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Doxorubicin Release from Core-Shell Type Nanoparticles of Poly(DL-lactide-co-glycolide)-Grafted Dextran

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In this study, we prepared core-shell type nanoparticles of a poly(DL-lactide-co-glycolide) (PLGA) grafted-dextran (DexLG) copolymer with varying graft ratio of PLGA. The synthesis of the DexLG copolymer was confirmed by ^{1}H nuclear magnetic resonance (NMR) spectroscopy. The DexLG copolymer was able to form nanoparticles in water by self-aggregating process, and their particle size was around 50 nm~300 nm according to the graft ratio of PLGA. Morphological observations using a transmission electron microscope (TEM) showed that the nanoparticles of the DexLG copolymer have uniformly spherical shapes. From fluorescence probe study using pyrene as a hydrophobic probe, critical association concentration (CAC) values determined from the fluorescence excitation spectra were increased as increase of DS of PLGA. ^{1}H-NMR spectroscopy using D_2O and DMSO approved that DexLG nanoparticles have core-shell structure, i.e. hydrophobic block PLGA consisted inner-core as a drug-incorporating domain and dextran consisted as a hydrated outershell. Drug release rate from DexLG nano-particles became faster in the presence of dextranase in spite of the release rate not being significantly changed at high graft ratio of PLGA. Core-shell type nanoparticles of DexLG copolymer can be used as a colonic drug carrier. In conclusion, size, morphology, and molecular structure of DexLG nanoparticles are available to consider as an oral drug targeting nanoparticles.

Key words: Doxorubicin, Dextran, Core-shell type nanoparticles, Poly(D,L-lactide-co-glycolide), Biodegradability, Colon delivery.

INTRODUCTION

Dextran is a polysaccharide consisting of glucose molecules coupled into long branched chains, mainly through the 1,6- and partly through the 1,3-glucosidic linkages. Dextrans are colloidal, hydrophilic and water-soluble substances, which are inert in biological systems and do not affect cell viability. Due to these properties, dextran has been extensively used as a drug carrier system, including for antidiabetics, antibiotics, anticancer drugs, peptides and enzymes (Akiyoshi *et al.*, 1998; Ichinose *et al.*, 2000; Nishikawa *et al.*, 1994). Especially, since dextran can be degraded by the colonic enzyme "dextranase", polymeric prodrugs or nanoparticles for colonic drug delivery can be designed (Molteni 1985). It was concluded that the dextran molecule could be degraded by microbial dextranases, which make the ester bond accessible to hydrolysis, thereby releasing the drugs.

Oral drug delivery system using biodegradable polymer have been extensively investigated for last few decades and it still remains one of the most promising routes of drug treatment (Andrianov and Payne, 1998; Chen and Langer, 1998; Norris *et al.*, 1998). Major obstacles to the oral drug delivery are the numerous barriers which prevent drug uptake. The main barriers are (a) the instability of bioactive agents introduced into the gastrointestinal tract, due to degradation by digestive enzymes, (b) the highly acidic pH of the gastric environment, (c) poor absorption of drugs across the intestinal lining (Norris *et al.*, 1998). To overcome these problems, colonic region have been considered for oral drug targeting due to their mild pH

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environment, ease to absorption of drug, longer transit time, and the presence of dextranase (Andrianov and Payne, 1998; Basit, 2005, Chen and Langer, 1998; Peppas and Robinson, 1995; Van Dijk-Wolthuis *et al.*, 1995).

In this study, we synthesized and characterized a PLGA-grafted dextran, for use as a nanoparticulate oral drug carrier. We expected that dextran would form the hydrophilic outer-shell, due to its solubility in water, while PLGA would form the inner core of the nanoparticle, due to its hydrophobic properties. Since the dextran domain can degrade in the colon, nanoparticles of DexLG copolymer can be used as oral drug carriers. Core-shell type nanoparticles of DexLG were characterized by ^{1}H-NMR spectroscopy, fluorescence spectroscopy, dynamic light scattering (DLS), and scanning electron microscopy (SEM).

MATERIALS AND METHODS

Materials

Dextran from *Leuconostoc mesenteroides* (average molecular weights : 77,000), dextranase, and doxorubicin (DOX) were purchased from Sigma Chem. Co. (St. Louis, U.S.A.). *N,N*-dicyclohexyl carbodiimide (DCC) and 4-(*N,N*-dimethylamino)pyridine (DMAP) were purchased from Aldrich Chemical Co. U.S.A. Poly(D,L-lactide-co-glycolide) (PLGA 5005) was purchased from Wako Pure Chem Co. Japan. The dialysis membranes with a molecular weight cutoff (MWCO) of 12,000 g/mol were purchased from Spectra/Pro™ Membranes. Dichloromethane (DCM) and dimethyl sulfoxide (DMSO) were of HPLC grade and used without further purification.

Synthesis of PLGA-g-dextran (DexLG) copolymer

The DexLG graft copolymer was synthesized by conjugating the carboxylic acid end of PLGA and the hydroxyl group of dextran using DCC as a coupling agent. For example, 770 mg of dextran and 100 mg of PLGA were dissolved separately in DMSO. 1.5 equivalent amount of DCC in DMSO solution were added to the PLGA/DMSO solution, which was then stirred for 30 min to activate the carboxyl group of the PLGA. The resulting solution was added to the Dextran/DMSO solution containing DMAP, and the reaction was allowed to continue at room temperature for 12 h. The reaction mixture was filtered off to remove the byproducts (dicyclohexylurea) and then placed into a dialysis tube. Dialysis was performed against deionized water for 3 days with exchange of water at every 3 h. After that dialyzed solution was lyophilized for 3 days and then solid product was precipitated in dichloromethane (DCM) to remove unreacted PLGA. Precipitation into DCM was repeated three times. The resulting product was dried in a vacuum oven for 2 days. After that, the dried product was dispersed in distilled water to remove unreacted dextran, with this procedure being repeated three times following lyophilization of it. The lyophilized DexLG was stored in a refrigerator at -20 until use. The copolymer recovery yield was higher than 70% (w/w) at all formulations.

^{1}H nuclear magnetic resonance spectroscopy (NMR) measurement

^{1}H-NMR spectra of the copolymers were measured in DMSO or D_2O using a 400 MHz NMR spectrometer (Varian 400 MHz NMR).

Elemental analysis

Degree of substitution of PLGA was estimated by elemental analysis using a Perkin-Elmer CHNS

Preparation of core-shell type nanoparticles

The nanoparticles were prepared by dialysis method. 40 mg of DexLG copolymer dissolved in 7 mL DMSO was introduced into dialysis tube (MWCO 12,000 g/mol) and dialyzed against deionized water for 1 day. The deionized water was exchanged every 1 h for the first 3 h, and 3 h for an additional 21 h. The dialyzed solution was then analyzed or lyophilized.

Transmission electron microscopy (TEM) observation

The morphology of the polymeric nanoparticles was observed using a TEM (JEOL JEM-2000 FX II, Japan). A drop of polymeric micelle suspension containing phosphotungstic acid (0.05% w/w) was placed onto a carbon film coated on a copper grid for TEM. Observation was done at 80 kV.

Particle size distribution measurement

Particle size of polymeric micelles were measured with a dynamic laser scattering spectrophotometer (DLS-7000, Otsuka Electonics Co. Japan). A sample solution prepared by dialysis method was used for particle size measurement (concentration: 0.1 wt.-%).

Fluorescence spectroscopy measurements

Fluorescence spectroscopy (Shimadzu RF-5301 PC spectrofluorophotometer, Shimadzu Co. Ltd., Japan) was performed to prove the potential for self-assembly formation of DexLG. DexLG nanoparticle suspension were prepared without drug as follows: 40 mg of various DexLG were dissolved in 7 mL of DMSO and dialyzed against distilled water up to 2 days as the same method described above. The resultant suspension was adjusted to various concentrations of the nanospheres.

The critical association concentration (CAC) of the DexLG

copolymer was estimated using pyrene as a hydrophobic probe (Wilhelm *et al.*, 1991; Kwon *et al.*, 1993). To prepare sample solutions, a known amount of pyrene in acetone was added to each of a series of 20 mL vials, and the acetone was evaporated. The final concentration of pyrene was 6.0×10^{-7} M. To each vial, 10 mL of various concentrations of the nanoparticle suspensions was added, and then heated for 3 h at 65°C. Equilibration of the pyrene and the DexLG nanoparticles were achieved by allowing the solutions to cool overnight at room temperature. The fluorescence excitation spectra were measured at emission wavelength of 390 nm. Excitation and emission bandwidths were 1.5 nm and 1.5 nm, respectively.

Drug loading procedure and release experiment

Preparation of drug-loaded nanoparticles was carried out as follows: 40 mg of DexLG copolymer and 10 mg of doxorubicin (DOX) were dissolved in 7 mL of DMSO and stirred magnetically for 3 h. After that, this solution was dialyzed using a molecular weight cut-off (MWCO) 12000 g/mol dialysis tube (Sigma Chemical Co., St. Louis, U.S.A.) against distilled water. The distilled water was exchanged every 1 h for 12 h. Then, the dialyzed solution was used for analysis, drug release test, or lyophilization.

Drug contents = [(drug weight in the nanoparticles)/(weight of nanoparticles)] × 100

Loading efficiency = [(Residual drug in the nanoparticle)/(initial feeding amount of drug)] × 100

The release experiment was carried out *in vitro* as follows: Dialyzed solution above mentioned adjusted a volume to 40 mL with deionized water (i.e. 1 mg polymer/mL water). 5 mL of this solution introduced into dialysis tube (MWCO: 12,000 g/mol) with or without dextranase (100 units/mL) were placed in 100 mL bottle with 95 ml of PBS, and the media stirred at 100 rpm and 37°C. At specific time intervals, the medium was taken for analysis of drug concentration. After that whole media was replaced with fresh PBS to prevent drug saturation. The concentration of the DOX released into PBS was determined using an UV-spectrophotometer (UV spectrophotometer 1201, Shimadzu Co. Japan) at 488 nm.

RESULTS AND DISCUSSION

Characterization of DexLG copolymer

Dextran consists of α-1,3 and α-1,6 glycosidic linkages and has unique properties, such as biodegradability at specific body sites, e.g. the colon. Due to its degradability in the colon, dextran is an ideal candidate for oral drug delivery systems. However, dextran itself cannot be used as a drug carrier due to its water solubility and therefore first needs to be rendered hydrophobic (Van Dijk-Wolthuis *et al.*, 1995). The synthetic scheme for the DexLG copolymer is shown in Fig. 1(a). Synthesized copolymer was purified using DCM and water to remove unreacted product, PLGA and dextran. Since the DexLG copolymer does not dissolved in DCM and PLGA can freely dissolved in DCM, un-reacted PLGA was removed by precipitation of DexLG copolymer into DCM. Subsequently, unreacted dextran was removed by precipitation of copolymer into water because of the DexLG copolymer does not ease dissolved in water. Therefore, unreacted product, dextran and PLGA itself, was not contained in the final product. At ^{1}H-NMR spectra, specific peaks of PLGA were shown at 1.5 ppm, 4.6~5.5 ppm and dextran were shown at 3.0~3.8 ppm, 4.5~5.0 ppm, respectively. As shown in Fig. 1(d), both of specific peak of dextran and PLGA were shown at ^{1}H-NMR spectra of DexLG graft copolymer.

To study molecular weight (M.W.) and degree of substitution (DS) of PLGA, elemental analysis was employed. At elemental analysis of carbon, hydrogen, and oxygen, evaluated M.W. of PLGA was 5,100, approximately. According to the increase of PLGA feeding ratio in the synthesis procedure, M.W. of DexLG copolymer was increased and DS of PLGA was also increased from 1.7 to 6.2 as summarized in Table I.

Characterization of core-shell type nanoparticles

It has been reported that microspheres or nanoparticles constitute one of the most promising oral drug delivery carriers, and which has the potential to enhance drug absorption (Allemann *et al.*, 1993; Andrianov and Payne, 1998; Carino *et al.*, 2000; Chen and Langer, 1998; Donini *et al.*, 2002; Norris *et al.*, 1998; Peppas and Robinson, 1995). Carino *et al.* (2000) reported that encapsulating insulin into nanospheres maintains its biological activity and that this form of insulin was able to control plasma glucose levels effectively. The gastrointestinal uptake of particulate carriers is reported to depend on the size of the particles and the surface properties (Desai *et al.*, 1996; Hussain *et al.*, 1997).

To make core-shell type nanoparticles, DexLG graft copolymer was dissolved in DMSO and the core-shell type nanoparticles were prepared by dialysis method against water. The morphology of core-shell type nanoparticles of DexLG copolymer was observed by SEM and the particle size was evaluated by DLS. As shown in Fig. 2, core-shell type nanoparticles of DexLG copolymer has spherical shapes in their morphology and particle size was around 50-200 nm. Fig. 3 shows the particle size distribution measured by DLS and the particle size was increased according to the increase of M.W. of DexLG copolymer as shown in Table I. These results indicated that DexLG copolymer was associated in the aqueous solution by hydro-

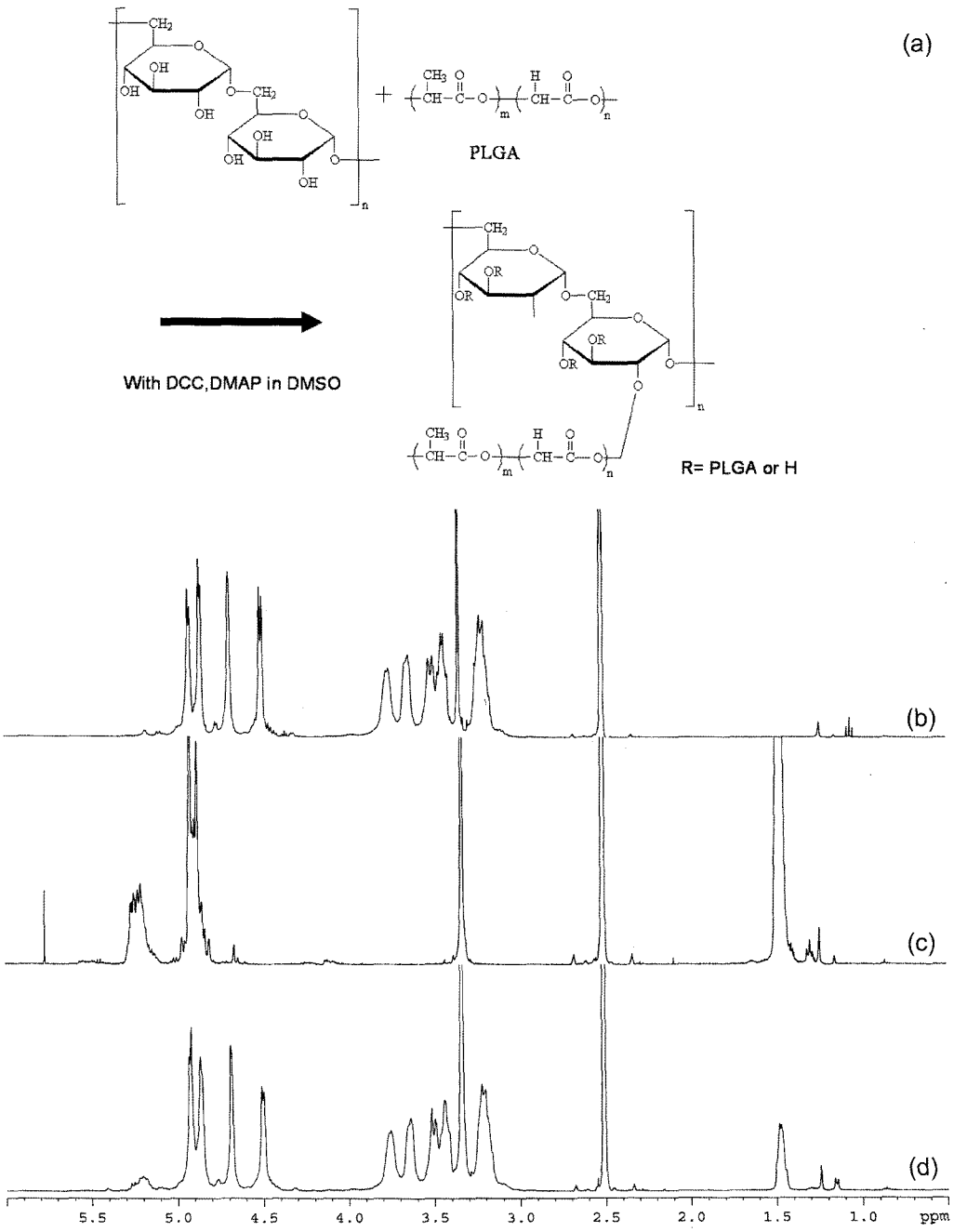

Fig. 1. Synthesis scheme of DexLG graft copolymer (a), ^{1}H-NMR spectra of dextran (b), PLGA (c), and DexLG-2 (d) in DMSO.

phobic properties of PLGA domain and made spherical nanoparticles. It is expected that DexLG nanoparticles were formed by a self-assembling process in an aqueous environment and has core-shell structures, i.e. hydrophobic inner-core formed by PLGA and dextran domain composed hydrated outershell in the core-shell type structure.

Generally, block and graft copolymers have amphiphilic characteristics in an aqueous environment and self-assembling properties (Gref *et al.*, 1994; Jeong *et al.*, 1998; Jung *et al.*, 2003; Kwon *et al.*, 1993) as described above. It was thought that the DexLG copolymer would also show amphiphilic characters in water and would form self-aggregating nanoparticles. To characterize the self-assembling behavior of the DexLG copolymer in aqueous solution, the fluorescence probe technique was employed, using pyrene as the hydrophobic probe. The fluorescence excitation spectra of pyrene at various concentrations of DexLG is shown in Fig. 4(a). The fluorescence intensity of pyrene was found to increase with increasing concentration of DexLG copolymer, which points to the self-

Table I. Characterization of PLGA-g-dextran copolymer

	M.W.[a]	DS[b]	CAC (g/L)[c]	Particle size (nm)
Dextran	77,000	-	-	-
DexLG-1	85,700	1.7	0.01	110±24.9
DexLG-2	96,400	3.8	0.006	173±40.8
DexLG-3	108,600	6.2	0.0052	230±59.6
PLGA	5,100	-	-	-

[a] Number-average molecular weight (M.W.) of DexLG was calculated from elemental analysis

[b] The degree of substitution (DS) of PLGA chains grafted per pullulan was estimated by subtracting the determined M_w of dextran from the M_w of DexLG graft copolymers and dividing by M_w of PLGA

[c] CAC was estimated from fluorescence spectroscopy measurements as explained in materials and methods.

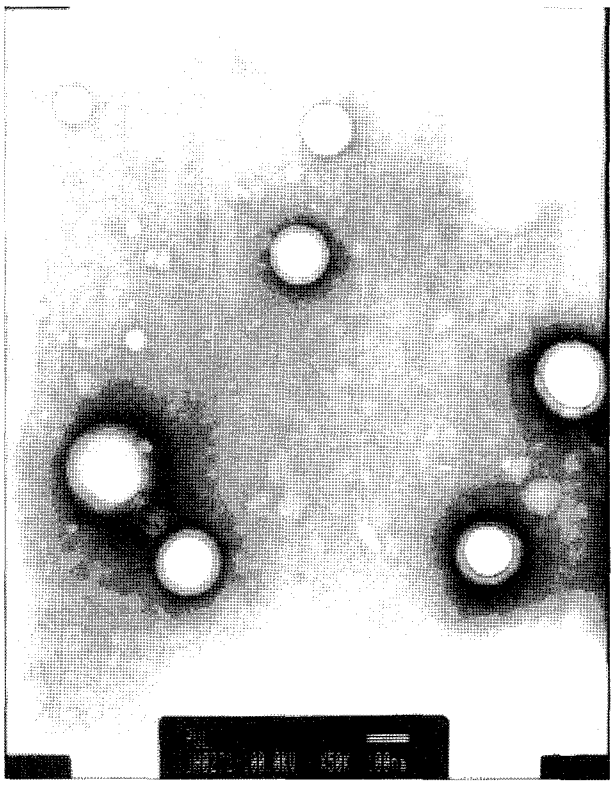

Fig. 2. TEM photographs of core-shell type nanoparticles of DexLG-1

assembly of the DexLG copolymer in water. In addition, a red shift was observed in the excitation spectra with increasing DexLG copolymer. It is thought that pyrene is preferentially solubilized into the core part of the core-shell type nanospheres. Fig. 4(b) shows the intensity ratio of I_{337}/I_{334} versus log c of DexLG copolymer for the pyrene excitation spectra. A flat region at extremely low concentration and a sigmoid change in the crossover region were observed. This result indicates that the signal change in the crossover region could be related to the CAC value of DexLG copolymer. The critical association concentration (CAC) values were determined from the fluorescence

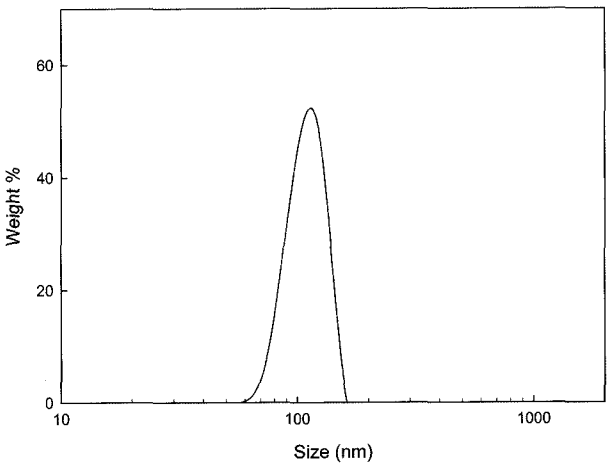

Fig. 3. Particle size distribution of core-shell type nanoparticles of dexLG-1

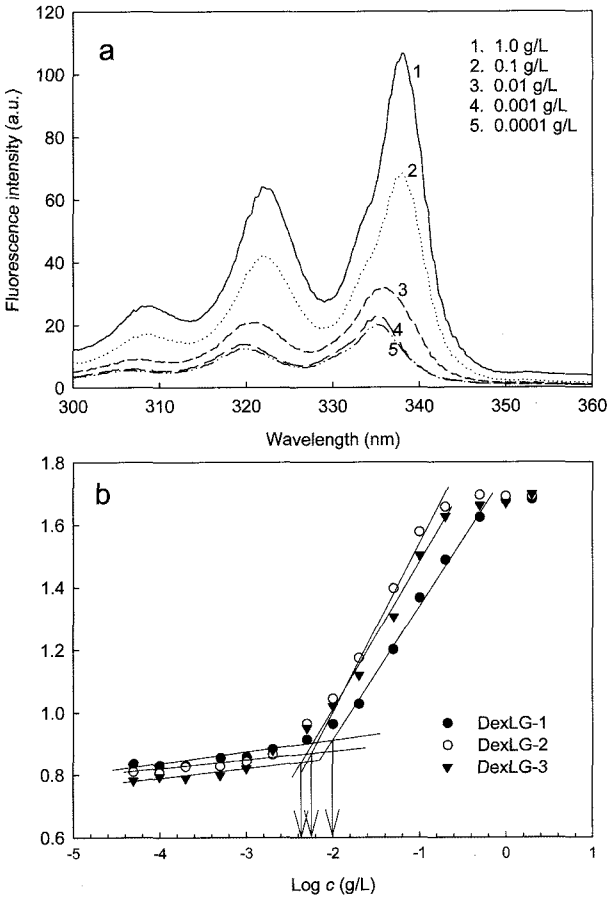

Fig. 4. Fluorescence excitation of pyrene (6.0×10^{-7} M) vs. the concentration of DexLG-2 copolymer in distilled water (λ_{em} = 390 nm) (a). Plots of the intensity ratios I_{337}/I_{334} from the pyrene excitation spectra vs. logc of the DexLG copolymers in distilled water (b).

excitation spectra and were found to be 0.01 g/L (DexLG-1 copolymer). It was suggested that the hydrophilic domain, dextran, plays the role of a flexible outershell of

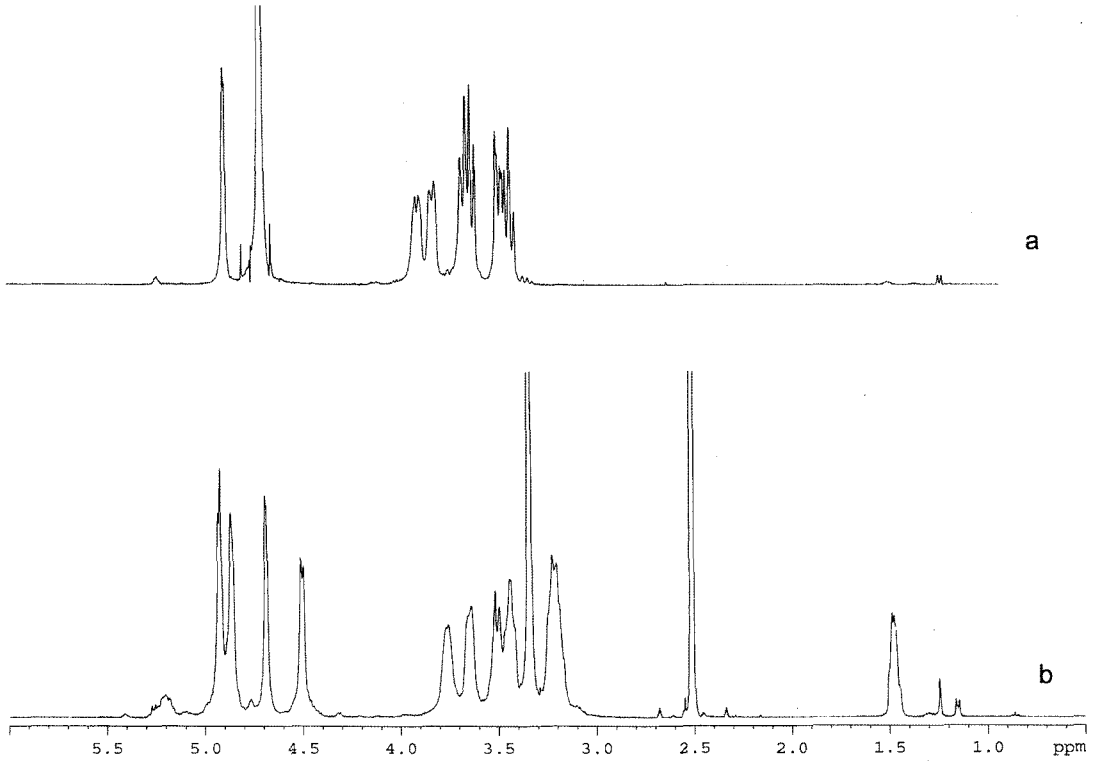

Fig. 5. ^{1}H-NMR spectra of core-shell type nanoparticles of DexLG-1 in D_2O (a), DMSO (b)

core-shell type nanoparticles in the aqueous environment and PLGA, the hydrophobic domain, forms the rigid inner core. As shown in Fig. 4(b), CAC values expressed as crossover region were decrease according to the increase of M.W. of DexLG copolymer, indicating that increase of hydrophobic domain, PLGA, makes DexLG copolymer ease to form core-shell type nanopaticles in an aqueous environment.

^{1}H-NMR spectra study of core-shell type nanoparticles of DexLG copolymer is another way to prove core-shell structure of nanoparticles. As shown in Fig. 5, ^{1}H-NMR spectra of core-shell type nanoparticles of DexLG copolymer at DMSO showed both peaks of dextran and PLGA (Fig. 5(b)) while specific peaks of dextran was only appeared at D_2O (Fig. 5(a)). These results indicated that the core of the nanoparticles composed of liphophilic PLGA and the shell was composed of dextran. This core-shell structure of nanoparticles can be considered to target colonic region because the surface-oriented structure of dextran will be able to ease the degradation of the dextran shell by colonic enzyme, dextranase.

Drug release from core-shell type nanoparticles of DexLG nanoparticles

From the experiment of the drug loading test, drug contents were increased according to the increased M.W. of DexLG copolymer from 5.7% (w/w) and 7.5% (w/w) as shown in Table II. Drug-incorporated DexLG nanoparticles showed more increased particle size than empty nanoparticles. The size of DexLG nanoparticles are suggested to acceptable oral drug delivery carriers because the efficiency of the intestinal uptake of drug-encapsulating particles is known to be dependent on the particle size (Desai *et al.*, 1996).

Table II. Characterization of core-shell type nanoparticles of DexLG copolymer

	DexLG/DOX weight ratio (mg/mg)	Drug contents (%, w/w)	Loading efficiency (%, w/w)	Particle size (nm)
DexLG-1	40/10	5.7	24.2	136.8±53.4
DexLG-2	40/10	7.1	30.6	212.1±15.1
DexLG-3	40/10	7.5	32.4	277.0±30.4

Since dextran can be degraded by dextranase presented in the colon, drug release from core-shell type nanoparticles of DexLG copolymer was tested in the presence or absence of dextranase. In Fig. 6, initial burst of drug release was shown for 1 day and then continuously released until day 4. The drug release became especially faster in the presence of enzyme than in the absence of enzymes. Furthermore, the higher the M.W. of DexLG copolymer the lower the release rate of the drug. The discrepancy of drug release rate in the presence of dextranase was decreased at higher DS of PLGA. These

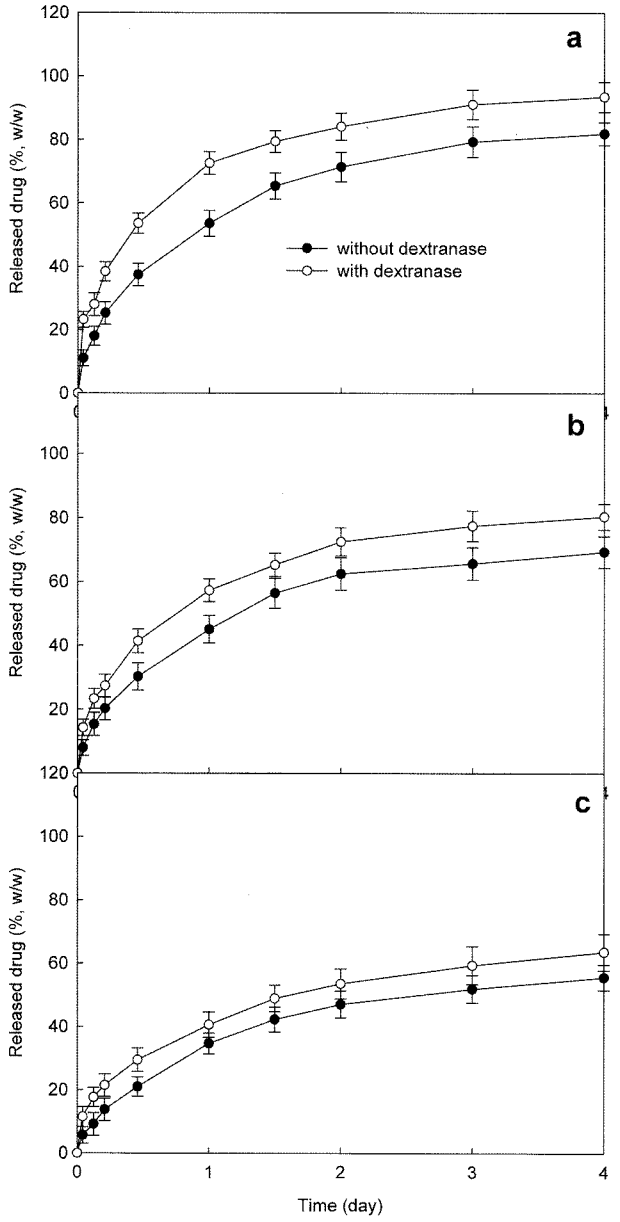

Fig. 6. Drug release from core-shell type nanoparticles of DexLG copolymer with and without dextranase

results showed that core-shell type nanoparticles of DexLG are considered to an acceptable vehicles for colonic drug delivery and higher dextran content in the copolymer is required to target colon.

CONCLUSION

The DexLG copolymer was synthesized by varying degree of PLGA graft ratio. The structure of the DexLG copolymer was confirmed by ^{1}H NMR spectroscopy. The DexLG copolymer was able to form nanoparticles in water by self-aggregating process, and their particle size was around 50 nm~300 nm according to the DS of PLGA. Morphology of DexLG nanoparticles was uniformly spherical shapes. From fluorescence probe study using pyrene as a hydrophobic probe, CAC values were increased as increase of DS of PLGA. Furthermore, core-shell structure of DexLG nanoparticles was approved by ^{1}H-NMR spectroscopy using D_2O and DMSO, i.e. inner-core of the nanoparticles are consisted of PLGA, while hydrated outershell consisted of dextran. Drug release rate from DexLG nano-particles became faster in the presence of dextranase. Core-shell type nanoparticles of DexLG copolymer can be used as an colonic drug carrier.

REFERNCES

Akiyoshi, K., Kobayashi, S., Shichibe, S., Mix, D., Baudys, M., Kim, S. W., and Sunamoto, J., Self-assembled hydrogel nanoparticle of cholesterol-bearing pullulan as a carrier of protein drugs: complexation and stabilization of insulin. *J. Control. Release.*, 54, 313-320 (1998).

Allemann, E., Gurny, R., and Doelker, E., Drug-loaded nanoparticles-preparation methods and drug targeting issues. *Europ. J. Pharm. Biopharm.*, 39, 173-191 (1993).

Andrianov, A. K. and Payne, L. G., Polymeric carriers for oral uptake of microparticulates. *Adv. Drug Del. Rev.*, 34, 155-170 (1998).

Basit, A. W., Advances in colonic drug delivery. *Drugs*, 65, 1991-2007 (2005).

Carino, G. P., Jacob, J. S., and Mathiowitz, E., Nanosphere based oral insulin delivery. *J. Control. Release.*, 65, 261-269 (2000).

Chen, H. and Langer, R., Oral particulate delivery: status and future trends. *Adv. Drug Del. Rev.*, 34, 339-350 (1998).

Desai, M. P., Labhasetwar, V., Amidon, G. L., and Levy, R. J., Gastrointestinal uptake of biodegradable microparticles: effect of particle size. *Pharm. Res.*, 13, 1838-1845 (1996).

Donini, C., Robinson, D. N., Colombo, P., Giordano, F., and Peppas, N. A., Preparation of poly(methacrylic acid-g-poly (ethylene glycol)) nanospheres from methacrylic monomers for pharmaceutical applications. *Int. J. Pharm.*, 245, 83-91 (2002).

Gref, R., Minamitake, Y., Peracchia, M. T., Trubetskoy, V., Torchilin, V., and Langer, R., Biodegradable long-circulating polymeric nanospheres. *Science*, 263, 1600-1603 (1994).

Hussain, N., Jani, P. U., and Florence, A. T., Enhanced oral uptake of tomato lectin-conjugated nanoparticles in the rat. *Pharm. Res.*, 14, 613-618 (1997).

Ichinose, K., Tomiyama, N., Nakashima, M., Ohya, Y., Ichikawa, M., Ouchi, T., and Kanematsu, T., Antitumor activity of dextran derivatives immobilizing platinum complex (II). *Anticancer Drugs*, 11, 33-38 (2000).

Jeong, Y. I., Cheon, J. B., Kim, S. H., Nah, J. W., Lee, Y. M., Sung, Y. K., Akaike, T., and Cho, C. S., Clonazepam release from core-shell type nanoparticles *in vitro*. *J. Control.*

Release., 51, 169-178 (1998).

Jung, S. W., Jeong, Y. I., and Kim, S. H., Characterization of hydrophobized pullulan with various hydrophobicities. *Int. J. Pharm.*, 254, 109-121 (2003).

Jung, S. W., Jeong, Y. I., Kim, Y.H., and Kim, S. W., Self-assembled nanoparticles of poly(ethylene glycol) grafted pullulan acetate as a novel drug carrier. *Arch. Pharm. Res.*, 27, 562-569 (2004).

Kwon, G. S., Naito M., Yokoyama, M., Okano, T., Sakurai, Y., and Kataoka, K., Polymeric micelles based on AB block copolymers of poly(ethylene oxide) and poly(β-benzyl L-aspartate). *Langmuir*, 9, 945-949 (1993).

Molteni L., Dextran and inulin conjugates as drug carriers. *Methods in Enzymolozy*, 112, 285-298 (1985).

Nishikawa, T., Akiyoshi, K., and Sunamoto, J., supramolecular assembly between nanoparticles of hydrophobized polysaccharide and soluble protein complexation between the self-aggregate of cholesterol-bearing pullulan and α-chymotrypsin. *Macromolecules*, 27, 7654-7659 (1994).

Norris, D. A., Puri, N., and Sinko, P. J., The effect of physical barriers and properties on the oral absorption of particulates. *Adv. Drug Del. Rev.*, 34, 135-154 (1998).

Peppas N. A. and Robinson J. R., Bioadhesives for optimization of drug delivery. *J. Drug Targeting*., 3, 183-184 (1995).

Van Dijk-Wolthuis, W. N. E., Franssen, O., Talsma, H., Van Steenbergen, M. J., Kettenes-Van den Bosch, J. J., and Hennink, W. E., Synthesis, characterization, and polymerization of glycidyl methacrylate derivatized dextran. *Macromolecules*, 28, 6317-6322 (1995).

Wilhelm, M., Zaho, C. L., Wang, Y., Xu, R., Winnik, M. A., Mura, J. L., Riess, G., and Croucher, M. D., Poly(styrene-ethylene oxide) block copolymer micelle formation in water: a fluorescence probe study. *Macromolecules*, 24, 1033-1040 (1991).