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pH-Dependent competition between N,S and N,N′ chelation in the reaction of $[Pt(en)(H_2O)_2]^{2+}$ (en = $H_2NCH_2CH_2NH_2$) with methionine-containing di- and tri-peptides

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Products of the reaction of the cisplatin analogue $[Pt(en)(H_2O)_2]^{2+}$ (en = ethane-1,2-diamine) with methionine (Hmet)-containing di- and tri-peptides in the range pH 2.5–11.0 have been separated by semi-preparative reversed-phase HPLC with perfluorinated carboxylic acids as ion-pairing agents. Structural characterisation of the (en)Pt^{II} complexes of met-Hxaa (met-Hgly, met-gly-Hgly) and xaa-Hmet (gly-Hmet, gly-met-Hgly, gly-gly-Hmet) peptides with respectively N-terminal methionine or glycine (Hgly) units was achieved by multinuclear NMR spectroscopy. The κ^2N (amino),*S* (thiother) and κ^2N (amino),*N*′ (amide) chelation modes are competitive for met-Hxaa peptides with the former mode predominating at pH < 8.7, the latter at higher pH. In contrast, κ^2N' (amide),*S* (thioether)-co-ordinated species are observed for xaa-Hmet peptides in acid solution with cleavage of a Pt–N (en) bond leading to competitive κ^3N (amino),*N*′ (amide),*S* (thioether) complexes for gly-Hmet and gly-met-Hgly. At pH > 7.4, the κ^2N,N' complexes dominate for these bioligands as for met-Hxaa peptides. This is not the case for gly-gly-Hmet, for which only one major species with κ^2N'' (amide),*S* (thioether) co-ordination is found at pH < 10.6.

Cisplatin, *cis*-$[PtCl_2(NH_3)_2]$, is a widely-used antitumour agent the mechanism of action of which appears to be based on an interaction with guanine bases of DNA.[1] A concentration-dependent nephrotoxicity of the drug has been ascribed to co-ordination of Pt^{II} by sulfur atoms of L-cysteine or L-methionine (L-Hmet) side chains in certain enzymes.[2–4] One of the few characterised metabolites of cisplatin is indeed the κ^2N,S co-ordinated methionato complex $[Pt(L\text{-met-}\kappa^2N,S)_2]$, which has been isolated from urine.[5,6] Reactions of cisplatin and *cis*-$[Pt(H_2O)_2(NH_3)_2]^{2+}$ with L-methionine in the range pH 2–7 have been studied in detail by Sadler and co-workers[7–9] using multinuclear NMR spectroscopy. At a 1:1 molar ratio the major products are $[Pt(L\text{-met-}\kappa^2N,S)(NH_3)_2]^+$, *cis*- and *trans*-$[PtCl(L\text{-met-}\kappa^2N,S)(NH_3)]$ and the three diastereomers of *cis*-$[Pt(L\text{-met-}\kappa^2N,S)_2]$. Employment of a 1:2 molar ratio affords the latter diastereomers together with minor quantities of the diastereomers of *trans*-$[Pt(L\text{-met-}\kappa^2N,S)_2]$.[8] Appleton *et al.*[10] have investigated the kinetics of reactions of *cis*-$[Pt(H_2O)_2(NH_3)_2]^{2+}$ with *S*-methyl-L-cysteine (L-Hmcys) and L-methionine. In strongly acidic solution at pH < 0.5 with a small excess of the platinum complex the initial product is a κ^2O,S chelate (two diastereomers) which slowly converts into the κ^2N,S co-ordinated complex. At a 1:3 molar ratio the reaction with L-methionine under these conditions affords *cis*-$[Pt(L\text{-Hmet-}\kappa S)_2(NH_3)_2]^{2+}$ as the major species.

Previous studies of the reaction of cisplatin or analogous platinum(II) complexes with methionine-containing peptides are rather limited. The κS co-ordinated dications of the type *cis*-$[Pt(peptide\text{-}\kappa S)_2(NH_3)_2]^{2+}$ and $[Pt(en)(peptide\text{-}\kappa S)_2]^{2+}$ (en = ethane-1,2-diamine) have been prepared using glycylmethionine (gly-Hmet) and alanylmethionine (ala-Hmet) at 1:2 molar ratio.[11–13] Treatment of *cis*-$[PtCl_2(NH_3)_2]$ with the dipeptide gly-Hmet at pH 5.5 leads to an efficient release of NH_3, which has been attributed to the formation of a κ^3N(amino),*N*′(amide),*S* complex.[14] This mode of co-ordination has been established by X-ray structural analysis for $[PtCl(gly\text{-met-}\kappa^3N,N',S)]$ with deprotonation of the amide NH being observed at a pH value as low as 2.5.[15] Ismail and Sadler have also reported a significant release of free en during the course of the reaction of *cis*-$[PtCl_2(en)]$ with *N*-acetyl-L-methionine.

The lack of systematic information on the interaction of platinum(II) complexes with methionine-containing peptides prompted the present study of the reaction of $[Pt(en)(H_2O)_2]^{2+}$ with di- and tri-peptides of the types met-Hxaa (met-Hgly, met-gly-Hgly) and xaa-Hmet (gly-Hmet, gly-met-Hgly, gly-gly-Hmet) in the range pH 2.5–11.0. Products were separated by semi-preparative ion-pairing reversed-phase HPLC and characterised by FAB mass spectrometry and multinuclear NMR spectroscopy (^1H, ^{13}C, ^{195}Pt). A potentiometric determination of the species distribution in the $[Pd(en)(H_2O)_2]^{2+}$–met-Hgly system (range pH 2.2–11.3) was carried out for comparison purposes.

Experimental

Materials

The complexes $[PtCl_2(en)]$[16,17] and $[PdCl_2(en)]$[18,19] were prepared in accordance with literature procedures. The amino acids L-Hmet, L-Hmcys, *N*-acetyl-L-methionine (L-Hamet) and *N*-acetyl-L-methionine methyl ester (L-amet-OMe) and the peptides met-Hgly, met-gly-Hgly, gly-Hmet, gly-met-Hgly and gly-gly-Hmet were obtained from Bachem Biochemica and used as received. The HPLC-grade methanol and acetonitrile were obtained from J. T. Baker and Riedel-de Haën. Respectively 4 and 15–40 mmol dm^{-3} stock solutions of $[Pt(en)(H_2O)_2][NO_3]_2$ for analytical and semi-preparative HPLC were prepared by stirring an aqueous solution of $[PtCl_2(en)]$ with the required equivalent quantity of $AgNO_3$ (1:1.95) for 15 h in the dark followed by centrifugation of the AgCl precipitate.

HPLC

The following equipment was employed: Merck L-6200A pump, Rheodyne 7125 sample injector, Merck L-4250 variable-wavelength detector. Integration and evaluation were performed with the Knauer Eurochrom 2000 software. Employment of the detector in a stopped-flow mode allowed UV spectra to be taken. Reversed-phase columns (25 × 0.4 cm inside diameter) packed with Nucleosil 100-C_{18} (5 μm, Macherey & Nagel) were employed for analytical separations. Semi-preparative work was carried out on 25 × 2 cm inside diameter reversed-phase columns (Nucleosil 100-C_{18}, 10 μm). Analytical

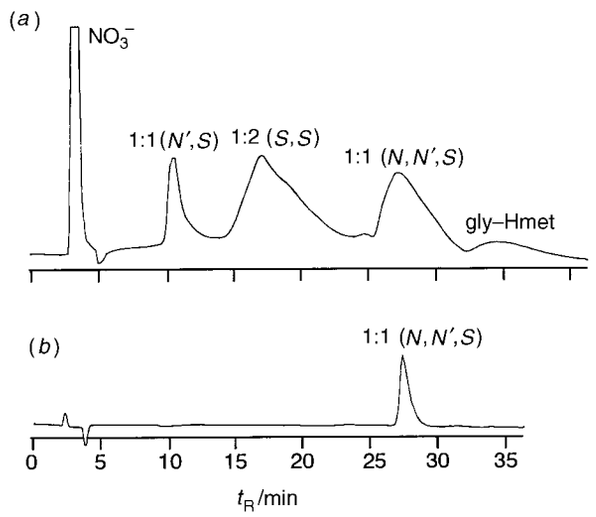


Fig. 1 (*a*) Semi-preparative chromatogram (mobile phase 100% water, ion-pairing agent 0.1% perfluoropropionic acid, pH 2.1, detection wavelength = 220 nm) for the 1:2 $[Pt(en)(H_2O)_2]^{2+}$–gly-Hmet reaction system. (*b*) Analytical HPLC trace of the isolated 1:1 (κ^3N,N',S) complex [same conditions as in (*a*)]

(0.8 mmol dm^{-3} per equivalent) and semi-preparative (5–10 mmol dm^{-3} per equivalent) reaction solutions (30–60 cm^3) were prepared at various Pt^{II}:peptide molar ratios (1:1, 1:2, 1:3, 2:1, 1:0 and 0:2). The initial pH was adjusted by addition of 0.1 mol dm^{-3} NaOH or 0.1 mol dm^{-3} HNO_3 and the solutions incubated for 3 d at 37 °C until the pH value and species distribution were constant. Samples were run isocratically with 90–100% water–10–0% acetonitrile (or methanol) as the mobile phase (pH 2.1 ± 0.1) in the presence of 0.1% (v/v) of the ion-pairing agent pentafluoropropionic acid. 0.124% (v/v) Heptafluorobutyric acid was occasionally more appropriate. The optimisation of the analytical and semi-preparative HPLC separation conditions for Pt^{II}–peptide systems has been discussed elsewhere.[20] Use of methanol (or tetrahydrofuran, thf) as the organic modifier is required for product mixtures containing significant concentrations of unreacted $[Pt(en)(H_2O)_2]^{2+}$, as on-column reaction with acetonitrile is possible.[21] Peak detection was generally performed at 220 nm; in cases in which unreacted peptide should not be registered, 254 nm was employed. For spectroscopic characterisation, 7–14 semi-preparative separations were carried out for 0.5–1.0 cm^3 portions of the reaction solutions, which were reduced in volume, centrifuged and made up to 7 cm^3 with water immediately before the separations. Respective fractions were then united and the solvent removed under vacuum to yield oily residues. These were treated with diethyl ether, which was subsequently removed to afford the required products as powders. The following complexes were isolated in this manner: $[Pt(en)(met\text{-}Hgly\text{-}\kappa^2N,S)][C_2F_5CO_2]_2$, $[Pt(en)(met\text{-}gly\text{-}Hgly\text{-}\kappa^2N,S)][C_2F_5CO_2]_2$, $[Pt(Hen\text{-}\kappa N)(gly\text{-}met\text{-}\kappa^3N,N',S)][C_2F_5CO_2]_2$, $[Pt(en)(gly\text{-}H_2met\text{-}\kappa S)_2][C_2F_5CO_2]_4$, $[Pt(en)(gly\text{-}gly\text{-}Hmet\text{-}\kappa^2N'',S)][C_2F_5CO_2]_2$ and $[Pt(en)(gly\text{-}gly\text{-}H_2met\text{-}\kappa S)_2][C_2F_5CO_2]_4$. Their purity was monitored by analytical HPLC and NMR spectroscopy. Fig. 1(*b*) provides a typical HPLC trace for $[Pt(Hen\text{-}\kappa N)(gly\text{-}met\text{-}\kappa^3N,N',S)][C_2F_5CO_2]_2$ separated from the 1:1 (κ^2N',S) and 1:2 (κS) products of a 1:2 mixture of $[Pt(en)(H_2O)_2]^{2+}$ and gly-Hmet by semi-preparative chromatography [Fig. 1(*a*)].

Mass spectral and NMR measurements

The FAB mass spectra were recorded on a Fisons VG Autospec instrument employing 3-nitrobenzyl alcohol as the matrix, NMR spectra at 297 K with a Bruker AM-400 spectrometer using 5 mm tubes and D_2O as the solvent. The chemical shift references were as follows: ^1H, sodium 3-(trimethylsilyl)tetradeuteriopropionate; ^{13}C, $CHCl_3$(external); ^{195}Pt, $K_2[PtCl_4]$–1 mol dm^{-3} NaCl(external). Reaction solutions for direct ^1H or ^{13}C NMR studies were prepared by adding 0.053 (1:1) or 0.106 mmol (1:2 ratio) of the peptide to 700 μl of an 80 mmol dm^{-3} stock solution of $[Pt(en)(D_2O)_2]^{2+}$ (0.056 mmol). After adjustment of the pH with 1 mol dm^{-3} NaOD, the solutions were incubated for 3 d at 37 °C and then poured into an NMR tube. These could be stored at 4 °C without further change in the species distribution. The pH values of the solutions in NMR tubes were measured with a micro glass electrode (Hamilton Minitrode 238100, length 180 mm).

Stability constant measurements

Potentiometric titrations were performed using 20 mmol dm^{-3} $[Pd(en)(H_2O)_2]^{2+}$ and 20 mmol dm^{-3} met-Hgly stock solutions at 4 mmol dm^{-3} (per equivalent) 1:1, 2:1, 1:0 and 0:1 reaction mixtures thereof with a fully automated microprocessor-controlled pH-titration unit (Metrohm 691 with Dosimat 665) in a thermostatted vessel at 25 ± 0.1 °C under nitrogen with carbonate-free 0.1 mol dm^{-3} NaOH. A background ionic strength of 0.15 mol dm^{-3} KNO_3 (p.a.) was employed for all titration solutions. A double-junction glass electrode (Metrohm 6.0219.100) containing a saturated KNO_3 solution between the membrane of the internal silver chloride reference electrode and an outer membrane was used to prevent diffusion of chloride ions into the titration solution, which could lead to formation of $[PdCl_2(en)]$. The pH meter was calibrated with standard buffer solutions (Riedel-de Haën; pH 4.008, 9.180). The use of such buffer solutions means that the measured pH value (pH_{meas}) will depend on the glass electrode, the junction potential, the background ionic strength I and the activity coefficient of H^+.[22,23] As proposed by Sigel *et al.*[23] the conversion factor A ($= pH_{meas} - p[H]_{calc/conc}$) for adjustments between the practical (pH_{meas}) and concentration ($p[H]_{calc/conc}$) scales was determined by pointwise evaluation of titration curves of 0.1 mol dm^{-3} HNO_3 (1 cm^3) and 0.15 mol dm^{-3} KNO_3 (25 cm^3) with 0.1 mol dm^{-3} NaOH. A value of $A = 0.09(1)$, which falls within the range of previously reported values,[22,23] was obtained on averaging all such calibrations performed in this work. Use of Gran plots provided an average pK_w value of 13.93(2) for these measurements. Protonation and stability constants were calculated with the program MINIQUAD.[24]

Results and Disucssion

After separation by ion-pairing reversed-phase HPLC, major products were characterised by FAB mass spectrometry and multinuclear one- and two-dimensional correlation spectroscopy (COSY) NMR methods. For cases in which analytical chromatograms confirmed the presence of only one major product, reaction solutions were prepared at the appropriate pH value for direct NMR measurements. Where necessary, ^1H and ^{13}C resonances were assigned on the basis of H–H and H–C COSY spectra.

Reaction of $[Pt(en)(H_2O)_2]^{2+}$ with the met-Hxaa peptides met-Hgly and met-gly-Hgly at a 1:1 molar ratio affords only one major product at pH < 7.0. A second important species appears on passing to alkaline solution and dominates at pH > 8.7. This state of affairs is illustrated by the three chromatograms presented in Fig. 2 for the $[Pt(en)(H_2O)_2]^{2+}$–met-Hgly system at equilibrium pH values of 3.31, 8.65 and 10.95. As a result of the kinetic inertness of platinum(II) complexes and the relatively short retention times involved, elution of the reaction mixtures at ambient temperature and at pH 2.1 does not lead to significant changes in species concentrations. This means that the species dependence on pH can be monitored for $[Pt(en)(H_2O)_2]^{2+}$–peptide reaction systems by HPLC, as demonstrated in Fig. 3. In this diagram the ordinate gives the peak area for a particular complex at the detection wavelength of 220 nm.

Table 1 Proton NMR chemical shifts δ (ppm) for (en)Pt^{II} complexes of met-Hxaa peptides

	κ^2N,S mode				κ^2N,N' mode		
	Hmcys	Hmet	met-Hgly	met-gly-Hgly	met-Hgly	met-gly-Hgly	met-gly-Hgly
α-CH	3.82 (dd)	3.74 (dd)	3.74 (dd)	3.75 (dd)	3.78 (m)	3.79 (m)	4.21 (t)
	4.04 (dd)	3.83 (dd)	3.82 (dd)	3.83 (dd)			
β-CH_2	3.13 (m)	2.36 (m)	2.25 (m)	2.24 (m)	2.05 (m)	2.05 (m)	2.21 (m)
	3.34 (m)	2.59 (m)	2.34 (m)	2.34 (m)	2.13 (m)	2.12 (m)	
			2.52 (m)	2.51 (m)			
			2.60 (m)	2.60 (m)			
γ-CH_2		3.08 (m)	3.09 (m)	3.09 (m)	2.80 (m)	2.76 (m)	2.67 (t)
δ-CH_3	2.64 (s)*	2.55 (s)	2.56 (s)	2.56 (s)	2.15 (s)	2.15 (s)	2.13 (s)
	2.67 (s)		2.57 (s)	2.57 (s)			
α′-CH_2			4.00 (s)	3.99 (d)	3.66 (d)	3.93 (d)	4.00 (d)
				4.06 (d)	3.99 (d)	4.18 (d)	4.10 (d)
α″-CH_2				4.00 (s)		3.79 (d)	3.77 (d)
						3.85 (d)	3.82 (d)
en CH_2	2.76 (m)	2.76 (m)	2.78 (m)	2.77 (m)	2.67 (m)	2.66 (m)	
pH	1.5	1.8	2.5	2.5	9.5	9.4	4.0

* γ-CH_3 for Hmcys.

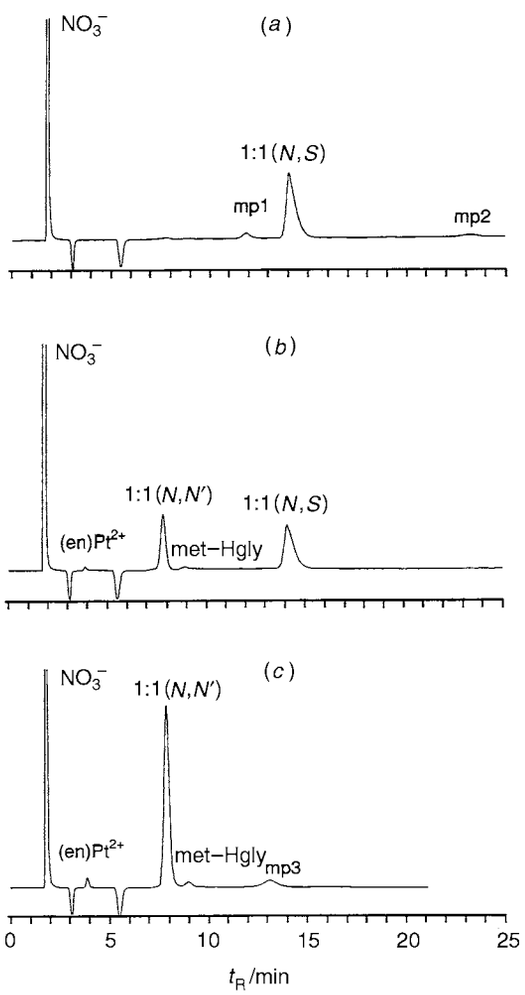


Fig. 2 Chromatograms (mobile phase 91% water–9% MeCN, ion-pairing agent 0.124% heptafluorobutyric acid, pH 2.1, detection wavelength = 220 nm) for the 1:1 $[Pt(en)(H_2O)_2]^{2+}$–met-Hgly reaction system (mp = minor product) at pH 3.31 (*a*), 8.65 (*b*) and 10.95 (*c*)

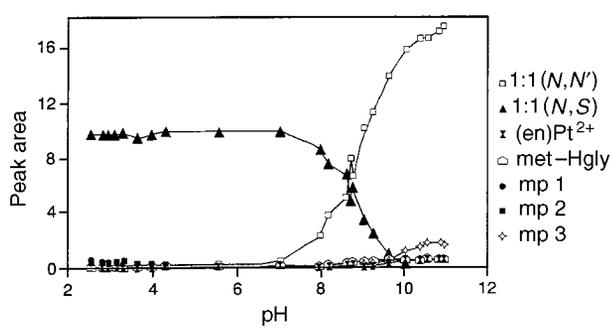
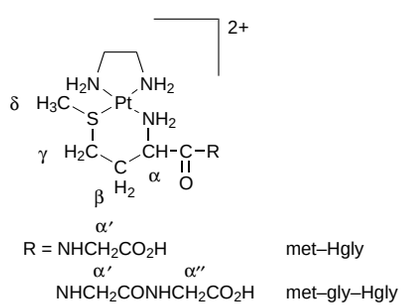


Fig. 3 Species distribution for the 1:1 $[Pt(en)(H_2O)_2]^{2+}$–met-Hgly reaction system as determined by HPLC (conditions as in Fig. 1) for the range pH 2.55–10.95



Scheme 1 The κ^2N,S-co-ordinated complexes for the 1:1 reaction systems $[Pt(en)(H_2O)_2]^{2+}$–met-Hxaa

Knowledge of the individual absorption coefficients would, of course, enable a quantitive evaluation of the equilibria involved. However, as it is reasonable to assume that the major species will exhibit similar absorption coefficients, Fig. 3 itself should provide an acceptable representation of their pH distribution. As will now be discussed, the major products for $[Pt(en)(H_2O)_2]^{2+}$–met-Hxaa at 1:1 molar ratio exhibit the κ^2N,S (pH < 8.7) and κ^2N,N' (pH > 8.7) co-ordination modes. Increasing the $[Pt(en)(H_2O)_2]^{2+}$:met-Hxaa ratio to 1:2 provides no further products and leads solely to an increase in the peak area of the free peptide in the resulting chromatogram. In addition to the κ^2N,S complex, two very minor products are observed for the met-Hxaa peptides in acid solution [*e.g.* Fig. 2(*a*)]. These are not found for chromatograms of alkaline reaction solutions [*e.g.* Fig. 2(*b*)]. At high pH values [Fig. 2(*c*)] small peaks for unreacted starting compounds and a further minor product can be identified.

The κ^2N,S co-ordinated complexes $[Pt(en)(met\text{-}Hgly\text{-}\kappa^2N,S)]^{2+}$ and $[Pt(en)(met\text{-}gly\text{-}Hgly\text{-}\kappa^2N,S)]^{2+}$ (Scheme 1) were separated by semi-preparative HPLC and characterised by FAB mass and multinuclear NMR spectroscopy. Their ^1H NMR signals are compared in Table 1 with those of the analogous complexes of Hmcys and Hmet, which may be obtained in high yield in acidic 1:1 reaction mixtures of $[Pt(en)(H_2O)_2]^{2+}$ and the respective amino acid. As may be monitored for met-gly-Hgly,

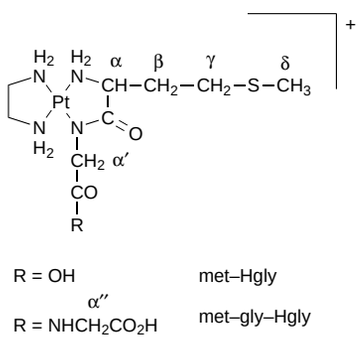
Table 2 Carbon-13 NMR chemical shifts δ (ppm) for [Pt(en)(met-gly-Hgly-κ²*N*,*S*)]²⁺ and met-gly-Hgly

	[Pt(en)(met-gly-Hgly-κ²*N*,*S*)]²⁺	met-gly-Hgly
α-C	56.9	52.8
	57.5	
β-C	30.7	30.2
	31.3	
γ-C	34.4	28.5
	34.5	
δ-C	21.8	14.3
	22.6	
CO	174.8	170.4
	175.2	
α′-C	43.8	42.8
CO′	174.3	170.8
α″-C	44.9	43.5
CO_2″H	175.9	176.7
en C	49.6	
	50.7	
CF_3	121.1	
CF_2	109.8	
CO_2^-	166.1	
pH	2.5	2.4

Table 3 Platinum-195 NMR chemical shifts δ (ppm) for (en)Pt^{II} complexes of met-Hxaa and xaa-Hmet peptides

Complex	δ	pH
[Pt(en)(Hmet-κ²*N*,*S*)]²⁺	−3298*	1.8
[Pt(en)(met-Hgly-κ²*N*,*S*)]²⁺	−3284	2.4
	−3298	
[Pt(en)(met-gly-Hgly-κ²*N*,*S*)]²⁺	−3284	2.4
	−3298	
[Pt(en)(gly-Hmet-κ²*N*′,*S*)]²⁺	−3206	2.1
	−3254	
[Pt(en)(gly-gly-Hmet-κ²*N*″,*S*)]²⁺	−3186	2.3
	−3230	
[Pt(Hen-κ*N*)(gly-met-κ³*N*,*N*′,*S*)]²⁺	−3069	2.1
	−3083	
[Pt(en)(Hamet-κ*S*)₂]²⁺	−3757	1.6

* Unresolved for the R_S and S_S diastereomers.



Scheme 2 The κ²*N*,*N*′-co-ordinated complexes for the 1:1 reaction systems [Pt(en)$(H_2O)_2$]²⁺–met-Hxaa

thioether co-ordination is confirmed for the κ²*N*,*S* complexes by the marked downfield shifts of the δ- and γ-protons, N (amino) co-ordination by the upfield shift of the α-proton at the N-terminal end of the peptides. Co-ordination of the thioether S atom introduces a chiral centre into the six-membered chelate ring, leading to the formation of two diastereomers, as has previously been reported for [Pt(Hmet-κ²*N*,*S*)$(NH_3)_2$]²⁺ and [Pt(Hmcys-κ²*N*,*S*)$(NH_3)_2$]²⁺, which exhibit ^{1}H NMR spectra similar to those of the analogous (en)Pt^{2+} complexes in Table 1.[8,10] A chair conformation has been established by X-ray structural analysis for the chelate ring in [$PtCl_2$(Hmet-κ²*N*,*S*)] and [$PdCl_2$(Hmet-κ²*N*,*S*)].[15,25,26] The diastereomers of the κ²*N*,*S* complexes of met-Hgly and met-gly-Hgly are both present in an approximately 2:1 ratio; their resonances coalesce at *ca.* 343 K.

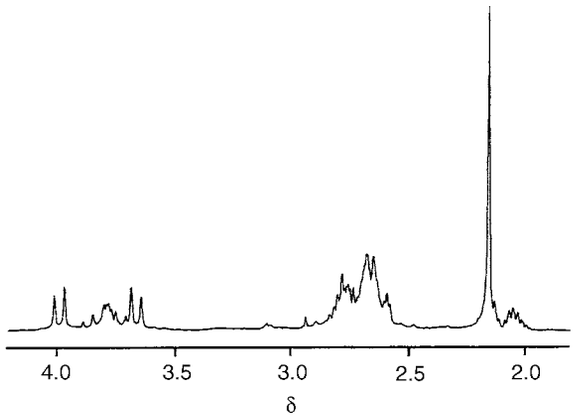


Fig. 4 Proton NMR spectrum of the 1:1 reaction mixture [Pt(en)$(D_2O)_2$]²⁺–met-Hgly at pH 9.5 [reference sodium (3-trimethylsilyl)tetradeuteriopropionate, δ 0.0]

Comparison of the ^{1}H NMR spectra of these separated complexes with those of the 1:1 reaction solutions [Pt(en)$(H_2O)_2$]²⁺–met-Hgly and [Pt(en)$(H_2O)_2$]²⁺–met-gly-Hgly points to the presence of a further resonance of low intensity at δ 3.37 in the latter solutions, which can be assigned to free [H_2en]²⁺. This observation suggests that the two minor products in the chromatograms of the [Pt(en)$(H_2O)_2$]²⁺–met-Hxaa systems in acid solutions could be 2:1 complexes of the type [Pt(met-Hxaa-κ²*N*,*S*)₂]²⁺. Further characterisation of these species was prevented by their low concentration.

Carbon-13 NMR data are presented for [Pt(en)(met-gly-Hgly-κ²*N*,*S*)]²⁺ and met-gly-Hgly in Table 2. As pentafluoropropionic acid was employed as the ion-pairing reagent in the semi-preparative HPLC separation, it also provides the counter ion $C_2F_5CO_2^-$ for the cationic metal complex. The κ²*N*,*S* co-ordination leads to marked shifts to lower frequency for the resonances of the α-, γ- and δ-C atoms. As also registered in the ^{1}H NMR spectrum (Table 1), the presence of diastereomers leads to the observation of two resonances for the atoms of the chelate ring and their immediate substituents. This is not the case for the more distant atoms of the glycine units, which also remain virtually unshifted in comparison to the free peptide. The ^{13}C chemical shifts are in close agreement with the data previously presented[10,25,27] for [Pt(Hmet-κ²*N*,*S*)$(NH_3)_2$]²⁺ and [$PtCl_2$(Hmet-κ²*N*,*S*)]. Further confirmation for the N_3S co-ordination sphere of the square-planar metal atom is provided by the ^{195}Pt NMR data presented in Table 3. The chemical shifts of the κ²*N*,*S*-co-ordinated complexes are similar to those of δ −3147/−3161 reported by Sadler and co-workers[8] for the R_S/S_S diastereomers of *cis*-[Pt(Hmet-κ²*N*,*S*)$(NH_3)_2$]²⁺ and the unresolved value of δ −3218 given by Appleton *et al.*[10] for the diastereomers of *cis*-[Pt(Hmcys-κ²*N*,*S*)$(NH_3)_2$]²⁺. Coupling constants ^3J[^{195}Pt–^{1}H(SCH_3)] of respectively 47.4, 47.9 and 47.4 Hz were determined for the κ²*N*,*S* complexes in Table 3.

Proton NMR spectra of the 1:1 reaction mixtures [Pt(en)$(D_2O)_2$]²⁺–met-Hxaa at pH 9.5 (met-Hgly, Fig. 4) and 9.4 (met-gly-Hgly) allow an unequivocal assignment of the κ²*N* (amino),*N*′ (amide) co-ordination mode (Scheme 2) to the respective major products in alkaline solution (pH > 8.7). The characteristic changes in the ^{1}H NMR peptide chemical shifts which occur on adoption of the κ²*N*,*N*′ co-ordination mode may be seen for met-gly-Hgly in Table 1. An effectively unchanged position for the δ-CH_3 resonance rules out thioether co-ordination in such complexes. κ²*N*,*N*′ Chelation is indicated by the pronounced shift of the α-CH signal to higher frequency and the large difference Δδ in the chemical shifts of the non-equivalent glycyl α′-CH_2 protons (AB system); Δδ increases from 0.10 for free met-gly-Hgly to 0.25 ppm for the κ²*N*,*N*′ complex. In contrast, the analogous difference for the C-terminal glycine α″-CH_2 protons remains virtually unchanged

Table 4 Summary of experimental parameters for the equilibrium system $[Pd(en)(H_2O)_2]^{2+}$–met-Hgly

Solution composition $c_M : c_L$	1:1, 2:1, 1:0 or 0:1
c_M/mol dm^{-3} (per equivalent)	0.004
c_L/mol dm^{-3} (per equivalent)	0.004
I/mol dm^{-3}, electrolyte	0.150, KNO_3
Experimental method	pH Titration, practical pH scale*
T/°C	25 ± 0.1
pH range	
$[Pd(en)(H_2O)_2]^{2+}$ protonation	4.35–11.30
met-Hgly protonation	2.85–11.30
$[Pd(en)(H_2O)_2]^{2+}$–met-Hgly complexation	2.20–11.30
Total number of data points	
$[Pd(en)(H_2O)_2]^{2+}$ protonation	800 (15 titrations)
met-Hgly protonation	583 (10 titrations)
$[Pd(en)(H_2O)_2]^{2+}$–met-Hgly complexation	539 (10 titrations)
Reliability indices R from MINIQUAD[24]	
$[Pd(en)(H_2O)_2]^{2+}$ protonation	0.0065
met-Hgly protonation	0.0160
$[Pd(en)(H_2O)_2]^{2+}$–met-Hgly complexation	0.0213
Protonation constants {M = $[Pd(en)(H_2O)_2]^{2+}$, L = met-gly$^-$}	
log $\beta_{MH_{-2}}$	−15.35 ± 0.01
log $\beta_{M_2H_{-2}}$	−8.36 ± 0.01
log β_{HL}	7.62 ± 0.01
log β_{H_2L}	10.96 ± 0.01
Stability constants	
log $\beta_{M(HL)}$	11.25 ± 0.03
log β_{ML}	8.29 ± 0.02
log $\beta_{MLH_{-1}}$	−0.38 ± 0.04
log $\beta_{M_2LH_{-1}}$	5.74 ± 0.04
log $\beta_{M_2LH_{-2}}$	−2.22 ± 0.04

* $A = pH_{meas} - p[H]_{calc/conc} = 0.09 \pm 0.01$.[22,23]

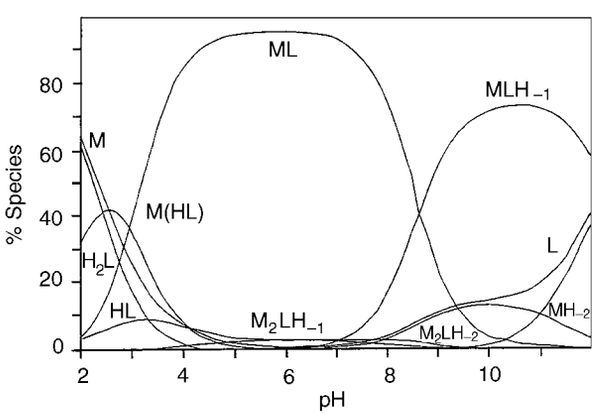


Fig. 5 Species distribution for the 1:1 $[Pd(en)(H_2O)_2]^{2+}$–met-Hgly reaction system (4 mmol dm^{-3}) as determined by potentiometry for the range pH 2.20–11.30

on complex formation (0.05 *vs.* 0.06 ppm). The same effect for the α′-CH_2 protons was reported by Kozlowski and co-workers[28] for the μ-1κ^2*N*,*N*′:2κ*S* bridged dimer formed in the 1:1 reaction between $K_2[PdCl_4]$ and met-Hgly at pH 7.1. These authors also postulated the presence of complexes of the type $[Pd(peptide\text{-}\kappa^2N,N')_2]$ (peptide = met-gly or gly-met) in 1:2 reaction solutions of $K_2[PdCl_4]$–peptide at pH > 13.

Platinum(II) and Pd^{II} have been reported to metallate amide N atoms at pH values as low as 2.[29] Our present studies indicate that the κ^2*N*,*N*′ chelation mode will suppress the κ^2*N*,*S* mode for (en)Pt^{II} complexes of met-Hxaa peptides at pH > 8.7. This finding prompted us to compare the pH dependence of the $[Pt(en)(H_2O)_2]^{2+}$–met-Hgly and $[Pd(en)(H_2O)_2]^{2+}$–met-Hgly reaction systems. Unfortunately, the kinetic lability of (en)Pd^{II} complexes leads to immediate on-column establishment of the species distribution for the eluent pH value (2.1), thereby preventing a pH-dependent HPLC separation. However, just this property is a prerequisite for potentiometric investigations and our results for the pH titration of the $[Pd(en)(H_2O)_2]^{2+}$–met-Hgly reaction system are summarised in Table 4. Whereas our protonation constants ($pK_{a1} = 3.34$, $pK_{a2} = 7.62$) for met-Hgly are in good agreement with literature values,[30–32] important differences were established for $[Pd(en)(H_2O)_2]^{2+}$, due to the fact that previous authors have neglected the dihydroxo complex $[Pd(OH)_2(en)]$ (MH_{-2}), which is present in significant concentrations at pH > 8. The graphical evaluation of the pH titration curve for a 2 mmol dm^{-3} $[Pd(en)(H_2O)_2]^{2+}$ solution led van Eldik and co-workers[33,34] to publish a log $\beta_{MH_{-1}}$ value of −5.6 ± 0.2 for $[Pd(OH)(en)(H_2O)]^+$. Further species were not considered. Lim and Martin[35] presented a log $\beta_{M_2H_{-2}}$ value of −8.33 for $[\{Pd(en)\}_2(\mu\text{-}OH)_2]^{2+}$, the only complex they proposed for a 10.5 mmol dm^{-3} (en)Pd^{II} solution. In a later evaluation of the same pH titration data, Martin[36] revised this log β value to −8.7 by including the species MH_{-1} (log β = −6.2) and $[\{Pd(en)\}_3(\mu\text{-}OH)_3]^{3+}$ (M_3H_{-3}, log β = −12.1). The findings of a recent ^{15}N NMR investigation by Appleton *et al.*[37] on a more concentrated $[Pd(en)(H_2O)_2]^{2+}$ solution (*ca.* 370 mmol dm^{-3}) are in striking contradiction to these potentiometric studies. The diaqua complex $[Pd(en)(H_2O)_2]^{2+}$ is present as the sole species at pH < 4; the dihydroxo species $[Pd(OH)_2(en)]$ plays a similar role at pH 12.3. Two resonances with an intensity ratio of 2.8:1 in neutral solution were assigned to the oligomeric complexes M_3H_{-3} and M_2H_{-2}.

Our titration data for 4 mmol dm^{-3} $[Pd(en)(H_2O)_2]^{2+}$ solutions in the range pH 4.35–11.30 allow the refinement of log β values for only two species, M_2H_{-2} (−8.36) and MH_{-2} (−15.35). The diaqua complex $[Pd(en)(H_2O)_2]^{2+}$ dominates at pH < 5.6, the dihydroxo complex $[Pd(OH)_2(en)]$ at pH > 9.7. The presence of the species M_3H_{-3}, M_4H_{-4} $[\{Pd(en)\}_4(\mu\text{-}OH)_4]^{4+}$, M_2H_{-1} $[\{Pd(en)(H_2O)\}_2(\mu\text{-}OH)]^{3+}$ and MH_{-1} cannot be confirmed at a high level of confidence. For instance, inclusion of the MH_{-1} species $[Pd(OH)(en)(H_2O)_2]^+$, as proposed by van Eldik and co-workers[33,34] and Martin,[36] only leads to a minor improvement of the *R* factor from 0.0065 to 0.0061. Refinement of this model leads to a log β value of −6.26 for MH_{-1} similar to that reported by Martin[36] and to a small change in the stability constant for the dominant dimeric species M_2H_{-2} (−8.43) with log β remaining unaffected for the dihydro complex (−15.35). It is reasonable to conclude that the tetrameric species and the trimeric species of Appleton *et al.*[37] will only be observed at much higher $[Pd(en)(H_2O)_2]^{2+}$ concentrations.

A species distribution for the 1:1 $[Pd(en)(H_2O)_2]^{2+}$–met-Hgly reaction system, as calculated on the basis of the log β values in Table 4, is presented in Fig. 5. Introduction of further species of the type $M_2(HL)$ or M_2L does not lead to a significant improvement in the *R* factor. Comparison of the respectively chromatographic and potentiometric distribution diagrams (Figs. 3 and 5) for the 1:1 reaction systems $[Pt(en)(H_2O)_2]^{2+}$–met-Hgly and $[Pd(en)(H_2O)_2]^{2+}$–met-Hgly indicates a similar pH dependence of N,S and N,N′ chelation for the Group 10 metals. It must be remembered, in this context, that the acidic conditions of the HPLC separation (pH 2.1) will lead to on-column protonation of species of the type ML (κ^2*N*,*S*) and MLH_{-1} (κ^2*N*,*N*′) at their carboxylate O atom with formation of M(HL) (κ^2*N*,*S*) and ML (κ^2*N*,*N*′). The κ^2*N*,*S* chelation in [Pd(en)(met-gly-κ^2*N*,*S*)]$^+$ (ML) leads to a pronounced increase in the pK_a value for amide deprotonation of ML (8.67) in comparison to the $[Pd(en)(H_2O)_2]^{2+}$–gly-Hgly reaction system, for which a value of 3.76 has been reported.[38] This large difference reflects the relative stability of the κ^2*N*,*S* chelate ring of the met-Hgly complex ML, which must be opened to yield the κ^2*N*,*N*′-co-ordinated species MLH_{-1} on deprotonation. As also observed for the $[Pt(en)(H_2O)_2]^{2+}$–met-Hgly system in the chromatogram in Fig. 2(*c*), the concentration of unreacted met-

Table 5 Proton NMR chemical shifts δ (ppm) for (en)Pt^{II} complexes of xaa-Hmet peptides

			$(\kappa S)_2$				
	κ^2N'',S gly-gly-Hmet	κ^3N,N',S gly-Hmet	Hamet*	gly-Hmet	gly-gly-Hmet	gly-gly-Hmet	gly-Hmet
α-CH/CH_2	3.88 (s)	3.47 (m)	4.54 (m)	3.73 (s)	3.72 (s)	3.72 (s)	3.72 (s)
α′-CH/CH_2	4.33 (d), 4.64 (d, AB)	4.13 (m)		4.52 (br)	3.87 (s)	3.87 (s)	4.44 (dd)
	4.37 (d), 4.60 (d, AB)	4.25 (m)					
α″-CH	4.79 (dd), 4.90 (dd)				4.48 (br)	4.33 (m)	
β-, β′- or β″-CH_2	2.0–2.6 (m)	1.83 (m)	2.15 (br)	2.09 (br)	2.07 (br)	1.86 (m)	1.88 (m)
		2.11 (m)	2.32 (br)	2.26 (br)	2.24 (br)	2.00 (m)	2.05 (m)
		2.27 (m)					
		2.34 (m)					
γ-, γ′- or γ″-CH_2	2.6–2.9 (m)	2.40 (m)	3.03 (br)	2.96 (br)	2.93 (br)	2.41 (m)	2.46 (m)
	3.20 (m)	2.77 (m)					
δ-, δ′- or δ″-CH_3	2.39 (s), 2.37 (s)	2.41 (s)	2.55 (s)	2.47 (s)	2.45 (s)	1.94 (s)	1.95 (s)
	2.53 (s), 2.51 (s)	2.45 (s)					
en CH_2	2.6–2.9 (m)	2.92 (m)	2.80 (s)	2.71 (s)	2.69 (s)		
		3.15 (m)					
pH	2.3	2.4	1.9	2.4	2.4	2.4	2.4

* δ 1.99 (s, 3 H, CH_3 of acetyl).

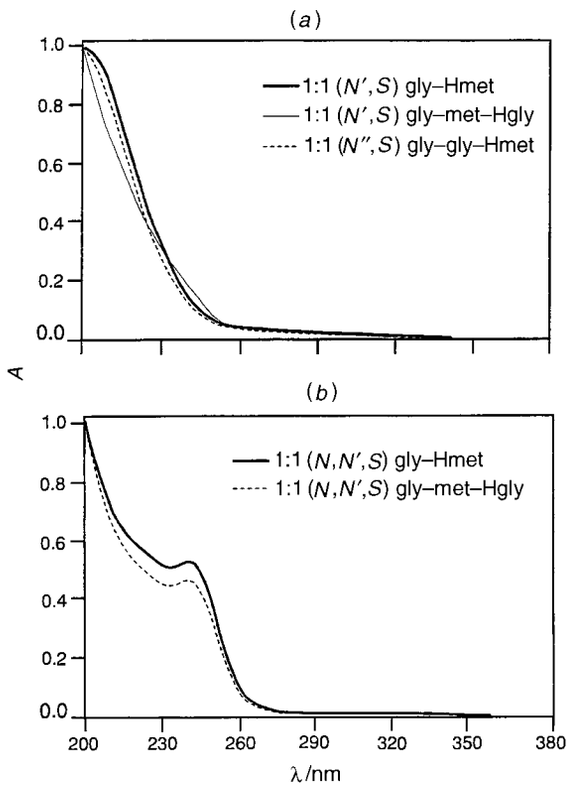
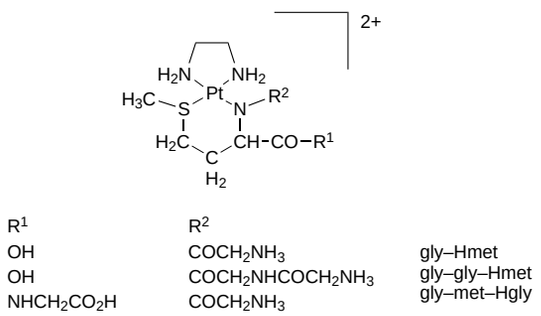


Fig. 6 The UV spectra for (*a*) κ^2N',S- and κ^2N'',S- and (*b*) κ^3N,N',S-co-ordinated complexes of xaa-Hmet peptides. The absorbances *A* were normalised



R^1	R^2	
OH	$COCH_2NH_3$	gly–Hmet
OH	$COCH_2NHCOCH_2NH_3$	gly–gly–Hmet
$NHCH_2CO_2H$	$COCH_2NH_3$	gly–met–Hgly

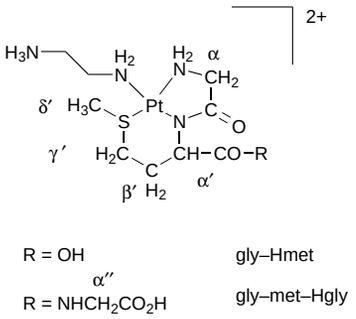
Scheme 3 The κ^2N',S- and κ^2N'',S-co-ordinated complexes for the 1:1 reaction systems $[Pt(en)(H_2O)_2]^{2+}$–xaa-Hmet

Hgly and (en)Pd^{II} species increases markedly at higher pH values. Fig. 5 indicates that the minor products of the reaction of $[Pt(en)(H_2O)_2]^{2+}$ with met-Hgly will most probably be 2:1 species.

Reaction of $[Pt(en)(H_2O)_2]^{2+}$ with the xaa-Hmet peptides gly-Hmet and gly-met-Hgly at a 1:1 molar ratio affords two major products for pH < 6.0. In contrast, only one important species appears in the chromatograms of gly-gly-Hmet and the model compound Hamet under analogous conditions. As will be discussed below, N (amide),S (thioether) chelation (Scheme 3) provides a major complex for all xaa-Hmet peptides in acid solution. A second dominant 1:1 species with tridentate κ^3N (amino),N' (amide),*S* (thioether) co-ordination is formed only by those xaa-Hmet peptides with their methionine unit adjacent to the N-terminal glycine fragment. An increase in the molar ratio to 1:2 leads to the appearance of a complex of the type $[Pt(en)(H_2peptide\text{-}\kappa S)_2]^{4+}$ in the chromatograms of all xaa-Hmet peptides investigated. Semi-preparative separations were successful for $[Pt(en)(gly\text{-}gly\text{-}Hmet\text{-}\kappa^2N'',S)]^{2+}$, $[Pt(Hen\text{-}\kappa N)(gly\text{-}met\text{-}\kappa^3N,N',S)]^{2+}$, $[Pt(en)(gly\text{-}H_2met\text{-}\kappa S)_2]^{4+}$ and $[Pt(en)(gly\text{-}gly\text{-}H_2met\text{-}\kappa S)_2]^{4+}$. The ^{195}Pt and ^1H NMR data for these complexes are in Tables 3 and 5 respectively.

The κ^2N',S-co-ordinated complexes of the other xaa-Hmet peptides studied in this work exhibit UV spectra effectively identical to that of $[Pt(en)(gly\text{-}gly\text{-}Hmet\text{-}\kappa^2N'',S)]^{2+}$ as depicted in Fig. 6(*a*). Thioether co-ordination in the latter complex is confirmed by the pronounced shift of the γ″-CH_2 and δ″-SCH_3 resonances to lower field (Table 5). Metallation of the amide N atom of the methionine unit has a similar effect on the resonance of the adjacent α″-CH proton. In contrast, the N-terminal α-CH_2 protons display a singlet at a position similar to that registered for the free peptide.

Of particular interest is the observation of two typical AB spectra with an intensity ratio of approximately 3:2 for the exocyclic α′-CH_2 protons of $[Pt(en)(gly\text{-}gly\text{-}Hmet\text{-}\kappa^2N'',S)]^{2+}$, a finding that is in accordance with the presence of isomers with the adjacent α′-CO function in either *syn* or *anti* position relative to the Pt–N″ (amide) bond. A similar intensity ratio is found for the resonances of the endocyclic α″-CH proton and the thioether δ″-CH_3 protons, with the latter signals being individually split in a 4:1 ratio by the presence of R_S and S_S diastereomers. Further confirmation of N (amide),S (thioether) chelation is provided by the ^{195}Pt NMR data for 1:1 reaction solutions of $[Pt(en)(D_2O)_2]^{2+}$ with respectively gly-Hmet and gly-gly-Hmet. Both exhibit resonances in a 3:2 ratio at approximately δ −3200 (Table 3), which allow unequivocal assignment of an N_3S co-ordination environment for the Pt atoms of the



Scheme 4 The κ^3N,N',S-co-ordinated complexes for the 1:1 reaction betweeen $[Pt(en)(H_2O)_2]^{2+}$ and gly-Hmet or gly-met-Hgly

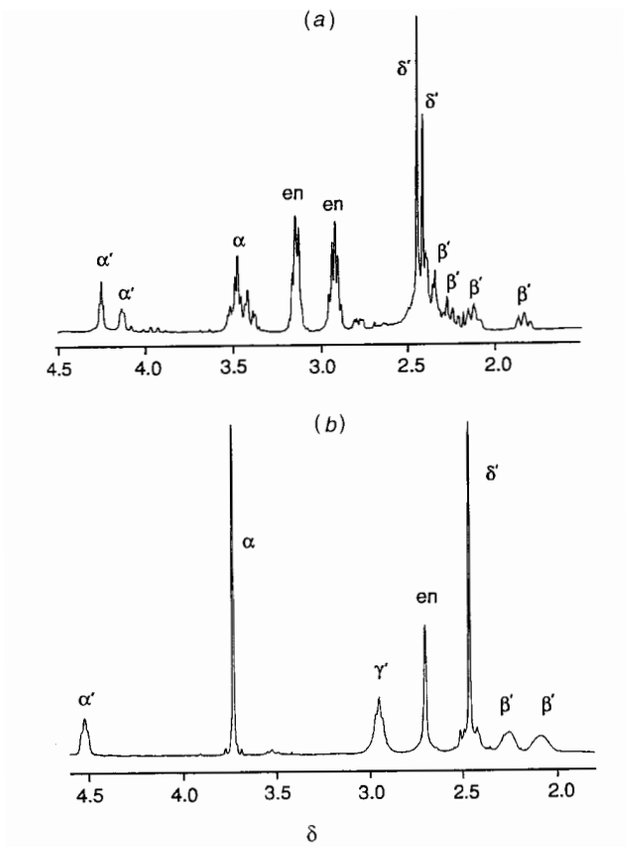


Fig. 7 Proton NMR spectra of (*a*) $[Pt(Hen\text{-}\kappa N)(gly\text{-}met\text{-}\kappa^3N,N',S)]^{2+}$ and (*b*) $[Pt(en)(gly\text{-}H_2met\text{-}\kappa S)_2]^{4+}$ at pH 2.4

isomers discussed above. Resolution of the R_S and S_S diastereomers was not possible. Contrasting ^{195}Pt NMR chemical shifts of about δ −2635 and −3660 have been previously registered for N_2SO and N_2S_2 co-ordination spheres.[10] As expected from the chromatographic separation, a second signal pair of higher intensity is observed in the ^{195}Pt NMR spectrum of the 1:1 $[Pt(en)(D_2O)_2]^{2+}$–gly-Hmet reaction mixture. The resonance positions (δ −3069, −3083) are in accordance with the N_3S environment provided by the κ^3N,N',S co-ordination mode, which is only possible for gly-Hmet and gly-met-Hxaa peptides. The complexes $[Pt(Hen\text{-}\kappa N)(gly\text{-}met\text{-}\kappa^3N,N',S)]^{2+}$ and $[Pt(Hen\text{-}\kappa N)(gly\text{-}met\text{-}gly\text{-}\kappa^3N,N',S)]^{2+}$ are eluted more slowly than their respective κ^2N',S complexes, which also display markedly different UV spectra (Fig. 6).

Conclusive evidence for the monodentate κ*N* en required by the κ^3N,N',S co-ordination mode (Scheme 4) is provided by the ^{1}H NMR spectrum (Fig. 7) of $[Pt(Hen\text{-}\kappa N)(gly\text{-}met\text{-}\kappa^3N,N',S)]^{2+}$, which was separated by semi-preparative liquid chromatography. The co-ordination of the amino and amide N atoms is confirmed by marked shifts of the α-CH_2 and α′-CH

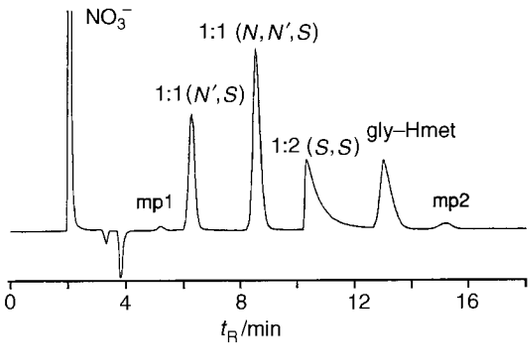


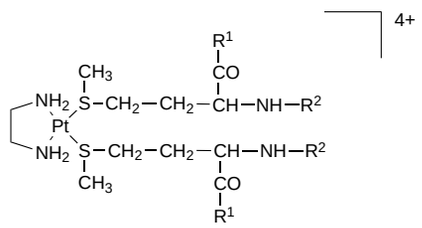
Fig. 8 Chromatogram (mobile phase 96% water–4% MeCN, ion-pairing agent 0.1% pentafluoropropionic acid, pH 2.1, detection wavelength = 220 nm) for the 1:3 $[Pt(en)(H_2O)_2]^{2+}$–gly-Hmet reaction system (mp = minor product) at pH 3.9

resonances to higher field (Table 5), that of the thioether S atom by a pronounced shift for δ′-CH_3 in the opposite direction. Resonances at δ 2.92 and 3.15 can be assigned to en CH_2 protons adjacent to respectively co-ordinated and non-co-ordinated N atoms. The presence of R_S and S_S diastereomers is indicated by the observation of two singlets for the δ′-CH_3 protons (δ 2.41, 2.45). Tridentate κ^3N,N',S co-ordinaiton has been confirmed by X-ray structural analysis for the complexes [PtCl(gly-met-κ^3N,N',S)] and [PdCl(gly-met-κ^3N,N',S)], which may be obtained by reaction of $K_2[PtCl_4]$ or $K_2[PdCl_4]$ with the peptide in acidic solution.[15,39] It is reasonable to assume that formation of κ^3N,N',S complexes will involve cleavage of the Pt–N (en) bond in the respective κ^2N',S complexes, a reaction which will be energetically favoured by the *trans* effect of the co-ordinated thioether S atom. Our results provide the first definitive evidence for a $\kappa^2N \longrightarrow \kappa^1N$ co-ordination change in (en)Pt^{II} complexes of amino acids or peptides. Ismail and Sadler[14] postulated the formation of a minor product with a 'dangling arm' en for the 5:1 reaction of $[Pt(en)(H_2O)_2]^{2+}$ with RNase. They also attributed the efficient release of NH_3 during the reaction of cisplatin with gly-Hmet to the formation of a κ^3N,N',S complex, for which, however, they provided no NMR data.[14]

Chromatographic studies of 1:2 and 1:3 reaction solutions of $[Pt(en)(H_2O)_2]^{2+}$ with the xaa-Hmet peptides, gly-Hmet, gly-met-Hgly and gly-gly-Hmet, show that a third major species of the type $[Pt(en)(H_2peptide\text{-}\kappa S)_2]^{4+}$ (Scheme 5) may be obtained on increasing the peptide concentration in acidic solution. The chromatogram for the 1:3 solution of $[Pt(en)(H_2O)_2]^{2+}$–gly-Hmet contains, in order of elution, peaks for a first minor product, the κ^2N',S, κ^3N,N',S and $(\kappa S)_2$ complexes, gly-Hmet and a second minor product (Fig. 8). Proton NMR data for the $(\kappa S)_2$ complexes of Hamet, gly-Hmet and gly-gly-Hmet are presented in Table 5. The complexes $[Pt(en)(Hgly\text{-}Hmet\text{-}\kappa S)_2][C_2F_5CO_2]_4$ and $[Pt(en)(Hgly\text{-}gly\text{-}Hmet\text{-}\kappa S)_2][C_2F_5CO_2]_4$ were separated by semi-preparative chromatography and characterised by FAB mass spectrometry. Thioether co-ordination is confirmed by the pronounced shift of the δ′- or δ″-CH_3 resonance to lower field for these complexes. In contrast, N (amino) or N (amide) binding can be ruled out by the effectively unchanged positions of the α-CH/CH_2 and α′-CH/CH_2 signals. The presence of two chiral S atoms in such 1:2 complexes means that three diastereomers (R_SR_S, R_SS_S and S_SS_S) will be possible. As depicted for $[Pt(en)(gly\text{-}H_2met\text{-}\kappa S)_2]^{4+}$ in Fig. 7(*b*), the presence of such diastereomers in solution leads to signal broadening for protons close to the chiral centre but not for the more distant α-CH_2 protons. A similar effect was observed by Appleton *et al.*[10] for *cis*-$[Pt(Hmet\text{-}\kappa S)_2(NH_3)_2]^{2+}$. As for the (en)$Pt^{II}$ complexes studied in this work, resolution of the diastereomer resonances was not possible. The ^{13}C NMR chemical shifts presented in Table 6 for the $(\kappa S)_2$ complexes of Hamet and gly-gly-Hmet are in good agreement with those obtained

Table 6 Carbon-13 NMR chemical shifts δ (ppm) for $[Pt(en)(Hamet\text{-}\kappa S)_2]^{2+}$ and $[Pt(en)(Hgly\text{-}gly\text{-}Hmet\text{-}\kappa S)_2][C_2F_5CO_2]_4$

	$(\kappa S)_2$		
	Hamet	gly-gly-Hmet	gly-gly-Hmet
α-C	54.3	40.6	41.1
β- or β″-C	31.8	28.9	31.5
γ- or γ″-C	38.1	35.1	30.1
δ- or δ″-C	22.5	19.7	14.6
CO		167.9	168.3
α′-C		42.4	42.9
CO′		171.4	170.9
CO_2H or $CO_2″H$	177.4	173.8	178.7
CH_3 of MeCO	25.0		
CO of MeCO	177.2		
en C	50.5	47.7	
CF_3		118.6	
CF_2		108.0	
CO_2^-		163.3	
pH	1.9	2.4	2.4



R^1	R^2	
OH	$COCH_2NH_3$	gly–Hmet
OH	$COCH_2NHCOCH_2NH_3$	gly–gly–Hmet
$NHCH_2CO_2H$	$COCH_2NH_3$	gly–met–Hgly

Scheme 5 The $(\kappa S)_2$-co-ordinated complexes for the 1:2 reaction systems $[Pt(en)(H_2O)_2]^{2+}$–xaa-Hmet

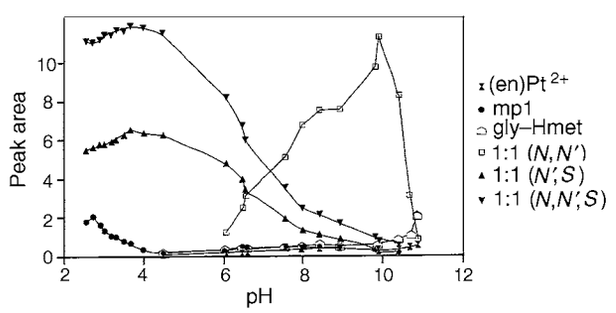
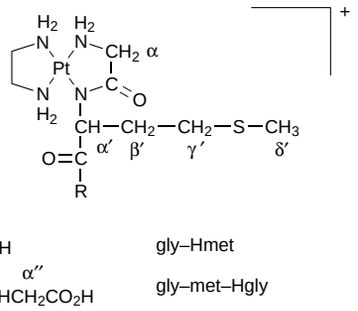


Fig. 9 Species distribution for the 1:1 $[Pt(en)(H_2O)_2]^{2+}$–gly-Hmet reaction system as determined by HPLC (mobile phase 98% water–2% MeCN, ion-pairing agent 0.1% pentafluoropropionic acid, pH 2.1, detection wavelength = 220 nm) for the range pH 2.58–10.89

for $[PtCl_3(Hamet\text{-}\kappa S)]^-$ by Kostic and co-workers,[40] who were also unable to resolve the diastereomers. Further confirmation of the N_2S_2 co-ordination sphere in the $(\kappa S)_2$ complexes is provided by the ^{195}Pt NMR shift of δ −3757 for $[Pt(en)(Hamet\text{-}\kappa S)_2]^{2+}$ (Table 3). Similar values of δ −3639 and −3685 were reported by Appleton *et al.*[10] for *cis*-$[Pt(Hmet\text{-}\kappa S)_2(NH_3)_2]^{2+}$ and assigned to the R_SR_S/S_SS_S and R_SS_S diastereomers. Resolution of the diastereomers was not observed for the Hamet complex studied in our work.

A similar species dependence on pH is found for a 1:1 solution of $[Pt(en)(H_2O)_2]^{2+}$ with the peptides gly-Hmet and gly-met-Hgly. As illustrated in Fig. 9 for gly-Hmet, a third major 1:1 product appears at intermediate pH values and dominates at pH > 7.4. Attempts to separate this new species by semi-preparative HPLC proved to be unsuccessful, so characterisation was restricted to ^{1}H NMR studies of appropriate reaction solutions at higher pH. Spectra recorded under such conditions exhibit broad overlapping resonances thereby preventing an unequivocal assignment. However S (thioether) co-ordination can be ruled out for the third major 1:1 species on the basis of the effectively unchanged position of the δ′-CH_3 resonance. Comparison of the species distrubutions for met-Hxaa and xaa-Hmet peptides (Figs. 3 and 9) suggest that the dominant 1:1 species for gly-Hmet and gly-met-Hgly in alkaline solution will also exhibit the κ^2N,N' co-ordination mode (Scheme 6) established for the former peptides.



Scheme 6 The κ^2N,N'-co-ordinated complexes for the 1:1 reaction between $[Pt(en)(H_2O)_2]^{2+}$ and gly-Hmet or gly-met-Hgly

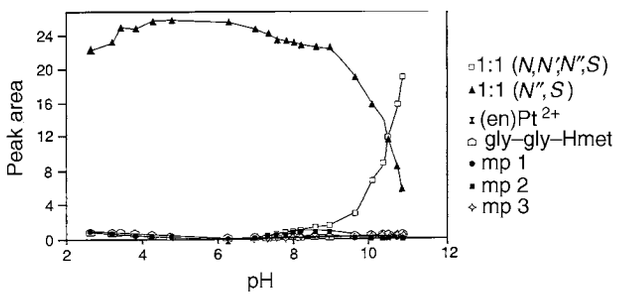


Fig. 10 Distribution of major species of the 1:1 $[Pt(en)(H_2O)_2]^{2+}$–gly-gly-Hmet reaction system as determined by HPLC (conditions as in Fig. 9) for the range pH 2.63–10.89

In contrast to gly-Hmet and gly-met-Hgly, only one major species (κ^2N'',S) can be observed for gly-gly-Hmet in acidic solution (Fig. 10). This complex continues to dominate in alkaline solution up to pH 10.6 with the $\kappa N,N'$ complex presumably only being present as one of a number of minor species in the range pH 6–11. It is reasonable to assume that the new major species in strongly alkaline solution (pH > 10.6) will contain two metallated amide N atoms. The presence of a large number of minor products at pH > 10.6 prevents an unequivocal assignment of the ^{1}H NMR signals in the reaction solution $[Pt(en)(D_2O)_2]^{2+}$–gly-gly-Hmet. Metallation of amide N atoms in peptides is generally accompanied by chelate-ring formation, with the second binding site being a previously co-ordinated terminal N or a donor atom of a side chain.[29] On this basis, the major 1:1 complex of the $[Pt(en)(H_2O)_2]^{2+}$–gly-gly-Hmet system at pH > 10.6 must exhibit either a κ^4N,N',N'',S or a κ^4N,N',N'',O co-ordination mode, both of which require full release of en. Supporting evidence for the former co-ordination mode is provided by the UV spectrum, which is identical to that of the first 1:1 product separated by reversed-phase HPLC from the reaction system $K_2[PtCl_4]$–gly-gly-Hmet (Fig. 11). The S (thioether) co-ordination is confirmed for this and a second 1:1 product present in a reaction solution at pH 2.0 by pronounced shifts of the δ″-CH_3 resonances to lower field.

The present findings demonstrate that κ^2N,S and κ^2N,N' chelation are competitive for the reaction of $[Pt(en)(H_2O)_2]^{2+}$ with met-Hxaa peptides. The former mode dominates for pH < 8.7, the latter in alkaline solution at higher pH values. In contrast κ^2N (amide),*S*-co-ordinated species are observed for xaa-Hmet

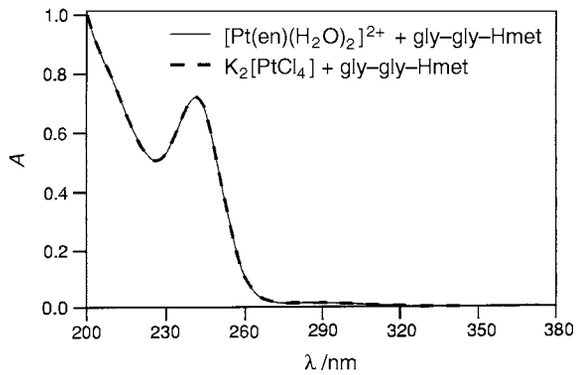


Fig. 11 The UV spectra of 1:1 complexes separated by HPLC from the reaction solutions of gly-gly-Hmet with (*a*) $[Pt(en)(H_2O)_2]^{2+}$ at pH 11.0 and (*b*) $K_2[PtCl_4]$ at pH 2.0. The absorbances *A* were normalised

peptides in acidic solution with cleavage of a Pt–N (en) bond leading to dominant κ^3N,N',S complexes for the peptides gly-Hmet and gly-met-Hgly with their methionine unit adjacent to the N-terminal glycine fragment. These latter peptides afford major κ^2N,N'-co-ordinated complexes in alkaline solution (pH < 11) as observed for met-Hxaa peptides. In contrast κ^2N'',S co-ordination is retained by gly-gly-Hmet under similar conditions.

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