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Mohamed M. Shoukry [a], Abdel Aziz Al-Najjar [a], Wafaa M. Hosny [a], Afaf E. Mahgoub [a], Afkar K. Abdelhadi [a] & Perihan A. Khalf-Alaa [a]

[a] Department of Chemistry, Faculty of Science, University of Cairo, Egypt

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Thermodynamics of the reactions of $[Pd(Et_4en)(H_2O)_2]^{2+}$ with ligands of biological significance: deactivation of based-drug by the sulfur-containing biomolecules

Mohamed. M. Shoukry*, Abdel Aziz Al-Najjar, Wafaa M. Hosny, Afaf E. Mahgoub, Afkar K. Abdelhadi and Perihan A. Khalf-Alaa

Department of Chemistry, Faculty of Science, University of Cairo, Egypt

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The reaction of $[Pd(Et_4en)(H_2O)_2]^{2+}$ ($Et_4en = N,N,N',N'$-tetraethylethylenediamine) with amino acids, peptides, dicarboxylic acids or DNA constituents was investigated at 37°C and 0.16 M ionic strength. The stoichiometry and stability of the complexes formed were determined and the binding centers of the ligands were assigned. The concentration distribution diagrams of the complexes were evaluated. The equilibrium constants for the displacement of representative coordinated ligands such as inosine, glycine or methionine by mercaptoethylamine were calculated and the concentration distribution diagrams of the various species were evaluated.

$Pd(Et_4En)Cl_2$ complex, where $Et_4En = N,N,N',N'$-tetraethylethylenediamine, was synthesized and characterized by elemental analysis and spectroscopic technique. The complex formation equilibria was investigated. The displacement of coordinated inosine (A) by cysteine (B) was studied.

$$[Pd(Et_4en)(A)]^+ + B^{2-} \underset{}{\overset{K_{eq}}{\rightleftharpoons}} [Pd(Et_4en)(B)] + A^-$$

The equilibrium constant for the displacement reaction given is given by

$$K_{eq}=[Pd(Et_4en)(B)][A^-]/[Pd(Et_4en)(A)^+][B^{2-}]$$

Keywords: equilibrium study; Pd(II); amino acids; peptides; dicarboxylic acids; DNA

1. Introduction

Cis-platin [*cis*-diamminedichloroplatinum(II)] mainly targets DNA by binding to N_7 of adjacent purines (*1–5*) of the same strand (*6–8*) or of opposite strands. The intra-strand adducts are thought to be the lesions that are responsible for cell death, but the mechanism is not entirely understood.

In solution, *cis*-A_2PtG_2 complexes (A_2 = two amines or a diamine and G_2 = two detached or tethered guanine bases) usually exhibit unrestricted rotation about the Pt–N_7 bond (*9*, *10*). The dynamic motion problem led to research designed to construct analogs of *cis*-platin with bulky ligands designed to reduce the dynamic motion by destabilizing the transition state for Pt–N_7 rotation. N,N'-Dimethyl-2,3-diaminobutane (Me_2dab) was the first carrier ligand used to

*Corresponding author. Email: shoukrymm@hotmail.com

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restrict the rotation about Pt-N_7 bond (*11*). The 2,2′-bipiperidine ligand (bip) is analogous to Me_2dab but is much more sterically hindered. The bip ligand was able to decrease the dynamic motion roughly a billion times with respect to $(NH_3)_2$ and ca. 100 times with respect to Me_2dab in *cis*-A_2PtG_2 complexes (*12–17*). This restricted rotation or dynamic motion stabilize the complex with DNA, which will enhance antitumour activity. In the present work, $Pd(Et_4En)Cl_2$ was synthesized and characterized. The amine used has four ethyl groups attached to the nitrogen atoms of ethylenediamine. These groups will restrict the dynamic motion as described above.

Pd(II) and Pt(II) amine complexes have the same structure and similar thermodynamic parameters. However, Pd(II) complexes are more labile than those of Pt(II) complexes. Therefore, Pd(II) complexes are good models for the analogous Pt(II) complexes in solution. Recent work in our laboratories focussed on the kinetics and equilibria of complex-formation reactions of *cis*-(diamine)palladium(II) complexes with DNA, the major target in chemotherapy of tumors, and biorelevant ligands such as amino acids, peptides, dicarboxylic acids and esters (*18–25*). The present investigation describes the equilibria associated with the interaction of $[Pd(Et_4en)(H_2O)_2]^{2+}$ with amino acids, peptides and DNA constituents. The sulfur ligands such as mercaptoethylamine have high affinity for Pd(II) and Pt(II) complexes. These ligands will compete with the DNA for the reaction with any antitumor agent. Therefore, it is of biological significance to calculate the equilibrium constants for the displacement reaction of model ligands such as inosine, glycine or methionine by mercaptoethylamine. These equilibrium constants may give a measure of the effectiveness of the antitumour agent.

2. Results and discussion

2.1. *Acid–base equilibria of $[Pd(Et_4en)(H_2O)_2]^{2+}$*

The $[Pd(Et_4en)(H_2O)_2]^{2+}$ 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 two species: **10-1** and **10-2** as given in reactions (1) and (2). The dimeric species **20-2** was detected (*26*, *27*) for a similar system. Trials were made to fit the potentiometric data assuming the formation of the dimeric species, but this resulted in a very poor fit to the data. This may be explained on the premise that the concentration range of the Pd(II) complex used did not allow the dimerization reaction.

$$\underset{\mathbf{100}}{[Pd(Et_4en)(H_2O)_2]^{2+}} \overset{K_{a1}}{\rightleftharpoons} \underset{\mathbf{10\text{-}1}}{[Pd(Et_4en)(H_2O)(OH)]^{+}} + H^{+} \quad (1)$$

$$\underset{\mathbf{10\text{-}1}}{[Pd(Et_4en)(H_2O)(OH)]^{+}} \overset{K_{a2}}{\rightleftharpoons} \underset{\mathbf{10\text{-}2}}{[Pd(Et_4en)(OH)_2]} + H^{+} \quad (2)$$

The pK_{a1} and pK_{a2} values for $[Pd(Et_4en)(H_2O)_2]^{2+}$ are 5.03 and 9.61, respectively.

The distribution diagram for $[Pd(Et_4en)(H_2O)_2]^{2+}$ and its hydrolyzed species reveals that the concentration of the monohydroxo species **10-1** increases with increasing pH, predominately in the pH range 6.5–7.5, and reaches a maximum concentration of 98%. 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 inert dihydroxo complex will be the major species, and consequently the ability of DNA to bind the Pd(amine) complex will decrease significantly.

2.2. *Amino acid complexes*

Analysis of the titration data for the Pd(Et_4en)–amino acid system showed the formation of 1:1 species. Ornithine, aspartic acid and mercaptoethylamine form in addition to 1:1 complexes, the monoprotonated species. The pK_a of the protonated complex (20) was calculated from Equation (3)

$$pK_a = \log \beta_{111} - \log \beta_{110}. \tag{3}$$

The pK_a values of the protonated species are 8.78 for ornithine, 3.70 for aspartic acid and 7.03 for mercaptoethylamine. The stability constants 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 centers, *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 centers. Aspartic acid has two carboxylic groups and one amino group as potential binding centers. It may coordinate either via the two carboxylate groups or by the amino group and one carboxylate group. The stability constant of its complex is in the range of those of amino acids. This suggests that aspartic acid coordinates via the amino and one carboxylate group. Serine has an extra binding center on the β-alcoholate group. This group was reported (*28*, *29*) to participate in metal complex formation. The potentiometric data are much better fitted assuming the formation of the complex species **110** and **11-1**. This reveals that the β-alcoholate group participates in complex formation through induced ionization of the alcoholic group forming the species **11-1**. The pK^H value of the β-alcoholate incorporated in the Pd(II) complex ($\log \beta_{110} - \log \beta_{11-1}$) is 8.29. This is consistent with the reaction scheme where the alcohol group in serine is coordinated to Pd(Et_4en)$^{2+}$ at higher pH. Due to the coordination of the alcohol group by donation of the electron pair on the oxygen to the metal center, the OH bond is considerably weakened and thus the ionization of a proton occurs at a lower pH.

The distribution diagram for the serine complex shows that the complex species with coefficients **110** reaches the maximum degree of formation (~94%) at pH 4.5–7.0, *i.e.* in the physiological pH range. However, the species **11-1** predominates after pH 8.5 and attains the maximum concentration of ~95% at pH ~10.

2.3. *Peptide complexes*

The potentiometric data for the peptide complexes were fitted on the basis of the formation of the complexes with stoichiometric coefficients **110** and **11-1** according to the following equilibria:

$$\underset{\mathbf{100}}{[Pd(Et_4en)(H_2O)_2]^{2+}} + L^- \overset{K}{\rightleftharpoons} \underset{\mathbf{110}}{[Pd(Et_4en)L]^+} + 2H_2O \tag{4}$$

$$\underset{\mathbf{110}}{[Pd(Et_4en)L]^+} \overset{K^H}{\rightleftharpoons} \underset{\mathbf{11\text{-}1}}{[Pd(Et_4en)LH_{-1}]} + H^+ \tag{5}$$

The **110** complex is formed via coordination of the amine and carbonyl groups. On increasing the pH, the coordination site should switch from the carbonyl oxygen to amide nitrogen with the release of the amide hydrogen forming the complex [Pd(Et_4en)(LH_{-1})]. Such changes in coordination centers are now well documented (30). The pK^H values of the amide groups incorporated in the Pd(II) complexes ($\log \beta_{110} - \log \beta_{11-1}$) are in the 4.60–9.00 range. It is noteworthy that the pK^H for glycinamide complex is lower than those of other peptides. This signifies that the more bulky substituent group on the peptide may serve to hinder the structural change in going from the

Table 1. Formation constants for amino acid complexes with $Pd(Et_4en)^{2+}$ at 37°C and 0.16 M $NaNO_3$.

System	M	L	H[a]	log β[b]	S[c]
$Pd(Et_4en)$-OH	1	0	−1	−5.03 (0.03)	2.3E−7
	1	0	−2	−14.64 (0.07)	
Glycine	0	1	1	9.20 (0.01)	3.8E−7
	1	1	0	10.33 (0.01)	1.1E−8
Alanine	0	1	1	9.27 (0.01)	1.9E−8
	1	1	0	11.00 (0.04)	2.1E−8
Proline	0	1	1	9.19 (0.01)	2.3E−8
	1	1	0	11.15 (0.03)	8.5E−8
β-Phenylalanine	0	1	1	8.74 (0.01)	4.1E−8
	1	1	0	9.93 (0.06)	1.1E−7
Methionine	0	1	1	8.76 (0.01)	4.0E−8
	1	1	0	9.94 (0.04)	2.0E−8
Serine	0	1	1	8.63 (0.01)	3.5E−8
	1	1	0	10.38 (0.07)	1.8E−7
	1	1	−1	2.09 (0.08)	
Histidine	0	1	1	8.84 (0.01)	2.0E−8
	0	1	2	14.74 (0.01)	
	1	1	0	11.61 (0.03)	3.1E−7
Mercaptoethylamine	0	1	1	10.85 (0.01)	4.9E8
	0	1	2	19.45 (0.01)	
	1	1	0	12.78 (0.08)	6.1E8
	1	1	1	19.81 (0.06)	
Aspartic acid	0	1	1	9.10 (0.01)	3.5E−7
	0	1	2	12.86 (0.01)	
	1	1	0	10.56 (0.04)	3.5E−9
	1	1	1	14.26 (0.05)	
Ornithine	0	1	1	10.14 (0.00)	2.0E−8
	0	1	2	18.77 (0.01)	
	1	1	0	11.47 (0.05)	1.2E−8
	1	1	1	20.25 (0.05)	

Notes: [a]M, L and H are the stoichiometric coefficient corresponding to $Pd(Et_4en)$, amino acid and H^+, respectively.
[b]Standard deviations are given in parentheses.
[c]Sum of the square of residuals.

protonated to the deprotonated complexes. The pK^H of the glutamine complex is markedly higher than those for other peptide complexes. This is ascribed to the formation of a eight-membered chelate ring, which would probably be more strained and therefore less favored.

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 $[Pd(Et_4en)(H_2O)_2]^{2+}$ in entirely different fashions. Glutaminate 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 behavior toward the palladium species. From the speciation diagram of glycylglycine complex, the $Pd(Et_4en)(L)^+$ (**110**) species starts to form at pH 2.0 and with increasing pH, its concentration increases reaching the maximum of 75% at pH 4.9. Further increase in pH is accompanied by a decrease in $Pd(Et_4en)(L)^+$ complex concentration and an increase in $Pd(Et_4en)(LH_{-1})$ complex formation.

2.4. *Dicarboxylic acid complexes*

In the $Pd(Et_4en)$–dicarboxylic acid system, the results showed the presence of the 1:1 species and its protonated form. The results show that the adipic acid complex is the least stable as the

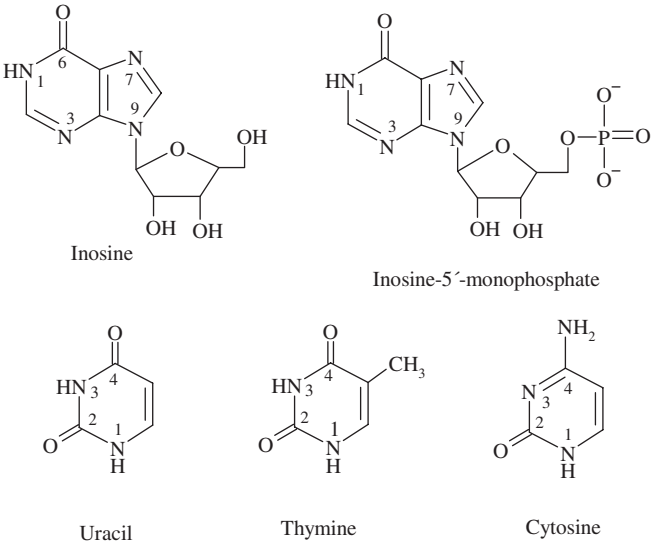
complex involves the formation of the least stable nine-membered chelate ring. The pK_a values of the protonated species for $[Pd(Et_4en)HL]^+$ is in the range 2.20–4.61. These values are lower than those for the HL^- ion. The lowering of the pK_a is due to acidification of the second carboxylic acid group upon coordination of Pd(II) to one carboxylate group (*31*).

From the concentration distribution diagram of the succinic acid complex, it is interesting to note that the mono-protonated species attains its maximum concentration of 57% at pH 3.8. This form has one coordination site available for binding to DNA. Such a species was documented to be the active form in the case of carboplatin (*32*).

2.5. *DNA complexes*

DNA models such as inosine, inosine-5′-monophosphate, uracil, thymine, cytosine (Scheme 1) form 1:1 and 1:2 complexes with the $Pd(Et_4en)^{2+}$ ion. However, inosine, inosine-5′-monophosphate, forms the protonated complex, in addition to the formation of 1:1 and 1:2 complexes. The pK_a value of the protonated inosine complex is 5.29. This value corresponds to N_1H. The lowering of this value with respect to that of free inosine (pK_a = 8.43) is due to acidification upon complex formation (*33*). Inosine-5′-monophosphate form mono- and diprotonated complexes. The pK_a values of the protonated species of the inosine-monophosphate (IMP) complex (**112**) are 3.28 (log β_{112} – log β_{111}) and 6.11 (log β_{111} – log β_{110}). The former pK_a value corresponds to the N_1H group and the second pK_a value to the $–PO_2(OH)$ group. The N_1H group was acidified upon complex formation, as a result of inductive effect, by 5.15 pK_a units. Acidification of the N_1H group upon complex formation is consistent with previous reports for the IMP complex (34). The phosphate group was not acidified significantly (acidified by 0.21 pK_a units) upon complex formation since it is far away from the coordination center. The IMP complex is more stable than that of inosine. This may be explained on the basis of different coulombic forces operated between the ions resulting from the negatively charged phosphate group. Hydrogen bonding between the phosphate group and exocyclic amine is also thought to contribute to the increased stability.

The pyrimidines uracil and thymine have basic nitrogen donor atoms (N_3). As a result of the high pK_a values of pyrimidines (pK_a > 9), the complexes formed predominat above pH 8.5.



Scheme 1. Structural formula of DNA models.

Table 2. Formation constants for peptide complexes with $Pd(Et_4en)^{2+}$ at 37°C and 0.16 M $NaNO_3$.

System	M	L	H[a]	log β[b]	pK_a	S[c]
Glycineamide	0	1	1	7.49 (0.00)		2.0E−9
	1	1	0	7.89 (0.06)	4.6	4.7E−7
	1	1	−1	3.29 (0.05)		
Glycylglycine	0	1	1	7.73 (0.01)		1.1E−8
	1	1	0	7.40 (0.02)		1.6E−7
	1	1	−1	1.16 (0.07)	6.24	
Glutamine	0	1	1	8.78 (0.01)		5.3E−8
	1	1	0	10.27 (0.09)		1.2E−7
	1	1	−1	1.27 (0.09)	9	
Aspargine	0	1	1	8.47 (0.01)		6.6E−8
	1	1	0	9.70 (0.04)		2.8E−8
	1	1	−1	1.57 (0.05)	8.13	

Notes: [a]M, L and H are the stoichiometric coefficient corresponding to $Pd(Et_4en)$, peptide and H^+, respectively.
[b]Standard deviations are given in parentheses.
[c]Sum of the square of residuals.

Cytosine undergoes N_3(*35*) protonation under mild acidic conditions. The value obtained for its protonation constant is 4.33. The lower value of the stability constant of its complex (Table 2) reflects the difference in the basicity of the donor site.

The concentration distribution diagram for the $Pd(Et_4en)^{2+}$–IMP system taken as a model for DNA binding (Figure 1) shows that in the physiological pH range the IMP complex (**110**) dominates with a maximum concentration of 60% and the hydrolyzed species have no contribution, *i.e.* the interaction between $Pd(Et_4en)^{2+}$ and IMP as a DNA constituent is feasible. The protonated species (**112**) exists in a concentration of 31% at pH 2.5 and the species (**111**) predominates with the maximum concentration of 82% at pH 5.0.

2.6. *Displacement reaction of coordinated inosine*

It was shown above that N-donor ligands such as DNA constituents have affinity for $[Pd(Et_4en)(H_2O)_2]^{2+}$, which may have important biological implications since the interaction

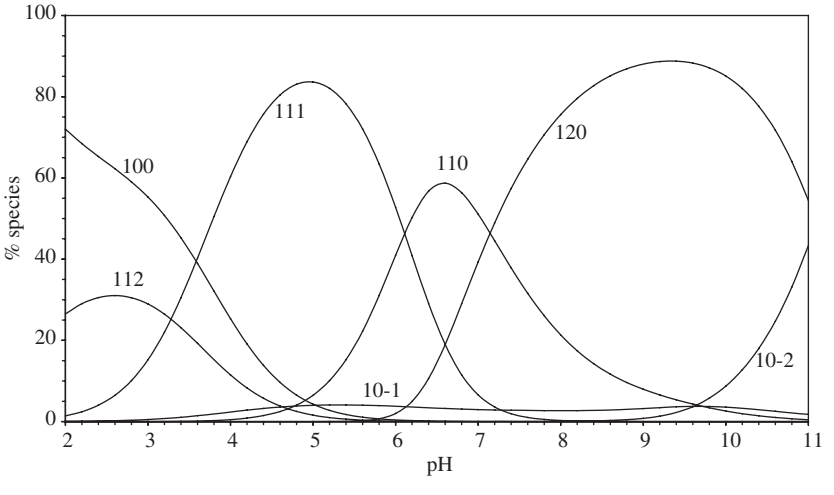


Figure 1. Concentration distribution of various species as a function of pH in the $Pd(Et_4en)$-5′-IMP system.

with DNA is thought to be responsible for the antitumor activity of related complexes. However, the preference of Pd(II) to coordinate to S-donor ligands suggests that Pd(II)–N adducts can easily be converted into Pd–S adducts (*36*). Consequently, the equilibrium constant for such conversion is of biological significance. Consider inosine as a typical DNA constituent (presented by HA) and mercaptoethylamine as a typical thiol ligand (presented by H_2B). The equilibria involved in complex formation and displacement reactions are:

$$\mathbf{HA} \rightleftharpoons \mathbf{H^+ + A^-}$$

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

$$\underset{\mathbf{(100)}}{\mathbf{[Pd(Et_4en)]^{2+}}} + \mathbf{A^-} \rightleftharpoons \underset{\mathbf{(110)}}{\mathbf{[Pd(Et_4en)A]^+}} \tag{6a}$$

$$\beta_{110}^{[Pd(Et_4en)A]+} = [Pd(Et_4en)A^+]/[Pd(Et_4en)^{2+}][A^-] \tag{6b}$$

$$\underset{\mathbf{(100)}}{\mathbf{[Pd(Et_4en)]^{2+}}} + \mathbf{B^{2-}} \rightleftharpoons \underset{\mathbf{(110)}}{\mathbf{[Pd(Et_4en)B]}} \tag{7a}$$

$$\beta_{110}^{[Pd(Et_4en)B]} = [Pd(Et_4en)B]/[Pd(Et_4en)^{2+}][B^{2-}] \tag{7b}$$

$$\mathbf{[Pd(Et_4en)(A)]^+ + B^{2-}} \overset{\mathbf{K_{eq}}}{\rightleftharpoons} \mathbf{[Pd(Et_4en)(B)] + A^-} \tag{8}$$

The equilibrium constant for the displacement reaction given in Equation (9) is given by

$$K_{eq} = \frac{[Pd(Et_4en)(B)][A^-]}{[Pd(Et_4en)(A)^+][B^{2-}]} \tag{9}$$

Substitution from Equations (6b) and (7b) in Equation (10) results in:

$$K_{eq} = \frac{\beta_{110}^{[Pd(Et_4en)B]}}{\beta_{110}^{[Pd(Et_4en)A]+}}, \tag{10}$$

log β_{110} values for $[Pd(Et_4en)(A)]^+$ and $[Pd(Et_4en)B]$ complexes taken from Table 2 amount to 6.00 and 12.78, respectively, and substitution in Equation (11) results in log $K_{eq} = 6.78$. In the same way, the equilibrium constants for the displacement of coordinated inosine by glycine and methionine are log $K_{eq} = 4.33$ and 3.94, respectively. These values clearly indicate how sulfide ligands such as mercaptoethylamine and, by analogy, glutathione are effective in displacing the DNA constituent, *i.e.* the main target in tumor chemotherapy.

3. Conclusion

By comparing stability constant of $[Pd(Et_4en)(H_2O)_2]^{2+}$ complexes with those of biorelevant ligands, it would be possible to evaluate the speciation of Pd(II) complexes in biological fluid. The competition between cysteine and DNA (inosine) for reaction with DNA was investigated. The equilibrium constant for the displacement of DNA by cysteine measures the deactivation of the Pt/Pd-based drug by the sulfur-containing biomolecules.

4. Experimental

4.1. *Materials and reagents*

All reagents were of analytical grade. K_2PdCl_4, and *N*,*N*,*N*′,*N*′-tetraethylethylendiamine were obtained from Aldrich. The amino acids and related compounds (glycine, alanine, β-phenylalanine, proline, serine, histidine, ornithine, methionine, aspartic acid and mercaptoethylamine) were provided by Sigma Chemical Co. The peptides used (glycinamide, glycylglycine, asparagine and glutamine) and the dibasic acids used (cyclobutane dicarboxylic acid, oxalic acid, malonic acid, succinic acid and adipic acid) were all provided by BDH-Biochemicals Ltd, Poole, England. The DNA constituents (inosine, inosine 5′-monophosphate, uracil, thymine, cytosine) were provided by Sigma Chemical Co. For equilibrium studies, $[Pd(Et_4en)Cl_2]$ was converted into the diaqua complex by treating it with two equivalents of $AgNO_3$ as described before (*23*). The ligands in the form of hydrochlorides were converted into the corresponding hydronitrates (*23*). Cytosine and the nucleotides were prepared in the protonated form with standard HNO_3 solution. All solutions were prepared in deionized water.

4.2. *Synthesis*

$Pd(Et_4en)Cl_2$ was prepared by dissolving K_2PdCl_4 (2 mmol) in 10 ml of water with stirring. The clear solution of $[PdCl_4]^{2-}$ was filtered and *N*,*N*,*N*′,*N*′-tetraethylethylenediamine (2 mmol), dissolved in 10 ml of H_2O was added dropwise to the stirred solution. The pH was adjusted to 2–3 by the addition of HCl and/or NaOH. A yellowish-brown precipitate of $[Pd(Et_4en)Cl_2]$ 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 diethylether. A yellow powder was obtained. Anal. Calcd. for $C_{10}H_{24}N_2PdCl_2$: C, 34.3; H, 6.9; N, 8.0. Found: C, 34.5; H, 6.5; N, 7.7. The IR spectrum of $Pd(Et_4en)Cl_2$ exhibits a strong NH absorption band in the range 3113–3207 cm^{-1}. δ (NH) bands are observed at 1580–1609 cm^{-1}. The Pd–N absorption was detected at 439 cm^{-1}.

4.3. *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 National Bureau Standard specification (*37*). 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). The pH was plotted against p[H]. The relationship pH-p[H] = 0.05 was observed. All titrations were carried out at 37.0°C ± 0.1°C in purified nitrogen atmosphere using a titration vessel described previously (*38*). A Colora Ultrathermostat was used for temperature control. IR spectra were measured on a 8001-PC FT-IR Shimadzu spectrophotometer using KBr pellets.

4.4. *Procedure and measuring technique*

The acid dissociation constants of the ligands were determined by titrating 1 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 $[Pd(Et_4en)(H_2O)_2]^{2+}$ were determined by titrating 1 mmol of the complex with standard 0.05 M NaOH solution. The formation constants of the complexes were determined by titrating solution mixtures of $[Pd(Et_4en)(H_2O)_2]^{2+}$ (1 mmol) and the ligand in the concentration ratio of 1:1 for

Table 3. Formation constants for dicarboxylic acid complexes with $Pd(Et_4en)^{2+}$ at 37°C and 0.16 M $NaNO_3$.

System	M	L	H[a]	log β[b]	S[c]
Cyclobutane dicarboxylic acid	0	1	1	5.54 (0.01)	9.6E−9
	0	1	2	8.77 (0.01)	
	1	1	0	7.94 (0.03)	1.2E−8
	1	1	1	10.59 (0.04)	
Oxalic acid	0	1	1	4.10 (0.01)	1.1E−7
	0	1	2	5.78 (0.06)	
	1	1	0	6.89 (0.01)	1.1E−7
	1	1	1	9.09 (0.02)	
Malonic acid	0	1	1	5.42 (0.01)	2.8E−8
	0	1	2	8.19 (0.01)	
	1	1	0	6.58 (0.04)	1.8E−7
	1	1	1	9.37 (0.07)	
Succinic acid	0	1	1	5.35 (0.00)	1.8E−8
	0	1	2	9.41 (0.01)	
	1	1	0	4.80 (0.05)	3.1E−7
	1	1	1	9.40 (0.07)	
Adipic acid	0	1	1	5.28 (0.01)	1.1E−7
	0	1	2	9.61 (0.01)	
	1	1	0	3.88 (0.01)	2.2E−8
	1	1	1	8.49 (0.02)	

Notes: [a]M, L and H are the stoichiometric coefficient corresponding to $Pd(Et_4en)$, dicarboxylic acid and H^+, respectively.
[b]Standard deviations are given in parentheses.
[c]Sum of the square of residuals.

Table 4. Formation constants for DNA complexes with $Pd(Et_4en)^{2+}$ at 37°C and 0.16 M $NaNO_3$.

System	M	L	H[a]	log β[b]	S[c]
Inosine	0	1	1	8.43 (0.01)	4.1E−08
	1	1	0	6.00 (0.06)	1.1E−08
	1	2	0	11.16 (0.01)	
	1	1	1	11.29 (0.04)	
Inosine 5′-mono-phosphate	0	1	1	8.95 (0.01)	4.8E−08
	0	1	2	15.27 (0.02)	
	0	1	3	17.10 (0.06)	
	1	1	0	8.64 (0.02)	1.2E−08
	1	2	0	13.96 (0.03)	
	1	1	1	14.75 (0.01)	
	1	1	2	18.03 (0.05)	
Uracil	0	1	1	8.83 (0.01)	1.2E−07
	1	1	0	6.45 (0.04)	3.8E−09
	1	2	0	12.54 (0.02)	
Thymine	0	1	1	9.95 (0.01)	7.6E−08
	1	1	0	6.16 (0.10)	3.0E−09
	1	2	0	12.38 (0.04)	
Cytosine	0	1	1	4.33 (0.00)	5.2E−08
	1	1	0	3.42 (0.09)	2.2E−08
	1	2	0	7.19 (0.02)	

Notes: [a]L and H are the stoichiometric coefficient corresponding to $Pd(Et_4en)$, DNA constituent and H^+, respectively.
[b]Standard deviations are given in parentheses.
[c]Sum of the square of residuals.

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 37°C and 0.16 M ionic strength (adjusted with $NaNO_3$), such a condition is similar to that existing in a biological system. A standard 0.05 M NaOH solution was used as the titrant.

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

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

where M, L and H stand for $[Pd(Et_4en)(H_2O)_2]^{2+}$ ion, ligand and proton, respectively. The calculations were performed using the computer program (*39*) 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 (*39*). Tables 1–4 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 (*40*) under the experimental condition used.

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