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Albumin Release from Biodegradable Hydrogels Composed of Dextran and Poly(Ethylene Glycol) Macromer

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Biodegradable hydrogels based on glycidyl methacrylate dextran (GMD) and dimethacrylate poly(ethylene glycol) (DMP) were proposed for colon-specific drug delivery. GMD was synthesized by coupling of glycidyl methacrylate with dextran in the presence of 4-(N,N-dimethylamino)pyridine (DMAP) using dimethylsulfoxide as a solvent. Methacrylate-terminated poly (ethylene glycol) (PEG) macromer was prepared by the reaction of PEG with methacryloyl chloride. GMD/DMP hydrogels were prepared by radical polymerization of phosphate buffer solution (0.1M, pH 7.4) of GMD and DMP, using ammonium peroxydisulfate (APS) and UV as initiating system. The synthetic GMD, DMP, and GMD/DMP hydrogels were characterized by fourier transform infrared (FT-IR) spectroscopy. The FITC-albumin loaded hydrogels were prepared by adding FITC-albumin solution before UV irradiation. Swelling capacity of GMD/DMP hydrogels was controlled not only by molecular weight of dextran, but also by incorporation ratio of DMP. Degradation of the hydrogels has been studied in vitro with dextranase. FITC-albumin release from the GMD/DMP hydrogels was affected by molecular weight of dextran and the presence of dextranase in the release medium.

Key words: Biodegradable, Hydrogel, Dextran, Poly(ethylene glycol), Colon-specific drug delivery

INTRODUCTION

Various bioactive proteins and peptides can now be produced in large quantities using biotechnological routes. The oral administration of peptide drugs is well known to be precluded by their digestion in the stomach and small intestine (Lee and Yamamoto, 1990). Therefore, proteinaceous drugs have to be delivered parenterally, but due to rapid elimination from the circulation, continuous or repeated administration is necessary to achieve a therapeutic effect. Consequently, there is a need for delivery systems for pharmaceutically active proteins. Delivery systems can be used to obtain a sustained (ideally zero-order) release of protein. A number of delivery systems for proteins are presently under investigation, among which liposomes (Crommelin and Schreier, 1994), polymeric nanoparticles and microspheres (Brannon-Peppas, 1995; Coevreur and Puisieux, 1993), implants made from biodegradable polymers and hydrogels. Biodegradable polymeric systems for controlled release drug delivery have been extensively studied because an invasive technique such as surgery is not required after their use (Heller, 1993; Okano *et al.*, 1994). Especially, biodegradable hydrogels have been represented as an attractive drug formulation because of their advantages such as biocompatibility, high responsibility for specific degradation, and a feasible approach to incorporate drugs into matrices (Kim *et al.*, 1992; Park *et al.*, 1993; Chen *et al.*, 1995; Kamath and Park, 1993).

Dextran is a polysaccharide consisting of glucose molecules coupled into long branched chains, mainly through a 1,6- and some through a 1,3-glucosidic linkages. Dextrans are colloidal, hydrophilic and water-soluble substances, inert in biological systems and do not affect cell viability. Because of these properties, dextrans have been studied as a carrier system for a variety of therapeutic agents including antidiabetics, antibiotics, anticancer drugs, peptides and enzymes (Molteni, 1979; Poznansky and Cleland, 1980). A special kind of microbial enzymes, dextranases, which are able to degrade the polysaccharide dextran, were found

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to be present in the colon. It was concluded that the dextran molecule was degraded by microbial dextranases making the ester bond accessible to hydrolysis, releasing the drug (Larsen *et al.*, 1989).

In this paper, the GMD/DMP hydrogels were synthesized and characterized in vitro. We reported on the use of GMD/DMP hydrogels for the controlled release of FITC-albumin. The factors affecting the FITC-albumin release were evaluated and possibilities to tailor the release by dextranase were studied.

MATERIALS AND METHODS

Materials

Dextran from *Leuconostoc mesenteroides* with average molecular weights of 70,000 and 39,000, PEG with average molecular weight of 8,000, dextranase from *Penicillium sp.* (EC 3.2.1.11, 3.6 U/mg solid), and FITC-albumin (bovine-fluorescein isothiocyanate) were obtained from Sigma Chemical Co.. APS and glycidyl methacrylate were purchased from Fluka AG, Buchs, Switzerland. Methacryloyl chloride and DMAP were obtained from Aldrich Chemical Co. Inc.. All other chemicals were reagent grade or above, and used without further purification.

Synthesis of GMD and DMP

GMD was synthesized and characterized essentially as previous report (Van Dijk-Wolthuis *et al.*, 1995). Briefly, dextran (50.0 g) was dissolved in dimethylsulfoxide (DMSO) (450 ml) in a stoppered 1 L round bottom flask under nitrogen atmosphere. After dissolution of DMAP (10.0 g), calculated amount of glycidyl methacrylate was added. The solution was stirred at room temperature for 48 h, and the reaction was stopped by adding an equimolar amount of concentrated HCl to neutralize the DMAP. The reaction mixture was transferred to a dialysis tube 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 before use.

Purified PEG (2×10^{-3} mol) was dissolved in 150 ml of benzene in a 500 ml round bottom flask and cooled to 0°C. DMP was synthesized by the method of our previous paper (Kim *et al.*, 1996). Briefly, methacryloyl chloride (90.57 ml) and triethylamine (0.49 ml) were added to the flask and stirred for 3 h at 80°C. The reaction mixture was filtered to remove triethylamine hydrochloride, the macromer was obtained by pouring the filtrate into an excess of n-hexane. Finally, it was dried at 40°C under reduced pressure for a day.

Preparation of GMD/DMP hydrogels and FITC-albumin loading

APS (10% of polymer weight) as a free radical initiator was added into the solution of GMD and DMP (weight ratio of 10:1, 10:5, and 10:10) in 0.1M phosphate buffer solution (PBS; pH 7.4). The solution was bubbled for 5 min with nitrogen, and injected into a mold to crosslink the macromers by LWUV lamp (Toshiba Chemical Lamp FL 20LB: wave range 300~400 nm, maximum intensity 360 nm) for 80 min. After UV irradiation, the mold was kept in a drying oven to remove the solvent. The dried hydrogels were immersed in deionized water to remove the unreacted matters. The GMD/DMP hydrogels subsequently immersed in ethanol to remove the water, and then residual solvent was removed under vacuum for 2 days. Fourier transform infrared spectroscopy (FT-IR, Nicolet, Magna IR 550) were examined to determine the synthesis of GMD, DMP and GMD/DMP hydrogels.

FITC-albumin was dissolved in PBS (0.1M, pH 7.4) and added to the mixed solution of GMD and DMP. After adding the calculated amount of APS, the solution was injected into a mold and irradiated by UV light. After the removal of unreacted substances, the GMD/DMP hydrogels were washed with ethanol and dried.

Swelling and enzymatic degradation of GMD/DMP hydrogels

The swelling behavior of the GMD/DMP hydrogels was established by measuring the weight of the hydrogels after wiping the excess water on the surface. The swelling degree (=water uptake) was calculated as (Ws-Wd)/Wd $\times$ 100, where Ws and Wd are swollen weight and dry weight of the hydrogels, respectively.

The degradation of GMD/DMP hydrogels by dextranase was examined in vitro. After equilibration in 0.1M PBS (pH 7.4), the hydrogels were mounted in a nylon mesh-bag attached to a thread for dipping into solutions and immersed into dextranase solution (concentration : 0.2 U/ml) with stirring to initiate the degradation. The degradation rate was estimated by measuring the residual weight of the hydrogel together with the nylon mesh-bag. Also, FITC-albumin release from the degraded hydrogel was estimated by measuring the absorbance at 493 nm by UV-VIS spectrophotometer.

FITC-albumin release from GMD/DMP hydrogels

FITC-albumin loaded GMD/DMP hydrogels were introduced into a bottle with 10 ml of 0.1M PBS (pH 7.4). The bottle was allowed to stir in a shaker whose temperature was maintained at 37°C. At predetermined time intervals, the first buffer solution of the whole volume was withdrawn and 10 ml of the second buffer solution was put into the bottle. The release of FITC-albumin from GMD/DMP hydrogels was estimated with UV-VIS spectrophotometer (Shimadzu, UV-1201, Japan) by measuring the absorbance at 493 nm.

RESULTS AND DISCUSSION

Synthesis and analysis of GMD, DMP, and GMD/DMP hydrogels

The synthesis of dextran derivatized with polymerizable groups has been previously described (Edman *et al.*, 1980). In their procedure, the incorporation of acrylate groups was low, and this can most likely be ascribed to the aqueous basic reaction conditions. Therefore, it was developed a novel and more efficient method to obtain dextran with polymerizable groups. In this procedure, dextran was reacted with glycidyl methacrylate in DMSO as a polar aprotic solvent. The coupling reaction of glycidyl methacrylate to dextran was studied in the presence of DMAP as a basic catalyst. The hydroxyl groups of dextran are polarized by the base and react subsequently with the less hindered methylene carbon of the epoxy group of glycidyl methacrylate (not shown in Fig.) (Van Dijk-Wolthuis *et al.*, 1995).

The synthetic evidence of GMD and DMP was observed by the FT-IR spectroscopy. The FT-IR spectra of dextran and GMD showed a broad absorption around 3,427 cm^{-1} due to many hydroxyl groups of dextran. At 1701 cm^{-1}, a new absorption was detected in the GMD due to the carbonyl group of ester carbonyl of glycidyl methacrylate. The FT-IR spectrum of the PEG showed an absorption band at 3,459 cm^{-1} due to the terminal hydroxyl group (Deng *et al.*, 1990; Kim *et al.*, 1996). This band became weak in the PEG macromer due to methacrylation. A new absorption was seen at 1,725 cm^{-1} in the DMP due to the carbonyl bond of methacryloyl group (Pavia *et al.*, 1979). The band at 2,889 cm^{-1} was attributed to the C-H stretch (Andini *et al.*, 1988) and was present in both polymers. Fig. 1 shows the results of FT-IR spectra of GMD (a), DMP (b), and the GMD/DMP hydrogels (c). The strong hydroxyl stretching vibration peak of 3,427 cm^{-1} is due to the dextran. The methyl peak near to carbonyl group of GMD is shown at 2,928 cm^{-1} and this is not changed by preparation of GMD/DMP hydrogels.

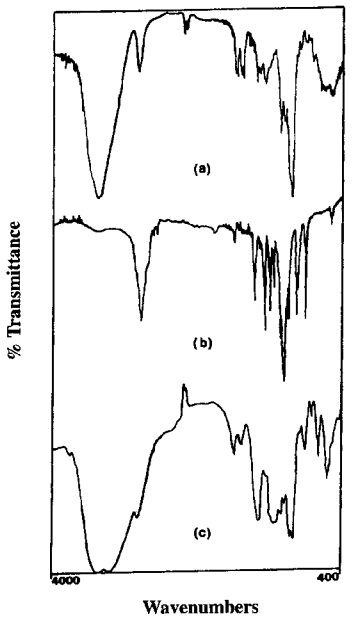

Fig. 1. FT-IR spectra of GMD (a), DMP (b), and GMD/DMP hydrogel (c).

Properties of GMD/DMP hydrogels

In the drug delivery systems using biodegradable hydrogels, the swelling capacity of hydrogels are very important factor because it controls swelling degree and drug release kinetics. This swelling capacity is altered by incorporation of PEG macromer to the dextran matrices. Since the swelling capacity of GMD matrices was relatively low and hardly controlled, the incorporation of DMP can improve the physico-chemical properties of dextran matrices such as drug release kinetics and degradation rate.

The swelling degree of GMD/DMP hydrogels as a function of molecular weight of dextran was shown in Fig. 2. Fig. 2 shows the effect of molecular weight of dextran on the swelling degree of GMD/DMP hydrogels. In higher molecular weight of dextran, the swelling degree becomes relatively restricted and decreased. This is probably due to the increased formation of entanglements, when high molecular weight of dextran is used. Fig. 3 shows the effect of incorporation ratio of DMP on the swelling of GMD/DMP hydrogel matrices. According to the incorporation of DMP is increased, the swelling is also expanded. This result indicated that the swelling capacity of GMD/DMP matrices can be controlled not only by molecular weight of dextran, but also by the incorporation ratio of DMP.

For evaluating the degradation rate of GMD/DMP hydrogels, matrices were immersed in 0.1M PBS (pH 7.4) containing dextranase (0.2 U/ml) and incubated in a thermostated

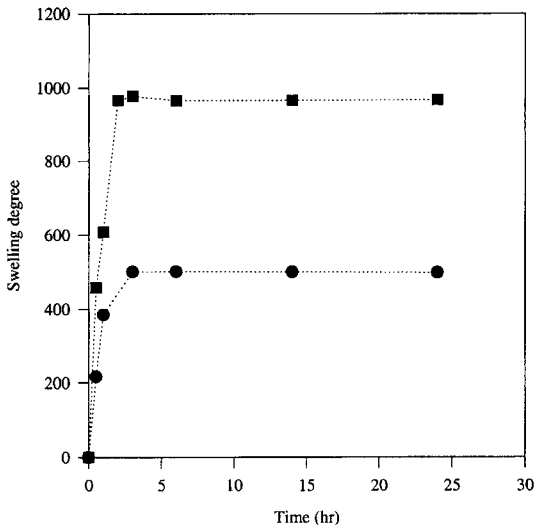

Fig. 2. Swelling degree of GMD/DMP hydrogels. The molecular weight of dextran was 70,000 (●) and 39,000 (■). The ratio of GMD:DMP was 10:1.

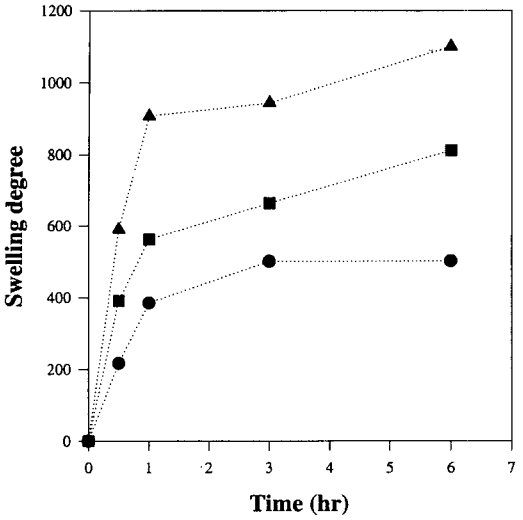

Fig. 3. Swelling degree of GMD/DMP hydrogels by different incorporation ratio of DMP (● , GMD:DMP=10:1; ■ , GMD:DMP =10:5; ▲ , GMD:DMP=10:10).The molecular weight of dextran was 70,000.

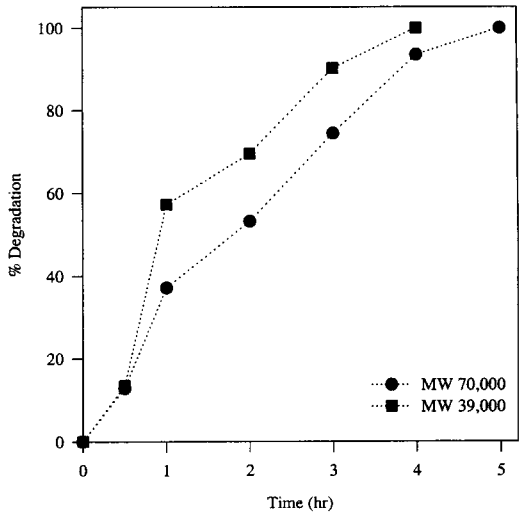

Fig. 4. Degradation of GMD/DMP hydrogels by dextranase at 37°C. The molecular weight of dextran was 70,000 (●) and 39,000 (■), respectively. The concentration of dextranase was 0.2 U/ml.

water bath at 37°C. Fig. 4 shows the effect of dextran molecular weight on the degradation of GMD/DMP hydrogel matrices. The degradability of the hydrogels was evaluated by the time to complete dissolution of the hydrogels. The higher molecular weight of dextran was induced slower degradation of hydrogel matrices. In the higher molecular weight of dextran, the swelling degree was low and the enzyme slowly penetrated into the hydrogel, resulting in a retarded association of the enzyme with the hydrogel.

FITC-albumin release from the GMD/DMP hydrogels

In Fig. 5, the release of albumin, as a model protein, from the GMD/DMP hydrogels was studied in 0.1 M PBS (pH 7.4), in the presence and absence of dextranase. The release rate was found to be dependent on the molecular weight and the existence of dextranase. When the dextranase was not present, the release rate was lower than the presence of dextranase and controlled by simple diffusion. Only about 25% of FITC-albumin was released after 24 h. When dextranase was present, the release of drug reaches 100% after 20 h at the 39,000 molecular weight and the release profile was more complex. In this case the release was at first diffusion controlled, then later degradation controlled. We found that FITC-albumin release from the higher molecular weight of dextran was slower than the lower molecular weight of dextran, and it can be explained as the difference of entanglements.

In conclusion, we have prepared hydrogels based on GMD and DMP by radical polymerization using APS and UV irradiation for initiation. The synthetic polymer of GMD, DMP, and GMD/DMP hydrogel was characterized by FT-IR measurement. Since the swelling capacity of

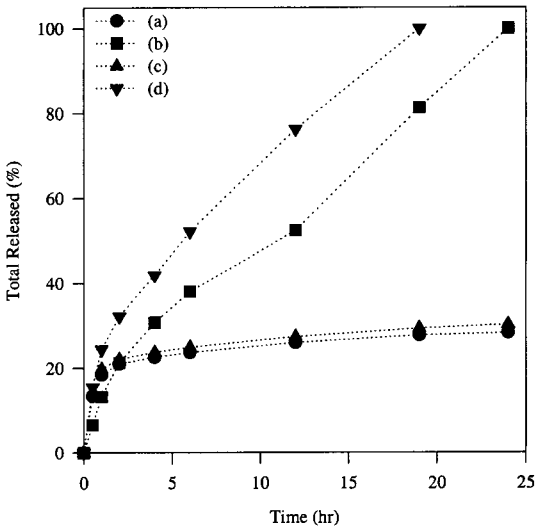

Fig. 5. Release profiles of FITC-albumin from GMD/DMP hydrogels. The molecular weight of dextran of (a) and (b) was 70,000, and that of (c) and (d) was 39,000. The sample (b) and (d) were examined with dextranase in the release medium.

GMD matrices was relatively low and hardly controlled, the incorporation of DMP can easily alter the physicochemical properties of dextran matrices such as drug release kinetics and degradation rate. The FITC-albumin release rate was found to be dependent on the molecular weight and the existence of dextranase. This hydrogel system is an attractive system for protein delivery because the materials have good biocompatibility with possibilities of manipulating the release characteristics of FITC-albumin. As an approach to drug delivery, peptide and protein drugs could be incorporated with this system to form a protective carrier.

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