Chem. Pharm. Bull. **42**(12) 2412—2417 (1994)

Mechanochemical Solid-State Polymerization. VII. The Nature of Hydrolysis of Novel Polymeric Prodrugs Prepared by Mechanochemical Copolymerization

Shin-ichi KONDO, Katsuyuki MURASE, and Masayuki KUZUYA*

Laboratory of Pharmaceutical Physical Chemistry, Gifu Pharmaceutical University, 5–6–1, Mitahora-Higashi, Gifu 502, Japan.

Received June 6, 1994; accepted August 22, 1994

Hydrophilic polymeric prodrugs have been prepared by the mechanochemical copolymerization of methacryloyl derivatives of bioactive compounds with acrylamide, which is a hydrophilic conventional vinyl monomer. The polymeric prodrugs are homogeneous in composition, since mechanochemical copolymerization is ideal copolymerization under the present experimental conditions. It is also shown that the polymeric prodrugs have a narrow molecular weight distribution.

The alkaline hydrolysis of polymeric prodrugs prepared by mechanochemical copolymerization has been examined in a heterogeneous system. Though the degree of hydrolysis of polymeric prodrugs increases with increasing the content of hydrophilic monomer in the copolymer, there exists a limit to the degree of hydrolysis attainable due to the retardation by the carboxylic group produced as a side chain of the polymer hydrolyzate. The rate of hydrolysis depended largely on the structural feature of the polymer hydrolyzate; the use of carbamoyl derivatives of bioactive compounds is valuable for improving the nature of hydrolysis, since the amino group formed with the progress of hydrolysis does not cause any retardation of the nucleophilic reaction of the hydroxyl anion.

Keywords polymeric prodrug; drug release; mechanochemical copolymerization; hydrolysis; carbamoyl derivative; 5-fluorouracil

Development of sustained- and controlled-release systems for drug delivery is one of the most active areas today in the entire field of drug research. Polymeric prodrugs, in which a drug is attached covalently to a polymer backbone and slowly released under appropriate conditions, possess unique properties distinct from those of the corresponding lower molecular weight prodrugs, and they can be utilized as a sustained-release delivery system. Thus, the chemotherapeutic utility of polymeric prodrugs has been the focus of intense research.[1)]

Ringsdorf has elaborated on the notion of a polymeric prodrug (Fig. 1). The backbone contains three essential units[2,3)]; the first is a device for controlling the physicochemical properties of the entire macromolecule, which mainly involves the hydrophilic–lipophilic balance, the electric charge, and the solubility of the system. The second is a device for bonding a drug. The drug must be covalently bonded to the polymer and must remain attached to it until the macromolecule reaches the desired site of action. The drug also must be detached from the parent polymer at the site of action. The release of a drug takes place by hydrolysis or by a specific enzymatic cleavage of the drug–polymer bond. The third functional unit of the polymer backbone is known as the "homing device." This unit guides the entire drug–polymer conjugate to the targeted tissue.

In principle, polymeric prodrugs can be prepared in two different ways.[3,4)] One approach is the substitution of reactive groups of polymers with functional groups of biologically active compounds. It is often observed, however, that some undesired side reactions also occur in such reactions, and the substitution reactions of this type sometimes do not proceed to completion due to severe steric hindrance by neighboring side chain groups, although such problems can be removed by spacing the reactive group of several carbon atoms from the polymer main chain. The other approach is the polymerization of drug molecules with polymerizable functional groups such as vinyl groups. This approach has the advantage that the monomer can be highly purified and then polymerized or copolymerized with any number of desirable comonomers. All these reactions, however, are usually carried out in the liquid state, and thus require the need for work-up of the reaction mixture, such as product separation and purification.

Kargin *et al.* first reported mechanochemical polymerization of solid-state monomers in 1959.[5)] Nevertheless, relatively little work has been done with mechanochemical polymerization in the solid state. Simionescu *et al.* speculated that the mechanism of initiation in mechanochemical polymerization involves a solid state single electron transfer (SSET) from the activated metal surface to the vinyl bond of a monomer,[6)] but no one has provided detailed experimental evidence, including the occurrence of SSET.

We have recently reported a detailed electron spin resonance(ESR) study on mechanochemical solid state polymerization of acrylamide,[7)] as well as the nature of the mechanoradical formation of several conventional

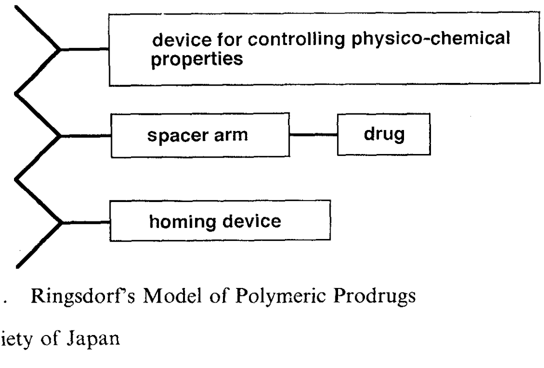

Fig. 1. Ringsdorf's Model of Polymeric Prodrugs

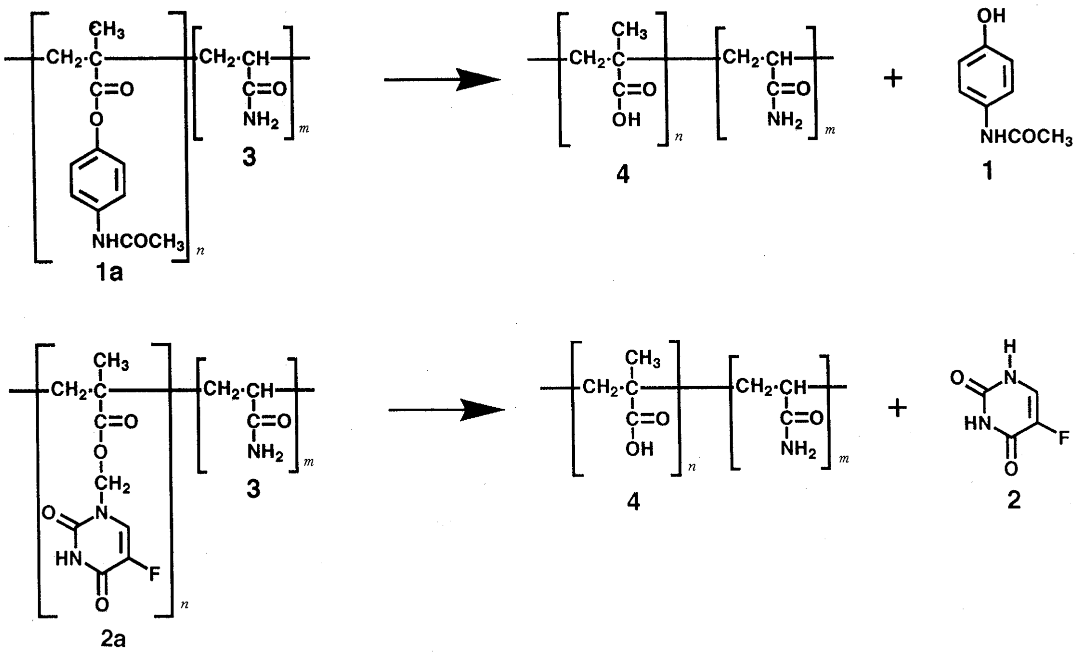

Fig. 2. Schematic Representation of Hydrolysis of Polymeric Prodrugs Containing Acetaminophen (**1**) or 5-Fluorouracil (**2**)

polymers.8) Part of the motivation for these investigations originated from the need for mechanoradical information related to our ESR studies of plasma-induced solid state radicals9) and their pharmaceutical applications.10) Subsequently, we have presented the first example of polymeric prodrugs prepared by the mechanochemical solid state polymerization of several methacryloyl vinyl monomers derived from bioactive compounds,11) and we discussed the alkaline hydrolysis of the above-mentioned mechanochemically polymerized prodrugs for drug release.12) We have also reported the first experimental evidence of the occurrence of SSET induced by applications of mechanical energy based on the observation of ESR of the reduced form of dipyridinium dications.13) Moreover, we have proposed the structural criteria for mechanochemical polymerizable vinyl monomers based on quantum chemical consideration.14)

Several important conclusions have been reached from a series of such studies. The monomers, prepared along the lines of structural criteria derived from the quantum chemical considerations, underwent simple mechanochemical solid state polymerizations to give the corresponding polymeric prodrugs essentially quantitatively.11,14) 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 have a narrow molecular weight distribution11) represented by M_w/M_n, which is of great value in pharmaceuticals for highly functionalized polymeric prodrugs. 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 they provide a novel and simple methodology for syntheses of polymeric prodrugs through a totally dry process. Homopolymers which contain bioactive compounds as a side chain, however, were not hydrolyzed in a buffer solution of pH 7 at 37 °C due to the hydrophobicity.13)

It is expected that the polymeric prodrugs which contain hydrophilic groups would be more easily hydrolyzed than the above-mentioned hydrophobic homopolymers. We report the nature of hydrolysis of the polymeric prodrugs prepared by the mechanochemical copolymerization of methacryloyl derivatives of acetaminophen (**1**) or 5-fluorouracil (**2**), whose slow-release preparation is desirable in treatment, with acrylamide (**3**), which is a hydrophilic vinyl monomer (Fig. 2).

Experimental

Materials Methacryloyl derivatives of bioactive compounds, *i.e.* *p*-methacryloyloxy acetanilide (**1a**), 1-methacryloyloxymethyl-5-fluorouracil (**2a**) were prepared according to the method previously reported.11) Acrylamide (**3**) supplied by Tokyo Kasei Co., Ltd. (Japan) is commercially available, and was purified by recrystallization from benzene and dried *in vacuo*. 1-(2-Methacryloyloxyethylcarbamoyl)-5-fluorouracil (**2b**) was prepared according to the literature.15)

Mechanochemical Copolymerization A mixture (100 mg) of various ratios of methacryloyl derivatives and **3** was mechanically fractured by ball milling in a stainless steel twin-shell blender at room temperature for a prescribed period according to the method previously reported.11) The rate of monomer consumption was monitored by the decay of the olefinic protons of monomers in the ^{1}H-NMR spectra of the fractured mixtures. The copolymer composition was determined by the ratio of residual methacryloyl derivatives and **3**, measured by the ^{1}H-NMR spectra of fractured mixtures and elemental analysis of the copolymer separated by reprecipitation of the mixtures.

Radical-Initiated Solution Polymerization A mixture (1 g) of various ratios of **1a** and **3** was dissolved in benzene (3 ml) with 2,2′-azobisisobutyronitrile (3 mg). The solution was warmed at 60 °C in a sealed glass-made tube under nitrogen for 3 h. After dimethylformamide (DMF)–H_2O (1 : 1, v/v) (20 ml) was added to the reaction mixture to dissolve the precipitated polymer, the solution was poured into a large amount of acetone. The precipitated polymer was collected and dried *in vacuo*. The yield ranged from 20 to 25%. The copolymer composition was determined by elemental analysis. The monomer reactivity ratio was calculated following the Mayo–Lewis equation. In this system, **1a** (M_1)–**3**(M_2), the value was $r_1 = 1.89 \pm 0.62$ and $r_2 = 0.046 \pm 0.078$.

Proton Nuclear Magnetic Resonance (^{1}H-NMR) Spectral Measurement ^{1}H-NMR spectra were recorded on a JEOL JNM-GX270

FT-NMR spectrometer in either dimethyl sulfoxide-d_6 (DMSO-d_6) or $CDCl_3$. Tetramethylsilane (TMS) was used as an internal standard. For determination of the rate of polymer conversion, the ^1H-NMR spectra of each of the fractured mixtures were taken after being exposed to air to quench the radicals.

Molecular Weight Measurement The molecular weight of the polymeric prodrugs was measured by 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 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. The molecular weight measured by KD-80M ranged from 500 to 20000000.

Method of Hydrolysis The hydrolysis of powdered polymeric prodrugs (5.0—6.0 mg) was conducted in 10 ml of a pH 8 phosphate buffer at 37 ± 0.2 °C in suspension. The reaction progress was monitored by high performance liquid chromatography (HPLC) under the following conditions: elution solvent, H_2O; flow rate, 0.5 ml/min; column, Asahi-pak ODP-50 and ODP-50G; column temperature, 37 °C; detector, ultraviolet (UV) 245 nm for **1** and 266 nm for **2**. Each of the drugs was separately confirmed to be stable under the prescribed conditions of hydrolysis. It was also confirmed that the pH of the phosphate buffer remained unchanged during the hydrolysis.

Results and Discussion

Copolymer Composition We first examined the composition of a copolymer in mechanochemical polymerization, which influences many of its important properties, such as solubility. Figure 3 shows the composition of **1a** in a copolymer with initial feed and reaction completion in the mechanochemical solid-state copolymerization of **1a** with **3**. It is seen that the composition of **1a** in the copolymer is equal to that in the initial feed regardless of the reaction progress. Thus, the mechanochemical solid-state copolymerization of **1a** with **3** is an ideal copolymerization under the present experimental conditions. We had also attempted the radical-initiated solution copolymerization of **1a** with **3**. It was shown that this reaction was not ideal copolymerization (see Experimental). It is often desirable to produce a polymer that is homogeneous in composition rather than one having a broad distribution of compositions.[16] Therefore, it is valuable that the copolymer, which is homogeneous in composition, is quantitatively obtained under the present experimental condition.

This result suggests that the rate of monomer consumption of **1a** must be equal to that of **3** under the present mechanochemical condition. We have compared the rate of monomer consumption in the mechanochemical polymerization of **1a** with that of **3** to confirm the view.

Figure 4 shows plots of the monomer consumption as a function of the duration of the vibratory milling of **1a** or **3**. It can be seen that the kinetics of monomer consumption exhibited a sigmoid curve, and that the profiles of both curves are similar to each other. The rate of monomer consumption closely relates to the radical concentration which participated in the polymerization. Thus, the above-mentioned result means that the radical concentrations are equal to each other in mechanochemical polymerization. It is considered that this would be responsible for the ideal copolymerization in the present reaction.

The profiles of the monomer consumption of **2a** also resemble those of **1a** and **3**. It can be predicted that the mechanochemical copolymerization of **2a** with **3** would be ideal copolymerization.

As described above, one of the most striking properties observed in polymers prepared by mechanochemical polymerization is that the resulting polymeric prodrugs have a narrow molecular weight distribution.[11] In order to confirm that the resulting copolymers possess the same feature, the molecular weight distribution of copolymers

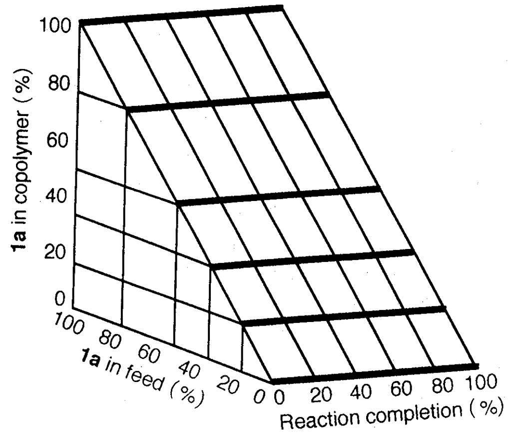

Fig. 3. Composition of **1a** in Copolymer with the Initial Monomer Feed and Reaction Completion for the System of Methacryloylacetaminophen (**1a**)–Acrylamide (**3**) in Mechanochemical Polymerization

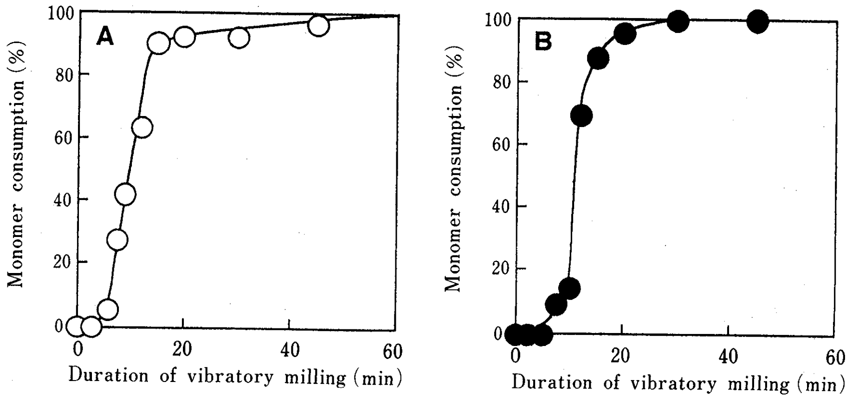

Fig. 4. Progressive Changes in Polymer Conversion (Monomer Consumption) with the Duration of Vibratory Milling

A: Methacryloylacetaminophen (**1a**). B: Acrylamide (**3**).

was measured by a GPC.

Polymeric prodrugs prepared by mechanochemical copolymerization have a narrow molecular weight distribution ($M_w/M_n = 1.09$), in contrast to those by radical-initiated solution copolymerization ($M_w/M_n = 3.20$), as shown in Fig. 5. It is very important that the polymeric prodrugs prepared by mechanochemical copolymerization have a narrow molecular weight distribution, which is of great value for highly functionalized polymeric prodrug syntheses.

Hydrolysis of Polymeric Prodrugs Prepared by Mechanochemical Copolymerization Figure 6 shows progressive changes in the hydrolysis of polymeric prodrugs prepared by mechanochemical copolymerization of **1a** or **2a** with **3** as a function of reaction time in a pH 8 phosphate buffer solution. No drug was released from the homopolymers of **1a** and **2a** to a detectable extent under the present condition.

It is seen from Fig. 6 that the degree of hydrolysis of polymeric prodrugs increases with an increase in the content of **3** in the copolymer, but that the reaction has ceased before its completion. As reported in a previous paper, carboxyl groups (**4**, Fig.2) are produced as a side chain of the polymer hydrolyzate of these polymeric prodrugs, which should be completely dissociated under the present basic media, causing the retardation of subsequent nucleophilic reactions.[14] We believe this is the essential reason for the incomplete hydrolysis. Such a pronounced effect on the retardation of the hydrolysis has further emerged in the case of the copolymer of **2a** with **3**, which contains **2**, as can be seen in Fig. 6B. The result can be interpreted by combined factors, such that the polymeric prodrug of **2a** not only produces a carboxyl group as a result of the hydrolysis in the polymer hydrolyzate, but also has a dissociable hydrogen ($pK_a = 7.3$)[17] at the 3-position of **2**.

Although the degree of hydrolysis of polymeric prodrugs increases with an increasing content of hydrophilic monomer in the copolymer, there exists a limit to the degree of hydrolysis attainable due to the retardation by the carboxylic group which is produced as a side chain of the polymer hydrolyzate.

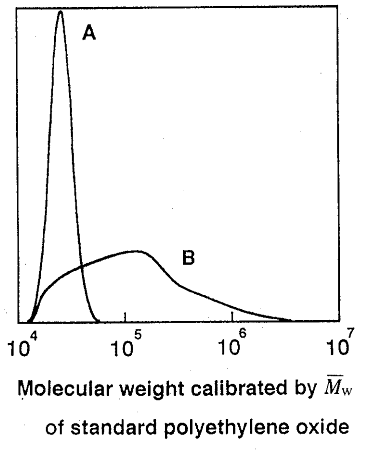

Fig. 5. GPC of Copolymer of Methacryloylacetaminophen (**1a**) with Acrylamide (**3**)

A: Mechanochemical polymerization. B: Radical initiated polymerization.

Influence of the Structural Feature of the Polymer Hydrolyzate on the Rate of Hydrolysis When the carboxyl groups are produced with progressing hydrolysis of polymeric prodrugs, the reaction ceases before its completion. It is considered that if amino or hydroxyl groups as a side chain of the polymer hydrolyzate are produced, the hydrolyses could proceed to 100% completion. It is well-known that 1-carbamoyl-5-fluorouracil derivatives are easily hydrolyzed to produce **2** and

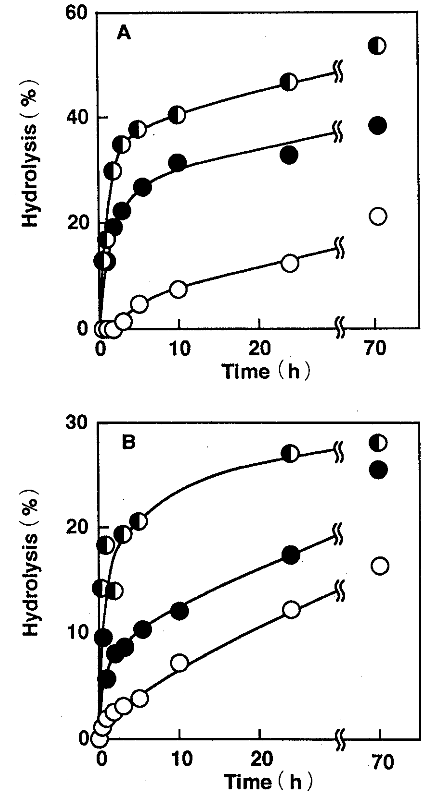

Fig. 6. Hydrolysis Profiles of Copolymer of Methacryloyl Derivatives with Acrylamide (**3**) by Mechanochemical Polymerization as a Function of Reaction Time

A: Copolymer of methacryloylacetaminophen (**1a**) with **3**. B: Copolymer of 1-methacryloyloxymethyl-5-fluorouracil (**2a**) with **3**. ○, 1 : 1; ●, 1 : 2; ◐, 1 : 5.

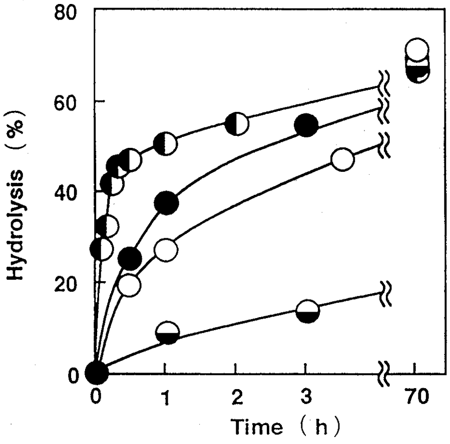

Fig. 7. Hydrolysis Profiles of Copolymer of 1-(2-Methacryloyloxyethylcarbamoyl)-5-fluorouracil (**2b**) with Acrylamide (**3**) by Mechanochemical Polymerization as a Function of Reaction Time

◒, homopolymer of **2b**; ○, copolymer of **2b** with **3** in 1 : 1; ●, copolymer of **2b** with **3** in 1 : 2; ◐, copolymer of **2b** with **3** in 1 : 5.

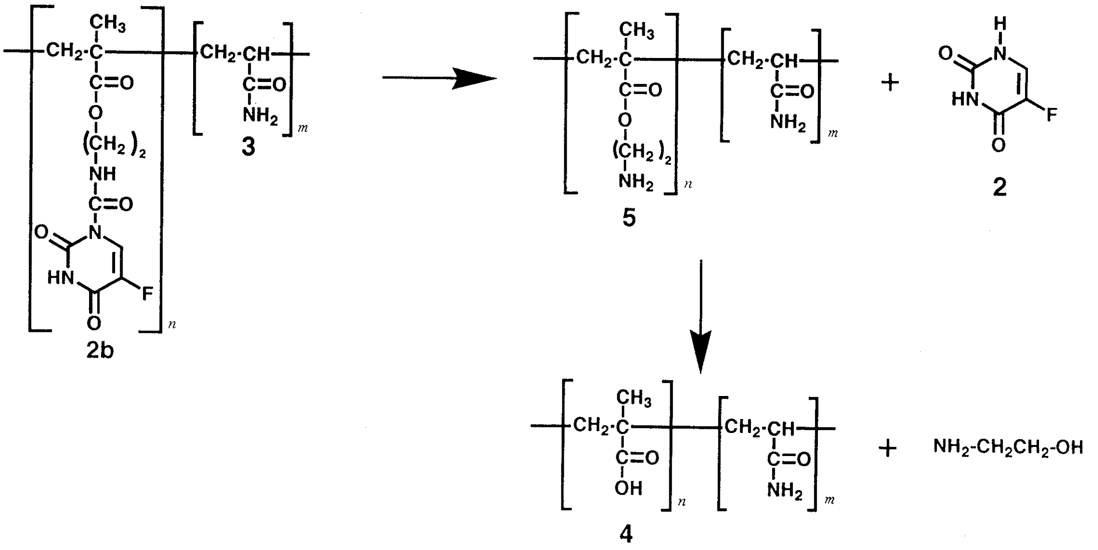

Fig. 8. Schematic Representation of the Hydrolysis of Polymeric Prodrugs Prepared by the Mechanochemical Copolymerization of 1-(2-Methacryloyloxyethylcarbamoyl)-5-fluorouracil (**2b**) with Acrylamide (**3**)

amines with decarboxylation.[18] We further investigated the hydrolysis of polymeric prodrugs prepared by the mechanochemical polymerization of 1-(2-methacryloyloxyethylcarbamoyl)-5-fluorouracil (**2b**).

It is seen from Fig. 7 that the homopolymer derived from **2b** is hydrolyzed more easily than the copolymer of **2a** with **3**, and that the rate of hydrolysis tends to gradually level off to *ca.* 70% completion.

It is also shown that the rate of hydrolysis of copolymers increases with an increasing content of **3** in the copolymer. Its degree of hydrolysis, however, is equal to that of the homopolymer. This can be rationalized in terms of the carbamoyl group being preferentially hydrolyzed to produce the 2-aminoethylester group (**5**), followed by the hydrolysis of the ester group, resulting in the production of a carboxyl group (**4**) as a side chain. (Fig. 8)

The hydrogen at the 3-position of the uracil ring of **2b** is also dissociated under the present basic media, so that it is considered that a pronounced effect on the retardation of hydrolysis would emerge in the case of the polymeric prodrug containing **2b** as well as **2a**. Figures 6B and 7 show that the rate of hydrolysis of polymeric prodrugs derived from **2b** is much faster than that from **2a**. It is known that the rate of hydrolysis of 1-carbamoyl-5-fluorouracil derivatives is about 1000 times greater than that of 1-acyloxymethyl-5-fluorouracils.[17,18] Thus, it is considered that the effect of the hydrogen at the 3-position of the uracil ring on the retardation of hydrolysis is negligible, since the carbamoyl group easily decomposes.

Conclusion

The conclusion drawn from the present study can be summarized as follows: Mechanochemical copolymerization under the present experimental conditions described in the Experimental section has shown that the resulting copolymer is homogeneous in composition (ideal copolymerization). It is also shown that the polymeric prodrugs prepared by mechanochemical copolymerization have a narrow molecular weight distribution.

Although the degree of hydrolysis of polymeric prodrugs containing **1a** or **2a** increases with an increase in the content of the hydrophilic monomer in the copolymer, there exists a limit to the degree of hydrolysis due to the retardation by the carboxylic group produced as a side chain of the polymer hydrolyzate. The rate of hydrolysis of polymeric prodrugs containing **2b** is much higher than that consisting of **2a** with **3**, since non-acidic 2-aminoethylester groups (**5**) are formed in the resulting polymer hydrolyzates. The hydrolysis of polymeric prodrugs containing **2b**, however, leveled off to *ca.* 70% progress in order to produce the carboxylic group (**4**) by the subsequent hydrolysis of the ester group. These results suggest that improvement of the hydrolysis must consider not only the introduction of a hydrophilic group, but also the structural features of the polymer hydrolyzate.

We are now actively elaborating on these initial studies, and the influence of a monomer sequence in a copolymeric prodrug on the rate of hydrolysis will be the subject of a forthcoming paper.

References and Notes

1) A. A. Sinkula, "Design of Prodrugs" ed. by H. Bundgaard, Elsevier Science Publishers, Amsterdam, 1985, pp. 157—176.
2) H. Ringsdorf, *J. Polym. Sci. Polym. Symp. Ed.*, **51**, 135 (1975).
3) H. N. Joshi, *Pharm. Technol.*, **12**, 118 (1988).
4) *a*) W. Scheler, *Makromol. Chem., Macromol. Symp.*, **12**, 1 (1987); *b*) J. Kalal, *ibid.*, **1**, 259 (1987).
5) V. A. Kargin, N. A. Plate, *Vysokomol. Soedin.*, **1**, 330 (1959).
6) C. Simionescu, C. V. Oprea, J. Nicoleanu, *Eur. Polym. J.*, **19**, 525 (1983).
7) M. Kuzuya, S. Kondo, A. Noguchi, N. Noda, *J. Polym. Sci., Polym. Chem.*, **29**, 489 (1991).
8) M. Kuzuya, S. Kondo, A. Noguchi, N. Noda, *J. Polym. Sci., Polym. Phys.*, **30**, 97 (1992).
9) *a*) M. Kuzuya, A. Koide, A. Ito, A. Noguchi, *Chem. Lett.*, **1989**, 555; *b*) M. Kuzuya, A. Noguchi, H. Ito, S. Kondo, N. Noda, *J. Polym. Sci., Polym. Chem.*, **29**, 1 (1991); *c*) M. Kuzuya, A. Noguchi, M. Ishikawa, A. Koide, K. Sawada, A. Ito, N. Noda, *J. Phys. Chem.*, **95**, 2398 (1991); *d*) M. Kuzuya, M. Ishikawa, A. Noguchi, H. Ito, K. Kamiya, T. Kawaguchi, *J. Mater. Chem.*, **1**, 387 (1991); *e*) M. Kuzuya, "Trends in Physical Chemistry," Vol. 2, Science

Research Integration, Trivandrum, 1991, p. 39; *f)* M. Kuzuya, H. Ito, S. Kondo, N. Noda, A. Noguchi, *Macromolecules*, **24**, 6612 (1991); *g)* M. Kuzuya, M. Ishikawa, A. Noguchi, K. Sawada, S. Kondo, *J. Polym. Sci., Polym. Chem.*, **30**, 379 (1992); *h)* M. Kuzuya, N. Noda, S. Kondo, K. Washino, A. Noguchi, *J. Am. Chem. Soc.*, **114**, 6505 (1992); *i)* M. Kuzuya, S. Kondo, H. Ito, A. Noguchi, *Appl. Sur. Sci.*, **60/61**, 416 (1992); *j)* M. Kuzuya, K. Sawada, T. Takagi, A. Noguchi, *Polymer J.*, **25**, 75 (1993); *k)* M. Kuzuya, K. Kamiya, Y. Yanagihara, Y. Matsuno, *Plasma Sources Sci. Technol.*, **2**, 51 (1993); *l)* M. Kuzuya, J. Niwa, H. Ito, *Macromolecules*, **26**, 1990 (1993); *m)* M. Kuzuya, J. Niwa, A. Noguchi, K. Xu, *J. Photopolym. Sci. Technol.*, **6**, 371 (1993).

10) *a)* M. Kuzuya, *Hyomen*, **27**, 885(1989); *b)* M. Kuzuya, A. Noguchi, H. Ito, M. Ishikawa, *DDS*, **6**, 119 (1991); *c)* M. Kuzuya, H. Ito, N. Noda, I. Yamakawa, S. Watanabe, *ibid.*, **6**, 437 (1991); *d)* M. Kuzuya, *J. Photopolym. Sci. Technol.*, **5**, 407 (1992); *e)* I. Yamakawa, S. Watanabe, Y. Matsuno, M. Kuzuya, *Biol. Pharm. Bull.*, **16**, 182 (1993); *f)* M. Ishikawa, Y. Matsuno, A. Noguchi, M. Kuzuya, *Chem. Pharm. Bull.*, **41**, 1626 (1993).

11) M. Kuzuya, S. Kondo, A. Noguchi, *Macromolecules*, **24**, 4047 (1991).

12) M. Kuzuya, S. Kondo, *Chem. Pharm. Bull.*, **39**, 3018 (1991).

13) M. Kuzuya, S. Kondo, K. Murase, *J. Phys. Chem.*, **97**, 7800 (1993).

14) S. Kondo, K. Murase, M. Kuzuya, *Chem. Pharm. Bull.*, **42**, 768 (1994).

15) *a)* N. M. Bortnick, U. S. Patent 2718516 (1959) [*Chem. Abstr.*, **50**, 1371*c* (1956)]; *b)* R. R. Hartsough, C. G. Gebelein, *Polym. Sci. Technol.* (Plenum), **32**, 115 (1985).

16) F. W. Billmeyer, Jr., "Textbook of Polymer Science," 3rd ed., John Wiley and Sons, Inc., New York, 1984, pp. 101—125.

17) A. Buur, H. Bundgaard, E. Falch, *Int. J. Pharm.*, **24**, 43 (1985).

18) A. Buur, H. Bundgaard, *Int. J. Pharm.*, **23**, 209 (1985).