Synthesis and Spectroscopic Studies of Potential Antiviral [Pt(2,2'-Bipyridyl)-(Amino Acid)]Cl Complexes

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Six platinum complexes of the formula [Pt(2,2'-bipyridyl)(amino acid)] Cl (where amino acids are glycine, alanine, leucine, serine, cysteine and methionine) have been synthesized by interaction of Pt(2,2'-bipyridyl)Cl_2 and appropriate amino acids. The conductivity data of these complexes confirm the above formulation by showing these complexes as 1:1 electrolytes. The ultraviolet spectra of these complexes have been interpreted in terms of charge transfer transition from platinum to π-antibonding orbitals of 2,2'-bipyridyl and of π–π transitions of 2,2'-bipyridyl. The ^1H NMR spectra of these complexes show that first four amino acids are bonded to platinum through nitrogen and oxygen atoms, whereas the latter two are bonded through nitrogen and sulfur atoms. The infrared data have further supported the above mode of binding of amino acids to platinum. All of these complexes (except leucine) complex show antiviral activities against tobacco mosaic virus in host plants like* Nicotiana glutinosa *and/or* Datura stramonium.

Introduction

The *cis*-dichlorodiamineplatinum(II) (*cis*-platin) is an effective antitumor agent, which is thought to bind to deoxyribonucleic acid (DNA) *in vivo* [1]. This behaviour of *cis*-platin has created great interest in aqueous chemistry of platinum [2], and in binding studies of platinum complexes to nucleic acids and their constituents [3]. Recently S. J. Lippard and coworkers [3, 4] have used [Pt(2,2',2''-terpyridyl)(2-mercaptoethanol)]$^+$, [Pt(2,2'-bipyridyl)-(ethylenediamine)]$^{2+}$ and related compounds as metallointercalation reagents in place of aromatic dyes such as proflavine, acridine and ethidium bromide. These platinum complexes are found to bind to double stranded DNA in an intercalative manner and stabilize the stacked complexes by additional hydrogen bonding between coordinated ligands such as 2-mercaptoethanol or ethylenediamine and the phosphate backbone of DNA. In view of the importance of noncovalent interactions in stabilizing the above and other systems [3–5], we have replaced the ethylenediamine by amino acids in the [Pt(2,2'-bipyridyl)(ethylenediamine]$^{2+}$ complex. In these amino acid derivatives, the various types of noncovalent interactions such as hydrogen bonding, ionic bonding and van der Waals' interaction between side chains of amino acids [6] and phosphate backbone or nucleoside of DNA molecule are possible for stabilizing the stacked complexes. In this report we describe the synthesis and characterization of six complexes of platinum of the formula [Pt(2,2'-bipyridyl)(amino acid)Cl (where amino acid = glycine, alanine, leucine, serine, cysteine or methionine).

Experimental

Starting Materials

Commercially available amino acids (BDH) and 2,2'-bipyridyl (abbreviated as bipy) of SISCO, India, were used without further purification. Potassium tetrachloroplatinate(II), K_2PtCl_4, was purchased from Strem Chemicals, U.S.A.

Synthesis of Platinum Compounds

Pt(bipy)Cl_2 was prepared according to the procedure given by Palocsay and Rund [7].

[Pt(bipy)(amino acid)] Cl

Pt(bipy)Cl_2 (0.1 m*M*) was suspended in 20 ml of methanol. The concentrated aqueous solution of sodium salt of appropriate amino acid (0.12 mM) was added with constant stirring. The solution was stirred at 40–45 °C for 24 hours. The resulting yellow solution was filtered to remove unreacted Pt(bipy)-Cl_2 and the filtrate was concentrated on a water bath to 1–2 ml. The yellow needle shaped crystals were obtained on cooling the concentrated solution to room temperature. These crystals were filtered, washed with a small amount of cold methanol and

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TABLE I. [Pt(bipy)(amino acid)]Cl Complexes with their Color, Analytical and Conductivity Data.

Compound	Color	Calculated		Found		Conductivity of 10^{-3} *M* solution (cm^2 ohm^{-1} mol^{-1})
		%C	%H	%C	%H	
[Pt(bipy)(gly)]Cl	Yellow	31.27	2.60	29.90	3.34	122
[Pt(bipy)(ala)]Cl	Yellow	32.87	2.95	32.35	3.77	120
[Pt(bipy)(leu)]Cl	Yellow	37.17	3.87	36.85	3.39	126
[Pt(bipy)(ser)]Cl	Orange–yellow	31.80	2.85	30.62	3.29	101
[Pt(bipy)(cys)]Cl	Orange–yellow	30.80	2.76	29.75	3.25	–
[Pt(bipy)(met)]Cl	Yellow	33.69	3.36	33.39	3.44	126

TABLE II. Electronic Spectra of [Pt(bipy)(amino acid)Cl Complexes.

Compound	Band maxima in kK[a]			
	Band 1	Band 2	Band 3	Band 4
$Pt(bipy)Cl_2$[b]	25.31	30.49, 31.55	35.34	–
	–	–	–	
$Pt(bipy)Cl_2$[c]	26.31	30.96, 32.15	37.45	39.68sh[d]
	–	–	–	–
[Pt(bipy)(gly)]Cl[c]	28.17	31.35, 32.47	33.67sh	40.0
	(0.24)[e]	(1.44) (1.04)	(0.62)	(2.00)
[Pt(bipy)(ala)]Cl[c]	28.98	31.75, 32.90	34.01sh	40.48
	–	(1.32) (0.95)	(0.57)	(1.92)
[Pt(bipy)(leu)]Cl[c]	28.98	31.75, 32.78	34.13sh	40.48
		(1.34) (0.96)	–	(1.96)
[Pt(bipy)(ser)]Cl[c]	28.73	31.64, 32.79	34.25sh	40.48
	(0.17)	(1.27) (0.93)	(0.53)	(1.82)
[Pt(bipy)(cys)]Cl[c]	28.57	31.05, 33.11	–	40.65
	(0.22)	(0.92) (1.20)	–	(1.58)
[Pt(bipy)(met)]Cl[c]	–	31.55, 32.68	–	41.32
		(1.40) (1.10)	–	(1.76)

[a]kK is 1×10^3 cm^{-1}. [b]DMF is used as a solvent. [c]Water (double distilled) is used as solvent. [d]sh is shoulder. [e]the extinction coefficients in 1 mol^{-1} cm^{-1} $\times 10^{-4}$ are given in parentheses.

finally with diethyl ether. The compound was dried in vacuum at room temperature. The yield was more than 80%.

Carbon and hydrogen analyses of the platinum complexes were carried out using standard methods.

Physical Measurements

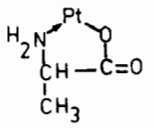
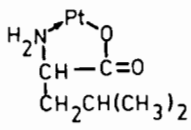
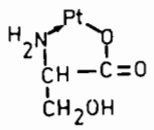
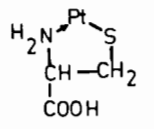
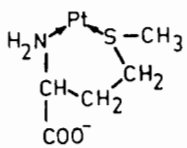
Conductivity measurements were carried out on a 'Systronic conductivity bridge 305' using a conductivity cell with a cell constant of 0.66. The solvent used was doubly distilled water.

Infra-red spectra of the platinum complexes were recorded on a Perkin-Elmer 237 B Infra-red spectrophotometer (4000 to 650 cm^{-1}) in nujol mull. Electronic absorption spectra of these complexes in doubly distilled water were recorded on a Perkin-Elmer 402 ultraviolet–visible spectrophotometer, in the range of 220 to 390 nm. ^1H NMR spectra were recorded on a Varian XL-100 NMR spectrometer in D_2O using DSS as internal standard.

Results and Discussion

The six amino acid complexes of platinum, of the general formula [Pt(bipy)(amino acid)]Cl, were prepared by interaction of $Pt(bipy)Cl_2$ with the sodium salt of amino acid in water–methanol mixture at 40–50 °C. The amino acids used were glycine (gly), alanine (ala), leucine (leu), serine (ser), cysteine (cys) and methionine (met). The above complexes were characterized by chemical analyses,

TABLE III. ^{1}H NMR Spectra of [Pt(bipy)(amino acid)]Cl Complexes.

Compound	Amino-acid Structure	$\delta_{H\text{-}6,6'}$[a]	$\delta_{H\text{-}4,4'}$	$\delta_{CH \text{ or } CH_2}$	$\delta_{C\text{-}CH}$ or $\delta_{S\text{-}CH}$	$^3J_{Pt\text{-}H}$[b] of ^{195}Pt–N–C–H fragment	$^3J_{Pt\text{-}H}$ of ^{195}Pt–S–C–H fragment
$Pt(bipy)Cl_2$	–	9.52	7.86	–	–	42.0 (^{3}JPt- H(6))	–
[Pt(bipy)(gly)]Cl		8.70	7.66	3.84 (–0.25)[c]	–	32.5	–
[Pt(bipy)(ala)]Cl		8.66	7.68	3.94 (–0.15)	1.57 (–0.08)	24.0	–
[Pt(bipy)(leu)]Cl		8.71	7.65	3.84 (–0.10)	1.00 (–0.05) CH_3 groups	29.0	–
[Pt(bipy)(ser)]Cl		8.53	7.68	4.10 (–0.07)	–	~24.0	–
[Pt(bipy)(cys)]Cl		8.66	7.55	3.87 (0.14)	2.68 (0.42)	52.0	38.0
[Pt(bipy)(met)]Cl		9.06	7.94	3.80 (0.09)	2.79 (–0.61) S–CH_3 group	66.0	50.0

[a]δ is chemical shift in ppm. [b]J is coupling constant in Hz. [c]The chemical shift differences are given in parentheses relative to corresponding amino acid. The positive sign denotes the upfield shift where as the negative sign denotes the downfield shift.

conductivity measurements and infra-red, ultraviolet–visible and ^1H NMR spectroscopy. The analytical and conductivity data of these platinum complexes with their colors are given in Table I. The analytical data are in good accordance with the formula [Pt(bipy)(amino acid)]Cl. The conductivity data are in agreement with the values for 1:1 electrolytes [8].

The amino acid complexes of platinum show several absorption maxima in the ultraviolet region. The positions of these maxima and their extinction coefficients are given in Table II. These spectra can be interpreted in terms of assignments of Pt(bipy)Cl_2 given by Gillard and coworkers [9]. The band 1 is assigned to charge transfer from platinum orbital to π-antibonding orbital of 2,2′-bipyridyl. The band 3 is assigned to charge transfer from same platinum orbital to a higher π-antibonding orbital of 2,2′-bipyridyl. The bands 2 and 4 are assigned to first and second internal π–π^* type transitions of 2,2′-bipyridyl respectively.

The ^1H NMR spectra of these platinum complexes have been recorded in D_2O. Table III lists the chemical shifts of H-6,6′ and H-4,4′ of 2,2′-bipyridyl and C–H proton and other protons of amino acids. The ^{195}Pt–N–C–H or ^{195}Pt–S–C–H coupling constants (whenever observed) are also given in Table III. The $^3J_{Pt-H}$ for Pt–N–C–H or Pt–S–C–H fragments depend on the dihedral angles as calculated by the Karplus method, and these variations could be accounted for in terms of conformation of coordinated ligand [10–13]. The [Pt(glycine)Cl_2]$^-$ and related complexes adopt an envelope conformation in solution with methyl substitution on the carbon atom, causing some puckering around a C–N bond of the chelate ring [10, 11]. The coordination of platinum invariably produces downfield shifts for ligand protons. The extent of the downfield shift is similar to corresponding protonation shifts and decreases similarly with distance from the coordinated site [10]. The H-6,6′ protons of 2,2′-bipyridyl in the amino acid derivatives of platinum are shifted to higher fields, and this could be explained in terms of stronger binding of amino acids than chloride ions to platinum [13]. The platinum complexes of glycine, alanine, leucine and serine show downfield shifts of C–H or CH_2 fragments as compared to free amino acids in zwitterion forms. This can be intepreted in terms of stronger binding of chelated amino acids to platinum. The large values of $^3J_{Pt-H}$ of ^{195}Pt–N–C–H in the above complexes confirm that nitrogen is bonded to platinum. The above complexes give a $\nu(COO^-)$ band in the region of 1640 to 1670 cm^{-1} of their infrared spectra, which can be interpreted in terms of ionized and coordinated $-COO^-$ group [14]. This gives further support to the above NMR results.

The cysteine in its platinum complex shows an upfield shift of S–CH_2 protons (0.42 ppm) as compared to free cysteine. This upfield shift of S–CH_2 protons is possibly interpreted in terms of ionization of the SH group and this ionized $-S^-$ group is involved in weak bonding to platinum. The 38 Hz $^3J_{Pt-H}$ of ^{195}Pt–S–C–H fragment further supports the bonding of sulfur to platinum. The 52 Hz $^3J_{Pt-H}$ of ^{195}Pt–N–C–H indicates that the nitrogen of the NH_2 group is also involved in forming a cysteine chelate complex. The upfield chemical shift of 0.14 ppm of C–H proton indicates that the hydrogen of –COOH group may be further involved in hydrogen bonding [10]. The infrared spectrum of cysteine complex shows a ν(COO) at 1725 cm^{-1} which further supports the presence of free –COOH group in the complex [14].

The methionine in its platinum complex shows a downfield shift of 0.61 ppm of $-CH_3$ group attached to sulfur, which clearly indicates that sulfur is bonded to platinum. The 50 Hz $^3J_{Pt-H}$ of ^{195}Pt–S–C–H in the complex further suggests that sulfur is bonded to platinum. The large 66 Hz $^3J_{Pt-H}$ of ^{195}Pt–N–C–CH indicates that the nitrogen of the NH_2 group is bonded to platinum and the complex has a chair conformation [12]. An upfield shift of 0.09 ppm of CH proton in the complex may be interpreted in terms of free $-COO^-$ group, possibly intermolecularly hydrogen-bonded to N–H proton(s). This is supported by the infrared spectra of the methionine complex which shows a 20 cm^{-1} high frequency shift of $\nu(COO^-)$, as compared to its value in free methionine in zwitterion form [14].

Recently the Pt(bipy)Cl_2 has been shown to be anticancer active [15]. The *cis*-platin shows a nephrotoxic effect which is due to coordination of protein-bound sulfhydryl groups of the kidney tubule cells to platinum [16]. The above cysteine and methionine complexes are good models of this *cis*-platin–protein interaction.

Preliminary screening of the above complexes against Tobacco mosaic virus in two host plants like *Nicotiana glutinosa* and *Datura stramonium*, both *in vivo* and *vitro*, has been carried out. The glycine, alanine and cysteine complexes are active *in vitro* but not *in vivo*. The methionine complex is very active *in vitro* but less active *in vivo*. The leucine complex is completely inactive whereas the serine complex is active only against *D. stramonium* virus. Dr. H. N. Verma and coworkers are conducting detailed screening of the above complexes for antiviral activity and these results will be published later.

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