Mechanochemical Solid-State Polymerization. VIII. Novel Composite Polymeric Prodrugs Prepared by Mechanochemical Polymerization in the Presence of Pharmaceutical Aids

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We carried out the mechanochemical polymerization of methacryloyl derivatives of acetoaminophen and 5-fluorouracil in the presence of lactose. The reaction proceeded readily and the polymeric prodrugs were quantitatively produced. This method produces powdered polymeric prodrugs in which fine particles of lactose are homogeneously dispersed, since the reaction proceeds quantitatively through a totally dry process. It is difficult to prepare such a powdered polymeric prodrug by conventional solution polymerization.

The rate of drug release of polymeric prodrugs increases with increasing content of lactose, as is shown to be true of the specific surface of polymeric prodrugs. These results suggest that lactose is homogeneously dispersed in powdered polymeric prodrugs.

The present method seems applicable to a wide variety of pharmaceutical aids. If one takes the physicochemical property of pharmaceutical aids into consideration, novel polymeric prodrugs with a variety of drug release rates can be synthesized simultaneously with mixing.

Key words polymeric prodrug; pharmaceutical aid; drug release; mechanochemical polymerization; 5-fluorouracil

We have reported the syntheses and the nature of drug release of novel polymeric prodrugs prepared by mechanochemical solid-state polymerization.[1] Several important conclusions were reached from a series of studies. The monomers prepared based on structural criteria derived from quantum chemical considerations underwent facile mechanochemical solid-state polymerizations to give the corresponding polymeric prodrugs essentially quantitatively.[1a,1b,1e] 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) represented by $\bar{M}_w/\bar{M}_n$, which is of great value in pharmaceuticals for highly functionalized polymeric prodrugs.[1b] Therefore, the present reactions seem applicable to a wide variety of vinyl monomers of an important class of bioactive compounds with different physicochemical properties, and provide a novel and simple methodology for syntheses of polymeric prodrugs through a totally dry process.

The rate of drug release of polymeric prodrugs prepared by mechanochemical solid-state polymerization depended largely on the content of the hydrophilic group as a side chain, the bonding type between the drug and polymer, and the structure of the spacer group.[1c,1f] We have also prepared hybrid polymeric prodrugs containing anticancer agents and contrast medium for chemoembolization, or anticancer agents and vitamins.[1g-1i]

Here, we studied the control of the rate of drug release from polymeric prodrugs to exploit the mechanochemical polymerization. Thus, since the mechanochemical polymerization quantitatively proceeds through a totally dry process, it is expected that the powdered polymeric prodrugs in which fine particles of lactose are homogeneously dispersed can be prepared by the present reaction

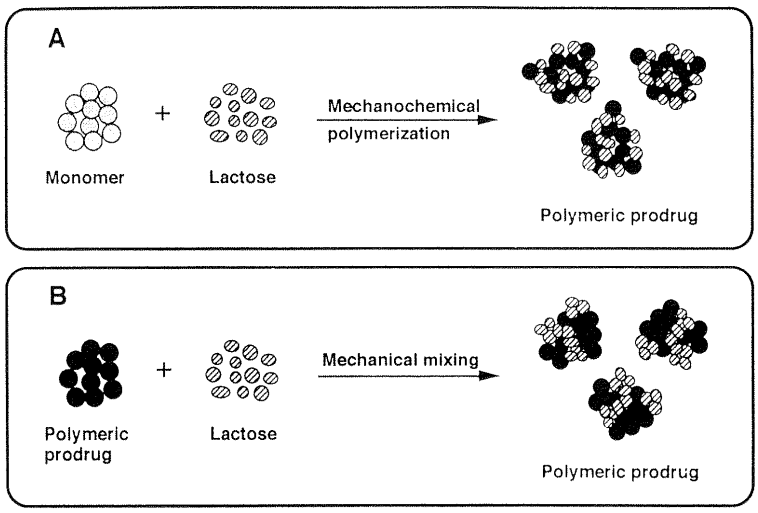

Fig. 1. Conceptual Illustration of the Synthesis of Polymeric Prodrugs Mixed with Lactose

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of solid-state vinyl monomer in the presence of lactose (Fig. 1-A). It is difficult to prepare such a polymeric prodrug by solution polymerization. Figure 1-B shows the preparation of blendmer, which is prepared by mixing lactose and polymeric prodrug. We provide detailed mechanistic implications of mechanochemical polymerization in the presence of lactose based on the ESR kinetics, the progressive changes in molecular weight, and the kinetics of polymer conversion. We also report the nature of drug release of such polymeric prodrugs, compared with the blendmer.

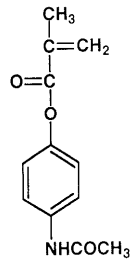

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Chart 1

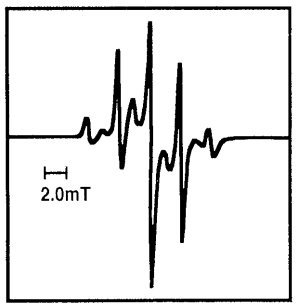

Fig. 2. ESR Spectrum Observed in the Mechanochemical Polymerization of **1** in the Presence of Lactose

Experimental

Materials Lactose (Hayashi Pure Chemical Industries, Ltd.) was screened with a 100 mesh sieve and dried *in vacuo* at 70 °C for 3 d. Methacryloyl derivatives, 4-acetoaminophenyl methacrylate (**1**) and 1-(2-methacryloyloxy)ethylcarbamoyl-5-fluorouracil (**2**), were prepared as described.[1b,1f] These monomers are thermally stable below 40 °C for a long period.[1b]

Mechanochemical Solid-State Polymerization A mixture (100 mg) of various ratios of methacryloyl derivatives (**1** or **2**) and lactose was mechanically fractured by ball milling in a stainless steel twin-shell blender at room temperature for 2 h in a vacuum glove box (Sanplatec Corp.) according to a method previously reported.[1b] Air in the vacuum glove box was replaced by purified nitrogen gas and then the remaining oxygen in this system was removed with a High Capacity Gas Purifier (Supelco, Inc.). The oxygen concentration was monitored with an oxygen analyzer (LC 750/PC-120, Toray Engineering Co., Ltd.) and kept below 20 ppm.

ESR Measurements The ESR spectra were recorded on a JEOL JES-RE1X spectrometer with X-band and 100-kHz field modulation, and the microwave power level was kept at less than 0.04 mW, since power levels higher than this began to produce saturation effects in the spectra. The spectral intensity was determined by double integration. The radical concentration (spin numbers/g) was calculated from the spectral intensities with the aid of a calibrated line obtained from the spectral intensities of poly(methyl methacrylate) (PMMA) samples impregnated with 1,1-diphenyl-2-picrylhydrazyl (DPPH).

Proton Nuclear Magnetic Resonance (^{1}H-NMR) Spectral Measurement ^{1}H-NMR spectra were recorded on a JEOL JNM-GX270 FT-NMR spectrometer in dimethylsulfoxide-d_6 (DMSO-d_6). The ^{1}H-NMR spectra of each of the fractured mixtures were taken after being exposed to air to quench the radicals.

Molecular Weight Measurement 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 CR-4A) under the following conditions: elution solvent, DMF containing 0.01 M LiBr; flow rate, 0.7 ml/min; column temperature, 40 °C. The calibration for the molecular weight determination was made by a standard specimen of polyethylene oxide.

Method of Hydrolysis The hydrolysis of powdered polymeric prodrugs (5.0—6.0 mg) was conducted in 20 ml of pH 6.8 phosphate buffer at 37 ± 0.2 °C in a heterogeneous system. Released 5-FU was periodically assayed by UV absorption spectrum (recording spectrophotometer UV-2200 (P/N 206-17000), Shimadzu Co.) at the wavelength of 266 nm.

Specific Surface Measurement The powdered polymeric prodrugs prepared in the presence of lactose were washed with water and dried *in vacuo* at 60 °C for 6 h. The specific surface of powdered polymeric prodrugs was measured by air permeability apparatus for specific surface area test (Lea-Nurse method) (Tsutsui Scientific Instruments Co., Ltd.).

Results and Discussion

ESR Kinetics of the Radicals Formed in Reaction of 1 in the Presence of Lactose To gain fundamental insight into the nature of mechanochemical polymerization in the presence of lactose, the detailed mechanistic implications of 4-acetoaminophenyl methacrylate (**1**) under the present conditions were explored as a representative example (Chart 1). The reason **1** was selected to do this study is that we have already reported the detailed mechanistic study of mechanochemical polymerization of **1** and there are many useful data compared with the reaction in the presence of lactose.

Figure 2 shows the ESR spectrum of the radical produced during the course of mechanochemical polymerization of **1** in the presence of lactose. As previously reported, virtually identical spectra have been observed in mechanochemical polymerization of **1** in the absence of lactose.[1b] It is now firmly established that the radical in the ESR spectra can be unambiguously assigned to a single end-chain radical (equivalent to a propagating radical) of methacrylic polymers.[2] We have already identified the mechanism of mechanochemical polymerization of methacryloyl derivatives of bioactive compounds.[1b] These radicals are mainly formed during the course of mechanochemical polymerization by the main-chain scission of polymeric prodrugs produced.

Figure 3 shows the progressive changes in the radical concentration of the ESR spectra in the course of the mechanochemical polymerization of **1** in the presence of lactose together with that in its absence. The two line features are seem to be similar in nature. The radical concentration at the maximum value in the presence of lactose is about 2.3 times higher than that in its absence.

Progressive Changes in Molecular Weight Figure 4 shows the progressive changes in the number average molecular weight ($\bar{M}_n$) of the polymeric prodrug formed in mechanochemical polymerization of **1** in the absence or presence of lactose. The maximum molecular weight appeared within a 5 min period of vibratory milling and the progressive changes exhibited a simple decay toward a limiting value. The molecular weight of the polymer formed in the presence of lactose, however, was lower than

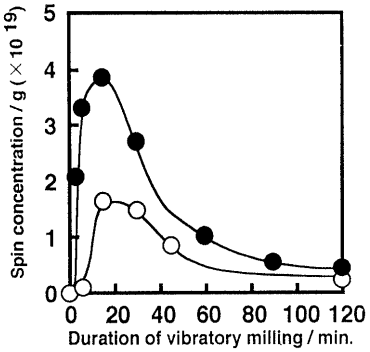

Fig. 3. Progressive Changes in Radical Concentration in the Course of Vibratory Milling

○, mechanochemical polymerization of **1**; ●, mechanochemical polymerization of **1** in the presence of lactose (50%).

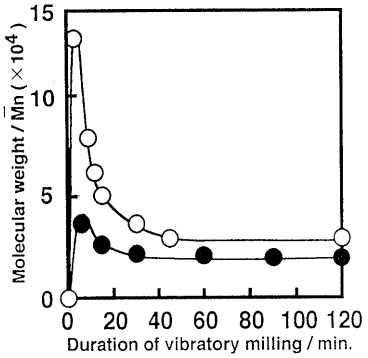

Fig. 4. Progressive Changes in Molecular Weight ($\bar{M}_n$) in the Course of Vibratory Milling

○, mechanochemical polymerization of **1**; ●, mechanochemical polymerization of **1** in the presence of lactose (50%).

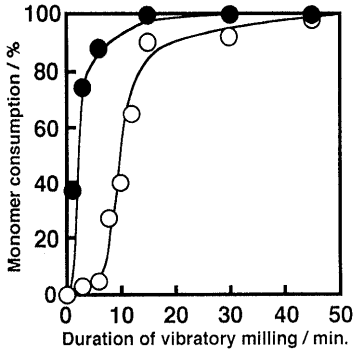

Fig. 5. Progressive Changes in Polymer Conversion (Monomer Consumption) in the Course of Vibratory Milling

○, mechanochemical polymerization of **1**; ●, mechanochemical polymerization of **1** in the presence of lactose (50%).

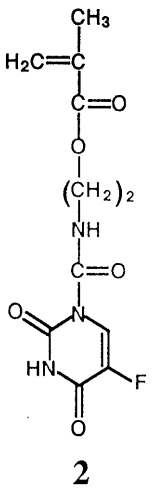

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Chart 2

that in its absence at any time. We also attempted the mechanical fracture of the mixture of lactose and the polymeric prodrug of **1**, whose molecular weight is the limiting value in the absence of lactose, for 2 h. The molecular weight of the resulting polymeric prodrug was equal to that prepared in the presence of lactose. These results show that the main-chain scission proceeds more readily in the presence than the absence of lactose. It is suggested that the feasibility of main-chain scission results from the many radicals observed in the presence of lactose.

It is also shown that the resulting polymeric prodrugs prepared by mechanochemical polymerization in the presence of lactose for 2 h are of lower heterogeneity ($\bar{M}_w/\bar{M}_n = 1.08$), which is of great value for highly functionalized polymeric prodrug syntheses.

Polymer Conversion Figure 5 shows the progressive changes of the monomer consumption as a function of the duration of the vibratory milling of **1**. The rate of monomer consumption was monitored by the decay of the olefinic protons of monomers as well as the corresponding growth of the alkyl protons of polymers in the ^{1}H-NMR spectra of the fractured mixtures.

The rate of monomer consumption in the presence of lactose is faster than that in its absence. It has already been determined that the mechanochemical polymerization involves a mechanoradical-initiated polymerization as a dominant process.[1b] Therefore, the higher radical concentration in the course of mechanochemical polymerization in the presence of lactose results from the steeper rate of disappearance of monomer.

The feasibility of main-chain scission is believed to depend on the hardness of lactose and polymeric prodrugs. The hardness of lactose was compared with that of PMMA, which is a conventional and methacrylic polymer. Vickers hardness numbers (Hv), an index of hardness, of lactose and PMMA were 535 MPa[3] and 204 MPa,[4] respectively. The hardness of lactose is more than 2.5 times that of PMMA. Lactose may be harder than polymeric prodrugs, hence the feasibility of main-chain scission, and thus the production of many radicals. Therefore, the mechanochemical polymerization in the presence of lactose progresses quickly.

Nature of Drug Release of Polymeric Prodrugs The methacryloyl derivative of 5-fluorouracil (**2**) was used as a monomer of bioactive compound to investigate the nature of drug release of polymeric prodrugs prepared by mechanochemical polymerization of various ratios of **2** and lactose (Chart 2).

Figure 6 shows progressive changes in the drug release of polymeric prodrugs as a function of reaction time. The number average molecular weights of these polymeric prodrugs were almost equal. It was also confirmed that the particle size distributions (number-size distributions) of the powdered polymeric prodrugs measured by Coulter

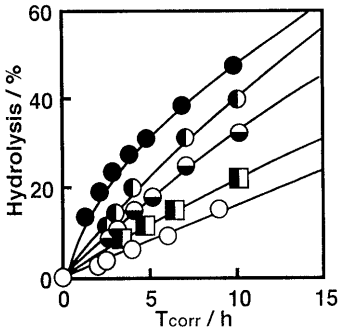

Fig. 6. Hydrolysis Profiles of Powdered Polymeric Prodrugs of **2** Prepared by Mechanochemical Polymerization in the Presence of Lactose

○, polymeric prodrug of **2**; ◒, polymeric prodrug of **2** containing lactose (25%); ◐, polymeric prodrug of **2** containing lactose (50%); ●, polymeric prodrug of **2** containing lactose (75%); ◧, blendmer containing lactose (50%).

Table 1. Specific Surface of Washed Polymeric Prodrugs of **2** Containing Lactose Measured by Lea-Nurse Method

Content of lactose (%)	Specific surface (cm^2/g)
0	4500
50	5500
75	5800
83	5900
50 (blendmer)	5200

Multisizer II (Coulter Electronics Limited) resembled one another.

As shown in Fig. 6, the rate of drug release of polymeric prodrugs increases with increasing content of lactose within this range. The acceleration of drug release is believed attributable to the increase of surface area of powdered polymeric prodrugs by the dissolution of lactose. Further acceleration of drug release, however, was not observed in the polymeric prodrugs containing more than 75% lactose. This result shows that there is a limiting value for the surface area of powdered polymeric prodrugs. There is not believed to be room for lactose to come into the powdered polymeric prodrug. Although the drug release of blendmer containing 50% lactose proceeds more readily than that of the polymeric prodrug of **2** (open circle), it progresses more slowly than that of the polymeric prodrug in the same composition prepared by the present method. This suggests that lactose is more homogeneously dispersed in the polymeric prodrug prepared by the present method.

As described above, the increase of surface area of powdered polymeric prodrug by the dissolution of lactose is viewed as important for the drug release. We attempted the quantitative analysis of dissolved lactose in the course of hydrolysis by the phenol-sulfuric acid method.[5] The lactose in the powdered polymeric prodrug was quantitatively released within 3 min. To confirm the increase of surface area of powdered polymeric prodrug by the lactose dissolution, the specific surface was measured by the Lea-Nurse method (see Experimental). Table 1 shows the specific surface of the polymeric prodrugs which were washed to remove the lactose.[6] The specific surface increases with increasing lactose content up to 75%. The

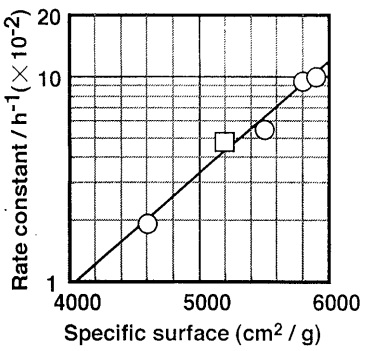

Fig. 7. Plot of the Apparent First-Order Rate Constants of Polymeric Prodrugs of **2** Containing Lactose against the Specific Surface

○, polymeric prodrug of **2**; □, blendmer of **2**.

specific surface of polymeric prodrug containing 83% lactose is close to that containing 75%. It is also shown that the specific surface of the blendmer is smaller than that of the corresponding polymeric prodrug prepared by the present method. This result suggests that the lactose is more homogeneously dispersed in the powdered polymeric prodrug prepared by the present method than the blendmer.

A previous study confirmed that the drug release of polymeric prodrugs obeyed the apparent first-order kinetics.[1i] Figure 7 shows the relationship between the apparent first-order rate constant and the specific surface as semilogarithmic plots. The apparent first-order rate constant is seen to exponentially increase with increase in the specific surface of powdered polymeric prodrugs. This suggests that the rate of drug release can be controlled by the content of lactose and that the surface area is an important factor in drug release.

Conclusion

The present study can be summarized as follows: We carried out the mechanochemical polymerization of methacryloyl derivatives of bioactive compounds in the presence of lactose. The reaction proceeded readily and the polymeric prodrugs were produced quantitatively. The resulting polymers were of lower heterogeneity, which is of great value for highly functionalized polymeric prodrug syntheses. The present method can be used to prepare powdered polymeric prodrugs in which fine particles of lactose are homogeneously dispersed, since the reaction proceeds quantitatively through a totally dry process. It is difficult to prepare such powdered polymeric prodrugs by conventional solution polymerization.

The rate of drug release of polymeric prodrugs increases with increasing content of lactose within 75%, and can therefore be controlled. These results appear attributable to the increases of the surface area of powdered polymeric prodrug by the dissolution of lactose. The lactose in the powdered polymeric prodrug was completely released within 3 min and the specific surface of washed polymeric prodrug increased as lactose content rose to 75%. The specific surface of the blendmer is smaller than that of the corresponding polymeric prodrug prepared by the present method. Thus, powdered polymeric prodrug in which the lactose is homogeneously dispersed can be prepared by

this method.

This technique seems applicable to a wide variety of pharmaceutical aids. If one takes the physicochemical property of pharmaceutical aids into consideration, novel polymeric prodrugs with a variety of drug release rates can be synthesized simultaneously with mixing.

References and Notes

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6) The particle size distributions of these polymeric prodrugs were measured by a Coulter Counter Multisizer II. These specific surface area calculated from the particle size distribution and particle density (1.2 g/cm^3), which was measured by pycnometer method, was about 4500 cm^2/g.