ELSEVIER

Coordination Chemistry Reviews
190–192 (1999) 171–184

COORDINATION CHEMISTRY REVIEWS

www.elsevier.com/locate/ccr

Studies on the interaction of histidyl containing peptides with palladium(II) and platinum(II) complex ions

Panayotis Tsiveriotis, Nick Hadjiliadis *

Department of Chemistry, Laboratory of Inorganic and General Chemistry, University of Ioannina, 451 10, Ioannina, Greece

Accepted 17 February 1999

Contents

* Corresponding author. Fax: +30-651-44831.
E-mail address: nhadjil@cc.uoi.gr (N. Hadjiliadis)

0010-8545/99/$ - see front matter
PII: S0010-8545(99)00076-4

Abstract

The results of our recent studies on the structure of the complexes formed by reaction of the peptides His-Ala, His-Gly-Ala, Pro-Gly-Ala-His and His-Pro-Gly-Ala-His with the complex anions $[Pd(dien)(D_2O)]^{2+}$, $[Pt(dien)(D_2O)]^{2+}$, $[Pd(en)(D_2O)_2]^{2+}$, *cis*-$[Pt(NH_3)_2(D_2O)_2]^{2+}$, $PdCl_4^{2-}$, $PtCl_4^{2-}$ in aqueous solutions at various pD's are reviewed. Multinuclear NMR in one and two dimensions proved to be the method of choice in studying the diamagnetic square planar complexes formed.

Keywords: Antitumour; Histidyl peptides; Palladium complexes; Platinum complexes; Oligopeptides

1. Introduction

Numerous studies on the antitumor properties of platinum(II) drugs concluded that their activity is due to DNA coordination [1]. The great toxicity of these drugs was ascribed to their interaction with sulfur atoms of cysteinyl or methionyl residues in proteins [2]. In this view, systematic studies have been carried out with the aim to determine the nature of such interactions [3].

However, considering the great affinity of platinum(II) for heterocyclic nitrogens like those of histidine imidazole ring, it is surprising that such interactions are not studied to the same extent as those with sulfur donor atoms of amino acid residues in proteins. Thus studies on the coordination behavior of histidine containing peptides to platinum(II) or palladium(II) compounds are scarce even though they are of key importance in the understanding of the mechanism of interaction of platinum drugs with histidyl imidazole nitrogens in proteins [4–6].

In reactions of palladium(II) with histidine peptides, imidazole N3 coordination is preferred when formation of chelate rings is possible [4,5]. These chelate rings involve also coordination of deprotonated amide nitrogens of histidyl and other residues before histidine in the sequence. In these reactions, deprotonation and subsequent coordination of imidazole N3 takes place in strongly acidic solutions.

Platinum(II) reactions with histidine peptides on the other hand, showed monodentate coordination of histidyl imidazole through either N1 or N3 of histidine or its simple derivatives when a salt of the metal with one labile ligand only was used. In some cases histidine imidazole is found to bridge two metal ions by simultaneous coordination of both N1 and N3 [6].

It is noticeable that studies on the interaction of platinum(II) and palladium(II) with peptides possessing histidine as the first amino acid in the sequence or with two histidyl residues did not exist in the literature, though they exist with other metal ions like copper(II) [7,8]. In this article we review our results on the interaction of complex ions like MCl_4^{2-}, $[M(dien)(D_2O)]^{2+}$ (M: Pt or Pd) $[Pd(en)(D_2O)_2]^{2+}$ and *cis*-$[Pt(NH_3)(D_2O)_2]^{2+}$ with the peptides His-Ala, His-Gly-Ala, Pro-Gly-Ala-His and His-Pro-Gly-Ala-His. In His-Ala and His-Gly-Ala [9], histidyl residue is the first in the sequence, while in Pro-Gly-Ala-His the histidyl residue is the last in the sequence. In His-Pro-Gly-Ala-His, the two histidyl residues are first and last in the sequence. They are separated from each other by three amino acid residues, one of which is proline, which possesses a ternary nitrogen acting as a brake point in coordination of peptides to metal ions [10]. The comparison of the results of the three first peptides, help to draw conclusions for the last one, a more realistic model of histidyl proteins.

2. Results

2.1. Reactions of His-Ala and His-Gly-Ala

2.1.1. The ligands His-Ala and His-Gly-Ala

Peak assignments in the ^{1}H- and ^{13}C-NMR spectra of the peptides at various pD's were based on homonuclear (COSY) and heteronuclear (COLOC) correlating bidimensional spectra. Results are summarized in Table 1.

Moreover ^{1}H-NMR/pD titration curves for both peptides allowed the determination by means of Henderson–Hasselbalch equation of the pK_a values for the carboxylate, imidazole and ammonium groups that deprotonate upon addition of base. (Table 2).

2.1.2. Reactions of His-Ala and His-Gly-Ala with $[M(dien)(D_2O)]^{2+}$ [M: Pd(II) or Pt(II)]

2.1.2.1. $[Pd(dien)(D_2O)]^{2+}$. As seen in Table 2, at pD 3.5 imidazole moieties of both peptides are totally protonated. Comparing however the ^{1}H- or ^{13}C-NMR spectra of 1:2 peptide:$[Pd(dien)(D_2O)]^{2+}$ mixtures at pD 3.5 we observe that the peptide is totally consumed and a new product is formed. At the spectra of these products only peaks from imidazole protons or carbons are shifted with respect to the free peptide (Table 1). Obviously total deprotonation of the imidazole moiety takes place, with subsequent binding of one Pd(dien) moiety per each of the two heterocyclic nitrogens [9] (Fig. 1).

Table 1
Selected ^{1}H-, ^{13}C- and ^{195}Pt-NMR data for the various compounds of His-Ala and His-Gly-Ala[a]

Compound	pD	His-α	Imidazole protons			^{195}Pt-NMR
			C2–H	C4–H	C5–H	
HA	3.5	4.16	8.51		7.29	
		(54.52)	(137.46)	(128.17)	(121.73)	
{[Pd(dien)$_2$μ-(N1,N3–HA)]} $^{4+}$	3.5	4.14	7.67		6.78	
		(54.16)	(140.62)	(136.96)	(129.51)	
[Pt(dien)(HA–O)]$^{3+}$	3.5	4.11	8.57		7.28	−2449
		(54.72)	(137.37)	(128.33)	(121.66)	
[Pt(dien)(N3–HA)]$^{+}$	8.5	3.56	7.77		6.98	−2863
[Pt(dien)(N1–HA)]$^{+}$	8.5	3.56	7.59		6.68	−2863
[Pd(en)NH$_2$,N3–HA)]$^{2+}$	1.5	3.45	7.49		6.86	
cis-[Pt(NH$_3$)$_2$(HA–O)$_2$]$^{4+}$	3.5	4.13	8.50		7.29	−2010
			(137.40)	(128.28)	(121.69)	
cis-[Pt(NH$_3$)$_2$(NH$_2$,N3–HA)]$^{+}$	8.5	3.41	7.68		6.91	−2682
cis-[Pd(NH$_2$,N3–HA)Cl$_2$]	1.5	3.31	7.81		6.81	
		(53.28)	(139.84)	(133.31)	(117.45)	
cis-[Pt(NH$_2$,N3–HA)Cl$_2$]	2.0	3.36	8.04		6.88	−2273
		(54.39)	(139.45)	(134.32)	(117.79)	
[Pt(NH$_2$,N3–HA)$_2$]	8.5	3.55	7.60		7.01	−2749
		3.62	7.54		6.97	
		(54.36)	(138.97)	(134.54)	(117.90)	
			(138.60)		(118.32)	
HGA	3.5	4.22	8.54		7.30	
		(54.89)	(137.49)	(128.46)	(121.57)	
{[Pd(dien)$_2$μ-(N1,N3–HGA)]}$^{4+}$	3.5	4.20	7.75		6.85	
		(55.20)	(141.06)	(137.21)	(129.94)	
[Pt(dien)(HGA–O)]$^{3+}$	3.5	4.22	8.53		7.28	−2452
		(54.89)	(137.43)	(128.39)	(128.39)	
[Pt(dien)(N3–HGA)]$^{+}$	8.5	3.54	7.73		6.95	−2880
[Pt(dien)(N1–HGA)]$^{+}$	8.5	3.54	7.55		6.84	−2880
[Pd(en)NH$_2$,N3–HGA)]$^{2+}$	1.5	3.47	7.50		6.88	
cis-[Pt(NH$_3$)$_2$(HGA–O)$_2$]$^{4+}$	3.5	4.19	8.50		7.27	−2010
		(54.87)	(137.42)	(128.37)	(121.57)	
cis-[Pt(NH$_3$)$_2$(NH$_2$,N3–HGA)]$^{+}$	8.5	3.49	7.65		6.89	−2686
cis-[Pd(NH$_2$,N3–HGA)Cl$_2$]	1.5	3.34	7.91		6.87	
		(53.70)	(139.93)	(133.56)	(117.27)	
cis-[Pt(NH$_2$,N3–HGA)Cl$_2$]	2.0	3.38	8.06		6.90	−2279
		54.82	(139.53)	(134.58)	(134.58)	
[Pt(NH$_2$,N3–HGA)$_2$]	8.5	3.57	7.82		6.95	−2750
		3.65	7.53			
		(54.96)	(139.97)	(134.95)	(134.95)	
			(138.90)			

[a] ^{13}C-NMR data are given in parentheses. The one letter abreviation is used for amino acids.

2.1.2.2. [Pt(dien)(D$_2$O)]$^{2+}$. At pD 3.5, [Pt(dien)(D$_2$O)]$^{2+}$ reacts with His-Ala or His-Gly-Ala through carboxylate oxygen as evidenced by ^{195}Pt- and ^{13}C-NMR spectra [9]. At the ^{195}Pt-NMR spectra of 1:1 peptide:[Pt(dien)(D$_2$O)]$^{2+}$ mixtures,

Table 2
pK_a values of His-Ala and His-Gly-Ala

Peptide	$-NH_3^+$	Imidazole$^+$	-COOH	Ref.
His-Ala	7.30	5.90	3.05	[9]
His-Gly-Ala	7.30	6.50	3.40	[9]

peaks appear at the region of -2450 ppm (Table 1) charactaristic of PtN_3O coordination [11] (-2547 ppm for free $[Pt(dien)(D_2O)]^{2+}$). In the ^{13}C-NMR spectra of these mixtures on the other hand, only peaks of carboxylate groups are shifted with respect to the free peptides [9] indicating carboxylate oxygen coordination.

At pD 8.5, Pt(dien) moiety shifts from carboxylate oxygens to imidazole nitrogens (either N1 or N3) to form a mixture of two linkage isomers [9]. New peaks appearing at the region of -2870 ppm in the ^{195}Pt-NMR spectra of the mixtures (Table 1) are characteristic of PtN_4 coordination [11]. At the ^1H-NMR spectra only peaks from imidazole protons are shifted with respect to the free peptide spectra. Thus two pairs of new peaks appear (Table 1) assigned on the basis of literature data to the imidazole protons of the two new products formed.

2.1.3. Reactions of His-Ala and His-Gly-Ala with $[Pd(en)(D_2O)_2]^{2+}$ and cis-$[Pt(NH_3)_2(D_2O)_2]^{2+}$

2.1.3.1. $[Pd(en)(D_2O)_2]^{2+}$. Reaction of $[Pd(en)(D_2O)_2]^{2+}$ with either His-Ala or His-Gly-Ala in strongly acidic aqueous solutions produced an insoluble precipitate. In the supernatant liquid a 1:1 adduct is present with the peptide coordinated to the metal ion through imidazole N3 and the terminal amino nitrogen to form a stable six membered chelate ring (Scheme 1), as the ^1H-NMR spectra revealed [9]. N3 rather N1 coordination is suggested by the larger shift (with respect to the free peptide at the same pD) of peaks from imidazole C2–H as compared to C5–H [9] (Table 1).

2.1.3.2. cis-$[Pt(NH_3)_2(D_2O)_2]^{2+}$. Peaks appearing at the region of -2010 ppm at the ^{195}Pt-NMR spectra of 2:1 peptide:*cis*-$[Pt(NH_3)_2(D_2O)_2]^{2+}$ at pD 3.5 indicate coordination of the peptides to Pt(II) through carboxylate oxygen [9,11] (Table 1). Furthermore at the ^{13}C-NMR spectra of these mixtures only peaks from alanyl carboxylate are shifted as compared to the free peptide spectra [9] thus confirming carboxylate group coordination.

Mixing of the peptides with the named complex ion in 1:1 ratio at pD 8.5 yields 1:1 adducts with the peptide coordinated to the metal ion through amine nitrogen and imidazole N3, forming stable six membered chelate rings [9]. The larger shift

observed for C2–H than for C5–H in the ^{1}H-NMR spectra indicates N3 coordination (Table 1).

Peaks appearing at the region of -2685 ppm in the ^{195}Pt-NMR spectra of the mixtures (Table 1) confirm PtN_4 coordination [9,11].

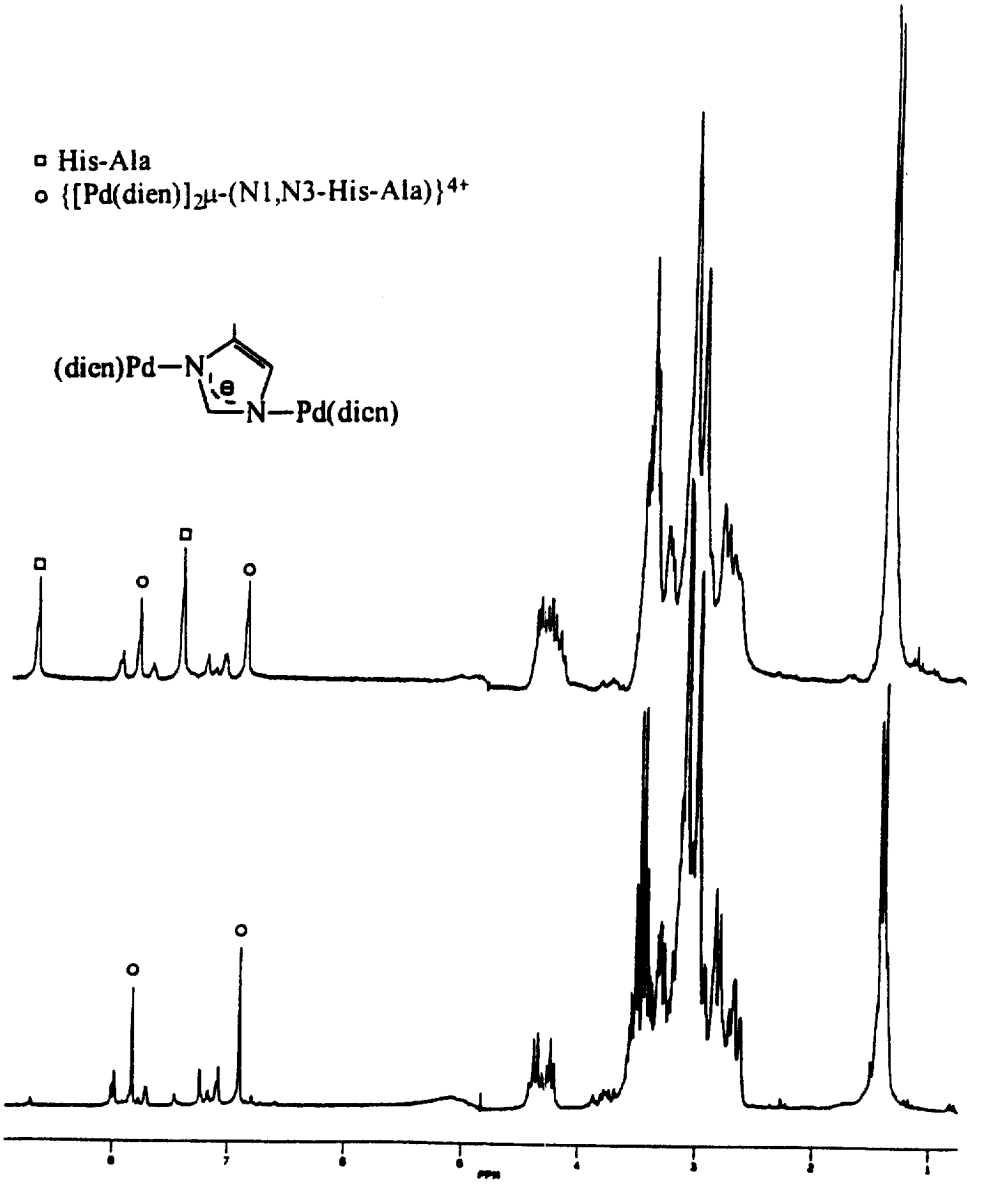
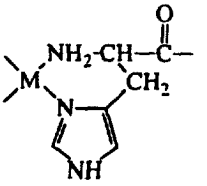


Fig. 1. ^{1}H-NMR spectra of 1:1 (up) and 1:2 (down) $[Pd(dien)(D_2O)]^{2+}$:His-Ala mixtures at pD 3.5. In the scheme is shown an imidazole ring bridging two Pd(dien) moieties.



Scheme 1.

2.1.4. Reactions of His-Ala and His-Gly-Ala with MCl_4^{2-} [M:Pd(II) or Pt(II)]

2.1.4.1. $PdCl_4^{2-}$. Reactions of the peptides with $PdCl_4^{2-}$ in acidic solutions (pD 1) yield 1:1 adducts. Based on the up field chemical shifts of C2–H and C5–H and of the α-histidyl protons, compared to the free peptide at the same pD, (Table 1) the formation of chelate rings through imidazole N3 and the histidyl amino nitrogens was also proposed [9].

This was further supported by the ^{13}C-NMR spectra of the compounds [9].

2.1.4.2. $PtCl_4^{2-}$. In acidic solutions (pD 3.5) both peptides react with Pt(II) through carboxylate oxygen as ^{13}C-NMR spectra reveal [9].

In 1:1 $PtCl_4^{2-}$:peptide mixtures at pD 5–6, 1:1 adducts are formed with the peptide coordinated to the metal through imidazole N3 and the terminal amino nitrogen [9], similar to the Pd(II) analogs. ^{13}C-NMR spectra further support the formation of this six membered chelate ring [9].

In slightly alkaline solutions however, 1:2:peptide adducts are formed. At the ^{1}H-NMR spectrum of 1:2 $PtCl_4^{2-}$:peptide mixtures at pD 8.5 we do not observe peaks from the free peptides after four days of reaction. However two pairs of peaks appear at the aromatic region of the spectra assigned to imidazole C2–H and C5–H, respectively of the new products formed. The larger shift of peaks from C2–H than from C5–H with respect to the free peptide (Table 1) suggests again imidazole N3 coordination. Moreover the upfield shift of histidyl α-protons at the spectra of the complexes as compared to spectra of the free peptides suggest again NH_2 coordination [9]. This is also supported by ^{13}C- and ^{195}Pt-NMR spectra [9,11] (Table 1). These two products formed were assigned to the two possible *cis–trans* isomers [9].

2.2. Reactions of Pro-Gly-Ala-His and His-Pro-Gly-Ala-His

2.2.1. The ligands Pro-Gly-Ala-His and His-Pro-Gly-Ala-His

^{1}H- and ^{13}C-NMR assignments of the free peptides at various pD's were performed by two dimensional homonuclear (COSY, TOCSY) and heteronuclear (HMQC, HMBC) techniques. The results are summarized in Table 3.

Analysis of the ^{1}H-NMR/pD titration curves also allowed us to determine protonation constants for the various groups of the peptides. Protonation constants for these peptides were calculated also by potentiometric techniques. The results are summarized in Table 4.

Table 3
Selected ^{1}H-, ^{13}C- and ^{195}Pt-NMR data for the various compounds[a]

Compound	pD	His^{1}Ca-H	Imidazole protons (carbons)						^{195}Pt-NMR
			C^{1}2-H	C^{1}4	C^{1}5-H	C$^{4/5}$2-H	C$^{4/5}$4	C$^{4/5}$5-H	
PGAH	2.0					8.62		7.32	
						(135.95)	(132.21)	(119.72)	
HPGAH	2.0	4.80	8.72		7.49	8.61		7.31	
		(53.35)	(137.45)	(127.99)	(121.89)	(135.98)	(132.13)	(119.75)	
[Pd(dien)(PGAH–N1)]$^{2+}$	3.5					7.72		6.74	
[Pd(dien)(PGAH–N3)]$^{2+}$	3.5					7.91		7.08	
{[Pd(dien)]$_2$μ-(PGAH–N1,N3)}$^{3+}$	7.0					7.16		6.60	
{[Pd(dien)]$_3$μ-(N1,N3–HPGAH–N1)}$^{5+}$	3.5		7.86		6.96	7.77		6.79	
{[Pd(dien)]$_3$μ-(N1,N3–HPGAH–N3)}$^{5+}$	3.5		7.86		6.96	7.96		7.12	
{[Pd(dien)]$_4$μ-(N1,N3–HPGAH–N1,N3)}$^{6+}$	7.0		7.86		6.96	7.12		6.57	
[Pt(dien)(PGAH–N1)]$^{2+}$	8.5					7.89		6.92	−2874
[Pt(dien)(PGAH–N3)]$^{2+}$	8.5					8.01		7.15	−2874
[Pt(dien)(HPGAH–N1)]$^{4+}$	2.5		8.70		7.50	7.88		6.89	−2872
[Pt(dien)(HPGAH–N3)]$^{4+}$	2.5		8.70		7.50	7.99		7.14	−2872
[Pt(dien)(N1–HPGAH)]$^{4+}$	2.5		[7.88]		6.75	8.61		7.32	−2872
[Pt(dien)(N3–HPGAH)]$^{4+}$	2.5		8.14		7.05	8.61		7.32	−2872
{[Pt(dien)]$_2$μ-(N1–HPGAH–N1)}$^{5+}$	2.5		[7.88]		6.75	7.88		6.89	−2872
{[Pt(dien)]$_2$μ-(N1–HPGAH–N3)}$^{5+}$	2.5		[7.88]		6.75	7.99		7.14	−2872
{[Pt(dien)]$_2$μ-(N3–HPGAH–N1)}$^{5+}$	2.5		8.14		7.05	7.88		6.89	−2872
{[Pt(dien)]$_2$μ-(N3–HPGAH–N3)}$^{5+}$	2.5		8.14		7.05	7.99		7.14	−2872
[Pd(en)(NH$_2$,N3–HPGAH)]$^{3+}$	1.2	3.67	7.72		7.08	8.62		7.32	
		(54.42)	(139.53)		(117.64)	(135.42)	(131.42)	(120.08)	
cis-[Pt(NH$_3$)$_2$(NH$_2$,N3–HPGAH)]$^{3+}$	2.5	3.74	8.02		7.20	8.61		7.30	−2685
		(54.05)	(139.22)		(117.81)	(135.69)	(132.25)	(119.98)	
cis-[Pd(NH$_2$,N3–HPGAH)Cl$_2$]$^{+}$	1.2	3.33	8.06		7.11	8.67		7.37	
		(54.21)	(140.02)	(133.84)	(117.12)	(136.21)	(132.32)	(120.28)	
cis-[Pt(NH$_2$,N3–HPGAH)Cl$_2$]$^{+}$	2.0	3.53	8.21		7.12	8.65		7.34	−2277
		(54.76)	(139.53)	(134.86)	(117.42)	(136.25)	(131.52)	(120.10)	

[a] The one letter abbreviation is used for the amino acids. ^{13}C-NMR chemical shifts are given in parenthese, ambigous data are given in brackets.

2.2.2. Reactions of Pro-Gly-Ala-His and His-Pro-Gly-Ala-His with $[M(dien)(D_2O)]^{2+}$ [M:Pd(II) or Pt(II)]

2.2.2.1. $[Pd(dien)(D_2O)]^{2+}$. Reaction of the tetrapeptide Pro-Gly-Ala-His with the complex ion $[Pd(dien)(D_2O)]^{2+}$ in acidic solution (pD 3.5) yielded a mixture of linkage isomers with Pd(dien) moiety bound to N1 or N3. Peaks appearing at 7.91 and 7.08 ppm in the ^{1}H-NMR spectrum of the mixture were attributed to the N3 bound isomer whereas peaks at 7.72 and 6.74 ppm were attributed to the N1 bound isomer (Table 3) on the basis of comparison with data from the His-Ala and His-Gly-Ala systems and other literature data [6,9,12].

When the pD of an 1:2 Pro-Gly-Ala-His:$[Pd(dien)(D_2O)]^{2+}$ was raised above 4.5 two new peaks appeared in the ^{1}H-NMR spectrum of the mixture at 7.16 and 6.60 ppm. On the basis of comparisons with similar systems of His or Ac-His these new peaks were assigned to imidazole protons of a product where imidazole ring bridges two Pd(dien) moieties bound to both N1 and N3 nitrogens [12] (Table 3).

In the ^{1}H-NMR spectra of acidic mixtures (pD 3.5) of the pentapeptide His-Pro-Gly-Ala-His with $[Pd(dien)(D_2O)]^{2+}$ in ratios of 1:3, new peaks appearing at 7.96, 7.86, 7.77, 7.12, 6.96 and 6.79 ppm were observed. Peak assignments were based on comparisons with analogous systems reported here (Table 3). Under these conditions we proposed the formation of a mixture of two linkage isomers with two Pd(dien) moieties bound to both histidyl-1-imidazole nitrogens with a third Pd(dien) moiety bound to either N1 or N3 of the histidyl-5-imidazole ring [12] (Scheme 2).

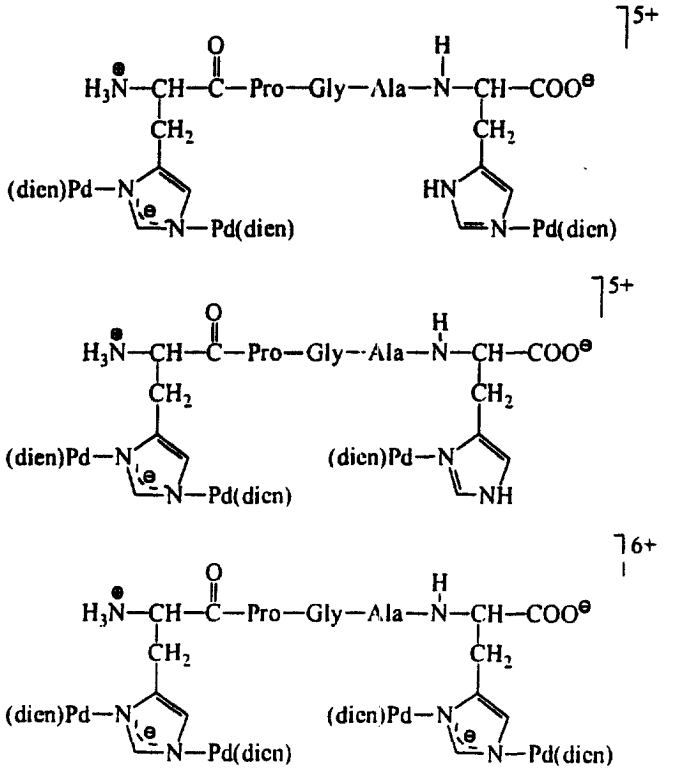
Increasing the quantity of $[Pd(dien)(D_2O)]^{2+}$ added and the pD to above 7, peaks at 7.77, 6.79, 7.96 and 7.12 ppm progressively disappear and two new peaks appeared at 7.12 and 6.57 ppm assigned to histidyl-5-imidazole C2–H and C5–H in a product where four Pd(dien) moieties are bound to all imidazole nitrogens of the peptide [12] (Table 3).

2.2.2.2. $[Pt(dien)(D_2O)]^{2+}$. In acidic solutions (pD 3.5) both Pro-Gly-Ala-His and His-Pro-Gly-Ala-His react with the named complex ion through carboxylate oxygen. Peaks appearing at -2502 ppm in the ^{195}Pt-NMR spectra of the mixtures indicate a PtN_3O coordination sphere for the complex formed [11,12].

Table 4
pK_a values of His-Ala and His-Gly-Ala[a]

Peptide	$-NH_3^+$	$Im^{(1)+}$	$Im^{(4/5)+}$	–COOH	Ref.
Pro-Gly-Ala-His	8.95		7.20	(2.76)	[12]
His-Pro-Gly-Ala-His	7.60	6.95	5.70	(2.52)	[12]

[a] Values in parentheses are calculated only by potentiometry [13,14].



Scheme 2.

In the ^{1}H-NMR spectrum of a 1:1 mixture of Pro-Gly-Ala-His with [Pt(dien)$(D_2O)]^{2+}$ at pD ca. 8.5 peaks at 7.89 and 8.01, and at 6.92 and 7.15 ppm for imidazole C2–H and C5–H protons, respectively are observed (Table 3). These were assigned to two linkage isomers where the peptide is bound to the metal ion through N1 or N3, respectively. A peak appearing in -2874 ppm at the ^{195}Pt-NMR spectum of the above mixture further confirm the proposed PtN_4 coordination mode [11,12] (Table 3).

His-Pro-Gly-Ala-His reacts also at pD 8.5 through imidazole nitrogens with [Pt(dien)$(D_2O)]^{2+}$. In the ^{1}H-NMR spectrum of an 1:1 His-Pro-Gly-Ala-His-[Pt(dien)$(D_2O)]^{2+}$ mixture at pD 8.5 new peaks at 8.14, 7.99, 7.88, 7.14, 7.05, 6.89, 6.75 and 5.17 ppm are observed. Based on comparisons with analogous systems reported in this paper we were able to assign these peaks to eight possible products [12] as seen in Table 3. Integration of the imidazole protons of the various linkage isomers, show that imidazole N3 coordination is preferred over N1 in the complexes with both peptides. ^{195}Pt-NMR is consisted with the formation of such products. Peaks appearing at -2872 ppm indicate PtN_4 coordination [11,12].

2.2.3. Reactions of Pro-Gly-Ala-His and His-Pro-Gly-Ala-His with $[Pd(en)(D_2O)_2]^{2+}$ and cis-$[Pt(NH_3)(D_2O)_2]^{2+}$

2.2.3.1. $[Pd(en)(D_2O)_2]^{2+}$. In the ^{1}H-NMR spectrum of mixtures of the tetrapeptide with $[Pd(en)(D_2O)_2]^{2+}$ at pD ca. 1.0, the prolyl α-proton is shifted from 4.42 ppm in the free peptide to 4.07 ppm. This shift indicates prolyl amine coordination. Moreover new peaks appear at 8.02, 7.85, 7.09 and 6.89 ppm were assigned to imidazole C2–H and C5–H protons in products formed by coordination of either N1 or N3 to the metal ion. This assigment was based on comparisons to the shifts of the tetrapeptide with the $[Pd(dien)(D_2O)]^{2+}$ system (Table 3). From these results however it was not possible to propose formation of chelate complexes or of compounds with oligomeric structure [13].

Reaction of the pentapeptide His-Pro-Gly-Ala-His with the named complex ion in strongly acidic solution (pD 1.2) yielded 1:1 adducts with the peptide coordinated to the metal through NH_2 and imidazole N3 of the histidyl-1 residue forming the known six membered stable chelate ring [12]. This is shown from the ^{1}H-NMR spectra of the reaction mixtures. The intensity of peaks from histidyl-1 protons decreases and new peaks appear at 7.71, 7.08 and 3.67 ppm assigned to imidazole C2–H, C5–H and α-protons of histidyl-1 residue (Table 3). These peaks are found upfield shifted by 0.69, 0.23 and 1.13 ppm, respectively compared to the spectrum of the free peptide at the same pD. The larger shift of the peak from C5–H and the shift of the peak from the α-proton suggest coordination of the peptide through imidazole N3 and amine nitrogens. ^{13}C-NMR data are in agreement with the proposed structure [12].

2.2.3.2. *cis*-$[Pt(NH_3)_2(D_2O)_2]^{2+}$. Although it is not possible to determine the structure of the products formed from the reaction of Pro-Gly-Ala-His with *cis*-$[Pt(NH_3)_2(D_2O)_2]^{2+}$ at pD 8.5, ^{1}H-NMR spectroscopy suggests involvement of imidazole nitrogens in coordination [13].

In alkaline solutions (pD 8.5) the pentapeptide His-Pro-Gly-Ala-His reacts with *cis*-$[Pt(NH_3)_2(D_2O)_2]^{2+}$ forming 1:1 adducts. As seen in the ^{1}H-NMR spectrum of a 1:1 His-Pro-Gly-Ala-His:*cis*-$[Pt(NH_3)(D_2O)_2]^{2+}$ mixture at pD 8.5 new peaks appear at 8.02, 7.20 and 3.74 ppm (Table 3) with a subsequent decrease in the intensity of peaks only from imidazole protons of the histidyl-1 residue. These new peaks were assigned to imidazole C2–H, C5–H and α-protons of the histidyl-1 residue, respectively in the new complex formed. The larger shift of the C2–H peak than that of C5–H peak compared to the free peptide in acidic solutions where both imidazole nitrogens are protonated, suggests imidazole N3 coordination. The upfield shift of the α-proton in the complex with respect to the free peptide also indicates coordination of the amino group. Thus a six membered chelate ring is also formed in this case [12].

^{13}C- and ^{195}Pt-NMR are also in agreement with the structure proposed [11,12] (Table 3).

2.2.4. Reactions of Pro-Gly-Ala-His and His-Pro-Gly-Ala-His with MCl_4^{2-} [M: Pd(II) or Pt(II)]

2.2.4.1. $PdCl_4^{2-}$. When the tetrapeptide Pro-Gly-Ala-His reacts with K_2PdCl_4 in a 1:1 ratio, different products are formed depending on the pD of the solution. Although the ^{1}H-NMR spectra of these solutions were very complicated we were able to determine the structure of the various products formed in combination with potentiometric techniques [14].

At pD < 1.2 a mixture of two 1:1 adducts is formed with the peptide coordinated to the metal ion through the amino nitrogen and either imidazole N1 or N3 [14]. At pD ca. 1.2–2.5 the bond between the metal ion and imidazole N1 dissociates and a new product is formed with the peptide coordinated to the metal through terminal amine, histidyl amine and imidazole N3 nitrogens [14]. At pD 3.76 the carboxylate group of this latter product deprotonates [14]. The products at pD > 4 however, were formed very slowly, so that potentiometric equilibrium techniques were not possible to apply and therefore not possible structures could be proposed.

Reaction of the pentapetide His-Pro-Gly-Ala-His with K_2PdCl_4 (1:1) in acidic solutions at pD 1.2 yielded an 1:1 adduct with the metal ion coordinated through amine and histidyl-1-imidazole N3 nitrogens forming a six membered chelate ring [12]. This is shown by the ^{1}H-NMR spectrum of the mixture where new peaks appear at 8.06, 7.11 and 3.33 ppm assigned to imidazole C2–H, C5–H and α-protons of histidyl-1 residue in the product formed (Table 3). The larger shift of the peak from imidazole C2–H than from C5–H and the shift of the peak from the α-proton of histidyl-1 residue in the complex compared to the one of the free peptide, suggest imidazole N3 and amine nitrogens coordination. ^{13}C-NMR also supports the structure proposed for the new complex.

At higher pD, coordination of histidyl-5 donor atoms was also observed but the structure of the products formed could not be further investigated due to the complexity of the spectra.

2.2.4.2. $PtCl_4^{2-}$. The complexity of the ^{1}H-NMR spectra of mixtures of the tetrapeptide Pro-Gly-Ala-His with $PtCl_4^{2-}$ in alkaline solutions did not allow detailed analysis of the structures of the species formed but give only indications of the involvement in bonding of imidazole nitrogens, and terminal amino groups [12].

The pentapeptide His-Pro-Gly-Ala-His reacts with $PtCl_4^{2-}$ in slightly acidic solutions (pD 5–6) to form 1:1 chelates. The peptide is bound again through N3 and the amine nitrogens as can be seen from the chemical shifts of the various imidazole and α-protons of the histidyl-1 residue, in the ^{1}H-NMR spectra (see Table 3). ^{13}C- and ^{195}Pt-NMR support the structure proposed for the complex formed [12]. In alkaline solutions, solid products of polymeric structures were formed as evidenced by the broad peaks appearing in their ^{1}H-NMR spectra in DMSO-d_6 solution.

3. Discussion

The present studies show that imidazole nitrogens are thermodynamically the most favorable coordination sites of the various peptides used. The preference for carboxylate oxygen observed in the reactions of the platinum complex ions may be the result of the kinetic control of these reactions and not of the thermodynamic stability of the products formed, since this preference is not observed in the reactions of the analogous palladium complex ions [6,9,12].

From the two imidazole nitrogens the most preferred coordination site is the N3 in all cases. This can be explained by the facts, first that N1 protonation is more extensive, and this implies a higher concentration of deprotonated N3. Second, the formation of a stable six membered chelate ring takes place through coordination imidazole N3 and amine nitrogen.

Whenever a second group of high basicity is provided by the ligand, this is coordinated in priority even when formation of a six membered chelate ring through N3 and amine nitrogen is possible. For example, in the case of the reactions of Pro-Gly-Ala-His with K_2PdCl_4 or $[Pd(en)(D_2O_2]^{2+}$ histidyl amide nitrogen do not react in priority because a terminal amine, a group of higher basicity, is provided by the ligand. In this case the six membered chelate ring is formed at higher pD's. Similar behavior was also observed in the reactions of the pentapeptide His-Pro-Gly-Ala-His where coordination takes place first through histidyl-1 donor groups of high basicity, providing also the possibility of formation of a six membered chelate ring. Coordination of histidyl-5 is not observed even though this offers the possibility of formation of more than one chelate rings. This should be due to the lower basicity of amide nitrogen of histidyl-5 residue. The possibility of formation of a six membered chelate ring which usually governs the coordinating behavior of simple histidine peptides, may be cancelled by the presence of a second anchoring group far enough from the imidazole residue.

The coordinating behavior of the peptides studied, in their reactions with $[Pd(dien)(D_2O)]^{2+}$ depend on the position of histidyl residue in the peptide sequence. When histidine is the amino terminal residue with its carboxylate involved in a peptide bond its imidazole ring totally deprotonates in strongly acidic solutions in the presence of $[Pd(dien)(D_2O)]^{2+}$. In these solutions imidazole ring bridges two Pd(dien) moieties simultaneously bound to both N1 and N3. On the other hand when histidine is the carboxylate terminal residue two linkage isomers are formed in their reactions with $[Pd(dien)(D_2O)]^{2+}$. In these isomers Pd(dien) moiety is bound to either N1 or N3. Imidazole ring bridges two Pd(dien) moieties only in neutral or alkaline solutions. This behavior is also observed in reactions of His or Ac-His [6].

In addition as seen in Table 5, when histidyl imidazole bridges two Pd(dien) moieties its protons are more shielded when the histidyl residue is the C terminal than when it is the N terminal amino acid. This difference might be the result of local electronic effects. The repulsive interaction of the net negative charge localized on the imidazole ring with that of the carboxylate group being in its vicinity may impose a relative orientation of the imidazole ring with respect to the carboxylate

Table 5
^{1}H-NMR data for imidazole protons in compounds where a histidyl imidazole ring bridges two Pd(dien) moieties[a]

Compound	C2–H	C5–H	Ref.
{[Pd(dien)]$_2$μ-(N1,N3–HA)}$^{4+}$	7.67	6.78	[10]
{[Pd(dien)]$_2$μ-(N1,N3–HGA)}$^{4+}$	7.75	6.85	[10]
{[Pd(dien)]$_2$μ-(Ac–H–N1,N3)}$^{2+}$	7.17	6.57	[6]
{[Pd(dien)]$_2$μ-(PGAH–N1,N3)}$^{3+}$	7.16	6.60	[13]
{[Pd(dien)]$_2$μ-(N1,N3–HPGAH–N1)}$^{3+}$	7.86	6.96	[13]
{[Pd(dien)]$_2$μ-(N1,N3–HPGAH–N3)}$^{3+}$	7.86	6.96	[13]
{[Pd(dien)]$_2$μ-(N1,N3–HPGAH-N1,N3)}$^{3+}$	7.86(Im1) 7.12(Im5)	6.96(Im1) 6.57(Im5)	[13]

[a] The one leter abbreviation is used for the amino acids.

group, causing the observed shielding of the imidazole ring protons. These local electronic effects may be the reason also of the observed difference in the reactivity of the imidazole ring nitrogens depending on the position of histidyl residues in the peptide sequence.

In the reactions of $[Pt(dien)(D_2O)]^{2+}$, in all cases one Pt(dien) moiety is bound per imidazole ring with N3 binding favored over N1. Formation of imidazole bridging complexes is observed only in strongly alkaline solutions and in the presence of a large excess of $[Pt(dien)(D_2O)]^{2+}$.

References

[1] S.J. Lippard, J.M. Berg, Principles of Bioinorganic Chemistry, University Science Books, Mill Valley, CA, 1994.
[2] R.F. Borch, M.E. Pleasants, Proc. Natl. Acad. Sci. USA 76 (1979) 6611.
[3] (a) K.J. Barnham, M.I. Djuran, P.D.S. Murdoch, P.J. Sadler, J. Chem. Soc. Dalton Trans. (1994) 721. (b) K.J. Barnham, M.I. Djuran, P.D.S. Murdoch, J.D. Ranford, J.P. Sadler, J. Chem. Soc Dalton Trans. (1995) 3721.
[4] (a) D.L. Rabenstein, A.A. Isab, M.M. Shoukry, Inorg. Chem. 21 (1982) 3234. (b) J.-P. Laussac, M. Pasdeloup, N. Hadjiliadis, J. Inorg. Biochem. 28 (1987) 227.
[5] M. Wienken, E. Zangrando, L. Randaccio, S. Menzer, B. Lippert, J. Chem. Soc. Dalton Trans. (1993) 3349
[6] T.G. Appleton, F.J. Pesch, M. Wienken, S. Menzer, B. Lippert, Inorg. Chem. 31 (1992) 4410.
[7] T. Kiss, Biocoordination Chemistry: Coordination Equilibria in Biologically Active Systems, Ellis Horwood, London, 1990, p. 56.
[8] (a) H. Aiba, A. Yokoyama, H. Tanaka, Bull. Chem. Soc. Jpn. 47 (1974) 136. (b) A. Yokoyama, H. Aiba, H. Tanaka, Bull. Chem. Soc. Jpn. 47 (1974) 112.
[9] P. Tsiveriotis, N. Hadjiliadis, G. Stavropoulos, Inorg. Chim. Acta 261 (1997) 83.
[10] I. Sovago, Biocoordination Chemistry: Coordination Equilibria in Biologically Active Systems, Ellis Horwood, London, 1990, p. 135.
[11] P.S. Pregosin, Coord. Chem. Rev. 44 (1982) 247.
[12] P. Tsiveriotis, N. Hadjiliadis, J. Chem. Soc. Dalton Trans. (1999) 459.
[13] P. Tsiveriotis, N. Hadjiliadis, unpublished results.
[14] P. Tsiveriotis, N. Hadjiliadis, I. Sovago, J. Chem. Soc. Dalton Trans. (1997) 4267.