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Dual mode bioreactions on polymer nanoparticles covered with phosphorylcholine group

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Abstract

We investigated the preparation of polymer nanoparticles covered with phosphorylcholine (PC) groups and the immobilization of proteins in order to observe dual mode bioreactions on the nanoparticles. For the surface modification on the nanoparticles, a water-soluble amphiphilic phospholipid polymer with PC groups as a hydrophilic moiety was synthesized. In this polymer, an active ester group, which can immobilize proteins, was introduced. Using the phospholipid polymer as a solubilizer, poly(L-lactic acid) nanoparticles were prepared from its methylene chloride solution in an aqueous medium by the solvent evaporation method. The diameter of the nanoparticles was ca. 200 nm and the surface was covered with the PC groups and active ester groups. Proteins could immobilize on the nanoparticles under mild conditions by the reaction between the active ester group and amino group in the proteins. Both an antibody and enzyme were immobilized on the nanoparticles and bioreactions such as the antigen/antibody reaction and enzymatic reaction were observed. When an antigen was added to the suspension of the nanoparticles, aggregation of the nanoparticles occurred and then they precipitated. Also, the enzymatic reaction proceeded well when the enzyme substrate was added to the suspension. Based on these results, we provided polymer nanoparticles functionalized with both the antibody and enzyme, and the dual mode bioreactions could occur. We concluded that the novel polymer nanoparticles could be used for nano-/micro-scaled diagnostic and medical treatment systems.

Keywords: Polymer nanoparticles; Phospholipid polymer; Bioconjugation; Bioreaction

1. Introduction

Polymer nanoparticles are widely used in the life science fields for separation technologies, histological studies, clinical diagnostic systems and drug delivery [1–3]. We are continuously investigating the preparation of polymer nanoparticles covered with phosphorylcholine (PC) groups to obtain excellent bio/blood compatibility and stability in an aqueous medium including plasma [4]. To cover the surface of the polymer nanoparticles, we prepared a water-soluble amphiphilic phospholipid polymer, poly(2-methacryloyloxyethyl phosphorylcholine (MPC)-*co*-*n*-butyl methacrylate (BMA)) (PMB) [5]. Since the PMB formed a polymer aggregate in the aqueous medium, it functioned as a good solubilizer for hydrophobic compounds. Thus, we could prepare polymer nanoparticles by solvent evaporation and interfacial precipitation techniques from an organic solvent containing a core polymer in aqueous medium containing the PMB. Moreover, the introduction of active ester units to the PMB was made possible for reactions with biomolecules [6]. We conjugated biomolecules such as a protein enzyme or antibody on the polymer nanoparticles and revealed the good performance of these biomolecules even if they were located on the solid surface [7,8].

Another viewpoint of biorecognition between the MPC polymer and living cells has been reported. On the cell membrane, carbohydrate and polysaccharide chains play an important role in molecular recognition from the outer medium and signal transport into the cells [9]. The incorporation of unnatural carbohydrates provides an opportunity to study the specific contributions of sialic acid and its *N*-acyl side chains to the sialic

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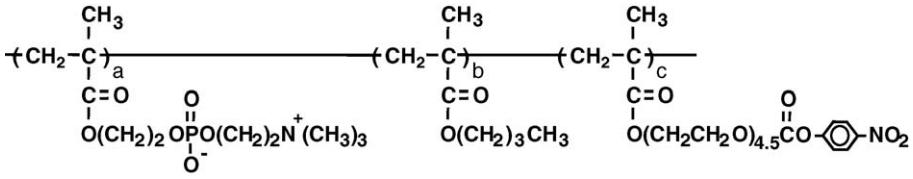

Fig. 1. Chemical structures of PMBN.

acid-dependent ligand–receptor interactions at a submolecular level. The MPC polymer surfaces with hydrazide groups, which can selectively react with unnatural ketone-containing carbohydrate as a cell surface tag, controlled the cell attachment [10].

From these fundamental research results, we propose novel diagnostic and medical treatment systems using polymer nanoparticles, that is, the polymer nanoparticles can selectively bind target cells, and enzymes conjugated on the polymer nanoparticles react with specific polysaccharide chains on the cell membrane. If the specific polysaccharide chains are digested by the enzymatic reaction, the cell cannot survive. In this study, the preparation of polymer nanoparticles covered with the PC groups and double bioconjugation with the antibody and enzyme on one polymer nanoparticle was carried out. Dual mode bioreactions, that is, aggregation of the polymer nanoparticles by the addition of an antigen and enzymatic reaction by the addition of an enzyme substrate were investigated.

2. Materials and methods

2.1. Synthesis of the MPC polymer

MPC was synthesized by a previously reported method [11]. BMA was reagent grade and used after vacuum distillation (bp 68.5 °C/32 mmHg). *p*-Nitrophenyloxycarbonyl polyethyleneglycol methacrylate (MEONP) was synthesized by a previously reported method [6]. Poly(MPC-*co*-BMA-*co*-MEONP) (PMBN) was synthesized by a conventional radical polymerization technique using 2,2′-azobisisobutyronitrile (AIBN) as an initiator [12]. The polymerization was carried out at 60 °C for 5 h. The reaction mixture was then poured into diethyl ether to precipitate the polymer, and then the polymer was collected and dried in vacuo. Using ^{1}H NMR, each mole fraction unit of PMBN was determined. Fig. 1 and Table 1 shows the chemical structures and synthetic results of the PMBN, respectively. All reagents and solvents were purified by conventional methods.

2.2. Preparation of polymer nanoparticles

The polymer nanoparticles having both PC groups and *p*-nitrophenyl ester groups on the surface were prepared by solvent evaporation and interfacial precipitation techniques in aqueous medium, the same method as previously reported [6]. A brief explanation is as follows. The PLA ($M_w = 2 \times 10^4$, Wako Pure Chemical Industries, Osaka, Japan) was used as a core polymer material. In a glass bottle, 40 mL of an aqueous solution containing the PMBN (10 mg/mL) was placed, and the resultant mixture stirred at 400 rpm with cooling in an ice bath. The PLA (20 mg) was dissolved in 2.0 mL of methylene chloride. The PLA solution was then dropped into a PMBN aqueous solution. The mixture was sonicated using a probe-type generator (Sonifier 250, Branson, USA) for 30 min and kept under reduced pressure for 2 h to evaporate the methylene chloride. The formed polymer nanoparticles (PMBN/PLA-NP) were fractionated by centrifugation at 10,300 × *g* at 4 °C for 30 min (Allegra^{TM} 21R Centrifuge, Beckman Coulter, Palo Alto, USA). The PMBN/PLA-NP as a precipitate was resuspended with distilled water and centrifuged again under the same conditions. This procedure was repeated three times to completely remove any free PMBN.

The particle size and size distribution of the PMBN/PLA-NP were determined by a dynamic light scattering measurement (DLS, Otsuka Electronics, Osaka, Japan) and observed using an atomic force microscope (AFM, SPI-3800, Seiko instrument, Chiba, Japan). The surface elemental analysis and surface potential measurement of the PMBN/PLA-NP were carried out by X-ray photoelectron spectroscopy (XPS, ESCA-200, Scienta, Uppsala, Sweden) and laser-doppler electrophoresis (ELS 8000, Otsuka Electronics), respectively. After 0.5 N NaOH was added to the PMBN/PLA-NP suspension, the PMBN/PLA-NP was precipitated by centrifugation. The UV adsorption of the supernatant was measured using a UV spectrophotometer (V-650, Jasco, Tokyo, Japan) at 400 nm to determine the amount of the active ester group on the PMBN/PLA-NP.

Table 1
Characterization of PMBN

	Monomer unit composition (mol%)						Time (h)	Yield (%)	M_w[b]	Solubility in water[c]
	In feed			In polymer[a]						
	MPC	BMA	MEONP	MPC	BMA	MEONP				
PMBN	40	55	10	32	62	6.0	5	78	6.2×10^4	++

[Monomer] = 1.0 mol/L, [AIBN] = 10 mmol/L. Reaction temperature at 60 °C. Precipitated by diethyl ether/chloroform = 8/2.

[a] Determined by ^{1}H NMR.

[b] Weight-averaged molecular weight(Mw) was determined by GPC in water/methanol = 3/7, PEO standard.

[c] Solubility was determined at 1 mg/mL polymer concentration.