Drug Release from the Enzyme-Degradable and pH-Sensitive Hydrogel Composed of Glycidyl Methacrylate Dextran and Poly(acrylic acid)

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Hydrogels composed of glycidyl methacrylate dextran (GMD) and poly(acrylic acid, PAA) were prepared by UV irradiation method for colon-specific drug delivery. GMD was synthesized by coupling of glycidyl methacrylate to dextran in the presence of 4-(N,N-dimethylamino)pyridine. GMD was photo-polymerized by ammonium peroxydisulfate as initiating system in phosphate-buffered solution (0.1 M, pH 7.4). And then, acrylic acid monomer was added and subsequently heat-polymerized by 2,2'-azobisisobutyronitrile as an initiator. The hydrogels exhibited high swelling ratio (about 20) at 37°C, and showed a pH-dependent swelling behavior. In addition, the swelling ratio of the hydrogel was remarkably enhanced to about 45 times in the presence of dextranase at pH 7.4. The swelling-deswelling behavior proceeded reversibly for the GMD/PAA hydrogels between pH 2 and pH 7.4. Release of 5-aminosalicylic acid from the GMD/PAA hydrogels was evaluated in simulated gastrointestinal pH fluids in the absence or presence of dextranase. We concluded that the hydrogels prepared could be used as a dual-sensitive drug carrier for sequential release in gastrointestinal tract.

Key words: Hydrogel, Dextran, Poly(acrylic acid), Colon-specific drug delivery, 5-Aminosalicylic acid

INTRODUCTION

Hydrogels are flexible, durable, and permeable to water, metabolites, and small molecules. In biomedical fields, the practical applications of hydrogels are diversely ranged from diagnostic apparatus to controlled drug delivery device (Peppas, 1987; Park et al., 1993; Hennink et al., 1997; Hennink et al., 2004). Biodegradable polymeric systems have been extensively studied for the controlled release of drug, because it does not require an invasive surgical technique after their use (Heller, 1993; Okano et al., 1994). Especially, biodegradable hydrogels have been represented as an attractive drug formulation due to their advantages such as biocompatibility, high responsibility for specific degradation, and a feasible approach to incorporate drugs into matrices (Peppas, 1987; Kim et al., 1992; Park et al., 1993; Kamath and Park, 1993; Yoshida

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et al., 1993; Chen et al., 1995; Hennink et al., 1997; Hennink et al., 2004).

Although zero-order drug release is significant for most drugs, there are many drugs that need to be delivered in a pulsatile manner (Kikuchi and Okano, 2002). The most widely used example is the delivery of therapeutic peptide or protein such as insulin (Shiino et al., 1994; Moriyama et al., 1999). Recently, a lot of researchers have prepared hydrogels with additional functions, such as the capability to swell, shrink, bend, or degrade in response to surrounding signals. These hydrogels with unique functions are called 'smart (or intelligent) hydrogels' (Takagi et al., 1993; Park and Park, 1999). The well-known smart hydrogels are those, which respond to changes in environmental conditions like temperature, pH, glucose, electric signal, light, pressure, specific ion, specific antigen, thrombin-induced infection, and so on (Qiu and Park, 2001).

Dextran is a biodegradable polysaccharide consisting of glucose molecules coupled to long branched chains, mainly through α-1,6 and some through 1,3 glycosidic linkages. Dextrans are hydrophilic water-soluble natural

polymers, inert in biological systems, do not affect cell viability, and are used as plasma expander. Dextran and its derivatives have excellent rheological properties, and have been widely used as carrier systems for various therapeutic agents including antibiotics, antidiabetics, anticancer drugs, peptides, and enzymes (Molteni, 1979; Poznansky and Cleland, 1980).

Dextran hydrogels have been studied to be the promising carrier for the colon-targeted drug delivery (Brondsted et al., 1995a, 1995b; Hovgaard and Brondsted, 1995). Because dextranases are present in the colon, and able to degrade the polysaccharide dextran (Sery and Hehre, 1956), polymeric prodrugs based on dextran were designed for colonic drug delivery with advantage of these enzymes (Larsen et al., 1989; Chourasia and Jain, 2004).

In this study, we synthesized an enzyme-degradable and pH-sensitive hydrogel composed of glycidyl methacrylate dextran (GMD) and poly(acrylic acid, PAA) for colon-specific drug delivery. The GMD/PAA hydrogel system can exhibit an enzyme-degradability of dextran chains in the presence of dextranase.

PAA is a pH-sensitive and well-known bioadhesive polymer, which sticks to hydrated mucosal cells coating the eye, nose, mouth, lungs, gastrointestinal tract, vagina, and anus. The pH-sensitive polymers could be used for enteric coating, site specific targeting, or tumor specific delivery (Dong and Hoffman, 1991; Siegel and Firestone, 1988; Brannon-Peppas and Peppas, 1989). From the specific characters of PAA, it can be expected that the GMD/PAA hydrogel pass through the stomach with low release of incorporated drug, and reside in the colon for a long time period with high release of therapeutic molecules.

5-Aminosalicylic acid (5-ASA) has been used for the treatment of gastrointestinal inflammatory diseases (e.g., ulcerative colitis), and was chosen as a model drug. This study reports the preparation and evaluation of the GMD/PAA hydrogel for colon-specific drug delivery in vitro.

MATERIALS AND METHODS

Materials

Dextran from Leuconostoc mesenteroides with average molecular weights of 70,000 and dextranase from Penicillium sp. (EC 3.2.1.11, 3.6 U/mg solid) were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Acrylic acid was purchased from Junsei Chemical Co. Ltd. (Tokyo, Japan) and purified by vacuum distillation. Ammonium peroxydisulfate (APS) and glycidyl methacrylate were purchased from Fluka AG (Buchs, Switzerland). 4-(N,N-dimethylamino)pyridine (DMAP) was obtained from Aldrich Chemical Co. Inc. (Milwaukee, WI, U.S.A.). 2,2'-Azobisisobutyronitrile (AIBN) was obtained from Poly-

sciences (Warrington, PA, U.S.A.). All other chemicals were reagent grade or above, and used without further purification.

Synthesis of GMD

GMD was synthesized according to the previous method (Van Dijk-Wolthuis et al., 1995; Kim et al., 2000). In summary, dextran (50.0 g) was dissolved in dimethylsulfoxide (DMSO, 450 mL) under nitrogen atmosphere. After dissolution of DMAP (10.0 g), glycidyl methacrylate was added. The solution was stirred at room temperature for 48 h, and the reaction stopped by adding an equimolar amount of concentrated HCl to neutralize the DMAP. The reaction mixture was transferred to a dialysis membrane and extensively dialyzed for 2 weeks against distilled water at 4°C. GMD was lyophilized, and the white fluffy product was stored at -20°C until use. Synthesized GMD was characterized by Fourier transform-infrared (FT-IR) spectroscopy (Nicolet, Magna IR 550).

Preparation of GMD/PAA hydrogel

APS was added to the solution of GMD in phosphate-buffered solution (PBS, 0.1 M, pH 7.4), and the solution was mixed well. GMD solution was polymerized by the UV irradiation for 80 min. Acrylic acid and AIBN was added into the solution, and polymerized at 80°C for 10 h. After polymerization, the product GMD/PAA hydrogel was washed with 1% $NaHCO_3$ solution to remove the unreacted acrylic acid monomer. The GMD/PAA hydrogel was washed with alcohol, and then dried at room temperature.

Measurement of swelling ratio

The swelling ratio was measured by weighing the GMD/PAA hydrogel after wiping the excess water on the surface. Swelling ratio was calculated as W_s/W_d, where W_s and W_d are wet weight and dry weight of the hydrogel, respectively.

The concentration of the dextranase was fixed at 2 U/mL, because there was no significant difference in the swelling ratio and the drug release in the range of 0.5~2 U/mL.

Drug loading and release studies in vitro

The drug was loaded by soaking the GMD/PAA hydrogel in a saturated solution of 5-ASA in order to achieve a stable drug loading in the hydrogel. Then, the 5-ASA-loaded hydrogel was dried in vacuum at room temperature. For the release studies, the hydrogel was introduced into a vial with 10 mL of 0.1 M PBS (pH 7.4). The vial was stirred in a shaker at 37°C, and at predetermined time intervals, whole volume of the release medium was replaced with fresh buffer solution. The

concentration of released 5-ASA was measured using a UV-VIS spectrophotometer (Shimadzu UV-1201, Japan) at 329 nm. All the experiments were repeated 3 times, and represented as mean ± S.D.

RESULTS AND DISCUSSION

Synthesis and characterization of the GMD/PAA hydrogel

The synthesis of GMD had been previously described (Kim et al., 2000). In this procedure, dextran was reacted with glycidyl methacrylate in DMSO in the presence of a base DMAP as a catalyst. The hydroxyl groups of dextran might be polarized by the base and react subsequently with the less hindered methylene carbon of the epoxy group of glycidyl methacrylate. In the FT-IR measurements, the broad absorption band at 3,427 cm^{-1} was detected at all spectra according to many hydroxyl groups of dextran. At 1,701 cm^{-1}, a new absorption band was observed in the GMD due to the ester carbonyl group of glycidyl methacrylate (data not shown).

Since the swelling of hydrogel was an important factor in regulating the permeability of drugs and the rates of enzymatic degradation (Pitarresi, 2003), the swelling ability of the GMD/PAA hydrogel had been assessed as a function of pH. Fig. 1 showed the swelling ratio of GMD/PAA hydrogel against the incubation time with various pH values. This result represented that the swelling of GMD/PAA hydrogel was dependent on the solution pH and increased with pH. Because PAA was a pH-sensitive polymer with pendant acidic groups to the polymer backbone which released protons in response to appropriate pH, it was thought that the increased electrostatic repulsion between negatively charged carboxyl groups and the osmotic effect by the increase in counter ions caused the increased hydrophilicity of the hydrogel
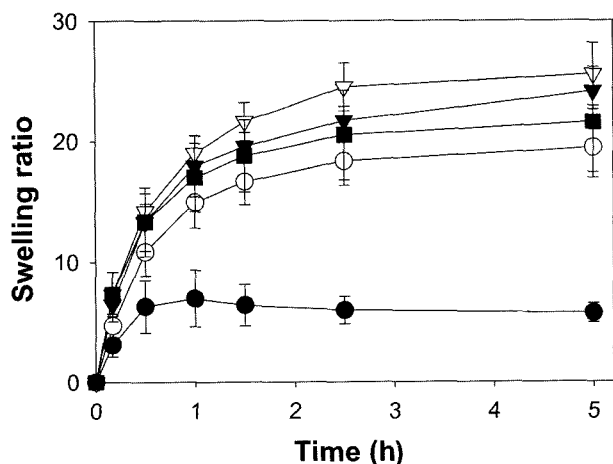
network and the up-regulation of swelling ratios at relatively high pH.

Hydrogel containing carboxylic acid groups showed a sudden or gradual change in dynamic and equilibrium swelling behavior as a result of changing the external pH (Zhang et al., 2004). In Fig. 2, the dramatic swelling shifts of the GMD/PAA hydrogel were represented by altering the medium pH. The oscillatory swelling was dependent on the pH, and showed the reversibility of swelling by repeated 3 cycles between pH 2 and pH 7.4. It might be considered that the reversible swelling fluctuation of the GMD/PAA hydrogel was the result from the effect of the hydrogen bonding formation and destruction by switching pH alternately.

The swelling of the GMD/PAA hydrogel was also affected by the presence of dextranase as shown in Fig. 3. The
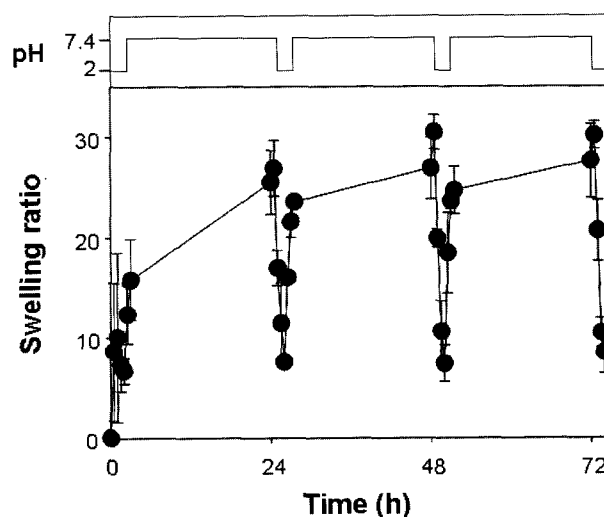
Fig. 2. Oscillatory swelling changes of the GMD/PAA hydrogel in response to pH. The buffer pH was switched by the fluctuation between pH 2 and pH 7.4.

Fig. 1. Effect of pH on the swelling ratio of the GMD/PAA hydrogel (● pH 2; ○ pH 4.4; ▼ pH 6.4; ▽ pH 7.1; ■ pH 7.4)
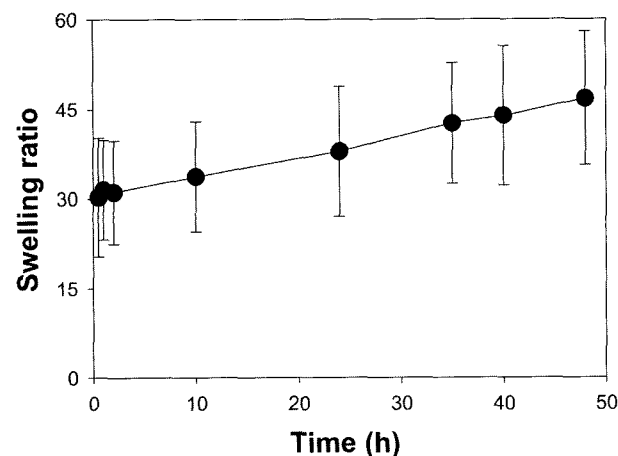
Fig. 3. Swelling of the GMD/PAA hydrogel in the presence of dextranase (2 U/mL) after the equilibration for 48 h in PBS (0.1M, pH 7.4)

hydrogel was pre-equilibrated in PBS (0.1 M, pH 7.4) for 48 h, and then moved into the medium treated with dextranase, which hydrolyzed α-1,6 glycosidic linkages (Hennink et al., 1997). The swelling ratio was increased more and more, which represented the degradation of dextran moiety in the GMD/PAA hydrogel by dextranase and relaxation of the hydrogel network following the degradation.

In addition, for evaluating the swelling of the GMD/PAA hydrogel in the gastrointestinal (GI) condition, we tested the swelling behavior by simulating with the proper pH in GI tract as shown in Fig. 4. The GMD/PAA hydrogel was sequentially soaked into the artificial gastric or intestinal pH solution (pH 2, pH 4.4, pH 6.4, pH 7.1, and pH 7.4) under considering the residence time of GI tract. Swelling of the GMD/PAA hydrogel in the stomach was minimal and the extent of swelling increased as the hydrogel passed down the intestinal tract due to the increase of pH, leading to ionization of the carboxyl groups (Qiu and Park,
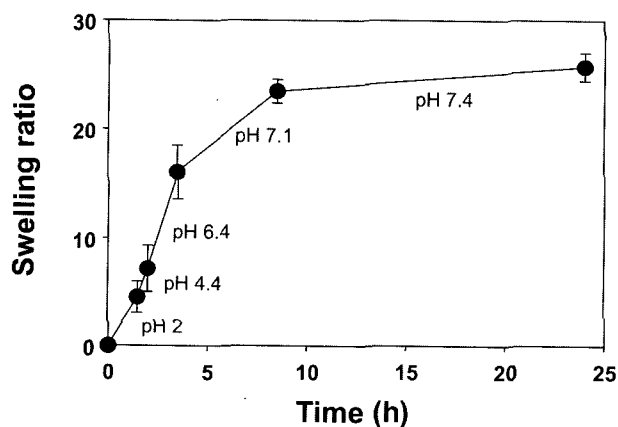

Fig. 6. 5-ASA release from the GMD/PAA hydrogel against simulated GI pH for 24 h. From the time of arrow, 2 U/mL of dextranase was added to the release medium.

2001). In the transition range from gastric to intestinal pH, the swelling was higher coincided with the result from Fig. 1.

In vitro drug release studies

As shown in Fig. 5, 5-ASA release was enhanced by 4~5 times with dextranase (2 U/mL) at pH 7.4 (0.1 M, PBS), and reached about 60% in 2 days in the presence of dextranase. From this result, it could be expected that the GMD/PAA hydrogel might represent the delayed release of drug in colon and the stimulation of drug release by the degradation of hydrogel matrix under the residence of dextranase.

With the same method in Fig. 4, the drug release was investigated by the sequential change of medium pH and additionally the dextranase in Fig. 6. The injection time of dextranase was shown as an arrow, which was expected that the hydrogel arrived at the colon around the time. The release content of 5-ASA was not significant in gastric and intestinal pH also. However, the addition of dextranase remarkably increased the release amount of drug from the GMD/PAA hydrogel, suggesting that the dextranase degraded the GMD/PAA hydrogel matrix by hydrolyzing glycosidic bonds and formed some paths to pass the drug molecules (Franssen and Hennink, 1997).

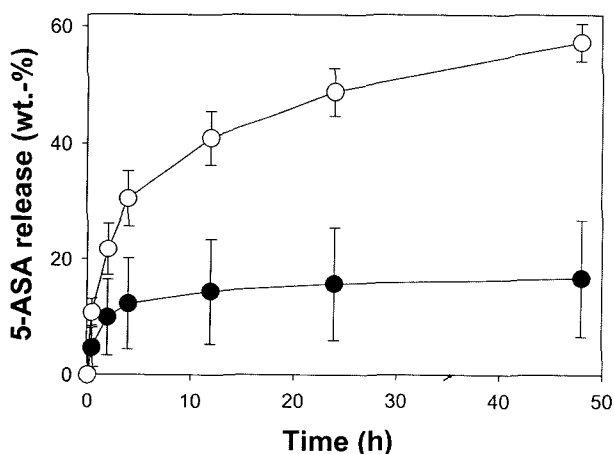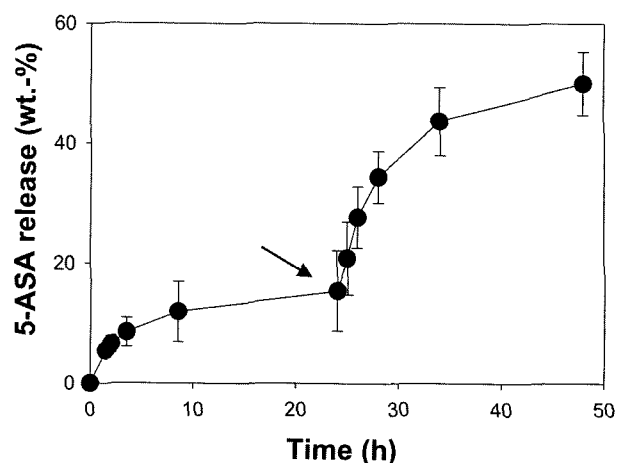**Fig. 4.** Swelling behavior of the GMD/PAA hydrogel in the simulated GI solution against pH

Fig. 5. 5-ASA release from the GMD/PAA hydrogel in the absence (●) and presence (○) of dextranase in PBS (0.1 M, pH 7.4). [Dextranase] = 2 U/mL.

CONCLUSION

A pH-sensitive and enzyme-degradable hydrogel was successfully prepared from GMD and PAA. The GMD/PAA hydrogel may be used as a novel colon-specific drug delivery device, due to the pH-sensitivity of PAA and enzymatic degradability of GMD. Moreover, it is possible that the hydrogel is used as a sustained release system in the colon using the mucoadhesive property of PAA.

ACKNOWLEDGEMENT

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