline{n}$-$C_5H_{11}NH_2$	N_2H_4	NH_3
2	0	$(CH_3)_2CO$	5.4	5.6	0	0
3	0	$(CH_3)_2CO$	1.1	1.4	0	0
2	2.0×10^{-4}	$(CH_3)_2CO$	90.1	75.0	0.5	20.3
2	2.0×10^{-4}	DMF	41.8	50.5	0.5	12.4
3	2.0×10^{-4}	$(CH_3)_2CO$	16.8	16.1	0	0

with those in the absence of MV^{2+}. In addition, the reduction catalyzed by cluster **2** gives considerable amounts of N_2H_4 and NH_3 as well as N_2 and $\underline{n}$-$C_5H_{11}NH_2$, while the reduction of $\underline{n}$-$C_5H_{11}N_3$ catalyzed by cluster **3** produces only $\underline{n}$-$C_5H_{11}NH_2$ and N_2 even in the presence of MV^{2+} (Table 2). Thus, **2** is superior to **3** as the catalyst for N_2ase model reactions under the present experimental conditions. A striking difference between **2** and **3** toward the reduction of $\underline{n}$-$C_5H_{11}N_3$ may be associated with the fact that $\underline{n}$-$C_5H_{11}N_3$ functions as an electron acceptor and donor to the Mo atom of the $MoFe_3S_4$ core and the Fe atom of the Fe_4S_4 core, respectively. This result is the first experimental support for the view that Mo of the MoFe-co may be the active site of N_2ase reactions.

ASSIMILATORY AND DISSIMILATORY REDUCTION OF NO_3^- AND NO_2^- WITH [Mo-Fe]/GCE (ref. 3 and 4)

The cyclic voltammogram of [Mo-Fe]/GCE in water (pH 10) containing $NaNO_3$ and an H_3PO_4-NaOH buffer (supporting electrolyte) shows the cathodic current stronger by about two times than that in the absence of $NaNO_3$ at -1.25 V vs. SCE, suggesting that [Mo-Fe]/GCE can reduce NO_3^-.

The controlled potential electrolysis with [Mo-Fe]/GCE at -1.25 V vs. SCE in H_2O (pH 10) containing $NaNO_3$ and H_3PO_4-NaOH buffer produces NO_2^-, NH_3, and H_2 catalytically (Fig. 2). The amount of NO_2^- formed increases with time for the initial 3 h, but thereafter remains almost constant. On the other hand, the amount of NH_3 increases linearly with time after a lapse of about 1 h. The current efficiencies for the formation of NO_2^-, NH_3, and H_2 were 7.7, 80.3, and 11.3%, respectively, during 5 h, suggesting that only the reductions of NO_3^- and protons take place under the present conditions. The turnover numbers for the formation of NO_2^- and NH_3 based on the amount of the cluster were 352 and 843, respectively, during 5 h. The saturation of NO_2^- in water at about 3 h after starting the reduction of NO_3^- demonstrates that NO_2^- is the first reduction product of NO_3^-. In fact, the reduction of NO_2^- with [Mo-Fe]/GCE under the electrolysis

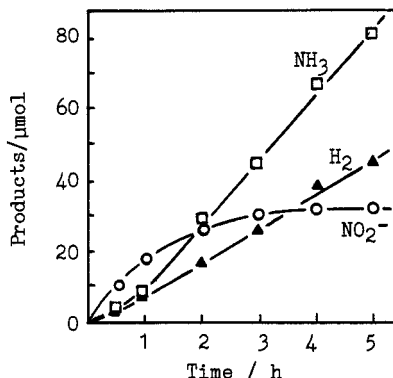Fig. 2. The reduction of NO_2^- (5.0×10^{-2} mol dm^{-3}) catalyzed by [Mo-Fe] (1.0×10^{-7} mol)/GCE under the electrolysis at -1.25 V vs. SCE in H_3PO_4-NaOH buffer (pH 10, 0.2 mol dm^{-3}).
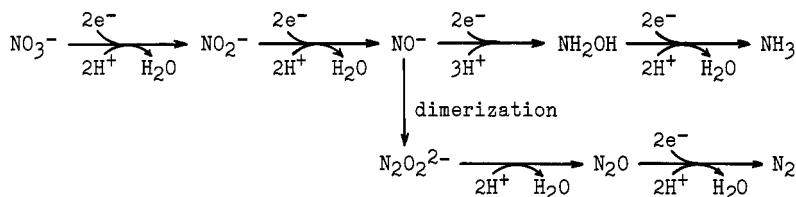
at -1.25 V $\underline{vs}$. SCE in H_2O (pH 10) containing $NaNO_2$ (5.0×10^{-1} mol dm^{-3}) and H_3PO_4-NaOH (0.2 mol dm^{-3}) produced NH_3 and H_2 catalytically with current efficiencies of 85.3 and 14.1%, respectively, during 3 h.

When the reduction of NO_2^- with the [Mo-Fe] modified glassy carbon disc of a ring-disc electrode with rotation 1000 r.p.m. was carried out at -1.25 V $\underline{vs}$. SCE in H_2O (pH 10), no detectable current flow was observed at the glassy carbon ring electrode on fixing the potential at a more negative value than +0.3 V $\underline{vs}$. SCE. An anodic current, however, began to flow at the ring electrode at a more positive potential than +0.3 V $\underline{vs}$. SCE and increased on shifting the potential to more positive values. This electrochemical oxidation behavior is consistent with that of NH_2OH at a glassy carbon ring electrode in H_2O at pH 10. Thus, NH_2OH should be an intermediate in the reduction of NO_2^- to NH_3.

The reduction of NO_2^- at -1.20 V $\underline{vs}$. SCE affords NH_3 together with H_2, being similar to that at -1.25 V $\underline{vs}$. SCE, while a small amount of N_2O as well as NH_3 is produced in the reduction conducted at -1.15 V $\underline{vs}$. SCE. A further anodic shift of the electrode potential to -1.10 V $\underline{vs}$. SCE results in a complete depression of the formation of NH_3, instead N_2O is formed as a reduction product. The alternation of the main product from NH_3 to N_2O in the reduction of NO_2^- suggests that both reductions proceed $\underline{via}$ a common reaction intermediate. A most plausible intermediate for the reduction of NO_2^- to NH_2OH is unstable NO^- (or HNO), which is known to dimerize rapidly in organic solvents, giving $N_2O_2^{2-}$ (or $H_2N_2O_2$). The $N_2O_2^{2-}$ anion, however, easily undergoes decomposition in H_2O to give N_2O. The present N_2O evolution in the reduction of NO_2^- may, therefore, arise from the preferential dimerization of NO^- to $N_2O_2^{2-}$ rather than the reduction of NO^- to NH_2OH owing to a decreasing ability of multi-electron reductions caused by the anodic shift of the [Mo-Fe]/GCE potential.

Possible reduction pathways of NO_3^- with [Mo-Fe]/GCE may be expressed as Scheme 2. The rate-determining step may be the reduction of NO_3^- to NO_2^-, since the latter is accumulated to some extent in the reaction mixture (Fig. 2) and NH_2OH is not identified in the reaction products. On the other hand, when the reduction of NO_2^- was conducted at -1.10 V $\underline{vs}$. SCE,

Scheme 2

$$NO_3^- \xrightarrow[2H^+ \ H_2O]{2e^-} NO_2^- \xrightarrow[2H^+ \ H_2O]{2e^-} NO^- \xrightarrow[3H^+]{2e^-} NH_2OH \xrightarrow[2H^+ \ H_2O]{2e^-} NH_3$$

$$NO^- \downarrow \text{dimerization}$$

$$N_2O_2^{2-} \xrightarrow[2H^+ \ H_2O]{} N_2O \xrightarrow[2H^+ \ H_2O]{2e^-} N_2$$

the first product NO^- dimerizes to $N_2O_2^{2-}$ before it is reduced to NH_2OH. The resulting $N_2O_2^{2-}$ anion easily undergoes decomposition in H_2O to evolve N_2O. Biologically dissimi-latory reduction of NO_2^- has been suggested to produce N_2 $\underline{via}$ N_2O. Although no N_2 evolution has taken place in the present NO_3^- and NO_2^- reductions, the controlled potential electroly-sis of an N_2O saturated aqueous solution (pH 10) with [Mo-Fe]/GCE at -1.25 V $\underline{vs}$. SCE has evolved N_2 catalytically with a 50% current efficiency. Thus, the [Mo-Fe]/GCE can simulate the assimilatory and dissimilatory reductions of NO_3^- and NO_2^-. Moreover, the reduction pathways of NO_3^- presented in Scheme 2 are consistent with those proposed for the reductions of NO_3^- by assimilatory and dissimilatory reductases.

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