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Synthesis and Drug-Releasing Behavior of Various Polymeric Prodrugs of PGE1 with PEG and Its Derivative as Polymer Carriers

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Abstract

Two polymeric prodrugs of PGE1 (prodrugs IVg and PNg) were newly synthesized. The drug conjugation proceeded in quantitative yield without decomposition of PGE1 to PGA1. With two types conjugates, PEG-PGE1 and PN-PGE1 with different spacer groups, we first discovered a possibility of slow release of PGE1 in blood circulatory system. PGE1 is conjugated with PEG and PN through the long alkylene spacers, and their availability as polymeric prodrugs is evaluated. Their drug-releasing behavior was examined both in phosphate buffer (pH=7.4) and rat plasma. Each prodrug was known to be highly stabile in the buffer solution. The drug-releasing rate became much faster in rat plasma than in the buffer solution due to the acceleration by the plasma enzymes. The drug-release was found to reach a plateau in rat plasma because the released PGE1 or its derivatives may be captured or decomposed by the plasma proteins. The slower drug-releasing rate of prodrug PNg in rat plasma is reasonably attributed to the molecular aggregation due to the hydrophobic bonding between the PGE1 moieties and spacers.

Key words : decomposition, blood circulatory system, drug-releasing, prostaglandin E1, prodrug, hydrophobic

I. INTRODUCTION

Prostaglandin E1(PGE1) has been noted as and effective pharmaceutical for medical treatment of various vascular disorders because of its excellent physiological activities such as antiplatelet aggregation and vasodilator effect [1]. However, it is extremely unstable and readily decomposes to PGA1 or PGB1 in contact with even weak acid, base and heat. Moreover, PGE1 is readily deactivated in blood, and its half-life time in blood is only 4 min [2]. Accordingly, a relatively large amount of PGE1 must be dosed to keep its effects for a certain period in spite of its high pharmacological potency. These dosage problems sometimes cause serious side effects to narrow the therapeutic use of PGE1. Until now, several drug delivery systems (DDS) have been developed for improving the dosage problems for PGE1. The most familiar and widely adopted is the use of lipo-PGE1 in which PGE1 is incorporated into lipid microspheres (LM) as liposome [3]. Inclusion of PGE1 in α-cyclodextrin (CD-PGE1) [4, 5] and PGE1-heparin conjugate [6, 7] are also used to stabilize PGE1 in blood circulatory system. A polymeric prodrug of PGE1 having a galactose residue for the cell-specific hepatic targeting has also been reported [8, 9]. However, none of these methods is effective for realizing the slow-release of PGE1 in blood circulatory system because of the easy decomposition of the PGE1 residue during the preparation and circulation of the prodrugs. Only lipo-PGE1 and CD-PGE1 have now gained for the therapeutic applications in spite of their less effectiveness. Therefore, it has been needed to develop a new method for conjugation PGE1 with appropriate carriers in which the reaction proceeds at mild conditions in quantitative conversion. It has also been requested to discover a new prodrug that can allow slow release of PGE1 in the blood circulatory system. Surprisingly, there has been no report thus far in regard to the polymeric prodrug of PGE1 using poly(ethylene glycol) (PEG) and its polyether derivatives as the polymer carriers, although they are the most widely as the used drug carriers. Since PEG has only two functional groups on the terminals, the amount of the pharmaceuticals attached to PEG is quite limited. However, for the pharmaceuticals with high potency such as PGE1 the highly biocompatibile PEG can be a good polymer carrier because the therapeutic effect can be gained with such a small quantity.

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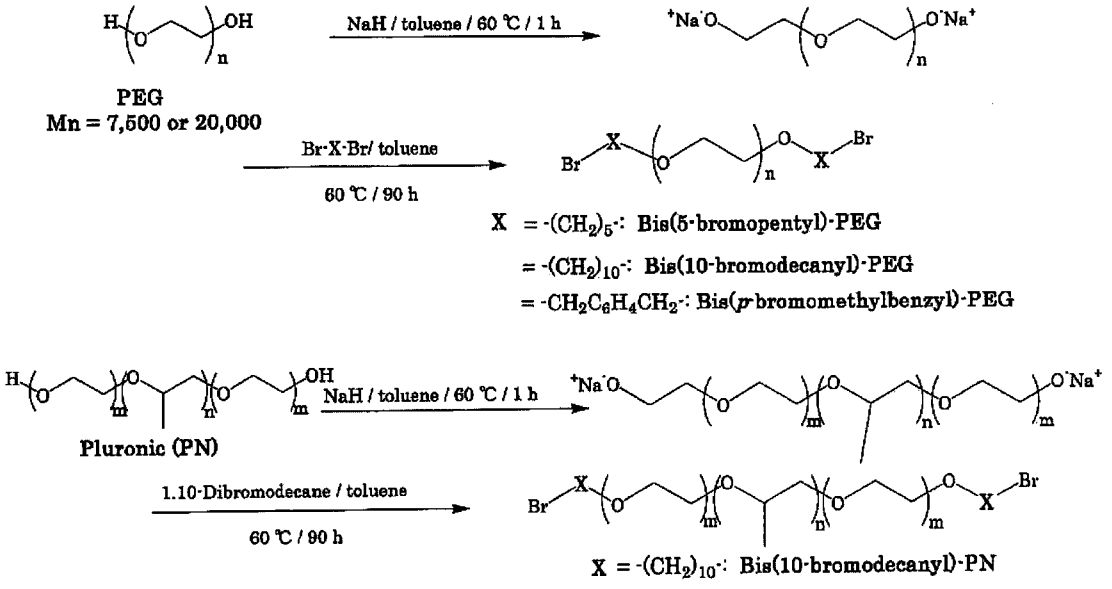


Scheme 1. Syntheses of bromo-terminated PEG and PN.

In this study, we synthesized new polymeric prodrugs of PGE1 by conjugation with PEG and its derivative [PN: a triblock copolymer poly(oxymethylene)-block-poly(oxypropylene)-block-poly(oxyethylene)(Pluronic F-108^{TM})] to demonstrate their drug-releasing behavior. With two types conjugates, PEG-PGE1 and PN-PGE1 with different spacer groups, we first discovered a possibility of slow release of PGE1 in blood circulatory system.

II. EXPERIMENTAL

A. Materials

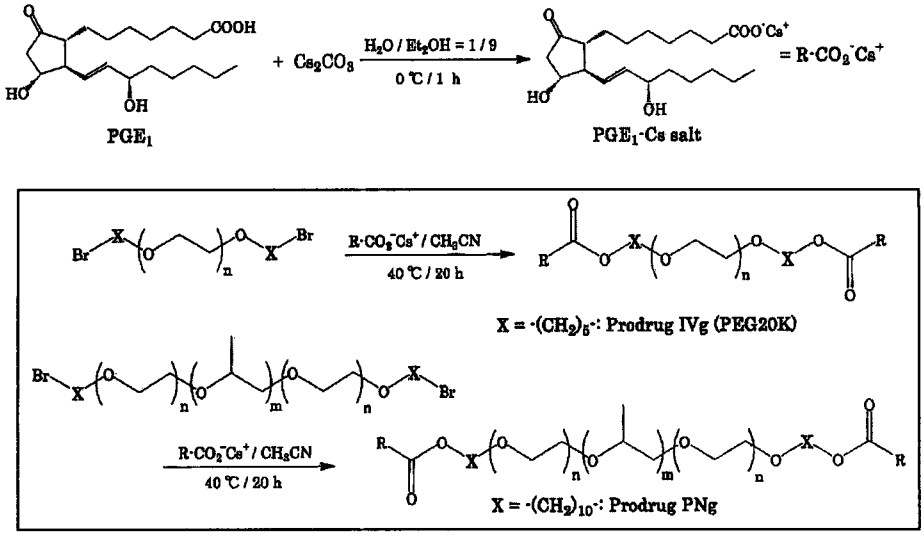
PEG with a number average molecular weight (Mn) of 20,000 was purchased from Wako Pure Chemical Industry (Osaka, Japan). Its molecular weight distribution in weight (Mw)/number (Mn) average molecular weight was 1.06. This PEG is abbreviated as PEG20K where the number represents their Mn value in kDa. Cesium carbonate (Cs_2CO_3), 1.5-dibromopentane, 1,10-dibromodecane, sodium hydride (NaH), magnesium chloride hexahydrate ($MaCl_2 \cdot 6H_2O$), and trifluoroacetic anhydride (TFAA) were of reagent grade and used as received. Toluene, benzene and acetonitrile were purified by distillation. Pyrene was purified by recrystallization from ethanol. Distilled water was further purified by filtration using a Milipore Milli Q system. PN with an oxyethylene/oxypropylene unit ratio of 20/80 was supplied by Asahi Denka Kogyo (Tokyo, Japan). Its Mn and Mw/Mn were 15,500 Da and 1.06, respectively. PGE1 and PGA1 as well as rat plasma were supplied by Ono Pharmaceutical Industry (Osaka, Japan).

B. Measurements

^{1}H-NMR spectra were recorded in $CDCl_3$ with tetramethylsilane (TMS) as the internal standard on a Brucker ARX 500 spectrometer operated at 500 MHz. Gel permeation chromatography (GPC) was performed on an analyzer composed of a Shimadzu LC-10AS pump, a Shimadzu RID-10A refractive index detector, a Shimadzu CTO-10A column oven (35 ℃), and a Shimadzu C-R4A chromatopac data processor. A Toso TSK gel G4500PWXL column was used together with a guard column, and the eluent was 0.02 M phosphate buffer (pH=7.4) containing 5% acetonitrile. The molecular weight was calibrated with the PEG standards. High performance liquid chromatography (HPLC) was performed on an analyzer composed of a Shimadzu LC-10AS pump, a Shimadzu SPD-10A UV-VIS detector (205 nm), a Shimadzu CTO-10A column oven (35℃), a Shimadzu C-R4A chromatopac data processor. A Nacalai PG10C18-500 reveraed-phase column was used, and the eluent was 0.02 M phosphate buffer (pH=5.0) containing 26 vol% acetonitrile.

C. Syntheses of Bromo-Terminated PEG

The reaction procedures are summarized in Scheme 1, where all the opreations were done in a nitrogen atmosphere unless otherwise noted. A typical example is as follows: a 2.0 g amount of PEG7.5K (0.34 mmol) was dissolved in a small volume of benzene and subjected to the lyophilization to



Scheme 2. Syntheses of various types of PGE1-conjugated prodrugs.

remove water. The lyophilized PEG was re-dissolved in 20 ml toluene and a 81 mg amount of (3.4 mmol) NaH suspended in 10 ml toluene was added to it at 0 ℃ under nitrogen. Then, the system was stirred at 60℃ for 1 h to complete the terminal alkoxide formation of PEG and cooled again to 0℃. To this system was added 7.7 g of 1,5-dibromopentane. After the addition, the mixture was stirred at 60℃ for 90 h. The resultant solution was filtered to remove the inorganic by-products, and the filtrate was reprecipitated into a large exess of diethyl ether to isolate the PEG products. The precipitates were filtered and dried under vacuum (71%).

Table 1. Chemical shift value of prodrugs IVg and PNg in $CDCl_3$

Proton	Coupling	Prodrugs(ppm)	
		IVg(1)	PNg(2)
CH$_3$ for PGE1	t	0.90	0.90
$CH_2CH(CH_3)O$ for PN	d		1.14
$-COOCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2O-$	m		1.28
CH$_2$ for PGE_1	q or m	1.31	1.31
$-COOCH_2CH_2CH_2CH_2CH_2O-$	m	1.31	
$-COOCH_2CH_2CH_2CH_2CH_2O-$	m	1.40	
$-COOCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2O-$	m		1.41
$-COOCH_2CH_2CH_2CH_2CH_2O-$	m	1.52	
CH$_2$ for PGE_1	m	1.62	1.59
$COCH_2CH(OH)$ for PGE_1	d	2.02	2.02
$-CH_2COO-$ for PGE_1	t	2.27	2.28
$COCH(CH)CH_2$ for PGE_1	q	2.64	2.64
$CH_2CH(CH_3)O$ for PN	m		3.40
$-COOCH_2CH_2CH_2CH_2CH_2O-$	t	3.49	
$CH_2CH(CH_3)O$ for PN	m		3.55
CH_2CH_2O for PEG	m	3.64	3.65
$-COOCH_2CH_2CH_2CH_2CH_2O-$	t	4.05	
$-COOCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2O-$	t		4.05
$-CH(OH)-$ for PGE_1	m	4.09	4.09
CH for PGE_1	t	5.72	5.72

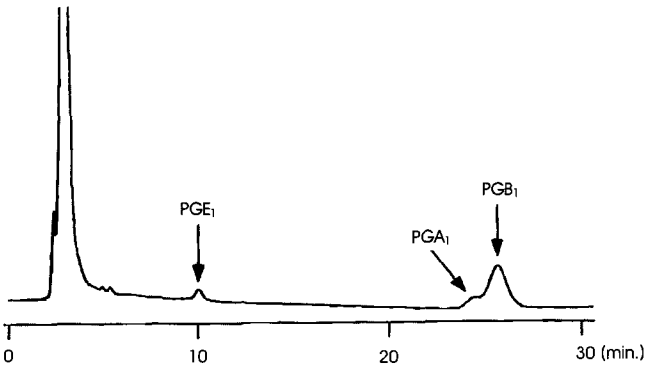


Fig. 1. A typical HPLC curve of the buffer solution of prodrug PNg after incubation in phosphate buffer for 130 h.

D. Syntheses of PEG-PGE1 Conjugates

The procedure (Scheme 2) was almost identical to that used for preparing the PEG-ibuprofen conjugates. The PGE1-Cs salt was prepared by the reaction of PGE1 (5.6×10^{-2} mmol) and cesium carbonate (0.24 mmol) in 1 ml of a water/ethanol (1/9 in vol.). The salt was less soluble in acetonitrile than the ibuprofen-Cs salt, and the coupling reaction with the bromo-terminated PEG (1.4×10^{-2} mol) was very slow. Therefore, the coupling reaction time was prolonged to 20 h for completion of the reaction. After the reaction being over, the reaction system was filtered to remove the abundant PGE1-Cs salt, and the filtrate was poured into 50 ml *t*-butanol containing 5 g magnesium chloride hexahydrate. The resultant suspension was stirred at 30 ℃ for 3 h to remove the residual PGE1-Cs salt by adsorption. The suspension was finally evaporated under vacuum, and the residue was treated with a dichloromethane/saturated aqueous NaCl mixture to extract the product in the dechloromethane layer. The separated dichloromethane layer was dried over sodium sulfate, filtered, and evaporated under vacuum. The residue was purified by reprecipitation using a benzene/diethyl ether (solvent/ precipitant) system. The finally obtained product was dried under vacuum.

The PGE1-PEG conjugate derived from the PEG 2 having a pentamethylene spacer is named as prodrug Ⅳg(1, 48%) and that derived from the PN 5 having a decamethylene spacer is named as prodrug PNg(2, 54%). Their ^{1}H-NMR spectra are summarized in Table 1 from which the percent introduction of PGE1 was known to be almost quantitative in each case.

E. Fluorescence Measurements

A stock solution of pyrene was prepared by dissolving a freshly recrystallized pyrene in ethanol in a concentration of 1.0×10^{-4} M. Each prodrug was dissolved in a distilled water to have concentrations of 0.001, 0.005, 0.01, 0.03, 0.05, 0.07, 0.08, 0.1, 0.2, and 0.6w%. A 100 μl portion of the stock solution of pyrene was charged into a reaction vial and dried up under a reduced pressure. Then, a 1 ml volume of the prodrug solution was charged into the vial and incubated at 37 ℃ for 24 h. The concentration of pyrene in the prodrug solution in the vial was constantly 10 μM in this condition. The prodrug solution in the vial was then subjected to the fluorescence measurement using a Shimadzu RF-5300PC spectrofluoro photometer. The fluorescence emission spectrum of pyrene was recorded in a wavelength range of 350~600 nm at an excitation wavelength of 388 nm. The intensities of the first emission band I_1(at 374 nm) and the third one I_3(at 385 nm) were determined and their ratio(I_1/I_3) was used as a measure of the polarity of the microenvironment where the hydrophobic pyrene molecule was located [10].

F. Drug-Releasing Study in Phosphate Buffer

Each of the prodrugs was dissolved in a 0.1 M phosphate buffer (pH = 7.4) to have a PGE1 concentration of 0.2 mg/ml. The solution was then incubated in a bath thermostated at 37 ℃, and a small portion of it was taken for the HPLC analysis at appropriate intervals from 0 to 200 h. Figure 1 shows a typical HPLC curve of the buffer solution of prodrug PNg after incubation for 130 h. In this curve, three peaks are shown around 10, 24, and 25 min in retention time due to the released PGE1, PGA1, and PGB1, respectively. From the area of these peaks, the amounts of the released prostaglandins (PGs) were calculated. The amount of each PG at the 100% releasing was determined by the calibration curve of the respective free PG at the same measurement conditions.

G. Drug-Releasing Study in Rat Plasma

Each prodrug was dissolved in a 0.1 M phosphate buffer (pH = 7.4) and mixed with nine times volume of rat plasma to have a final PGE1 concentration of 0.2 mg/ml. The plasma solution was incubated at 37 ℃ for 7h. A 50 μl portion of this

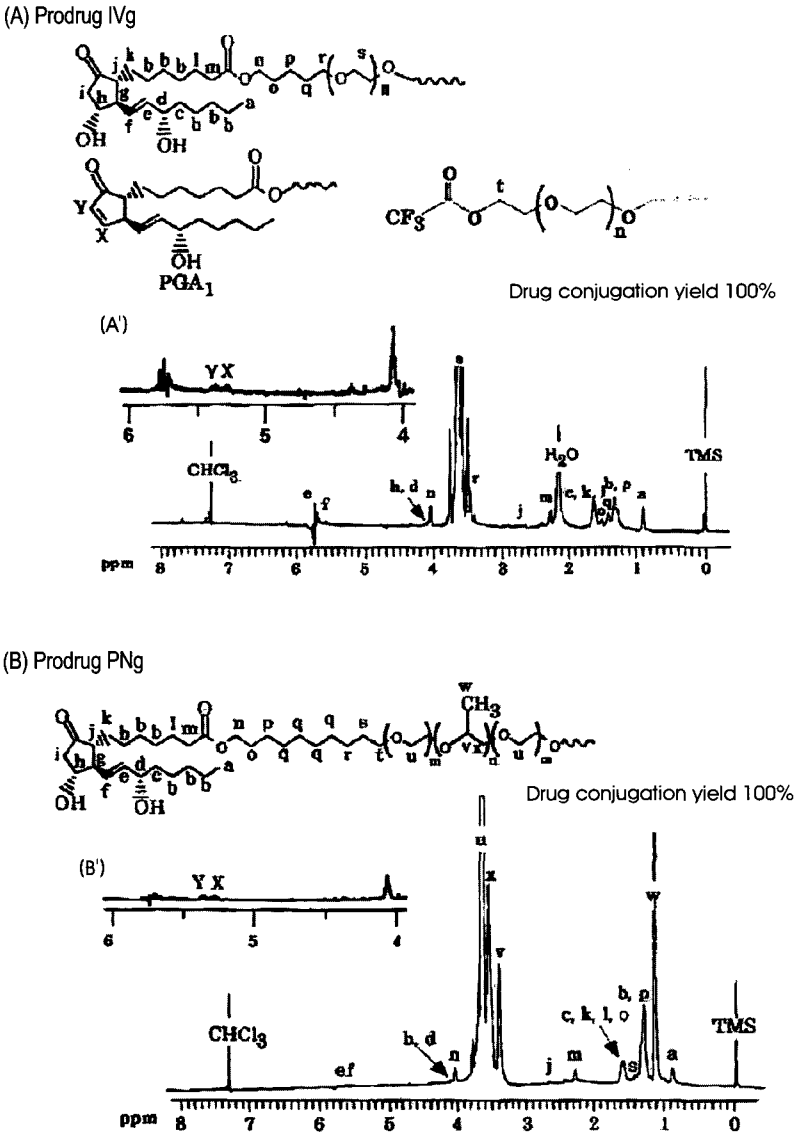


Fig. 2. 500 MHz ^{1}H-NMR spectra of prodrugs IVg (A) and PNg (B). The partial spectra of (A') and (B') are for the corresponding samples treated with TFAn.

plasma solution was taken out from time to time and charged into a 200 μl of ethanol to deactivate the enzymes involved. After ultrafiltration, a small portion of the filtrate was subjected to the HPLC analysis mentioned above.

III. RESULTS AND DISCUSSION

A. Preparation of PGE1-Conjugated Prodrugs

Two polymeric prodrugs of PGE1 (prodrugs IVg and PNg) were newly synthesized according to Scheme 2. Both reactions proceeded under mild conditions. Figure 2 (A) shows the ^{1}H-NMR spectrum of prodrug IVg having pentamethylene spacers. A small signal (n) at δ4.05 ppm is assigned to the oxymethylene groups that are linked with PGE1. It is known that the β-methylene signal of the bromo- terminated PEG at δ 1.85 ppm shifted to δ1.6 ppm in the Figure 2 (A). This fact supports the quantitative conjugation of PGE1 at the terminals of the bromo-terminated PEG. The enlarged spectrum (A') around δ4~6 ppm is for the reaction product with trifluoroacetic anhydride (TFAn). This trifluoroacetylation made no signal to appear around δ4.5 ppm, suggesting that the conjugation reaction proceeded without hydrolysis of the ester groups. The two small signals (X and Y) around δ5.3 ppm in the enlarged spectrum (A') are assigned to the methyne protons on the 5-membered cyclic moiety of PGA1 that was produced by decomposition of PGE1 by the action of TFAn.

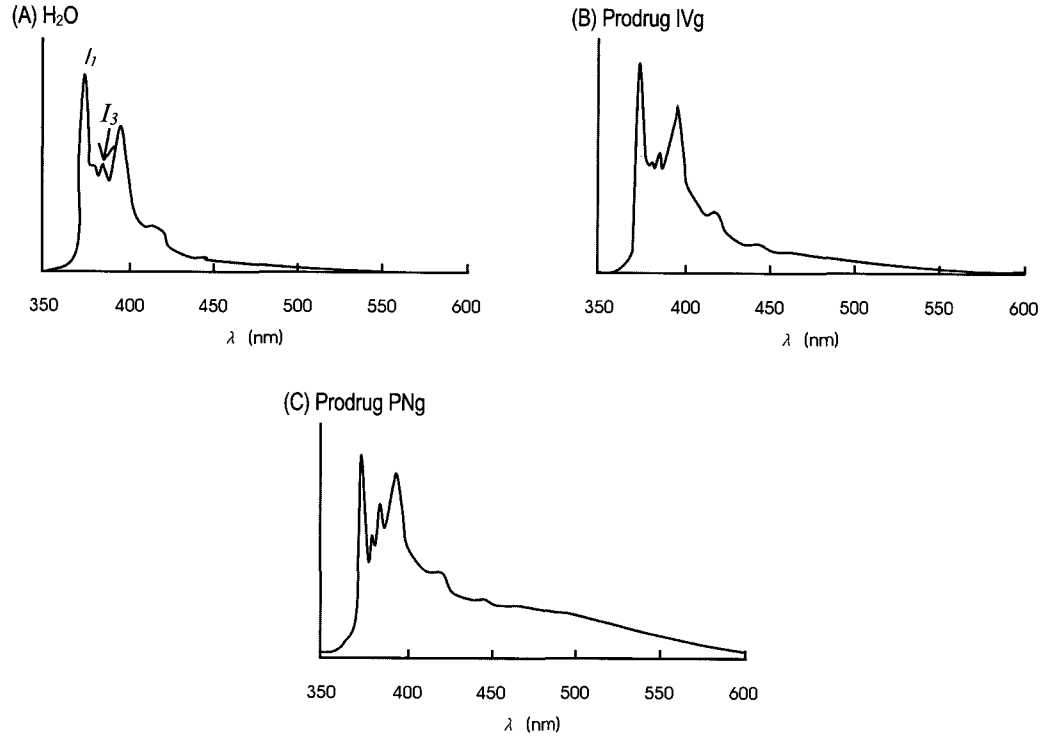


Fig. 3. Fluorescence emission spectra of pyrene solubilized in aqueous solutions of 0.6w% prodrugs; [pyrene] = 10 μM.

Since the original spectrum (A) dose not show any of these signals, it is supported that no decomposition from PGE1 to PGA1 occurred during the conjugation reaction.

Figure 2 (B) shows the ^{1}H-NMR spectrum of prodrug PNg having a PN carrier with a decametylene spacer. The signal assignment shown in the figure supports the quantitative conjugation of PGE1 with no decomposition from PGE1 to PGA1 during the conjugation reaction.

B. Molecular Aggregation of the Prodrugs

Figure 3 shows the fluorescence emission spectra of pyrene in the aqueous solutions of the prodrugs as compared with the spectrum in water. Pyrene has been utilized as a probe (chromophore) for studying the microheterogeneity of such solution systems as micellar [10] and polyamphiphile [11, 12] solutions. At the higher concentrations of pyrene, more than one chromophoric probe molecule can occupy the same micellar particle to likely form an excimer. In fact, the excimer peak was observed at 480 nm in the spectrum of prodrug PNg, but no excimer peak was observed in that of prodrug IVg.

Table 2 summarizes the I_1/I_3 ratio in the aqueous solutions of the prodrugs IVg and PNg as compared with those in water

Table 2. I_1/I_3 Rations of various aqueous solutions [a].

Solute	I_1/I_3
None	1.83
PEG20K	1.82
Pluronic(PN)	1.56
Prodrug IVg	1.72
Prodrug PNg	1.31
NaLS[b]	1.1410
TritonX−100[c]	1.3210
SDS[d]	1.1410

[a] Containing 0.6w% solute and 10 μ M of pyrene.
[b] Sodium lauryl sulfate
[c] Polyoxyethylene octylphenyl ether
[d] Sodium dodecyl sulfate

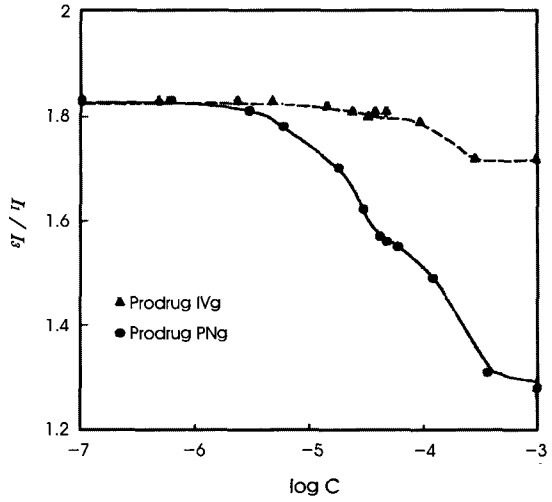


Fig. 4. I_1/I_3 vs. the prodrug concentration (C) for prodrugs IVg and PNg.

and aqueous solutions of the original PEG and PN as well as several ordinary surfactants [10]. The ratio in the aqueous solution of PEG20K was 1.82, while the ratio was 1.56 in the aqueous solution of PN. Therefore, the fully hydrophilic PEG is completely hydrated to form no hydrophobic pocket, while the somewhat hydrophobic PN aggregates by itself through hydrophobic interaction in aqueous environment to form a hydrophobic pocket. In the aqueous solution of prodrug IVg, the ratio was 1.72 being close to the ratio in water. Consequently, it is considered that the prodrug IVg can not form the hydrophobic pocket by the aggregation of the spacers and PGE1 moieties.

Contrarily, in the aqueous solution of prodrug PNg the ratio was 1.31 which is similar to the ratios observed in the typical micellar solutions. Therefore, it is known that prodrug PNg readily aggregates in water to form the hydrophobic pocket. The ratio is, however, slightly higher than those of the corresponding ibuprofen-conjugated prodrug PN, because the hydrophobic nature of PGE1 is lower than that of ibuprofen.

Figure 4 shows the variation of I_1/I_3 as a function of the concentration of prodrugs IVg and PNg (log scale). It was previously reported [13, 14, 15, 16] that pyrene is captured in the hydrophobic pocket above the critical micelle concentration (CMC) of a surfactant to bring a decrease in I_1/I_3. Judging from the curves in Figure 4, it was known that the CMC value of prodrugs IVg and PNg is between 10^{-4} and 10^{-3} M, but that prodrug IVg scarcely forms the hydrophobic environment.

C. Drug-Releasing Behavior of the Prodrugs in Vitro

Prior to subjecting the PGE1 prodrugs to the in vivo test, their stability and drug-releasing behavior were investigated in buffer solution and in rat plasma.

D. Drug-Releasing Behavior in Phosphate Buffer

Drug-releasing behavior of prodrugs IVg and PNg was studied in phosphate buffer (pH = 7.4) at 37 ℃. Figure 5 shows the

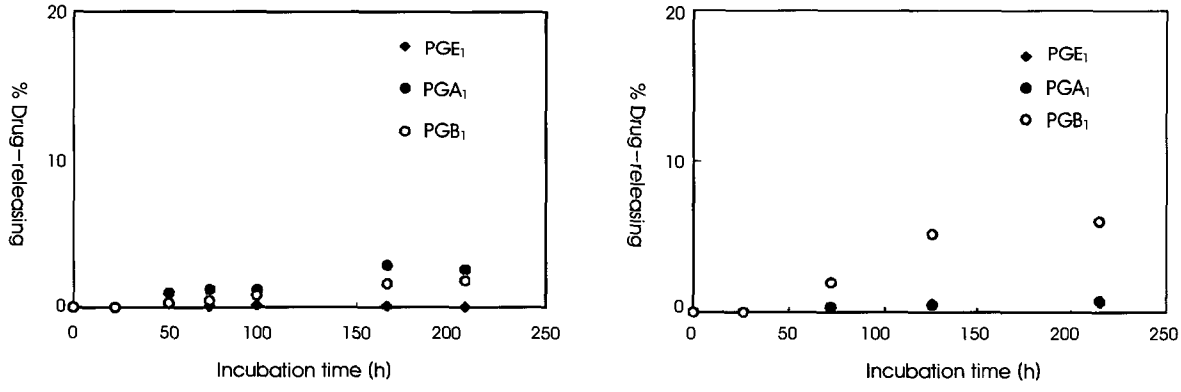


Fig. 5. Drug-releasing profile of PGE1-conjugated prodrugs in phosphate buffer (pH = 7.4) with respect to each PGs.

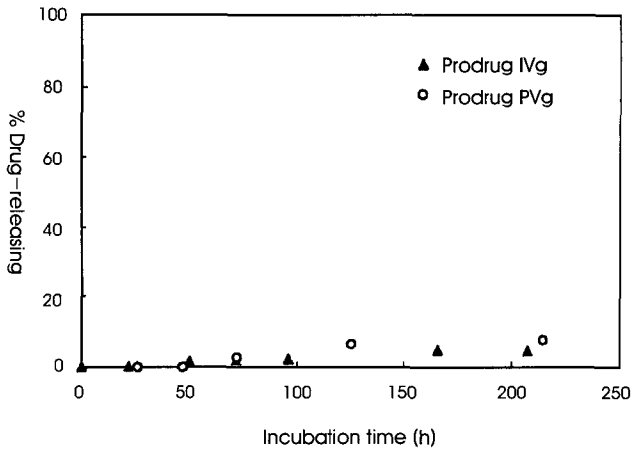


Fig. 6. Overall drug-releasing profiles in total amount of PGE1, PGA1, and PGB1 for prodrugs IVg and PNg in phosphate buffer (pH = 7.4).

typical drug-releasing profiles of both prodrugs. In addition to PGE1, PGA1 and PGB1 were detected because of fast degradation of PGE1. Since the incubation time was too long as compared with the ordinary life-time of PGE1, the ratios of PGA1 and PGB1 relative to PGE1 became larger. Two possibilities are considered for this decomposition. In one possibility, the PGE1 moieties conjugated with polymer carriers are first decomposed to PGA1 and PGB1 and then released. In another possibility, PGE1 released from the prodrugs is decomposed to PGA1 and PGB1. Since the concentration in situ, the decomposition is thought to follow after the PGE1 release.

Figure 6 shows the overall PGE1-releases for both prodrugs IVg and PNg. Each prodrug showed a very slow release. These drug-releasing profiles could be interpreted by the simple first order kinetic analysis assuming the first order rate constant as k1. Table 3 summarizes the k1 values and the half-life times ($t_{1/2}$) of the overall PGE1-release. The values for prodrugs Ig and IIg that were reported before are also involved. The k1 value of prodrug IVg is almost identical to that of ibuprofen-conjugated prodrug IV, while the value of prodrug PNg is about ten times as large as that of ibuprofen-conjugated prodrug PN. On the other hand, the drug-releasing rate of prodrug PNg is slightly faster than that of prodrug IVg, although the aggregation of prodrug PNg is stronger than prodrug IVg as noted in section "molecular aggregation of the prodrugs". Close examination of the releasing profile of profile of prodrug PNg reveals that no release is shown within the initial 50 h where prodrug IVg shows some release. It can therefore be presumed that the release from prodrug PNg is driven as a consequence of destruction of the aggregation. PGE1 having two hydroxyl groups is more hydrophilic than ibuprofen, and the hydrophobic microenvironment involving PGE1 is not so stable to render the linkage more highly susceptible to hydrolysis.

E. Drug-Releasing Behavior in Rat Plasma

In order to verify the therapeutic applicability of the prodrugs IVg and PNg, their drug-releasing behavior was examined in rat plasma. The drug-release was monitored by the increases in concentration of PGA1 and PGB1 in addition to PGE1, because of the immediate decomposition of PGE1 that was also observed in phosphate buffer. The releasing profiles with respect to the three PG derivatives are shown in

Table 3. Rate constants k1 and half-life times($t_{1/2}$) of drug-releasing of prodrugs IVg and PNg in phosphate buffer (pH=7.4).

Prodrug type	$k_1(h^{-1})$	$t_{1/2}(h)$
Ig	$k_1 = 4.9 \times 10^{-3}$	1.4×102 [a]
	$k_2 = 6.3 \times 10^{-3}$	1.1×102 [b]
IIg	3.6×10^{-3}	1.9×10^2
IVg	2.0×10^{-4}	3.4×10^3
PNg	3.8×10^{-4}	1.8×10^3

The concentration : 0.2 mg/ml relative to the amount of PGE_1.
[a] First-stage. [b] Second-stage.

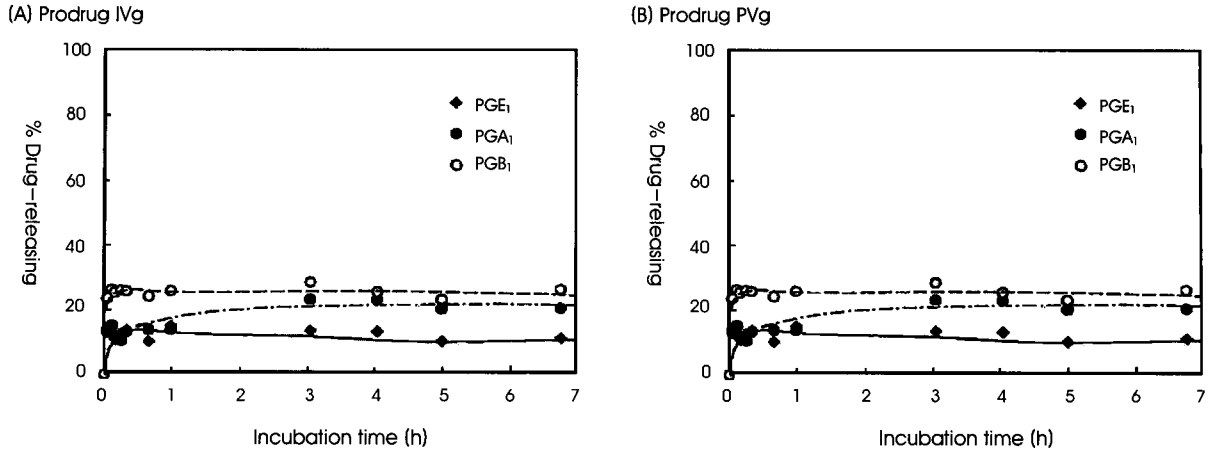


Fig. 7. Drug-releasing profiles of PGE1-conjugated prodrugs in rat plasma with respect to the three PG derivatives.

Figure 7, and the overall releasing profiles of the conjugated PGE1 are shown in Figure 8. The drug-releasing rates for both prodrugs became much faster in rat plasma than in buffer solution because of the action of the hydrolysis enzymes involved in rat plasma. Prodrug IVg exhibited a brust release in the beginning with decomposition from PGE1 to PGA1 and PGB1. The initial drug-release from prodrug PNg, on the other hand, became much slower in spite of the similar decomposition of PGE1. The drug-release from prodrug IVg reached a plateau only 3 min after the incubation when about 50% of the charged drug had been released. The drug-release from prodrug PNg also reached a plateau 30 min after the incubation at a 30% releasing level.

The initial "burst-releasing" was successfully relieved as compared with prodrug IVg. This difference in releasing behavior is also attributed to the difference in the molecular aggregation of the prodrugs that has been described earlier. In prodrug PNg, aggregating strongly, the bonding between the long methylene spacers and PGE1 is stable in the internal domain of the aggregates to receive slow hydrolysis. Their weaker bonding formed in the external domain of the aggregates can receive faster hydrolysis to give the initial fast release.

The maximum percentage of the overall drug-release from prodrugs IVg and PNg were 64% and 38% after 3~4 h, respectively. When an identical amount of the free PGE1 was

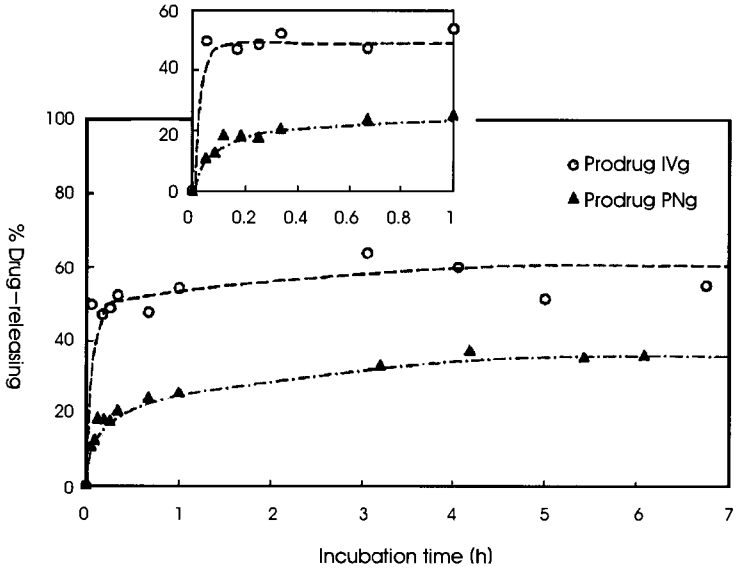


Fig. 8. Overall drug-releasing profiles in total amount of PGE1, PGA1, and PGB1 for prodrugs IVg and PNg in rat plasma. The initial profiles are enlarged in the upper diagram.

incubated in rat plasma at 37 ℃ for 1 h, 30% of the feed PGE1 was lost other than its conversion to PGA1 and PGB1. This fact indicates that PGE1 or its derivatives may be adsorbed by the plasma proteins or further decomposed to unknown products. If this adsorption or decomposition is taken into consideration, the maximum release from prodrug Ⅳg is understandable, but the overall drug-release from prodrug PNg is too smaller than expected. The very slow release noted in the plateau stage of the releasing profile suggests that the remaining conjugated PGE1 is firmly trapped in the hydrophobic pocket and can hardly be attacked by the plasma enzymes. Judging from this excellent retention of PGE1 in the hydrophobic pocket made by the aggregation, prodrug PNg is quite effective for allowing the slow-release of PGE1.

Ⅳ. CONCLUSIONS

Both PGE1-conjugated prodrugs Ⅳg and PNg were synthesized by the nucleopholic substitution of the bromo-treminated PEG and PN with PGE1-Cs salt at mild reaction conditions. The drug conjugation proceeded in quantitative yield without decomposition of PGE1 to PGA1. The I_1/I_3 ratio in the aqueous solutions of the prodrugs Ⅳg and PNg as compared with those in water and aqueous solutions of the original PEG and PN as well as several ordinary surfactants. The ratio in the aqueous solution of PEG20K was 1.82, while the ratio was 1.56 in the aqueous solution of PN. In the aqueous solution of prodrug PNg the ratio was 1.31 which is similar to the ratios observed in the typical micellar solutions. Therefore, it is known that prodrug PNg readily aggregates in water to form the hydrophobic pocket. The variation of I_1/I_3 as a function of the concentration of prodrugs Ⅳg and PNg (log scale). It was that pyrene is captured in the hydrophobic pocket above the critical micelle concentration (CMC) of a surfactant to bring a decrease in I_1/I_3. Judging from the curves it was known that the CMC value of prodrugs Ⅳg and PNg is between 10^{-4} and 10^{-3} M, but that prodrug Ⅳg scarcely forms the hydrophobic environment.

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