Mixed-ligand Complexes of Palladium(II). Part 1. Diaqua(ethylenediamine)palladium(II) Complexes of Glycylglycine and Glycinamide

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Potentiometric titrations of diaqua(ethylenediamine)palladium(II) with glycylglycine and glycinamide in 0.1 mol dm^{-3} $K[NO_3]$ have been carried out. The results are consistent with the equilibria (i) and (ii) where en = NH_2CH_2-CH_2NH_2, Gly-GlyO = $NH_2CH_2CONHCH_2CO_2^-$, and Gly(NH_2) = $NH_2CH_2CONH_2$, log K_1 and pK_a' are 9.60 and 3.76 for Gly-GlyO$^-$ and 8.64 and 2.47 for Gly(NH_2).

$$[Pd(en)(OH_2)_2]^{2+} + \text{Gly-GlyO}^- \overset{K_1}{\rightleftharpoons} [Pd(en)(\text{Gly-GlyO})]^+ \overset{K_a}{\rightleftharpoons} [Pd(en)(\text{Gly-GlyO})'] + H^+ \quad (i)$$

$$[Pd(en)(OH_2)_2]^{2+} + \text{Gly}(NH_2) \overset{K_1}{\rightleftharpoons} [Pd(en)\{\text{Gly}(NH_2)\}]^{2+} \overset{K_a'}{\rightleftharpoons} [Pd(en)\{\text{Gly}(NH_2)\}']^+ + H^+ \quad (ii)$$

cis-DICHLOROPLATINUM(II) amine complexes have been extensively studied because of their known anti-tumour property. It was pointed out recently that, even though the neutral dichloro-complexes may be required for their passage through cell membranes, the charged diaqua-complexes derived from them are likely to be the active intermediates within the cells.[1] In this connection it is interesting to note that Rosenberg and his co-workers [2,3] have reported the synthesis of a large number of blue platinum complexes of nucleic acid bases and related material with diaquaplatinum(II) amine complexes as starting material. These blue complexes showed potent anti-tumour property as well as being less toxic than the dichloro-complexes.

Comparative studies have shown that diaqua(ethylenediamine)platinum(II) and diaqua(ethylenediamine)-palladium(II) give entirely different titration curves with sodium hydroxide.[1] Whereas the titration curve for $[Pt(en)(OH_2)_2]^{2+}$ corresponds to that of a simple dibasic acid with overlapping pK_a values, the palladium complex dimerises on addition of base according to equilibrium (1)

$$2[Pd(en)(OH_2)_2]^{2+} \rightleftharpoons [Pd_2(en)_2(OH)_2]^{2+} + 2H^+ \quad (1)$$

with a sharp inflexion in the titration curve occurring at the point where exactly one equivalent of base is added. The platinum complex dimerises only with difficulty under special conditions.

Despite this difference, the reactions of these complexes towards nucleosides are very similar. The results of an n.m.r. study of the diaquapalladium system greatly facilitate the interpretation of the platinum system which reacts very slowly and requires drastic conditions such as prolonged heating to achieve equilibrium.[4] Little is known about the reactions of these diaqua-complexes with ligands other than the nucleic acid derivatives. In view of the great affinity of palladium and platinum for nitrogen and sulphur ligands, it is of interest to investigate their reactions with amino acids and peptides and other material derived from proteins. At present there is no evidence to rule out the probability that platinum complexes derive their anti-tumour property from their interactions with some enzymes connected with cell division. The results of a potentiometric study of $[Pd(en)(OH_2)][NO_3]_2$ with glycylglycine (Gly-Gly) and glycinamide [Gly(NH_2)] are now reported.

EXPERIMENTAL

Materials.—The starting material $[PdCl_2(en)]$ was prepared as reported in the literature.[5] The conversion of $[PdCl_2(en)]$ into $[Pd(en)(OH_2)_2][NO_3]_2$ has been described previously.[1] Puriss grade glycylglycine and glycinamide hydrochloride were obtained from Fluka. The glycinamide hydrochloride was converted into the nitrate just before use by stirring it with one equivalent of $Ag[NO_3]$ in water, filtering, and making the filtrate up to the desired volume in a standard flask. The concentration was checked by titrating samples with sodium hydroxide. Carbon-dioxide free sodium hydroxide solution was prepared by diluting a saturated solution under a nitrogen atmosphere. All the other chemicals used were of AnalaR grade quality. Distilled water was passed through a mixed-bed ion-exchange column before use.

E.M.F. Measurement.—Potentiometric titrations were carried out in a jacketed glass vessel (capacity, 100 ml) maintained at 25 °C by circulating water from a constant-temperature bath (Haake NK22). All the titrations were carried out in a 0.1 mol dm^{-3} $K[NO_3]$ medium. The e.m.f.s of test solutions were measured on an Orion 801 pH meter equipped with a glass electrode (Arthur H. Thomas, type 4136 B20) and a special double-junction calomel reference electrode (Arthur H. Thomas, no. 4092-H10), the inner tube of which contained saturated KCl and the outer tube of which was filled with 0.1 mol dm^{-3} $K[NO_3]$. This reference electrode minimises diffusion of chloride ions into the test solution; such diffusion may interfere with the titration.[1] Oxygen-free nitrogen gas was presaturated by passing it through an aspirator containing 0.1 mol dm^{-1} $K[NO_3]$ solution at 25 °C before bubbling through the solutions throughout titrations. Sodium hydroxide solution was delivered from a 10-ml burette (KIMAX type 17115F) through a three-way Teflon stopcock, one arm of which was connected to a storage bottle. The top of the burette and the storage bottle were protected with guard tubes containing Ascarite to prevent CO_2 contamination.

Measured e.m.f. values were converted into pH values by standardising the electrode system with standard buffers of

[1] M. C. Lim and R. B. Martin, *J. Inorg. Nuclear Chem.*, in the press.

[2] B. Rosenberg, *Cancer Chemotherapy Reports, Part* 1, 1975, **59**, 589.

[3] J. P. Davidson, P. J. Faber, R. G. Fischer Hr., S. Mansy, H. J. Peresie, B. Rosenberg, and A. L. Van Camp, *Cancer Chemotherapy Report, Part* 1, 1975, **59**, 287.

[4] M. C. Lim and R. B. Martin, *J. Inorg. Nuclear Chem.*, in the press.

[5] H. D. K. Drew, F. W. Pinkard, G. H. Preston, and A. W. Wardlaw, *J. Chem. Soc.*, 1932, 1895.

TABLE 1

Titration of $[Pd(en)(OH_2)_2]^{2+}$ and glycylglycine in 1 : 1 molar ratio. Total initial concentration of complex = 5.900×10^{-3} mol dm^{-3}

No.	Volume of Na[OH] / ml	pH	10^3[Pd(en)$(OH_2)_2^{2+}$] / mol dm^{-3}	10^3[Pd(en)(Gly-GlyO)$^+$] / mol dm^{-3}	10^3[Pd(en)(Gly-GlyO)'] / mol dm^{-3}	10^9[Gly-GlyO$^-$] / mol dm^{-3}	log K_1
1	0.00	2.567	1.463	4.225	0.211	0.667	9.63
2	0.50	2.612	1.364	4.269	0.237	0.749	9.62
3	1.00	2.658	1.258	4.316	0.267	0.836	9.61
4	1.50	2.709	1.150	4.359	0.302	0.937	9.60
5	2.00	2.762	1.040	4.398	0.345	1.050	9.60
6	2.50	2.819	0.926	4.433	0.396	1.172	9.61
7	3.00	2.888	0.833	4.430	0.465	1.379	9.58
8	3.50	2.957	0.723	4.432	0.545	1.555	9.59
9	4.00	3.035	0.619	4.406	0.648	1.777	9.60
10	4.50	3.120	0.517	4.351	0.777	2.017	9.62
11	5.00	3.220	0.439	4.228	0.952	2.430	9.59
12	5.50	3.325	0.363	4.063	1.165	2.864	9.59
13	6.00	3.434	0.291	3.854	1.420	3.257	9.61
14	6.50	3.546	0.226	3.597	1.716	3.585	9.64
15	7.00	3.667	0.182	3.269	2.061	4.147	9.63
16	7.50	3.797	0.159	2.881	2.448	5.211	9.54
17	8.00	3.925	0.126	2.493	2.843	5.914	9.52

pK_a' (trial) = 3.76 ± 0.01; log K_1 (average) = 9.60 ± 0.03.

pH 4.008 and 6.865 prepared according to Bates.[6] Conversion of pH values into $[H^+]$ with the aid of the Davies equation has been described.[7] E.m.f. readings of test solutions were steady until *ca.* 1.5 equivalents of base per ligand had been added. On further addition of base the readings tended to drift towards lower pH and as much as half an hour was required to obtain steady readings (possibly due to slight hydrolysis). However, the end-point corresponded to the addition of exactly two equivalents of base.

Calculations.—A typical titration curve for a solution containing a 1 : 1 molar ratio of palladium starting material to ligand showed a sharp inflexion point when two equivalents of Na[OH] had been added. The following equations were found to hold:

For glycylglycine

$$^+HGly\text{-}Gly \overset{K_A}{\rightleftharpoons} {}^+HGly\text{-}GlyO^- + H^+ \quad (2)$$

$$^+HGly\text{-}GlyO^- \overset{K_B}{\rightleftharpoons} Gly\text{-}GlyO^- + H^+ \quad (3)$$

$$[Pd(en)(OH_2)_2]^{2+} + Gly\text{-}GlyO^- \overset{K_1}{\rightleftharpoons} [Pd(en)(Gly\text{-}GlyO)]^+ \quad (4)$$

$$[Pd(en)(Gly\text{-}GlyO)]^+ \overset{K_a'}{\rightleftharpoons} [Pd(en)(Gly\text{-}GlyO)'] + H^+ \quad (5)$$

For glycinamide

$$^+HGly(NH_2) \overset{K_B}{\rightleftharpoons} Gly(NH_2) + H^+ \quad (6)$$

$$[Pd(en)(OH_2)_2]^{2+} + Gly(NH_2) \overset{K_1}{\rightleftharpoons} [Pd(en)\{Gly(NH_2)\}]^{2+} \quad (7)$$

$$[Pd(en)\{Gly(NH_2)\}]^{2+} \overset{K_a'}{\rightleftharpoons} [Pd(en)\{Gly(NH_2)\}']^+ + H^+ \quad (8)$$

The pK_A and pK_B values for glycylglycine and pK_B for glycinamide have been determined previously.[7] The method for determining K_1 and K_a' has been described in detail.[7,8] All the numerical calculations were made with the aid of an IBM 1130 computer. Relevant results are shown in Tables 1—3.

DISCUSSION

From Table 3 it is seen that both ligands formed very strong complexes with the palladium species. The large values of log K_1 suggest that both ligands are bidentate in the complexes. In analogy with the extensively studied complexes of Cu^{II} and Ni^{II} with simple peptides, it is likely that in the present case the ligands co-ordinate to the central metal ion through the terminal amino-nitrogen and the oxygen atom of the carbonyl group.[9] The resulting structures are expected to be planar, in common with most palladium complexes. The glycylglycine complex is more stable than the glycinamide complex by a factor of 10 as shown by their respective log K_1 values. It is most unlikely that the extra stability is due to the co-ordination of the carboxyl group in Gly-GlyO$^-$ to the central metal ion since such a structure would require the Pd^{II} to be five-co-ordinate and the resulting chelate ring would also be very large. The most likely explanation lies in the fact that whereas the Gly-GlyO$^-$ carries a negative charge, $Gly(NH_2)$ is neutral. The electrostatic interactions between the Gly-GlyO$^-$ and the dipositively charged $[Pd(en)]^{2+}$ would result in a lowering of free energy of formation. Unlike the case of copper(II) complexes of peptides where a large number of *X*-ray single-crystal structures are available and which often help in confirming or rejecting structures proposed for the same complexes in solution, there are no comparable data for palladium complexes.[9]

From the pK_a' values it is seen that the amide groups in these two complexes undergo very facile deprotonation. Indeed the pK_a' values are some of the lowest yet reported for deprotonation of amide groups of this type

[6] R. G. Bates, 'Determination of pH,' Wiley, New York, 1963.

[7] A. P. Brunetti, M. C. Lim, and G. H. Nancollas, *J. Amer. Chem. Soc.*, 1968, **90**, 5120.

[8] M. C. Lim, Ph.D. Thesis, State University of New York, Buffalo, 1970.

[9] H. C. Freeman, *Adv. Protein Chemistry*, 1968, **22**, 257.

TABLE 2

Titration of $[Pd(en)(OH_2)_2]^{2+}$ and glycinamide in 1 : 1 molar ratio. Total initial concentration of complex = 5.900×10^{-3} mol dm^{-3}

No.	Volume of Na[OH]	pH	$10^3[Pd(en)(OH_2)_2^{2+}]$ mol dm^{-3}	$10^3[Pd(en)\{Gly(NH_2)\}^{2+}]$ mol dm^{-3}	$10^3[Pd(en)\{Gly(NH_2)\}'^{+}]$ mol dm^{-3}	$10^9[Gly(NH_2)]$ mol dm^{-3}	log K_1
1	0.00	2.353	1.779	2.578	1.542	2.832	8.71
2	0.50	2.393	1.780	2.470	1.619	3.105	8.65
3	1.00	2.429	1.722	2.404	1.714	3.266	8.63
4	1.50	2.466	1.647	1.346	1.818	5.395	8.62
5	2.00	2.504	1.571	2.282	1.930	3.532	8.61
6	2.50	2.540	1.467	2.234	2.054	3.582	8.63
7	3.00	2.582	1.387	2.157	2.183	2.724	8.62
8	3.50	2.623	1.292	2.085	2.322	3.813	8.62
9	4.00	2.670	1.214	1.990	2.467	3.986	8.61
10	4.50	2.715	1.111	1.909	2.624	4.041	8.63
11	5.00	2.761	1.005	1.823	2.790	4.060	8.65
12	5.50	2.815	0.917	1.711	2.963	4.182	8.65
13	6.00	2.870	0.820	1.599	3.146	4.237	8.66
14	6.50	2.932	0.732	1.470	3.337	4.353	8.66
15	7.00	3.000	0.642	1.334	3.537	4.445	8.67
16	7.50	3.079	0.565	1.176	3.746	4.681	8.65
17	8.00	3.168	0.483	1.016	3.963	4.874	8.63

pK_a' (trial) = 2.47 ± 0.01; log K_1 (average) = 8.64 ± 0.02.

in any metal complexes. In simple copper(II) peptides the pK_a' values are usually between 4 and 5, while in mixed-ligand complexes they are larger.[9,10] The low pK_a' values in the present systems undoubtedly arise because of the large affinity of palladium for nitrogen-type ligands, since on deprotonation the co-ordination sites in these complexes switch from carbonyl oxygen to nitrogen. Such changes in co-ordination centres are now very well documented.[9] In view of the greater affinity of platinum for nitrogen, one would predict even lower pK_a' values for platinum complexes of the same ligands. No values are yet available for comparison.

It is interesting to note that the pK_a' value for Gly-(NH_2) is lower than that for Gly-GlyO$^-$ even though the log K_1 values are in the opposite order. The reason for this is not entirely clear at the moment. It has been shown from c.d. and n.m.r. studies on $[Pd(en)(Ala\text{-}GlyO)]^+$ (Ala = alanyl) at high pH that the carboxyl group of the peptide is not co-ordinated.[11] Thus there is no difference in the co-ordination centres in Gly-GlyO$^-$ and $Gly(NH_2)$ in the deprotonated complex. It is possible to speculate that the more bulky substituent group on the nitrogen in Gly-GlyO$^-$ may serve to hinder the structural change in going from the protonated to the deprotonated species. This would favour the smaller $Gly(NH_2)$ complex. However, there is no conclusive evidence to show that this is the case. In this connection it is perhaps interesting to note that recently Sigel[10] showed that in mixed-ligand complexes involving 2,2′-bipyridyl-copper(II) and Gly-GlyO$^-$ and $Gly(NH_2)$ the pK_a' for both complexes are very similar (*ca.* 7.7), whereas in their simple complexes the pK_a' for Gly-GlyO$^-$ is much lower (*ca.* 4.0). It was proposed that this is due to Gly-GlyO$^-$ acting as a bidentate ligand in the mixed-ligand complex while it is tridentate in the simple complex, $Gly(NH_2)$ remaining bidentate in both situations. It has been shown by *X*-ray diffraction of a single crystal of the mixed-ligand complex $[Cu(phen)(Gly\text{-}GlyO)]^+$ (phen = 1,10-phenanthroline) that in this complex the peptide is actually tridentate in the solid state. Comparison of the absorption spectra of the solid and in solution both in aqueous and in non-aqueous solvents suggests that the same structure

TABLE 3

Stability and dissociation constants of $[Pd(en)(Gly\text{-}GlyO)]^+$ and $[Pd(en)\{Gly(NH_2)\}]^{2+}$ at 0.5 mol dm^{-3} K[NO_3] and 25 °C

	Glycylglycine	Glycinamide
log K_1	9.60	8.64
pK_a'	3.76	2.47

persists in solution.[12] In view of the relatively scanty data for mixed-ligand complexes of this type, it is difficult to outline specific factors which can determine the magnitude of such pK_a values.

The present study shows clearly that diaquapalladium(II) amine complexes can form strong bonds with peptides and promote facile deprotonation of the peptide group. In view of the great affinity of Pt^{II} for nitrogen ligands, it is expected to show the same reactions. In combination with data on the stability constants of such diaqua-complexes with nucleosides and nucleotides, it would be possible to calculate the equilibrium distribution of the metal species in situations where both 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.

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[10] H. Sigel, *Inorg. Chem.*, 1975, **1**, 1535.
[11] T. P. Pitner, E. W. Wilson, jun., and R. B. Martin, *Inorg. Chem.*, 1972, **11**, 738.
[12] M. C. Lim, E. Sinn, and R. B. Martin, *Inorg. Chem.*, 1976, **15**, 807.