

Figure 2. Pressure dependence of the 300-MHz proton NMR spectra of horse heart ferric cyanomyoglobin at 30 °C, pH 7.8 in 0.1 M Tris-HCl-H₂O buffer solution. The peaks a, b, and e are exchangeable proton signals. The numbering of the heme methyl groups is shown in Figure 1.

downfield and upfield, respectively, while the proximal histidyl NH peak at 16.0 ppm remained unchanged. It is therefore likely that the pressure-induced shifts of vinyl C₂H for the natural myoglobin and the pyrrole protons for deuteriomoglobin are not caused by changes in the electronic structure of the heme but rather by the local structural alterations of the heme periphery resulting from the nonbonded interactions with nearby amino acid residues. We have also examined high-pressure NMR of other hemoproteins such as cyano horseradish peroxidase.^{1b} No pressure-induced spectral changes were observed. Use of different buffer solutions led to the same results.

The distal histidyl N₃H proton is located near the heme iron closely enough to experience substantial pseudocontact shift. Decrease in the downfield paramagnetic shift for this resonance upon pressurization could be due to dislocation of this histidyl imidazole group in a way that the iron-N₃H distance increases and/or the N₃H moves far off the nodal axis of the heme plane. This structural change could occur by a swing away of the imidazole group from the distal histidine upon pressure-induced structural changes. According to an X-ray analysis of sperm whale cyanometmyoglobin,⁹ the distal histidyl N₃H proton is hydrogen bonded to the coordinated cyanide.^{6c} Some changes in this hydrogen bond interaction and in tautomeric equilibrium of the distal histidyl imidazole ring could not be ruled out as causes for the pressure-induced shift of the N₃H resonance. If this local structural change in the distal side of the heme is induced by pressure for other myoglobin derivatives, the ligand-exchange phenomena at the iron sixth coordination position are expected to be modulated by pressure. Our recent finding that the pK of the acid-alkaline transition of aquometmyoglobin is substantially changed at high pressure² could be interpreted in terms of this structural alteration. The hydrogen bond of the iron-bound water with the distal histidine in the acid form may be affected by this structural change upon pressurization and eventually lead to a change in pK associated with deprotonation of this bound water

to produce the hydroxyl group at the iron sixth site (alkaline form).

In summary the present high-pressure NMR study of cyanometmyoglobin revealed that the pressure effects are localized to a particular region of the protein, especially the heme distal side.

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Electroreduction of Carbon Dioxide Catalyzed by Iron-Sulfur Clusters [Fe₄S₄(SR)₄]²⁻

Meguru Tezuka,* Tatsuhiko Yajima, and Atsuhiko Tsuchiya

*The Saitama Institute of Technology
Okabe, Saitama 369-02, Japan*

Yoichi Matsumoto, Yasuzo Uchida, and Masanobu Hidai*

*Department of Industrial Chemistry
Faculty of Engineering
The University of Tokyo, Hongo, Tokyo 113, Japan*

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Much attention has been focused on converting the cheap carbon resource carbon dioxide into organic substances. Carbon dioxide is electrolytically reduced to various organic acids, the distribution of which crucially depends on the reaction conditions such as electrode materials, solvent systems, and operational parameters.¹ Recently, Savéant and his co-worker² shed some mechanistic light on the product distribution. One of the problems

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Table I. Electroreduction of CO₂ in DMF Solution^a

run	cluster ^b	E, V (vs. SCE)	η, % ^c		
			oxalate	formate	CO
1		-2.4	73	12	13
2	1	-1.7		59	2
3	1	-1.9		71	1
4	1	-2.1		81	1
5	2	-2.0		93	1
6 ^d		-2.4	48	28	18
7 ^d	1	-1.8		40	
8 ^e		-2.4	7	60	30
9 ^e	1	-1.7		34	
10 ^f		-2.1	10	29	51
11 ^f	1	-2.0	13	33	5
12 ^g		-2.0	14	16	69
13 ^g	1	-2.0	14	21	6

^a 3 mF of electricity passed through CO₂-saturated 0.1 M TBAT/DMF solution. ^b Concentration 2 mM. ^c Current efficiency based on the assumption that each product requires two electrons for its formation. ^d 1 volume % of water added. ^e 5 volume % of water added. ^f 5 mol % of TBAT replaced by CTMAT. ^g 10 mol % of TBAT replaced by CTMAT.

in the CO₂ reduction comes from its large overpotential. Indeed, potentials far more negative than -2 V vs. SCE are required in the preparative-scale electrolyses. A great deal of effort has, therefore, been devoted to searching for catalysts to reduce the potential. The transition-metal complexes of tetraazamacrocycles including phthalocyanines or tetraphenylporphyrins have been found to act as electrocatalysts for CO₂ reduction in aqueous systems.³ In this paper, we report that a quite different type of complex, the tetranuclear iron-sulfur cluster [Fe₄S₄(SR)₄]²⁻, catalyzes the electroreduction of CO₂ in nonaqueous DMF solution. The iron-sulfur clusters were first synthesized by Holm and co-workers⁴ as analogues of the 4-Fe active sites in iron-sulfur proteins, which play a significant role in the electron-transfer reactions occurring in living systems. Actually, it was demonstrated that the clusters catalyzed the hydrogenation of acetylenes.⁵

The clusters, [Fe₄S₄(SR)₄]²⁻ (R = CH₂C₆H₅ (1) or C₆H₅ (2)), were prepared as tetraalkylammonium salts according to the procedure in ref 4. A cylindrical gastight cell was employed as a reactor, where cathode and anode chambers were separated by a glass frit from each other. Controlled-potential electrolyses (CPE) were performed in a CO₂-saturated 0.1 M tetrabutylammonium tetrafluoroborate (TBAT)/DMF solution (50 mL) on mercury pool cathode (16 cm²), referred to SCE, at room temperature. After each run, the gas phase and electrolytic solution were analyzed by means of gas chromatography and isotachopheresis.⁶

The CPE results are summarized in Table I. In the absence of a cluster, CO₂ was substantially reduced only at the potentials not less negative than -2.4 V vs. SCE, while the presence of 1 allowed the reduction to take place at more positive potentials. The potential shift amounted to approximately 0.7 V. Besides, it should be noted that the reduction proceeded catalytically, since the product current efficiency of 50% corresponds to 15 electrons per catalyst molecule. With respect to the cluster 2, the results were essentially same.

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(6) For theory and applications of isotachopheresis, see: "Analytical Isotachopheresis"; Everaerts, F. M., Ed.; Elsevier: Amsterdam, 1981. Formate and oxalate were analyzed with Shimadzu capillary tube isotachopheric analyzer IP-2A by using histidine + histidine monohydrochloride and n-caproic acid as leading and terminating electrolytes, respectively. The PU values of formate and oxalate were 0.20 and 0.07.

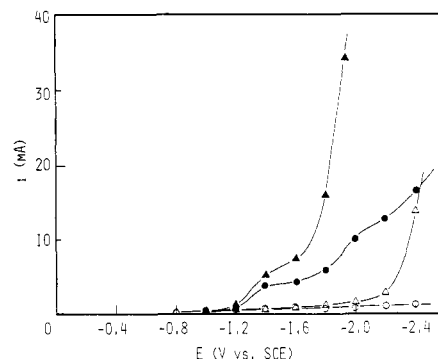


Figure 1. Current-potential relationship in CPE of CO₂: (●) with the cluster 1 under N₂; (▲) with the cluster 1 under CO₂; (○) without the cluster under N₂; (△) without the cluster under CO₂.

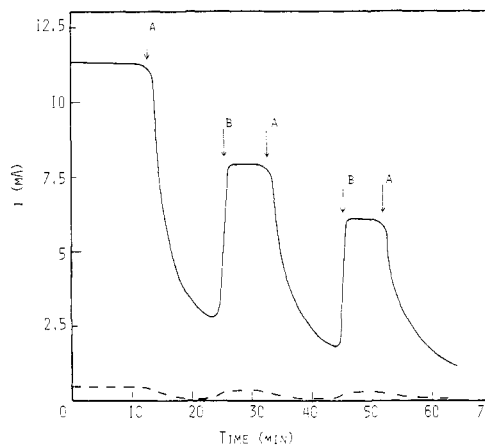


Figure 2. Controlled-potential electrolyses at -1.7 V vs. SCE: (A) N₂ introduced; (B) CO₂ introduced; (—) with 2 mM of cluster 1; (---) without the cluster.

The redox properties of a series of iron-sulfur clusters have been studied extensively.⁷ They are reduced consecutively to trianions and then tetraanions through reversible or quasi-reversible one-electron steps in nonaqueous solutions. Compatible results were also obtained in our CPE experiments. As shown in Figure 1, the cluster 1 gave an *i*-*E* curve indicating the successive reduction of the cluster under N₂, whereas the presence of CO₂ caused a remarkable increase in current within the potential range where 1 could be reduced to the tetraanion. The current held on over several hours, showing that the catalyst remained active therein. When N₂ was introduced into the electrolyte to purge CO₂ in the course of the CPE experiments, the current was gradually decreased (Figure 2). The subsequent replacement of N₂ by CO₂ put back the current to the intrinsic value. This sequence was repeatable as long as most of the cluster was not consumed through its own electrochemical reactions under N₂.⁸ These findings strongly suggest that the tetraanion is involved in the reduction of CO₂.⁹

Without the catalysts, oxalate was predominantly formed together with small quantities of formate and carbon monoxide, the latter implying the concurrent formation of carbonate in an equivalent amount. Addition of water or partial replacement of the supporting electrolyte (TBAT) by cetyltrimethylammonium tetrafluoroborate (CTMAT), a common surfactant, favored formation of formate or CO, respectively, at the expense of oxalate.

(7) DePamphilis, B. V.; Averill, B. A.; Herskovitz, T.; Que, L., Jr.; Holm, R. H. *J. Am. Chem. Soc.* **1974**, *96*, 4159-4167.

(8) In ref 7, it is described that the reduction of the clusters to the tetraanions were followed by relatively rapid decomposition under inert atmosphere in DMF and CH₃CN solutions.

(9) Participation of the tetraanions of Fe-S clusters in the addition of mercaptans to isocyanides or H-D exchange reaction are reported: (a) Schwartz, A.; Van Tamelen, E. E. *J. Am. Chem. Soc.* **1977**, *99*, 3189-3191 (b) Tanaka, M.; Tanaka, K.; Tanaka, T. *Chem. Lett.* **1982**, 767-770.

The product variation can be consistently interpreted according to the pathways proposed by Savéant and his co-worker² that CO₂ reduction involves three competitive pathways: (a) oxalate formation through self-coupling of CO₂⁻; (b) formate formation through protonation of CO₂⁻; (c) CO formation via oxygen-carbon coupling of CO₂⁻ with CO₂. The latter two might become prevalent by addition of water or a surfactant to be adsorbed onto the electrode.

By contrast, formate was obtained preferentially in the presence of the clusters even in a dry solution. This supports the view that CO₂⁻ would be generated through the electron transfer from the reduced clusters to CO₂ in the bulk solution, not on the electrode, and consequently undergo the protonation rather than the self-coupling. The hydrogen atom in the formate produced seems to be derived, at least in part, from tetraalkylammonium ion.¹⁰ In fact, considerable amounts of hydrocarbons consisting mainly of C₃ were detected in the gas analyses after CPE with the clusters. These hydrocarbons were undetectable in such cases where oxalate was predominantly formed.

Works are now in progress to elucidate the reaction mechanism.

Registry No. 1, 52349-82-3; 2, 52325-39-0; CO₂, 124-38-9; CO, 630-08-0; TBAT, 429-42-5; CTMAT, 73257-08-6; oxalate, 338-70-5; formate, 71-47-6.

(10) Evidences that tetraethylammonium ion works as proton donor in the reaction with O₂⁻, giving rise to ethylene and triethylamine, are provided: Feroci, G.; Roffia, S. *J. Electroanal. Chem.* 1976, 71, 191-198.

H/D Secondary Isotope Effects in the Thermal Decomposition of a 1,2-Diaza-1-cyclobutene. Mechanism of Thermolysis

Henrik Olsen*

Laboratory for Organic Chemistry
Swiss Federal Institute of Technology
CH-8006 Zürich, Switzerland

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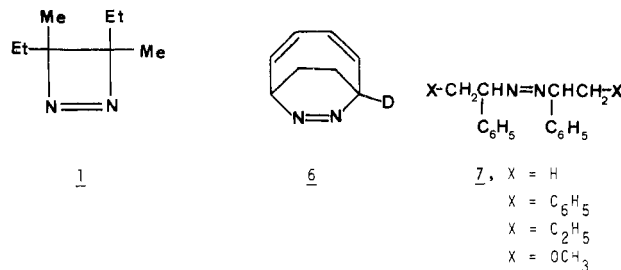
There has been considerable interest for some time in the mechanism of the thermal deazetation of substituted 1,2-diaza-1-cyclobutenes because of the variety of possible decomposition pathways.¹ Aside from a stepwise process involving a biradical-like species residing in a minimum or at the saddle point on the potential surface, synchronous loss of nitrogen by a [2_s(olefin) + 2_s(N₂)], [2_a(olefin) + 2_s(N₂)], [2_s(olefin) + 2_a(N₂)], or [2_s(olefin) + "partial"2_a(N₂)] process is conceivable.^{1d,2} Studies of the thermal decomposition of *meso*- and *dl*-diazetines **1** excluded the possibility of synchronous loss of N₂ by a concerted [2_a(olefin) + 2_s(N₂)] process, indicated the unimportance of electronically excited states in this highly exothermic reaction, and placed severe constraints on the lifetime of a diradical derivable by a stepwise cleavage.^{1d} In an attempt to learn more about the pyrolytic

* Present address: The Technological Institute, Gregersensvej, DK-2630 Tåstrup, Denmark.

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(2) In contrast to the cyclobutane → ethylene decomposition, where the [σ₂ + σ₂] pathway is allowed, the [2_s(olefin) + 2_s(N₂)] process is forbidden. Thus the diazacyclobutene σ₋(A), σ₊(S), π_{NN}(A), n₊(S), and n₋(A) orbitals cannot transform into the olefin π_{CC}(S) and nitrogen π_{NN}(S), π_{NN}(A), n₊(S), and n₋(A) orbitals, if orbital symmetry is to be conserved by using a 2-fold axis of symmetry. Since this pathway furthermore involves a very strained transition state, it must be considered less likely than the [2_s(olefin) + 2_s(N₂)] path.

decomposition of diazeties, we undertook the determination of the α secondary deuterium isotope effects in the previously studied pyrolysis of bicycle **2**, which delivers norbornene and N₂ in a clean reaction.^{1a}



Diazeties **2-d₁** and **2-d₂**, with respectively one and two deuterium atoms in the bridgehead positions α to nitrogen, were synthesized as outlined in Scheme 1.³ Oxidative hydrolysis⁴ of adduct **3⁵** results in quantitative conversion to azo-N-oxide **4**, which rapidly exchanges the bridgehead proton α to oxidized nitrogen for deuterium in CD₃ONa/CD₃OD at 50 °C to give **5**.^{6,7} Reduction with lithium aluminum hydride yielded the diazetyne-*d₁* **2-d₁**; mp 127-128 °C; λ_{max}(isooctane) 359.1 nm (ε 277), 348.6 (273). Saponification of **3** followed by cupric bromide oxidation in situ and decomposition of the resulting metal complex with aqueous ammonium hydroxide led to **2**. The latter readily undergoes H → D exchange at 100 °C in CD₃ONa/CD₃OD at the bridgehead protons α to nitrogen to give diazetyne-*d₂* **2-d₂**; mp 126.5-127.5 °C, λ_{max}(isooctane) 359.1 nm (ε 255), 348.6 (254). NMR analysis gave 95.4 ± 1.4 and 190.0 ± 1.0 atom % of D for **2-d₁** and **2-d₂**, respectively.⁸ The activation parameters for the decomposition of **2** in isooctane are similar to those observed by Lemal et al. in the gas phase (ΔH[‡]_{isooctane} = 35.3 ± 0.6 kcal mol⁻¹, ΔS[‡]_{isooctane} = 6.4 ± 1.4 eu; ΔH[‡]_{gas phase} = 33.7 ± 0.5 kcal mol⁻¹, ΔS[‡]_{gas phase} = 3.0 ± 1.0 eu).^{1a} The isotope effects determined by simultaneously observing the rates of disappearance of **2**, **2-d₁**, and **2-d₂** at 140.65 °C in isooctane are listed in Table I.⁹

Several groups have reported that the replacement of hydrogen by deuterium on the carbon atom α to nitrogen of an azoalkane increases ΔG[‡] when the carbon-nitrogen bond is breaking.¹⁰ For five-membered cyclic azoalkanes (pyrazolines) these effects have

(3) All new compounds exhibited consistent spectroscopic properties and yielded satisfactory elemental analyses.

(4) Olsen, H.; Snyder, J. P. *J. Am. Chem. Soc.* 1977, 99, 1524-1536.

(5) Prepared by a [2 + 2 + 2] cycloaddition of MTAD and quadricyclane followed by hydrogenation over palladium-charcoal.

(6) The ¹H NMR spectrum of azo-N-oxide **4** shows the bridgehead protons α to nitrogen as two multiplets at δ 5.04 and 3.71. The signal at lowest field disappears after treatment with CD₃ONa/CD₃OD.

(7) Attempts to exchange the bridgehead proton α to unoxidized nitrogen at higher temperatures (100 °C) resulted in decomposition.

(8) The NMR measurements involved a minimum of five independent determinations of the proton ratios for labeled and unlabeled compounds by integration following repetitive scanning with a Varian XL-100 spectrometer. The error is given by the standard deviation.

(9) The rate of decomposition was determined by following the disappearance of the absorption at 359.1 nm with a Cary 17 spectrometer. The kinetic experiments were carried out by preparing approximately 0.008 M solutions of the azo compounds **2**, **2-d₁**, and **2-d₂** in isooctane (Fluka for UV spectroscopy, distilled before use) and syringing 4-mL aliquots into 10-mm quartz cuvettes (carefully base washed before use) equipped with Pyrex necks for sample introduction. The samples were degassed by three freeze-thaw cycles and sealed off under low pressure (10⁻⁷ torr). The samples were heated at 140.65 ± 0.05 °C over several half-lives (up to 5) and periodically monitored at room temperature after the reaction was quenched by sudden chilling (liquid N₂). Unimolecular rate constants were obtained by least-squares analysis of ln(A_∞ - A_t) vs. time. Correlation coefficients of 0.99998 were routinely obtained. ¹H NMR investigations of the decomposition products from **2-d₁** and **2-d₂** showed that no deuterium scrambling had taken place.

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