Interaction of *cis*-$Pd(guo)_2Cl_2$ with Amino Acids

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Abstract

The reactions of the complex *cis*-$Pd(guo)_2Cl_2$ with the sodium salts of the amino acids gly, L-ala, L-val, L-ileu, L-pro and L-phe, were studied in methanolic solutions. Complexes of the general formulae *cis*-$[Pd(guo)_2(amac)]Cl$, with the amino acids coordinated to Pd(II) with the N atom of their amino groups and the O atom of their carboxylate groups were isolated from these reactions. These were further reacted with dilute hydrochloric acid to produce complexes of the formulae *cis*-$[Pd(guo)_2(amacH)Cl]Cl$, with the amino acid coordinated only through the $-NH_2$ group. All the isolated complexes in the solid state were characterized with elemental analysis, conductivity measurements, IR and ^1H NMR spectra. Many isomers corresponding to 'head to head', 'head to tail' and 'tail to tail' orientations of the nucleosides, with two major ones called 'closed' and 'opened' forms in 1:1 ratio, with strong and weak inter- or intra-molecular ligand–ligand hydrophobic interactions were observed with ^1H NMR, in D_2O solutions, for the complexes. In DMSO-d_6 the 'opened' form with weak ligand–ligand interactions is favored (~80–90%). Possible ligand–ligand interactions are detected in the ^1H NMR spectra of the compounds. The *anti* conformation of the sugar moiety of guo is found to increase in the 'closed' forms of the *cis* ternary complexes, indicating that the model DNA–Pt–protein crosslink may be responsible for the toxicity of the platinum drugs, increasing with increase of the DNA–protein hydrophobic interactions.

Introduction

The significance of DNA–protein interactions that take place *in vivo* through the interactions of metal ions, has been recognized in many cases [1, 2]. The antitumor drug *cis*-DDP, which exhibits its antitumor action through a direct interaction with DNA during its replication, is known together with its *trans* congener, to form DNA–Pt–protein crosslinks in the body [3–7]. The significance of such crosslinks is not presently well understood.

Such crosslinks caused by Pt(II) drugs can be studied *in vitro* by making the simplest models of them, constituted by the metal or the analogous Pd(II) and nucleobases or nucleosides–nucleotides and amino acid–peptides.

Studying such models, we recently reported the ternary complexes formed from the interactions of *cis*-$Pt(ino)_2Cl_2$ [8, 9], together with a preliminary account of the interaction of *cis*-$Pd(guo)_2Cl_2$ with amino acids of increasing aliphatic side chain, e.g. gly, L-ala, L-val, L-ileu, L-pro and L-phe [8]. In the case of the Pd(II) complexes of guo and amino acids, we had noticed the presence of many rotational or other isomers in D_2O solutions, with ^1H NMR spectra.

In the present paper we report details of these interactions, trying to identify the various species existing in solution, to detect ligand–ligand interactions and correlate the results with other similar systems [10, 11].

Results and Discussion

Ternary complexes of Pd(II) with guo and am-acH, were synthesized as follows. After the synthesis of the starting material *cis*-$Pd(guo)_2Cl_2$, according to a known method [12], a similar method to the one employed for the corresponding Pt(II) complexes [8, 9] was employed, consisting of treatment of the starting Pd(II) complex, with the sodium salt of the amino acids, in methanolic solutions. The amino acids used were: glycine (glyH), L-alanine (alaH), L-proline (proH), L-valine (valH), L-isoleucine (ileuH) and L-phenylalanine (pheH).

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$$cis\text{-}Pd(guo)_2Cl_2 + amacNa \xrightarrow{MeOH}$$

$$cis\text{-}[Pd(guo)_2(amac)]Cl + NaCl \quad (1)$$

When the *cis*-$[Pd(guo)_2(amac)]Cl$ complexes were treated with dilute solutions of hydrochloric acid, they underwent the reaction

$$cis\text{-}[Pd(guo)_2(amac)]Cl + HCl \longrightarrow$$

$$cis\text{-}[Pd(guo)_2(amacH)Cl]Cl \quad (2)$$

The elemental analysis of the complexes given in Table 1, agree with the assigned formulae.

The molar conductance values of the first series of complexes correspond to 1:1 electrolytes in aqueous solutions, while they are somewhat larger in the second series, increasing further with time, due to both the chloride and the hydrolysis of the free –COOH group of the amino acid (see Table 1).

IR Spectra

The characteristic IR bands of the complexes are given in Table 2.

The region of 2800–3600 cm^{-1} is covered by a very strong and broad band with various submaxima, due to the νNH of guo and the amino acids, the νOH of the sugar of guo and the aliphatic and aromatic νCH motions. Deuteration of the complexes results to the appearance of a new strong and broad band at 2300–2550 cm^{-1}, due to νN–D and νO–D motions ($\nu NH_2/\nu ND_2 = 1.33$).

The region of 1500–1700 cm^{-1} for all the complexes is characterized by a strong band at about 1690 cm^{-1}, due to the free νC=O of the 6th position of guo [10, 12] (see Table 2). Bands attributable to the ring νC=C and νC=N motions of guo are also shown almost invariably at about 1590 and 1530 cm^{-1} for all the complexes.

The $\nu^{\alpha}_{COO^-}$ of the coordinated carboxylate group coincides with the δNH_2 of guo at 1629 cm^{-1} for the complex *cis*-$[Pd(guo)_2(gly)]Cl$, at 1635 cm^{-1} for *cis*-$[Pd(guo)_2(ala)]Cl$ and *cis*-$[Pd(guo)_2(phe)]Cl$, at 1638 cm^{-1} for *cis*-$[Pd(guo)_2(pro)]Cl$ and *cis*-$[Pd(guo)_2(val)]Cl$ and at 1637 cm^{-1} for *cis*-$[Pd(guo)_2(ileu)]Cl$. This band diminishes in intensity upon deuteration of the complexes, or in the second series of complexes of the type *cis*-$[Pd(guo)_2(amacH)Cl]Cl$ where the protonated free carboxylate group (–COOH) contributes to the increase of the intensity of the band near 1690 cm^{-1}, due also to the νC=O of the carbonyl group at the 6th position of guo. These findings give good indications for the bidentate coordination ($-NH_2$, $-COO^-$) of the amino acids in the first series of complexes and for the monodentate ($-NH_2$) coordination in the second [8, 9], the latter also supported by the appearance of a medium band, due to the νPd–Cl near 330 cm^{-1} and absent in the first series of complexes.

The $\Delta\nu = \nu^{\alpha}_{COO} - \nu^{\delta}_{COO}$ is found at 217 cm^{-1} for the complex *cis*-$[Pd(guo)_2(gly)]Cl$, 220 cm^{-1} for *cis*-$[Pd(guo)_2(ala)]Cl$ and *cis*-$[Pd(guo)_2(val)]Cl$, 224 cm^{-1} for *cis*-$[Pd(guo)_2(pro)]Cl$, 225 cm^{-1} for *cis*-$[Pd(guo)_2(phe)]Cl$ and 239 cm^{-1} for *cis*-$[Pd(guo)_2(ileu)]Cl$. This difference, which reflects the degree of covalency of the metal–oxygen bond [13], follows the order ileu > phe > pro > val ~ ala > gly, e.g. increases with the bulkier side substituent of the amino acid. A similar behavior was also found with analogous Pt(II) complexes [14].

Free guo shows medium intensity bands at 824 and 801 cm^{-1} [15–17]. These two bands were found to be associated with the percentage of the $C_{3'}$-*endo* and $C_{2'}$-*endo* conformation of the sugar. Thus, the intensity of the first decreases and of the second increases as the $C_{3'}$-*endo* conformation of the sugar increases upon coordination of Pt(II) with guo at N_7 [15–17]. Similar behavior is observed in all the complexes of the present study, showing a decrease of the band at 820 cm^{-1} and an increase of the one at 798 cm^{-1} (see Table 2), compared to free guo. This indicates an increase of the $C_{3'}$-*endo* conformation of

TABLE 1. Elemental analysis and conductivity values of the complexes

Complexes	Pd (%)		Cl (%)		C (%)		N (%)		H (%)		Λ_M ($cm^2\ \Omega^{-1}\ mol^{-1}$)
	Calc.	Found	Calc.	Found	Calc.	Found	Calc.	Found	Calc.	Found	
cis-$[(guo)_2Pd(gly)]Cl$	13.60	13.71	4.53	4.25	33.74	33.70	19.68	19.62	3.83	3.80	119.0
cis-$[(guo)_2Pd(ala)]Cl$	13.36	13.55	4.45	4.34	34.65	34.60	19.33	19.31	4.02	4.22	121.0
cis-$[(guo)_2Pd(pro)]Cl$	12.94	13.08	4.31	4.20	36.48	36.30	18.72	18.57	4.13	4.26	111.0
cis-$[(guo)_2Pd(val)]Cl$	12.91	12.82	4.30	4.19	36.39	36.25	18.68	18.54	4.36	4.55	95.2
cis-$[(guo)_2Pd(ileu)]Cl$	12.69	12.85	4.23	4.00	37.20	37.00	18.40	18.20	4.60	4.68	93.8
cis-$[(guo)_2Pd(phe)]Cl$	12.19	12.40	4.06	4.00	39.89	39.80	17.65	17.52	4.24	4.33	122.0
cis-$[(guo)_2Pd(alaH)Cl]Cl$	12.77	12.96	8.51	8.19	33.13	33.00	18.49	18.30	3.96	4.06	168.0
cis-$[(guo)_2Pd(proH)Cl]Cl$	12.39	12.55	8.25	7.89	34.93	34.80	17.93	17.70	4.07	4.17	165.0
cis-$[(guo)_2Pd(valH)Cl]Cl$	12.36	12.48	8.23	7.94	34.85	34.62	17.89	17.68	4.30	3.41	147.8
cis-$[(guo)_2Pd(ileuH)Cl]Cl$	12.16	12.22	8.10	7.88	35.68	35.49	17.60	17.51	4.45	4.62	122.8

TABLE 2. Characteristic IR bands of the complexes

Complexes	ν_α(NH)	ν_s(NH)	ν(CH)	ν_α(C=O) of guo	ν_α(C=O) of amacH	δ(NH_2)	ν(C=C, C=N)	δ_s(CH_3)	ρ_w(CH_2)	δ(CH)	ρ_t(CH_2)	Sugar conformation	ν_α(COO^-) – ν_s(COO^-)	vPd–Cl
cis-[$(guo)_2$Pd(gly)]Cl	3210b	3160b	2930sh	1690s	1629b	1629b	1597s		1324sh		914m	820sh	217	
							1528s					802m		
cis-[$(guo)_2$Pd(ala)]Cl	3230b	3120b	2940sh	1695s	1635b	1635b	1590s	1385m		1280w		821sh	220	
							1540s					799m		
cis-[$(guo)_2$Pd(pro)]Cl			2940sh	1690s	1638b		1590s		1330sh		905w	820sh	224	
							1533s					798m		
cis-[$(guo)_2$Pd(val)]Cl	3230b	3140b	2950w	1691s	1638b	1638b	1590s	1370sh		1252sh		823sh	220	
							1534s					799m		
cis-[$(guo)_2$Pd(ileu)]Cl	3230b	3140b	2960w	1695s	1637b	1637b	1590s	1380m	1330w			821sh	239	
							1540s					798m		
cis-[$(guo)_2$Pd(phe)]Cl	3240b	3140b		1690s	1635b	1635b	1590s					820sh	225	
							1540s					797w		
cis-[$(guo)_2$Pd(alaH)Cl]Cl	3230b	3120b	2942sh	1698s	1700s	1630b	1590s	1385m		1278w		820sh		340w
							1539s					795m		
cis-[$(guo)_2$Pd(proH)Cl]Cl			2940sh	1695s	1700s		1589s		1330sh		906w	820sh		335w
							1534s					797m		
cis-[$(guo)_2$Pd(valH)Cl]Cl	3230b	3140b	2948w	1690s	1798s	1630b	1589s	1370sh		1250sh		820sh		339sh
							1534s					799m		
cis-[$(guo)_2$Pd(ileuH)Cl]Cl	3230b	3140b	2950m	1695s	1700s	1629b	1589s	1380sh	1330sh	1260w		820sh		340sh
							1534s					798w		
cis-$(guo)_2PdCl_2$				1702s			1590s					822sh		335w
							1540s					800m		
guo				1730s			1630s					823m		
							1565s					800w		

b = broad; sh = shoulder; s = strong; m = medium; w = weak.

the sugar in the complexes, also confirmed with the ^{1}H NMR spectra (see below).

Other characteristic bands of the compounds with their assignments can also be seen in Table 2.

^{1}H NMR Spectra

The ^{1}H NMR spectra of the compounds have been taken in D_2O and DMSO-d_6 solutions and are given in Tables 3 and 4 respectively. The chemical shifts of the complexes are compared with the ones of the free amino acids in their zwitterionic forms and are given in parentheses. Negative values correspond to chemical shifts of the protons of the coordinated ligands downfield as compared to the free ones, while positive values correspond to upfield shifts.

The ^{1}H NMR spectra of the complexes of both series *cis*-[Pd(guo)$_2$(amac)]Cl and *cis*-[Pd(guo)$_2$-(amacH)Cl]Cl showed the presence of two major isomers, besides a few minor ones, which are more in the second series with the monodentate amino acid. An attempt was made to characterize the various isomers observed and this is outlined in detail in the case of the complexes *cis*-[Pd(guo)$_2$(val)]Cl and *cis*-[Pd(guo)$_2$(valH)Cl]Cl.

The H_8 proton of guo in the ^{1}H NMR spectrum of the complex *cis*-[Pd(guo)$_2$(val)]Cl in D_2O, shows three resonances at 8.4287, 8.4080 and 8.1218 ppm, all downfield shifted, as compared to free guo, by 0.4420, 0.4210 and 0.1350 ppm respectively, with a relative intensity of 1:0.2:0.9. This result confirms the retention of the Pd–N_7 bonds in the ternary complex [12]. The two major peaks at 8.4287 and 8.1218 ppm, in a 1:0.9 relative intensity, are assigned to two different isomers with strong and weak interactions of the aliphatic side chain of the chelated amino acid and the aromatic rings of guo respectively, in equilibrium. These are the so called 'closed' and 'opened' forms observed also in similar M–nucleotide–amino acid ternary systems by Sigel *et al.* [1, 18, 19] (see Fig. 1). They were also observed in the ternary systems *trans*-[Pd(ino)$_2$-(peptide)$_2$]Cl$_2$ [10].

'Closed' and 'opened' form isomers have also been assigned in the complex *cis*-[Pd(guo)$_2$(ala)]Cl, presenting two peaks at 8.4123 and 8.1656 ppm in D_2O solutions, in about a 1:1 ratio of relative intensities. The position of the methyl group of ala may justify a hydrophobic interaction with the aromatic rings of guo, only when the latter are oriented in a 'head to head' position. The phenomenon of the presence of the 'closed' and 'opened' isomeric forms, however, with strong and weak ligand–ligand interactions can also be intermolecular [20], since the distance between the guo molecules and the methyl group is quite large even with such an orientation [14], as is also evidenced with molecular models.

The peak at 8.4080 ppm of *cis*-[Pd(guo)$_2$(val)]Cl, on the other hand, is undoubtedly due to rotational isomers around the Pd–N_7 bond, which may give rise to relative orientations of the two guo molecules, such as 'head to head', 'head to tail' and 'tail to tail' [8, 21–26]. The two peaks are coalesced at 50 °C with $\Delta G^{\neq} = 72.8$ kJ/mol.

In DMSO-d_6 solutions only two resonances are observed for the H_8 of guo in the complex *cis*-[Pd(guo)$_2$(val)]Cl, in 1:6.3 relative intensity at 8.6010 and 8.0440 ppm respectively. They are assigned to the 'closed' and 'opened' forms. A relatively higher degree of intramolecular guo–guo interaction could justify the smaller downfield shift (8.0440 ppm) of the H_8 in the 'opened' form. The latter is favored in DMSO-d_6 solution, since the intermolecular stacking effects are minimized [10]. The downfield shifts of the H_8 protons of both isomers, as compared to the free guo (Table 4), shows again the retention of the Pd–N_7 coordination [12].

The H_8 of the 'opened' form of *cis*-[Pd(guo)$_2$-(val)]Cl (Fig. 2(a)) in D_2O solutions, shown at 8.1218 ppm in $c = 2.72 \times 10^{-3}$ mol/l, shifts more upfield with increasing concentration, due to the increase of the intermolecular stacking effect of the aromatic rings of guo. The H_8 of the 'closed' form on the other hand (Fig. 2(b)) shows exactly the opposite effect; this can be explained with the increase of the intermolecular hydrophobic interactions between the aliphatic side chain of the amino acid and the aromatic rings of guo, with increasing concentration.

The valine protons, on the other hand, are as follows: The α-CH proton of the amino acid shows two doublets at 3.5965 and 3.6384 ppm, the first almost unshifted (−0.005 ppm) and the second shifted downfield by −0.037 ppm, relative to the zwitterionic form of the val [27] (see Table 3). Two bands with opposite shifts (upfield, downfield), compared to the zwitterionic form of the amino acid show also the protons of γ and δ carbons of val while the β protons show a multiple resonance centered at 2.2441 ppm, upfield by +0.0164 ppm. The upfield shifts of the methyl protons are +0.024 and +0.025 ppm respectively for the γ and δ protons and the corresponding downfield shifts are −0.091 and −0.097 ppm (see Table 3). They are assigned to the 'closed' and 'opened' forms respectively. Since the two methyl groups of the amino acid should lie closer to the guo molecules in the 'closed' form, the largest upfield shifts observed for their protons are justified [1, 18].

The complex *cis*-[Pd(guo)$_2$(valH)Cl]Cl in D_2O, shows five resonances for the H_8 of guo, at 8.5589, 8.4496, 8.4296, 8.3207 and 8.1384 ppm, all shifted downfield compared to the free ligand. This confirms again the retention of the Pd–N_7 bonds [12]. The three bands at 8.4496, 8.4296 and 8.3207 ppm are assigned to the rotational isomers around the Pd–N_7

TABLE 3. ^1H NMR chemical shifts (ppm) of the complexes in D_2O solutions

Complexes	Guanosine protons							Amino acid protons				
	H_8	$H_{1'}$	$H_{2'}$	$H_{3'}$	$H_{4'}$	$H_{5'}$	$H_{5''}$	αCH	βCH	γCH	δCH	εCH
Guanosine [36]	7.9870	5.8950	4.7120	4.3940	4.2150	3.8450	3.8450					
cis-$(guo)_2PdCl_2$ [12]	9.1700 (−1.183)	6.0700										
cis-[$(guo)_2$Pd(gly)]Cl	8.4252 (−0.438)	5.8224d		4.2882				3.5399 (+0.002)				
	8.1800 (−0.193)	5.8654d						3.5755 (−0.033)				
	7.9699											
cis-[$(guo)_2$Pd(ala)]Cl	8.4123 (−0.425)	5.8245d	4.5105t	4.2973t				3.6281	1.4682 (+0.010)			
	8.1656 (−0.179)	5.8719d	4.5871t	4.3410t					1.5084d (−0.030)			
cis-[$(guo)_2$Pd(pro)]Cl	8.4986 (−0.5116)											
	8.3469 (−0.359)	5.8326d	4.5054t	4.2769t					2.1390 (+0.294)	1.6839 (+0.345)	2.8017t (+0.559)	
	8.2788 (−0.292)	5.8499d	4.5635t	4.3168t					2.2876 (+0.046)		3.0362t (+0.324)	
cis-[$(guo)_2$Pd(val)]Cl	8.4287 (−0.442)	5.8067d	4.5038t	4.2787t	4.1409	3.7254	3.7254	3.5965d (−0.005)		1.0141 (+0.024)	0.9625 (+0.025)	
	8.4080 (−0.421)								2.2441m (+0.016)			
	8.1218 (−0.135)	5.8879d	4.6051t	4.3531t	4.1930	3.8253	3.8253	3.6384d (−0.037)		1.1276 (−0.091)	1.0851 (−0.097)	
cis-$(guo)_2$Pd(ileu)]Cl	8.4319 (−0.445)	5.8119d	4.5023t	4.2776	4.1482	3.7209	3.7209	3.6667d (−0.015)	1.9355 (+0.030)	1.3079 (+0.053)	0.9733 (+0.028)	0.8778 (+0.055)
	8.4109 (−0.424)											
	8.1219 (−0.135)	5.8842d	4.5998	4.3517	4.1839	3.8226	3.8226					
cis-[$(guo)_2$Pd(proH)Cl]Cl	8.5450 (−0.558)											
	8.4192 (−0.432)								2.0621 (+0.271)	1.7046m (+0.324)	2.8157 (+0.545)	

(continued)

TABLE 3. *(continued)*

Complexes	Guanosine protons							Amino acid protons				
	H_8	$H_{1'}$	$H_{2'}$	$H_{3'}$	$H_{4'}$	$H_{5'}$	$H_{5''}$	αCH	βCH	γCH	δCH	ϵCH
	8.3468								2.3061		3.0328	
	(−0.360)								(+0.027)		(+0.328)	
	8.3048											
	(−0.318)											
	8.2716											
	(−0.284)											
cis-[(guo)$_2$Pd(valH)Cl]Cl	8.5589											
	(−0.572)											
	8.4496	5.7703										
	(−0.463)											
	8.4296	5.8258							2.2394m		1.1086	
	(−0.443)											
	8.3207	5.8971										
	(−0.333)											
	8.1384											
	(−0.151)											
cis-[(guo)$_2$Pd(ileuH)Cl]Cl	8.5606											
	(−0.0574)											
	8.4492	5.7713										
	(−0.462)											
	8.4312	5.8280							1.9417m	1.4859m	1.0149	0.9306
	(−0.444)								(+0.023)	(−0.036)	(−0.014)	(+0.003)
	8.3232	5.8995										
	(−0.336)											
	8.1335											
	(−0.146)											

d = doublet; t = triplet; m = multiplet.

TABLE 4. ^{1}H NMR chemical shifts (ppm) of the complexes in DMSO-d_6 solutions

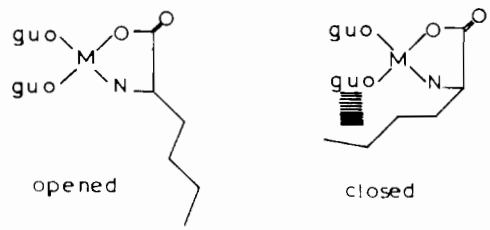
Complexes	Guanosine protons							Amino acid protons					
	H_8	$H_{1'}$	$H_{2'}$	$H_{3'}$	$H_{4'}$	$H_{5'}$	$H_{5''}$	NH	αCH	βCH	γCH	δCH	εCH
Guanosine	7.8000	5.8950											
cis-[(guo)$_2$Pd(ala)]Cl	8.5800												
	(−0.780)												
	8.0420	5.8095	4.5150	4.1955	3.9903			10.8980		1.3940d			
	(−0.242)									1.3140d			
cis-[(guo)$_2$Pd(pro)]Cl	8.6916												
	(−0.892)												
	8.6060							11.2470					
	(−0.806)												
	8.0626	5.8060	4.5130	4.2057	3.9890			10.8541		2.0543	1.6799m	2.9038m	
	(−0.203)												
cis-[(guo)$_2$Pd(val)]Cl	8.6010												
	(−0.801)												
	8.0440	5.8125	4.5120	4.2035	3.9893			10.7820		2.2000m	1.1030m		
	(−0.244)												
cis-[(guo)$_2$Pd(ileu)]Cl	8.6092							11.2050					
	(−0.809)												
	8.0546	5.8058	4.5074	4.1952	3.9805	3.7041	3.6548	10.7886		1.8895m	1.5337	1.0679	0.9960
	(−0.255)												
cis-[(guo)$_2$Pd(alaH)Cl]Cl	8.5830							11.2370					
	(−0.783)									1.3920d			
	8.0420	5.8110	4.5140	4.2090	3.9840			10.8490		1.3100d			
	(−0.242)												
cis-[(guo)$_2$Pd(proH)Cl]Cl	8.6290												
	(−0.829)												
	8.5830							11.2240					
	(−0.783)												
	8.0450	5.8110	4.5090	4.1865	3.9905			10.8420		2.0120m			
	(−0.245)												
cis-[(guo)$_2$Pd(valH)Cl]Cl	8.6975												
	(−0.898)												
	8.6108							11.2540					
	(−0.811)												
	8.4855												
	(−0.686)												

(continued)

TABLE 4. *(continued)*

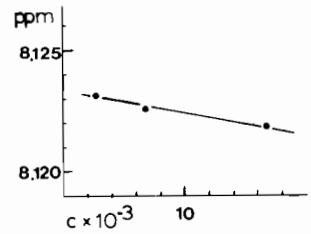
Complexes	Guanosine protons							Amino acid protons					
	H_8	H_1'	H_2'	H_3'	H_4'	H_5'	H_5''	NH	αCH	βCH	γCH	δCH	εCH
	8.3666												
	(−0.567)												
	8.0630	5.8076	4.5232	4.2000	3.9858			10.8538		2.1868		1.0646	
	(−0.263)												
cis-[(guo)$_2$Pd(ileuH)Cl]Cl	8.6854												
	(−0.885)												
	8.6094							11.2100					
	(−0.809)												
	8.4850												
	(−0.685)												
	8.3567												
	(−0.557)												
	8.0562	5.8060	5.5079	4.1958	3.9879			10.7921		1.8800m	1.5090m	1.0668	0.9946
	(−0.256)												

d = doublet; m = multiplet.

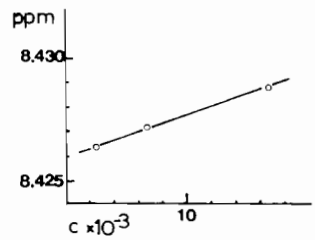


(a) (b)

Fig. 1. The 'opened' (a) and 'closed' (b) forms of the guo–M–amac ternary systems.



(a)



(b)

Fig. 2. Chemical shift of the H_8 proton of the 'opened' (a) and 'closed' (b) forms of the complex *cis*-[Pd(guo)$_2$(val)]Cl, as a function of concentration.

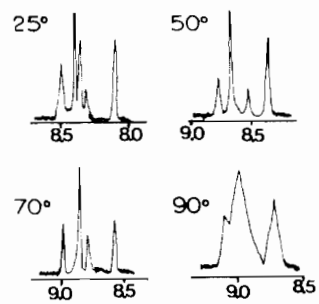


Fig. 3. The region of the H_8 proton in the ^{1}H NMR spectrum of the complex *cis*-[Pd(guo)$_2$(valH)Cl]Cl, as a function of temperature.

bonds of the two guo molecules corresponding to their relative orientations ('head to head', 'head to tail' and 'tail to tail') [8, 21–26] of the 'closed' form. This is confirmed with the spectra taken at 50 °C where the first two bands coalesce with $\Delta G^{\neq} = 72.86$ kJ/mol. All the three bands coalesce to one at 90 °C, with $\Delta G^{\neq} = 74.4$ kJ/mol (Fig. 3).

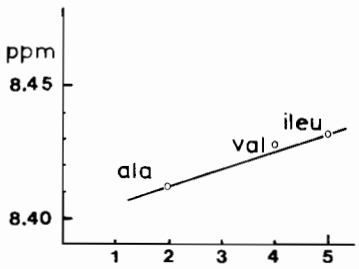


Fig. 4. Chemical shift of the H_8 proton of the 'closed' form of the complexes *cis*-[Pd(guo)$_2$(amac)]Cl, where amac is L-ala, L-val and L-ileu.

The resonance at 8.5589 ppm is assigned to the hydrolysis product *cis*-[Pd(guo)$_2$(valH)(H$_2$O)]Cl$_2$, which is then converted to *cis*-[Pd(guo)(guo-H$^+$)-(valH)]Cl containing a possible N_7O_6 chelate of guo [12, 28, 29]. It should be noted that at pH = 6.2 the *cis*-[Pd(guo)$_2$(val)]Cl precipitates out the complex of the empirical formulae *cis*-[Pd(guo)(guo-H$^+$)(val)]. Finally the 8.1384 ppm resonance may be due to the 'opened' isomer.

In DMSO-d_6 solutions five resonances are observed at 8.6975, 8.6108, 8.4855, 8.3666 and 8.0630 ppm in an intensity ratio 0.4:1:0.2:0.3:7.5, all shifted downfield, compared to free guo, implying Pd–N_7 bonds. The assignment of the bands is the same as in D_2O solutions. The band at 8.0630 ppm which increases in intensity in DMSO-d_6, is assigned to the 'opened' form.

The behavior of the other complexes of both series is similar. It is worthwhile noting that the chemical shifts of the H_8 proton of the 'closed' form, observed more downfield than the corresponding 'opened' form in D_2O solutions, in the chelated complexes of the amino acids with aliphatic side chain, e.g. ala, val, ileu, increase with an increase of the aliphatic side chain (Fig. 4), reflecting a stronger ligand–ligand hydrophobic interaction [1]. The more upfield shifted H_8 of the 'opened' form, on the other hand, shifts slightly upfield with an increase of the aliphatic side chain of the amino acids.

Conformations around the Cα–Cβ Bond of the Amino Acids

The percentage of the three possible conformations around the Cα–Cβ bond in the complexes *cis*-[Pd(guo)$_2$(val)]Cl and *cis*-[Pd(guo)$_2$(ileu)]Cl (Fig. 5) can be calculated from their ^{1}H NMR spectra [30–33]. The results are included in Table 5.

The percentage of the g + h conformers directing the aliphatic side chains of the amino acids towards the metal ion, is thus 89% in the 'closed' form of the complex *cis*-[Pd(guo)$_2$(val)]Cl and 83% in the 'opened' form. In the complex *cis*-[Pd(guo)$_2$(ileu)]Cl the percentage of only the 'opened' form could be calculated and found to be more than 90%. In complexes of Pd^{2+} with dipeptides containing val and ileu [20, 30], the sum of g + h isomers was also found larger than 90%. The same percentage was also found in the ternary systems IMP or GMP–Pd–dipeptide [20], while in the complex GHL–Pd–nucleotide, the h isomer was more than 80% [11].

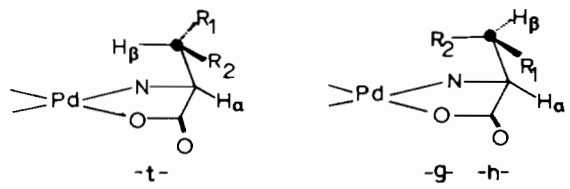


Fig. 5. The h, g and t conformers of L-val and L-ileu.

TABLE 5. Percentages of the t, g and h rotational isomers of the complexes with L-val and L-ileu

Complexes	J_{AB}	t (%)	(g + h) (%)	Solvent
cis-[(guo)$_2$Pd(val)]Cl 'closed'	3.60	11.0	89.0	D_2O
'open'	4.32	17.0	83.0	
cis-[(guo)$_2$Pd(ileu)]Cl 'open'	3.45	10.0	90.0	D_2O

The existence or not of hydrophobic interactions between the aliphatic side chain of the amino acids and the aromatic rings of guo, in the same complex molecule, is therefore determined by the relative position of the two *cis*-guo molecules. If they are oriented 'tail to tail', the two guo molecules are far from the aliphatic side chains of the amino acids ('opened' form) and the interactions between them are minimum, while in a 'head to head' orientation, the two ligands are closer together and their interaction possible ('closed' form). The ligands however, may also interact intermolecularly [20].

Finally the largest percentage of the h + g isomers in the ileu complex, reflects the stronger ligand–ligand interaction in this complex, possessing a longer side chain than val.

Sugar Conformations

The percentage of the ^{3}E conformation of the sugar moiety of guo, being 38% in the free ligand [34], is known to increase by about 10% upon coordination with Pt(II) or Pd(II) at N_7 [10, 15, 16]. The same is true with ino [9]. A slight decrease was further observed in the ternary systems of *cis*-[Pt(ino)$_2$(amac)]Cl upon amino acid coordination [9] which was larger in the amino acids with longer aliphatic side chains. A similar trend was also observed here for the systems *cis*-[Pd(guo)$_2$(amac)]Cl in D_2O solutions, being 55% for the gly derivative, 53% for the ala and pro, 50% and 49% for the 'closed' and 'opened' forms respectively of both the val and ileu derivatives. In DMSO-d_6 solutions on the other

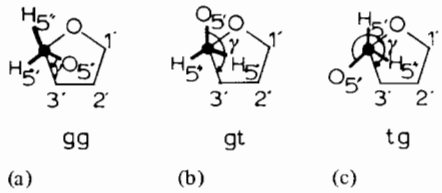


Fig. 6. Sugar conformation around the $C_{4'}$–$C_{5'}$ bond; (a) gg, (b) gt and (c) tg.

hand, the percentage of the ^3E conformation is comparable to the one of the free ligand, varying from 38–40% in the complexes of the present study and the systems *trans*-$[Pd(guo)_2(dipeptide)_2]Cl_2$ [10].

The conformation around the $C_{4'}$–$C_{5'}$ bond of the sugar moiety, described with the gg, gt and tg conformers (Fig. 6) was calculated only for the complexes *cis*-$[Pd(guo)_2(val)]Cl$ and *cis*-$[Pd(guo)_2(ileu)]Cl$, where the values of the coupling constants $^3J(HH)$ could be estimated and the Carplus equation subsequently applied [35–38]. The results were 75% and 77% gg conformer for the 'closed' form and 65% and 68% for the 'opened' forms of the two complexes respectively, in D_2O solutions. A 69% gg conformer is retained upon coordination of *cis*-DDP with guo [15, 17], as well as in the series *trans*-$[Pd(guo)_2(dipeptide)_2]Cl_2$ [10].

The chemical shift of the $H_{2'}$ proton of the nucleosides is related to the relative percentage of the *syn–anti* conformation of the sugar moiety and was observed at 5.073 ppm in t-Bu^{8}guo with a supposed 100% *syn* and at 4.608 ppm in the *cis*-$Pt(NH_3)_2{}^7(G_P^{5'})_2{}^{2+}$ with a supposed 100% *anti* conformation [17]. The relative ratio of the *syn–anti* conformation of nucleosides was then calculated from their observed H_2' chemical shifts [17] and the relation [39]

$$\delta_{obs} = P_{syn}\delta_{syn} + P_{anti}\delta_{anti} \quad (3)$$

In the present case however, the $H_{2'}$ proton of the sugar moiety of guo is always found more upfield than the value of 4.608 ppm of the *cis*-$Pt(NH_3)_2{}^7(G_P^{5'})_2{}^{2+}$ with a supposed 100% *anti* conformation [17], for both the 'closed' and 'opened' forms, with the former even more upfield (see Table 3). The relation (3) cannot therefore be applied and it can only be said that the *anti* conformation predominates and it increases linearly with the increasing aliphatic side chain of the amino acid (see Fig. 7), in the 'closed' form of the complexes. In the 'opened' form, on the other hand, although again the *anti* conformation is favored, its ratio does not depend on the amino acid (Table 3).

Finally, in DMSO-d_6 the chemical shift of the $H_{2'}$ values observed, is at almost constant frequency (Table 4) for all the complexes, though again more upfield than the value of 4.608 ppm of the complex

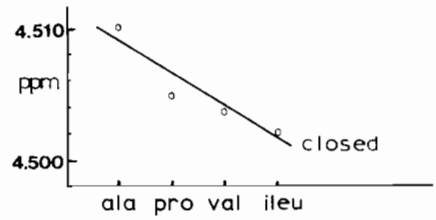


Fig. 7. Variation of the *anti* conformation of sugar in the complexes *cis*-$[Pd(guo)_2(amac)]Cl$, based on the chemical shift of the $H_{2'}$ proton.

cis-$Pt(NH_3)_2{}^7(G_P^{5'})_2{}^{2+}$ [17]. These results are similar to the ones observed for the systems *trans*-$[Pd(guo)_2(dipeptide)]Cl_2$ [10], though the relative ratio of the *anti* conformation was larger in these last systems.

Conclusions

The *cis* ternary complexes of the types *cis*-$[Pd(guo)_2(amac)]Cl$ and *cis*-$[Pd(guo)_2(valH)Cl]Cl$, with amino acids of increasing aliphatic side chain show:

(i) Two main isomers with strong and weak ligand–ligand interactions, called 'closed' and 'opened' forms respectively, in D_2O solutions. These interactions can be inter- or intramolecular and decrease in DMSO-d_6 solutions, where the 'opened' form is favored.

(ii) The increase of the aliphatic side chain of the amino acids results in an increase of the percentage of the 'closed' form and to a decrease of the 'opened' form (see Fig. 8).

(iii) The hydrophobic interactions between the protons of the aliphatic side chain and the aromatic rings of guo in D_2O solutions increase with increasing distance of the protons from the bonding site with Pd(II), contrary to what is observed in the series *trans*-$[Pd(guo)_2(dipeptide)_2]Cl_2$ [10].

(iv) The ^3E conformation of the sugar moiety in the complexes *cis*-$[Pd(guo)_2(amac)]Cl$ decreases slightly upon coordination of amino acids with increasing aliphatic side chain, in D_2O. In DMSO-d_6, the percentage of ^3E is comparable to the one of the free ligand and does not depend upon the amino acid.

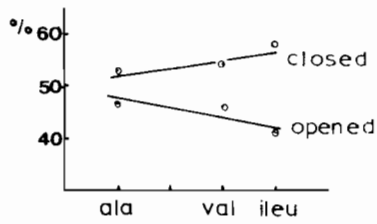


Fig. 8. Percentages of the 'closed' and 'opened' forms of the complexes *cis*-$[Pd(guo)_2(amac)]Cl$ with L-ala, L-val and L-ileu.

(v) The gg conformer increases by 6–8% in the 'closed' form as compared to the free ligand and decreases by 1–4% in the 'opened' form.

(vi) The *anti* conformation of the sugar of guo increases with an increase of the aliphatic side chain of the amino acid in the 'closed' form of the complexes. The *anti* conformation of the 'closed' form is larger than the one of the 'opened' form.

(vii) The relative ratio of the *anti* conformation in these systems appears to be less than in the systems *trans*-[Pd(guo)$_2$(dipeptide)$_2$]Cl$_2$ [10].

In the DNA–Pt–protein model, it could be said that the *anti* conformation increases with an increase in the DNA–protein hydrophobic interaction, while the ^{3}E conformation of the sugar decreases, though to a lesser extent. The toxic effects of the platinum drugs could therefore be due to such DNA–Pt–protein crosslinks.

Experimental

Materials

Glycine, L-alanine, L-valine, L-proline, L-isoleucine, L-phenylalanine and guanosine were purchased from Aldrich Chemical Company and Sigma Chemical Company. Palladium chloride was purchased from Degusa A.G. (F.R.G.). They were all used without further purification.

Methods

(i) The elemental analyses of Pd and Cl were performed in our laboratory, those of C, H and N in the Laboratoire de Chimie de Coordination in Toulouse. (ii) The conductivity measurements were performed in an E365B Conductoscope, Metrohm Ltd., Herisau, Switzerland. (iii) The IR spectra were recorded on a Perkin-Elmer model 580 spectrophotometer. (iv) The ^{1}H NMR spectra were obtained on a Brucker WM-250 spectrometer equipped with an Aspect 3000 computer. ^{1}H NMR chemical shifts were measured in parts per million with DSS or TMS as internal reference.

Preparation of the Compounds

The preparation of the starting complex *cis*-Pd(guo)$_2$Cl$_2$ was carried out according to a literature method [12]. The sodium salts of the amino acids were prepared by mixing equivalent amounts of each one of them with 0.1 NaOH solution and allowing the water to evaporate slowly in the water bath, washing the white residue with acetone and drying it at 110 °C under vacuum.

Preparation of cis-[Pd(guo)$_2$(amac)]Cl

One mmol of *cis*-Pd(guo)$_2$Cl$_2$ was mixed in the solid state with 1:1 mmol of the corresponding sodium salt of the amino acid (amacNa) and 150 ml of methanol were added. The suspension was stirred for 8 h at 25 °C, filtered and the yellow filtrate obtained. This was evaporated to dryness and the residue dissolved in DMF, filtered and reprecipitated with a mixture of acetone and ether (1:1), in excess. The light yellow precipitate was filtered and washed with acetone and ether. It was then recrystallized in a mixture of ethanol:water (8:2). After subsequent drying in a dessicator at room temperature, followed by drying at 110 °C under vacuum, in the presence of CaCl$_2$, the yield varied from 40–75%, depending on the amino acid.

Preparation of cis-[Pd(guo)$_2$(amacH)Cl]Cl

One mmol of the complex *cis*-[Pd(guo)$_2$(amac)]Cl was dissolved in an equivalent amount of a dilute HCl solution (0.1 N) and left to react at room temperature under stirring, for a few minutes. After filtration the complex was precipitated with an excess of isopropanol. The precipitated complex was filtered and washed with acetone and ether. It was then dried, first in a dessicator at room temperature, followed by drying at 110 °C under vacuum, in the presence of CaCl$_2$. Yield 80–85%.

Preparation of the Deuterated Derivatives

One hundred mg of the corresponding complex were dissolved in a few ml of D$_2$O and stirred for 5 min at room temperature. The deuterated complex was then reprecipitated with acetone and ether, filtered and dried *in vacuo*.

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