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Coordination Chemistry of Peptides. Part I. Metal Complexes of *Cyclo*-L-methionyl-L-methionine

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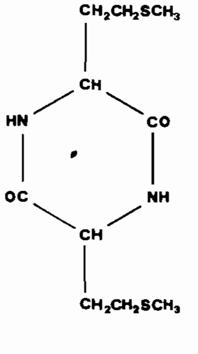
We report here studies on the complexation of some transition metals with *cyclo*-L-methionyl-L-methionine (CMT). These studies were intended to provide models of metal–protein interactions at methionine residues. The cyclic dipeptide CMT seems to be a better model ligand than methionine itself or linear methionine peptides, where end effects and greater stereochemical flexibility are present.



(*CMT*)

The ^1H NMR spectrum of CMT in $Me_2SO\text{-}d_6$ shows the following features: a broad signal at δ = 8.21 (NH), a pseudotriplet centered at δ = 3.94 (CH), a sharp singlet at δ = 2.02 (CH_3), and a complex resonance with major peaks gathered about δ = 2.5 and δ = 2.0 (CH_2). Metal complexation of CMT has been studied looking at the NH, CH and CH_3 resonances. Chemical shift data are collected in Table I. The observed spectra are consistent in all the cases with maintenance of the ligand chemical structure.

The proton spectrum of the complex $Pt(CMT)Cl_2$ shows downfield shift of the NH, CH and CH_3 resonances relative to free CMT. The greatest shift is observed for the methyl resonance (*ca.* 0.5 ppm). The CH_3 signal is superimposed onto the $Me_2SO\text{-}d_5$ resonance and flanked by ^{195}Pt satellites ($|^3J(^{195}Pt\text{–}^1H)|$ = 51.5 Hz). These results indicate binding of the dipeptide to platinum(II) through the side chain sulfur atoms. Similar values of $^3J(^{195}Pt\text{–}1H)$ have been found for thioether complexes of platinum(II) [1]. In addition to the resonances referred to in Table I, less intense resonances are observed in the spectrum of $Pt(CMT)Cl_2$ at $\delta \sim 8.2$ (NH), $\delta \sim 4.0$ (CH), and δ = 2.04 (CH_3). ^{195}Pt satellites are not observed for the latter resonance. The above observations are accounted for by the presence of coordination equilibria, slow in the NMR time scale, involving partial substitution of thioether groups by $Me_2SO\text{-}d_6$.

The rather small shift of the NH proton resonance observed upon coordination (*ca.* 0.1 ppm) and the relative intensity of the signal are in agreement with lack of direct interaction between the metal and the amide nitrogen. Metal complexation at the amide nitrogen atom could be expected if dissociation of the peptide proton had occurred [2]. The other basic sites of CMT where metal coordination may take place are the acyl oxygen atoms. This binding mode seems to be absent in the platinum complex, since the carbonyl ^{13}C resonance is found to occur at δ_C = 167.7 in both complexed and free CMT (sulfoxide solution). Metal coordination of the amide oxygen is expected to cause upfield shift of the carbonyl ^{13}C resonance [3]. Binding of CMT to platinum(II) through the O (acyl) atom in the solid state cannot

TABLE 1. Proton chemical shifts of CMT (δ, $Me_2SO\text{-}d_6$ solutions).

	NH	CH	CH_3
CMT 0.38 *M*	8.21	3.94	2.02
$Pt(CMT)Cl_2$ 0.11 *M*	8.33	4.05	2.54[a]
$Pd(CMT)Cl_2$ 0.04 *M*	8.31	4.00	2.26
$Pd(Me_2SO)_2Cl_2$ 0.2 *M*–CMT 0.2 *M*	8.32	4.03	2.31
$Pd(Me_2SO)_2Cl_2$ 0.19 *M*–CMT 0.38 *M*	8.23	3.96	2.16
$Rh(Me_2SO\text{-}d_6)_3Cl_3$ 0.09 *M*–CMT 0.09 *M*	8.30	4.03	2.54
$(Et_4N)[AuCl_4]$ 0.26 *M*–CMT 0.13 *M*	8.30	4.03	2.54
$(AuCl)_{1.5}(CMT)$ 0.03 *M*	8.32	4.07	2.54
$Cu(Me_2SO)_2Cl_2$ 0.011 *M*–CMT 0.27 *M*	8.20	3.94	1.97[b]

[a] $|^3J(^{195}Pt\text{–}^1H)|$ = 51.5 Hz. [b] Linewidth *ca.* 20 Hz.

be ruled out, but evidence from the behaviour of sulfoxide solutions (NMR and conductivity) points to a structure of Pt(CMT)Cl_2 where the four metal coordination sites are occupied by two chloride ions and two S (thioether) atoms each CMT molecule possibly bridging two $PtCl_2$ moieties in a polymeric arrangement. The molar conductivity of freshly prepared $10^{-3}M$ solutions of Pt(CMT)Cl_2 in Me_2SO is 0.7 S cm^2 mol^{-1}; the conductivity is increased to 3.0 after 18 hr and to 3.7 after 42 hr. Values in the range 23–42 S cm^2 mol^{-1} are characteristic for 10^{-3}-*M* solutions of 1:1 electrolytes in this solvent [4].

The proton spectra of the complex Pd(CMT)Cl_2 and of equimolar mixtures of $Pd(Me_2SO)_2Cl_2$ and CMT display downfield shift of the NH, CH and CH_3 resonances with respect to the free ligand. The methyl signal is rather broad and its chemical shift is intermediate between the values found for Pt(CMT)Cl_2 and uncomplexed CMT. These results are consistent with coordination equilibria of CMT involving exchange of thioether groups between free and coordinated sites (intermediate exchange conditions). Accordingly, if excess of CMT with respect to palladium is added, a single rather broad CH_3 signal is observed at higher field than the equimolar mixture (see Table I). The molar conductivity of $10^{-3}M$ Pd(CMT)Cl_2 in Me_2SO is 2.8 S cm^2 mol^{-1}. For the palladium complex a structure can be suggested similar to that proposed for the platinum complex. Methionine has been reported to coordinate palladium(II) through both the S (thioether) and N (amino) atoms to form chelate rings [5]. Coordination *via* both S and N and *via* S only has been described for methionine–platinum(II) complexes [2, 6].

The proton spectra of the systems $Rh(Me_2SO\text{-}d_6)_3Cl_3$–CMT and $(Et_4N)[AuCl_4]$–CMT and of the complex $(AuCl)_{1.5}$(CMT) show similar shifts of NH, CH and CH_3 resonances relative to free CMT. The observed chemical shifts are very close to the values observed for coordination of CMT to platinum(II), indicating complexation of the dipeptide with rhodium(III), gold(III) and gold(I) through the S (thioether) atoms. It is pertinent to note that coordination of methionine to rhodium(III) through N and O has been proposed [7]. The compound $(AuCl)_{1.5}$(CMT) can be considered as a mixture of the complexes (AuCl)(CMT) and $(AuCl)_2$(CMT), where CMT acts as a monofunctional and as a difunctional ligand, respectively. The molar conductivity of the gold(I) complex in Me_2SO (9.5 S cm^2 mol^{-1}, $10^{-3}M$ solution) shows rather extensive ionic dissociation, due to chloride replacement by free thioether groups of CMT and/or by the solvent. Therefore, the observed spectrum of the gold(I)–CMT system must be looked at as the time averaged spectrum of uncharged and cationic adducts.

The interaction of CMT with copper(II) has been studied by measuring the proton spectra of CMT solutions to which small quantities of $Cu(Me_2SO)_2Cl_2$ were added (0.5–4%). Under these conditions linewidths and chemical shifts of NH and CH resonances are not appreciably affected, whereas small upfield shift and considerable broadening of the CH_3 resonance are observed (see Table I). Broadening is also observed for the signals of HDO contained in the solvent and of $Me_2SO\text{-}h_6$. The upfield shift of the CMT methyl signal is increased as the amount of added copper is increased. The above observations are consistent with coordination of paramagnetic copper(II) ions to CMT through S (thioether) atoms and with conditions of fast exchange between metal bonded CMT and the free ligand present in a large excess. Copper(II) complexes with methionine or methionine containing peptides have been previously found to involve nitrogen and oxygen donors [8, 9]. Only recently has evidence been reported for Cu–S bond formation in aqueous methionine dipeptide–copper(II) systems frozen at liquid nitrogen temperature [9]. The Cu–S bond has been proposed to be of importance in some blue proteins [10].

Experimental

Cyclo-*L-methionyl-L-methionine*

This compound was prepared according to Nitecki *et al.* [11] from *tert*-butyloxycarbonyl-L-methionyl-L-methionine methyl ester [12]. *Anal.* Found: C, 45.94; H, 7.26; N, 10.66; S, 24.21. Calcd. for $C_{10}H_{18}N_2O_2S_2$: C, 45.73; H, 6.92; N, 10.67; S, 24.45%.

Pt(CMT)Cl_2

The complex *cis*-$[Pt(MeCN)_2Cl_2]$ and CMT in the molar ratio 1:1 were dissolved in 2,2,2-trifluoroethanol. After a week dichloromethane was added to the gelatinous mixture formed and the solvents removed under vacuum. The yellow residue was washed with boiling ethanol and CH_2Cl_2 and dried under vacuum. *Anal.* Found: C, 22.71; H, 3.57; N, 4.98. Calcd. for $C_{10}H_{18}Cl_2N_2O_2S_2Pt$: C, 22.73; H, 3.43, N, 5.30%.

Pd(CMT)Cl_2

An equimolar concentrated solution of $Pd(Me_2SO)_2Cl_2$ and CMT in Me_2SO was allowed to stand for two days. The yellow precipitate formed was washed with Me_2SO and CH_2Cl_2 and dried under vacuum. *Anal.* Found: C, 27.50; H, 4.12; N, 6.66. Calcd. for $C_{10}H_{18}Cl_2N_2O_2S_2Pd$: C, 27.32; H, 4.13; N, 6.37%.

$(AuCl)_{1.5}$(CMT)

To a saturated solution of $(Et_4N)[AuCl_4]$ in ethanol an equimolar solution of CMT in hot ethanol was added. After one day the orange precipitate formed was filtered off and treated with refluxing ethanol until all the solid was dissolved to give a colourless solution. The white solid separated after cooling was washed with ethanol and CH_2Cl_2 and dried under vacuum. *Anal.* Found: C, 20.18; H, 3.06; N, 4.60. Calcd. for $C_{10}H_{18}Cl_{1.5}N_2O_2S_2Au_{1.5}$: C, 19.66; H, 2.97; N, 4.58%. The stoichiometry of the compound was confirmed by analyses of samples from different preparations and by proton counts (NMR).

Other complexes used as metal ion sources for NMR measurements or as starting materials for preparations were obtained according to literature methods.

Measurements

NMR spectra were recorded on a Bruker WP-60 spectrometer operating at 14.09 kG in the pulsed FT mode. Resonances from the solvent were used as internal references and chemical shift data referred to tetramethylsilane. In each case the solutions were allowed to stand for appropriate time intervals before measurements in order to ensure equilibration. Conductivity measurements were performed using a Conductivity Meter CDM3-Radiometer.

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