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Synthesis and thermodynamics of the reactions of $[Pd(Eten)(H_2O)_2]^{2+}$ with ligands of biological significane with reference to the antitumour activity

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Abstract

$Pd(Eten)Cl_2$ complex, where Eten = N-ethylethylenediamine , was synthesized and characterized by elemental analysis and spectroscopic techniques. The stoichiometry and stability of the complexes formed between $[Pd(Eten)(H_2O)_2]^{2+}$ and various biologically relevant ligands as adenine, adenosine, adenosine-5'-monophosphate and some selected peptides were investigated at 25ºC and 0.1 M ionic strength. The speciation diagrams of the complexes formed in solutions are evaluated. Thermodynamic parameters for Pd(Eten)-glycylglycine complexes were estimated.

Keywords: palladium diamine complexes / bioligands / equilibrium constants.

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1. Introduction

The use of platinum coordination compounds in cancer chemotherapy has been extensively investigated following the discovery of the therapeutic properties of cisplatin [cis-diamminedichloroplatinum(II)] by Rosenberg et al. [1,2]. Cisplatin is one of the most widely utilized antitumour drugs, exhibiting high efficacy against solid tumours, particularly testicular and ovarian cancer [3–8]. Despite the remarkable success of cisplatin, several problems have been found in clinical use. First, cisplatin treatment is often accompanied by severe side effects, including cumulative toxicities of nephrotoxicity, neurotoxicity, and emetogenesis [5,6,9–11]. In addition, cisplatin activity is limited to a relatively narrow range of tumours as a result of inherent or treatmentinduced tumour resistance [6,12]. 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 general structures and thermodynamic properties. However, the former complexes are five orders of magnitude more reactive than their platinum counterparts. Therefore, 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 (diamine)palladium(II) complexes with bio-relevant ligands as amino acids, peptides and dicarboxylic acids and esters [13–17]. As an extension of the research conducted in our laboratory, the Palladium(II) complex with N-ethylethylenediamine (Eten) was investigated. The ethyl

group will create steric hindrance for the incoming ligand. This will slow down the reactivity of the complexes to the same level as its platinum-amine analogues.

Also, the ethyl group may undergo hydrophobic interaction with DNA, such effect may favour the interaction with DNA, which is the main target for the antitumour agent. The present investigation describes the synthesis and characterization of $Pd(Eten)Cl_2$ complex . The interaction of $Pd(Eten)(H_2O)_2^{2+}$ with adenine as typical example of DNA, the main target in the tumour therapy is investigated . Also the interaction of the Pd(II) complex with biorelevant ligands as peptides is studied.

2. Experimental

2.1. Materials

The complex $[Pd(Eten)Cl_2]$ was prepared by heating $PdCl_2$ (0.1773 g; 1.0 mmol) and KCl (0.1491 g; 2.0 mmol) in the least amount of water to 70oC with stirring. The clear solution of $[PdCl4]^{2-}$ solution was cooled to 25ºC, filtered and N-ethylethylenediamine (0.0881 g; 1.0 mmol), was added to the stirred solution. The pH of the solution was adjusted to 2-3 by addition of HCl. The solution was evaporated to a small volume (20ml) under vacuum then an orange crystalline precipitate of $[Pd(Eten)Cl_2]$ was formed on cold. The precipitate was filtered off and washed with H_2O. An orange crystalline precipitate was obtained; yield 92%. For $C_4H_{12}N_2PdCl_2$ (F.wt=265.48) (Anal. Found: C, 18.04; H, 5.40; N, 10.29. Calc.: C, 18.08; H, 4.52; N, 10.55 %.)

The complex was converted in solution into the diaqua form by treating it with 2 equivalents of $AgNO_3$, as described elsewhere [18]. The ligands in the hydrochloride form were converted to the corresponding hydronitrate form using the procedure described before. The peptides studied are glycinamide, glycylglycine, glycylleucine, asparginne and glutamine. These materials were all obtained from Sigma Chem. Co. Adenine, adenosine, adenosine-5'-monophosphate were supplied by BDH-Biochemicals Ltd. The structural formula of the ligands were given in scheme 1.

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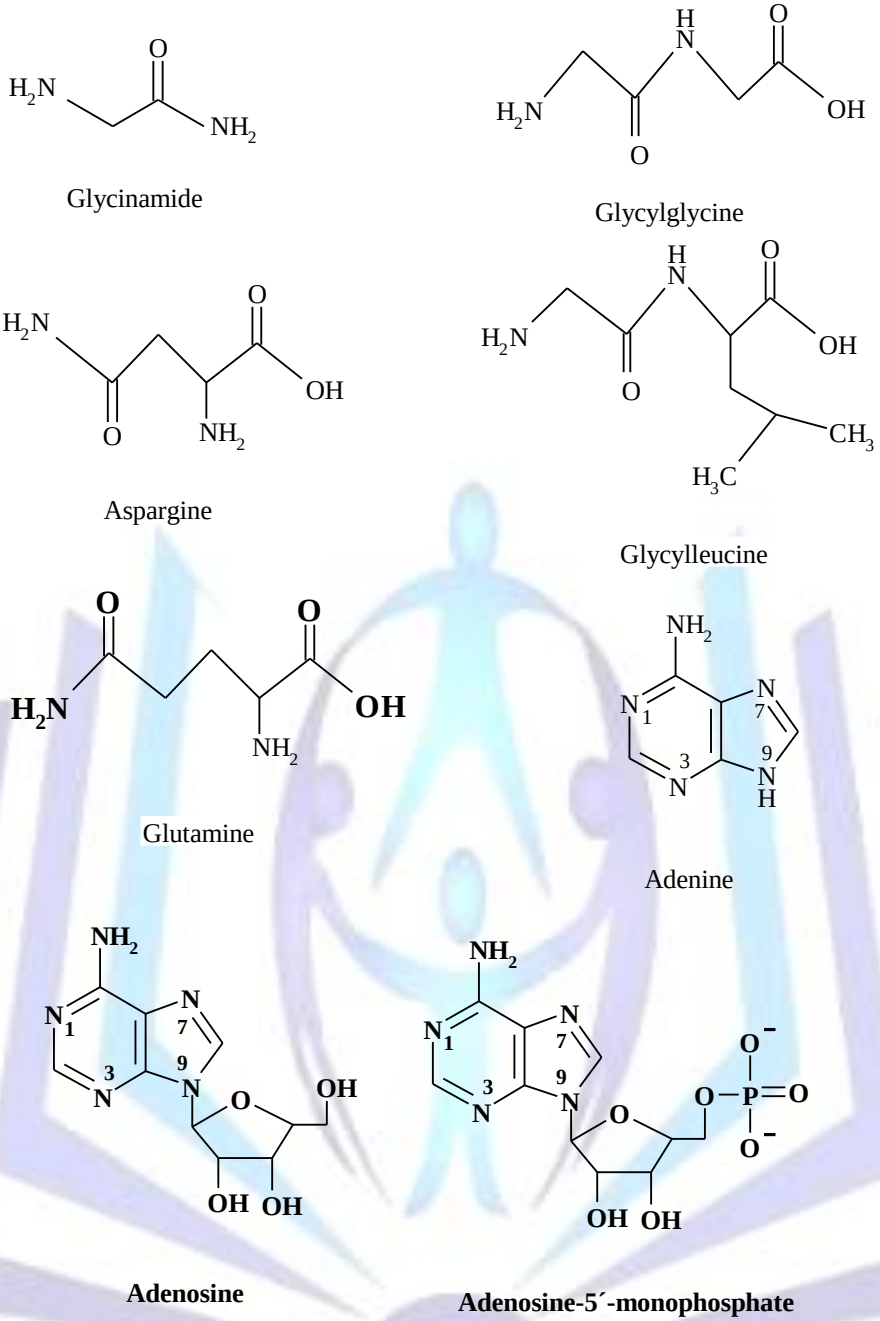

Scheme 1: structural formula of the investigated ligands.

2.2. Potentiometric measurements

Potentiometric measurements were performed using a Metrohm 751 Titroprocessor. The electrode and titroprocessor were calibrated with standard buffer solutions prepared according to NBS specifications [19]. 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 (0.05M) at 25 ˚C. The pH was plotted against p[H]. The relationship pH– p[H] = 0.05 was observed. IR spectrum was measured on a 8001-PC FT-IR Shimadzu spectrophotometer using KBr pellets.

The acid dissociation constants of the ligands were determined by titrating 0.625 mmol samples of each with standard NaOH solutions. The acid dissociation constants of the coordinated water molecules in $[Pd(Eten)(H_2O)_2]^{2+}$ were determined by titrating 1mmole of the complex with standard 0.05M NaOH solution. The formation constants of the

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complexes were determined by titrating solution mixtures of $[Pd(Eten)(H_2O)_2]^{2+}$ (1 mmole) and the ligand in the concentration ratio of 1 : 1 (Pd:peptide) for peptides and 1:2 for adenine. Adenine, adenosine and adenosine-5'-monophoshate were converted into its protonated form with standard HNO_3 solutions). 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 0.05M NaOH solution was used as titrant.

The equilibrium constants evaluated from the titration data are defined by Eq. (1) and Eq. (2)

$$pM + qL + rH \rightleftharpoons (M)_p(L)_q(H)_r \quad (1)$$

$$\beta_{pqr} = [(M)_p(L)_q(H)_r] / [M]^p [L]^q [H]^r \quad (2)$$

Where the charges are omitted for simplicity.

M, L and H represent $[Pd(Eten)(H_2O)_2]^{2+}$, ligand and proton, respectively. The calculations were performed using the program MINIQUAD-75 [20]. Stoichiometric and stability constants of the complexes were determined by fitting various possible composition models. The selected model gave the best statistical fit and was chemically consistent with the titration data without giving any systematic drift in the magnitude of various residuals, as described elsewhere [20]. The stability constants of the complexes formed in solution are given in Tables 1–3. Distribution diagrams were obtained using the program SPECIES [21].

3. Results and Discussion

3.1. Characterization of the solid complex

The analytical data indicates that the complex is of 1:1 stoichiometry and of formula $Pd(Eten)Cl_2$. The IR spectrum of the $Pd(Eten)Cl_2$ complex exhibits bands in the region 3300 - 3400 cm^{-1}, attributed to stretching vibrations of NH_2 group. The complex exhibits bands for (NH_2) bending at 1465 and 1562 cm^{-1} and bands for the stretching vibration corresponding to Pd-N at 480 and 523 cm^{-1} [22].

3.2. Equilibrium studies

3.2.1 Acid-base equilibria of the ligands and $[Pd(Eten)(H_2O)_2]^{2+}$ complex

The acid dissociation constants of the ligands were determined under the same experimental conditions of ionic strength and temperature used to study the Pd(II) complexes. The acid–base equilibria of $[Pd(Eten)(H_2O)_2]^{2+}$ given in Eq. (3a, 3b and 3c) were investigated and the equilibrium constants were determined and given in Table 1. These values were taken into account in determining the stability constants of the Pd(II) complexes.

$$[Pd(Eten)(H_2O)_2]^{2+} \rightleftharpoons [Pd(Eten)(H_2O)(OH)]^{+} + H^{+} \quad (3a)$$
100 10-1

$$[Pd(Eten)(H_2O)(OH)]^{+} \rightleftharpoons [Pd(Eten)(OH)_2] + H^{+} \quad (3b)$$
10-1 10-2

$$2[Pd(Eten)(H2O)(OH)]+ \overset{K_{dimer}}{\rightleftharpoons} [Pd(Eten)(\mu\text{-}OH)]_2^{2+} + H^{+} \quad (3c)$$
10-1 20-2

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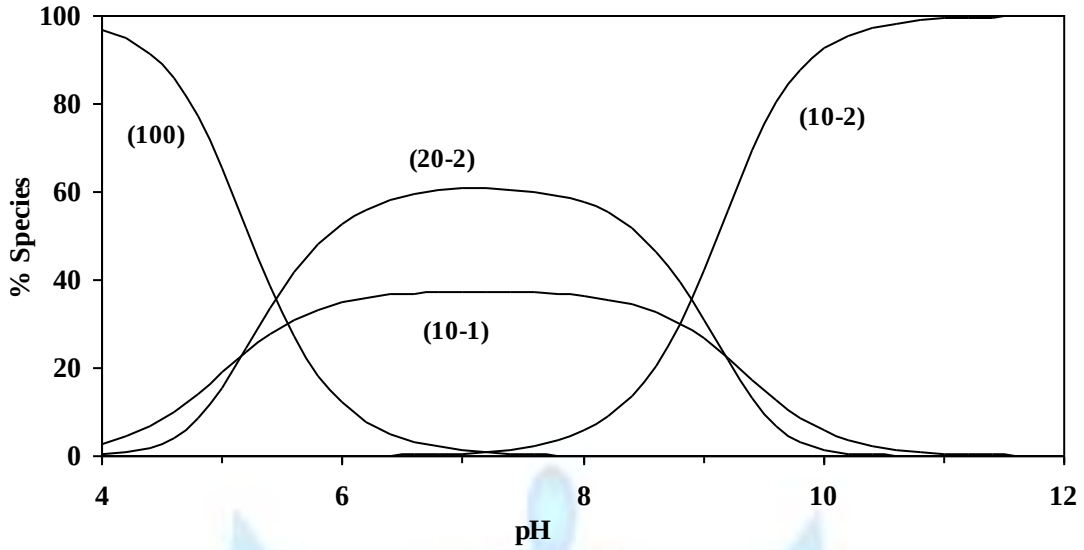
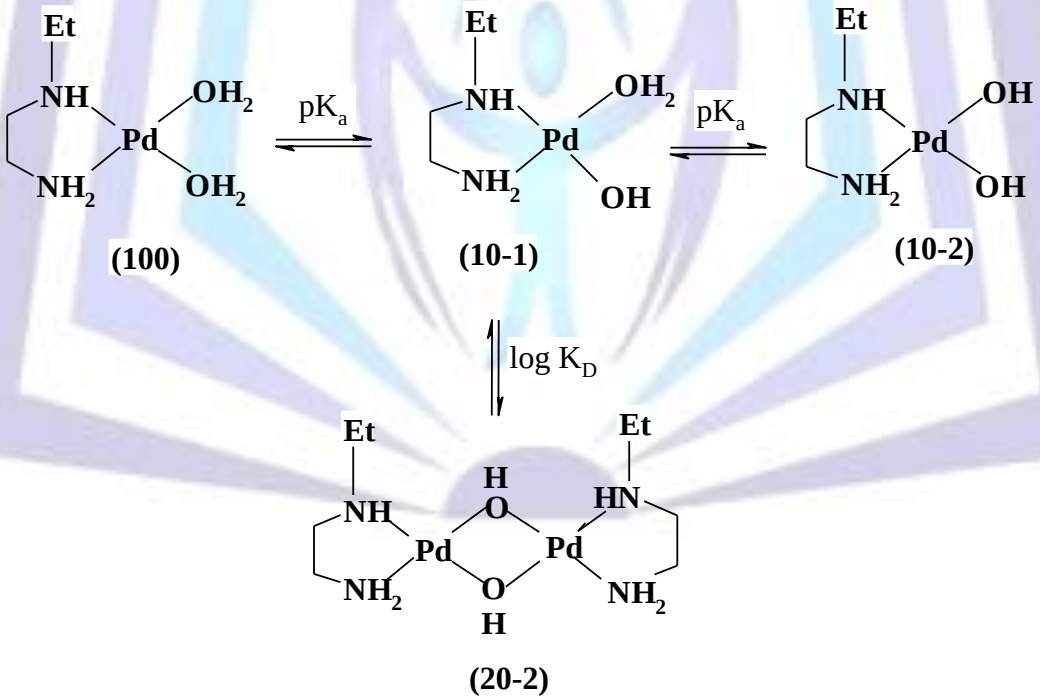


Fig. 1. Concentration distribution diagram of various species as a function of pH in the hydrolysis of $Pd(Eten)(H_2O)^{2+}$

The oligomerisation of cis-aqua-hydroxo-Pd(II) and Pt(II) complexes is well known because the water is a good leaving group in complexes of both metal ions [23]. The formation constants values obtained in this work agree reasonably well with the corresponding data previously reported for, related diamino-palladium(II) [24]. The hydrolysis reactions of $[Pd(Eten)(H_2O)_2]^{2+}$ may be summarized as shown (scheme 2):



Scheme 2. Hydrolysis of $[Pd(Eten)(H_2O)_2]^{2+}$ complex.

The species, (10-1) and (10-2) are formed as a result of deprotonation of the two coordinated water molecules, as given in equations (1), (2). The third species, (20-2), is formed as a result of dimerization of (10-1) species according to equation (3c). The equilibrium constant of dimerization reaction can be calculated using the relationship.

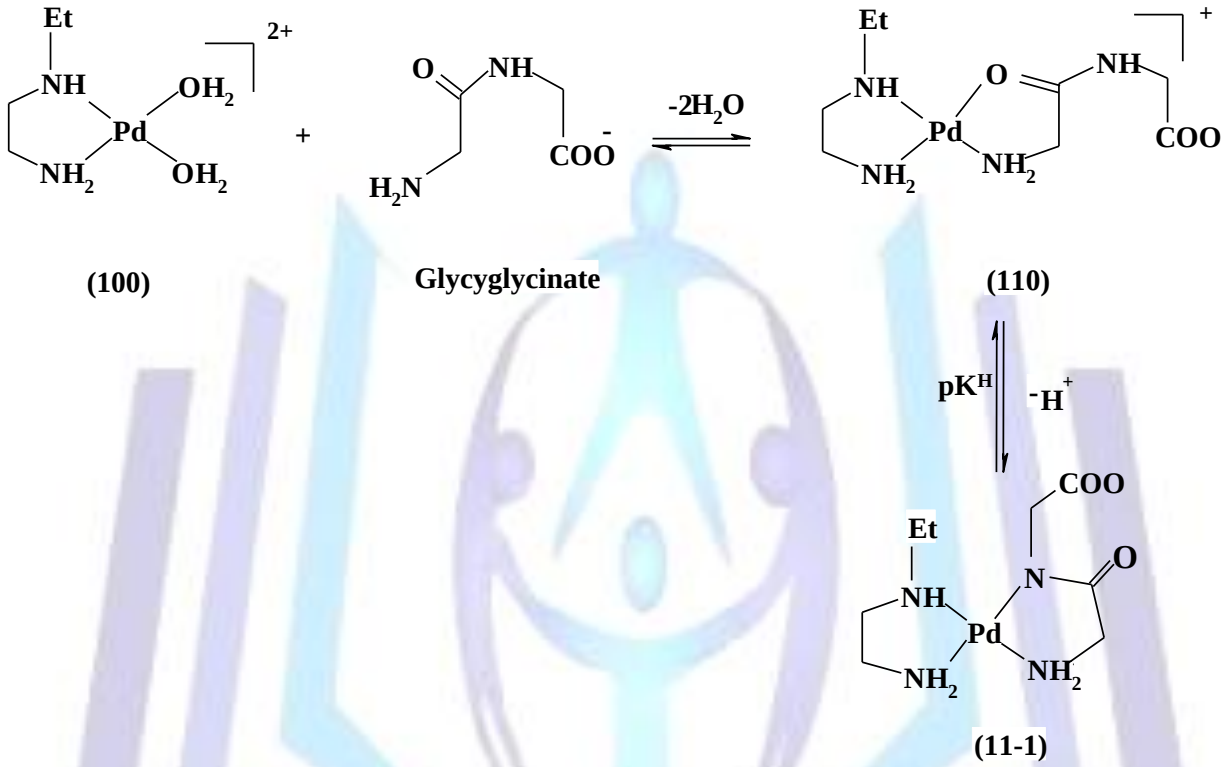
$$LogK_{dimer} = log\beta_{20\text{-}2} - 2log\beta_{10\text{-}1}$$

$$= -7.84 - 2(-5.42) = 3.00$$

The concentration distribution diagram for $[Pd(Eten)(H_2O)_2]^{2+}$ and its hydrolyzed species is shown in Fig. 1. The concentrations of the monohydroxo species (10-1) and the dimeric species, (20-2), increase with increasing pH. These species are the main hydrolyzed forms with percentage of 36% and 62% respectively at pH ca. 7., i.e. they are the main species present under physiological conditions. A further increase in pH is accompanied by an increase in the dihydroxo species concentration, which is the main species above pH 9.

3.2.2 Peptide complexes

The potentiometric data of Pd(II)-peptide system has been fitted considering various models. The compex formation equilibria is given in scheme 3. It is found that glycylglycine (HL), taken as a representative example of peptide, reacts with $Pd(Eten)(H_2O)_2^{2+}$ as a bidentate ligand through the amino and carboxyl groups to form $Pd(Eten)(L)^+$, (110 species) . At higher pH, $Pd(Eten)(LH_{-1})$ is formed by loss of proton from the amide group (11-1 species) ,see Scheme 3..



Scheme 3: Complex formation equilibria of glycylglycine complexes.

The stability constants ($\log\beta_{110}$) of glycylglycine and glycinamide complexes are higher than that of glycylleucine. This is attributed to steric effect of substituent of glycylleucine. Aspargine and glutamine complexes have the highest stability constant. This may be explained on the premise that the α-amino group of aspargine and glutamine are more basic than those of other peptides.

Peptides with Pd(II) are known for promoting ionization of the peptide linkage [25,26] with pK_H value calculated by:

$$pK_H = \log\beta_{110} - \log\beta_{11-1}$$

It is noteworthy that pKH 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 structural change in going from protonated (110) to deprotonated complex (11-1). The pK_H of the glutamine complex, on the other hand is exceptionally higher than the others. This due to the formation of seven membered chelate ring, which would be more strained and less favored. Therefore, under physiological condition (pH ~ 7.4), glutamine would coordinate in the protonated form, whereas glycinamide would preferably coordinate in the deprotonated form.

The concentration distribution diagrams indicate that all peptides form the complex species 110 at low pH, and thus prevent the hydrolysis of Pd(II) i.e. hydrolysed species 10-1 and 10-2 are either not formed or formed in very low concentration. The deprotonation of the peptide NH group starts at pH ~ 3 for glycinamide and glycylglycine complexes and at pH 5 for glycylleucine complex. However the NH ionization starts at pH 6.5 and pH 8 for aspargine and glutamine complexes respectively.

Protonated adenine has two pKa's at 4.03 and 9.29, corresponding to the N1 and N9 sites, respectively. Hodgson [27] and Marzilli [28] discussed both solution and solid complex studies and concluded that N9 is the coordination site of adenine in the palladium(II) complex. Adenosine like the purine riboside undergoes N1 protonation as shown by UV, IR and ^{15}N-NMR spectroscopic measurements. The stability constants $\log\beta_{110}$ and $\log\beta_{120}$ of adenosine 5`-monophosphate are slightly higher than those of adenosine although they both coordinate through N1.

This may due to the doubly negative charge on 5`-AMP. The concentration distribution diagram of 5'-AMP complexes is given in Fig. 2.

Table 1. Formation constants for complexes of $[Pd(Eten)(H_2O)_2]^{2+}$ with peptides and some DNA constituents.

System	P	q	r[a]	Log β[b]	S[c]
Glycinamide	0	1	1	7.50(0.01)	3.1E-8
	1	1	0	7.93(0.07)	9.9E-7
	1	1	-1	4.05(0.05)	
Glycylglycine	0	1	1	7.94(0.01)	2.5E-8
	1	1	0	7.91(0.03)	3.3E-7
	1	1	-1	3.97(0.02)	
Glycylleucine	0	1	1	7.96(0.01)	2.0E-7
	1	1	0	8.77(0.04)	4.9E-7
	1	1	-1	2.12(0.01)	
Glutamine	0	1	1	9.06(0.01)	1.1E-8
	1	1	0	11.91(0.03)	6.7E-7
	1	1	-1	2.54(0.03)	
Aspargine	0	1	1	8.55(0.01)	5.9E-8
	1	1	0	9.30(0.01)	5.1E-8
	1	1	-1	1.39(0.04)	
Adenine	0	1	1	9.29(0.01)	9.4E-8
	0	1	2	13.32(0.02)	
	1	1	0	13.05(0.02)	4.2E-8
	1	2	0	17.56(0.05)	
	1	1	1	16.36(0.07)	
Adenosine	0	1	1	3.60(0.01)	7.5E-8
	1	1	0	4.58(0.08)	4.2E-10
	1	2	0	7.21(0.03)	
Adenosine 5`-monophosphate	0	1	1	6.04(0.01)	2.6E-8
	0	1	2	9.32(0.02)	
	1	1	0	5.77(0.03)	4.8E-8
	1	2	0	8.66(0.09)	
	1	1	1	11.38(0.01)	

[a]p, q and r are the stoichiometric coefficient corresponding to $[Pd(Eten)(H_2O)_2]^{2+}$, peptide or DNA and H^+ respectively; [b]Standard deviations are given in parentheses. [c]Sum of square of residuals.

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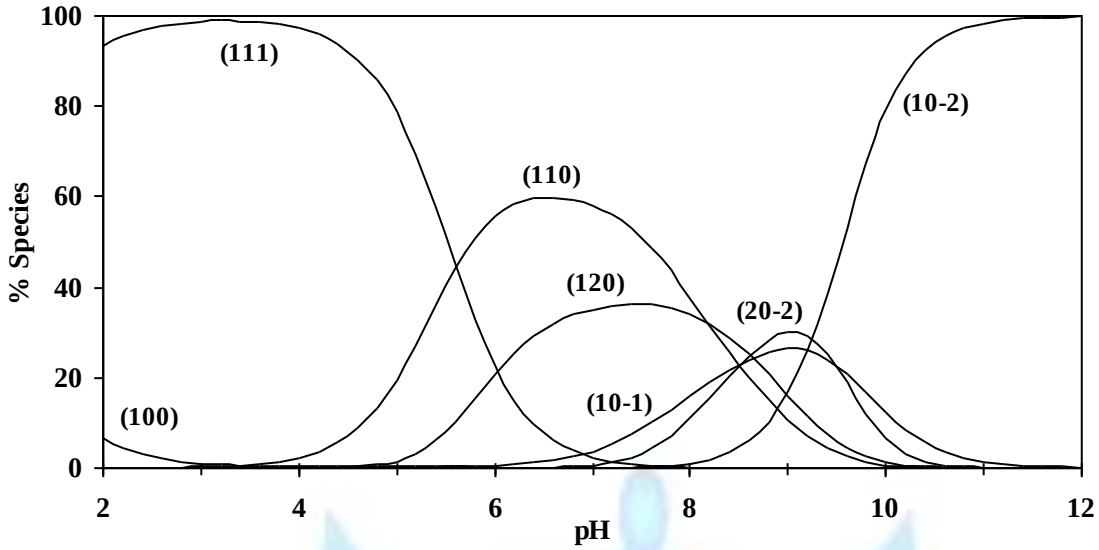

Fig. 2. Concentration distribution of various species as a function of pH in the $Pd(Eten)(H_2O)_2]^{2+}$- Adenosine-5'-monophoshae system

3.2.3. Effect of Temperature on the Complex Formation Equilibria of Pd(Eten)-GlycylGlycine complex

The hydrolysis constants of $[Pd(Eten)(H_2O)_2]^{2+}$, the protonation constants of glycylglycine and the formation constants of $[Pd(Eten)(H_2O)_2]^{2+}$ - glycylglycine complexes are calculated at different temperatures and 0.1 N ionic strength Tables (2,3). The thermodynamic parameters ΔH^o and ΔS^o were obtained by linear least square fit of logK versus 1/T leading to an intercept $\Delta S^o/2.303R$ and a slope of $-\Delta H^o/2.303R$. The results obtained are summarized in Table (4) and explained as follows:

a) The formation constant for the hydrolyzed species of $[Pd(Eten)(H_2O)(OH)]^+$, βOH, taken as an example, can be calculated from:

$$\log \beta_{OH} = pK_w + \log \beta_{10-1}$$

The hydrolysis reaction (1), Table (4), are accompanied by endothermic liberation of ordered water of hydration from the reactants to form bulk water of greater disorder and are accompanied by significant increase in entropy. However, the ΔH values can be considered as the net summation of two opposing effects, namely the exothermic hydrolysis reaction and the endothermic liberation of ordered water of hydration. The hydrolysis reactions (1) and (2) are exothermic reactions. Reaction (1) comprises attraction between doubly positive ion and OH^- while reaction (2) comprises attraction between mono-positive ion and OH^-. So, reaction (1) is more exothermic than reaction (2). Also, the first water molecule to be removed in reaction (1) is more bound than the second water molecule in reaction (2), this results in ΔS^o of reaction (1) to be more than that of reaction (2). Reaction (3), dimerization reaction is an exothermic reaction.

b) The protonation reaction (4) of glycylglycine is exothermic with negative ΔH^o value, because it is neutralization reaction.

c) The complexation reactions (5) and (6) are exothermic reactions with negative ΔH values. This is attributed to the charge neutralization between positive $Pd(N\text{-}N)^{2+}$ and negative ligands.

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Table 2. Formation constants of the hydrolyzed species of $[Pd(Eten)(H_2O)_2]^{2+}$ at different temperatures and 0.1 M ionic strength.

Temp. °C	M	L	H[a]	Log β[b]	S[c]
15	1	0	-1	-5.66(0.01)	7.9E-8
	1	0	-2	-14.89(0.03)	
	2	0	-2	-8.31(0.01)	
20	1	0	-1	-5.55(0.02)	9.0E-8
	1	0	-2	-14.62(0.04)	
	2	0	-2	-8.08(0.01)	
25	1	0	-1	-5.42(0.04)	6.7E-8
	1	0	-2	-14.34(0.04)	
	2	0	-2	-7.84(0.01)	
30	1	0	-1	-5.34(0.04)	3.4E-7
	1	0	-2	-14.13(0.06)	
	2	0	-2	-7.69(0.05)	
35	1	0	-1	-5.22(0.02)	7.9E-8
	1	0	-2	-13.89(0.01)	
	2	0	-2	-7.61(0.03)	

[a]M, L and H are the stoichiometric coefficient corresponding to $[Pd(Eten)(H_2O)_2]^{2+}$, ligand and H^+ respectively. [b]Standard deviations are given in parentheses. [c]Sum of square of residuals.

Table 3. Formation constants for complex of $[Pd(Eten)(H_2O)_2]^{2+}$ complexes with glycylglycine at different temperatures and 0.1 M ionic strength.

Temp °C	M	L	H[a]	logβ[b]	S[c]
15	0	1	1	8.13(0.01)	7.8E-8
	1	1	0	8.34(0.03)	2.1E-7
	1	1	-1	4.36(0.01)	
20	0	1	1	8.04(0.01)	9.4E-8
	1	1	0	8.13(0.02)	1.7E-7
	1	1	-1	4.17(0.01)	
25	0	1	1	7.94(0.01)	2.5E-8
	1	1	0	7.91(0.03)	3.3E-7
	1	1	-1	3.97(0.02)	
30	0	1	1	7.87(0.01)	6.8E-8
	1	1	0	7.76(0.02)	1.5E-7
	1	1	-1	3.84(0.01)	
35	0	1	1	7.81(0.01)	5.6E-8
	1	1	0	7.57(0.03)	2.3E-7
	1	1	-1	3.68(0.01)	

[a]M, L and H are the stoichiometric coefficient corresponding to $[Pd(Eten)(H_2O)_2]^{2+}$, glycylglycine and H^+ respectively. [b]Standard deviations are given in parentheses. [c]Sum of square of residuals.

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Table 4. Thermodynamic parameters for the equilibria of Pd(Eten)-glycylglycine complexes.

Equilibrium[a]	ΔH° kJMol^{-1}	ΔS° JK^{-1} Mol^{-1}
$[Pd(Eten)(H_2O)_2]^{2+}$		
1) $^{b}M(H_2O)_2^{2+} + OH^- \rightleftharpoons M(H_2O)(OH)^+ + H_2O$	-20.06	96.64
2) $M(H_2O)(OH)^+ + OH^- \rightleftharpoons M(OH)_2 + H_2O$	-9.49	65.18
3) $2M(H_2O)(OH)^+ + 2OH^- \rightleftharpoons M_2(H_2O)_2(OH)_2^{2+} + 2H_2O$	-13.06	12.99
Pd(Eten)-Glycylglycine		
4) $L^- + H^+ \rightleftharpoons LH$	-27.57	59.76
5) $M(H_2O)_2 + L \rightleftharpoons ML$	-64.96	66.00
6) $ML \rightleftharpoons MLH_{-1} + H^+$	-7.46	50.31

[a] Stepwise formation constants, [b]M denote $[Pd(Eten)(H_2O)_2]^{2+}$ and L denotes glycylglycine

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