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Specific Chelation of Tetrapeptide containing Two Cysteine Residues to Palladium(II)

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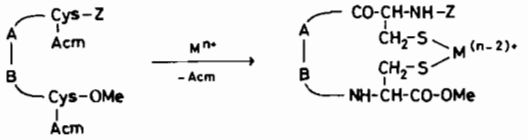
A peptide sequence, -Cys–A–B–Cys-, exists at the metal binding sites of many important metalloproteins, *e.g.* rubredoxin [1], alcohol dehydrogenase [2], and asparatate carbamoyl transferase [3]. Rydon *et al.* [4] studied spectrochemically the complexation of $FeCl_3$ with synthetic sequential oligopeptides, Ac–Gly(-Gly-Cys-Gly-)$_n$Gly–NH_2 (n = 4). The solution species of such complexes exhibit the visible spectral pattern very similar to that of rubredoxin. However, it was extremely thermally unstable, being different from the behavior of the native rubredoxin. It is still unclear if the peptide backbone of the complex prefers a conformation involved in the chelation of Cys–A–B–Cys to one metal. Our current interest is concerned with the tendency of the chelation. Conformational restriction caused by combination of steric effects of the two amino acid residues, A and B, may induce the peptide to take hair-pin turn conformation and to accommodate transition metal ions by the S,S-chelation at the two cysteine thiolato groups. Now, evidence is presented for such an S,S-chelated metal complex by the careful interpretation of the spectral data especially for the Pd(II) complex, which prefers to a square planar geometry.



Reaction of Na_2PdCl_4 with Z-Cys(Acm)–Ala–Ala–Cys(Acm)–OMe in Me_2SO-d_6 gave brown-red precipitates, $Na_2[PdCl_2$(S,S'-Z-cys–Ala–Ala–cys–OMe)]. M.p. (dec.) 180 °C. The ^1H NMR spectra of the original S-blocked peptide, Z-Cys(Acm)–Ala–Ala–Cys(Acm)–OMe (Acm = acetoamidomethyl), the di-mercury(II) complex, and the square planar diamagnetic Pd(II) complex are taken in Me_2-SO-d_6 and the NH region of these complexes are compared in Fig. 1. Close examination of the NH region of these spectra reveals remarkable chemical shift differences between the NH and $C_\alpha H$ signals of Hg(II) and Pd(II) complexes. The assignment of the NH signals of Z-Cys, Ala, and Cys-OMe was accomplished by careful decoupling experiments. Two NH protons of two Ala residues are deshielded from two adjacent amide planes with each other. The NH proton of Cys-OMe shifts to up-field by the shielding due to the vicinity of the sulfur lone pair electrons. Weak shielding of the vicinal proton by the sulphur lone pair electrons of [2,7-Cystine]-gramicidin S has been reported by Schwyzer [5]. Also the NH proton of Z-Cys is shielded by the adja-

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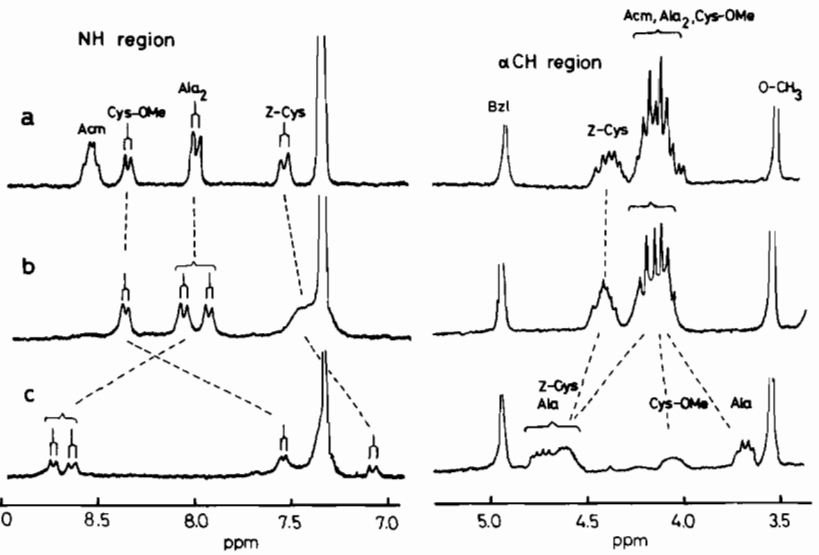

Fig. 1. ^1H NMR spectra (220 MHz) of a) Z-Cys(Acm)–Ala–Ala–Cys(Acm)–OMe, b) $(HgCl)_2$(S,S-Z-cys–Ala–Ala–cys–OMe), and c) $[PdCl_2$(S,S-Z-cys–Ala–Ala–cys–OMe)$]^{2-}$ in Me_2SO-d_6 (31 °C).

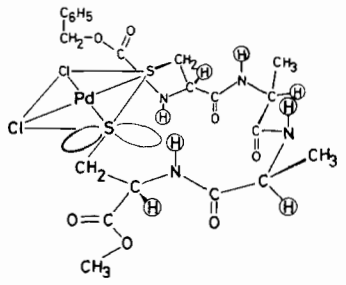

Fig. 2. Proposed structure of $[PdCl_2(S,S\text{-Z-cys–Ala–Ala–cys–OMe})]^{2-}$ complex.

cent amide and the urethane planes. The observed change of chemical shifts of the NH protons may be rationalized by invoking the anisotropic shielding effects of neighboring -Co–NH- moiety. A CPK model constructed by the chemical shifts of NH and αCH protons allows easy *cis*-chelation with the two cysteine thiolate groups as shown in Fig. 2.

For the purpose of meaningful comparison, we have also examined various oligopeptides containing Cys residues. For example, a tripeptide with one cysteine thiolate group, Z-Ala–Ala–Cys–OMe, was allowed to form Hg(II) and Pd(II) complexes and the solution structures were investigated by the ^{1}H NMR spectra. Absence of any appreciable chemical shift differences was observed between the signals of the Hg(II) and the Pd(II) complexes. A related tripeptide, Z-Cys–Ala–Cys–OMe, was also examined similarly by the ^{1}H NMR. Here the Pd(II) complex exhibits no sharp signals assignable to the NH groups. The observed broad ^{1}H NMR signals are ascribed to the inability of the tripeptide to assume conformations leading to a stable S,S-chelated structure in solution.

The S,S-chelation in the $PdCl_2$(S,S-Z-cys–Ala–Ala–cys–OMe)$^{2-}$ (abbr: $[PdCl_2(S,S\text{-tetpep})]^{2-}$) in Me_2SO was also supported by the CD spectrum which is quite different from those of $[PdCl_2(S\text{-Z-Ala–cys–OMe})_2]^{2-}$, $PdCl_2(S\text{-Z-Ala–Ala–cys–OMe})]^{2-}$, and $[PdCl_2(S,S\text{-Z-cys–Ala–cys–OMe})]^{2-}$.

To confirm the *cis*-chelation of the tetrapeptide dianion (S,S-tetpep) various molar equivalents of dipyridyl were added to a solution of $PdCl_2(S,S\text{-tetpep})^{2-}$, while observing the CD spectra. The original CD pattern changed on the 1:1 ration but exhibited no further change on increasing amounts of dipyridyl. Disproportionation to $[Pd(S,S\text{-tetpep})_2]^{2-}$ and $[Pd(dipy)_2]^{2+}$ was ruled out by comparison with the CD spectrum of the performed $[Pd(S,S\text{-tetpep})_2]^{2-}$. Addition of toluene-3,4-dithiol-(S,S-tol) the $[PdCl_2(S,S\text{-tetpep})]^{2-}$ gave a CD extremum of considerable strength at 400 nm at the 1:1 ratio by indicating the presence of a mixed ligand complex, $[Pd(S,S\text{-tol})(S,S\text{-tetpep})]^{2-}$. These CD evidences support the *cis*-chelation of the tetrapeptide dianion to Pd(II). The bi- or polynuclear structures with bridging S,S-tetpep ligand are less probable on consideration of highly diluted state of the labile Pd(II) peptide complex at the CD measurement and the concentration independence of NH chemical shift of ^{1}H NMR spectra. The complex, $PdCl_2(S,S\text{-tetpep})^{2-}$, was not sufficiently soluble even in Me_2SO to warrant accurate determination of its molecular weight by cryoscopy.

The Pd(II) complex of Z-Cys–Val–Val–Cys–OMe having more bulky amino acid residues between two cysteine residues were also synthesized by the same procedure. Interpretation of ^{1}H NMR spectra of the original blocked Z-Cys(Scm)–Val–Val–Cys-(Acm)–OMe, the Hg(II) complex, and the Pd(II) complex indicates that the peptide chelation to Pd(II) is practically absent.

Such S,S-chelation as involved in the cys–Ala–Ala–cys residue should depend on the conformational fitness controlled by steric effects of two amino acid residues between two cysteine residues. This chelating tendency with two thiolate groups of terminal residues is considered to be important for the understanding of the chemical implication of the cys–A–B–cys sequence involved in active sites of some metalloenzymes. The conformational effect upon the chelating tendency to various transition metal ions which have octahedral or tetrahedral geometry is now being investigated.

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