Preparation of Novel Polysaccharide Nanoparticles by the Self-assembly of Amphiphilic Pectins and Their Protein-encapsulation Ability

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Novel polysaccharide-based nanoparticles were successfully prepared by the self-assembly of amphiphilic pectins, which were easily synthesized by the reaction of pectins with L-phenylalanine ethyl ester, and these nanoparticles were able to retain entrapped ovalbumin for one week without any significant leakage.

The development of nanoparticles constructed by the self-assembly of polymeric systems has gained considerable interest.[1] In particular, biodegradable and biocompatible nanoparticles have attracted much attention in the biomedical field, since they can be utilized as efficient drug carriers.[2] There has been growing interest in natural polysaccharide-based nanoparticles because of their excellent biocompatibility and biodegradability.[3]

Pectins, poly(α-1,4-D-galacturonic acid)s with a variety of methyl-esterification degrees, are a component of the cell wall in plants and have been utilized as a gelator in many foods.[4] By utilizing their ability to cross-link with Ca^{2+}, several kinds of particles such as pectin–chitosan composite gel particles[5] and pectin microcapsules[6] have been constructed. On the other hand, to the best of our knowledge, there has been no report on nanoparticles formed by the self-assembly of amphiphilic pectins in aqueous media. Such nanoparticles can be expected to exhibit drug release behavior unlike conventional pectin particles based on their cross-linking with Ca^{2+}. In addition, these nanoparticles could be easily fabricated by the self-assembly of amphiphilic pectins, which can be synthesized by the simple reaction of the pectin carboxyl groups with the appropriate hydrophobic groups.[7] Herein, we report the preparation of novel polysaccharide-based nanoparicles via the self-assembly of amphiphilic pectins and their encapsulation ability towards ovalbumin (OVA).

L-Phenylalanine-grafted pectins (pectin-*graft*-L-PAEs), were chosen as the amphiphilic pectins. They were prepared by the reaction of the carboxylic groups of the pectins with the amino groups of L-phenylalanine ethyl ester (L-PAE) in water (Figure 1). Three types of pectins whose methylation degrees were 25% (P-25), 65% (P-65), and 94% (P-94), respectively, were used as the starting materials.[8] The pectins (0.25 unit mmol) were reacted with excess L-PAE (3.75 mmol) in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (1 mmol) and *N*-hydroxybenzotriazole (HOBt) (1 mmol) in pH 6.8 aqueous solution (20 mL) at ambient temperature for 24 h. The reaction mixture was dialyzed for 3 days against water using a dialysis membrane (50 kDa molecular weight cut-off), and then lyophilized for 3 days to give the pectin-*graft*-L-PAEs. The pectin-*graft*-L-PAEs thus obtained were characterized by their ^{1}H NMR and IR spectra. The ^{1}H NMR spectra showed that almost all of the carboxylic groups of the pectins reacted with L-PAE to generate the corresponding amphiphilic pectins. It is noteworthy that these amphiphilic polymers formed the nanoparticles by simply stirring them (5 mg) in water (1 mL) for 10 min at 20 °C. Dynamic light scattering (DLS) measurements revealed that the mean diameters (C.V.)[9] of the nanoparticles formed from the P-25-*graft*-L-PAE, P-65-*graft*-L-PAE, and P-94-*graft*-L-PAE were 250 nm (40%), 400 nm (39%), and 230 nm (36%), respectively. Figure 2 shows the transmission electron microscopic (TEM) and scanning electron microscopic (SEM) images of nanoparticles formed from the P-94-*graft*-L-PAE. These images indicate that the P-94-*graft*-L-PAE formed almost spherical nanoparticles in aqueous media. The particle diameters estimated from the TEM images (<100 nm) are smaller than those estimated from the DLS measurements, possibly due to shrinkage of the particles during the sample drying process for the TEM studies. P-94-*graft*-L-PAE nanoparticles were stably dispersed in water for more than one week. It was also observed that these nanoparticles were easily redispersed into aqueous media while retaining almost the same particle sizes after the lyophilization.[10]

The encapsulation ability of the pectin-*graft*-L-PAE nanoparticles towards proteins was examined. Fluorescein-4-isothiocyanate-labeled ovalbumin (FITC-OVA)[11] was chosen as a model protein. The encapsulation of FITC-OVA into pectin-*graft*-L-PAE nanoparticles was carried out as follows: pectin-*graft*-L-PAE (10 mg) in DMSO solution (1 mL) was mixed with different amounts of FITC-OVA (0, 0.5, 1.0, 2.0, and 4.0 mg) in 0.05 M phosphate buffer (pH 7.4), and then the resultant solution

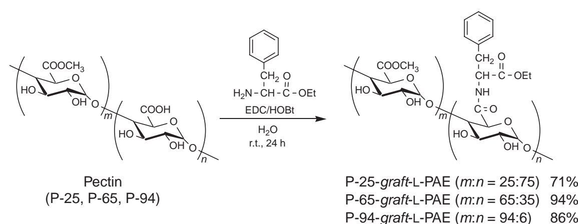

Figure 1. Preparation of pectin-*graft*-L-PAE.

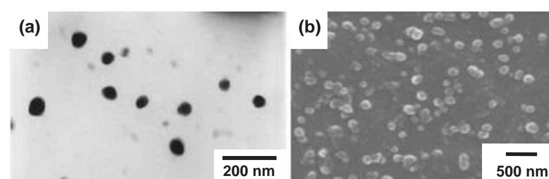

Figure 2. TEM (a) and SEM (b) images of nanoparticles formed from P-94-*graft*-L-PAE.

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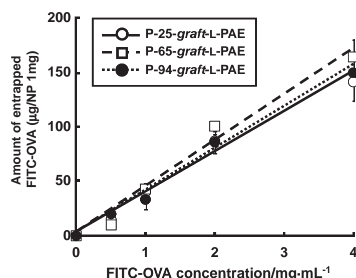

Figure 3. Plots of the amounts of FITC-OVA entrapped into the pectin-*graft*-L-PAE nanoparticles against the FITC-OVA feed concentration.

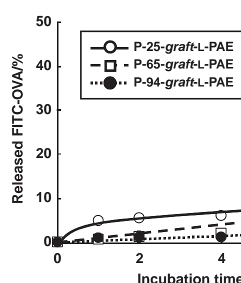

Figure 4. Release behavior of the entrapped FITC-OVA from the pectin-*graft*-L-PAE nanoparticles (at 37 °C, pH 7.4).

was dialyzed against water for 3 days to remove the DMSO. The precipitate was separated from the solution by centrifugation, and then washed with phosphate buffer (1 mL) twice to give the FITC-OVA-entrapped nanoparticles. The amounts of FITC-OVA entrapped into the nanoparticles were determined by measuring the fluorescence intensity of the FITC-OVA released after the collapse of the nanoparticles by treatment with sodium dodecylsulfate (SDS). Figure 3 shows the amounts of FITC-OVA entrapped into the pectin-*graft*-L-PAE nanoparticles as a function of the FITC-OVA feed concentration. The entrapped FITC-OVA amounts increased linearly with an increase in the FITC-OVA feed concentration. There was no difference in the increment between the different types of amphiphilic pectins. These findings suggest that in all of the pectin nanoparticles examined here, the encapsulation amounts of FITC-OVA can be easily controlled by changing the FITC-OVA feed concentration. When the entrapped FITC-OVA amount was less than 150 μg per 1 mg of nanoparticle, the resulting nanoparticles were stably dispersed in aqueous media. On the other hand, the encapsulation of FITC-OVA at more than 200 μg per 1 mg of nanoparticles caused some aggregation of the nanoparticles. The release behavior of the entrapped FITC-OVA in 0.05 M phosphate buffer (pH 7.4) is shown in Figure 4. Interestingly, the encapsulated FITC-OVA was retained without any significant leakage within all of the pectin nanoparticles examined here. In particular, the amount of FITC-OVA released from the P-94-*graft*-L-PAE nanoparticle was less than 3% even after one week of storage.[12] This phenomenon may be explained by considering the higher stability of the FITC-OVA-entrapped P-94-*graft*-L-PAE nanoparticle in the phosphate buffer. These results clearly show that these pectin nanoparticles have a high ability to retain the entrapped proteins during storage.

In conclusion, we have demonstrated that novel polysaccharide-based nanoparticles were successfully prepared by the self-assembly of amphiphilic pectins, which were easily synthesized by the reaction of pectins with L-phenylalanine ethyl ester. These particles retained entrapped ovalbumin for one week without any significant leakage. Since pectin is a biocompatible polymer with no antigenicity, these pectin-derived nanoparticles are potentially applicable as highly biocompatible protein carriers. The encapsulation of different types of proteins into the pectin nanoparticles and their controlled release are now under investigation.

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8 The weight-average molecular weights (M_w) of P-25, P-65, and P-94 are 8.0×10^4, 18×10^4, and 8.6×10^4, respectively.

9 Coefficient of variation (C.V.) = standard deviation/mean diameter.

10 The mean diameters (C.V.) of the nanoparticles after the lyophilization are as follows: 290 nm (38%) for the P-25-*graft*-L-PAE nanoparticle, 450 nm (35%) for P-65-*graft*-L-PAE nanoparticle, and 250 nm (36%) for P-94-*graft*-L-PAE.

11 Ovalbumin (OVA) is a protein with a molecular weight of about 4.5×10^3 and an isoelectric point of 4.6.

12 The mean diameter (382 nm, C.V. = 39%) of the FITC-OVA-entrapped P-94-*graft*-L-PAE nanoparticles (36 μg of FITC-OVA entrapped per 1 mg of nanoparticle) was retained during one week of storage.

13 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

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