Synthesis of Water-Soluble Polymeric Prodrugs Possessing 4-Methylcatechol Derivatives by Mechanochemical Solid-State Copolymerization and Nature of Drug Release

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In this study we synthesized the water-soluble polymeric prodrugs possessing a 4-methylcatechol (4MC) derivative as a side chain by mechanochemical solid-state copolymerization. 1-Benzoyl-4-methylcatechol (Bz4MC) was selected as a model compound of 4MC, and its methacryloyl derivative (1) was synthesized. 6-*O*-Methacryloyl- β -galactose (2) was also prepared as a water-soluble monomer. The mechanochemical solid-state copolymerization of 1 and 2 was carried out to obtain the water-soluble polymeric prodrug possessing the Bz4MC as a side chain. The mechanochemical copolymerization of 1 and 2 proceeded to completion, and the polymeric prodrug produced possessed a narrow molecular weight distribution. Three kinds of polymeric prodrugs, whose compositions were different from one another, were hydrolyzed *in vitro*. The hydrolysis of these polymeric prodrugs proceeded to completion. The rate constants of hydrolysis decreased with increasing the mole fraction of 1 in polymeric prodrug. It was suggested that the rate constant of hydrolysis could be controlled by the composition, the mole fraction of 1 in the polymeric prodrug.

Key words mechanochemical polymerization; polymeric prodrug; nerve growth factor (NGF); 4-methylcatechol; sustained release

Although evidence indicates that nerve growth factor (NGF) may have promise as a therapeutic agent for neurodegenerative diseases like Alzheimer's,^{1,2)} its potential use in such a central nervous system (CNS) disorder is prevented by its inability to cross the blood-brain barrier (BBB). A variety of low molecular weight compounds has been found as a NGF stimulator.^{3–9)} Among them, 4-methylcatechol (4MC) is a potent NGF stimulator both *in vivo*^{10,11)} and *in vitro*.^{12–14)} 4MC, however, could not penetrate into the brain for lacking sufficient lipophilicity. Several dihydropyridine derivatives of 4MC were synthesized as a potential brain selective targetry forms by Kourounakis *et al.*¹⁵⁾ to increase the lipophilicity and the BBB permeability of 4MC.

Major advancements have been made in the understanding of Alzheimer's disease. It has been suggested that the integrity of BBB may be compromised in Alzheimer's disease.^{16,17)} Immunohistological studies have demonstrated the presence of serum proteins in the cerebrovascular amyloid in Alzheimer's patients suggesting abnormal BBB permeability.^{18–20)} Therefore, it is expected that the polymeric prodrugs, which possess 4MC or its derivative as a side chain, can passively be delivered into the diseased part of the brain in Alzheimer's patients. It is also known that 4MC is easily oxidized *in vivo*. The polymeric prodrugs possessing 4MC would be useful in terms of the stabilization and sustained release of 4MC.

We have reported the synthesis and nature of drug release of novel polymeric prodrugs prepared by mechanochemical solid-state polymerization.^{21–33)} Several important conclusions were reached from a series of such studies. The monomers prepared on the basis of the structural criteria derived from quantum chemical considerations underwent facile mechanochemical solid-state polymerizations to give corresponding polymeric prodrugs essentially quantitatively.^{21,22,25)} Thus, this method eliminates the need for any work-up of the reaction mixture. One of the most striking

properties observed in such polymers is that the resulting polymeric prodrugs are of very low heterogeneity (narrow molecular weight distribution), which is of great value in pharmaceuticals for highly functionalized polymeric prodrugs.²²⁾ It was also shown that the resulting copolymer under the appropriate experimental conditions was homogeneous in composition (ideal copolymerization).²⁶⁾ Therefore, the present reactions seem to be applicable to a wide variety of vinyl monomers of an important bioactive compound with different physicochemical properties and provide a novel and simple methodology for syntheses of polymeric prodrugs through a totally dry process.

In order to gain a fundamental insight to develop a polymeric prodrug that can passively be delivered to the disease parts of brain by the peripheral administration and slowly release 4MC derivatives, we synthesized the polymeric prodrugs possessing 4MC derivatives as a side chain by mechanochemical solid-state polymerization. Thus, 1-benzoyl-4-methylcatechol (Bz4MC) was selected as a model compound of 4MC derivatives. The mechanochemical solid-state polymerization of methacryloyl derivatives of Bz4MC and water-soluble monomers, 6-*O*-methacryloyl- β -galactose, was carried out to obtain water-soluble polymeric prodrugs. The nature of drug release from the polymeric prodrugs formed was examined *in vitro*.

Experimental

Ultraviolet spectral measurements were performed with a Shimadzu Recording Spectrophotometer UV-3100 using 1-cm quartz cells. ¹H-NMR spectra were run on a JEOL JNM-GX270 FT-NMR spectrometer using tetramethylsilane as an internal or external standard. Reading of pH was carried out on a TOA pH meter HM-16S at room temperature. Melting points were measured on a capillary melting-point apparatus and uncorrected. IR spectra were obtained on a Perkin Elmer 1650 FT-IR spectrometer. MS spectra were obtained on a JEOL JMS-SX102A mass spectrometer.

Materials Acrylamide supplied by Tokyo Kasei Kogyo Co., Ltd. (Japan) was commercially available. It was purified by recrystallization from benzene and dried *in vacuo*. 2-Methacryloyloxyethyl isocyanate, Bz4MC

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and 1,2:3,4-di-*O*-isopropylidene- α -D-galactopyranose were synthesized according to the literatures,^{31,34,35} respectively.

1-Benzoyl-2-methacryloyloxyethylcarbamoyl-4-methylcatechol (1) 2-Methacryloyloxyethylisocyanate (0.40 g, 2.6 mmol) was added to a solution of Bz4MC (0.50 g, 2.2 mmol) and triethylamine (0.26 g, 2.6 mmol) in dry acetonitrile (15 ml). The reaction mixture was stirred at room temperature overnight. The reaction mixture was filtered and evaporated *in vacuo*. The residue was dissolved in CHCl_3 (30 ml). The CHCl_3 solution was washed fully with H_2O , dried over MgSO_4 , and evaporated *in vacuo*. The residue was chromatographed over silica gel (Wakogel C300) in CHCl_3 as an eluent. The eluted product was further recrystallized from cyclohexane and cyclohexanone (1 : 1) to yield 0.20 g (24%) of **1**: mp 69–70 °C. IR (KBr) cm^{-1} : 3315 (–CONH–), 1741, 1708 (–COO–), 1637 (vinyl group). $^1\text{H-NMR}$ (CDCl_3) δ : 1.88 (3H, s, – CH_3 of methacryloyl group), 2.37 (3H, s, – CH_3 of 4-methylcatechol), 3.42–3.50 (2H, m, – CH_2OCO –), 4.10–4.18 (2H, m, – NHCH_2 –), 5.54 (1H, s, vinyl group), 6.03 (1H, s, vinyl group), 7.05–7.17 (3H, m, aromatic ring of 4-methylcatechol), 7.45–7.67 (3H, m, *meta*- and *para*-H of benzoyl group), 8.16–8.18 (2H, m, *ortho*-H of benzoyl group). UV λ_{max} (EtOH) nm (ϵ): 230.9 (17700). EI-MS m/z : 383 (M^+). Anal. Calcd for $\text{C}_{21}\text{H}_{21}\text{NO}_6$: C, 65.79; H, 5.52; N, 3.65. Found: C, 65.65; H, 5.59; N, 3.67.

6-*O*-Methacryloyl-D-galactose (2) Methacryloyl chloride (1.6 g, 15.4 mmol) was added to a solution of 1,2:3,4-di-*O*-isopropylidene- α -D-galactopyranose (4.0 g, 15.4 mmol) and triethylamine (1.6 g, 15.4 mmol) in dry acetonitrile (60 ml). The reaction mixture was stirred at room temperature overnight. The reaction mixture was filtered and evaporated *in vacuo*. The residue was dissolved in CHCl_3 (300 ml). The CHCl_3 solution was washed with H_2O , dried over MgSO_4 , and evaporated *in vacuo* to obtain an oily substance. This oily substance (3.0 g) and 4-methoxyphenol (0.15 g, 1.2 mmol) were dissolved in formic acid (23.75 ml) and then water (5.9 ml) was added to the solution. The reaction mixture was stirred at 20 °C for 20 h. Water (30 ml) was added to the reaction mixture. This solution was evaporated *in vacuo* and EtOH (42 ml) was added to the residue. The EtOH solution was filtered and evaporated *in vacuo*. The residue was added to 10 times the ether. The precipitate was collected and recrystallized from EtOH to yield 0.80 g (38%) of **2**: mp 85–87 °C. IR (KBr) cm^{-1} : 3350 (–OH), 1700 (–COO–), 1640 (vinyl group). $^1\text{H-NMR}$ (CDCl_3) δ : 1.88 (3H, s, – CH_3), 2.55–3.69 (4H, br, –OH), 4.14 (2H, m, – CH_2 –), 4.37–4.94 (4H, m, 2, 3, 4, and 5-position of galactose), 5.69 (1H, s, vinyl group), 6.03 (1H, s, vinyl group), 6.23 (1H, m, 1-position of galactose). EI-MS m/z : 248 (M^+). Anal. Calcd for $\text{C}_{10}\text{H}_{16}\text{O}_7 \cdot 0.5\text{H}_2\text{O}$: C, 46.53; H, 6.68. Found: C, 46.75; H, 6.98.

Mechanochemical Solid-State Copolymerization A mixture of **1** and **2** was mechanically fractured under anaerobic conditions (*e.g.* in nitrogen) by ball milling (6.0 mm ϕ , 890 mg) in a stainless steel twin-shell blender (7.8 mm ϕ , 24 mm long) at room temperature for 2 h at 60 Hz according to the method previously reported.²² Residual oxygen in this system was removed with Model 1000 Oxygen Trap (Chromatography Research Supplies) coupled with Indicating Oxygen Trap (Chromatography Research Supplies). The oxygen concentration was monitored with Oxygen analyzer (Toray Engineering Co., Ltd., LC 750/PC-120) and kept below 10 ppm. Copolymerization was made on the mixture of **1** and **2** ranging in mole fraction of **1** from 0.05 to 0.5. Mechanochemical copolymerization of **1** and acrylamide was performed in the same way as described above.

Solubility of Polymeric Prodrugs Five kinds of polymeric prodrugs, in

which the mole fraction of **1** was 0.05, 0.1, 0.2, 0.3 and 0.4, were used. The polymeric prodrug powder (5.0 mg) was added to distilled water (10 ml). The solution containing the polymeric prodrug powder was shaken for 15 min at room temperature. It was observed whether the polymeric prodrug powder dissolved or not.

Molecular Weight Measurement The molecular weight of polymeric prodrugs was measured with a gel permeation chromatograph (GPC; Shimadzu LC-6A) equipped with a refractive index detector (Shimadzu, RID-6A), gel column (Shodex, KD-800M and KD-80M), and a data analyzer (Shimadzu, Chromatopac C-R4A), under the following conditions: elution solvent, DMF containing 10 mmol of LiBr; flow rate, 0.7 ml/min; column temperature, 40 °C. Calibration was carried out with a standard specimen of poly(ethylene oxide).

Method of Hydrolysis The hydrolysis of polymeric prodrugs (20–25 mg) was conducted in the mixture of pH 7.4 phosphate buffer (50 mM KH_2PO_4 and 40 mM NaOH) (10 ml) and CH_3CN (10 ml) at 37 ± 0.2 °C. The pH of the solution unchanged before and after hydrolysis. Released 4MC and Bz4MC were periodically assayed by using a HPLC procedure. HPLC was performed with a Shodex Asahipak ODP-50 (4.6 i.d. $\times$ 250 mm) and ODP-50G (4.6 i.d. $\times$ 10 mm), and eluted with a mixture of pH 7.4 phosphate buffer– CH_3CN (1 : 1) at a flow rate of 0.5 ml/min.

Results and Discussion

Characterization of Polymeric Prodrugs Synthesized by Mechanochemical Solid-State Copolymerization We selected Bz4MC as a model compound of 4MC derivatives. 1-Benzoyl-2-methacryloyloxyethylcarbamoyl-4-methylcatechol (**1**) was specially synthesized as a model monomer of Bz4MC (Fig. 1). 6-*O*-Methacryloyl-D-galactose (**2**) was also prepared as a water-soluble monomer.

We carried out the mechanochemical solid-state copolymerization of **1** and **2** to obtain a polymeric prodrug, **Poly I**. The mechanochemical copolymerization of **1** and acrylamide (AAm), which is a conventional water-soluble monomer, was also carried out, and **Poly II** was obtained (Fig. 2). The polymer conversion was determined by observing the disappearance of vinyl protons and the appearance of the corresponding alkyl protons of the polymer in the $^1\text{H-NMR}$ spectra of the resultant powders (Chart 1). The mechanochemical solid-state copolymerization of these monomers proceeded to completion to give the corresponding polymeric prodrugs. This eliminates the need for any work-up such as is required in reactions in the liquid state.

We observed the behavior of dissolution of the polymeric prodrugs in water. **Poly I (0.1)** denotes the **Poly I** in which the mole fraction of **1** is 0.1. **Poly I (0.05)**, **(0.1)** and **(0.2)** dissolved in water at room temperature. **Poly I (0.3)** swelled in water but did not dissolve. **Poly I (0.4)** and **(0.5)** were also

Fig. 1. Structures of Bz4MC, 1-Benzoyl-2-methacryloyloxyethyl-4-methylcatechol (**1**) and 6-*O*-Methacryloyl-D-galactose (**2**)

Fig. 2. Structures of **Poly I** and **II** Prepared by Mechanochemical Solid-State Polymerization

Chart 1. NMR Spectra of **Poly I** (0.1)

insoluble in water. On the other hand, **Poly II** (0.05) dissolved in water but the other **Poly II**s, such as **Poly II** (0.1), were insoluble. The molecular weight of **Poly I** was similar to those of **Poly II**, and the number average molecular weight ($\bar{M}_n$) was *ca.* 30000. These results suggest that monomer **2** is superior to AAm in terms of synthesis of water-soluble polymeric prodrug.

Table 1 shows the $\bar{M}_n$ and heterogeneity ($\bar{M}_w/\bar{M}_n$, $\bar{M}_w$ is weight-average molecular weight) of **Poly I**. The value in the parentheses denotes the mole fraction of **1** in **Poly I**. The number average molecular weight was similar to one another. It was also shown that these polymeric prodrugs were of lower heterogeneity. In general, the polymer prepared by conventional radical-initiated solution polymerization possesses a broad molecular weight distribution, and its heterogeneity is more than 2. The low heterogeneity is very important in nature due to avoiding the scatter of the behavior in the body and the rate of drug release of polymeric prodrugs by molecular weight.

Nature of Drug Release from Polymeric Prodrugs *in Vitro* Bz4MC is a hydrophobic compound and slightly soluble in water. We carried out the hydrolysis of **Poly I** in the mixture of acetonitrile and pH 7.4 phosphate buffer solution (1 : 1) to prevent the saturation of Bz4MC.

Figure 3 shows the progressive changes in the concentration of Bz4MC and 4MC in the course of the hydrolysis of

Poly I. In the case of **Poly I** (0.1) the concentration of Bz4MC continues to increase for *ca.* 5 h and then tends to decrease toward x-axis. The similar result was obtained for **Poly I** (0.05) and (0.2). The concentration of 4MC increased with increasing the reaction time. These results suggest that Bz4MC released was hydrolyzed to produce 4MC. The total amount of Bz4MC released, area under the curve shown in Fig. 3, and the maximum concentration of 4MC indicated that the hydrolysis of a polymeric prodrug proceeded to completion.

Chart 2 shows the hydrolysis of **Poly I** and the subsequent reaction of Bz4MC. The rate constants of hydrolysis of polymeric prodrug and Bz4MC are defined as k_1 and k_2 , respectively. Two rate equations are written

$$-d[P]/dt = k_1 [P] \quad (1)$$

$$d[D]/dt = k_1 [P] - k_2 [D] \quad (2)$$

where $[P]$ = amount of Bz4MC bonded to the polymeric prodrug per unit volume at time t and $[D]$ = concentration of free Bz4MC at time t . These equations give

$$[D] = k_1 P_0 (\exp(-k_1 t) - \exp(-k_2 t)) / (k_1 - k_2) \quad (3)$$

where P_0 = total amount of Bz4MC in the polymeric prodrugs per unit volume at $t=0$.

The theoretical curves (solid curves) shown in Fig. 3 were obtained by fitting iteratively the rate constants, k_1 and k_2 , with the obtained data of hydrolysis using a nonlinear least-squares method. The values of the rate constants, k_1 and k_2 , are listed in Table 2. The curve well fitted with the experimental data. It was shown that Eq. 3 and Chart 1 properly described the hydrolysis kinetics.

It was shown in Table 2 that the value of k_1 decreased with increasing the mole fraction of **1** in **Poly I**. The value of k_2 was almost equal to one another. We separately examined the hydrolysis of Bz4MC under the same condition. The rate constant of the hydrolysis of Bz4MC was 0.110 h^{-1} and almost equal to the value of k_2 shown in Table 2. As shown in Table 1, the molecular weight and heterogeneity of **Poly I** were similar to one another. Therefore, the difference of the value of k_1 can be ascribed to the composition of polymeric prodrug, the mole fraction of **1**. This result suggests that the rate constant of hydrolysis can be controlled by the mole fraction of **1**.

Fig. 3. Progressive Changes in the Concentration of Bz4MC (○) and 4MC (●) in the Course of Hydrolysis of **Poly I** in a Solution of a pH 7.4 Phosphate Buffer and Acetonitrile

Chart 2

Table 1. Molecular Weight and Heterogeneity of **Poly I**

Compound	Number average molecular weight ($\bar{M}_n$)	Heterogeneity ($\bar{M}_w/\bar{M}_n$)
Poly I (0.05)	29000	1.16
Poly I (0.1)	28000	1.10
Poly I (0.2)	28000	1.10

Table 2. Rate Constants of Hydrolysis Derived from Eq. 3

	k_1 (h ⁻¹)	k_2 (h ⁻¹)	Correlation coefficient
Poly I (0.05)	0.585	0.115	0.990
Poly I (0.1)	0.299	0.118	0.995
Poly I (0.2)	0.140	0.116	0.983

Conclusions

The conclusion drawn from the present study can be summarized as follows: The benzoyl derivative of 4MC (Bz4MC) was selected as a model compound of 4MC. We carried out the mechanochemical solid-state copolymerization of the vinyl monomer of Bz4MC with the hydrophilic vinyl monomer. The mechanochemical copolymerization proceeded to completion, and the polymeric prodrugs obtained

possessed narrow molecular weight distributions (low heterogeneity). This feature is of great value for highly functionalized polymeric prodrug.

The hydrolysis of polymeric prodrug proceeded to completion. It was shown that Bz4MC released was further hydrolyzed under the present experimental condition to produce 4MC. It can be suggested that the rate constant of hydrolysis could be controlled by the composition, the mole fraction of **1** in **Poly I**. The relationship between the rate constants of hydrolysis and copolymer structure (e.g. the composition and molecular weight) will be the subject of a forthcoming paper.

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