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Synthesis and Antitumor Activity of Polyphosphazene/Methoxy-Poly(ethylene glycol)/(Diamine)platinum(II) Conjugates

Sang Beom LEE, Soo-Chang SONG,† Jung-Il JIN,* and Youn Soo SOHN

Inorganic Chemistry Laboratory, Korea Institute of Science and Technology, Seoul 130-650, Korea
**Department of Chemistry, Korea University, Seoul 136-701, Korea*

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ABSTRACT: The antitumor platinum moiety, (dach)Pt^{2+} (dach = *trans*(±)-1,2-diaminocyclohexane), was introduced to a poly(organophosphazene) using dicarboxylate spacers, glutamate or aspartate. After characterization of the polymeric conjugates by means of multinuclear (^1H, ^{31}P) NMR spectroscopy, elemental analysis, and gel-permeation chromatography (GPC), their *in vitro* hydrolytic behavior was examined in buffer solutions at different pHs and temperatures by monitoring with GPC. Hydrolytic properties of the conjugates were dependent on pH and temperature of the polymer solutions, and their degradation occurred more rapidly at lower pH and higher temperature. The antitumor activity of the conjugates was evaluated both *in vitro* and *in vivo* against the murine leukemia L1210 cell line and found to be dependent on the structures of the conjugates, and some of them exhibited higher *in vivo* activity than cisplatin and carboplatin.

KEY WORDS Anticancer Drug / Polymer-Drug Conjugates / Polyphosphazene/Methoxy-Poly(ethylene glycol)/(Diamine)platinum(II) Conjugates / Controlled Release /

Cisplatin (*cis*-diamminedichloroplatinum(II)) is widely used for the chemotherapy of various tumors of ovary, bladder, head and neck, and testicle.[1-3] However, its toxic side effects including nephrotoxicity and neurotoxicity are a serious problem.[4] Following its administration, cisplatin is rapidly and irreversibly bound to plasma proteins with greater than 90% loss in the first 2 h, which is a reason for decrease of its drug efficiency and nephrotoxicity.[5] The second generation platinum drug, carboplatin (*cis*-diammine(1,1-cyclobutanedicarboxylato)platinum(II)), exhibits remarkably lowered toxicity and low protein binding, but is inferior to cisplatin in antitumor activity.[6,7] There is an urgent need for the development of a more active but less toxic platinum compound.

Poly(organophosphazenes) prepared by replacement of the chlorine atoms in poly(dichlorophosphazene) with organic nucleophiles such as amines or alkoxides are potential polymeric carriers for biologically active agents. By suitable choice of organic substituents, *e.g.*, amino acid esters, biodegradable and/or biocompatible poly(organophosphazenes) can be designed, which are decomposed to non-toxic hydrolytic products. Bioactive agents may be linked covalently or coordinatively to the polyphosphazene back-bone by using proper spacer groups.

Poly(ethylene glycol) (PEG) is one of the most important materials in the biomedical field and has been extensively studied for nearly 30 years because of its unique properties such as high hydrophilicity, biocompatability, thermosensive solubility, high mobility, and so on. It has been known that PEG-modified polymer surface exhibits high antithrombogenicity due to its ability to suppress plasma protein binding.[8] Modification of proteins with PEG has been studied in order to reduce immunogenicity and increase plasma half-life.[9] A number of PEG copolymers has been studied as thermosensitive polymers.[10]

A major direction in the drug delivery research concerning development of antitumor agent today is focused on how to reduce toxicity of existing drugs without loss of therapeutic activity. Polymer–anticancer drug conjugation has been a major approach to reduce toxicity with increased therapeutic efficacy of the drugs. The advantages of polymeric anticancer drugs have been explained in terms of controlled release and EPR (enhanced permeability and retention) effect.[11-14] Several papers reported introduction of platinum compounds into polymeric carriers.[15,16] The antitumor activity of polymer-platinum conjugates is dependent on many factors such as, properties of polymeric carriers, molecular size of the conjugates, and coordination mode of the platinum compound to polymers, etc. We have studied poly(organophosphazenes) with methoxy-PEG[17-19] and polyphosphazene–(diamine)platinum(II) conjugates.[20,21] In the present work aiming to design a polymeric platinum drug with increased plasma half-life and low plasma protein binding, we have synthesized polyphosphazene/MPEG/(diamine)platinum(II) conjugates and studied their hydrolytic properties and antitumor activity.

EXPERIMENTAL

Materials

Potassium tetrachloroplatinate(II) (Kojima) and hexachlorocyclotriphosphazene (Aldrich) were used without purification. Ethyl esters of L-aspartic and L-glutamic acids were prepared by the literature method.[22] Methoxy-diethylene glycol (MDEG) and methoxy-poly(ethylene glycol) (MPEG) with molecular weights of 350 and 750 were dried azeotropically with benzene, followed by vacuum drying, and then stored over molecular sieve 4A. Tetrahydrofuran (THF) was dried by boiling at reflux over sodium metal and distilled under a nitrogen atmosphere. (dach)$Pt(NO_3)_2$ was

† To whom correspondence should be addressed.

prepared by the literature method.[23] The low molecular weight ($M_w = 10^4$) polydichlorophosphazene was obtained by the author's procedure.[24]

General Procedure for the Synthesis of Polyphosphazene/MPEG/(Diamine)platinum(II) Conjugates

A typical synthetic procedure of polymeric conjugates is shown in Scheme 1. Poly(organophosphazenes) (**III**) with MPEG and a dicarboxylic amino acid ester were prepared by stepwise reaction of poly(dichlorophosphazene) with sodium salt of MPEG and amino acid ester, as was mentioned earlier.[17] The ethyl group of the amino acid in polymer (**III**) was removed by treatment with NaOH: To a solution of the polymer (**III**) (1.65 mmol) dissolved in methanol (20 mL) was added a solution of excess sodium hydroxide (6.60 mmol) in methanol (20 mL). The reaction mixture was precipitated again in ether after stirring for 2 h, and dried in vacuo to obtain polymer (**IV**). To a solution of polymer (**IV**) (1.72 mmol) in distilled water (20 mL) was added an equimolar aqueous solution (20 mL) of (dach)$Pt(NO_3)_2$. The reaction mixture was stirred at room temperature for 1 h, and dialyzed with distilled water for 1 day using a cellulose acetate tubing (mol. wt. cutoff: 3500). The dialyzed solution was freeze-dried to obtain the final polymeric product (**V**).

Poly[(*MDEG*)(L-*asparto*-(*trans*(±)-*1,2-diaminocyclohexane*)*platinum*)*phosphazene*] (**1**). MDEG (17.3 mmol) and L-aspartic acid ethyl ester (35.5 mmol) were used. Overall yield: 63%. ^{1}H NMR(D_2O), δ(ppm): 1.1—1.3(b, 4H, dach C-4, C-5 protons), 1.5 (b, 2H, dach C-3 protons), 2.1 (b, 2H, dach C-6 protons), 2.3 (b, 2H, dach C-1, C-2 protons), 2.6 (b, 2H, aspartate α-C protons), 3.4 (m, 3H, MDEG methyl protons), 3.6—3.9 (b, 7H, aspartate α-C, MDEG ethylene protons), 4.2(b, 2H, MDEG C-2 protons).

Poly[(*MPEG350*)(L-*asparto*-(*trans*(±)-*1,2-diaminocyclohexane*)platinum)*phosphazene*] (**2**). MPEG (17.3 mmol) and L-aspartic acid ethyl ester (35.5 mmol) were used. Overall yield: 66%. ^{1}H NMR (D_2O), δ (ppm): 1.1—1.3 (b, 4H, dach C-4, C-5 protons), 1.5 (b, 2H, dach C-3 protons), 2.1 (b, 2H, dach C-6 protons), 2.3 (b, 2H, dach C-1, C-2 protons), 2.6 (b, 2H, aspartate β-C protons), 3.4 (m, 3H, MPEG methyl protons), 3.6—3.9 (b, 27H, aspartate α-C, MPEG ethylene protons), 4.2 (b, 2H, MPEG C-2 protons).

Poly[(*MDEG*)(L-*glutamato*-(*trans*(±)-*1,2-diaminocyclohexane*)*platinum*)*phosphazene*] (**3**). MDEG (17.3 mmol) and L-glutamic acid ethyl ester (35.5 mmol) were used. Overall yield: 73%. ^{1}H NMR (D_2O), δ (ppm): 1.1—1.3(b, 4H, dach C-4, C-5 protons), 1.5 (b, 2H, dach C-3 protons), 2.1 (b, 4H, glutamate β-C, dach C-6 protons), 2.3 (b, 4H, glutamate γ-C, dach C-1, C-2 protons), 3.4 (m, 3H, MDEG methyl protons), 3.6—3.9 (b, 7H, glutamate α-C, MDEG ethylene protons), 4.2 (b, 2H, MDEG C-2 protons).

Poly[(*MPEG350*)(L-*glutamato*-(*trans*(±)-*1,2-diaminocyclohexane*)*platinum*)*phosphazene*] (**4**). MPEG (17.3 mmol) and L-glutamic acid ethyl ester (35.5 mmol) were used. Overall yield: 72%. ^{1}H NMR (D_2O), δ (ppm): 1.1—1.3 (b, 4H, dach C-4, C-5 protons), 1.5 (b, 2H, dach C-3 protons), 2.1 (b, 4H, glutamate β-C, dach C-6 protons), 2.3 (b, 4H, glutamate γ-C, dach C-1, C-2 protons), 3.4 (m, 3H, MPEG methyl protons), 3.6—3.9 (b, 27H, glutamate α-C, MPEG ethylene protons), 4.2 (b, 2H, MPEG C-2 protons).

Poly[(*MPEG750*)(L-*glutamato*-(*trans*(±)-*1,2-diaminocyclohexane*)*platinum*)*phosphazene*] (**5**). MPEG (17.3 mmol) and L-glutamic acid ethyl ester (35.5 mmol) were used. Overall yield: 62%. ^{1}H NMR (D_2O), δ (ppm): 1.1—1.3 (b, 4H, dach C-4, C-5 protons), 1.5 (b, 2H, dach C-3 protons), 2.1(b, 4H, glutamate β-C, dach C-6 protons), 2.3 (b, 4H, glutamate γ-C, dach C-1, C-2 protons), 3.4 (m, 3H, MPEG methyl protons), 3.6—3.9 (b, 63H, glutamate α-C, MPEG ethylene protons), 4.2 (b, 2H, MPEG C-2 protons).

Instruments

Elemental analysis was carried out with a Fisons 1108 CHNS Microanalyzer and Polyscan 61E ICP. ^{1}H NMR measurements were made with a Varian Gemini-300 spectrometer operating at 300 MHz in the Fourier transform mode. Proton-decoupled ^{31}P NMR spectra were measured with the same spectrometer operating at 121.4 MHz using triphenyl phosphate as an external standard. Gel-permeation chromatography was carried out to measure the weight-averaged molecular weight (M_w) of the polymers using a Waters Associates HPLC/GPC 150C unit and two Ultrahydrogel columns (Ultrahydrogel Linear and 250) connected in line at a flow rate of 0.8 mL min^{-1} at 35°C. Poly(ethylene oxides) (M_w: 600, 900, 1470, 7100, 12600, 23000, 46000, 95000) were used as standards to calibrate the column.

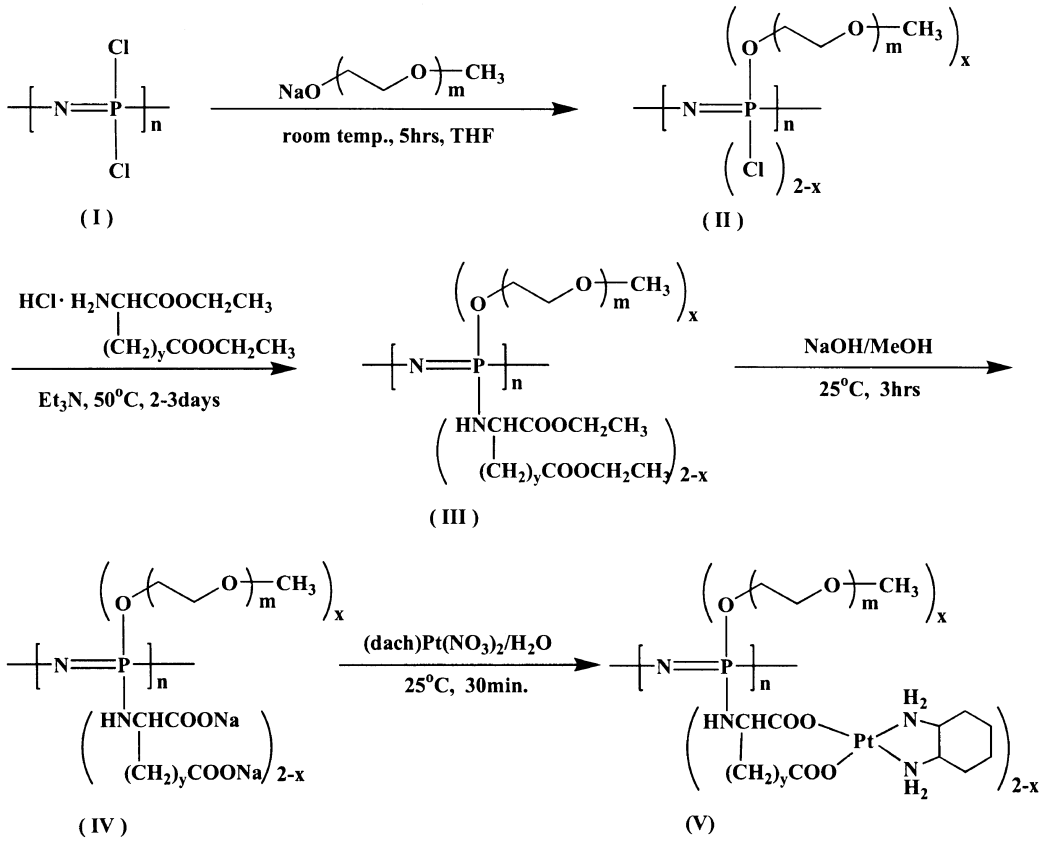
In Vitro Hydrolytic Studies

Time-dependent degradation of the polymer conjugates was examined in buffer solutions at different pHs and temperatures. Polymer conjugates (80 mg) were dissolved in 2 mL of buffer solutions (0.5 M, acetate pH 5, Tris pH 7.4, and carbonate pH 10) and incubated in a water bath at different temperatures (5, 37, and 50°C). Time-dependent hydrolytic behavior of the conjugates was monitored by GPC.

Antitumor Activity

For *in vitro* assay against the murine leukemia L1210 cell line, cells maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum were adjusted to 1×10^6 cells mL^{-1} and distributed to 24 well tissue culture plates (0.5 mL well^{-1}). Test compounds were serially diluted and added to wells (0.5 mL well^{-1}). Following 48 h incubation in a 5% CO_2 atmosphere at 37°C, cell counts were determined with a Coulter Model ZM cell count. Cell growth in the presence of polymeric conjugates was expressed as a percentage of growth in untreated control wells and the concentration of platinum in the compound producing 50% inhibition of cell growth was determined (ED_{50}).

In vivo assay was carried out using the ascite cell form of L1210 lymphoid leukemia obtained from BDA/2 donor mice bearing 3- to 5-day tumor growth. L1210 leukemia cells (10^6) were inoculated *i.p.* in BDF mice (6—8 week old, 20—25 g; 8 mice per group), and 24 h later, compounds were administered *i.p.* on days 1, 5,



m = 2 (DEG), 7 (MPEG350), 16 (MPEG750)

x = 0 ~ 2

y = 1 (Asp), 2 (Glu)

Scheme 1.

and 9. Mortality was recorded and mean survival time was calculated for each group. *In vivo* activity of the polymeric conjugates was expressed as survival effect (T/C), with T as mean survival time of the drug treated mice and C that of control mice.

RESULTS AND DISCUSSION

Synthesis and Characterization

Polyphosphazene/MPEG/(diamine)platinum(II) conjugates were synthesized according to the reaction Scheme 1. Poly(organophosphazenes) (**III**) bearing MPEG and L-aspartic acid ethyl ester as side groups were prepared by two step reactions: Poly(dichlorophosphazene) was reacted with sodium salt of MPEG, and then the remaining chlorine atoms were substituted by L-aspartic acid ester. The mole ratio of MPEG to L-aspartic acid ester in polymer (**III**) was estimated from the integration ratio of the methyl protons of aspartic acid ethyl ester to the methoxy protons of MPEG appearing at 1.3 and 3.4 ppm, repectively and found to be in the range of 0.4/0.6—0.46/0.54, as shown in Figure 1(a). The ester group of L-aspartatic acid in the polymer was completely removed by treatment with NaOH in methanol resulting in polymer (**IV**), which was confirmed by the ^{1}H NMR spectra of poly(organophosphazenes) (**III**) and (**IV**) as shown in Figure 1. The signal of methyl protons at 1.3 ppm of L-aspartatic acid ethyl ester disappeared and the resonance of β-methylene protons of L-aspartatic acid appearing at 2.96 ppm shifted to 2.6 ppm. For introducing (diamine)platinum(II) anticancer drug, (dach)Pt$(NO_3)_2$ was reacted with poly(organophospazene) (**IV**) in aqueous solution to obtain polyphosphazene/MPEG/(diamine)platinum(II) conjugates. Complexation of platinum moiety to the dicarboxylate ligands on the polymer back-bone was usually resulted in 70% to 80% yield. A typical ^{1}H NMR spectrum of polyphosphazene/MPEG/(diamine)platinum(II) conjugate is shown in Figure 1(c). The resonance of cyclohexane protons of the carrier amine ligand coordinated to platinum appeared in the range of 1.1—2.8 ppm and that of methoxy protons of MPEG at 3.43 ppm.

The ^{31}P NMR spectrum of polyphosphazene derivative (**III**) in Figure 2(a) showed one single sharp peak at 19.4 ppm, which indicates that the two chlorine atoms of poly(dichlorophosphazene) were completely substituted stepwise by MPEG and L-aspartic acid ethyl ester. On the other hand, a broad peak was observed in the polyphosphazene derivative with MPEG and L-glutamic acid ethyl ester(spectrum not shown). It appears that the chlorine atoms of poly(dichlorophosphazene) may not have been completely replaced during the second substitution reaction by L-glutamic acid ethyl

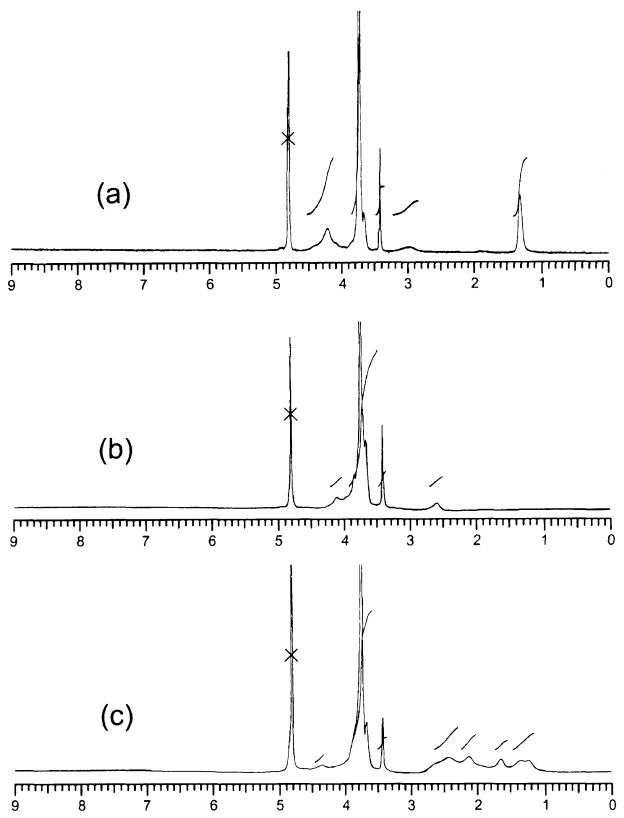


Figure 1. ^{1}H NMR spectra of polyphosphazene derivatives: (**III**) (a), (**IV**) (b), and (**V**) (c).

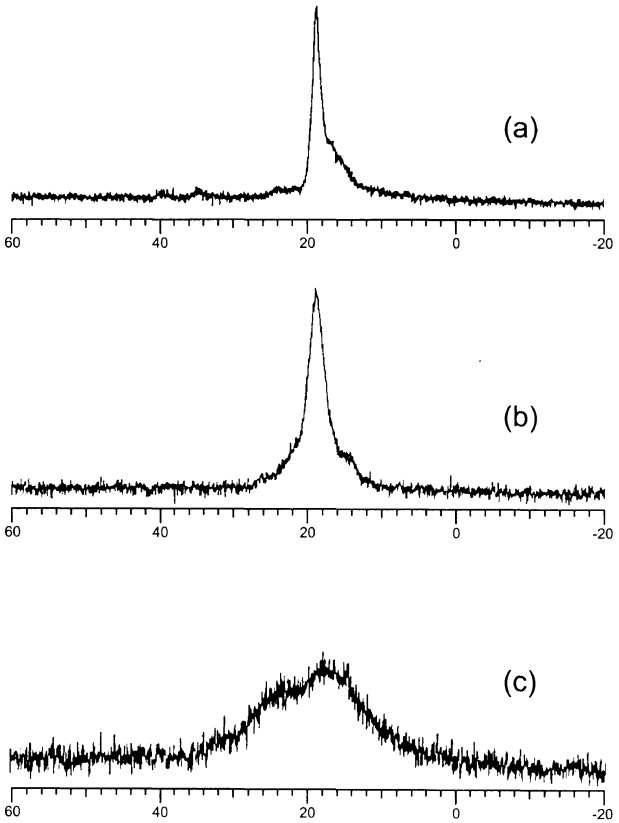


Figure 2. ^{31}P NMR spectra of polyphosphazene derivatives: (**III**) (a), (**IV**) (b), and (**V**) (c).

ester because of its bulkiness, and the residual chlorine was hydrolyzed by water during purification process. From the results of elemental analysis, the mole fractions of the hydroxyl group in the polymeric conjugates **3**, **4**, and **5** were estimated to be 0.13, 0.12, and 0.16, respectively. The ^{31}P NMR spectra of the polyphosphazene derivatives exhibited different spectral patterns before and after introduction of the platinum moiety. Polyphosphazenes (**III**) and (**IV**) exhibited relatively sharp single resonance with chemical shift at 19.4 ppm whereas polyphosphazene (**VI**) showed broad resonance, as shown in Figure 2. Such results seem due to variety of coordination mode of platinum moiety, degradation of polyphosphazene back-bone during introduction of platinum moiety, and shielding effect by bulkier platinum moiety. Previously, we studied by ^{195}Pt NMR the coordination mode of (diamine)platimnum(II) conjugated to a polyphophazene bearing L-glutamate ligand as a spacer, and it was suggested that the (diamine)-platinum moiety was conjugated through (*O*,*O*) coordination mode to the dicarboxylate group of the glutamate linked to polyphosphazene, but the detached (diamine)platinum glutamate underwent (*O*,*N*) isomerization.[20] The coordination mode of the (diamine)platinum moiety is probably the same in the present polymeric conjugates because the same dicarboxylate spacer was used. The chemical environment of phosphorus atoms can be changed by degradation of the polymeric conjugates, which is probably one reason for broadness of the ^{31}P NMR resonance.

In order to study the relationship between polymer structure and activity, different polymeric conjugates were synthesized using different molecular weight MPEG and poly(dichlorophosphazene) as well as different kinds of dicarboxylate spacers. The characteristics of the polymeric conjugates are listed in Table I. All the polyphosphazene/MPEG/(diamine)platinum(II) conjugates were obtained as yellow powders which are soluble in water and polar organic solvents such as methanol, *N*,*N*-dimethylformamide (DMF) and dimethyl sulfoxide (DMSO), but insoluble in most other organic solvents.

Hydrolytic Properties

Hydrolytic properties of the present polymeric conjugates were investigated in buffer solutions at different pHs and temperatures by means of GPC. Figure 3 shows the time-dependent molecular weight decrease of polymeric conjugates **2** and **4** in different buffer solutions (acetate pH 5, tris pH 7.4, carbonate pH 10) at 37°C. The hydrolysis rate of polymeric conjugate **2** was dependent on pH of the buffer solutions, and the rate of molecular weight decrease was in the order of pH 5 > 7 > 10. There are two possibilities for molecular weight decrease of the present polymeric conjugates, that is, cleavage of polyphosphazene back-bone or dissociation of the (diamine)platinum moiety from the polymer back-bone. The hydrolytic degradation of poly(organphosphazenes) bearing amino acid esters as a side group has been explained in terms of carboxylic acid catalyzed degradation.[25,26] It has been proposed that the carboxylic acid group generated by hydrolysis of the pendant ester group may attack the phosphorus atom of the polymer back-bone resulting in back-bone cleavage, and the final products of hydrolysis are ammonia,

Table I. Characteristics of polyphosphazene-(diamine)platinum conjugates

Compound	Formula[a]	M_w ($\times 10^3$)[b]	PDI[b]	Elemental analysis	% C	% H	% N	% P	% Pt
1	$[NP(DEG)_{0.93}(L\text{-}Asp\cdot Pt(DACH))_{0.86}(L\text{-}Asp\cdot Na_2)_{0.21}]_n$	3.8	1.55	Calcd	30.01	4.92	9.41	5.49	29.75
				Found	29.07	4.69	9.87	5.32	28.35
2	$[NP(MPEG350)_{0.82}(L\text{-}Asp\cdot Pt(DACH))_{0.88}(L\text{-}Asp\cdot Na_2)_{0.30}]_n$	11.6	1.27	Calcd	35.19	5.76	7.25	4.07	22.56
				Found	34.02	5.42	7.61	4.25	21.93
3	$[NP(DEG)_{0.82}(L\text{-}Glu\cdot Pt(DACH))_{0.78}(L\text{-}Glu\cdot Na_2)_{0.27}(OH)_{0.13}]_n$	5.1	1.43	Calcd	31.35	5.33	9.40	5.76	27.87
				Found	32.31	5.47	9.27	5.81	27.41
4	$[NP(MPEG350)_{0.81}(L\text{-}Glu\cdot Pt(DACH))_{0.85}(L\text{-}Glu\cdot Na_2)_{0.22}(OH)_{0.12}]_n$	13.5	1.31	Calcd	36.20	4.65	7.34	3.83	23.11
				Found	36.19	4.59	7.41	3.71	24.70
5	$[NP(MPEG750)_{0.92}(L\text{-}Glu\cdot Pt(DACH))_{0.67}(L\text{-}Glu\cdot Na_2)_{0.25}(OH)_{0.16}]_n$	9.8	1.39	Calcd	43.31	6.62	4.46	2.74	12.09
				Found	42.27	6.72	4.51	2.69	12.60

[a] Compositions of compounds were determined by elemental analysis and ^1H NMR. [b] Molecular weight and polydispersity index (PDI) were measured by GPC.

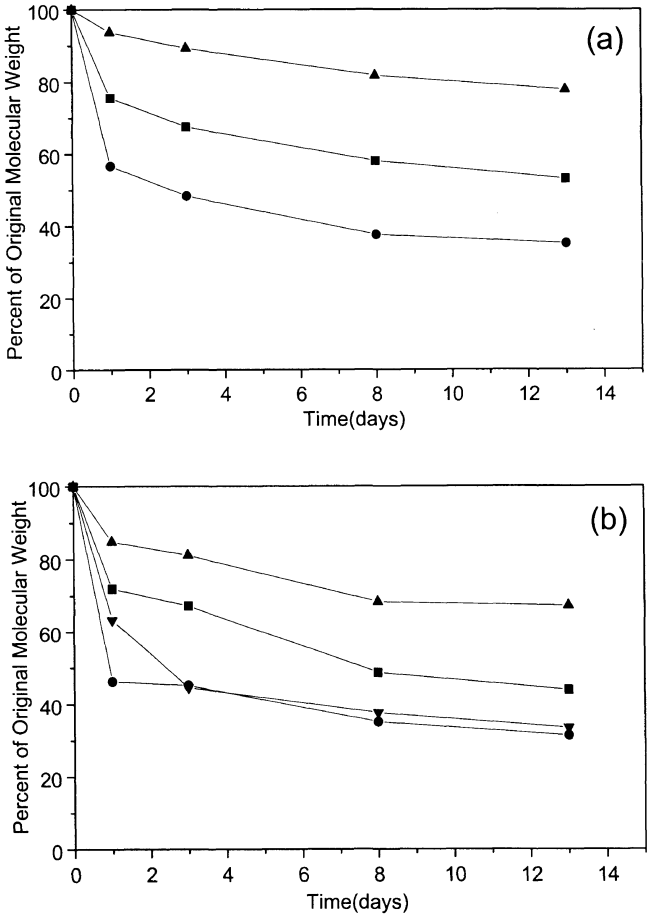


Figure 3. Time-dependent molecular weight decrease of polymeric conjugates **2** (a) and **4** (b) in different buffer solutions (0.5 M, acetate pH 5 (●), Tris pH 7.4 (■), and carbonate pH 10 (▲)) at 37°C, and of polymeric conjugate **5** in Tris pH 7.4 at 37°C (▼).

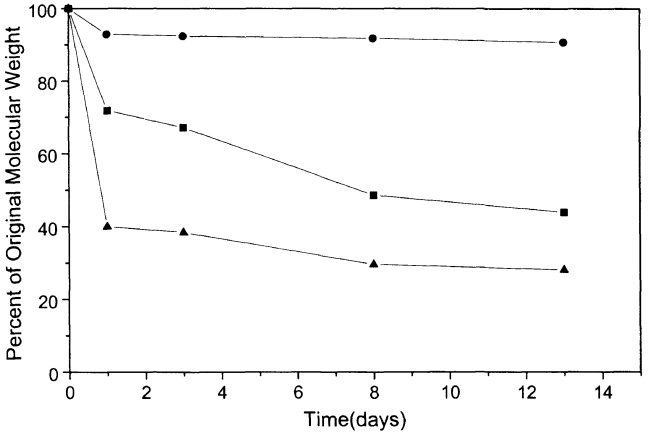


Figure 4. Time-dependent molecular weight decrease of polymeric conjugate **2** in tris buffer solution with pH 7.4 at different solution temperatures (5°C (●), 37°C (■), and 50°C (▲)).

phosphates and corresponding substituents. It was also pointed out that the poly(organophosphazenes) with MPEG and amino acid esters were degraded by the same mechanism and the rate of hydrolysis of the polymer was dependent on the number of the free carboxylic acid moieties.[18] Now the afore-mentioned observation that the molecular weight of the polymeric conjugate **2** decreased rapidly with decreasing pH of the buffer solutions may be explained by the same acid catalyzed degradation mechanism. The number of free carboxylic acid moieties generated by hydrolysis of the dicarboxylate spacer may increase with decreasing pH of the buffer solution, and therefore, the free carboxylic acid catalyzes the cleavage of the polymer back bone. The release of (diamine)platinum moieties conjugated to the polyphosphazene back-bone through a dicarboxylate spacer was studied in our previous work, which showed that the platinum moieties released from the polymer back-bone in a controlled rate and the rate was faster both in the acidic and basic buffer solutions than in the neutral.[21] Consequently, the molecular weight decrease of the polymeric conjugates seems mainly due to cleavage of the polyphosphazene back-bone. The pH-dependent molecular weight decrease of the polymeric conjugate **4** was similar to that of the polymeric conjugate **2**, and difference between the polymeric conjugates **2** and **4** in the rate of degradation was not significant. From these results, it may be presumed that anticancer (diamine)-platinum(II) moieties are released mainly in a complex form chelated by the dicarboxylate spacer.

The effect of solution temperature on the degradation of the polymeric conjugate **2** is shown in Figure 4. Increase of the solution temperature greatly accelerated degradation as reflected by the rapid molecular weight decrease of the conjugate **2** at 50°C, whereas the same conjugate was rather resistant to hydrolysis at 5°C.

Table II. *In vitro* and *in vivo* activity against the leukemia L1210 cell line of polyphosphazene-(diamine)platinum conjugates

Compound	*In vitro* ED_{50} (μM)[a]	*In vivo* (*i.p.*)	
		Dose/mg kg^{-1}	T/C/%
1	4.4	60	146
		30	138
2	2.0	60	196
		30	232
3	1.7	60	104
		30	154
4	0.7	60	103
		30	>222
5	1.0	60	155
		40	148
		20	135
Cisplatin	0.7	4	163
Carboplatin	5.9	40	168

[a] Concentration of platinum.

Antitumor Activity

Antitumor activity of all polyphosphazene/MPEG/(diamine)platinum(II) conjugates was estimated *in vitro* and *in vivo* using the murine leukemia L1210 cell line and the results are listed in Table II. The *in vitro* antitumor actitvity of the polymeric conjugates was comparable to or less cytotoxic than cisplatin, but more cytotoxic than carboplatin. The *in vivo* antitumor activity of the polymeric conjugates was dependent on the structures of the conjugates and, particularly, MPEG chain length in the polymeric conjugates greatly affected antitumor activity. Polymeric conjugates **2** and **4** with MPEG350 exhibited higher *in vivo* activity than cisplatin and carboplatin, whereas conjugates **1** and **3** with DEG and **5** with MPEG750 showed less *in vivo* antitumor activity. The low activity of the conjugate with the longer pendant MPEG750 seems due to its high hydrophilicity and/or faster degradation than those of polymeric conjugates **2** and **4** (data not shown), because the more hydrophilic molecule is less permeable to cell membrane and rapid degradation may cause decrease in the plasma half-life. On the other hand, the reason for low activity of conjugates **1** and **3** is not clear, but their low molecular weight which may give rise to decrease of their plasma half-life could be the reason. Differences in the activity between the conjugates of aspartate and glutamate as spacers for (diamine)platinum(II) were not significant.

CONCLUSIONS

Anticancer (diamine)platinum(II) moiety was successfully conjugated to poly(organophosphazene) bearing MPEG through a dicarboxylate spacer. The hydrolytic degradation of polymeric conjugates was affected by pH and temperature of the polymer solution, and lower pH and higher temperature gave rise to faster degradation. *In vivo* antitumor activity was dependent on the structures of the conjugates; conjugates **2** and **4** with MPEG350 exhibited higher activity than cisplatin and carboplatin, possibly due to the controlled release of the platinum moiety from the polymer back-bone.

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