Syntheses of 2Fe-2S Ferredoxin Model Complexes of Cys-Containing Oligopeptides by Reaction with $Fe_2S_2^{2+}$ Ion or by Sulfide Incorporation

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(Received September 12, 1983)

The 2Fe-2S ferredoxin model complexes of two peptide ligands, Z-L-Ala-L-Cys-OMe (Z=benzyloxycarbonyl) and Z-L-Cys-L-Ala-L-Ala-L-Cys-OMe, were synthesized from $[NMe_4]_2[Fe_2S_2Cl_4]$ by addition of triethylamine. These were characterized by absorption, CD, and MCD spectra in dimethyl sulfoxide or *N,N*-dimethylformamide. Incorporation of inorganic sulfide to the iron(III) complex, $[Fe(Z\text{-cys-Ala-Ala-cys-OMe})_2]^-$ (rubredoxin models), results in the selective formation of a 2Fe-2S cluster by a controlling ability of the peptides.

The 2Fe-2S type of iron-sulfur proteins plays important roles in biochemical reactions, *e.g.* photosynthesis, and have been the subjects of many biological and physicochemical studies.[1] The structure of the active site in oxidized *Spirulina platensis* ferredoxin which is responsible for photosynthetic electron transfer in blue-green algae was confirmed by an X-ray analysis.[2] Three cysteinyl thiolates in one characteristic Cys-A-B-C-D-Cys-X-Y-Cys sequence bind a $[Fe_2S_2]^{2+}$ core.

Structural analogs of 2-Fe ferredoxins, such as $[Fe_2S_2(S_2\text{-}o\text{-xyl})_2]^{2-}$ (S_2-*o*-xyl=*o*-xylene-α,α'-dithiolate) were synthesized and the structure was confirmed crystallographically by Mayerle *et al.*[3] Such analogs have extremely low redox potentials, for example at −1.50 V (*vs.* SCE) in *N,N*-dimethylformamide (DMF) for $[Fe_2S_2(S_2\text{-}o\text{-xyl})_2]^{2-}$,[4] while spinach ferredoxin exhibits one at −0.42 V (*vs.* NHE).[5] The positive shift of the redox potentials of native 2Fe-2S ferredoxins is apparent. Probably the peptide sequence is responsible for this.

An important problem exists in the incorporation of S^{2-} into $[Fe(SR)_4]^-$ to give a 2Fe-2S type, since 2-Fe ferredoxins are formed from 1-Fe iron proteins in nature. Rydon *et al.* reported that the incorporation of S^{2-} and Fe^{3+} in dimethyl sulfoxide (DMSO) into denatured rubredoxin results in the formation of an Fe_4S_4 complex.[6] They also reported the formation of an Fe_4S_4 type complex from Ac-Gly_2-Cys-$(Gly_2\text{-Cys})_n$Gly-NH_2 (n=0—3), iron(III) chloride, and sodium sulfide in DMSO.[7] However, Sugiura *et al.* observed the formation of an Fe_2S_2 complex by the reaction among HS-$(CH_2)_n$SH, iron(III) chloride, and S^{2-}.[8] The difference in these studies is probably attributable to the structure of thiolate ligands; Cys-containing peptide or alkanedithiolate. The Gly-Gly peptide sequence used by Christou *et al.*[6] does not contribute to the bending of the peptide chains which facilitates chelation by the two cysteinyl thiolates. Therefore, we are interested in the synthesis of an Fe_2S_2 complex of Z-Cys-Ala-Ala-Cys-OMe by the reaction of $[Fe_2S_2Cl_4]^{2-}$ with the peptide. The Ala-Ala sequence, interposed between the two cysteinyl residues, promotes the formation of a hairpin turn conformation and enables to chelate to a tetrahedral iron(III) ion. The formation of the tetrapeptide chelate has already been confirmed for palladium(II) and iron(III) ions.[9] We also examined incorporation of S^{2-} into the oxidized rubredoxin analog of a Cys-X-Y-Cys peptide. Recently, Coucouvanis *et al.*[10] reported the synthesis of $[Fe_2S_2\{Ac\text{-}Gly_2\text{-}(cys\text{-}Gly_2)_2NH_2\}]^{2-}$ by the same procedure as mentioned above, but they have not examined the incorporation of S^{2-} into their complexes.

Experimental

All procedures were carried out under argon atmosphere.

Materials. All solvents were purified by distillation before use. The syntheses of Z-Ala-Cys-OMe and Z-Cys-Ala-Ala-Cys-OMe will be reported elsewhere.[11].

Syntheses of $[NMe_4]_2[Fe_2S_2(Z\text{-cys-Ala-Ala-cys-OMe})_2]$ and $[NMe_4]_2[Fe_2S_2(Z\text{-Ala-cys-OMe})_4]$. $[NMe_4]_2[Fe_2S_2Cl_4]$ was prepared by the reaction of $[NMe_4]_2[Fe_2S_2(S_2\text{-}o\text{-xyl})_2]$ (500 mg, 7.6×10^{-4} mol) with PhCOCl (0.46 cm^3, 4×10^{-3} mol) according to the procedure reported by Wong *et al.*[12] The 2Fe-2S complex of Z-Cys-Ala-Ala-Cys-OMe (**1**) was synthesized by the addition of triethylamine (2×10^{-3} cm^3, 1.43×10^{-5} mol) to a solution of $[NMe_4]_2[Fe_2S_2Cl_4]$ (1.7 mg, 3.7×10^{-6} mol) and Z-Cys-Ala-Ala-Cys-OMe (4.2 mg, 8.2×10^{-6} mol) in 2.0 cm^3 of *N,N*-dimethylformamide (DMF). The solution was concentrated *in vacuo* after 5 h. The crude product was washed with methanol and dried *in vacuo*.

$[NMe_4]_2[Fe_2S_2(Z\text{-Ala-cys-OMe})_4]$ (**2**) was synthesized by the reaction of Z-Ala-Cys-OMe (6.6 mg, 1.94×10^{-5} mol) with $[NMe_4]_2[Fe_2S_2Cl_4]$ (2.0 mg, 4.3×10^{-6} mol), followed by the addition of triethylamine (3×10^{-3} cm^3, 2.1×10^{-5} mol) in 2.0 cm^3 of DMF as mentioned above.

Incorporation of S^{2-} to Iron(III) Complex of the Cys-containing Peptides. A solution of $[Fe(Cys\text{-peptide})_4]^-$ was prepared by the same procedure reported previuosly.[13] Iron(III) chloride (3.7 mg, 2.3×10^{-5} mol) and Z-Ala-Cys-OMe (31.7 mg, 9.3×10^{-5} mol) or Z-Cys-Ala-Ala-Cys-OMe (24.2 mg, 4.7×10^{-5} mol) were dissolved in 2 cm^3 of DMSO. The addition of triethylamine (7×10^{-3} cm^3, 5×10^{-5} mol) to the solution gave a deep red-violet solution. Quick addition of Na_2S (1.8 mg, 2.3×10^{-5} mol) resulted in rapid development of a black color. The solution was characterized by absorption, CD, and EPR spectra. The values of ε for absorption and $\Delta\varepsilon$ for CD and MCD are based upon the molar concentration of $Fe_2S_2^{2+}$ ion.

Physical Measurement. Absorption spectra were measured on a JASCO UVIDEC-5A in visible region. CD and MCD spectra were recorded on a JASCO J-40 spectrometer equipped with electromagnets. The magnetic field was calibrated by using a $K_3[Fe(CN)_6]$ aqueous solution, $\Delta\varepsilon_M$ of 3.0 at 420 nm. EPR spectra were obtained at 77 K on a JEOL JESFE1X with 100 kHz magnetic field modulation. The *g*-value (g=1.981) was standardized by using Mn^{2+}. Cyclic voltammetries were measured with a Yanaco-P8-CV equipped with a function generator Yanaco Model FG-1218. Sample solutions were 10^{-3} mol dm^{-3} in DMF containing 0.05

mol dm^{-3} $[N(n\text{-}Bu)_4][ClO_4]$ as a supporting electrolyte. The voltammograms were recorded at 25°C *vs.* a saturated calomel electrode (SCE) as the reference.

Results and Discussion

Syntheses and Spectral Characterization of $[Fe_2S_2(Z\text{-}Ala\text{-}cys\text{-}OMe)_4]^{2-}$ *and* $[Fe_2S_2(Z\text{-}cys\text{-}Ala\text{-}Ala\text{-}cys\text{-}OMe)_2]^{2-}$. The Cys-containing peptide complexes possessing an Fe_2S_2 core were prepared quantitatively by the addition of triethylamine to a solution containing the corresponding peptides and $[Fe_2S_2Cl_4]^{2-}$. An attempt to isolate these complexes as crystals has been unsucessful due to its instability in air. Only a solid material was obtained.

Figure 1 shows the absorption and CD spectra of $[NMe_4]_2[Fe_2S_2(Z\text{-cys-Ala-Ala-cys-OMe})_2]$ (**1**) and $[NMe_4]_2[Fe_2S_2(Z\text{-Ala-cys-OMe})_4]$ (**2**), and the absorption spectra of $[NMe_4]_2[Fe_2S_2Cl_4]$. **1** exhibits three characteristic absorptions due to the core, $Fe_2S_2^{2+}$, at 325 nm (ε:11 100), 414 nm (ε:8 760), and 445 nm (ε:7 150) similar to those of oxidized native 2Fe-2S ferredoxin, while **2** shows only a broad absorption at 450—400 nm. Both complexes provide a strong absorption at 310 nm with similar intensity. Such three characteristic absorptions were reported for native 2Fe-2S ferredoxins and an alkanedithiolate complex. The 2Fe-2S complex of $Ac\text{-}Gly_2\text{-}(Cys\text{-}Gly_2)_2\text{-}NH_2$ was reported to exhibit absorption maxima at 333, 423, and 458 nm.[10] The oxidized native 2Fe-2S ferredoxin was reported to show three absorption maxima at 323—325 nm (ε:12 000—15 000), 423 nm (ε:9 700), and 466 nm (ε: 8 520) in visible region.[14] Absorption maxima at 294 nm (ε:14 500), 338 nm (ε:16 200), 414 nm (ε:17 000), and 590 nm (ε:4 800) with a shoulder at around 455 nm in DMF were observed in the oxidized 2Fe-2S model complex, $[Fe_2S_2(S_2\text{-}o\text{-}xyl)_2]^{2-}$.[3,4]

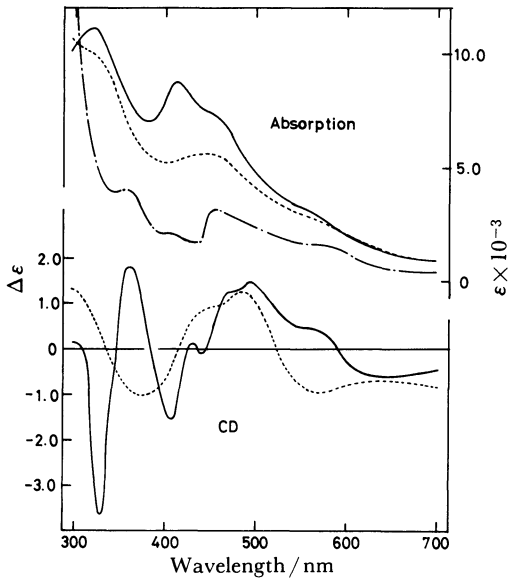

Fig. 1. Absorption and CD spectra of $[Fe_2S_2(Z\text{-Ala-cys-OMe})_4]^{2-}$ ----, $[Fe_2S_2(Z\text{-cys-Ala-Ala-cys-OMe})_2]^{2-}$ ——, $[Fe_2S_2Cl_4]^{2-}$ -·-·- in DMF.

CD spectra of **1** and **2** are quite different from that of the native 2Fe-2S ferredoxin as listed in Table 1. Well-defined troughs at 332, 408, and 660 nm and peaks at 363, 470, and 495 nm were observed in the DMF solution of **1**. The low values of Δε observed for **1** and **2** are distinct from the observed high extrema of native 2Fe-2S ferredoxins with the characteristic peptide sequence. The higher Δε values of **1** at 363 nm (Δε:+1.94) and 332 nm (Δε:−3.61) than those of **2** indicate that chelation by the tetrapeptide leads to the fixation of the peptide conformation where chiral effect of cysteine residues is not averaged.

As listed in Table 2, the MCD spectrum of **1** indicates typical peaks at 486 nm ($\Delta\varepsilon_M$:+0.58) and 526 nm ($\Delta\varepsilon_M$:+0.62) and a broad shoulder at 560 nm ($\Delta\varepsilon_M$:+0.54) of an oxidized 2Fe-2S core reported by Stephens *et al.* for 2Fe-2S ferredoxins of *Spirulina maxima* and putidaredoxin.[14]

Positive MCD transitions at 480 nm ($\Delta\varepsilon_M$:+0.76), and 566 nm ($\Delta\varepsilon_M$:+0.21) in **1** are also observed as well as the oxidized native 2Fe-2S ferredoxin. Unfortunately, assignments of the MCD transitions also in **1** or oxidized native 2Fe-2S ferredoxin were not possible because of the complexity of the energy levels of the Fe_2S_2 core. However, by comparing the native 2Fe-2S ferredoxins with 1Fe or 4Fe-4S ferredoxins, Stephens *et al.* reported that the native 2Fe-2S ferredoxins exhibit a characteristic shape with a positive MCD transition at 486 nm ($\Delta\varepsilon_M$:+0.58).[14] Generally, the MCD spectra of 4Fe-4S ferredoxins have the strongest peak ($\Delta\varepsilon_M$: +1.0—+1.8) at 380—390 nm. However, in the case of the model complex **1**, the MCD peak at 380—385 nm was weak ($\Delta\varepsilon_M$:+0.06) and the strongest MCD peak was observed at 480 nm ($\Delta\varepsilon_M$:+0.76) indicating presence of a 2Fe-2S core.

Table 1. CD spectra of $[Fe_2S_2(Z\text{-cys-Ala-Ala-cys-OMe})_2]^{2-}$, $[Fe_2S_2(Z\text{-Ala-cys-OMe})_4]^{2-}$, and related systems in DMF

Complex	CD extrema λ/nm(Δε)			
$[Fe_2S_2(Z\text{-cys-Ala-Ala-cys-OMe})_2]^{2-}$ (**1**)	660(−0.43)	495(+1.50)	470(+1.23)	448(−0.06)
	433(+0.20)	408(−1.57)	363(+1.94)	332(−3.61)
Fe^{III}/Z-Cys-Ala-Ala-Cys-OMe/Na_2S (1:2:1)[a]	622(−0.63)	563(+0.04)	547(−0.10)	477(+1.39)
	450(+0.62)	444(+0.64)	401(−2.19)	366(+1.14)
	331(−2.71)	303(−0.77)		
$[Fe_2S_2(Z\text{-Ala-cys-OMe})_4]^{2-}$ (**2**)	570(−0.98)	488(+1.30)	450sh(+0.83)	380(−1.03)
Fe^{III}/Z-Ala-Cys-OMe/Na_2S (1:4:1)[a]	526(−0.16)	456(+0.10)	376(−0.35)	318(+0.19)
Native 2Fe-2S ferredoxin[b]	619(−0.05)	602(+0.05)	553(−5.71)	510sh(−2.93)
(*S. maxima*)	429(+19.79)	381(−5.57)	359(+1.93)	344sh(−0.93)

a) In Me_2SO. b) In H_2O.

TABLE 2. CD SPECTRA OF $[Fe_2S_2(Z\text{-cys-Ala-Ala-cys-OMe})_2]^{2-}$, AND RELATED SYSTEMS

Complex	MCD extrema λ/nm(Δε$_M$)			
$[Fe_2S_2(Z\text{-cys-Ala-Ala-cys-OMe})_2]^{2-}$ (**1**)	664(−0.44)	566sh(+0.21)	480(+0.76)	430(−0.18)
	376(+0.32)			
Fe^{III}/Z-Cys-Ala-Ala-Cys-OMe/Na_2S (1:2:1)[a]	685(+0.06)	664(+0.07)	638(+0.05)	561(+0.14)
	544(+0.13)	525sh(+0.16)	508sh(+0.19)	482(+0.23)
	419(+0.03)	400(+0.08)	383sh(+0.07)	362(+0.06)
	332(+0.27)			
Native 2Fe-2S ferredoxin[b]	560sh(+0.54)	526(+0.62)	486(+0.58)	444(−0.08)
(*S.maxima*)	424(+0.20)	377(−0.54)	360(−0.30)	352(−0.31)
	328(+0.23)			

a) In Me_2SO. b) In H_2O.

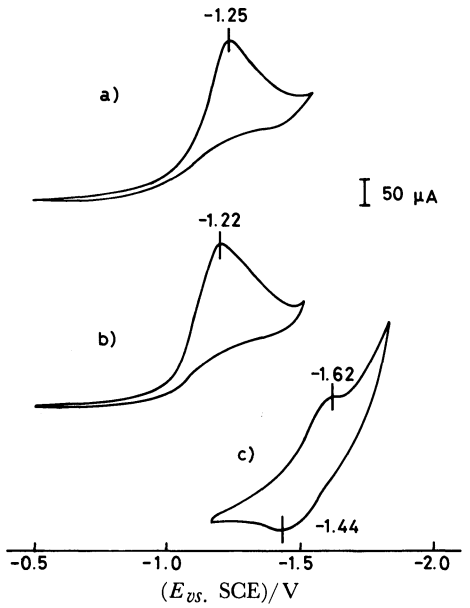

Fig. 2. Cyclic voltammograms of a) $[Fe_2S_2(Z\text{-Ala-cys-OMe})_4]^{2-}$, b) $[Fe_2S_2(Z\text{-cys-Ala-Ala-cys-OMe})_2]^{2-}$, and c) $[Fe_2S_2(S_2\text{-}o\text{-xyl})_2]^{2-}$ in DMF.

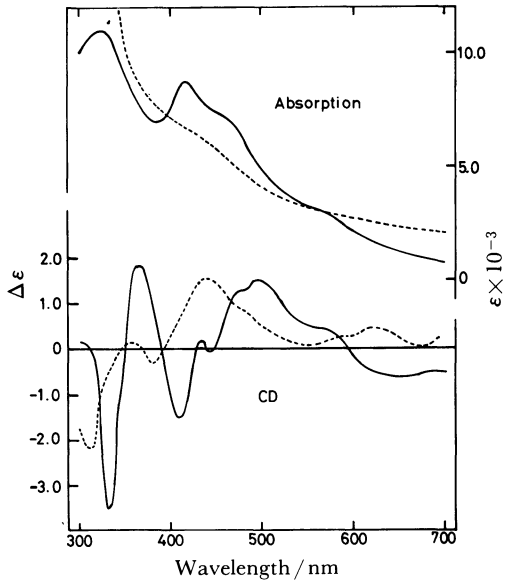

Fig. 3. Absorption and CD spectra of $[Fe_2S_2(Z\text{-cys-Ala-Ala-cys-OMe})_2]^{2-}$ —— and its reduced species with 10 equiv of dithionite complex of 18-crown-6 ---- in DMF.

Electrochemical and Chemical Reduction of $[Fe_2S_2(Z\text{-cys-Ala-Ala-cys-OMe})_2]^{2-}$. Importance of the peptide sequence in the stability of redox couple (2-/3-) was investigated by cyclic voltammetry. Figure 2 shows the cyclic voltammograms of **1** and **2** in DMF at room temperature. Although a reduction peak was observed at −1.22 V(*vs.* SCE) for **1** and −1.25 V(*vs.* SCE) for **2**, no oxidation peak has been recognized. At a low temperature (−12°C), an oxidation peak for **1** was observed ($E_{p,a}$:−1.23 V, $E_{p,c}$:−0.83 V, $i_{p,c}/i_{p,a}$=1/20), while it was undetectable for **2**. The results indicate that both synthetic complexes are readily decomposed by electrochemical reduction and suggest that the chelating peptide such as Z-Cys-Ala-Ala-Cys-OMe has a potential ability to maintain the $[Fe_2S_2]^+$ ion during the redox couple. A stable redox couple of $[Fe_2S_2(S_2\text{-}o\text{-xyl})_2]^{2-}$ in acetonitrile/hexamethylphosphoric triamide(HMPA) (7:3 v/v) was found by Mascharak *et al.*[4] Actually, at the same concentration (10^{-3} mol dm^{-3}) in DMF, a quasi-reversible redox couple ($i_{p,c}/i_{p,a}\simeq 1$) of $[Fe_2S_2(S_2\text{-}o\text{-xyl})_2]^{2-}$ was detected at −1.62 V($E_{p,a}$) and −1.44 V($E_{p,c}$) in fair agreement with their values in the different solvents. **1** or **2** exhibited a positive shift (ΔV=0.37—0.40 V) of the reduction peak in the cyclic voltammogram as compared with the reduction peak of $[Fe_2S_2(S_2\text{-}o\text{-xyl})_2]^{2-}$. Therefore, the reduction of **1** and **2** with $Na_2S_2O_4$ was examined. The reduction of a model complex, $[Fe_2S_2(S_2\text{-}o\text{-xyl})_2]^{2-}$, has been known to require sodium acenaphthylenide in HMPA.[4] Figure 3 shows the visible spectrum of **1** reduced by $Na_2S_2O_4$ complexed with 18-crown-6 in DMF.[15] The spectrum indicates the formation of a $[Fe_4S_4]^{2+}$ core possessing a characteristic absorption maximum at 420 nm (ε:6 600) in DMF. Also the CD spectrum of the reduced species of **1** exhibits a different pattern from that of **1**. The CD maximum at 440 nm (Δε:+1.57) corresponds to that of $[Fe_4S_4(Z\text{-cys-Gly-Ala-cys-OMe})_2]^{2-}$.[16] The reduction of **2** by $Na_2S_2O_4$ provided the same type of a product having $[Fe_4S_4]^{2+}$ that was detected by an absorption maximum at 420 nm (ε:4 820). The formation of $[Fe_4S_4X_4]^{2-}$ (X=SR, Cl) by the reduction of $[Fe_2S_2X_4]^{2-}$ was reported by Cambray *et al.*[17] and Wong *et al.*,[12] respectively, except for $[Fe_2S_2(S_2\text{-}o\text{-xyl})_2]^{2-}$. Thus, binuclear-to-tetranuclear conversions have been found to take place by chemical means, which are reflected by the irreversibility of the cyclic voltammograms of **1** and **2** at room temperature. The *o*-xylene-α,α′-dithiolate ligand prevents the $[Fe_2S_2]^+$ core from the reductive conversion to the $[Fe_4S_4]^{2+}$ core. The results suggest that chelation of the

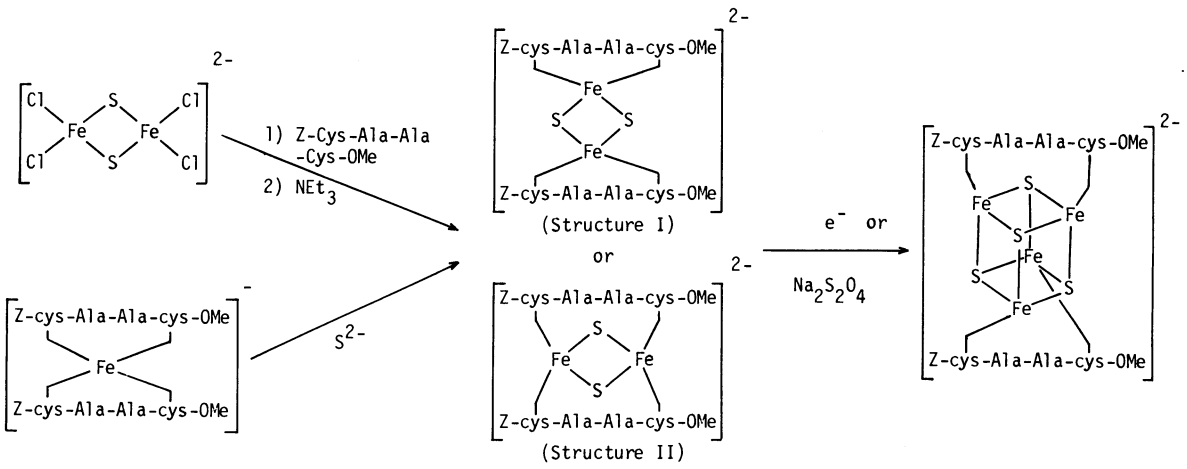

Fig. 4. Syntheses and reactions of the 2Fe-2S complexes of Z-Cys-Ala-Ala-Cys-OMe.

tetrapeptide is involved not for one iron(III) (structure I in Fig. 4) but for two iron(III) ions in a FeS_2Fe system (structure II) and that the alkanedithiolate ligand is more suitable for the chelation to one iron(III) ion. The coordination of Z-Cys-Ala-Ala-Cys-OMe in the structure II corresponds to that of the native Cys-X-Y-Cys sequence as reported with the X-ray analysis.[2] It is plausible that the complex having the structure II easily associates to the 4Fe-4S type by reduction.

Fe^{III}/Cys-containing peptides/S^{2-} Complexes. The formation of iron-sulfur clusters by incorporation of inorganic sulfide(S^{2-}) into Fe^{III}/Cys-containing peptide complexes was studied. On the basis of the investigation on iron-sulfur protein models, Sugiura *et al.*[8] and Cambray *et al.*[17] have found the selective formation of 2Fe-2S complexes from iron(III) complexes of simple thiolate ligands by incorporation of inorganic sulfide. On the other hand, Christou *et al.* reported the formation of a 4Fe-4S complex by the incorporation of inorganic sulfide into iron(III) complexes of Ac-Gly$_2$-(cys-Gly$_2$)$_4$-NH$_2$.[7] Thus, it is interesting to determine which type of complexes is formed by the incorporation of inorganic sulfide into the present Fe^{III}/peptide complexes. The peptide ligand probably controls the incorporation and the stability of the products.

The addition of an equimolar amount of Na_2S to a solution of Fe^{III}/Cys-containing peptide(1:4) complexes results in the rapid development of black color. We have already established that Fe^{III}/Cys-containing peptide(1:4) complexes provide a $Fe(S\text{-}Cys)_4$ core as a model of rubredoxin, especially in the case of macro-ring chelation by Z-Cys-Ala-Ala-Cys-OMe.[18] Tables 1 and 2 list the CD and MCD spectra of the products of the sulfide incorporation, *i.e.* Fe^{III}/Cys-containing peptide/S^{2-} complexes. The absorption spectrum of each of these complexes had maxima at 300 and 415 nm and a weak shoulder around 450 nm, similar to those of **1**. On the other hand, 4Fe-4S ferredoxin model complexes, $[Fe_4S_4(SR)_4]^{2-}$, show two absorption maxima around 300 and 410 nm in DMF or in DMSO/H_2O (8:2 v/v).[19] Sugiura *et al.* reported that the product formed between Fe^{III}/alkanethiolate complexes with inorganic sulfide has absorption maxima at 330, 410, and 450 nm and there is a considerable similarity in spectra between their complexes and the 2Fe-2S ferredoxins.[8] They revealed also that reconstituted adrenodoxins exhibit an absorption maximum at 410 nm with a weak shoulder at 450 nm which is due to a 2Fe-2S cluster.[20] From the comparison of these data with our results, a major component of an iron-sulfur cluster involved in the system, Fe^{III}/peptide complexes/S^{2-}, is suggested to be a 2Fe-2S cluster. The CD (Table 1) and MCD (Table 2) spectra of the Fe^{III}/Cys-containing/S^{2-} system were more highly structured than the corresponding absorption spectra(Table 1) and these are useful for identification of the complexes. In the case of the Fe^{III}/Z-Cys-Ala-Ala-Cys-OMe/S^{2-} system, CD troughs at 331 nm ($\Delta\varepsilon$:−2.71) and 401 nm ($\Delta\varepsilon$:−2.19), and peaks at 366 nm ($\Delta\varepsilon$:+1.14) and 477 nm ($\Delta\varepsilon$:+1.39) were observed, which correspond to those of **1** (Table 1), though the positions of absorption maxima are somewhat ambiguous. In order to compare with the above CD spectrum, $[Fe_4S_4(Z\text{-cys-Ala-Ala-cys-OMe})_2]^{2-}$ was synthesized by a ligand exchange reaction of $[(n\text{-}Bu)_4N]_2[Fe_4S_4(S\text{-}i\text{-}Pr)_4]$ with Z-Cys-Ala-Ala-Cys-OMe in solution.[16] The 4Fe-4S complex in DMF provides two CD troughs at 330 nm ($\Delta\varepsilon$:−0.8) and 400 nm ($\Delta\varepsilon$:−0.7), and two peaks at 360 nm ($\Delta\varepsilon$:+0.2) and 430 nm ($\Delta\varepsilon$:+0.9). The CD spectra of the Fe^{III}/Z-Cys-Ala-Ala-Cys-OMe/Na_2S (1:2:1) system indicate the preferential formation of an 2Fe-2S cluster although being not consistent completely with the CD extrema of **1**. The inconsistency between their CD extrema seems to be caused by the contamination of the 4Fe-4S cluster. The CD spectrum of the Fe^{III}/Z-Ala-Cys-OMe/S^{2-} complex was completely different from that of $[NMe_4]_2[Fe_2S_2(Z\text{-Ala-cys-OMe})_4]$ (Table 1) The low $\Delta\varepsilon$ values suggest that the Fe^{III}/Z-Ala-Cys-OMe/S^{2-} system results in the preferential formation of the 4Fe-4S cluster.

Recently the MCD spectra of ferredoxins of various 2Fe-2S and 4Fe-4S types have been reported in detailed.[11] Those data indicated that the 4Fe-4S ferredoxin in both $Fe_4S_4^{2+}$ and $Fe_4S_4^{+}$ states exhibit only positive MCD bands in all the region from 300 to 2000

nm, whereas the 2Fe–2S ferredoxins show positive and negative bands. The MCD spectra were used to establish whether the structure of the iron-sulfur centers in the peptide complexes is like 2Fe–2S or 4Fe–4S type. The tetrapeptide complex, Fe^{III}/Z-Cys-Ala-Ala-Cys-OMe/S^{2-} (1:2:1), exhibited the strongest peak at 482 nm ($\Delta\varepsilon_M$:+0.23) due to the 2Fe–2S cluster (Table 2). Therefore, the absorption, CD, and MCD results indicate that a 2Fe–2S cluster forms predominantly in the Fe^{III}/tetrapeptide/S^{2-} system and small amounts of other types of clusters are contaminated.

The 2Fe–2S cluster in these peptide complexes was also established by the analysis of the EPR spectra of the Fe^{III}/Cys-containing peptide/S^{2-} system. The EPR signals at g=4.18 of the peptide complexes disappeared completely on the addition of S^{2-} and new signals appeared at g=2.04, 2.00, and 1.98; g_{av}=2.01 for the tetrapeptide complex and at g_{av}=2.06 for the dipeptide complex. Laskowski *et al.* reported that a 4Fe–4S ferredoxin analog, $[Fe_4S_4(SCH_2Ph)_4]^{3-}$, exhibits two EPR signals at g=2.04 and 1.93 in acetonitrile below 15 K.[21] The EPR data of the reduced 2Fe–2S ferredoxin analog are not available at present. From these data and the fact that the EPR signals of the peptide complexes with inorganic sulfide are measurable at 77 K, it is apparent that the tetranuclear cluster are not preferentially formed at least by the simple addition of inorganic sulfide to Fe^{III}/Cys-containing peptide(1:4) complexes.

Thus, a major part of iron-sulfur clusters in the Fe^{III}/Cys-containing peptide/S^{2-} system is the 2Fe–2S type, which is different from the previous study of Rydon *et al.* on iron(III) complexes of sequential oligopeptide, Ac-Gly_2-(Cys-Gly_2)$_n$-NH_2.[7] Probably their peptides, having more than two Cys residues and Gly-Gly residues interposed between the two Cys residues, do not have any controlling power for the selective formation of a 2Fe–2S type ferredoxin core.

We thank Professor Yukio Sugiura for useful discussion on the characterization of 2Fe–2S complexes. The present work was supported by a Grant-in-Aid (No. 56430014) from the Ministry of Education, Science and Culture.

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