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Thermodynamic and structural characterisation of the complexes formed in the reaction of $[Pd(en)(H_2O)_2]^{2+}$ and $[Pd(pic)(H_2O)_2]^{2+}$ with *N*-alkyl nucleobases and *N*-acetyl amino acids

Zoltán Nagy and Imre Sóvágó*

Department of Inorganic and Analytical Chemistry, University of Debrecen, H-4010 Debrecen, Hungary. E-mail: sovago@delfin.klte.hu

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Potentiometric and ^{1}H NMR studies have been performed on the interaction of $[Pd(en)(H_2O)_2]^{2+}$ and $[Pd(pic)(H_2O)_2]^{2+}$ (pic = 2-picolylamine) with *N*-alkyl nucleobases and *N*-acetyl amino acids. The ligands 1-methylthymine, 1-methyluracil and uridine formed only mono and bis(ligand) complexes in the whole pH range (2 to 10) *via* the co-ordination of N(3) donor atoms of pyrimidine rings. In the case of 1-methylcytosine (MeC), *N*-acetyl-L-histidine (AcHis) and *N*-acetylhistamine (AcHm) polynuclear complexes containing a deprotonated exocyclic amino group of MeC or a fully deprotonated imidazole of AcHis or AcHm were also formed. In the $[Pd(pic)(H_2O)_2]^{2+}$–MeC and AcHis/AcHm systems various isomeric species including the *cis–trans* and linkage isomers were detected by NMR measurements. The thermodynamic equilibrium constants of the complexes of $[Pd(pic)(H_2O)_2]^{2+}$ with nitrogen donors were generally higher than those of $[Pd(en)(H_2O)_2]^{2+}$. The differences in the complex formation reactions of $[Pd(en)(H_2O)_2]^{2+}$ and $[Pd(pic)(H_2O)_2]^{2+}$ were especially high for *N*-acetyl-L-methionine. The high *trans*-effect of the thioether donor function of AcMet resulted in the liberation of free ethylenediamine in the $[Pd(en)(H_2O)_2]^{2+}$–AcMet system, while bidentate (S,O) co-ordination of AcMet was proposed for $[Pd(pic)(H_2O)_2]^{2+}$ in acidic solutions. The hydrolytic reactions of the complexes were also followed. The formation of dihydroxo complexes $[Pd(en)(OH)_2]$ and $[Pd(pic)(OH)_2]$ was characteristic in alkaline solutions, while the existence of a dimeric–trimeric and monomeric–dimeric equilibrium was suggested in the neutral pH range for the monohydroxo complexes of $[Pd(en)(H_2O)_2]^{2+}$ and $[Pd(pic)(H_2O)_2]^{2+}$, respectively.

Introduction

It is generally accepted that the antitumor activity of various platinum containing drugs is related to the platination of DNA, most commonly *via* binding to guanine.[1,2] The kinetic lability of the platinum–sulfur bond is, however, much higher than that of platinum–nitrogen bonds, thus Pt–S bonded intermediates can play a significant role both in the transport and the toxicity of various platinum containing drugs.[3] The intra- or intermolecular competitions between the thioether sulfur and purine or pyrimidine nitrogen donor functions have been studied by several authors and in spite of the high thermodynamic stability of the Pt–N bonded species the kinetic preference for the formation of Pt–S bonds was generally observed.[4–6] As a consequence, the biological activity of the anticancer drugs is very much influenced by both the kinetic and thermodynamic properties of the various platinum complexes. The slow formation kinetics, however, generally rules out the possibility of stability constant determinations for platinum complexes. The complex formation processes and co-ordination geometry of palladium(II) are very similar to those of platinum(II) and palladium(II) ions can be used to mimic the binding properties of platinum(II). In a previous paper we reported both thermodynamic and kinetic parameters for the palladium(II) complexes formed in the reaction of $[Pd(dien)]^{2+}$ with various nitrogen and thioether sulfur donors. In the case of the $[Pd(dien)]^{2+}$–AcMet–MeC system the results provided clear evidence for the existence of Pd–S bonded metastable intermediates at physiological pH, but the thermodynamic equilibrium was described by the predominant formation of Pd–N bonded complexes.[7]

Comparison of the stability constants of various palladium(II) complexes obtained in the previous study led to another important conclusion. Namely, the affinity of palladium(II) for thioether binding was very much influenced by the presence of the other donor groups in the co-ordination sphere of the metal ion. For instance $[Pd(dien)]^{2+}$ and some dipeptide complexes of palladium(II) (*e.g.* $[Pd(H_{-1}L)]$, where L = GlyAla or other common dipeptides) had much higher affinity for thioether binding than those of $[Pd(terpy)]^{2+}$ or $[Pd(H_{-1}GlyMet)]$.[7] The reduced affinity of thioether binding of $[Pd(terpy)]^{2+}$ and $[Pd(H_{-1}GlyMet)]$ was accompanied by increased tendency for hydrolysis and these differences were explained by electronic and steric effects. On the other hand, significant differences were reported in the complex formation processes of $[Pd(dien)]^{2+}$ and $[Pd(terpy)]^{2+}$ with nitrogen donors, too.[8] The formation of a dimeric species co-ordinated *via* the pyrimidine-N3 and deprotonated exocyclic amino groups of 1-methylcytosine (MeC) was much favored in the case of $[Pd(terpy)]^{2+}$ and it was explained by the stacking interaction between terpy residues.[9] These studies revealed that complex formation processes of co-ordinatively unsaturated palladium(II) complexes are influenced by the other donor functions already present in the co-ordination sphere of the metal ion. In the previous papers we reported equilibrium, kinetic and structural parameters for the monofunctional palladium(II) species including $[Pd(dien)]^{2+}$ $[Pd(terpy)]^{2+}$ and $[Pd(H_{-1}L)]$ complexes of dipeptides.[7,8,10] In the continuation of these studies the results obtained on the palladium(II) complexes of strongly co-ordinating bidentate ligands $[Pd(en)(H_2O)_2]^{2+}$ and $[Pd(pic)(H_2O)_2]^{2+}$ (pic = 2-picolylamine) will be discussed.

The outstanding biological activity of cisplatin resulted in a huge number of publications on the platinum(II) complexes with two free co-ordination sites. These studies covered mainly

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the structural and kinetic description of platinum(II) complexes and the most important conclusions have already been reviewed by several authors.[1,11,12] Thermodynamic equilibrium studies are scarce for platinum(II) species, but palladium(II) complexes were used as appropriate models for the determination of metal ion speciation in solution. The interaction of $[Pd(en)(H_2O)_2]^{2+}$ and its derivatives have been thoroughly studied with nucleobases, amino acids and some other sulfur or nitrogen donors.[13–20] These results made a significant contribution to the identification of the binding sites of various bioligands as a function of the pH and metal ion to ligand ratio. However, it is also obvious from the results that the metal ion speciation under biological conditions is a very sensitive function of the kinetic and thermodynamic parameters of various complexes. Moreover, these parameters are significantly influenced by the substituents of the bidentate nitrogen donors.[21–26]

In this paper we report the results of combined potentiometric and ^{1}H NMR studies on the interaction of $[Pd(en)(H_2O)_2]^{2+}$ and $[Pd(pic)(H_2O)_2]^{2+}$ with monodentate ligands containing nitrogen or sulfur donor atoms. The ligands represent the side chain residues of nucleic acids and proteins and include the *N*-alkyl nucleobases or nucleosides (MeC, MeUH, MeTH and uridine) and *N*-acetyl amino acids (AcLys, AcHis, AcHm and AcMet).

Experimental

Materials

The binary palladium(II) complexes $[Pd(en)(H_2O)_2]^{2+}$ and $[Pd(pic)(H_2O)_2]^{2+}$ (abbreviated later as [Pd(en)] and [Pd(pic)] or 'M') were prepared from $K_2[PdCl_4]$ (Fluka) using ethylenediamine (en, Sigma) or 2-picolylamine (pic, Fluka), respectively, under acidic conditions to avoid hydrolysis. The appropriate chloride salts, $[Pd(en)Cl_2]$ and $[Pd(pic)Cl_2]$, were first precipitated, and transformed to the soluble nitrates with $AgNO_3$ (Fluka). The palladium content of the stock solutions of $[Pd(en)(H_2O)_2](NO_3)_2$ and $[Pd(pic)(H_2O)_2](NO_3)_2$ was checked by atomic absorption spectroscopy. The *N*-alkyl nucleobases 1-methylcytosine (MeC), 1-methylthymine (MeTH), 1-methyluracil (MeUH) and uridine were purchased from Sigma. The *N*-acetyl amino acids *N*-acetyl-L-lysine (AcLys), *N*-acetyl-L-methionine (AcMet), *N*-acetyl-L-histidine (AcHis) and *N*-acetylhistamine (AcHm) were the product of Sigma and used without further purification. The concentrations of the ligands were checked by potentiometric titrations.

Potentiometric measurements

The stability contants of the ternary complexes were determined by pH-metric titrations of samples containing one of the bifunctional palladium(II) species $[Pd(en)(H_2O)_2]^{2+}$ and $[Pd(pic)(H_2O)_2]^{2+}$ and the ligand 'L' in 1 : 1 and 1 : 2 ratios. The very high thermodynamic stability constants of the ethylenediamine complexes of palladium(II) result in the complete formation of the species $[Pd(en)(H_2O)_2]^{2+}$ even under very acidic conditions (pH < 2), while the relatively high ratio of the stepwise stability constants suppresses the bis(ligand) complex formation in equimolar solution.[27] As a consequence, all the palladium(II) ions are present in the form of $[Pd(en)(H_2O)_2]^{2+}$ and $[Pd(pic)(H_2O)_2]^{2+}$ in the equimolar solutions of palladium and the nitrogen donors (en and pic) above pH 1. Hydrolysis of these species starts under slightly acidic conditions, thus before titration a known amount of nitric acid was added to the samples to suppress hydrolytic reactions. The total concentration of the samples ranged from 1×10^{-3} to 10×10^{-3} mol dm^{-3}. Argon was bubbled through the samples to ensure the absence of oxygen and carbon dioxide and for stirring the solutions. All pH-metric measurements were carried out in 10 cm^3 samples at 298 K, at a constant ionic strength of 0.2 mol dm^{-3} KNO_3. Measurements were made with a Radiometer PHM93 pH-meter equipped with a Metrohm 6.0219.100 double junction electrode to avoid the formation of chloro complexes. Carbonate free potassium hydroxide of known concentration was used for titrations with the help of a Metrohm 715 Dosimat automatic burette. The pH readings were converted to hydrogen ion concentration[28] and the stability constants were calculated by means of a general computational program (PSEQUAD).[29] The stability constants were defined by the equations:

$$pM + qH + rL \rightleftharpoons M_pH_qL_r \qquad \log \beta_{pqr} = \frac{[M_pH_qL_r]}{[M]^p[H]^q[L]^r} \tag{1}$$

where 'M' stands for $[Pd(en)]^{2+}$ or $[Pd(pic)]^{2+}$. The standard deviations of the equilibrium data are shown in parentheses in the appropriate Tables (the charges are omitted in eqn. (1) and in Tables 2 to 4, because of the different charges of the various ligands).

NMR measurements

Proton magnetic resonance spectra of the free ligand and the mixed ligand palladium(II) complexes were recorded on a Bruker AM360 spectrometer in D_2O using tetramethylammonium tetrafluoroborate (3.18 ppm) as internal reference. The concentration of the NMR samples varied between 5 and 20 mmol dm^{-3} and the pD values were determined by use of a combined electrode (Metrohm 6.0243.100) and addition of 0.4 to the pH-meter readings.

Results and discussion

Hydrolytic reactions of $[Pd(en)(H_2O)_2]^{2+}$ and $[Pd(pic)(H_2O)_2]^{2+}$

The hydrolytic reactions of unsaturated platinum(II) complexes are among the most important issues which should be considered under physiological conditions. As a consequence, hydrolysis of cisplatin and its derivatives has been thoroughly studied in both solution and the solid state.[30–35] It is clear from these studies that hydrolysis of cisplatin and other *cis*-diamineplatinum(II) species cannot be described by the formation of simple monomeric dihydroxo complexes, but various polynuclear hydroxo bridged species are also formed.[32–35] The very slow formation kinetics, however, hampers the determination of stability constants for platinum(II), but the corresponding palladium(II) complexes can be used as the appropriate model compounds.[30,31] The hydrolysis of $[Pd(en)(H_2O)_2]^{2+}$ has been the most extensively studied. In addition to the common mono- and bis-hydroxo complexes, ($[Pd(en)(H_2O)(OH)]^+$ and $[Pd(en)(OH)_2]$), the formation of di- and tri-meric hydroxo bridged species has also been suggested[30,31,36] and the existence of polynuclear species was proven by NMR studies, too.[19] However, in the different polynuclear complexes, $[Pd_n(en)_n(OH)_n]^{n+}$, the stoichiometric ratio of the various components is the same hence it is difficult to determine the molar ratio of the different oligomers. As a consequence, the formation of di- and trinuclear complexes has been neglected by several authors, which results in some contradiction in the literature data.[21,30,37,38] Hydrolytic reactions of $[Pd(pic)(H_2O)_2]^{2+}$ have been much less studied and the formation of mononuclear complexes was only proposed.[26]

We performed potentiometric and ^{1}H NMR measurements for the elucidation of the hydrolytic equilibria of $[Pd(en)(H_2O)_2]^{2+}$ and $[Pd(pic)(H_2O)_2]^{2+}$. The stability constants of the hydroxo complexes are included in Table 1, while the corresponding speciation curves are demonstrated by Fig. 1.

It is clear from Table 1 that our model for the hydrolysis of $[Pd(en)(H_2O)_2]^{2+}$ is very similar to that published by Martin

Table 1 Stability constants of the hydroxo complexes of [Pd(en)-$(H_2O)_2]^{2+}$ and $[Pd(pic)(H_2O)_2]^{2+}$ (T = 298 K, I = 0.2 mol dm^{-3} KNO_3)

M	$[Pd(en)]^{2+}$	$[Pd(pic)]^{2+}$
$[MH_{-1}]^{+}$	—	−5.00(4)
$[M_2H_{-1}]^{3+}$	−3.04(4)	−2.28(8)
$[M_2H_{-2}]^{2+}$	−8.41(2)	−6.59(2)
$[M_3H_{-3}]^{3+}$	−11.80(8)	—
$[MH_{-2}]$	−15.21(2)	−13.79(2)

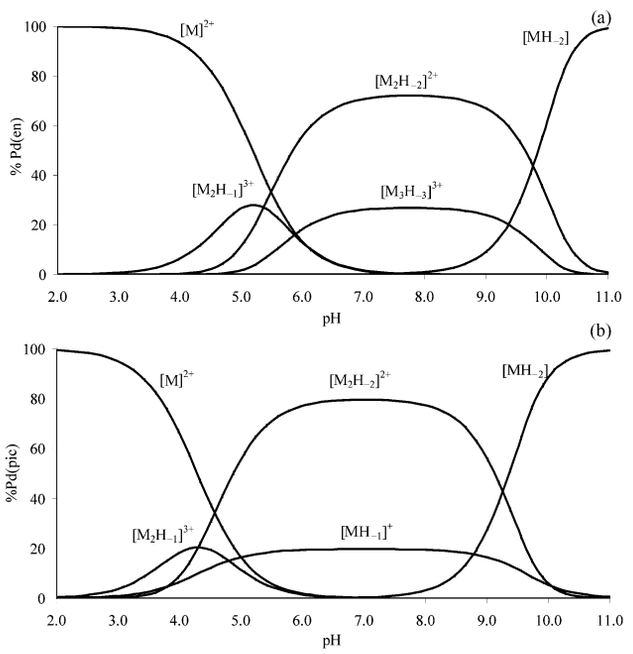


Fig. 1 Concentration distribution of the species formed in the hydrolytic reactions of $[Pd(en)(H_2O)_2]^{2+}$ and $[Pd(pic)(H_2O)_2]^{2+}$ with increasing pH. "M" stands for $[Pd(en)]^{2+}$ (a) and $[Pd(pic)]^{2+}$ (b), $c_M = 4 \times 10^{-3}$ mol dm^{-3}.

et al.[30,31] Fig. 1 reveals that dimeric $[\{Pd(en)(OH)\}_2]^{2+}$, trimeric $[\{Pd(en)(OH)\}_3]^{3+}$ and dihydroxo $[Pd(en)(OH)_2]$ complexes are the major species in the millimolar concentration range. The monohydroxo complex $[Pd(en)(H_2O)(OH)]^{+}$ is probably also present, but its concentration is too low for either potentiometric or NMR detection. The major difference between our and the previous literature studies is that we proved the existence of the monohydroxo bridged dimeric species $[\{Pd(en)\}_2(OH)]^{3+}$ and $[\{Pd(pic)\}_2(OH)]^{3+}$, too. This is the first species formed in the hydrolytic reactions, therefore its consideration is important in any complex formation reactions. It is important to note that similar monohydroxo bridged complexes have already been suggested in ternary systems containing nucleobases[17] supporting the formation of $[\{Pd(en)\}_2(OH)]^{3+}$ in the binary systems, too. On the other hand, the concentration of the monohydroxo bridged dimers is generally very low in diluted solutions as demonstrated by Fig. 1.

The comparison of the stability constants in Table 1 and the speciation curves in Fig. 1. reveals two significant differences between the hydrolytic reactions of $[Pd(en)(H_2O)_2]^{2+}$ and $[Pd(pic)(H_2O)_2]^{2+}$. One of these differences is reflected in the different ratio of the polynuclear complexes. Namely, in the case of $[Pd(pic)]^{2+}$ we were not able to detect any formation of trimeric species, but the monomer was present in measurable concentration. The reduced tendency to polymerize of $[Pd(pic)]^{2+}$ is probably due to steric requirements caused by the bulky pyridine moiety. On the other hand, stability constants of the hydroxo complexes are higher for $[Pd(pic)]^{2+}$ suggesting that the presence of aromatic residues increases the affinity of palladium(II) for hydrolysis. A similar observation has already been reported for the hydrolytic reactions of the monofunctional palladium(II) complexes, $[Pd(dien)]^{2+}$ and $[Pd(terpy)]^{2+}$, the latter having the higher affinity for hydroxo complex formation.[7,8] The existence of the major hydroxo complexes has been proven by NMR measurements and it is demonstrated by Fig. 2.

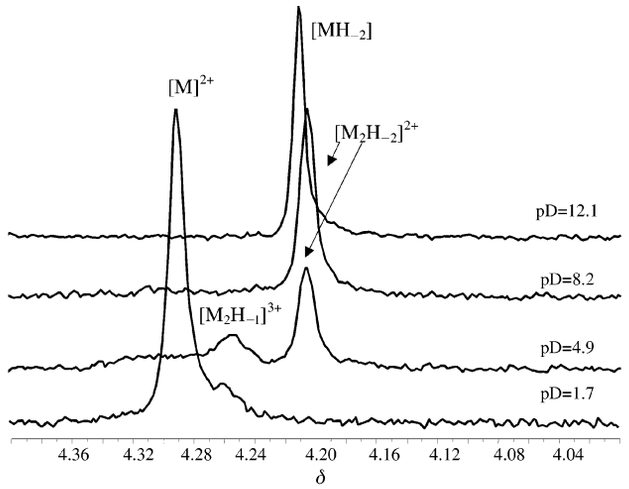


Fig. 2 ^{1}H NMR spectra of CH_2 protons of the samples containing $[Pd(pic)(H_2O)_2]^{2+}$ and its hydrolysed species at different pH values ($c_M = 10^{-2}$ mol dm^{-3}).

The NMR spectra in Fig. 2 indicate that $[Pd(pic)(H_2O)_2]^{2+}$ can be a single species only under strongly acidic conditions (pH < 2). The formation of mono and dihydroxo bridged dimers overlaps in the pH range 3 to 6 the latter being the major species around physiological pH. The species $[Pd(pic)(H_2O)(OH)]^{+}$ is probably also present in the same pH range, but its concentration is decreased by the increase of the total palladium(II) concentration, thus it cannot be detected in the concentration range used for NMR measurements (>10 mmol dm^{-3}). Further increase of pH results in a small change in the CH_2 proton resonances, which corresponds to the formation of $[Pd(pic)(OH)_2]$ and $[Pd(en)(OH)_2]$.

Complexes of *N*-alkyl nucleobases

Stability constants of the complexes of *N*-alkyl pyrimidine bases formed in the reaction with $[Pd(en)(H_2O)_2]^{2+}$ and $[Pd(pic)(H_2O)_2]^{2+}$ were determined by pH-potentiometric titrations of samples containing the metal ion and the ligands in 1 : 1 and 1 : 2 ratios. The purine bases (derivatives of adenine and guanine) were not involved in this study, because purine bases have two co-ordination sites available for metal binding and it results in polynuclear complex formation with bifunctional palladium(II) species.[8,16,31] The equilibrium data obtained for the complexes of 1-methyluracil (MeUH), 1-methylthymine (MeTH), 1-methylcytosine (MeC) and uridine are collected in Tables 2 and 3, for $[Pd(en)(H_2O)_2]^{2+}$ and $[Pd(pic)(H_2O)_2]^{2+}$, respectively.

One of the major comments which should be made in connection with the data in Tables 2 and 3, is that the pH-metric titration curves were recorded over a wide pH range (2 to 10) for uridine, MeTH and MeUH, while equilibrium was not reached even in 30 minutes for any titration points above pH 5 in the case of MeC. The differences between the complex formation processes of MeC and related ligands can be explained by the deprotonation and metal ion co-ordination of the exocyclic amino group of MeC above pH 5. In the case of monofunctional palladium species (*e.g.* $[Pd(dien)]^{2+}$ and $[Pd(terpy)]^{2+}$) the formation of these complexes have already been well characterised in both solution[8] and the solid state.[9] For the bifunctional palladium(II) species, however, the stoichiometries of the complexes formed with the deprotonated bidentate ligands are much more complicated and the complex formation

Table 2 Stability constants (log β_{pqr}) of the complexes formed in the reaction of $[Pd(en)]^{2+}$ (= "M") with derivatives of pyrimidine bases (L) (T = 298 K, I = 0.2 mol dm^{-3} KNO_3)

Species	Uridine	MeUH	MeTH	MeC
[HL]	9.09(2)	9.54(4)	9.99(2)	4.64(3)
[ML]	8.98(2)	9.07(1)	9.05(1)	6.13(7)
$[ML_2]$	14.80(9)	14.88(4)	14.76(2)	11.44(5)
$[MH_{-1}L]$	1.31(3)	0.54(6)	0.61(2)	0.44(6)
$[M_2H_{-1}L_2]$	12.14(10)	12.58(15)	12.70(7)	10.41(11)
$pK - \log K_1$	0.11	0.47	0.94	−1.49
$\log(K_1/K_2)$	3.16	3.26	3.34	0.82
$pK(ML \rightarrow MH_{-1}L)$	7.67	8.53	8.44	5.69

Table 3 Stability constants (log β_{pqr}) of the complexes formed in the reaction of $[Pd(pic)]^{2+}$ (= "M") with derivatives of pyrimidine bases (L) (T = 298 K, I = 0.2 mol dm^{-3} KNO_3)

Species	Uridine	MeUH	MeTH	MeC
[HL]	9.09(2)	9.54(4)	9.99(2)	4.64(3)
[ML]	9.20(2)	9.57(1)	9.56(1)	8.07(10)
$[ML_2]$	15.09(5)	15.73(4)	15.40(3)	13.35(9)
$[MH_{-1}L]$	1.26(9)	1.84(6)	1.56(4)	2.85(7)
$[M_2H_{-1}L_2]$	13.82(20)	14.58(17)	14.30(9)	14.06(9)
$pK - \log K_1$	−0.11	−0.03	0.43	−3.40
$\log(K_1/K_2)$	3.31	3.41	3.72	2.79
$pK(ML \rightarrow MH_{-1}L)$	7.94	7.73	8.00	5.22

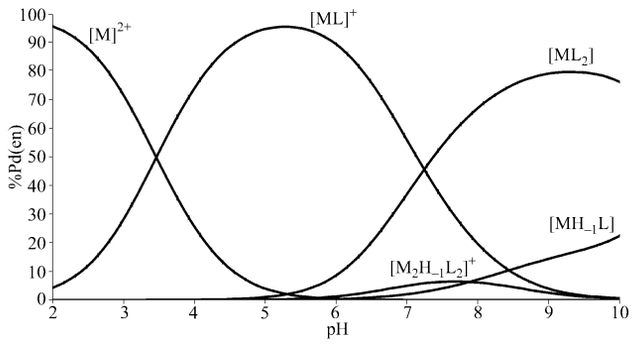


Fig. 3 Concentration distribution of the species formed in the reactions of $[Pd(en)(H_2O)_2]^{2+}$ (M) with MeTH (LH) ($c_{[Pd(en)]} = 2 \times 10^{-3}$, $c_{MeTH} = 4 \times 10^{-3}$ mol dm^{-3}).

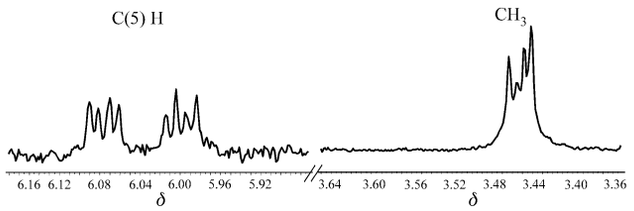


Fig. 4 ^{1}H NMR spectra of the samples containing [Pd(pic)] and MeC in equimolar concentration at pD = 2.6.

always overlaps with metal ion hydrolysis. Several features of these reactions have already been clarified by Häring and Martin[17] in the [Pd(en)]–cytidine system using ^{1}H NMR measurements. The complete equilibrium evaluation of the potentiometric measurements, however, is hampered by the overlapping deprotonation and hydroxo complex formation reactions.

Apart from the effect of the exocyclic amino group above pH 5 the complex formation processes of the four ligands in Tables 2 and 3 are very similar to each other and the speciation is demonstrated by Fig. 3 for the [Pd(en)]–MeTH system.

It is clear from Tables 2 and 3 and demonstrated by Fig. 3 that [ML] and $[ML_2]$ are the major species with all the ligands and the stability constants are in reasonable agreement with those reported for the [Pd(en)]–uridine and –cytidine systems on the basis of NMR measurements.[17] On the other hand, the NMR measurements provide an unambiguous proof that metal binding takes place *via* the N(3) donors of pyrimidine rings in the complexes of both [Pd(en)] and [Pd(pic)]. The species $[M_2H_{-1}L_2]$ is present in low concentration and it can be considered as a monohydroxo bridged dimer (*e.g.* $[\{Pd(en)(MeU^-)\}_2(OH)]^+$, while $[MH_{-1}L]$ is a simple mixed hydroxo complex (*e.g.* $[Pd(en)(MeU^-)(OH)]$).

Careful analysis of the data in Tables 2 and 3 however, reveals several differences between the complexes of MeC and the other ligands and between the complexes of [Pd(en)] and [Pd(pic)]. Namely, the $pK - \log K_1$ values which correspond to the relative stability of the complexes of various ligands reveal the outstanding metal binding ability of MeC in all cases. In the [Pd(en)]–MeC system the increased stability is reflected in the equilibrium data of both mono and bis complexes, which suggests hydrogen bond formation between the co-ordinated ligands. The formation of an intramolecular hydrogen bond between the exocyclic amino group of one ligand and the C(2)O carbonyl oxygen of the other ligand has already been reported in the *cis*-diamminebis(1-methylcytosine)platinum(II) complex containing the two MeC residues in a *head to tail* arrangement.[39] The existence of similar hydrogen bonded structures was not observed in the corresponding *trans*-bis(1-methylcytosine)-palladium(II) complexes,[40,41] but hydrogen bond formation between the amine ligands and the co-ordinated nucleobase has been suggested.[11,42] The [ML] species formed in the [Pd(pic)]–MeC system has especially high stability, which can be attributed to the hydrogen bond formation between the amino group of 2-picolylamine and the carbonyl oxygen of co-ordinated MeC. The increased stability of the species [ML], however, slightly suppresses the bis(ligand) complex formation in this system which is reflected in the increased ratio of the stepwise stability constants caused by the bulky pyridine residue. The complex formation processes of MeUH, MeTH and uridine are always characterised by high $\log(K_1/K_2)$ ratios suggesting that there is no significant interaction between the co-ordinated ligands.

The differences between the stability constants of MeC complexes of [Pd(en)] and [Pd(pic)] were well demonstrated by the NMR measurements. Namely, the proton resonances of both en and MeC protons in the [Pd(en)]–MeC (1 : 1) system show the existence of three different species: the "free" metal ion $[Pd(en)(H_2O)_2]^{2+}$, the 1 : 1 complex, and the bis(ligand) complex around pD 2.6, supporting the low ratio of the stepwise stability constants. However, the proton resonances of the free ligand and the bis(ligand) complex cannot be observed in the equimolar solutions of [Pd(pic)] and MeC under the same conditions. It is in agreement with the increased stability of the species [ML] and the increased ratio of the stepwise stability constants. Four different sets of C(5)H and C(6)H doublets and CH_3 singlets of MeC can, however, be assigned in these solutions. The C(6) resonances of MeC overlap with those of the pyridine residue, but the C(5)H and CH_3 resonances can be easily identified as shown by Fig. 4.

The appearance of four sets of NMR peaks suggests the existence of four isomeric forms of [ML] (= $[Pd(pic)(MeC)(H_2O)]^{2+}$). Two of the isomers can be easily identified as the *cis* and *trans* configurations of [ML], while the other two isomers come from the hindered rotation of MeC residues around the Pd–N(3) bond as shown in Scheme 1. In the [Pd(en)]–MeC system these isomers cannot exist and only a well resolved doublet and a singlet was obtained for the C(5)H and CH_3 resonances of MeC, respectively.

The resolution of the NMR peaks in Fig. 4 has a significant temperature dependence suggesting the existence of rotamers in solution. Fig. 5 shows the NMR spectra obtained in the solution of [Pd(pic)] and MeC in a 1 : 2 ratio at pD = 5. Both C(5)H and CH_3 resonances of MeC and the singlet from CH_2

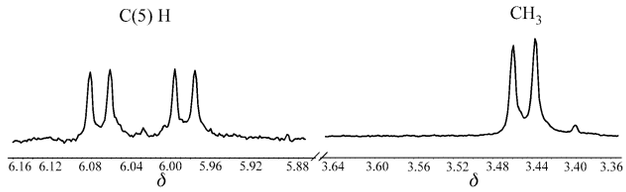


Fig. 5 ^{1}H NMR spectra of the samples containing [Pd(pic)] and MeC in a 1 : 2 ratio at pD = 5.

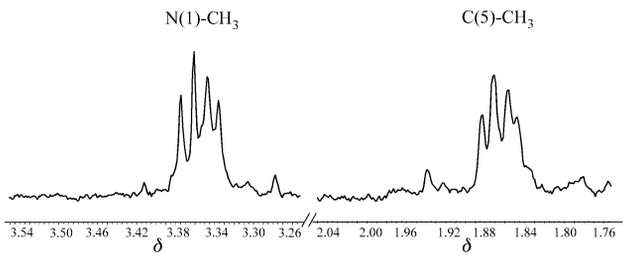
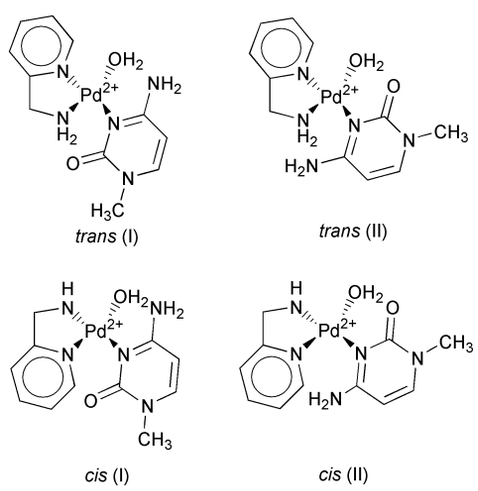


Fig. 6 ^{1}H NMR spectra of the samples containing [Pd(pic)] and MeTH in equimolar concentration at pD = 6.25.



Scheme 1

protons of picolylamine indicate the existence of the bis(ligand) complex as a single species, but containing the MeC in two different environments caused by the different ligands in *trans* positions. In all probability the *head to tail* isomer is present in overwhelming concentration, because it is stabilised by hydrogen bonds between the exocyclic amino groups and the carbonyl oxygen donors.

Further increase of pH results in the upfield shift of the proton resonances of MeC, which corresponds to the deprotonation and co-ordination of the exocyclic amino group as discussed above.

NMR spectra obtained with the other nucleobases also provided nice evidence for the existence of isomers both in mono and bis(ligand) complexes. It is best represented by the [Pd(pic)]–MeTH system, where the resonances of the C(6)H protons overlap with those of the pyridine residue, but both N(1)- and C(5)-methyl resonances are well separated. Fig. 6 shows the NMR spectra obtained in the equimolar solution of [Pd(pic)] and MeTH at pD = 6.25. The complex [ML] is present as a single species at this pD value and the four sets of methyl resonances correspond to the same isomers as shown in Scheme 1.

The pK values obtained for the formation of the species [$MH_{-1}L$] show again a significant difference in the complex formation processes of MeC and the other three ligands. Namely, in the case of MeTH, MeUH and uridine [$MH_{-1}L$] mixed hydroxo complex is definitely formed and pK values correspond well to those of other 3N co-ordinated palladium(II) complexes.[7,31] The value obtained for MeC is, however, significantly lower supporting the proposal that the new base consumption process around pH 5 is not hydrolysis, but deprotonation and co-ordination of the exocyclic amino group.

The comparison of all data in Tables 2 and 3 leads to the conclusion that stability constants of the nucleobase complexes of [Pd(pic)] are always higher than those of [Pd(en)]. The differences are generally rather small and similar tendencies were reported for the complexes of [Pd(dien)] and [Pd(terpy)].[8] These observations provide further support that simultaneous co-ordination of aliphatic and aromatic nitrogen donors is not favored in the mixed ligand complexes of palladium(II) as has already been suggested for mixed ligand complexes of copper(II).[43,44]

Complexes of *N*-acetyl amino acids containing nitrogen donors

The results of the previous studies on the mixed ligand complexes of $[Pd(dien)]^{2+}$ and $[Pd(terpy)]^{2+}$ with AcLys indicated that the ε-amino group of the lysyl residue is not an important binding site in multicomponent systems.[8] This was explained by the high pK value of the side chain amino group (pK = 10.40), which shifts complex formation reactions into the pH range of hydrolytic reactions. As a consequence, the mixed ligand complex $[Pd(terpy)(AcLys)]^{+}$ has not been detected at all, hydrolysis being the preferred reaction with aromatic nitrogen donors. Similar conclusions can be drawn from the potentiometric data obtained for the reaction of $[Pd(en)(H_2O)_2]^{2+}$ and $[Pd(pic)(H_2O)_2]^{2+}$ with AcLys. Formation of hydroxo complexes of the bifunctional palladium(II) species starts in the acidic pH range, thus co-ordination of the monodentate amino group cannot compete with hydrolysis. It does not mean that the amino group would not be an effective binding site for bifunctional palladium(II) species, because there are a lot of examples of the co-ordination of amino groups in chelates and in non-aqueous solutions or in the solid state.[12] Both potentiometric and NMR data prove, however, that monodentate binding of the ε-amino group of the lysyl residue cannot compete with hydrolysis; only the hydroxo complexes and free ligand can be detected in the solution containing $[Pd(en)(H_2O)_2]^{2+}$ or $[Pd(pic)(H_2O)_2]^{2+}$ and AcLys.

Imidazole nitrogen donor atoms are among the most common metal binding sites in proteins.[45] As a consequence, the M(II)–N(1)/N(3)(Im) bonded complexes were detected as the major species in the physiological pH range in the mixed ligand complexes of both palladium(II)[8] and copper(II).[46] In the continuation of these studies now we report the stability constants and structural considerations on the mixed ligand complexes of *N*-acetyl-L-histidine (AcHis) and *N*-acetylhistamine (AcHm) with bifunctional palladium(II) species. The stability constants were determined by potentiometric measurements and are collected in Table 4 together with the equilibrium data of *N*-acetyl-L-methionine (AcMet).

Before the interpretation of the results in Table 4 it should be emphasized that the potentiometric titration curves were used for calculation in the pH range 2–4.5 only, because equilibration of the titration points cannot be reached at higher pH values. An extra base consuming process starts in this pH range, which should correspond to the deprotonation and co-ordination of both nitrogen donors of imidazole *via* N(1)–N(3) bridging. This process has already been well clarified for monofunctional palladium(II) species and the formation of a dinuclear complex, [$M_2H_{-1}L$] was suggested.[8] $[Pd(en)(H_2O)_2]^{2+}$ and $[Pd(pic)(H_2O)_2]^{2+}$, however, have two free co-ordination sites, which result in more complicated polynuclear complex formation and these reactions overlap with hydrolysis. As a consequence, the potentiometric measurements cannot provide unambiguous proof for the stoichiometry of the imidazole bridged polynuclear complexes. The same problems have already been mentioned in connection with the complexes

Table 4 Stability constants of the mixed ligand complexes formed in the reaction of AcHis, AcHm and AcMet (L) with $[Pd(en)(H_2O)_2]^{2+}$ and $[Pd(pic)(H_2O)_2]^{2+}$ (M) ($T = 298$ K, $I = 0.2$ mol dm^{-3})

	AcHis		AcHm		AcMet
Species	[Pd(en)]	[Pd(pic)]	[Pd(en)]	[Pd(pic)]	[Pd(pic)]
[HL]		7.04(2)		7.07(5)	3.34(2)
[H_2L]		9.89(3)		—	—
[MHL]	10.56(5)	10.92(3)	—	—	—
[ML]	8.46(2)	8.87(1)	8.06(3)	8.43(3)	9.63(15)
[MH_2L_2]	21.25(2)	21.29(2)	—	—	21.77(20)
[MHL_2]	18.53(3)	18.40(4)	—	—	19.95(15)
[ML_2]	15.19(2)	14.79(3)	15.06(2)	15.79(2)	16.51(20)
pK − log K_1	−1.42	−1.83	−0.99	−1.36	−6.29
pK(MH_2L_2)	2.72	2.89	—	—	1.82
pK(MHL_2)	3.34	3.61	—	—	3.44
$\log(K_1/K_2)^H$	0.13	0.55	—	—	—
$\log(K_1/K_2)$	1.73	2.95	1.06	1.07	2.75

of MeC in the previous paragraph, where the deprotonation of the exocyclic amino group resulted in polynuclear complex formation.

Comparison of the data in Table 4 clearly indicates that imidazole N(1) or N(3) nitrogen donor atoms are important binding sites for the interaction with $[Pd(en)(H_2O)_2]^{2+}$ and $[Pd(pic)(H_2O)_2]^{2+}$. Below pH 4 this interaction results in the formation of simple mono and bis(ligand) complexes with the stoichiometry of [ML] and [ML_2]. In the case of AcHis protonated complexes are also present, in which the carboxylic groups are protonated and not co-ordinated. This is reflected in the pK values of the protonated complexes, which are similar to those of the free ligand (pK = 2.85 for carboxylic group of AcHis). The lowest values (2.10 and 2.05) obtained for the species [MHL] suggest that carboxylate groups of AcHis also interact with the metal ion after deprotonation of the 1 : 1 complexes. This effect is also reflected in the stability constants of the [ML] complexes. It can be seen from Table 4 that [ML] complexes of AcHis always have higher thermodynamic stability than those of AcHm and this probably comes from the involvement of carboxylate residues in metal binding. This interaction results in a seven-membered chelate, which is generally not stable, but it can contribute to the thermodynamic stability of the AcHis complexes. Obviously, this weak binding will slightly suppress the formation of bis(ligand) complexes, which is reflected in the higher ratio of the stepwise stability constants of AcHis. It is also obvious from the data in Table 4 that the complexes of [Pd(pic)] always have slightly higher stability constants than those of [Pd(en)]. Similar observations were reported for the complexes of MeC and the other nucleobases in Tables 2 and 3, supporting the idea that the presence of the pyridyl residue in the co-ordination sphere of palladium(II) slightly increases the affinity for binding of nucleobases. From the comparison of the data in Tables 2 to 4 it is also important to note that there are several similarities in the complex formation processes of MeC and the imidazole containing ligands. The formation of mononuclear complexes is followed by deprotonation of another donor function and this process starts between pH 4 and 6 in both cases. The deprotonation belongs to the exocyclic amino group of MeC or the pyrrole type NH groups of imidazole ligands and results in polynuclear complex formation in both cases. Both MeC and AcHm (or AcHis) have outstanding affinity for palladium(II) binding, which is reflected in the very low pK − log K_1 values and the low ratios of the stepwise stability constants.

The above mentioned conclusions were supported by the NMR measurements which also gave clear evidence for the existence of linkage isomers and rotamers both for the mono and bis complexes. In the [Pd(en)]–AcHm system the imidazole C(2)H and C(5)H proton resonances provide a simple possibility for the assignment of various isomers, which is demonstrated by Fig. 7.

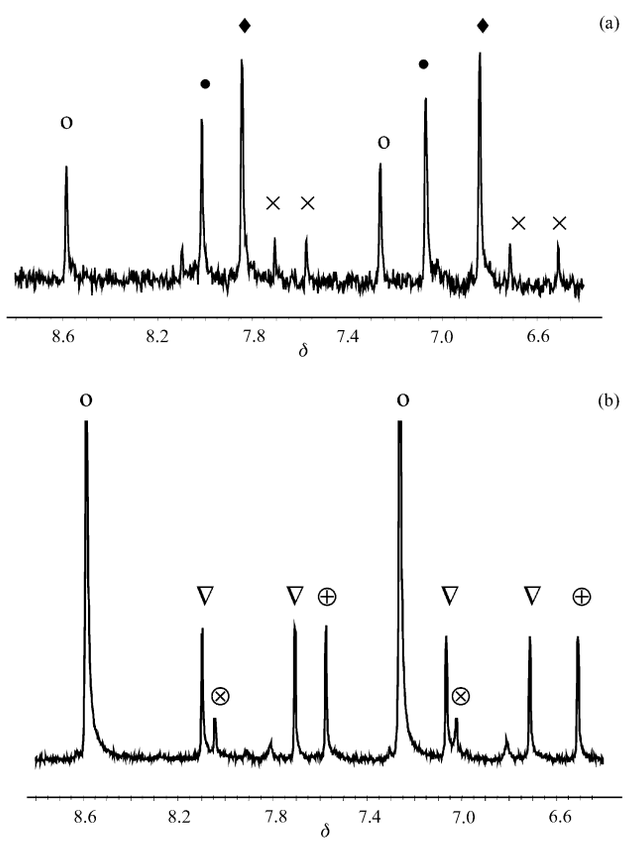
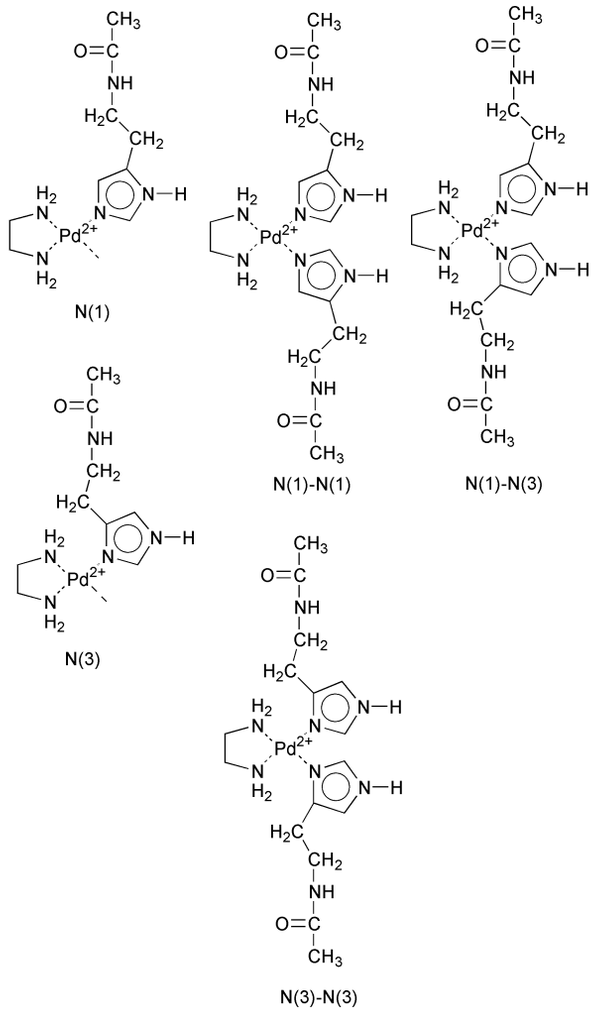


Fig. 7 ^{1}H NMR spectra of the samples containing [Pd(en)] and AcHm. (a) $c_M = c_{AcHm} = 10^{-2}$ mol dm^{-3}. (b) $c_M = 10^{-2}$ mol dm^{-3}, $c_{AcHm} = 8 \times 10^{-2}$ mol dm^{-3}. (The assignment of NMR peaks is discussed in the text.)

Spectrum (a) in Fig. 7 was obtained in equimolar solutions of [Pd(en)] and AcHm at pD 1.8. The proton resonances indicate the presence of [ML] as the major species with the free ligand (o) and the bis(ligand) complex (×) in low concentration. This is in agreement with the low pH, where the complex formation has not been completed yet, and with the low ratio of the stepwise stability constants (see Table 4). Two sets of NMR peaks for the major species can be assigned under these conditions, which correspond to the existence of Pd–N(1) (◆) and Pd–N(3) (●) linkage isomers of [ML] (see Scheme 2). The ratio of the isomers is around 55% to 45% supporting some preference for N(1) binding. The dependence of the molar ratios of the linkage isomers on the structure of imidazole ligands and on the other donor sites around palladium(II) have



Scheme 2

already been reported by several authors.[8,12,47] Spectrum 7(b) was obtained in the presence of a high excess of AcHm at pD = 2.2 to suppress hydrolysis and polynuclear complex formation *via* imidazole bridging. Proton resonances of the free ligand (o) and four different sets of NMR peaks from the bis(ligand) complexes were detected under these conditions. The relative intensities of the four different peaks make it possible to propose the assignment of the appropriate linkage isomers of bis(ligand) complexes.

The linkage isomers can be described as $Pd\{N(1)\}_2$, $Pd\{N(3)\}_2$ and $Pd\{N(1)–N(3)\}$ species as shown by Scheme 2. These linkage isomers should give four different sets of proton resonances of which the mixed species $Pd\{N(1)–N(3)\}$ ($\bigtriangledown$) has two peaks with the same intensity. By comparison with the chemical shifts of the 1 : 1 species it can be concluded that the peaks with the smaller upfield shift for the protonated ligands correspond to $Pd\{N(3)\}_2$ ($\otimes$) and the other to $Pd\{N(1)\}_2$ ($\oplus$) linkage isomers. It is also clear from the intensities of the NMR peaks in Fig. 7(b) that the mixed isomer is the preferred form in solution (≈60%), while the $Pd\{N(1)\}_2$ and $Pd\{N(3)\}_2$ linkage isomers are present in lower concentrations (≈30% and ≈10%, respectively). Very similar sets of proton resonances were detected in the [Pd(en)]–AcHis system. The ratio of the various isomers, however, slightly changed, the mixed species being the most preferred again (≈66%), while $Pd\{N(1)\}_2$ and $Pd\{N(3)\}_2$ linkage isomers were present in comparable concentrations (≈20% and ≈14%, respectively).

The NMR spectra of the samples containing [Pd(pic)] and AcHm, or AcHis under the same conditions are much more complicated. This is due to the increased number of isomeric species. In the case of the 1 : 1 complexes at least four different isomers should be assigned, which correspond to the *cis–trans* and N(1)/N(3) isomers. The number of isomers is further increased if the hindered rotation of the imidazole ligand is considered, which results in the existence of various rotamers as already discussed for MeC. On the other hand, the proton resonances of imidazole protons significantly overlap with those of pyridine moieties. As a consequence, the clear assignment of the NMR resonances of the independent isomeric forms was not possible in this case.

Metal complexes of *N*-acetyl-L-methionine (AcMet)

Thioether sulfur atoms are among the most common binding sites for palladium(II) and their substitution reactions take place *via* a much faster reaction than with nitrogen donor ligands. In a previous paper we reported the thermodynamic, kinetic and structural characterisation of the complexes formed in the reaction of AcMet with monofunctional palladium(II) species.[7] Now in this paper the results obtained for the interaction of AcMet with the bifunctional palladium(II) species $[Pd(en)(H_2O)_2]^{2+}$ and $[Pd(pic)(H_2O)_2]^{2+}$ are discussed.

In the case of the thioether ligands the first problem is to find an appropriate method for the determination of stability constants. The common pH-potentiometric titrations cannot be applied, because thioether donor functions are not protonated in aqueous media. In the [Pd(dien)]–AcMet system a competitive method was applied for stability constant determination using uridine (or other nitrogen donors) as the competitive ligands. This method, however, cannot be applied to the [Pd(en)]–AcMet–uridine system, because both ligands can bind at the free co-ordination sites and the ratio of the mixed species cannot be assessed. However, if we apply only the thioether ligands the sulfur atoms can occupy all free co-ordination sites around the metal ion and p*K* values of the non-co-ordinated carboxylic groups can be determined from pH-metric studies. Therefore we performed potentiometric and NMR measurements in [Pd(en)]–AcMet and [Pd(pic)]–AcMet systems.

The comparison of the potentiometric titration curves and the appropriate proton NMR spectra revealed very significant differences in the complex formation processes of [Pd(en)] and [Pd(pic)] with thioether ligands. In the previous study the interaction of [Pd(dien)] and [Pd(terpy)] with AcMet was described by the same speciation, although the thermodynamic stability of [Pd(dien)] complexes was higher than those of [Pd(terpy)].[7] The differences between the thioether complexes of [Pd(en)] and [Pd(pic)] were, however, much more pronounced and influenced even the speciation and stoichiometry of the complexes. The most striking difference between the two metal species at strongly acidic conditions (pD < 3) is that the NMR peaks of free (fully protonated) ethylenediamine can be observed in the [Pd(en)]–AcMet system, while free 2-picolylamine cannot be detected at any metal ion to ligand ratio or pD values. If the ratio of [Pd(en)] and AcMet exceeds 1 : 2 and the pD is below 2 all the ethylenediamine is liberated and all the AcMet is bonded *via* the thioether residue. This observation can be explained by the high *trans*-effect of the thioether donors, which results in the decomposition of the bifunctional metal species $[Pd(en)(H_2O)_2]^{2+}$. The metal ion, of course, will bind the thioether ligand and the NMR spectra did not indicate proton resonances of any unco-ordinated S–CH_3 residue of AcMet (2.100 ppm for [HL] and 2.113 ppm for [L^-]). Instead, very broad NMR signals are obtained between 2.3 ppm and 2.5 ppm, which correspond to the formation of thioether bridged polynuclear complexes. Thus, the interaction of [Pd(en)] and AcMet is described by the following equilibrium:

$$[Pd(en)]^{2+} + 2\ AcMetH \rightleftharpoons [\{Pd(AcMet)_2\}_n] + [H_2en]^{2+} \quad (2)$$

Obviously, the strong chelator ligand 'en' will shift this equilibrium in the direction of the lower arrow by increasing pH and the non-co-ordinated ethylenediamine disappears by pD ≈ 4.0. The proton resonance of all S-methyl groups appears at 2.498 ppm at this pH suggesting that [Pd(en)(AcMet)$_2$] with monodentate thioether co-ordination is the major species under these conditions. Further increase of pH results in a significant change in the Ac-methyl proton resonances. The upfield shift of these protons (0.115 ppm) strongly suggests the deprotonation and co-ordination of the acetamido nitrogen donor atom ($-NHCOCH_3$). Thus, in slightly acidic and neutral solutions the interaction of [Pd(en)] and AcMet is described by eqn. (3):

$$[Pd(en)]^{2+} + AcMet \rightleftharpoons [Pd(en)(AcMetH_{-1})]^{+} + H^{+} \quad (3)$$

The complex [Pd(en)(AcMetH$_{-1}$)]$^+$ contains two bidentate chelating ligands, in which ethylenediamine forms five-membered (N,N), while AcMet forms six-membered (N^-,S) chelates. The presence of the two chelating ligands may result in the "disproportionation" of the mixed ligand complex according to eqn. (4):

$$2\,[Pd(en)(AcMetH_{-1})]^{+} \rightleftharpoons [Pd(en)_2]^{2+} + [Pd(AcMetH_{-1})_2] \quad (4)$$

In agreement with this assumption the CH_2 resonances of ethylenediamine can be observed also at 2.741 ppm, characteristic of the species [Pd(en)$_2$]$^{2+}$. Further increase of pH does not result in changes in the NMR spectra indicating that the (S,N^-) chelate of AcMet also remains intact in alkaline solutions. On the other hand, these results provide further support that thioether ligands can form thermodynamically stable species in chelates,[48] although monodentate thioether ligands are easily substituted by nitrogen donors or hydroxo complexes in basic media.[7]

It has already been mentioned that NMR spectra do not give any indication for the liberation of picolylamine in the [Pd(pic)]–AcMet system. Thus, in agreement with our previous expectations, only two coordination sites of [Pd(pic)] are available for binding of the thioether function of AcMet ligand. As a consequence, the potentiometric measurements can be used for determination of equilibrium parameters and these data are collected in Table 4. It can be seen from Table 4 that the presence of the species [ML], [ML$_2$], [MH$_2$L$_2$] and [MHL$_2$] gave the best fitting below pH 4. The species [MHL] was rejected by computer calculation even in equimolar solutions indicating that carboxylate residues of the co-ordinated ligands should be deprotonated in very acidic media. This suggests that the species [ML] contains a bidentate (S,O^-) chelating ligand. Thioether and carboxylate residues of AcMet form a seven-membered chelate, which is not stable enough to hinder bis(ligand) complex formation, but enhances the thermodynamic stability of the mono complex. The NMR spectra obtained in the equimolar solutions of [Pd(pic)] and AcMet provide further indirect proof for the existence of the (S,O^-) chelate. Namely, two independent sets of NMR peaks can be assigned for both S- and Ac-methyl groups, which should correspond to the *cis* and *trans* configurations of (S,O^-) chelates. The ratio of these peaks does not depend on pH and shows that one of the isomers is present in higher concentration. The NMR spectra taken of the solutions containing a two-fold excess of AcMet do not indicate the presence of any free ligand, in agreement with bis(ligand) complex formation in very acidic media. The proton resonance of the Ac-methyl group is, however, characterised by a single peak supporting the presence of equivalent side chains. The chemical shift of this peak changes from 2.052 ppm to 2.038 ppm in the pH range 2 to 4, corresponding to the deprotonation of the free carboxylic groups in [MH$_2$L$_2$]. Further increase of pH results in significant modification of the NMR spectra again, which corresponds to the formation of (S,N^-) bonded chelates, similarly to the [Pd(en)]–AcMet system discussed in the previous paragraph.

Concerning the stability constants of AcMet complexes reported in Table 4 it should be noted that [Pd(pic)] has a much higher affinity for thioether binding than the various monofunctional palladium(II) species. For instance, log $K = 5.61$ was reported for the interaction of [Pd(dien)] with AcMet.[7] The increase in the stability of [ML] is four orders of magnitude, which can most probably be explained by the formation of the (S,O^-) chelate. On the other hand, this chelation is responsible for the reduced stability of the bis(ligand) complex reflected in the relatively high ratio of stepwise stability constants. At the same time it is important to note that the speciation curves obtained by the data collected in Table 4 can be used only in a narrow pH range (2 to 4), because deprotonation of the other donor functions (pyrrole type NH and acetamido NH for AcHis and AcMet, respectively) takes place above pH 4. Finally it should be emphasized that these data provide further evidence on the role of Pt–S bonds in the biological transport of platinum containing anticancer agents. Monodentate binding of thioether ligands is kinetically preferred, while the formation of (S,N)-bonded chelates is favoured both kinetically and thermodynamically.

Acknowledgements

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