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Complex-formation reactions and stability constants for mixed-ligand complexes of diaqua(2-picolylamine)palladium(II) with some bio-relevant ligands

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Received 6th December 2002, Accepted 22nd January 2003
First published as an Advance Article on the web 25th February 2003

The complex-formation reactions of $[Pd(Pic)(H_2O)_2]^{2+}$ (pic = 2-Picolylamine) with selected bio-relevant ligands, containing different functional groups, were investigated. Stoichiometry and stability constants for the complexes formed are reported. The results show the formation of 1 : 1 complexes with amino acids and dicarboxylic acids. The effect of chelate ring size of the dicarboxylic acid complexes on their stability constants was examined. Peptides form both 1 : 1 complexes and the corresponding deprotonated amide species. Structural effects of the peptide on the amide deprotonation were investigated. DNA pyrimidinic constituents, such as uracil, uridine, thymidine and thymine, form 1 : 1 and 1 : 2 complexes, whereas purinic constituents, such as inosine 5′-monophosphate (5′-IMP) and guanosine 5′-monophosphate (5′-GMP), form only 1 : 1 complexes. Both DNA constituents and cyclobutane dicarboxylate (CBDCA) react with $[Pd(Pic)(H_2O)_2]^{2+}$ forming [Pd(Pic)(CBDCA-O)DNA], where (CBDCA-O) represents cyclobutane dicarboxylate coordinated by one carboxylate oxygen atom. The concentration distribution of the complexes in solution was evaluated. The effect of dioxane on the acid dissociation constants of CBDCA and the formation constant of its complex with $Pd(Pic)^{2+}$ is reported. The effect of increasing chloride concentration on the formation of the CBDCA complex was investigated.

Introduction

cis-$Pt(NH_3)_2Cl_2$ (cisplatin) is well known as an anti-tumour drug.[1,2] However, there are still difficulties related to its use, because of numerous side effects and its toxicity. Several methods have been developed during the past ten years which have considerably reduced these side effects. Cisplatin is not very soluble in water and tends to hydrolyse at neutral pH. Replacement of the chloro ligands by carboxylate groups in carboplatin,[3] *cis*-diammine(1,1-cyclobutanedicarboxylate)-platinum(II), reduces the toxicity and increases the solubility in water. Some investigators have suggested that carboplatin is merely a pro-drug for cisplatin,[4] whereas others have postulated a ring opening reaction[5] of carboplatin followed by the reaction with guanosine 5′-monophosphate to form $[Pt(NH_3)_2$-(CBDCA-O)(5′-GMP)]. The ring opening reaction with DNA constituents was investigated kinetically in a study of the reaction of [Pd(amine)(CBDCA)] with inosine 5′-monophosphate.[6]

In order to avoid the inert substitution behaviour of Pt(II) complexes, and on the basis of the remarkable analogy between the coordination chemistry of Pt(II) and Pd(II) complexes, a series of labile Pd(II) complexes have proved useful as models to obtain a reasonable picture of the thermodynamics of the reactions for closely related Pt(II) complexes.

Recent work in our laboratories focused on the equilibria[7–10] and kinetics[11–14] of complex-formation and ligand substitution reactions of model *cis*-bis(amine)palladium(II) complexes with amino acids, peptides and DNA constituents. In the present study, we investigated the thermodynamic behaviour of Pd(II) complexes with a bidentate amine ligand having a hetero-aromatic nitrogen base (pyridine) that possesses π-acceptor properties, which is believed to be involved in π–π stacking effects with amino acids, peptides and DNA constituents. The ring opening of chelated CBDCA and monodentate co-ordination of DNA constituents in the Pd(Pic)–(CBDCA-O)–DNA system were studied. The effects of chloride ion concentration and the dielectric constant of the medium on the formation of Pd(Pic)–CBDCA (as a representative example) were also investigated.

Experimental

The complex $[Pd(Pic)Cl_2]$ was prepared as described before.[11] The diaqua complex $[Pd(Pic)(H_2O)_2]^{2+}$ was prepared in solution by stirring the chloro complex with two equivalents of $AgNO_3$ overnight (with careful protection from light). The precipitated AgCl was removed by filtration and the filtrate made up to the desired volume in a standard volumetric flask. The ligands in the form of hydrochlorides were converted to the corresponding hydronitrate in the same way as described above. The ligands used were glycine, alanine, phenylalanine, valine, proline, threonine, isoleucine, serine, aspartic acid, methionine, *S*-methylcysteine, histamine·2HCl, histidine, ornithine·HCl, methylamine·HCl, ethanolamine·HCl, glycinamide, glycylglycine, leucylalanine, glycylalanine, glycylleucine, glycylvaline, glutamine, aspargine, uracil, uridine, thymine, thymidine, guanosine 5′-monophosphate, inosine 5′-monophosphate, 1,1-cyclobutanedicarboxylic acid, oxalic acid, malonic acid, succinic acid and adipic acid. These materials were obtained from Sigma Chemicals.

Potentiometric measurements were peformed using a Metrohm 686 titroprocessor equipped with a 665 dosimat. The electrode and titroprocessor were calibrated with standard buffer solutions prepared according to NBS specifications.[15] The pH meter readings were converted into hydrogen ion concentration by titrating a standard acid solution (0.01 M), the ionic strength of which was adjusted to 0.1 M with $NaNO_3$, with standard base (0.10 M) at 25 °C. The pH is plotted against p[H]. The relationship pH − p[H] = 0.05 was observed. $[OH^-]$ value was calculated using a pK_w value of 13.997.[16]

Procedure and measuring technique

The acid dissociation constants of the ligands were determined by titrating a 0.1 mM solution of each with standard NaOH solution. The acid dissociation constants of the coordinated water molecules in $[Pd(Pic)(H_2O)_2)]^{2+}$ were determined by titrating a 0.1 mM solution of the complex with NaOH. The formation constants of the complexes were determined by

DOI: 10.1039/b212104b

titrating solution mixtures of $[Pd(Pic)(H_2O)_2]^{2+}$ (0.1 mM) and the ligand in concentration ratios of 1 : 1 for dicarboxylic acids, amino acids and peptides, and for concentration ratios of 1 : 1 and 1 : 2 (metal : ligand) for DNA constituents. The formation constants of Pd(Pic)(CBDCA-O)(DNA) were determined by titrating solution mixtures of $[Pd(Pic)(H_2O)_2]^{2+}$ (0.1 mM), $CBDCAH_2$ and DNA constituents in a concentration ratio of 1 : 1 : 1. The titration solution mixtures had a volume of 40 ml. The titrations were carried out at 25 °C by circulating thermostated water through the double-wall titration vessel, and under a slow and constant stream of N_2 over the test solutions. The ionic strength was adjusted to 0.1 M by using of $NaNO_3$. A 0.10 M NaOH solution was used as titrant. The equilibrium constants for the species of the general formula $M_lL_pH_q$ (M = [Pd(Pic)], L = amino acid, peptide or DNA constituent), and for the species $M_{l'}(CBDCA\text{-}O)_{p'}(DNA)_{q'}H_{r'}$, were calculated using the computer program[17] MINIQUAD-75. The stoichiometry and stability constants of the complexes formed were determined by trying various possible composition models. The model selected gave the best statistical fit and was chemically consistent with the titration data without giving any systematic drifts in the magnitudes of various residuals, as described elsewhere.[17] The results are summarized in Tables 1 to 4. The species distribution diagrams were obtained using the program SPECIES[18] under the experimental conditions employed.

Results and discussion

The acid dissociation constants of the ligands were determined under the experimental conditions of 25 °C and a constant ionic strength of 0.1 M, which were also used to determine the stability constants of the Pd(II) complexes. The values obtained are consistent with data reported in the literature.[19]

Acid–base equilibria of $[Pd(Pic)(H_2O)_2]^{2+}$

The $[Pd(Pic)(H_2O)_2]^{2+}$ ion may undergo hydrolysis. Its acid–base chemistry was characterized by fitting the potentiometric data to various acid–base models. The best fit model was found to be consistent with species of the compositions 10−1, 10−2 and 20−2, as given in reactions (1) to (3). The dimeric species 20−1 reported by Nagy *et al.*[20] was rejected. This may be explained on the premise that the concentration range of $Pd(Pic)^{2+}$ used in this investigation is too low to allow the formation of this species.

$$\underset{100}{\left[Pd(pic)(H_2O)_2\right]^{2+}} \rightleftharpoons \underset{10-1}{\left[Pd(pic)(H_2O)(OH)\right]^{+}} + H^+ \quad (1)$$

$$\underset{10-1}{\left[Pd(pic)(H_2O)(OH)\right]^{+}} \rightleftharpoons \underset{10-2}{\left[Pd(pic)(OH)_2\right]} + H^+ \quad (2)$$

$$2\underset{10-1}{\left[Pd(pic)(H_2O)(OH)\right]^{+}} \rightleftharpoons \underset{20-2}{\left[Pd(pic)(OH)_2Pd(pic)\right]^{2+}} + 2H_2O \quad (3)$$

The pK_{a1} and pK_{a2} values were found to be 4.81 and 8.46, respectively. The pK_{a1} value is intermediate between those for the $Pd(en)^{2+}$ and $Pd(bpy)^{2+}$ complexes,[21] since picolylamine has one pyridine ring that has π-acceptor properties, leading to an increase in the electrophilicity of the Pd ion and consequently a decrease in the pK_a of the coordinated water molecule. The equilibrium constant for the dimerization reaction (3) can be calculated with eq. (4) and amounts to 3.17.

$$\log K_{dimer} = \log\beta_{20-2} - 2\log\beta_{10-1} \quad (4)$$

The distribution diagram for $[Pd(Pic)(H_2O)_2]^{2+}$ and its hydrolysed species is shown in Fig. 1. The concentrations of the monohydroxo species 10−1, and the dimeric species 20−2, increase with increasing pH, predominating in the pH range 6 to 7, with formation percentages of *ca.* 40 and 60% for the monohydroxo (10−1) and the dimeric species (20−2), repectively, *i.e.* they are the main species present in solution in the physiological pH range. A further increase in pH is accompanied by an increase in the dihydroxo species, which is the main species above a pH of *ca.* 10.

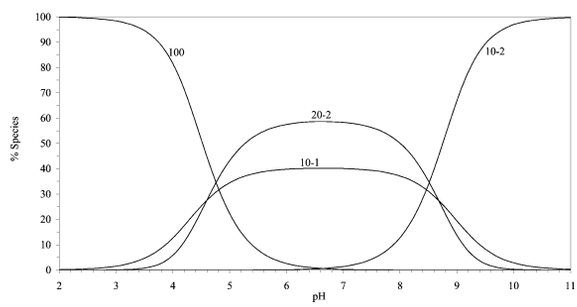

Fig. 1 Species distribution of various species as a function of pH in the $Pd(Pic)(H_2O)_2$ system.

Complex-formation equilibria involving dicarboxylic acids

Analysis of the titration data for the Pd(Pic)–dicarboxylic acid system showed the formation of the 1 : 1 species and its protonated form. The results (Table 1) show that the cyclobutanedicarboxylic acid complex has the highest stability constant and that of adipic acid (forming an eight-membered chelate ring) has the smallest stability constant. This may be explained on the premise that the six-membered ring is more favoured energetically than the eight- and seven-membered rings (as in the adipic acid and succinic acid complexes, respectively). It is interesting to note that CBDCA has a higher stability constant than malonic acid, although they both form six-membered chelate rings. This may be due to the higher pK_a values of the former than the latter dicarboxylic acid. The stability constant of the CBDCA complex with $[Pd(Pic)(H_2O)_2]^{2+}$ (110) is higher than that for $[Pd(aliphatic\ diamine)(H_2O)_2]^{2+}$ and lower than that for $[Pd(bpy)(H_2O)_2]^{2+}$.[21] Also, the protonated species were not observed in similar Pd(II) complexes of CBDCA and aliphatic amines.[8] The higher stability of the complex with CBDCA and the stabilization of the protonated species may be attributed to the π-acceptor property of the pyridine ring. The pK_a of the protonated species is 2.82, a value lower than that of free H-$CBDCA^-$, which indicates acidification upon first chelation to Pd(II) through one carboxylate group by 2.86 pH units (5.68 to 2.82). The pK_a value of this protonated species for the corresponding bipyridyl complex[6] was estimated previously from UV-Vis measurements to be *ca.* 2.5 at 25 °C and 0.1 M ionic strength. This lowering is due to the π-acceptor property of the two pyridine rings of bipyridyl. The distribution diagram, Fig. 2, shows that the 111 species is stable only at low pH, *i.e.* less than *ca.* 4. The main species in the physiological pH range is the ring-closed form, 110, which reaches a maximum concentration of 98% in the pH range 5 to 7.

Complex-formation equilibria involving amino acids

Analyses of pH titration data for Pd(Pic)–amino acid systems showed the formation of 1 : 1 complexes with stability constants larger than for the corresponding monodentate methylamine complex. This indicates that amino acids bind through the amino and carboxylate groups. Histidine is a tridentate ligand having amino, imidazole and carboxylate groups as binding sites. With $[Pd(Pic)(H_2O)_2]^{2+}$, only two of the three binding sites are involved in complex formation. Hence,

Table 1 Formation constant of $M_lL_pH_q$ species

System	l	p	q	Logβ^b	S^c
Pd(Pic)-OH	1	0	−1	−4.81 (0.07)	2.5×10^{-7}
	1	0	−2	−13.27 (0.02)	
	2	0	−2	−6.45 (0.09)	
Glycine	0	1	1	9.60 (0.01)	1.5×10^{-7}
	0	1	2	11.93 (0.03)	
	1	1	0	9.95 (0.03)	6.1×10^{-7}
Alanine	0	1	1	9.69 (0.01)	9.2×10^{-8}
	0	1	2	11.88 (0.02)	
	1	1	0	10.89 (0.03)	3.1×10^{-7}
Phenylalanine	0	1	1	9.12 (0.01)	8.0×10^{-8}
	0	1	2	11.01 (0.03)	
	1	1	0	11.05 (0.01)	1.1×10^{-7}
Valine	0	1	1	9.57 (0.01)	9.9×10^{-8}
	0	1	2	11.70 (0.03)	
	1	1	0	10.33 (0.02)	2.1×10^{-7}
Proline	0	1	1	10.52 (0.01)	4.4×10^{-8}
	0	1	2	12.03 (0.03)	
	1	1	0	11.16 (0.03)	5.8×10^{-7}
Threonine	0	1	1	9.06 (0.01)	7.9×10^{-8}
	0	1	2	11.03 (0.02)	
	1	1	0	10.40 (0.03)	2.6×10^{-7}
Isoleucine	0	1	1	9.76 (0.00)	3.4×10^{-8}
	0	1	2	12.22 (0.01)	
	1	1	0	11.76 (0.05)	1.8×10^{-8}
Methionine	0	1	1	9.10 (0.01)	8.9×10^{-8}
	0	1	2	11.08 (0.03)	
	1	1	0	9.49 (0.01)	8.4×10^{-8}
Histidine	0	1	1	9.88 (0.01)	2.4×10^{-8}
	0	1	2	15.97 (0.01)	
	1	1	0	13.19 (0.03)	8.1×10^{-7}
Aspartic acid	0	1	1	9.68 (0.01)	3.8×10^{-8}
	0	1	2	13.25 (0.01)	
	1	1	0	10.02 (0.06)	3.9×10^{-6}
Serine	0	1	1	9.14 (0.01)	1.7×10^{-8}
	0	1	2	11.40 (0.01)	
	1	1	0	11.35 (0.09)	7.5×10^{-7}
	1	1	−1	3.05 (0.10)	
S-Methylcysteine	0	1	1	8.51 (0.01)	3.4×10^{-8}
	1	1	0	10.52 (0.03)	9.9×10^{-9}
Histidine	0	1	1	9.53 (0.01)	1.8×10^{-7}
	0	1	2	15.81 (0.03)	
	0	1	3	17.81 (0.06)	
	1	1	0	13.36 (0.01)	5.6×10^{-7}
Ornithine	0	1	1	10.58 (0.00)	1.0×10^{-8}
	0	1	2	19.43 (0.02)	
	0	1	3	21.39 (0.02)	
	1	1	0	13.13 (0.02)	3.3×10^{-8}
	1	1	1	20.54 (0.02)	
Methylamine	0	1	1	10.03 (0.04)	4.4×10^{-7}
	1	1	0	7.32 (0.03)	1.8×10^{-7}
	1	2	0	13.45 (0.05)	
Ethanolamine	0	1	1	9.07 (0.02)	4.7×10^{-8}
	1	1	0	7.36 (0.06)	7.4×10^{-8}
	1	1	−1	2.45 (0.03)	
Glycinamide	0	1	1	7.88 (0.00)	4.5×10^{-8}
	1	1	0	9.30 (0.05)	7.7×10^{-8}
	1	1	−1	5.73 (0.04)	
Glycylglycine	0	1	1	7.97 (0.00)	4.0×10^{-8}
	1	1	0	8.29 (0.03)	2.6×10^{-7}
	1	1	−1	4.37 (0.02)	
Leucylalanine	0	1	1	8.03 (0.00)	3.1×10^{-8}
	1	1	0	7.59 (0.06)	1.9×10^{-6}
	1	1	−1	2.11 (0.10)	
Glycylalanine	0	1	1	8.04 (0.00)	4.4×10^{-8}
	1	1	0	8.31 (0.05)	9.15×10^{-7}
	1	1	−1	3.08 (0.06)	
Glycylleucine	0	1	1	8.13 (0.00)	4.1×10^{-8}
	1	1	0	8.22 (0.04)	7.84×10^{-7}
	1	1	−1	3.06 (0.05)	
Glycylvaline	0	1	1	8.13 (0.00)	9.3×10^{-9}
	1	1	0	7.73 (0.04)	7.5×10^{-7}
	1	1	−1	2.39 (0.05)	
Glutamine	0	1	1	8.92 (0.00)	3.0×10^{-8}
	1	1	0	10.02 (0.05)	3.3×10^{-7}
	1	1	−1	0.36 (0.09)	
Aspargine	0	1	1	8.55 (0.02)	5.9×10^{-8}
	1	1	0	10.06 (0.07)	5.2×10^{-8}

Table 1 (*Contd.*)

System	*l*	*p*	*q*	Logβ^b	S^c
	1	1	−1	2.65 (0.07)	
1,1-Cyclobutanedicarboxylic acid	0	1	1	5.68 (0.004)	1.66×10^{-8}
	0	1	2	8.80 (0.006)	
	1	1	0	8.09 (0.04)	4.59×10^{-7}
	1	1	1	10.91 (0.04)	
Uracil	0	1	1	9.28 (0.006)	2.4×10^{-8}
	1	1	0	9.17 (0.01)	7.4×10^{-8}
	1	2	0	15.98 (0.02)	
Uridine	0	1	1	9.01 (0.01)	1.1×10^{-7}
	1	1	0	9.00 (0.02)	1.7×10^{-7}
	1	2	0	14.90 (0.03)	
Thymidine	0	1	1	9.50 (0.00)	8.7×10^{-8}
	1	1	0	9.17 (0.01)	7.9×10^{-8}
	1	2	0	15.21 (0.03)	
Thymine	0	1	1	9.58 (0.00)	8.1×10^{-8}
	1	1	0	8.96 (0.01)	3.3×10^{-8}
	1	1	0	15.62 (0.03)	
Guanosine 5′-monophosphate	0	1	1	9.28 (0.008)	6.55×10^{-8}
	0	1	2	15.41 (0.01)	
	1	1	0	10.83 (0.04)	6.8×10^{-8}
	1	1	1	17.35 (0.04)	
	1	1	2	21.01 (0.04)	
Inosine 5′-monophosphate	0	1	1	9.21 (0.006)	4.26×10^{-8}
	0	1	2	15.21 (0.01)	
	1	1	0	10.42 (0.01)	5.25×10^{-8}
	1	1	1	16.46 (0.02)	
	1	1	2	18.73 (0.08)	
Oxalic acid	0	1	1	3.93 (0.002)	1.28×10^{-9}
	0	1	2	5.66 (0.01)	
	1	1	0	6.87 (0.09)	7.3×10^{-8}
Malonic acid	0	1	1	5.21 (0.004)	1.9×10^{-8}
	0	1	2	7.61 (0.01)	
	1	1	0	6.78 (0.02)	4.5×10^{-7}
	1	1	1	9.24 (0.04)	
Succinic acid	0	1	1	5.25 (0.01)	1.87×10^{-7}
	0	1	2	9.27 (0.01)	
	1	1	1	5.06 (0.007)	1.3×10^{-8}
	1	1	1	9.25 (0.01)	
Adipic acid	0	1	1	4.96 (0.003)	9.84×10^{-9}
	0	1	2	9.08 (0.003)	
	1	1	0	4.57 (0.01)	4.19×10^{-8}
	1	1	1	8.87 (0.01)	

[a] *l*, *p* and *q* are the stoichiometric coefficients corresponding to $Pd(Pic)^{2+}$, amino acid, dicarboxylic acid, and DNA or peptide and H^+, respectively. The coefficient −1 refers to loss of H^+ from the coordinated water molecule, or from the coordinated hydroxyl groups of serine and ethanolamine, or from the coordinated amide group of the peptide and DNA or peptide and H^+, respectively. [b] Standard deviations are given in parentheses. [c] Sum of square of residuals.

Table 2 Formation constants for mixed ligand complexes of $[Pd(Pic)(H_2O)_2]$ with cyclobutanedicarboxylic acid and some DNA units at 25 °C and 0.1 M ionic strength

System	*l*′	*p*′	*q*′	*r*′ [a]	Logβ^b	S^c
Uracil	1	1	1	0	14.18 (0.08)	1.1×10^{-6}
Uridine	1	1	1	0	14.18 (0.05)	1.2×10^{-6}
Thymine	1	1	1	0	14.34 (0.08)	3.8×10^{-6}
Guanosine monophosphate	1	1	1	0	15.07 (0.05)	1.48×10^{-6}
	1	1	1	1	21.58 (0.08)	
Inosine monophosphate	1	1	1	0	14.65 (0.03)	3.7×10^{-7}
	1	1	1	1	20.86 (0.09)	

[a] *l*′, *p*′, *q*′ and *r*′ are the stoichiometric coefficients corresponding to $Pd(Pic)^{2+}$, cyclobutanedicarboxylic acid, DNA subunits and H^+, respectively. [b] Standard deviations are given in parentheses. [c] Sum of square of residuals.

histidine coordinates in either a glycine-like or a histidine-like mode. The stability constant of the histidine complex is in fair agreement with that of histamine and higher than those of amino acids. This indicates that histidine interacts with the Pd(II) complex in the same way as histamine does. Serine has an extra binding centre on the β-alcoholate group. This group was reported [22] to participate in transition metal ion complex-formation reactions. The titration curve for the serine complex is much lower than the curves for the other amino acid complexes in the region followed by the complete formation of the (110) complex. Also, the potentiometric data are fitted much better when the formation of the complex species with stoichiometric coefficients 110 and 11−1 is assumed. The pK_a value of the alcoholate group incorporated in the Pd(II) complex ($\log\beta_{110} - \log\beta_{11-1}$) is 8.30. This value is lower than that of the Pd(*N*,*N*′-dimethylethylenediamine)–serine complex (8.43).[7] This may be due the π-acceptor property of the pyridine ring, which increases the electrophilicity of the Pd(II) ion and consequently decreases the pK_a value of the coordinated alcoholate group. The distribution diagram for the serine complex is given in

Table 3 Effect of chloride ion concentration on the formation constants of Pd(Pic)–CBDCA at 25 °C

Chloride ion conc./M	*l*	*p*	*q*[a]	Logβ[b]	*S*[c]
0.05	1	1	0	3.95 (0.09)	9.75×10^{-7}
	1	0	−1	−7.31 (0.01)	6.1×10^{-8}
	1	0	−2	−16.46 (0.02)	
0.1	1	1	0	3.56 (0.06)	7.4×10^{-7}
	1	0	−1	−7.9 (0.007)	1.04×10^{-8}
	1	0	−2	−17.26 (0.01)	
0.15	1	1	0	3.3 (0.08)	5.46×10^{-7}
	1	0	−1	−7.93 (0.04)	2.2×10^{-7}
	1	0	−2	−17.34 (0.05)	
0.2	1	1	0	3.00 (0.07)	2.92×10^{-7}
	1	0	−1	−8.21 (0.01)	3.9×10^{-8}
	1	0	−2	−17.71 (0.01)	
0.25	1	1	0	2.62 (0.09)	1.88×10^{-7}
	1	0	−1	−8.39 (0.01)	5.95×10^{-8}
	1	0	−2	−18.00 (0.02)	

[a] *l*, *p* and *q* are the stoichiometric coefficients corresponding to Pd(Pic), CBDCA and H^+, respectively. [b] Standard deviations are given in parentheses. [c] Sum of square of residuals.

Table 4 Effect of dioxane on the formation constant of Pd(Pic)–CBDCA at 25 °C

% Dioxane	*l*	*p*	*q*[a]	Logβ[b]	*S*[c]
12.5	0	1	1	6.16 (0.01)	1.4×10^{-7}
	0	1	2	9.58 (0.02)	
	1	1	0	8.93 (0.09)	2.8×10^{-7}
	1	0	−1	−4.42 (0.03)	
	1	0	−2	−13.11 (0.06)	2.1×10^{-7}
25	0	1	1	6.57 (0.01)	2.5×10^{-8}
	0	1	2	10.38 (0.02)	
	1	1	0	9.36 (0.03)	3.1×10^{-7}
	1	0	−1	−4.7 (0.01)	
	1	0	−2	−13.81 (0.02)	3.4×10^{-8}
37.5	0	1	1	7.06 (0.01)	2.4×10^{-8}
	0	1	2	11.28 (0.01)	
	1	1	0	9.89 (0.01)	1.6×10^{-7}
	1	0	−1	−4.73 (0.01)	
	1	0	−2	−14.14 (0.03)	4.8×10^{-8}
50	0	1	1	7.63 (0.03)	2.2×10^{-8}
	0	1	2	12.31 (0.03)	
	1	1	0	10.58 (0.02)	3.4×10^{-7}
	1	0	−1	−4.9 (0.02)	
	1	0	−2	−14.68 (0.05)	6.7×10^{-7}
62.5	0	1	1	8.09 (0.05)	1.03×10^{-8}
	0	1	2	13.07 (0.05)	
	1	1	0	10.99 (0.04)	1.72×10^{-6}
	1	0	−1	−4.98 (0.03)	
	1	0	−2	−14.99 (0.05)	7.92×10^{-7}

[a] *l*, *p* and *q* are the stoichiometric coefficients corresponding to Pd(Pic), cyclobutanedicarboxylic acid and H^+ respectively. [b] Standard deviations are given in parentheses. [c] Sum of square of residuals.

Fig. 3. The complex species with coefficients 110 reaches the maximum degree of formation (99%) at pH 4 to 6.5, *i.e.* in the physiological pH range. However, the species 11−1 attains a maximum concentration of 98% at pH *ca.* 10.

Ethanolamine forms the complex species 110 and 11−1. The log β_{110} value for ethanolamine is smaller than those for the amino acids. This may be attributed to the weaker coordinating tendency of an alcohol group compared to a carboxylate group. Charge effects will also be important since the alcohol is neutral, whereas the carboxylate group is negatively charged. The pK_a value of the coordinated alcohol group in the ethanolamine complex (4.91) is considerably smaller than that of the serine complex. This is consistent with the reaction scheme where the alcohol group in ethanolamine is coordinated to the palladium centre, whereas the OH group in serine remains attached prior to deprotonation. Due to the donation

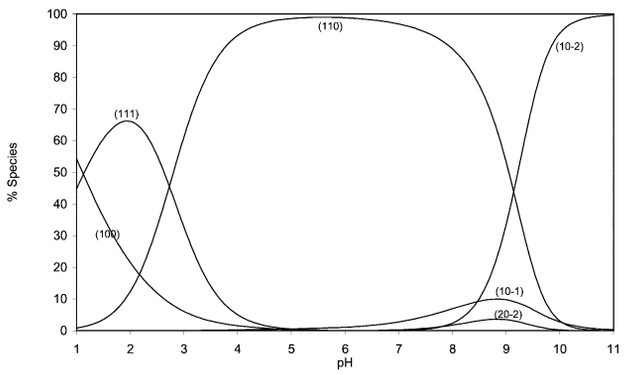

Fig. 2 Concentration distribution of various species as a function of pH in the Pd(Pic)–CBDCA system.

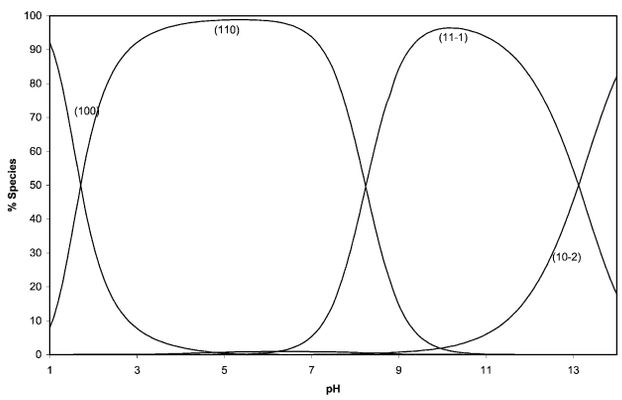

Fig. 3 Concentration distribution of various species as a function of pH in the Pd(Pic)–serine system.

of the electron pair on oxygen to the metal centre, the OH bond can be considerably weakened and the ionization of a proton occurs at fairly low pH.

Phenylalanine forms a more stable complex than alanine, although the amino group of the former is less basic than that of the latter. This may be due to some stacking interactions between the phenyl group of phenylalanine and the pyridine ring of picolylamine. This will contribute to the stabilization of the formed complex.

S-Methylcysteine has the lowest pK_a value (8.51) among the amino acids studied. Its complex has a higher stability constant than that for amino acids such as glycine. This may be taken as evidence that the sulfur atom is participating in the complex-formation process. Also, *S*-methylcysteine forms a more stable complex than methionine, plausibly due to the fact that the five-membered chelate ring in the former complex is energetically more favoured than the six-membered chelate ring in the latter complex.

Aspartic acid has two carboxylic and one amino group as potential chelating centres. It may coordinate either *via* the two carboxylate groups or by the amino and one carboxylate group. The stability constant of the aspartic acid complex is in the range of those for amino acids. This may reveal that aspartic acid coordinates *via* the amino and one carboxylate group. Also, ornithine has two amino and one carboxylic groups. Its stability constant is higher than those of amino acids, which indicates binding of ornithine *via* the two amino groups. This formulation is supported by the high affinity of Pd(II) to nitrogen donor centres.

Complex-formation equilibria involving peptides

For the Pd(Pic)–peptide system the potentiometric data were fitted to various models. The most acceptable model was found to be consistent with the formation of complexes with

stoichiometric coefficients 110 and 11−1 according to eq. (5) and (6).

$$\left[\mathrm{Pd(Pic)(H_2O)_2}\right]^{2+} + \mathrm{L^-} \underset{}{\overset{K_1}{\rightleftharpoons}} \underset{110}{\left[\mathrm{Pd(Pic)L}\right]^{+}} + 2\mathrm{H_2O} \quad (5)$$

$$\underset{110}{\left[\mathrm{Pd(Pic)L}\right]^{+}} \overset{\mathrm{p}K_a}{\rightleftharpoons} \underset{11-1}{\left[\mathrm{Pd(Pic)LH_{-1}}\right]} + \mathrm{H^+} \quad (6)$$

The 110 complex is formed *via* coordination of the amino and carbonyl groups. Upon deprotonation of the amide group, the coordination sites could switch from the carbonyl oxygen to the amide nitrogen such that the 11−1 complex is formed. Such changes in coordination modes are well documented.[23] The glutamine complex is more stable than the glycinamide complex, presumably due to the fact that glutamine carries a negative charge, whereas glycinamide is neutral. The electrostatic interaction between the glutaminate and the positively charged Pd(II) complex would result in a lowering of the free energy of formation. The pK_a values of the amide group incorporated in the Pd(II) complex ($\log\beta_{110} - \log\beta_{11-1}$) are in the range of 3.57–9.66. The low pK_a values in the present study are probably due to the high affinity of Pd(II) to nitrogen donor ligands. It is interesting to note that the pK_a value for the glycinamide complex is lower than those for other peptides. This can be explained on the basis that the more bulky substituents on the peptide may hinder the structural changes when going from the protonated to the deprotonated complexes. The pK_a of the glutamine complex is exceptionally higher than those for the other peptide complexes. This is ascribed to the formation of a seven-membered chelate ring which is more strained and therefore less favoured. The distribution diagram for the Pd(Pic)–glycylglycine system is given in Fig. 4. [Pd(Pic)L]$^+$ (110) (glycylglycine = HL), starts to form at pH 1.0, its concentration increases with increasing pH and reaches a maximum of 54% at pH 3.4. A further increase in pH is accompanied by a decrease in [Pd(Pic)L]$^+$ concentration and an increase in [Pd(Pic)LH$_{-1}$] (11−1) concentration, reaching a maximum of 100% at pH 6, *i.e.* in the physiological pH range the deprotonated species (11−1) predominates.

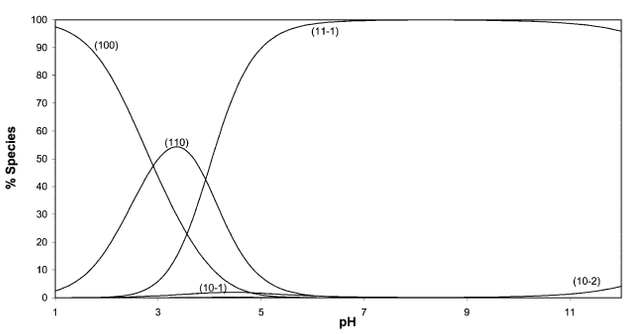

Fig. 4 Concentration distribution of various species as a function of pH in the Pd(Pic)–glycylglycine system.

Complex-formation equilibria involving DNA constituents

The pyrimidines, uracil, uridine and thymine have only basic nitrogen donor atoms (N_3–C_4O group). They form 1 : 1 and 1 : 2 complexes with $[Pd(Pic)(H_2O)_2]^{2+}$. The thymidine complexes are more stable than those of uridine, most probably owing to the high basicity of the N_3 group of thymidine resulting from the extra electron donating methyl group. As a result of the high pK_a values of pyrimidines (p$K_a \approx 9$) and the fact that they are monodentates, the complexes are formed only above pH 6, supporting the view that the negatively charged nitrogen donors of pyrimidine bases are important binding sites in the neutral and slightly basic pH ranges. The purines, inosine 5′-monophosphate and guanosine 5′-monophosphate form 110, 111 and 112 complexes. The protonated species are formed in the acidic pH range and correspond to the N_7 coordinated complex, where the N_1 nitrogen and the phosphate group are protonated. The pK_a values of the protonated species of the IMP complex (112) are 2.27 ($\log\beta_{112} - \log\beta_{111}$) and 6.04 ($\log\beta_{111} - \log\beta_{110}$). The corresponding values for GMP are 3.66 and 6.52. The former pK_a value for IMP and GMP corresponds to the N_1H group and the second pK_a value to the –PO_2(OH) group. The N_1H groups were acidified upon complex formation by 6.94 (9.21 − 2.27) and 5.62 (9.28 − 3.66) pK units for IMP and GMP, respectively. Acidification of the N_1H group upon complex formation is consistent with previous reports for IMP and GMP complexes.[24] The phosphate group was not acidified upon complex formation since it is far away from the coordination centre. The IMP and GMP complexes are more stable than those of the pyrimidines. The extra stabilization can be explained on the basis of different coulombic forces operating between the ions resulting from the negatively charged phosphate group. Hydrogen bonding between the phosphate and the exocyclic amine is also thought to contribute to the higher stability of the nucleotides over that of the nucleosides.[25] It is interesting to compare our earlier kinetic results[11] with those of the present study. The kinetic study was done in an acidic pH range. Under this condition Pd(Pic)$^{2+}$ binds to IMP through the N_7 site, leaving the N_1 site and the phosphate groups protonated. The stability constant (K) of the species formed under this condition is calculated using eq. (7).

$$\log K = \log\beta_{112} - \log\beta_{012} \quad (7)$$

The log$K$ value was found to be 3.52. This is comparable with the value obtained from the kinetic investigation (log$K$ = 2.09). The difference can be related to different experimental conditions (the kinetic study was performed at 10 °C and an ionic strength of 0.5 M), techniques employed and the acidity range selected for the kinetic measurements, where more than one Pd^{II} complex and/or IMP acid–base forms may contribute to the kinetic result.

Ring-opening of [Pd(Pic)(CBDCA)] and the formation of [Pd(Pic)(CBDCA-O)(DNA)]

The potentiometric data for the system consisting of [Pd(Pic)$(H_2O)_2]^{2+}$, CBDCA and DNA constituents such as uracil, uridine, thymine, IMP or GMP, were fitted assuming different models. The accepted model for pyrimidines is consistent with the formation of the 1110 species. The accepted model for IMP and GMP was found to consist of 1110 and 1111 species. These results were further verified by comparing the experimental potentiometric data with the theoretically calculated (simulated) curve. Fig. 5 presents such a comparison for the IMP system, from which it follows that the experimental data coincide with the theoretical curve. This supports the formation of the quaternary complex. The pK_a values of the protonated

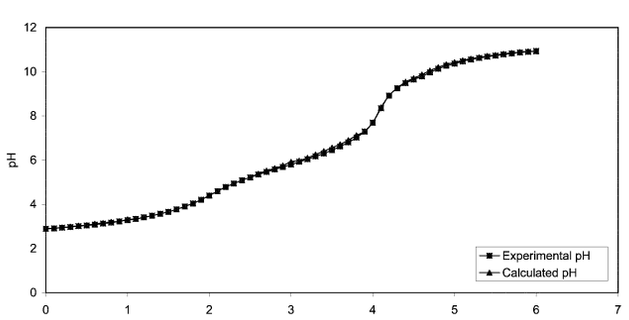

Fig. 5 Potentiometric titration curve for the Pd(Pic)–CBDCA–inosine monophosphate system.

complexes are 6.21 and 6.51 for the IMP and GMP complexes, respectively. These are most probably due to the phosphate groups. It is interesting to note that the quaternary complexes of IMP and GMP are more stable than those of the pyrimidines. This may be explained on the premise that the cyclobutane ring forms a close hydrophobic contact with the purine rings of IMP and GMP. Such hydrophobic contacts may contribute to the stabilization of the quaternary complexes. The same finding was obtained from an NMR investigation of the carboplatin–GMP complex.[26] It should be recognized that further studies are necessary to elucidate the ring-opening reaction of chelated CBDCA by DNA consitituents, especially multinuclear NMR measurements. These aspects will be considered in future investigations.

The speciation diagram obtained for the Pd(Pic)–CBDCA–IMP system is shown in Fig. 6. The Pd(Pic)–CBDCA species (1100) predominates at 73% at pH = 4.3. The Pd(Pic)–IMP species (1010) reaches the maximum concentration of 16% at pH = 7.4. The quaternary species Pd(Pic)–CBDCA–IMP (1110) attains a maximum of 80% in the pH range 7.6–9. This reveals that in the physiological pH range the ring opening of chelated CBDCA by DNA is quite feasible.

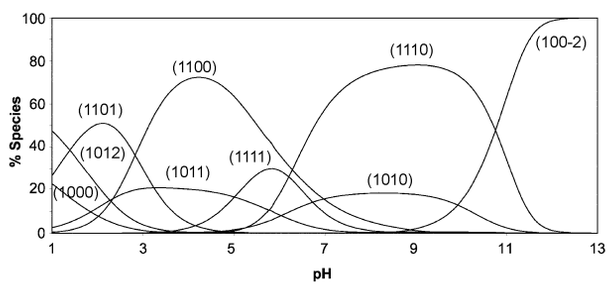

Fig. 6 Concentration distribution of various species as a function of pH in the Pd(Pic)–CBDCA–IMP system.

Effect of chloride ion concentration

The equilibrium constants for the Pd(Pic)–CBDCA complex obtained for different chloride ion concentrations at a constant ionic strength of 0.30 M are summarized in Table 3. The stability constants of the Pd(Pic)–CBDCA complex tend to decrease on increasing the chloride ion concentration. This can be accounted for on the basis that the concentrations of the active species, the mono- and the diaqua complexes, decrease with increasing $[Cl^-]$, which in turn will affect the stability of the complexes formed.

Effect of solvent

In order to characterize the formation equilibria of the Pd(Pic)–CBDCA complex in dioxane–water solutions, all other equilibria involved, *viz.* acid–base equilibria of CBDCA and $[Pd(Pic)(H_2O)_2]^{2+}$, have to be studied in the same solvent. The equilibrium constants are reported in Table 4. The hydrolysis of the $Pd(Pic)^{2+}$ complex in dioxane–water solution leads to the formation of mono- and dihydroxy species. The dihydroxo bridged dimer was not detected. The pK_a values of CBDCA and those of the coordinated water molecules in $[Pd(Pic)(H_2O)_2]^{2+}$ increase linearly with increasing dioxane concentration. This may be correlated with the ability of a solvent of relatively low dielectric constant to increase the electrostatic attraction between the proton and ligand anion in case of CBDCA, and that between a proton and the hydrolysed form of Pd(II) species. The variation in stability constant of the $Pd(Pic)^{2+}$ complex with CBDCA as a function of solvent composition is shown in Fig. 7. The stability constant for the Pd(Pic)–CBDCA complex increases linearly with increasing dioxane concentration. This is explained in terms of complex formation involving oppositely charged ions as in the Pd(Pic)–CBDCA complex, which is favoured by the low dielectric constant of the medium, *i.e.* with increasing dioxane concentration.

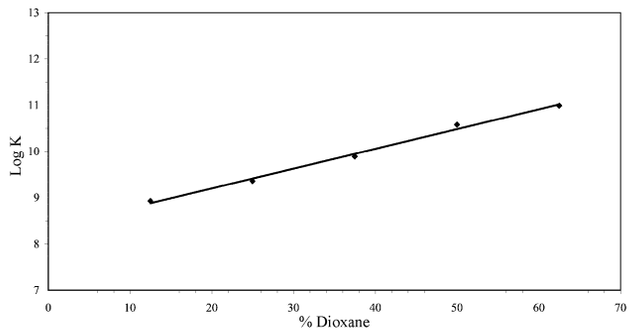

Fig. 7 Effect of dioxane on the stability constant of the Pd(Pic)–CBDCA system.

Conclusions

The present investigation describes the formation equilibria of $[Pd(Pic)(H_2O)_2]^{2+}$ with ligands of biological significance. From a combination of stability constant data of such diaqua complexes with dicarboxylic acids, amino acids, peptides and DNA constituents, it will in principle be possible to calculate the equilibrium distribution of the metal species in biological fluids where all types of ligands are present simultaneously. This would form a clear basis for understanding the mode of action of such metal species under physiological conditions.

From the above results it may be concluded that CBDCA among the dicarboxylic acid ligands forms the most stable complex, which is inconsistent with the fact that CBDCA complexes have the highest anti-tumour activity. In addition, CBDCA forms the ring opened mono-protonated complex which may interact with DNA, *i.e.* the main target for antitumour agents.

Amino acids form highly stable complexes, the substituent on the α-carbon atom has a significant effect on the stability of the formed complex. The thioether group in *S*-methylcysteine increases the stability constant of its complex as a result of the stronger donor properties of the sulfur atom. The imidazole group in histidine increases the stability of the complex due to high affinity of Pd^{II} for the nitrogen donor group. On the other hand the extra carboxylic group in aspartic acid does not contribute to the stability of the complex formed as the additional carboxylate group is not competing with the amino group during complex formation. The β-alcoholate group on the side chain of the amino acid serine was found to play an essential role in the function of a number of proteolytic enzymes, *e.g.* chymotrypsin and subtilisin.[27] $[Pd(Pic)(H_2O)_2]^{2+}$ promotes the ionization of the alcohol group of serine. The pK_a of the alcoholate group incorporated in the Pd(II) complex is 8.3, which indicates that the participation of the OH group in complex formation is not contributing significantly in the physiological pH range.

The present study shows clearly that the $[Pd(Pic)(H_2O)_2]^{2+}$ complex can form strong bonds with peptides and promote facile deprotonation of the peptide. The relative magnitudes of the pK_a values of the Pd(II) complexes with peptides have interesting biological implications. Under normal physiological conditions (pH 6–7), the peptides would coordinate to $[Pd(Pic)(H_2O)_2]^{2+}$ in entirely different ways. Glutaminate exists solely in its protonated form, whereas the other peptides are present entirely in the deprotonated form. Also, slight differences in the side chain of the peptides seem to produce dramatic differences in their behaviour toward the Pd(II) complex.

Anti-tumour Pt(II) amines are usually administrated as *cis*-dichloro complexes. This form persists in human blood plasma because of its high chloride content (0.1 M). The net zero charge on the complex facilitates its passage through cell walls.

Within many cells, the chloride ion concentration is much lower (only *ca.* 4 mM). A realistic extrapolation of the present study to biologically relevant conditions will require information on the effect of the chloride concentration on the reported stability constants. The reactivity of CBDCA toward the different Pd(II) species increases markedly when chloride ligands in $Pd(Pic)Cl_2$ are replaced successively by one and two water molecules. A similar qualitative conclusion was reached Lim and Martin in the case of $Pt(en)Cl_2$, based on the equilibrium distribution of $Pt^{II}(en)$ and on the rates of reactions of pyridine with $Pt^{II}(dien)$ complexes.[28]

Traditionally, water has been considered as the solvent that best represents biological conditions. Although this is a general assumption, a lower polarity has been detected in some biochemical micro-environments, such as active sites of enzymes and side chains in proteins.[29–33] It was suggested that these properties approximately correspond to those (or can be simulated by those) existing in the water/dioxane mixtures. Consequently, by way of example, a study of the Pd(Pic)–CBDCA complex-formation equilibria in dioxane–water solutions of different compositions could be of biological significance. The results show that the formation of the Pd(Pic)–CBDCA complex will be more favoured in biological environments of lower dielectric constant.

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