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IRON-SULFUR CLUSTER MICELLE AS A FERREDOXIN MODEL

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Iron-sulfur clusters having hydrophilic and hydrophobic L-cysteine derivative as their components were prepared. Electronic and CD spectra of these clusters as well as their oxidation-reduction potentials are very similar to those of native enzymes. These clusters incorporated in the micelles show satisfactory stability and electron transfer ability.

After Holm's successful preparation of the first artificial iron-sulfer cluster[1], a variety of artificial iron sulfur clusters have been prepared to gain further insights into chemistry of ferredoxin and related enzymes[2]. However, application of these artificial clusters to the catalytic electron transfer *in water* has achieved least successes, mainly due to their inproper oxidation-reduction potentials and also instability in water.

We now wish to report that artificial iron-sulfur cluster $(n\text{-}Bu_4N)_2[Fe_4S_4(SCH_2CH(CO_2Na)NHCOC_{17}H_{35}\text{-}L)_4]$ **1** incorporated into a CTAB micelle behaves quite nicely in water: electronic and CD spectra are very similar to native ferredoxin, satisfactorily stable in water, oxidation-reduction potential is appropriate, solubility (as micelles) is sufficiently high and electron uptake rate from the bulk aqueous phase is reasonably fast.

Preparation of **1** was carried out according to usual cluster exchange reaction[3] between 80 mg of $L\text{-}HSCH_2CH(CO_2Na)NHCOC_{17}H_{35}$[4] and 50 mg of $(n\text{-}Bu_4N)_2[Fe_4S_4(t\text{-}BuS)_4]$ **2**[1] in 5 mL DMF at 50° under Ar. After 1 hour, the solution was condensed to 4 mL and was further stirred for 2 hr at 50°. On cooling the solution, red-brown precipitates were formed, which were collected and reprecipitated from DMF/benzene-hexane. 400 MHz ^1H NMR δ 13.7, 14.3 (doublet like, SCH_2), δ 7.0 (broad-singlet like, methine) in DMSO d_6[5]. Physical and chemical properties of **1** and its analog $(n\text{-}Bu_4N)_2[Fe_4S_4(SCH_2CH(CO_2Na)NHAc\text{-}L)_4]$ **3**[6] are listed in Table 1. Cyclic voltammetry of **1** in 0.1 M $n\text{-}Bu_4N\cdot ClO_4$-DMF solution at 20° vs a Ag^+/Ag electrode gave E to be -0.88 V (NHE), suggesting E in water to be ca. -0.5 V, close to H_2/H^+ potential[7]. Aqueous micelles of **1** were prepared by the addition of 50 μL of a DMF solution of **1** (ca. 0.6 mg) to the 2.0 mL of an aqueous solution of CTAB (15 mg) at 30° under vigorous stirring. A micellar solution of **1** showed electronic absorptions at 300 (ε,

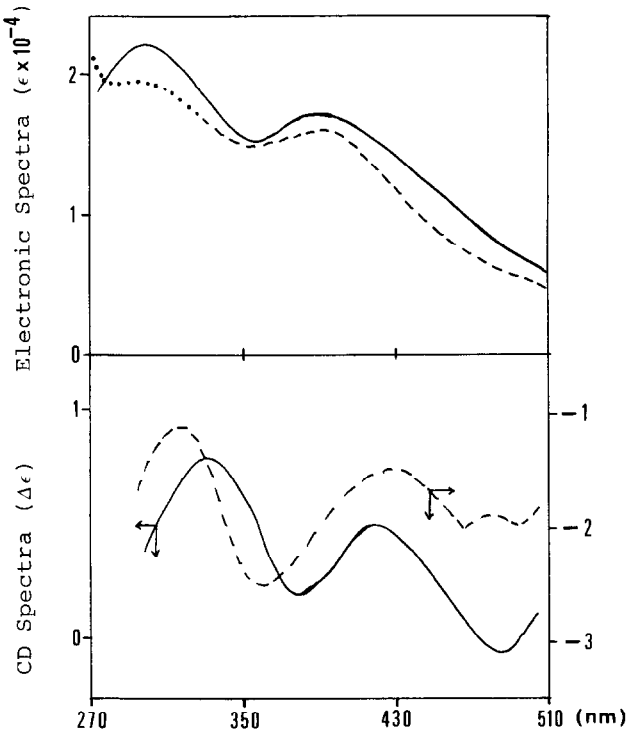
Table 1 Stabilities and Reduction Rates of Ox.-States of Fe-S Cluster Micelles

Fe-S Cluster	Ox.-State[a] $10^4 \cdot k_{dec.}(S^{-1})$	$10^4 \cdot k_{red.}(S^{-1})$[b]	E1/2[e] V vs NHE	Solubility[g] (M)
1	0.0014	2600	-0.88	8×10^{-4}
3	0.014	3200	-0.90	$> 7 \times 10^{-5}$
	26000[c]	not measured[c]		
2	0.025		-1.18	$> 7 \times 10^{-5}$
	$80 > k_{dec.} + k_{red.}$			
4	0.032	920	-0.86[f]	8×10^{-5}
	$0.8 > k_{red.} + k_{dec.}$[d]			

a) based on the characteristic absorption changes of Fe-S clusters at 400nm (1, 2, 3) and 460nm (4), 20mM CTAB, 100mM Tris-Cl, [Fe-S Cluster] ca. 0.1mM, pH=8.5 at 25° b) [$Na_2S_2O_4$] ca. 1mM, the rate of $10^4 k_{dec.}(S^{-1})$ of red.-states were over 9.0 (1) and 200 (3), respectively. c) 10% H_2O-90% DMF, 10mM Tris-Cl, pH=8.5 at 25° d) 10% Triton X-100, 10mM Tris-Cl, pH=8.5 at 25° e) in 0.1 M $n\text{-}Bu_4N \cdot ClO_4$-DMF at 20° f) reference 10) g) To the 2mL of 100mM Tris-Cl buffer solution (pH=8.5) of CTAB (20mM) was added the 100μL of DMF solution of Fe-S cluster (ca. 20mM) at 40°, followed by filtration by 0.45μm filter at 25°.

2.2×10^4) and 396 nm (ε, 1.7×10^4) at pH 8.5 and CD absorptions at 340, 384, 420, 468 and 530 nm. Both spectra are very similar to those of native ferredoxin from *Bacillus stearothermophilus*[8] (Fig 1). Another significant characteristic of the micellar solution of 1 was its stability against undesired hydrolytic decomposition[9]. As shown in Table 1, the decomposition rate of 1/CTAB is ca 1.4×10^{-7} sec^{-1} at pH 8.5, being ca 2×10^7 fold slower than the corresponding water soluble cluster 3 in 90 % aqueous DMF. Therefore, micellar 1 can be used without addition of excess thiolate anion, which is necessary for stabilization of a water soluble cluster like 3 (or 2)[9]. Such stability is also gained by CTAB micelle incorporation of a strongly hydrophobic cluster $(n\text{-}Bu_4N)_2[Fe_4S_4(S\text{-}C_6H_4\text{-}C_{12}H_{25})_4]$ 4[10]. However, solubility of 4 is ca 10 fold lower than that of 1 in CTAB micelles. Moreover, electron uptake rate of 4/CTAB from bulk aq $Na_2S_2O_4$ is ca 3 fold slower than 1/CTAB. Maximum overall electron influx from a bulk aqueous $Na_2S_2O_4$ to 1·micellar interior, which is given by solubility of an electron transport catalyst (1 - 4) multiplied by an electron uptake rate constant, is therefore 30 fold larger for 1·micelles than 4·micelles.

Fig. 1 Electronic and CD spectra



——, 1 (6·n-Bu_4N^+ Salt), CTAB 20 mM, Tris-Cl 100 mM, pH=8.5 at 25°
----, Fd. from *Bacillus stearothermophilus*[8)]

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