Mo(IV) Complexes of Cysteine-Containing Peptides and their Interaction with 4Fe Ferredoxin Model Complexes

NORIKAZU UEYAMA, MICHIO NAKATA, AKIRA NAKAMURA*, MASATO KAMATA and SEI OTSUKA

Department of Macromolecular Science, Faculty of Science, Osaka University, Toyonaka, Osaka 560 and Department of Chemistry, Faculty of Engineering Science, Osaka University, Toyonaka, Osaka 560, Japan

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$Mo(Ac\text{-}cys\text{-}OH)_4$, $Mo(S\text{-}tBu)_2(Z\text{-}Ala\text{-}cys\text{-}OMe)_2$ (Z = carbobenzoxy), and $Mo(Z\text{-}cys\text{-}Ala\text{-}Ala\text{-}cys\text{-}OMe)_2$ were prepared from $Mo(S\text{-}tBu)_4$ and the corresponding peptides using a ligand exchange method. The Mo(IV) complexes were characterized by ^1H NMR, visible, and CD spectra. Reaction of these complexes with 4Fe ferredoxin model complexes, $[Fe_4S_4(SR)_4]^{2-}$, was examined by CD spectroscopy to reveal ligand exchange between these complexes. Formation of a Mo–Fe mixed cluster was implied by the CD results.

Introduction

The molybdenum site of nitrogenase has been inferred by EXAFS to involve four to five sulfur atoms and two or three iron atoms at close proximity [1]. Spectroscopic as well as catalytic properties of model compounds containing low-valent Mo with thiolato and sulfido ligands are thus of interest. Recently a variety of such Mo complexes was prepared and characterized. Among these, a highly reactive tetrathiolato Mo(IV) complex, $Mo(S\text{-}t\text{-}Bu)_4$, with a distorted T_d structure was synthesized to provide an important starting material for the preparation of a variety of new thiolato complexes of Mo (I, II, and IV) [2, 3]. Mo(IV) complexes of cysteine and cysteine-containing peptides are thus of interest as better models of the Mo site of reduced xanthine oxidase or the Mo-cofactor leading to the FeMo-cofactor of nitrogenase.

Utilizing facile ligand exchange reactions of $Mo(S\text{-}t\text{-}Bu)_4$ we have prepared Mo(IV) complexes of Ac-CysOH, Z-Ala-Cys-OMe (Z = carbobenzoxy) and Z-Cys-Ala-Ala-Cys-OMe where coordination of the free amino group at the N-terminal is blocked to enforce thiolato coordination to Mo(IV). The particular tetrapeptide, Z-Cys-Ala-Ala-Cys-OMe, was chosen since it was previously found to form stable macroring chelates with Pd(II) [4] and Fe(III) [5]. The interaction of the peptide complexes with 4-Fe ferredoxin model compounds was examined by CD spectroscopy in order to gain information on the MoFe site of nitrogenase from the synthetic side.

Experimental

Materials

All solvents were dried by appropriate methods under argon. Acetyl cysteine (Ac-Cys-OH) was a gift from Sun Orient Chemical Co. The syntheses of Z-Ala-Cys-OMe and Z-Cys-Ala-Ala-Cys-OMe will be described elsewhere. $Mo(S\text{-}tBu)_4$ was prepared using the method of Otsuka *et al.* [2]. $[nBu_4N]_2[Fe_4S_4(S\text{-}iPr)_4]$ was prepared according to the method of Holm *et al.* [6].

General Procedures

All reactions and spectral measurements were carried out under argon since Mo(IV) thiolate complexes are all air-sensitive.

Reaction of $Mo(S\text{-}tBu)_4$ with Ac-Cys-OH

To a solution of $Mo(S\text{-}tBu)_4$ (11.5 mg, 0.025 mmol) in 1 ml of methanol, Ac-Cys-OH (15 mg, 0.100 mmol) was added at 40 °C. The solvent and the released t-butylthiol were removed under reduced pressure after 5 min. The residue was washed with 2 ml of acetonitrile. The brown air-sensitive powder obtained was dried under reduced pressure and was characterized by CD and ^1H NMR spectra.

Reaction of $Mo(S\text{-}tBu)_4$ with Z-Ala-Cys-OMe

Z-Ala-Cys-OMe (75 mg, 0.22 mmol) was added to a solution of $Mo(S\text{-}tBu)_4$ (25 mg, 0.055 mmol) in 2 ml of dimethylsulfoxide. The solvent and the released t-butylthiol were removed under reduced pressure to

*Author to whom correspondence should be addressed.

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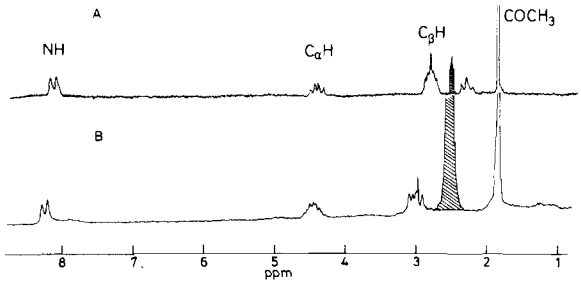

Fig. 1. ^{1}H NMR spectra of A) N-acetyl-L-cysteine and B) Mo(Ac-cys-OH)$_4$ in DMSO-d$_6$ at 31 °C.

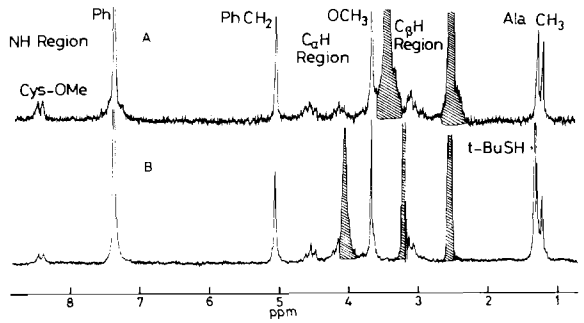

Fig. 2. ^{1}H NMR spectra of A) Z-Ala-Cys-OMe and B) Mo(S-tBu)$_2$(Z-Ala-cys-OMe)$_2$ in DMSO-d$_6$ at 31 °C.

give a brown powder which was examined by ^{1}H NMR and CD spectra.

Reaction of Mo(S-tBu)$_4$ with Z-Cys-Ala-Ala-Cys-OMe

The reaction was carried out as mentioned above for the reaction of Mo(S-tBu)$_4$ with Z-Ala-Cys-OMe.

Reaction of [Fe$_4$S$_4$(S-iPr)$_4$]$^{2-}$ with the Cys-Containing Peptides

The ligand-substitution reactions were carried out according to the method of Holm *et al.* [7]. [Fe$_4$S$_4$-(S-iPr)$_2$(Z-Ala-cys-OMe)$_2$]$^{2-}$ was prepared by adding two equivalents of the peptide to a solution (0.09 *M*) of [n-Bu$_4$N]$_2$[Fe$_4$S$_4$(S-iPr)$_4$] in DMSO. The i-propylthiol formed was removed under reduced pressure. [Fe$_4$S$_4$(S-iPr)$_2$(Z-cys-Ala-Ala-cys-OMe)]$^{2-}$ was prepared by adding a stoichiometric amount of ferredoxin model compound to the tetrapeptide. The reactions of Mo(IV) complexes of Cys-containing peptides with a variety of ferredoxin model compounds were performed by addition of an equimolar amount of [Fe$_4$S$_4$(SR)$_4$]$^{2-}$ (SR = S-iPr, Ac-cys-OH, Z-Ala-cys-OMe) to solutions of Mo(IV)/AcCys-OH, Z-Ala-Cys-OMe, or Z-Cys-Ala-Ala-Cys-OMe systems in DMSO. These reactions were monitored by CD-spectral changes.

Physical Measurements

Proton NMR spectra were recorded at 100 MHz on a Varian XL-100 spectrometer. Absorption spectra were measured on a JASCO UVIDEC-5A. CD spectra were obtained on a JASCO J-40 spectrometer.

Results and Discussion

Ligand Exchange Reaction of Mo(S-tBu)$_4$ with Cys-Containing Peptide

Figure 1 shows the ^{1}H NMR evidence for formation of Mo(Ac-Cys-OH)$_4$ by the ligand exchange reaction. No detectable peak for the S-tBu group at 1.30

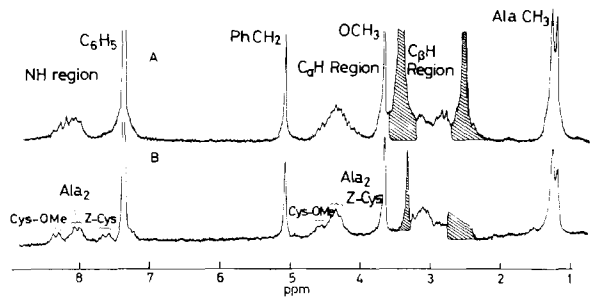

Fig. 3. ^{1}H NMR spectra of A) Z-Cys-Ala-Ala-Cys-OMe and B) Mo(Z-cys-Ala-Ala-cys-OMe)$_2$ in DMSO-d$_6$ at 31 °C.

ppm was observed when the exchange was complete. Disappearance of the triplet peaks due to the SH group of Ac-Cys-OH at 2.5 ppm (Fig. 1-A) also indicates the formation of Mo(Ac-cys-OH)$_4$ with four Mo–S bonds.

If the carbonyl group of the Mo(IV)/Ac-Cys-OH complex were involved in the coordination, the complex would have a higher coordination number than 4 by 6-membered chelate formation. However, the NH–CH coupling constant of the Mo(IV) complex which reflects the dihedral angles of an NH–CH bond [8] was 8.0 Hz (in DMF), identical with the value (8.0 Hz in DMF) for free Ac-Cys-OH. The NH–CH coupling constant of [Mo$_2$O$_2$(μ-S)$_2$(Ac-cys-O$^-$)$_2$]$^{2-}$ which has an S,O-chelation, was 8.2 Hz in H$_2$O, different from the value (7.8 Hz in H$_2$O) for free Ac-Cys-OH. This is also supported by the same value of the NH–CH coupling constant of HgCl-(Ac-cys-OH) in DMSO-d$_6$. Therefore, the carbonyl groups of the Ac-Cys-OH moieties in Mo(Ac-cys-OH)$_4$ do not coordinate to the Mo(IV) ion.

The Mo(IV) Complex of Z-Ala-Cys-OMe

^{1}H NMR spectra of the Mo(IV)/Z-Ala-Cys-OMe complex are shown in Fig. 2. Although four equivalents of the peptide thiolates were added to Mo(S-tBu)$_4$, two t-butylthiolato groups still remain. The

TABLE I. Chemical Shifts and Coupling Constants of the NH Peaks of Z-Cys-Ala-Ala-Cys-OMe Metal Complexes in DMSO-d_6.

Compounds	Chemical shifts, ppm ($J_{N\alpha}$ Hz)			
	Z-Cys NH	Ala_2 NH		Cys-OMe NH
The free peptide	a broad peak around 8.1			
Pd(II)[a]	7.05 (8.0)	8.65 (6.2)	8.56 (7.5)	7.56 (5.5)
Mo(IV)[b]	7.6 (8.5)	8.0 (8.7)		8.3 (7.6)

[a]Reference 4. At 220 MHz. [b]At 100 MHz.

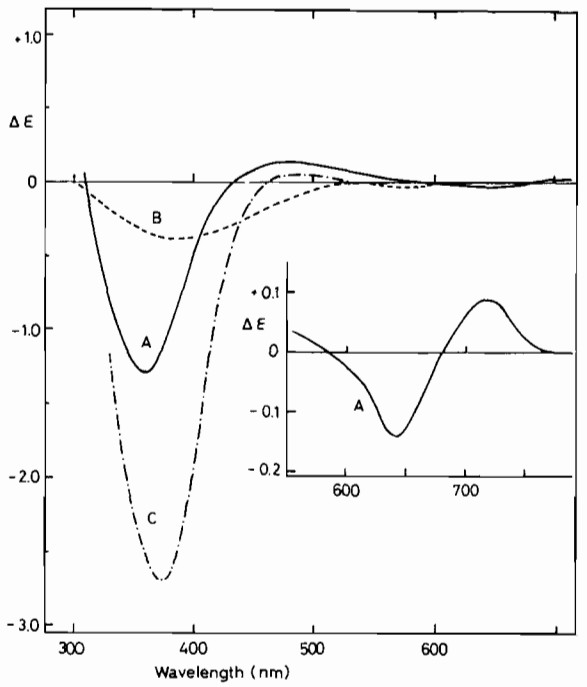

Fig. 4. CD spectra of A) $Mo(Ac\text{-}cys\text{-}OH)_4$, B) (A) + $[Fe_4S_4(S\text{-}i\text{-}Pr)_4][n\text{-}Bu_4N]_2$ (1:1), and C) (A) + $[Fe_4S_4(Ac\text{-}cys\text{-}OH)_4]^{2-}$ (1:1) in DMSO at 25 °C. The inset shows the CD spectrum of A enlarged in the 550–750 region.

NMR results indicate the formation of $Mo(S\text{-}tBu)_2(Z\text{-}Ala\text{-}cys\text{-}OMe)_2$. Bulkiness of the peptide restricts the exchange for only two thiolate ligands. Neither of the NH protons of the Ala and Cys-OMe residues were deprotonated, since two doublet peaks due to two amide groups were observed at 7.36 ppm and 8.43 ppm, overlapped with the peak of the phenyl group of the carbobenzoxy protecting group. The latter peaks was clearly observable at upfield positions when some $CDCl_3$ was added as a diluent.

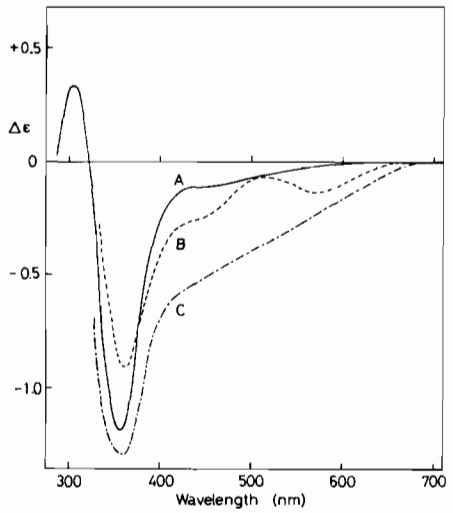

Fig. 5. CD spectra of A) $Mo(S\text{-}t\text{-}Bu)_2(Z\text{-}Ala\text{-}cys\text{-}OMe)_2$, B) (A) + $[Fe_4S_4(S\text{-}i\text{-}Pr)_4][n\text{-}Bu_4N]_2$ (1:1), and C) (A) + $[Fe_4S_4(Z\text{-}Ala\text{-}cys\text{-}OMe)_4]^{2-}$ in DMSO at 25 °C.

The Mo(IV) Complex of Z-Cys-Ala-Ala-Cys-OMe

This tetrapeptide coordinates with two thiolates to the Pd(II) ion forming a macrocyclic complex [4]. Figure 3 shows the ^1H NMR spectra of the tetrapeptide and its Mo(IV) complex. Complete exchange of four t-butylthiolate ions with two equivalents of the peptide was observed. The results suggest that the tetrapeptide is more sterically compact than two molecules of Z-Ala-Cys-OMe. Table I lists the chemical shifts and the coupling constants of the NH peaks of Z-Cys-Ala-Ala-Cys-OMe and the metal complexes therefrom. The tetrapeptide coordinates to a square planar Pd(II) with a *cis*-chelation [4]. The NMR peaks due to the NH groups of the Mo(IV) peptide complex were observed in a relatively wide region, compared with those of the free peptide. The observed wide peaks arise from the magnetic anisotropy of the amide planes which is influenced by loose peptide conformations. The observed NMR data seem to indicate chelation of the peptide to the Mo(IV) ion. The chelation of the tetrapeptide to a tetrahedral Mo(IV) allows a somewhat loose conformation, while that for the square planar Pd(II) shows a tightly restricted one. The data at hand do not completely establish the chelation by the tetrapeptide to Mo(IV) in the present case. Further work is required.

CD Spectroscopic Investigations

The CD spectrum (see Fig. 4) of $Mo(Ac\text{-}cys\text{-}OH)_4$ showed negative extrema at 360 and 640 nm and positive ones at 470 and 710 nm. Another positive extremum also exists at the shorter wavelength side of 300 nm. A similar CD pattern was observed for $Mo(S\text{-}t\text{-}Bu)_2(Z\text{-}Ala\text{-}cys\text{-}OMe)_2$ which showed a posi-

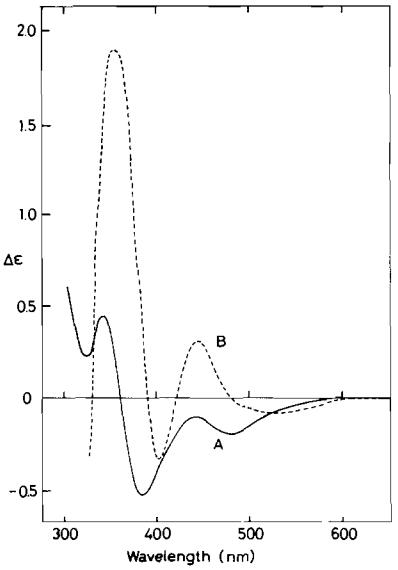

Fig. 6. CD spectra of A) $Mo(Z\text{-}cys\text{-}Ala\text{-}Ala\text{-}cys\text{-}OMe)_2$, and B) (A) + $[Fe_4S_4(S\text{-}i\text{-}Pr)_4]\,[n\text{-}Bu_4N]_2$ (1:1) in DMSO at 25 °C.

tive one at 306 nm and a negative one at 380 nm. The strong CD peaks at 300–380 nm may be due to ligand-to-metal charge transfer absorption. Most of the metal complexes having Z-cysteine thiolato ligands showed a strong negative peak at their ligand-to-metal charge transfer bands. Therefore the feature is taken as evidence for L-cysteine thiolato coordination. The weak CD extrema at 600–750 nm (Fig. 4, inset) seem to correspond to the visible band observed for $Mo(S\text{-}t\text{-}Bu)_4$. The diamagnetism and the chiroptical data suggest a distorted tetrahedral structure at the metal just as found for the parent $Mo(S\text{-}t\text{-}Bu)_4$. The bulkiness of cys-containing peptides evaluated by construction of CPK models supports the above structure.

The relative orientation of asymmetric carbons and peptide linkages seems to influence the CD pattern of d–d transition when the patterns at 400–700 nm (see Figs. 4 and 5) are compared. In the case of Fe(III) complexes of the tetrapeptide, we have observed remarkable similarity to native 1-Fe ferredoxin, oxidized rubredoxin, even though the peptide sequence is different [5]. This is considered to be due to the similarity of peptide conformations around the coordinating cysteinates.

Similarity in the CD patterns (Figs. 4 and 5) of the Mo(IV) peptide complexes results from conformational preference of the peptide ligands. However, the CD pattern of $Mo(Z\text{-}cys\text{-}Ala\text{-}Ala\text{-}cys\text{-}OMe)_2$ is quite different, as seen in Fig. 6. This is interpreted to be due to chelation of the tetrapeptide in contrast to the monodentate character of Ac-Cys-OH and Z-Ala-Cys-OMe.

Reaction of Mo(IV) Cysteine-Containing Peptides with 4-Fe Ferredoxin(fd) Model Complexes

Since Mo(IV) tetrathiolato complexes are labile, their reaction with 4-Fe fd models has been investigated to obtain novel MoFe mixed metal clusters. Owing to the multitude of strong visible absorption bands of these thiolato complexes, examination of the reaction by visible spectroscopy gave no definite chemical conclusion as to the structure. Thermal lability and air instability prevented purification through chromatography or crystallization.

We have taken advantage of solution CD spectra for investigation of the interaction of Mo(IV)–cysteine-containing peptide thiolato complexes with 4-Fe fd model complexes. Figure 4 shows CD spectral changes upon mixing $Mo(Ac\text{-}cys\text{-}OH)_4$ with $[Fe_4S_4(S\text{-}i\text{-}Pr)_4]^{2-}$ or $[Fe_4S_4(Ac\text{-}cys\text{-}OH)_4]^{2-}$. The peak positions and the CD intensity changed after mixing with $[Fe_4S_4(S\text{-}i\text{-}Pr)_4]^{2-}$ to suggest the formation of new chromophores. The reduced CD intensity was ascribed to the thiolato ligand exchange between Mo and Fe. To eliminate this exchange, $[Fe_4S_4(Ac\text{-}cys\text{-}OH)_4]^{2-}$ was used and the intensity at 385 nm was found to increase as shown in Fig. 4. Similarly $Mo(Z\text{-}Ala\text{-}cys\text{-}OMe)_2(S\text{-}t\text{-}Bu)_2$ was examined for the interaction with the fd-models and a qualitatively similar result was obtained (see Fig. 5). Here, the intensity at the 400–600 nm region shows a remarkable change. It is important that 4-Fe fd models containing cysteine thiolato or similar peptide ligands show generally weak CD intensities ($\Delta\epsilon$ value: 0.1–0.3) at the 400–700 nm region. The observed CD spectral change was remarkable when the chelating tetrapeptide was used for the Mo(IV) component. As shown in Fig. 6, a strongly positive CD extremum was found at 360 nm together with a weaker one at 445 nm. The spectral pattern may be compared with the CD spectrum of MoFe protein [9] from reduced *Azotobacter vinelandii* or *Klebsiella pneumoniae* where a MoFe mixed cluster is thought to be coordinated through cysteine thiolato groups of the protein [10]. Strongly positive peaks exist at 370 and 460 nm which are obscured by overlapping bands. These features are qualitatively similar to what was found for our tetrapeptide complex shown in Fig. 6.

Thus, formation of a new metal chromophore, from Mo(IV) thiolates and fd-model complexes, is apparent from the present CD spectra. When the lability at the Fe–thiolate bonding of 4-Fe fd models is considered, some solution structures shown in Fig. 7 are probable. The Fe_4S_4 core is kept under the reaction conditions of our experiment to favor the Fe_4Mo structure with the chelating tetrapeptide on the Mo(IV).

The lability of $Mo(S\text{-}R)_4$ was important for the reaction with fd models when comparison was made with a less labile doubly-sulfur bridged binuclear

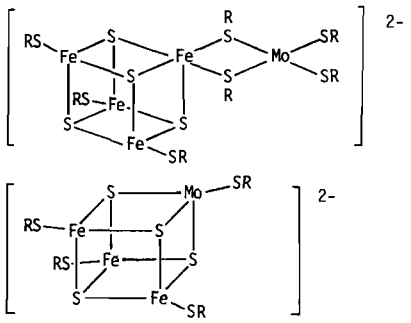

Fig. 7. Possible structures of Mo–Fe complexes derived from the Mo(IV)/thiolate complex and ferredoxin model complex.

Mo(V) complex, $Mo_2O_2(\mu\text{-}S)_2(cys\text{-}OMe)_2$. Here, no change in the CD spectrum was observed when it was mixed with $[Fe_4S_4(S\text{-}i\text{-}Pr)_4]^{2-}$ in DMF. Absence of reaction has already been confirmed with $Mo_2O_2S_2(Et_2dtc)_2$ of similar core structure. Remarkably high activation of catalysis of Mo(IV) thiolato complexes with the addition of 4-Fe fd-models in the reduction of acetylene [11] also implies MoFe mixed cluster formation as one possible catalytic species.

Recently sulfido-bridged binuclear Fe–Mo complexes of two peptides, Ac-Gly_2-(Cys-Gly_2)$_n$ Cys-Gly_2-NH_2 were prepared and characterized by visible spectrum [12]. The peptides are found to coordinate only on the Fe ion. A chelating structure similar to our Mo(IV) tetrapeptide complexes has also been proposed.

The importance of peptide chelation in the construction of characteristic environments around the metal ions in metalloenzymes is thus apparent.

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