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STERICALLY HINDERED FERREDOXIN COMPLEXES AS A MODEL OF HIGH POTENTIAL IRON-SULFUR PROTEINS

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4Fe-4S ferredoxin model complexes, $[NEt_4]_2[Fe_4S_4(Z\text{-cys-Ile-Ala-OMe})_4]$, $[N(n\text{-Bu})_4]_2[Fe_4S_4(2,4,6\text{-trimethylbenezenethiolato})_4]$, and $[NMe_4]_2[Fe_4S_4(2,4,6\text{-triisopropylbenzenethiolato})_4]$ having highly sterically hindered ligands exhibit an electrochemically quasi-reversible redox couple of 2-/1- at +0.12 V, +0.02 V, and -0.03 V vs. SCE, respectively, but an irreversible redox couple of 3-/2-. The results suggest that $[Fe_4S_4(SR)_4]^{1-}$ is stabilized with protection by hydrophobic bulkiness, but that $[Fe_4S_4(SR)_4]^{3-}$ is destabilized by the bulkiness.

High potential iron-sulfur protein (HiPIP) from Chromatium virosum has a $[Fe_4S_4(S\text{-cys})_4]^{2-/1-}$ core and exhibits a redox couple at +0.35 V (NHE).[1)] Construction of a model of HiPIP has been one of the attractive targets of bioinorganic chemistry. Depamphilis et al. found an irreversible anodic process at -0.124 V (SCE) for $[Fe_4S_4(S\text{-}t\text{-Bu})_4]^{2-}$ in DMF.[2)] Recently, Mascharak et al. reported that in the cyclic voltammetry of this process a cathodic peak (-0.155 V vs. SCE) as well as the anodic peak (-0.085 V vs. SCE) was found as an irreversible electron transfer in DMF.[3)] Origin of the reversible redox couple of 2-/1- in native HiPIP is still unsolved. Previously we reported that a NH---S hydrogen bonding between cysteinyl thiolate and alanyl NH in $[Fe_4S_4(Z\text{-cys-Gly-Ala-OMe})_4]^{2-}$ (Z=benzyloxycarbonyl)[4)] causes the positive shift of redox potential for 3-/2-.[5)] The model complex did not exhibit any redox couple for 2-/1- in cyclic voltammetry.

$[NEt_4]_2[Fe_4S_4(Z\text{-cys-Ile-Ala-OMe})_4]$ (**1**) was synthesized from $[Fe_4S_4(S\text{-}\mathit{t}\text{-Bu})_4]^{2-}$ and Z-Cys-Ile-Ala-OMe by the same method reported by Holm's group.6) The sequence, -Cys-Ile-Ala-, is a part of hydrophobic amino acid sequences around an active site of *P. aerogenes* ferredoxin.7) **1** exhibited an irreversible redox couple of 3-/2- at ca. -1.0 V (SCE) and a quasi-reversible redox couple (E_p) of 2-/1- at +0.12 V ($E_{p,a}$= +0.20 V, $E_{p,c}$= +0.04 V; $i_{p,c}/i_{p,a} \simeq 1.0$) in DMF as shown in Fig. 1-a. The sterically demanding amino acid sequence such as Cys-Ile-Ala prevents the formation of NH---S hydrogen bonding by the side chain of Ile residue which is unsuitable for making a hairpin turn conformation. From the results of X-ray analysis for *P. aerogenes* ferredoxin and HiPIP, Adman et al. pointed out that HiPIP has the smaller numbers of NH---S hydrogen bonds than those in other ferredoxins.7) Sherian et al. discussed that the reduced state of an Fe_4S_4 core in HiPIP was stabilized by the NH---S hydrogen bonds, whereas the oxidized state was also stabilized by the another geometry of the NH---S hydrogen bonds.8,9) Our results of the redox potential of **1** indicate that a sterically demanding sequence around the Cys residue is required for the stability of the oxidation state (1-).

We synthesized two model complexes having simple bulky arylthiolato ligands such as 2,4,6-trimethylbenzenethiolato (tmbt) or 2,4,6-triisopropylbenzenethiolato (tibt) ligands. $[N(\mathit{n}\text{-Bu})_4]_2[Fe_4S_4(tmbt)_4]$ (**2**) 10) was synthesized by the same procedure reported by Averill et al.11) A bulky arylthiolate ligand has been employed for the successful stabilization of a mononuclear iron(III) complex such as $[NEt_4][Fe(2,3,5,6\text{-tetramethylbenzenethiolato})_4]$.12) $[NMe_4]_2[Fe_4S_4(tibt)_4]$ (**3**) was prepared by the ligand exchange method as mentioned above for the synthesis of **1**. The existence of $Fe_4S_4^{2+}$ core in **1**, **2**, or **3** was confirmed by the core extrusion method reported by Gillum et al.13)

The characteristic absorptions of **2** and **3** in DMF were found at 412 nm (ε: 22500) and 413 nm (ε: 26900), respectively. These absorption maxima are quite different from those of ferredoxin model complexes having ring-substituted thiophenolato ligands studied by Holm's group.14,15) They reported that $[NMe_4]_2[Fe_4S_4(S\text{-}\mathit{p}\text{-tol})_4]$ exhibited an absorption maximum at 460 nm (ε: 18300) in DMF.16) The observed difference in the position of the absorption maxima indicates that the coordinating sulfurs of **2** or **3** are extremely hindered by the bulkiness of two methyl groups or two isopropyl groups substituted at the 2,6-positions of the phenyl group. Otherwise, the alkyl substituents at these positions should contribute to an electron donation to the phenyl ring resulting in the red shift of the absorption maxima.

In electrochemical studies, **2** in DMF exhibits an irreversible redox couple (Ep) of 2-/1- about +0.02 V (SCE) ($E_{p,a}$= +0.08 V, $E_{p,c}$= -0.04 V) and a quasi-reversible couple of 3-/2- at -1.27 V (SCE) ($E_{p,a}$= -1.17 V, $E_{p,c}$= -1.37 V; $i_{p,c}/i_{p,a}$= 0.8) as shown in Fig. 1. **3** with the bulky thiolates provides a quasi-reversible couple of 2-/1- at -0.03 V (SCE) ($E_{p,a}$= +0.01 V, $E_{p,c}$= -0.07 V; $i_{p,c}/i_{p,a} \simeq 1.0$) and an irreversible couple of 3-/2- about -1.38 V (SCE). For the unstable redox couple (3-/2-), the following reason can be speculated. A reduced Fe_4S_4 core (3-) is unstable unless stabilized by solvation with protic solvents through hydrogen bonding. The intramolecular NH---S hydrogen bonds were found to stabilize the redox couple in native ferredoxin[1)] and the model complex.[5)] Recently, Tanaka et al. reported that the redox potentials (3-/2-) of $[Fe_4S_4(SC_6H_4R\text{-}\underline{p})_4]^{2-}$ (R = $\underline{n}\text{-}C_4H_9$, $\underline{n}\text{-}C_8H_{17}$, and $\underline{n}\text{-}C_{12}H_{25}$) shifted to more positive side at low pH in aqueous micellar solutions.[16)]

Our present results suggest that an essential requisite for the stability of oxidation state of native HiPIP is a protection of $Fe_4S_4^{3+}$ cluster against decomposition and that such a protection is accomplished by bulkiness and hydrophobic environment around the Fe_4S_4 core.

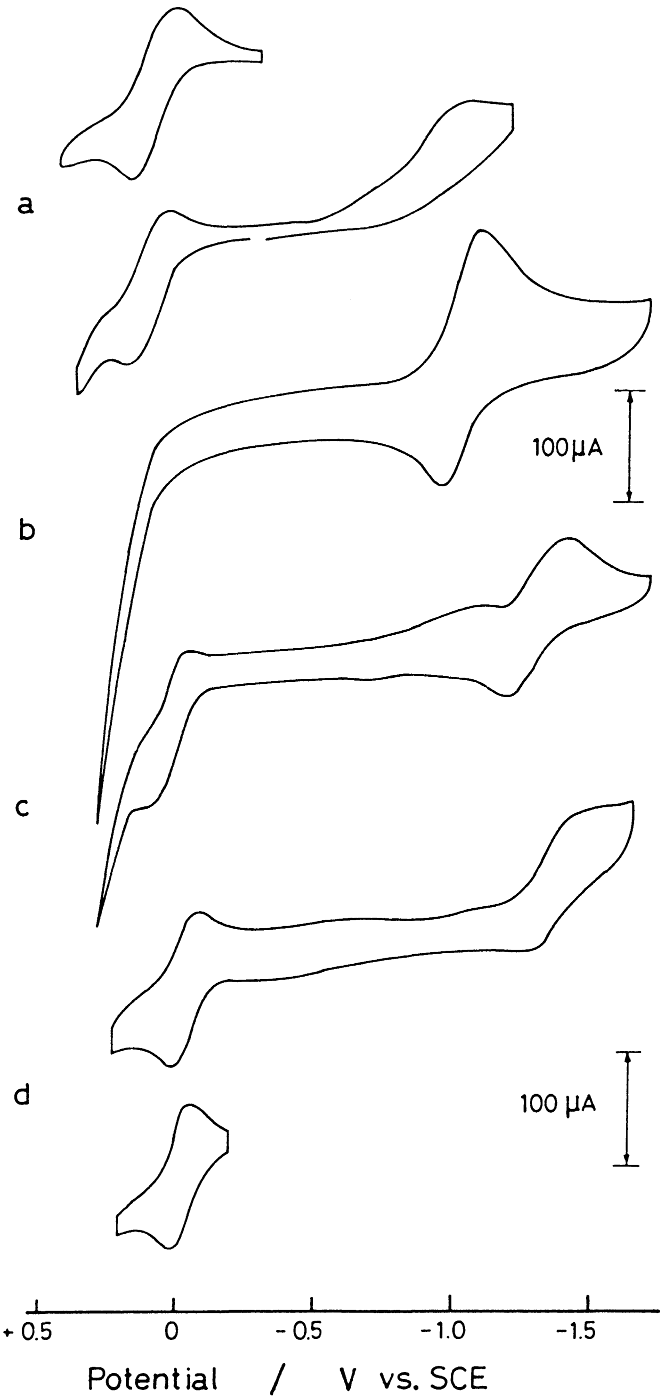

Fig. 1. Cyclic voltammograms of a) $[N(\underline{n}\text{-}Bu)_4]_2[Fe_4S_4(\text{Z-cys-Ile-Ala-OMe})_4]$, b) $[NEt_4]_2[Fe_4S_4(S\text{-}Ph)_4]$, c) $[N(\underline{n}\text{-}Bu)_4]_2[Fe_4S_4($2,4,6-trimethylbenzenethiolato$)_4]$, and d) $[NMe_4]_2[Fe_4S_4($2,4,6-triisopropylbenzenethiolato$)_4]$ in DMF at glassy carbon electrode, supporting electroyte $N(\underline{n}\text{-}Bu)_4ClO_4$, 0.1 M, and scanning rate, 100 mV s^{-1}.

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