Spectroscopic and Potentiometric Studies on the Interaction of *trans*-$[(MeH_2N)_2Pt(mcyt)_2PdCl]NO_3$ (mcyt = 1-methylcytosinate) with Derivatives of Amino Acids

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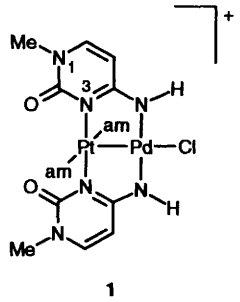
The interaction of the mixed-metal complex *trans*-$[(MeH_2N)_2Pt(mcyt)_2PdCl]NO_3$ (mcyt = 1-methylcytosinate) with various derivatives of amino acids mimicking the side-chain metal binding sites of proteins were studied by ^1H NMR, spectrophotometric and potentiometric methods. The derivatives included *N*-acetylamino acids (*N*-acetyl-methionine, -cysteine, -lysine, -histidine and -histamine), amino acids (glycine and methionine) and dipeptides (Gly-Met and Gly-Lys). The co-ordination of independent thioether, amino or imidazole nitrogen side-chain donor groups resulted in the formation of stable mono- or di-nuclear adducts. The formation of dinuclear complexes was characteristic of dipeptides and *N*-acetyl-histidine and -histamine. The existence of linkage isomers was demonstrated in the latter case, the Pd–N(3) (imidazole) isomers being more favoured. The possibility of stable chelate formation with palladium(II) (*e.g.* S,O for *N*-acetylcysteine and S,N for methionine) significantly enhanced the decomposition of the mixed-metal complex, leading to *trans*-$[Pt(NH_2Me)_2(Hmcyt)_2]^{2+}$.

The association of proteins and nucleic acids may arise from electrostatic, stacking or hydrogen-bonding interactions between the various amino acid side chains or peptide linkages of proteins and constituents of nucleic acids. There is, however, an increasing number of examples, which show that metal ions can also induce the interactions of proteins and nucleic acids.[1] In addition to divalent cations of $3d^5$–$3d^{10}$ essential transition elements, the corresponding complexes of palladium(II) and platinum(II) might have a biological significance in this respect. The slow formation kinetics of palladium(II) and especially platinum(II) complexes, however, makes it difficult to characterize all features of the possible interactions between side-chain residues of amino acids and these metal ions in solution.[2,3]

Three-co-ordinated palladium(II) complexes of peptides and triamines have served in many studies as ideal 'monofunctional metal ions' for examining the binding properties of various organic ligands including nucleobases,[4–9] amines[10–12] or amino acids and derivatives.[13,14]

In a previous paper[15a] the preparation and structure of a new mixed-metal complex *trans*-$[(MeH_2N)_2Pt(mcyt)_2PdCl]NO_3$ **1** (mcyt = 1-methylcytosinate) was reported. It contains a strong Pt→Pd dative bond and the co-ordination planes of the two metal ions are perpendicular to each other.[15a,b] The palladium(II) has one free co-ordination site. As has been demonstrated, the chloride in **1** can be substituted by a large variety of ligands, including nucleobases.[15a] As a consequence, **1** can be used, similarly to $[Pd(dien)Cl]^+$ (dien = diethylenetriamine), to study the binding properties of palladium(II) (present in a different chemical environment) towards various organic ligands. In this paper the interactions with biomolecules have been extended to amino acids and derivatives and include *N*-acetylamino acids [*N*-acetyl-L-methionine, -L-cysteine, -L-lysine, -L-histidine and -histamine (histamine = imidazole-4-ethanamine)], the simplest models with which to mimic the side-chain residues of proteins. Taking into account the biological significance of the direct metal ion–amino acid or –peptide interactions, reactions between complex **1** and amino acids (glycine and L-methionine) and simple dipeptides (glycyl-L-lysine and glycyl-L-methionine) were also studied.



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am = NH_2Me

Experimental

Starting Materials.—Complex **1** was prepared and its purity checked by IR and NMR spectra as described previously.[15a] The analytical grade amino acids, peptides and *N*-acetylamino acids were obtained from Bachem and used without further purification. From ^1H NMR spectroscopy, no evidence for oxidation of *N*-acetyl-L-cysteine was observed, even though oxygen was not rigorously excluded.

Instrumentation.—Proton NMR spectra were recorded on a Bruker AC200 FT spectrometer in D_2O (with sodium 3-(trimethylsilyl)propanesulfonate as internal reference). pD Values were determined by use of a glass electrode and addition of 0.4 to the pH-meter reading. Absorption spectra of the complexes were recorded on a Beckman ACTA MIV UV/VIS spectrophotometer between 350 and 800 nm.

The pH-metric titrations were performed in 5 cm^3 samples in the concentration range 2×10^{-3}–4×10^{-3} mol dm^{-3} at complex **1** to ligand ratios of 1:1 and 2:1. Argon was bubbled

through the samples to ensure the absence of oxygen and carbon dioxide, and for stirring of the solutions. All pH-metric measurements were carried out at 298 K and at a constant ionic strength of 0.2 mol dm^{-3} (KNO_3). Measurements were made with a Radiometer pHM 84 pH-meter equipped with a GK2421C combined electrode and an ABU 13 automata burette containing carbonate-free potassium hydroxide in known concentration. The pH readings were converted into hydrogen-ion concentration[16] and stability constants were calculated by means of a general computational program (PSEQUAD).[17]

Results and Discussion

Amino nitrogen atoms of amino acids are converted into amide nitrogens in the corresponding *N*-acetyl derivatives, a group which generally does not take part in metal-ion co-ordination.[18] Thus, the various side-chain donor groups of *N*-acetylamino acids are the best candidates to mimic the independent binding site of amino acid side chains in proteins. The ligands containing thioether sulfur, amino or imidazole nitrogen-donor atoms generally form rather stable complexes with palladium(II)[3] and, as a consequence, the chloro complex **1** was not converted into the aqua species, but the equilibrium (1) was monitored

$$[(MeH_2N)_2Pt(mcyt)_2PdCl]^+ + A \rightleftharpoons [(MeH_2N)_2Pt(mcyt)_2PdA]^{2+} + Cl^- \quad (1)$$

potentiometrically and by visible and ^1H NMR spectroscopy. As was discussed previously[8,15] the methyl resonances of the platinum-bonded methylamine and 1-methylcytosinate ligands are not very sensitive to the change of the co-ordination sphere of palladium(II) in the mixed-metal complex. Thus, during the NMR measurements, development of reaction (1) was followed *via* the C(5)H and C(6)H protons of 1-methylcytosinate and the appropriate proton resonances of the ligands.

Replacement of chloride ion by a sulfur- or nitrogen-donor atom is also accompanied by a colour change. Namely, the original olive-green complex **1** turns orange with nitrogen donors and intense reddish brown with sulfur donors. Removal of palladium(II) from the species results in the formation of the colourless complex *trans*-$[Pt(NH_2Me)_2(Hmcyt)_2][NO_3]_2$. These colour changes take place within seconds after mixing the components and the rates of ligand-exchange processes are in the slow-exchange limit of the ^1H NMR spectra for nitrogen co-ordination and in the fast-exchange limit for sulfur co-ordination. As a consequence, the free and co-ordinated forms of the thioether ligands and **1** give only an averaged signal, but the binding can easily be detected from the chemical shift of the SMe resonances. In the case of nitrogen donors, the bonded and free compounds give independent signals, which makes the determination of the stoichiometric composition of the complexes easier. The ^1H NMR chemical shifts of the C(5) and C(6) protons of 1-methylcytosine and the appropriate proton resonances of the ligands are collected in Tables 1 and 2, respectively. Spectral parameters are included in Table 3, while the stability constants of the complexes obtained from pH-metric studies are in Table 4.

Interaction of Complex **1** *with* N-*Acetylamino Acids.*—(*i*) N-*Acetyl*-L-*methionine* (Ac-Met). The reaction of complex **1** with *N*-acetyl-L-methionine results in a significant change in colour (from olive-green to reddish brown) and is accompanied by a characteristic change of the NMR spectra of both 1-methylcytosine and *N*-acetylmethionine. Stability-constant determinations *via* pH-metric measurements cannot be performed for the thioether ligands, because this group does not take part in proton-competition processes in the measurable pH range. Absorption spectra of the solutions are most intense in

Table 1 Proton NMR data (δ) for aromatic 1-methylcytosinato protons in $[(MeH_2N)_2Pt(mcyt)_2PdA]^+$ complexes

		mcyt		
Ligand (A)	Stoichiometry	C(5)H	C(6)H	Binding mode
Cl^-	1:1	5.562	7.018	Pd–Cl
		5.600	7.056	
Ac-Met	1:1	5.685	7.118	Pd–S
		5.723	7.157	
Ac-Lys	1:1	5.623	7.066	Pd–NH_2
		5.661	7.104	
Ac-Hist	1:1	5.498	7.058	Pd–N(3)
		5.536	7.096	
		5.588		Pd–N(1)
		5.636		
	2:1	5.519		Pd_2[N(1), N(3)]
		5.557		
Ac-His	1:1	5.505	7.046	Pd–N(3)
		5.543	7.084	
		5.608		Pd–N(1)
		5.648		
	2:1	5.523		Pd_2[N(1), N(3)]
		5.565		
Gly	1:1	5.633	7.060	Pd–NH_2
		5.671	7.098	
Met	1:1	5.690	7.123	Pd–S
		5.729	7.161	
Gly-Lys	1:1 and	5.628	7.065	Pd–NH_2
	2:1	5.666	7.104	
Gly-Met	1:1	5.689	7.120	Pd–S
		5.727	7.158	
	2:1	5.64	7.08	Pd–NH_2
		5.68	7.11	

Table 2 Proton NMR data (δ) for amino acid protons

Ligand (A)	Proton	Free	Complexed	Binding mode
Ac-Met	SCH_3	2.099	2.346	Pd–thioether
Met	SCH_3	2.122	2.381	Pd–thioether
Gly-Met	SCH_3	2.104	2.333	Pd–thioether
	CH_2 (Gly)	3.413	3.494	Pd–N (amino)
			3.528	
Ac-Hist	Imidazole C(2), C(5)	7.666, 6.910	8.035, —*	Pd–N(3)
			7.905, 6.910	Pd–N(1)
			7.492, 6.812	Pd_2[N(1), N(3)]
Ac-His	Imidazole C(2), C(5)	7.944, 7.013	8.039, —*	Pd–N(3)
			7.904, 6.90	Pd–N(1)
			7.498, 6.801	Pd_2[N(1), N(3)]
Gly	CH_2	3.377	3.264	Pd–NH_2

* The imidazole C(5)H resonance was not identified because of superposition with C(6)H of 1-methylcytosinate.

equimolar solutions of **1** and Ac-Met, suggesting the formation of a 1:1 adduct. The ^{1}H NMR parameters of the complex depend neither on the pH nor on an excess of amino acid, suggesting again the formation of a 1:1 complex. Proton resonances of the sulfur-bonded methyl groups of Ac-Met show a significant downfield shift (0.227 ppm), while the methyl resonances of the acetyl group are not affected by complex formation. Protonation or deprotonation of the carboxylate group of Ac-Met does not influence the proton resonances of the complex, suggesting that thioether is the only binding site. Small and time-dependent spectral changes are observed at high pH values (pH 11), probably due to hydrolysis of the mixed-metal complex.

(*ii*) N-*Acetyl*-L-*lysine* (Ac-Lys). The (log) protonation constant of the ε-amino group of the lysyl residue is 10.40. Thus spectral changes are not observed when complex **1** is mixed with the fully protonated form of Ac-Lys. Specifically, no effect on the α-CH proton is seen in the ^{1}H NMR spectrum. After addition of base (above pH 5) the samples turn orange, and characteristic shifts of the C(5) and C(6) proton resonances are observed. Methyl protons of the acetyl residues are not affected, again suggesting that the ε-amino group is the only binding site of the compound. The pH-metric titration curves can best be fitted by the assumption of a 1:1 species (see Table 4), in agreement with the spectrophotometric and NMR results.

(*iii*) N-*Acetyl-histamine* (Ac-Hist) *and* -L-*histidine* (Ac-His). Imidazole nitrogen-donor atoms of histidyl residues represent the most common binding sites for metal ions in proteins. As a consequence, the metal-ion complexes of histidine and its derivatives have been widely studied.[19,20] The amino and imidazole-N(3) donors of histidine and histamine can readily form six-membered chelate rings, which result in enhanced stability of the metal complexes. The amino group is not available for metal-ion co-ordination in the *N*-acetyl derivatives, thus only the imidazole nitrogens can interact with metal ions. Data for the interactions of Pd(dien) with Ac-His have recently been published and the co-ordination of both N(1) and N(3) nitrogens, hence the existence of linkage isomers, has been demonstrated.[13]

Table 3 Parameters of visible spectra of complexes formed in the reaction of complex **1** with various ligands (A)

		'Peak'		'Shoulder'	
Ligand (A)	Species **1**:A	λ/nm	ε/dm^3 mol^{-1} cm^{-1}	λ/nm	ε/dm^3 mol^{-1} cm^{-1}
	1	453	473	575	112
Ac-Met	1:1	410	1579	550	158
Ac-Lys	1:1	402	599	500	153
Gly	1:1	405	624	500	144
Ac-Hist	1:1	401	573	500	133
	2:1	403	863	500	163
Ac-His	1:1	400	567	500	134
	2:1	405	884	525	129
Gly-Met	1:1	408	1552	525	148
	2:1	408	1046	550	121
Gly-Lys	1:1	400	582	500	137
	2:1	406	560	500	137

Our spectroscopic and potentiometric studies on the interaction of Ac-Hist and Ac-His with complex **1** lead to similar conclusions. As can be seen from Tables 1 and 2, the existence of three different species is detected with both derivatives. The ligand-exchange processes are relatively fast, but still in the slow-exchange limit of the ^{1}H NMR spectra, which allows identification of the C(2) and C(5) proton resonances of the free and Pd-bonded imidazole residues. Several NMR spectra of the **1**–Ac-Hist system are shown in Fig. 1.

As can be seen from Fig. 1, in equimolar solution the complex formation is around 50% at pD 2.60 and is complete at pD 7.0. The C(2) and C(5) protons of Pd-bonded imidazole have two pairs of signals suggesting the existence of linkage isomers (see also Table 2). Applying the assignment of aromatic His resonances used in a variety of complexes of Pt and Pd,[13] the ratio of N(3)- and N(1)-bonded isomers is about 6 and 8:1 for Ac-Hist and Ac-His, respectively. This number differs significantly from the ratio obtained in the Pd(dien)–Ac-His system, where both isomers are present in comparable concentrations.[13] The difference stems from the fact that the co-ordination of N(1) of imidazole to the bulky complex **1** is sterically less favourable than that of N(3). In the latter case the C=O group of the acetyl residue is in a position to form a hydrogen bond with the cytosinato NH of **1**. The different ratios of linkage isomers clearly demonstrate that binding of N(1) or N(3) of imidazole in a non-chelated form is significantly affected by the chemical environments of both the imidazole residue and the metal ions.

A third pair of resonances from the imidazole protons appears at high pH values and becomes the predominant species in the presence of an excess of complex **1**, as can be seen from Fig. 1. Free *N*-acetylamino acid cannot be detected, not even at pD 4.90 in solutions containing **1** and the acid in 2:1 ratio. Thus the existence of these NMR resonances can best be interpreted in terms of formation of a bridged tetranuclear (PtPd)N(1)–N(3)(PtPd) species with both derivatives.

These conclusions are supported by the potentiometric and spectrophotometric results. It can be seen from Table 3 that the formation of the 1:1 adducts is accompanied by a significant blue shift of the absorption spectra and a small increase in molar absorption coefficient. The formation of bridged species will not affect the location of the absorption bands, but it results in a significant increase in molar absorption coefficients. Fig. 2 shows the species distribution curves obtained in the **1**–Ac-His system at different ratios. It can be seen that in equimolar solutions and in the acidic pH range the 1:1 complexes (MAH and MA) predominate. According to the NMR data they are mainly N(3)-bonded species, but the N(1)-bonded linkage isomers are also present. The species MAH contains the carboxylate group of Ac-His in protonated form. A pK = 3.01 is obtained for deprotonation of the complex, a value slightly higher than that of free Ac-His (pK = 2.88). It can also be observed from Fig. 2 that the dinuclear complex (M_2AH_{-1}) with Pd binding both to N(1) and N(3) is present not only in 2:1, but also in equimolar solutions. It suggests the existence of a co-operativity during complex formation with Ac-Hist and Ac-His. The log K values for the 1:1 species are very similar to those of

Table 4 Stability constants (log β_{pqr})[a] of the complexes formed in the reaction of complex **1** with various ligands [298 K, I = 0.2 mol dm^{-3} (KNO_3)]

Ligand	HA	H_2A	MAH	MA	M_2AH_{-1}	pK − log K (HA − MA)
Ac-Lys	10.40	13.70	—	7.35	—	3.05
Gly	9.59	11.91	—	6.95	—	2.64
Ac-Hist	7.03	—	—	6.76	4.21	0.27
Ac-His[b]	7.04	9.92	10.09	7.08	4.46	−0.04
Gly-Lys[c]	10.71	18.91	16.48	—	—	2.43

[a] $\beta_{pqr} = [(PtPd)_pA_qH_r]/[PtPd]^p[A]^q[H]^r$ where PtPd is denoted by M. [b] $pK_{MA}^{MAH} = 3.01$. [c] log β = 21.91 for H_3A, 13.16 for M_2A.

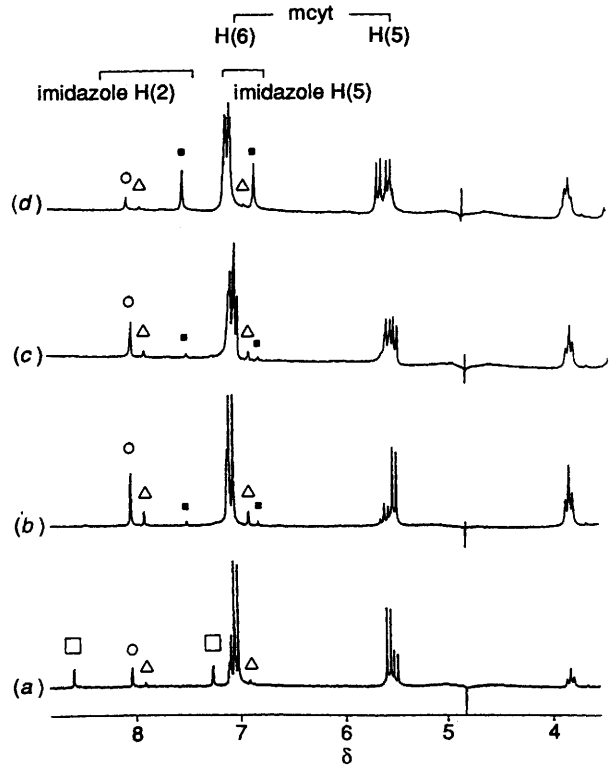

Fig. 1 Proton NMR spectra (δ 4–9, D_2O, water peak suppressed) of the complex **1**–Ac-Hist system at different ratios and pD values: (*a*) 1 : 1, pD 2.60; (*b*) 1 : 1, pD 7.00; (*c*) 2 : 1, pD 4.90; (*d*) 2 : 1, pD 8.50. The imidazole H(2) and H(5) protons are assigned as follows: (□), free Ac-Hist; (○), N(3)-linkage isomer; (△), N(1)-linkage isomer; (■), N(1),N(3)-bridged species. The imidazole C(5)H resonances of the N(3) linkage isomer are superimposed by the C(6)H resonances of the 1-methylcytosinate ligands at around δ 7

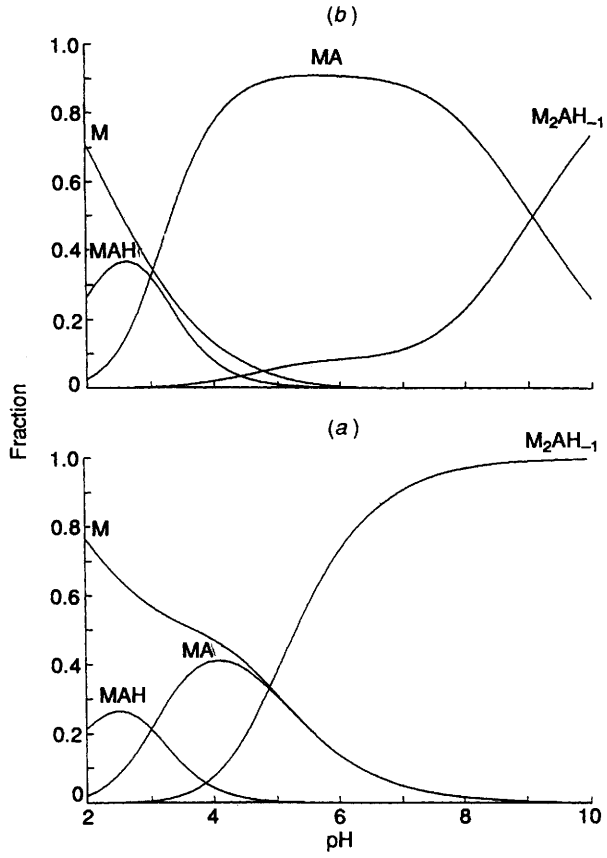

Fig. 2 Species distribution of the complexes present in the **1**–Ac-His system at different ratios: (*a*) $c_1 = 8 \times 10^{-3}$, $c_{Ac\text{-}His} = 4 \times 10^{-3}$ mol dm^{-3}; (*b*) $c_1 = c_{Ac\text{-}His} = 4 \times 10^{-3}$ mol dm^{-3}

the complexes with amino nitrogen co-ordination (Ac-Lys and -Gly). However, if one takes into account the low pK values of imidazole (see pK − log K values in Table 4), then it becomes evident that binding of Pd^{II} to imidazole nitrogens is favoured.

At high pH (> 8) a slow decomposition reaction can be observed which leads to *trans*-$[Pt(NH_2Me)_2(Hmcyt)_2]^{2+}$. The removal of palladium(II) from the mixed-metal complex is more pronounced in the presence of an excess of ligand. It suggests that the slow formation of palladium(II) bis(ligand) complexes co-ordinated *via* the imidazole N(3) and deprotonated amide nitrogens might be responsible for breakage of the structure of the mixed-metal complex. Similarly, deprotonation and co-ordination of the amide nitrogens of Ac-Hist and Ac-His was reported to occur in several mixed-ligand complexes of copper(II).[21]

(*iv*) *N-Acetyl-L-cysteine* (Ac-Cys). Ligands containing sulfhydryl groups generally form very stable complexes with transition-metal ions, and various polynuclear species are also formed.[22,23] Metal-ion-promoted deprotonation and co-ordination of the amide nitrogen was not reported to occur in the palladium(II)–Ac-Cys system,[24] but in contrast to the other *N*-acetylamino acids involved in this study the side-chain sulfur atom is now in a chelatable position with the carboxylate group (six-membered chelate). As a consequence, Ac-Cys initiates a rapid decomposition [removal of palladium(II)] of the mixed-metal complex. Mixing of **1** and Ac-Cys in equimolar ratio results in the formation of *ca.* 50% *trans*-$[Pt(NH_2Me)_2(Hmcyt)_2]^{2+}$, while the remaining part of complex **1** has similar NMR parameters to those of thioether-containing ligands. This tentatively suggests that the palladium(II) complex of Ac-Cys co-ordinates to **1** *via* sulfur bridges.

Removal of Pd from the mixed-metal complex becomes complete (even at pH 2.0) when Ac-Cys is present in a two-fold excess. It is probably due to the formation of the stable bis complex between palladium(II) and Ac-Cys *via* the co-ordination of sulfur and carboxylate oxygen donors.

The remaining *trans*-$[Pt(NH_2Me)_2(Hmcyt)_2]^{2+}$ species is not decomposed by Ac-Cys but stays intact. There is, however, a ligand-rotation process following the removal of Pd from **1**: the two 1-methylcytosine rings, which are in a head-to-head orientation immediately following the substitution of Pd, rearrange to an equilibrium with the head-to-tail rotamer[15,25] being preferred. Since this process is relatively slow (half-life *ca.* 2 h at room temperature), we are presently attempting also to crystallize the head-to-head rotamer and determine its crystal structure.

Interaction of Complex **1** *with Amino Acids.*—(*i*) *Glycine.* Amino acids, including the simplest one glycine, generally form stable bis complexes with palladium(II) even in the acidic pH range.[26] Accordingly, it is surprising to observe that mixing of **1** with the monoprotonated form of glycine (HA) does not result in any spectral change, only the free mixed-metal complex and glycine being detected. Upon addition of base (pD > 5.0) the formation of a mononuclear species can be observed, having almost the same NMR chemical shifts and visible spectra as those of Ac-Lys. On the basis of the spectral parameters it can be concluded that glycine co-ordinates in a monodentate manner to **1** *via* the amino group and that this is accompanied by an unusual upfield shift of the CH_2 glycine proton resonances (see Table 2). The stability constant for the resulting complex is included in Table 4 and the log K value is similar again to that of Ac-Lys. The smaller value obtained for pK − log K suggests, however, that the binding of glycine is slightly favoured compared to that of Ac-Lys. This can probably be explained by the steric requirements of the bulky lysyl side chain.

At high pH (> 9) and in the presence of an excess of glycine, a

slow decomposition of the mixed-metal complex is observed, probably due to the formation of the thermodynamically more favoured bis(glycinato)palladium(II) complex.

(ii) L-*Methionine*. Compared to the thiolate sulfur of cysteine, the thioether side chain of methionine is generally a poorer binding site for transition-metal ions, except for palladium(II) and some other soft metal ions such as platinum(II).[27] As a consequence, the interaction of complex **1** with methionine is completely different from that of glycine. Co-ordination of the thioether residue takes place in very acidic media (pD < 2) as can be seen from the NMR parameters in Tables 1 and 2. The amino group is protonated in the mononuclear complex, but can form a stable six-membered chelate ring with the thioether group, which results in a significantly enhanced decomposition of the mixed-metal complex. The removal of palladium(II) is relatively slow below pH 7, but occurs almost instantly at pH > 8 where deprotonation of the ammonium group takes place.

Interaction of Complex **1** *with Dipeptides*.—*(i) Glycyl*-L-*lysine* (Gly-Lys). It is well known that palladium(II) readily forms stable complexes with dipeptides, which, in the absence of sulfur or imidazole N side-chain donor groups, are co-ordinated *via* the terminal amino, deprotonated amide nitrogen and carboxylate oxygen donors.[28] Thermodynamic equilibrium data on palladium(II) peptide complexes are scarce, however, due to the relatively slow formation of the complexes, which makes the stability constant determinations difficult.

The dipeptide Gly-Lys contains two amino groups, but they are not in the position to form a chelate with palladium(II). As a consequence, it does not promote the decomposition of the mixed-metal complex, but its interaction is similar to those of glycine or Ac-Lys. Namely, mixing of **1** with an equimolar amount of doubly protonated Gly-Lys (H_2A) does not result in any spectral change. Upon addition of base a 1:1 species is formed, suggesting co-ordination of the terminal amino group of the dipeptide and this is supported by the proton resonances of the CH_2 glycyl residues (see Table 2). However, it should be mentioned that the existence of linkage isomers in slightly basic solution cannot be excluded. The co-ordination of the two amino groups has the same effect on the cytosine proton resonances, while the aliphatic protons of the lysyl residue are not well resolved enough to calculate the ratio of the linkage isomers.

Independent signals of free and palladium(II)-bonded peptide molecules show that ligand-exchange processes are in the slow-exchange limit of the NMR time-scale. In spite of this the NMR parameters of free complex **1** cannot be obtained in solutions containing a two-fold excess of **1**. This observation can only be explained by the formation of dinuclear complexes, in which both amino groups of the peptide are co-ordinated to palladium(II).

The pH-metric equilibrium studies support these findings, because titration curves can only be fitted under the assumption that species MAH, MA and M_2A are present. It should be emphasized that the dinuclear complex M_2A exists again not only in solutions containing an excess of **1**, but also when the components are present in a 1:1 ratio, suggesting a co-operativity in binding or **1** with nitrogen donors. The parameters of the visible absorption spectra are very similar to each other and to those of glycine or Ar-Lys due to involvement of the same binding sites in the various complexes (Pt, Pd, amino N).

The NMR signals of the Pd^{II}-free platinum complex can be observed at elevated pH values, but decomposition of the mixed-metal complex in equimolar solutions and at pD 9.0 at room temperature does not exceed 2% in 1 d.

(ii) Glycyl-L-*methionine* (Gly-Met). The results obtained for the interaction of complex **1** with Ac-Met have already indicated that the thioether side chains of amino acids form stable complexes with palladium(II). As a consequence, mixing of **1** with an equimolar amount of Gly-Met results in a reddish brown solution having similar spectral parameters to those of Ac-Met or Met. Proton resonances and the visible spectra of the complex are not affected by increasing pH, suggesting that the amino group does not take part in complex formation in the 1:1 species. Upon deprotonation of the ammonium group (pH > 8) a rather rapid decomposition of the mixed-metal complex is observed, however. Decomposition is complete within several hours, which probably is best explained by the formation of the (NH_2,N^-,S)-co-ordinated palladium(II)–Gly-Met complex.[3]

In acidic solution and in the presence of an excess of complex **1**, broad averaged NMR signals are detected, suggesting the existence of free and thioether-co-ordinated mixed-metal complexes. Upon increasing the pH of the 2:1 mixture a pair of the proton resonances of **1** grows in at pD 7.5, which probably corresponds to the formation of a dinuclear complex containing both palladium(II)–thioether and –amino nitrogen bonds. At the same time the co-ordination of **1** at amino nitrogen results in an increased lifetime of the mixed-metal complex. The molar absorption coefficient of the dinuclear complex (see Table 3) is just the average of those characteristic of amino nitrogen and thioether binding. This suggests the existence of two independent binding sites in the molecule.

Conclusion

The mixed-metal complex *trans*-$[(MeH_2N)_2Pt(mcyt)_2PdCl]NO_3$ **1** contains palladium(II) in a square-planar geometry, with a strong platinum(II)–palladium(II) dative bond. The fourth co-ordination site of palladium(II) is occupied by chloride ion and it can easily be replaced by various monofunctional ligands. As a consequence, the mixed-metal complex, similarly to $[Pd(dien)Cl]^+$ or to palladium(II)–dipeptide[8] complexes, can be used to study the ligating properties of various compounds toward palladium(II). On the other hand, it should be considered that in these molecules the palladium(II) ions are present in chelate systems, while only independent binding sites (Pt^{II} and two cytosine-NH^- groups) are available for palladium(II) in the mixed-metal complex.

The results of combined ^1H NMR, visible spectroscopic and potentiometric studies reported in this paper, however, clearly demonstrate that the palladium(II) ion of complex **1** is present in a stable chemical environment and **1** can be used to mimic the binding properties of palladium(II) for a large variety of organic ligands. *N*-Acetylcysteine and methionine proved to be the most effective to break the Pt–Pd and Pd–N bonds and remove palladium(II) from the mixed-metal complex *via* formation of bis(amino acid) palladium complexes. In the case of these ligands the co-ordinated sulfur atoms are in a position to form chelates involving another functional group of the molecule (carboxylate and amino group for Ac-Cys and methionine, respectively). Thus, it can be concluded that compounds which are able to form stable chelates in a kinetically rapid process with palladium(II) will significantly accelerate the decomposition of the mixed-metal complex.

The interaction of complex **1** with the other *N*-acetylamino acids (especially Ac-Lys and Ac-Met) results in the formation of stable mononuclear complexes without breaking the Pt–Pd and Pd–N bonds. The ligating donor groups of these molecules (amino and thioether groups, respectively) are far from any other donor atom in the same molecule and simple monodentate co-ordination can be expected.

The compounds containing imidazole residues (Ac-Hist and Ac-His) and glycine and dipeptides (Gly-Lys and Gly-Met) also form stable adducts with complex **1**, having quite a long lifetime in the physiological pH range. Most probably these complexes are not in thermodynamic equilibrium, but they represent long-lived species of metastable compounds due to the combined effects of thermodynamic and kinetic parameters. Namely, the dipeptides should form rather stable chelates with palladium(II) in the acidic pH range (< 4). If $[PdCl_4]^{2-}$ is treated with dipeptides this process takes place, albeit relatively slowly. In the case of **1** both the thermodynamic stability and relative

inertness of palladium(II) are significantly increased as compared to those in $[PdCl_4]^{2-}$. The existence of a metastable state is supported by the fact that the opposite reactions [transfer of palladium(II) from a palladium peptide complex to the corresponding platinum complex] cannot be achieved.

Another important observation from the present results is the existence of tetranuclear complexes and linkage isomers. The imidazole nitrogens of Ac-Hist and Ac-His cannot take part in the formation of chelate rings. Accordingly, both N(3) and N(1) have the possibility of co-ordination. During the interaction of complex **1** with Ac-Hist or Ac-His, formation of the N(3)-bonded complexes is favoured, but taking into account previous literature data [13] it can be concluded that the ratio of linkage isomers is very sensitive to the chemical environments of both the metal ions and the imidazole-containing ligands.

In the case of dipeptides the donor groups, which are rather far from each other (*e.g.* two amino groups for Gly-Lys and one amino and one thioether group for Gly-Met), behave as independent binding sites. In the presence of an excess of complex **1** the formation of 2:1 complexes is consequently observed. In the case of Gly-Lys and especially for Ac-Hist and Ac-His, the 2:1 complexes exist in solutions containing **1** and the amino acid in equimolar concentrations, again indicating a co-operativity of the various binding sites [N(1) and N(3) for Ac-Hist and Ac-His and two amino groups for Gly-Lys]. The existence of these di- or poly-nuclear species represents a promising model for metal ion–protein interaction and indicates that similar studies should be performed on more complicated peptide molecules and eventually proteins. As to the latter, a possible application of **1** as an isomorphous replacement stain for certain amino acid side chains can be foreseen.

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