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Equilibrium Studies on Complex-Formation Reactions of Pd[(2-(2-aminoethyl)pyridine)$(H_2O)_2]^{2+}$ with Ligands of Biological Significance and Displacement Reactions of DNA Constituents

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The [Pd(AEP)Cl_2] complex was synthesized and characterized, where AEP = 2-(2-aminoethyl)pyridine. The stoichiometry and stability constants of the complexes formed between various biologically relevant ligands (amino acids, peptides, DNA constituents and dicarboxylic acids) and [Pd(AEP)$(H_2O)_2]^{2+}$ were investigated at 25 °C and 0.1 M ionic strength. The equilibrium constants for the substitution of representative coordinated ligands such as inosine, glycine or methionine by cysteine were calculated and the concentration distribution diagrams of the various species evaluated. The kinetics of base hydrolysis of free and coordinated amino acid esters was investigated. The effects of the medium dielectric constant and temperature on the kinetics of base hydrolysis of the glycine methyl ester in the presence of the [Pd(AEP)$(H_2O)_2]^{2+}$ complex were studied and the activation parameters $\Delta H^{\neq}$ and $\Delta S^{\neq}$ for the hydrolysis process were determined.

Introduction

Cisplatin [*cis*-diamminedichloroplatinum(II)] is one of the most active antitumour agents in clinical use.[1] However, it has a narrow spectrum of activity, and its clinical use is limited by undesirable side effects, including nephrotoxicity, ototoxicity, neurotoxicity, nausea, vomiting and myelosuppression.[2,3] In the search for new platinum anticancer drugs, great efforts are devoted to the design of complexes more efficient and less toxic than the reference drugs already in clinical use. For this purpose, the rational design of complexes and the study of relevant structure–activity relationships have been extended to families of new compounds having high structural diversity.

Pd^{II} and Pt^{II} amine complexes have the same structure, with a five orders of magnitude higher reactivity in the case of Pd^{II} complexes, but similar thermodynamic parameters. Pd^{II} complexes are good models for the analogous Pt^{II} complexes in solution. Recent work in our laboratories focused on the equilibria of complex-formation reactions of *cis*-(diamine)palladium(II) complexes with DNA, the major target in chemotherapy of tumours, and biorelevant ligands as amino acids, peptides and dicarboxylic acids and esters.[4–7] In the present study, we have investigated the thermodynamic behaviour of Pd^{II} complexes with a bidentate amine ligand having a heteroaromatic nitrogen base (pyridine) that possesses π-acceptor properties, which is believed to favour the interaction with DNA and consequently increase the effectiveness of the drug. Because most of the amines previously studied form five-membered chelate rings, the present investigation aimed to use AEP [2-(2-aminoethyl)pyridine] as amine forming a six-membered chelate ring. The increase in the chelate-ring size will increase the bite angle, which will have an effect of increasing the steric interaction between the guanines in the *cis*-Pt(diamine)G_2 adduct, thereby slowing down the rotation of the guanines about the Pt–N_7 bonds.[8,9] Such a restriction may stabilize the DNA adduct. This is in line with the finding that *cis*-Pt(1,4-DACH)Cl_2, where a seven-membered chelate ring is formed, is more active than cisplatin and oxaliplatin in several in vivo and in vitro tests.[10] For these reasons we have performed a systematic study of the complex formations of [Pd(AEP)$(H_2O)_2]^{2+}$ with various biologically relevant ligands and also studied the displacement reactions of some DNA constituents bound to this complex.

Results and Discussion

The acid-dissociation constants of the ligands were determined under the experimental conditions 25 °C and constant 0.1 M ionic strength (adjusted with $NaNO_3$), which were also used for determining the stability constants of the Pd^{II} complexes.

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Hydrolysis of $[Pd(AEP)(H_2O)_2]^{2+}$

The $[Pd(AEP)(H_2O)_2]^{2+}$ 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 the formation of three species: 10–1, 10–2 and 20–1, as given in reactions (1)–(3). Trials were made to fit the potentiometric data assuming the formation of the hydroxo-bridged dimer, 20–1, but this resulted in a very poor fit to the data.[11]

$$\underset{100}{[Pd(AEP)(H_2O)_2]^{2+}} \overset{pK_{a1}}{\rightleftharpoons} \underset{10\text{-}1}{[Pd(AEP)(H_2O)(OH)]^{+}} + H^{+} \quad (1)$$

$$\underset{10\text{-}1}{[Pd(AEP)(H_2O)(OH)]^{+}} \overset{pK_{a2}}{\rightleftharpoons} \underset{10\text{-}2}{[Pd(AEP)(OH)_2]} + H^{+} \quad (2)$$

$$\underset{100}{[Pd(AEP)(H_2O)_2]^{2+}} + \underset{10\text{-}1}{[Pd(AEP)(H_2O)(OH)]^{+}} \overset{K_{dimer}}{\rightleftharpoons} \underset{20\text{-}1}{[(AEP)Pd(H_2O)Pd(OH)Pd(OH_2)(AEP)]^{3+}} + H_2O \quad (3)$$

The pK_{a1} and pK_{a2} values for $[Pd(AEP)(H_2O)_2]^{2+}$ are 4.59 and 9.71, respectively. The equilibrium constant for the rapid dimerization reaction (3) can be calculated by Equation (4) and amounts to 3.15.

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

The distribution diagrams for $[Pd(AEP)(H_2O)_2]^{2+}$ and its hydrolyzed species are shown in Figure 1. The concentration of the monohydroxo species 10–1, and the dimeric species 20–1 increase with increasing pH, predominate at pH 7.4 and 4.6, and reach a maximum concentration of 99 and 38%, respectively. A further increase in pH is accompanied by an increase in the dihydroxo species (10–2), which is the main species above pH ≈ 9.6. This reveals that in the physiological pH range, i.e. at pH 6–7, the monohydroxo complex (10–1) predominates and can interact with the DNA subunits. At higher pH the dihydroxo complex, the less labile species, will be the major species in solution, and consequently the ability of DNA to bind the Pd(amine) complex will decrease significantly.

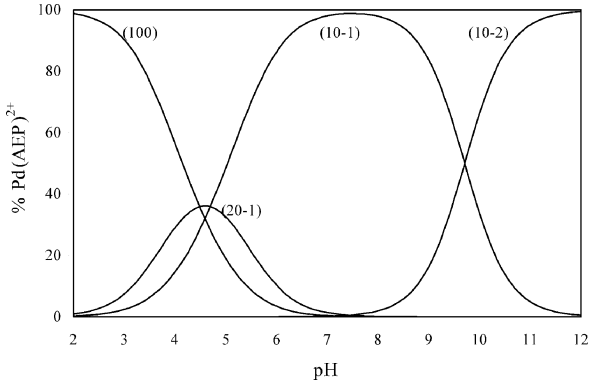


Figure 1. Concentration distribution of various species as a function of pH for the Pd(AEP)-OH system [1.25 mM Pd(AEP)].

Complex-Formation Equilibria Involving Amino Acids

Analysis of the titration data for the Pd(AEP)-amino acid system showed the formation of 1:1 species. Histidine, ornithine, lysine, glutamic acid and aspartic acid form in addition to 1:1 complexes, the monoprotonated species. The pK_a of the protonated complex was calculated from Equation (5).

$$pK_a = \log\beta_{111} - \log\beta_{110} \quad (5)$$

The pK_a values of the protonated species are 2.95 for histidine, 7.26 for ornithine, 8.87 for lysine, 4.08 for glutamic acid and 3.49 for aspartic acid. The stability constant of the histidine, ornithine, and lysine complexes are higher than those of simple amino acids. This indicates that these amino acids coordinate via the two nitrogen centres, i.e. imidazole and amino groups in the case of histidine, and by two amino groups in the case of ornithine and lysine. This is in line with the strong affinity of Pd^{II} for nitrogen donor centres. Aspartic acid and glutamic acid have two carboxylic and one amino group as potential chelating centres. It may coordinate either via the two carboxylate groups or by the amino group and one carboxylate group. The stability constants of their complexes are in the range of those for amino acids. This may reveal that aspartic acid and glutamic acid coordinate via the amino and one carboxylate group. Serine and threonine have an extra binding centre on the β-alcoholate group. Their titration curves are lower than those of simple amino acid complexes in the region $2 \leq a \leq 3$ (a = number of moles of base added per mole of ligand). This reveals that the β-alcoholate group participates in complex formation. In addition, the potentiometric data is much better fitted assuming the formation of the complex species 110 and 11–1. The pK^H value of the β-alcoholate incorporated in the Pd^{II} complex ($\log\beta_{110} - \log\beta_{11-1}$) is 8.30 and 8.10 for serine and threonine, respectively. In addition, ethanolamine forms the complex species 110, 120 and 11–1, and the $\log\beta_{110}$ value for ethanolamine is smaller than those for amino acids. This may be due to the coordination of ethanolamine at low pH through the amino group, while in the case of serine and threonine the coordination is through amino and carboxylate groups. The pK^H value of the coordinated alcohol group in ethanolamine (6.28) is smaller than those of serine and therionine. This is attributed to the tendency of ethanolamine to coordinate through the OH group at lower pH to form a five-member chelate ring (Table 1).

The distribution diagram for the serine complex is given in Figure 2. The complex species with coefficients 110 reaches the maximum degree of formation (ca. 95%) at pH 4.5 to 7, i.e. in the physiological pH range. However, the species 11–1 predominates after pH 8.3 and attains the maximum concentration of about 95% at pH ≈ 10.

Peptide Complexes

The potentiometric data for the peptide complexes were fitted on the basis of formation of the complexes with stoichiometric coefficients 110 and 11–1.

Table 1. Formation constants for complexes of $[Pd(AEP)(H_2O)^2]^{2+}$ with amino acids at 25 °C and 0.1 M ionic strength.

Ligand	M L H[a]	logβ[b]	Ligand	M L H[a]	logβ[b]
OH^-	1 0 –1	–4.59(0.01)	Glycine	0 1 1	9.61(0.02)
	1 0 –2	–14.30(0.02)		0 1 2	12.02(0.03)
	2 0 –1	–1.44(0.04)		1 1 0	10.33(0.02)
Alanine	0 1 1	9.71(0.01)	β-Phenylalanine	0 1 1	9.12(0.01)
	0 1 2	12.17(0.02)		0 1 2	11.01(0.03)
	1 1 0	10.46(0.02)		1 1 0	9.86(0.02)
γ-Aminobutyric acid	0 1 1	9.97(0.00)	β-Alanine	0 1 1	10.11(0.02)
	0 1 2	13.60(0.02)		0 1 2	13.75(0.03)
	1 1 0	7.81(0.03)		1 1 0	9.81(0.02)
	1 1 1	13.29(0.04)		1 1 1	13.37(0.04)
Valine	0 1 1	9.51(0.01)	Proline	0 1 1	10.49(0.01)
	0 1 2	11.82(0.02)		0 1 2	12.35(0.05)
	1 1 0	10.22(0.03)		1 1 0	10.81(0.10)
Isoleucine	0 1 1	9.76(0.01)	Histamine	0 1 1	9.69(0.01)
	0 1 2	12.22(0.02)		0 1 2	15.75(0.02)
	1 1 0	10.56(0.02)		1 1 0	12.85(0.08)
Histidine	0 1 1	9.15(0.01)	Ethanolamine	0 1 1	9.46(0.01)
	0 1 2	15.30(0.02)		1 1 0	8.45(0.02)
	0 1 3	17.00(0.06)		1 2 0	14.10(0.04)
	1 1 0	13.37(0.01)		1 1 –1	2.17(0.04)
	1 1 1	16.32(0.03)			(pK^H = 6.28)
Serine	0 1 1	9.14(0.01)	Threonine	0 1 1	9.06(0.01)
	0 1 2	11.40(0.02)		0 1 2	11.03(0.02)
	1 1 0	10.34(0.01)		1 1 0	10.29(0.07)
	1 1 –1	2.04(0.02)		1 1 –1	2.19(0.08)
		(pK^H = 8.30)			(pK^H = 8.10)
Ornithine	0 1 1	10.58(0.02)	Lysine	0 1 1	10.56(0.02)
	0 1 2	19.43(0.02)		0 1 2	19.75(0.03)
	0 1 3	21.39(0.03)		0 1 3	21.48(0.03)
	1 1 0	13.27(0.05)		1 1 0	10.62(0.05)
	1 1 1	20.53(0.04)		1 1 1	19.49(0.02)
Aspartic acid	0 1 1	9.61(0.02)	Glutamic acid	0 1 1	9.54(0.02)
	0 1 2	13.33(0.03)		0 1 2	13.65(0.03)
	0 1 3	15.26(0.03)		0 1 3	15.83(0.05)
	1 1 0	8.93(0.03)		1 1 0	9.19(0.03)
	1 1 1	12.42(0.09)		1 1 1	13.27(0.02)
	1 1 2	16.08(0.02)		1 1 2	15.79(0.03)
S-Methylcysteine	0 1 1	8.65(0.02)	Methionine	0 1 1	9.12(0.02)
	0 1 2	10.61(0.02)		0 1 2	11.39(0.03)
	1 1 0	9.16(0.01)		1 1 0	9.08(0.04)
Hydroxyproline	0 1 1	9.40(0.01)	Methylamine	0 1 1	10.43(0.01)
	0 1 2	11.39(0.02)		1 1 0	7.71(0.06)
	1 1 0	10.16(0.03)		1 2 0	14.53(0.04)
Cysteine	0 1 1	10.36(0.01)			
	0 1 2	18.61(0.02)			
	0 1 3	20.62(0.02)			
	1 1 0	15.11(0.03)			
	1 1 1	19.20(0.04)			

[a] M, L and H are the stoichiometric coefficients corresponding to Pd(AEP), amino acid, and H^+, respectively; the coefficient –1, refers to a proton loss. [b] logβ of Pd(AEP)-amino acids. Standard deviations are given in parentheses; sum of square of residuals are less than 5E–7, pK^H = logβ_{110} – logβ_{11-1}.

$$[Pd(AEP)(H_2O)_2]^{2+} + L^- \underset{}{\overset{K}{\rightleftharpoons}} [Pd(AEP)L]^+ + 2H_2O$$
$$100 \qquad\qquad 110$$

$$[Pd(AEP)L]^+ \overset{K^H}{\rightleftharpoons} [Pd(AEP)LH_{-1}] + H^+$$
$$110 \qquad\qquad 11\text{-}1$$

The 110 complex is formed via coordination of the amine and carbonyl groups. On increasing the pH, the amide groups undergo deprotonation and the complex [Pd(AEP)-LH_{-1}], (11–1) is formed. The pK^H values of the amide groups incorporated in the Pd^{II} complexes (logβ_{110} – logβ_{11-1}) are in the 3.85–8.64 range. The pK^H of the gluta-

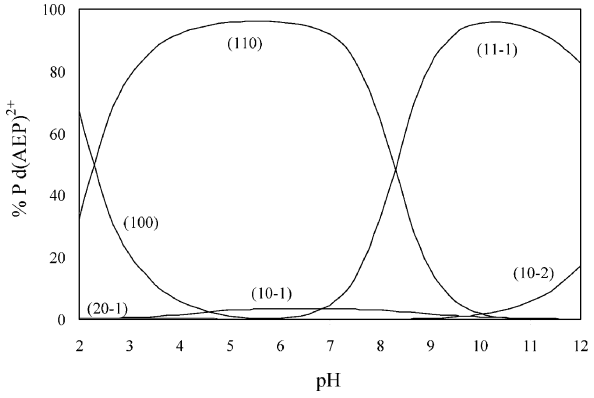


Figure 2. Concentration distribution of various species as a function of pH for the Pd(AEP)-serine system [1.25 mM Pd(AEP) and serine].

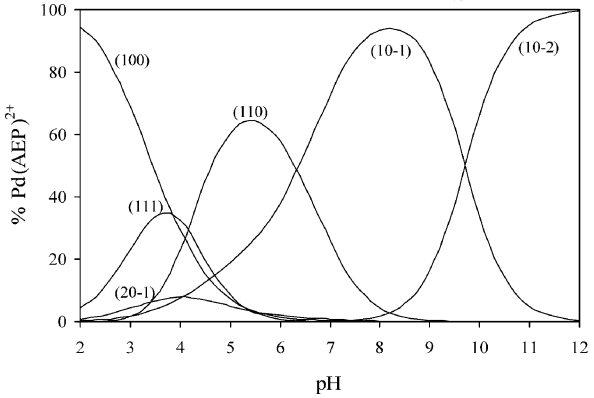


Figure 3. Concentration distribution of various species as a function of pH for the Pd(AEP)-succinic acid system [1.25 mM Pd(AEP) and succinic acid].

mine complex is markedly higher than those for peptide complexes, Table 2. This is ascribed to the formation of a seven-membered chelate ring, which would probably be more strained and therefore less favoured.

The relative magnitude of the pK^H values of the Pd^{II} complexes with peptides has interesting biological implications. Under normal physiological conditions (pH 6–7) the peptide would coordinate to $[Pd(AEP)(H_2O)_2]^{2+}$ in entirely different fashions. Glutamate would exist solely in the protonated form, whereas the other peptides would be present entirely in the deprotonated form. In addition, the slight difference in the side chain of the peptides produces dramatic differences in their behaviour toward the palladium species.

Dicarboxylic Acid Complexes

In the Pd(AEP)-dicarboxylic acid system, the computer analysis of the pH titration data showed the presence of the 1:1 species and its protonated form. The results in Table 2 show that the adipic acid complex is the least stable as the complex involves the formation of the least stable eight-membered chelate ring. The pK_a values of the protonated species for $[Pd(AEP)HL]^+$ is in the range (1.65–4.37). These values are lower than those for the HL^- ion, Table 2. The lowering of the pK_a is due to acidification of the second carboxylic acid group upon coordination of Pd^{II} to one carboxylate group.

From the concentration-distribution diagram of the succinic acid complex given in Figure 3 as a representative example, it is interesting to note that the mono-protonated species attains its maximum concentration of 37% at pH 3.8. This form has one coordination site available for binding to DNA. The mono-coordinated species was documented to be the active form in the case of carboplatin.[12–14] However, the displacement of chelated CBDCA by DNA (as 5′-GMP) can not be ruled out.[15]

DNA Complexes

DNA constituents such as guanosine, adenosine, cytosine, uracil and thymidine, form 1:1 and 1:2 complexes with the $Pd(AEP)^{2+}$ ion. However, inosine, adenine and nucleotides such as inosine-5′-monophosphate, guanosine-5′-monophosphate, cytidine-5′-monophosphate and uridine-5′-monophosphate form the monoprotonated complex, in addition to the formation of 1:1 and 1:2 complexes. The pK_a value of the protonated inosine complex is 4.11. This value corresponds to N_1H. The lowering of this value with respect to that of free inosine (pK_a = 8.80) is due to acidification upon complex formation. The pK_a values of the protonated nucleotide complexes are 4.75, 5.93, 4.97 and 6.11 for inosine-5′-monophosphate, guanosine-5′-monophos-

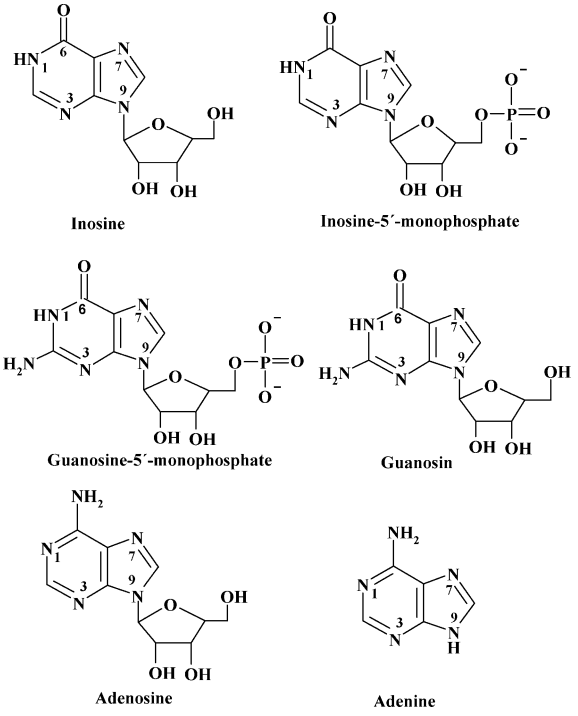


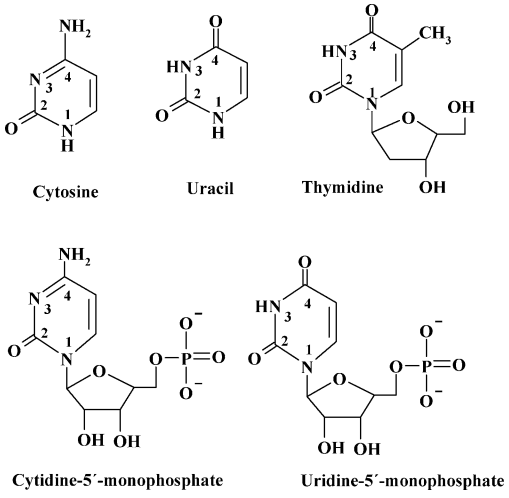
Table 2. Formation constants for complexes of $[Pd(AEP)(H_2O)_2]^{2+}$ with peptides, dibasic acids and DNA units at 25 °C and 0.1 M ionic strength.

Ligand	M L H[a]	logβ[b]	Ligand	M L H[a]	logβ[b]
Glycinamide			Cyclobutane-1,1-dicarboxylic acid		
	0 1 1	7.88(0.02)		0 1 1	5.57(0.01)
	1 1 0	8.01(0.02)		0 1 2	8.57(0.01)
	1 1 –1	4.16(0.01)		1 1 0	7.11(0.02)
		(pK^H = 3.85)		1 1 1	8.76(0.05)
Glycylglycine			Succinic acid		
	0 1 1	7.97(0.01)		0 1 1	5.54(0.02)
	0 1 2	11.01(0.01)		0 1 2	9.57(0.03)
	1 1 0	8.20(0.01)		1 1 0	5.01(0.00)
	1 1 –1	3.56(0.08)		1 1 1	9.16(0.01)
		(pK^H = 4.64)			
Aspargine			Malonic acid		
	0 1 1	8.55(0.01)		0 1 1	5.25(0.02)
	0 1 2	10.79(0.03)		0 1 2	7.85(0.03)
	1 1 0	9.42(0.02)		1 1 0	5.68(0.02)
	1 1 –1	1.90(0.04)		1 1 1	8.15(0.06)
		(pK^H = 7.52)			
Glycylleucine			Adipic acid		
	0 1 1	8.13(0.01)		0 1 1	5.45(0.03)
	1 1 0	7.75(0.03)		0 1 2	9.64(0.04)
	1 1 –1	2.18(0.08)		1 1 0	4.29(0.00)
		(pK^H = 5.57)		1 1 1	8.66(0.00)
Glutamine			Oxalic acid		
	0 1 1	9.00(0.01)		0 1 1	3.96(0.02)
	0 1 2	11.19(0.02)		0 1 2	5.67(0.03)
	1 1 0	9.11(0.02)		1 1 0	5.84(0.07)
	1 1 –1	0.47(0.05)		1 1 1	8.06(0.07)
		(pK^H = 8.64)			
Inosine			Fumaric acid		
	0 1 1	8.80(0.02)		0 1 1	4.47(0.04)
	1 1 0	7.78(0.03)		0 1 2	6.93(0.04)
	1 2 0	11.64(0.05)		1 1 0	4.44(0.04)
	1 1 1	11.89(0.05)		1 1 1	7.94(0.06)
Inosine-5′-mono-phosphate			Cytidine-5′-monophosphate		
	0 1 1	9.02(0.02)		0 1 1	6.32(0.02)
	0 1 2	15.24(0.03)		0 1 2	10.82(0.03)
	1 1 0	9.18(0.03)		1 1 0	5.89(0.07)
	1 2 0	16.35(0.04)		1 2 0	8.57(0.09)
	1 1 1	13.93(0.04)		1 1 1	10.86(0.05)
Adenine			Uridine-5′-monophosphate		
	0 1 1	9.65(0.03)		0 1 1	9.53(0.01)
	0 1 2	13.90(0.04)		0 1 2	15.62(0.02)
	1 1 0	9.47(0.11)		1 1 0	9.64(0.01)
	1 2 0	14.05(0.02)		1 2 0	14.22(0.02)
	1 1 1	18.56(0.02)		1 1 1	15.75(0.04)
Cytosine			Uracil		
	0 1 1	4.65(0.02)		0 1 1	9.18(0.01)
	1 1 0	5.98(0.03)		1 1 0	8.63(0.04)
	1 2 0	8.91(0.04)		1 2 0	14.47(0.08)
Guanosine			Guanosine-5′-monophosphate		
	0 1 1	9.13(0.01)		0 1 1	9.48(0.02)
	0 1 2	11.11(0.02)		0 1 2	15.82(0.03)
	1 1 0	10.48(0.06)		1 1 0	9.23(0.06)
	1 2 0	19.03(0.06)		1 2 0	13.44(0.19)
				1 1 1	15.16(0.01)
Adenosine			Thymidine		
	0 1 1	3.60(0.01)		0 1 1	9.55(0.04)
	1 1 0	2.84(0.04)		1 1 0	8.25(0.08)
	1 2 0	5.25(0.00)		1 2 0	13.72(0.04)

[a] M, L and H are the stoichiometric coefficients corresponding to Pd(N-N), ligands, and H^+, respectively; the coefficient –1, refers to a proton loss. [b] logβ of Pd(AEP) ligands; standard deviations are given in parentheses; sum of square of residuals are less than 5 e^{-7}, pK^H = logβ_{110} – logβ_{11-1}.

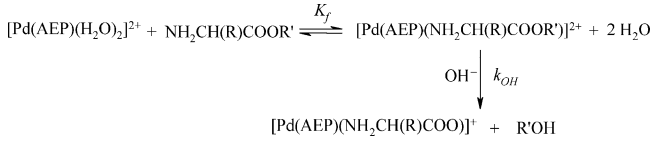
phate, cytidine-5′-monophosphate and uridine-5′-monophosphate complexes, respectively.

The pyrimidines uracil, uridine-5′-monophosphate and thymidine, have basic nitrogen donor atoms (N_3) as a result of the high pK_a values of pyrimidines (pK_a > 9) and complex formation predominates above pH 8.5. Both cytosine and cytidine-5′-monophosphate undergo N_3 protonation under mild acidic conditions. The values obtained for their protonation constants are 4.65 and 4.50, respectively. The lower values of the stability constants of their complexes, Table 2, reflect the difference in the basicity of the donor site.



Kinetics of Hydrolysis of Amino Acid Esters

The hydrolysis of amino acid esters can be presented as shown in Scheme 1.



Scheme 1.

The equilibrium constant for complex formation (K_f) is sufficiently large that coordination of the ester is readily accomplished. The kinetic data, viz. the volume of base added to keep the pH constant vs. time, could be fitted by a single exponential function as shown in Figure 4. A plot of $k_{obsd.}$ vs. hydroxide ion concentration is linear as shown in Figure 5 (for experimental data see Table 3) and follows the rate expression $k_{obsd.} = k_o + k_{OH}[OH^-]$, where k_o presents the rate constant for the water-catalyzed pathway and k_{OH} the rate constant for the base-catalyzed pathway. The linear dependence of the rate constant on the OH^- concentration is consistent with direct attack of OH^- ion on the coordinated ester carbonyl group. The catalysis ratio $C = k_{OH}/k_{OH}^{ester}$, where k_{OH}^{ester} represents the rate constant for the hydrolysis of the free amino acid ester, is given in Table 4. The catalysis ratio for coordinated glycine methyl ester equals 2.2×10^5. A catalysis ratio of such high value is consistent with the structural formula **I** for the mixed-ligand complex, in which there is direct interaction between the Pd^{II} ion and the ester carbonyl group.[16,19] The relatively low catalysis ratio, viz. 32.4 for histidine methyl ester and 91.0 for methionine methyl ester, suggests that the ester carbonyl group is not bound to the Pd^{II} ion. The histidine methyl ester complex is expected to have structure **II** in which the donor atoms are the amino group and unprotonated nitrogen of the imidazole ring. A similar situation **III** is likely with the methionine methyl ester where sulfur and amine groups act as donors. The degree of catalysis in histidine and methionine methyl ester complexes is probably due to activation by induction through the coordinated nitrogen atoms.

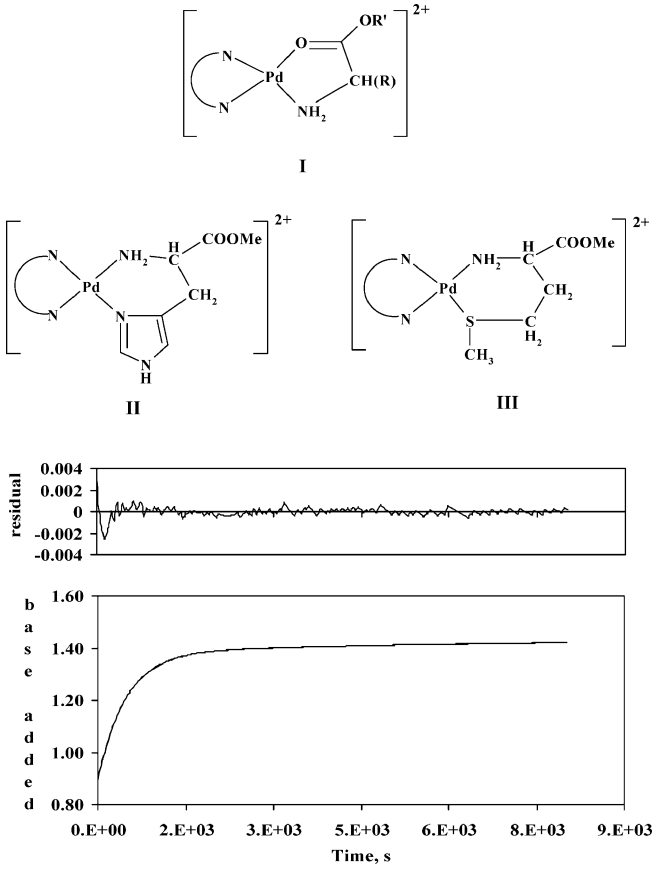


Figure 4. Typical time dependence of the volume of base added, fitted to a single exponential, for the hydrolysis of [Pd(AEP)GlyOMe]$^{2+}$ at 25 °C and 0.1 M ionic strength.

Activation parameters were determined for the hydrolysis of [Pd(AEP)(GlyOMe)]$^{2+}$. The temperature dependence of the rate constants k_o and k_{OH} is summarized in Table 5 and displayed in Figure 6. For k_{OH}, $\Delta H^{\neq} = 13.6 \pm 0.2$ kJ mol^{-1} and $\Delta S^{\neq} = -95.2 \pm 0.6$ J K^{-1} mol^{-1}, whereas for k_o, $\Delta H^{\neq} = 28.5 \pm 0.4$ kJ mol^{-1} and $\Delta S^{\neq} = -241 \pm 2$ J K^{-1} mol^{-1}. The activation parameters for k_o must be regarded as somewhat approximate as these rate constants were determined from an extrapolation of the experimental data. For base hydrolysis of the free glycine methyl ester, the activation parameters were reported[16] to be $\Delta H^{\neq} = 39.7$ kJ mol^{-1} and $\Delta S^{\neq} = -117$ J K^{-1} mol^{-1}. The enhanced rate for base hydrolysis

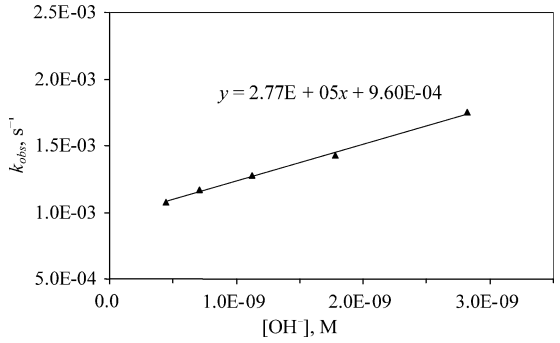


Figure 5. Plot of $k_{obsd.}$ vs. hydroxide ion concentration for the hydrolysis of $[Pd(AEP)GlyOMe]^{2+}$ at 25 °C.

Table 3. Kinetic data for hydrolysis of $[Pd(AEP)(ester)]^{2+}$ at 25 °C and 0.1 M ionic strength.

Ester	pH	$[OH^-]$/M	$k_{obsd.}$ [s^{-1}]
Glycine methyl ester			
	4.6	4.47E–10	1.08E–03
	4.8	7.08E–10	1.17E–03
	5.0	1.12E–09	1.28E–03
	5.2	1.78E–09	1.43E–03
	5.4	2.82E–09	1.75E–03
Methionine methyl ester			
	8.8	7.59E–06	4.95E–04
	9.0	1.20E–05	7.67E–04
	9.2	1.91E–05	1.39E–03
	9.4	3.02E–05	1.88E–03
	9.6	4.79E–05	3.36E–03
Histidine methyl ester			
	8.8	7.59E–06	1.70E–04
	9.0	1.20E–05	3.00E–04
	9.2	1.91E–05	4.72E–04
	9.4	3.02E–05	6.68E–04
	9.6	4.79E–05	1.00E–03

Table 4. Kinetic data for the hydrolysis of $[Pd(AEP)(ester)]^{2+}$ at 25 °C and 0.1 M ionic strength.

Ester	k_{OH} [$M^{-1}s^{-1}$]	k_o [s^{-1}]	$k_{OH}^{(ester)}$[a] [$M^{-1}s^{-1}$]	$k_{OH}/k_{OH}^{(ester)}$
Glycine methyl ester	2.77E+05	1.73E–05	1.28	2.16E+05
Methionine methyl ester	6.98E+01	9.27E–07	0.767	9.10E+01
Histidine methyl ester	2.01E+01	9.42E–07	0.62	3.24E+01

[a] Data taken from ref.[16].

of the ester coordinated to the metal centre is therefore mainly due to a decrease in $\Delta H^{\neq}$. The negative activation entropies point at the role of bond formation during the water- and hydroxide-assisted hydrolysis reactions.[17] A comparative value for k_{OH} at 25 °C for the hydrolysis of the glycine methyl ester incorporated in the $[Pd(en)L]^{2+}$ complex is 6.3×10^4 $M^{-1}s^{-1}$.[16] This value is lower than that obtained in the present study. This may be due to the π-acceptor property of the AEP ligand, which will increase the stability of the glycine methyl ester complex. Assuming that the stability constant is conceived as one of the factors that determine the donor acceptor interaction between glycine methyl ester and Pd^{II}, the complex which binds the ester more tightly will withdraw more electron density from the ester carbonyl group, making it more susceptible to attack by OH^-. This will lead to an increase in the corresponding rate constant.

Table 5. Kinetic data for the hydrolysis of $[Pd(AEP)(GlyOMe)]^{2+}$ as a function of temperature in aqueous solution.[a]

T [°C]	k_{OH} [$M^{-1}s^{-1}$]	k_o [s^{-1}]
15	2.22E+05	9.50E–06
20	2.43E+05	1.26E–05
25	2.77E+05	1.70E–05
30	3.01E+05	1.98E–05
35	3.76E+05	2.16E–05

[a] pK_w = 14.25, 14.07, 13.90, 13.74, 13.69 at 15, 20, 25, 30 and 35 °C (ref.[26]).

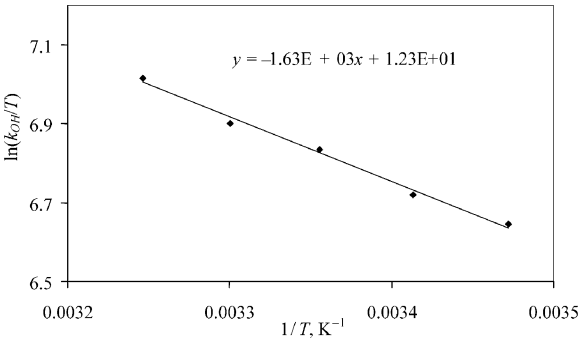


Figure 6. Effect of temperature on k_{OH} for the hydrolysis of $[Pd(AEP)GlyOMe]^{2+}$.

The rate constants for the hydrolysis of $[Pd(AEP)(GlyOMe)]^{2+}$ were determined in various dioxane/water solutions of different composition and are reported in Table 6. The rate constant k_{OH} increases with increasing concentration of dioxane. This may be accounted for on the basis that as the dioxane content increases the dielectric constant of the solution decreases. This will favour the interaction of the negatively charged OH^- ion with the electropositive carbonyl carbon atom of the ester. Consequently, the hydrolysis will proceed faster. The investigation of amino acid esters in dioxane/water solutions is of biological significance since it is known that solutions in a biochemical micro-environment such as active sites of enzymes and side chains in proteins have dielectric constant values of 30–50.[18–20] These properties approximately correspond to those of dioxane/water solution mixtures.

Table 6. Kinetic data for the hydrolysis of $[Pd(AEP)(GlyOMe)]^{2+}$ in dioxane/water solutions of different composition at 25 °C and 0.1 M ionic strength.

Dioxane (%)	k_{OH}	k_{H_2O}
12.5	3.47E+05	2.72E–05
25.0	4.41E+05	3.41E–05
37.5	5.46E+05	4.33E–05
50.0	6.63E+05	5.53E–05

Displacement Reaction of Coordinated Inosine

It was shown above that N-donor ligands such as DNA constituents have affinity for $[Pd(AEP)(H_2O)_2]^{2+}$, which may have important biological implications because the interaction with DNA is thought to be responsible for the antitumour activity of related complexes. However, the preference of Pd^{II} to coordinate to S-donor ligands was demonstrated as shown in Tables 1 and 2. These results suggest that Pd^{II}–N adducts can easily be converted into Pd–S adducts. Consequently, the equilibrium constant for such conversion is of biological significance. Consider inosine as a typical DNA constituent (presented by HA) and cysteine as a typical thiol ligand (presented by H_2B). The equilibria involved in complex formation and displacement reactions are as shown.

$$HA \rightleftharpoons H^+ + A^-$$

$$H_2B \rightleftharpoons 2H^+ + B^{2-}$$

$$\underset{100}{[Pd(AEP)]^{2+}} + A^- \rightleftharpoons \underset{110}{[Pd(AEP)A]^+} \quad (6a)$$

$$\beta_{110}^{[Pd(AEP)A]^+} = [Pd(AEP)A^+]/[Pd(AEP)^{2+}][A^-] \quad (6b)$$

$$\underset{100}{[Pd(AEP)]^{2+}} + B^{2-} \rightleftharpoons \underset{110}{[Pd(AEP)B]} \quad (7a)$$

$$\beta_{110}^{[Pd(AEP)B]} = [Pd(AEP)B]/[Pd(AEP)^{2+}][B^{2-}] \quad (7b)$$

$$[Pd(AEP)(A)]^+ + B^{2-} \overset{K_{eq}}{\rightleftharpoons} [Pd(AEP)(B)] + A^- \quad (8)$$

The equilibrium constant for the displacement reaction given in Equation (8) is given by

$$K_{eq} = [Pd(AEP)(B)][A^-]/[Pd(AEP)(A)^+][B^{2-}] \quad (9)$$

Substitution from Equations (6b) and (7b) in Equation (9) results in

$$K_{eq} = \beta_{110}^{[Pd(AEP)B]}/\beta_{110}^{[Pd(AEP)A]+} \quad (10)$$

The 100 species, $[Pd(AEP)(H_2O)_2]^{2+}$, is represented in the above equations as $[Pd(AEP)]^{2+}$ for simplicity reasons. $\log\beta_{110}$ values for $[Pd(AEP)(A)]^+$ and [Pd(AEP)B] complexes taken from Table 2 amount to 7.78 and 15.11, respectively, and by substitution in Equation (10) results in $\log K_{eq}$ = 7.33. In the same way the equilibrium constants for the displacement of coordinated inosine by glycine and *S*-methylcysteine are $\log K_{eq}$ = 2.55 and 1.38, respectively. These values clearly indicate how sulfhydryl ligands such as cysteine and by analogy glutathione are effective in displacing the DNA constituent, i.e., the main target in tumour chemotherapy. Chelated cyclobutanedicarboxylate may undergo displacement reaction with inosine. $\log K_{eq}$ for such a reaction was calculated as described above and amounts to 0.68. The low value of the equilibrium constant for the displacement reaction of coordinated cyclobutanedicarboxylate by inosine is of biological significance since it is in line with the finding that carboplatin interacts with DNA through ring opening of chelated CBDCA and not through displacement of CBDCA.

Experimental Section

Materials: $PdCl_2$, 2-(2-aminoethyl)pyridine and 1,1-cyclobutanedicarboxylic acid were obtained from Aldrich. The amino acids and related compounds (glycine, alanine, β-alanine, γ-aminobutyric acid, β-phenylalanine, valine, proline, hydroxyproline, isoleucine, ethanolamine·HCl, serine, histidine, histamine dihydrochloride, ornithine, lysine, cysteine, methionine, threonine, *S*-methylcysteine, aspartic acid glutamic acid and methylamine·HCl) were provided by Sigma Chemical Co. The peptides used (glycinamide, glycylglycine, glycylleucine, asparagine and glutamine) and the dibasic acids used (1,1-cyclobutanedicarboxylic acid, malonic acid, oxalic acid, succinic acid, adipic acid and fumaric acid) were all provided by BDH Biochemicals Ltd., Poole, England. The DNA units (inosine, inosine 5′-monophosphate, adenine, guanosine, guanosine 5′-monophosphate, adenosine, cytosine, thymidine, cytidine 5′-monophosphate, uracil and uridine 5′-monophosphate) were provided by Sigma Chemical Co. The studied amino acid esters (glycine methyl ester, histidine methyl ester and methionine methyl ester) were obtained from Sigma Chem. Co. For equilibrium studies, [Pd(AEP)-Cl_2] was converted into the diaqua complex by treating it with two equivalents of $AgNO_3$ as described before.[21] The ligands in the form of hydrochlorides were converted into the corresponding hydronitrates. Cytosine, guanosine and the nucleotides were prepared in the protonated form with standard HNO_3 solution. All solutions were prepared in deionized water.

Synthesis: $[Pd(AEP)Cl_2]$ was prepared by heating $PdCl_2$ (0.50 g; 2.82 mmol) and KCl (0.4205 g; 5.64 mmol) in the least amount of water to 70 °C for 30 min with stirring. The clear solution of $[PdCl_4]^{2-}$ was cooled to 25 °C, filtered and 2-(2-aminoethyl)pyridine (0.3445 g; 2.82 mmol), dissolved in 10 mL of H_2O was added drop wise to the stirred solution. The pH was adjusted to 2–3 by the addition of HCl and/or NaOH. A yellow precipitate of $[Pd(AEP)Cl_2]$ was formed and stirred for a further 30 min at 50 °C. After filtering off the precipitate, it was thoroughly washed with H_2O, ethanol and ethyl ether. 0.719 g, 2.40 mmol (yield, 85%) of yellow powder was obtained. $C_7H_{10}Cl_2N_2Pd$ (299.48): calcd. C 28.07, H 3.37, N 9.35; found C 27.9, H 3.3, N 9.2. The IR spectrum of $Pd(AEP)Cl_2$ exhibits strong NH absorption band in the range 3100–3250 cm^{-1}. δ(NH) bands are observed at 1580–1620 cm^{-1}. The Pd–N absorption was detected at 460 cm^{-1}.

Apparatus: Potentiometric titrations were performed with a Metrohm 686 titroprocessor equipped with a 665 Dosimat. The titroprocessor and electrode were calibrated with standard buffer solutions, prepared according to NBS specification.[22] All titrations were carried out at 25.0 ± 0.1 °C in purified nitrogen atmosphere using a titration vessel described previously.[23] IR spectra were measured on a 8001-PC FT-IR Shimadzu spectrophotometer using KBr pellets.

Procedure and Measuring Technique: The acid-dissociation constants of the ligands were determined by titrating 1.25 mmol samples of each with standard NaOH solutions. Ligands were converted into their protonated form with standard HNO_3 solutions. The acid-dissociation constants of the coordinated water molecules in $[Pd(AEP)(H_2O)_2]^{2+}$ were determined by titrating 1.25 mmol of complex with standard 0.05 M NaOH solution. The formation constants of the complexes were determined by titrating solution mixtures of $[Pd(AEP)(H_2O)_2]^{2+}$ (1.25 mmol) and the ligand in the concentration ratio of 1:1 for amino acids, peptides and dicarboxylic acids and in the ratio of 1:2 (Pd/ligand) for the DNA constituents. The titrated solution mixtures each had a volume of 40 mL and the titrations were carried out at 25 °C and 0.1 M ionic strength (adjusted with $NaNO_3$). A standard 0.05 M NaOH solution was

used as titrant. The pH meter readings were converted to hydrogen ion concentration by titrating a standard HNO_3 solution (0.01 M), the ionic strength of which was adjusted to 0.1 M with $NaNO_3$, with standard NaOH (0.05 M) at 25 °C. The pH was plotted against p[H]. The relationship pH – p[H] = 0.05 was observed.

The species formed were characterized by the general equilibrium

$$pM + qL + rH \rightleftharpoons (M)_p(L)_q(H)_r$$

for which the formation constants are given by

$$\beta_{pqr} = \frac{[(M)_p(L)_q(H)_r]}{[M]^p[L]^q[H]^r}$$

M, L, and H stand for the $[Pd(AEP)(H_2O)_2]^{2+}$ ion, ligand, and proton, respectively. The calculations were performed using the computer program[24] MINIQUAD-75. The stoichiometry and stability constants of the complexes formed were determined by trying various possible composition models for the systems studied. The model selected was that which gave the best statistical fit and was chemically consistent with the magnitudes of various residuals, as described elsewhere.[24] Tables 1 and 2 list the stability constants together with their standard deviations and the sum of the squares of the residuals derived from the MINIQUAD output. The concentration distribution diagrams were obtained with the program SPECIES[25] under the experimental condition used.

The hydrolysis kinetics of the complexed ester was monitored by pH-stat technique,[23,26] by using the titroprocessor operating in the SET mode. The hydrolysis was investigated using an aqueous solution (40 mL) containing a mixture of $[Pd(AEP)(H_2O)_2]^{2+}$ (1.25 mmol) and amino acid ester (1.25 mmol), and the ionic strength was adjusted to 0.1 M with $NaNO_3$. The pH of the mixture was progressively raised to the desired value. The reaction was monitored by addition of NaOH solution to maintain the given pH. The data fitting was performed with the OLIS KINFIT set of programs[27] as described previously.[21] The pK_w values were determined in different organic solvent/water mixtures as described previously.[28,29] For this purpose, various amounts of standard NaOH solution were added to 0.1 M $NaNO_3$ solution. $[OH^-]$ was calculated from the amount of base added, and $[H^+]$ was calculated from the pH value.

Acknowledgments

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