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Short communication

Atomic force microscopy imaging of novel self-assembling pectin–liposome nanocomplexes

Pornsak Sriamornsak [a,b,*], Nartaya Thirawong [a,b], Jurairat Nunthanid [a,b], Satit Puttipipatkhachorn [c], Jringjai Thongborisute [d], Hirofumi Takeuchi [d]

[a] Department of Pharmaceutical Technology, Faculty of Pharmacy, Silpakorn University, Nakhon Pathom 73000, Thailand
[b] Pharmaceutical Biopolymer Group (PBiG), Faculty of Pharmacy, Silpakorn University, Nakhon Pathom 73000, Thailand
[c] Department of Manufacturing Pharmacy, Faculty of Pharmacy, Mahidol University, Bangkok 10400, Thailand
[d] Laboratory of Pharmaceutical Engineering, Gifu Pharmaceutical University, 5-6-1 Mitahora-Higashi, Gifu 502 8585, Japan

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Abstract

Self-assembling pectin–liposome nanocomplexes (PLNs) were prepared by a simple mixing of cationic liposomes with pectin solution. Nanostructures of liposomes, pectin, and PLNs were observed by atomic force microscopy (AFM). The AFM images of pectin show a chain-like structure with a small number of branches while those of liposomes show a spherical form. The AFM images also provided a direct evidence for association of cationic liposomes on the pectin chain. This was confirmed by the FTIR analysis.

Keywords: Atomic force microscopy (AFM); Liposomes; Nanocomplex; Pectin; Self-assembly

1. Introduction

Polyelectrolyte complexes (PECs) are formed by the electrostatic interaction between a polyelectrolyte and oppositely charged polyelectrolyte in aqueous solution. PECs have been long investigated from the standpoint of the polyacid–polybase interaction, stoichiometry, self-assembly (Ohkawa, Takahashi, & Yamamoto, 2000), and have numerous applications such as membranes, coatings, surfactants, and microcapsules (Hales & Pochan, 2006). In addition, the formation of PECs to the formation of building blocks is expected to find important applications in nanoscience. Owing to the practical and economical aspects for the rational use of PECs, as well as the theoretical interest of such systems in soft condensed matter science, nanochemistry, biology, and medicine, a better understanding of the key factors controlling the complexation between oppositely charged macromolecules is therefore important.

Recently, the interactions between charged lipids and oppositely charged biopolymers have been studied (Antonietti & Wenzel, 1998; Raviv et al., 2005). The highly ordered structures formed by polyelectrolyte–lipid complexes are of great interest in material sciences as templates and building blocks for hierarchical supramolecular assembly (Antonietti & Wenzel, 1998). Takeuchi, Yamamoto, Niwa, Hino, and Kawashima (1994) reported the preparation of chitosan-coated liposomes, in which the chitosan (i.e. cationic polysaccharide) is believed to cover the surface of the liposomes (i.e. vesicles composed of one or more phospholipid bilayers) by forming the ion-complex with dicetylphosphate in the liposomal formulation. Diebold et al. (2007) described the complexes between liposomes and chitosan nanoparticles which formed by combining those components. However, the structures formed when

* Corresponding author. Address: Department of Pharmaceutical Technology, Faculty of Pharmacy, Silpakorn University, Nakhon Pathom 73000, Thailand. Tel.: +66 34 253912x2317; fax: +66 34 255801.
E-mail address: pornsak@email.pharm.su.ac.th (P. Sriamornsak).

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charged liposomes are complexed with oppositely charged electrolytes have not been morphologically examined and yet to be understood.

Microscopic analysis at the single molecule level can be achieved by scanning probe methods such as atomic force microscopy (AFM). It has evident advantages for the detection and characterization of heterogeneous populations with regards to the amount of material required for a study as well as to the level of information that can be acquired. AFM is capable of resolving individual molecules so that differences in size (length, diameter, etc.) and conformation (stiffness, aggregation, and association, mode of adsorption to a substrate, etc.) between neighboring polymers can be visualized directly, thus making possible the characterization of the heterogeneity of a molecular population at the level of single polymers. AFM has become a standard tool for the characterization of heterogeneity at the nanoscale, finding applications in every aspect of surface and interface science (Hansma et al., 1997). In the field of single biomolecular characterization, AFM imaging has been used to identify the existence of extended branches in pectin (Morris et al., 2001; Round, Rigby, MacDougall, Ring, & Morris, 2001).

Pectin is a naturally occurring water-soluble polysaccharide which is found in the cell wall of most plants. Though it is a heterogeneous polysaccharide, pectin contains linear chains of (1–4)-linked α-D-galacturonic acid residues (Rolin, 1993). The galacturonic acids have carboxyl groups, some of which are naturally presented as methyl esters and others which are reacted with ammonia to produce carboxamide groups. The degree of esterification (DE) and degree of amidation (DA), which are both expressed as a percentage of carboxyl groups (esterified or amidated), are an important means to classify pectin. Due to its biocompatibility, biodegradability, and non toxicity, pectin represents an attractive biopolymer for a variety of pharmaceutical and biomedical applications. Pectin has shown promise in engineering drug carriers for oral drug delivery (Sriamornsak, 1998; Sriamornsak, Sungthongjeen, & Puttipipatkhachorn, 2007; Sriamornsak, Thirawong, & Puttipipatkhachorn, 2005). Chemically, the structure of pectin is full of carboxyl groups. This may allow the interaction between pectin and the oppositely charged membranes or liposomes. The objective of this study was then to examine the morphological arrangement of pectin–liposome nanocomplexes (PLNs) using different types of pectin by AFM images.

2. Materials and methods

2.1. Materials

Different types of pectin were donated by Herbstreith & Fox KG (Germany). High methoxy pectin, type CU201 (referred as CU201) contains 70% DE and MW of 200 kDa. Low methoxy pectin, type CU701 (referred as CU701) has 38% DE and MW of 80 kDa while low methoxy amidated pectin, type CU020 (referred as CU020) has 29% DE, 20% DA and MW of 150 kDa. Distearoylphosphatidylcholine (DSPC) and stearylamine (SA) were purchased from Oil & Fats (Japan) and Tokyo Kasei (Japan), respectively. Cholesterol (Chol) was supplied from Sigma Chemical Co. (USA). All other chemicals were of analytical grade and were used as received without further purification.

2.2. Preparation of cationic liposomes and self-assembling pectin–liposomes nanocomplexes (PLNs)

Cationic multilamellar liposomes were prepared by the hydration method of lipid film as described previously by Takeuchi, Matsui, Yamamoto, and Kawashima (2003) with some modifications. In brief, the mixture of DSPC, SA, and Chol in molar ratio of 8:0.2:1 was dissolved in a small amount of chloroform. The solution was placed in a rotary evaporator at 40 °C until a thin film was obtained then allowed to stand over night in vacuum chamber to ensure completely solvent removal. The thin film was hydrated with phosphate buffer (pH 6.8, 66.7 mM) by vortexing to obtain multilamellar liposomes. In order to reduce particle size, the multilamellar liposomes were then passed through an extruder (LipoFast-Pneumatic, Avestin, Canada) using 0.2 μm polycarbonate membrane filters (Nucleopore®, Whatman, USA) to generate submicron-sized liposomes. In order to prepare PLNs, an equal volume (i.e. 2 mL) of the liposomal suspension and pectin solution (at the concentration of 1.0% w/v in 66.7 mM phosphate buffer solution, pH 6.8) was mixed by vortexing to obtain 0.5% w/v of pectin as a final concentration.

2.3. Atomic force microscopy (AFM)

For imaging, samples were diluted with filtered deionized water to 2–4 μg/mL. An aliquot (2 μL) of the diluted sample solutions was immediately spread on freshly cleaved mica surfaces. The sample was then allowed to dry at ambient temperature (25 °C) for 20 min before imaging at a scan speed of 2 Hz. Tapping mode was carried out using a multimode NanoScope IIIa AFM (Digital Instruments, USA) equipped with Phosphorus (n) doped Si (Veeco, model RTESP) cantilever with a quoted spring constant of 20–80 N/m. Several images of different zones were examined since AFM images are generally limited to small scanned areas. Height mode was used for image analysis. The correction of the images by commercial image processing software (Adobe Photoshop, Version 6.0.1, Adobe Systems Inc., USA) with glowing edges or finding edges mode enabled a reduction of the noise, as shown on the right-hand side of the original AFM images.

2.4. FTIR spectroscopic analysis

The FTIR spectra of the liposomes, SA and their physical mixture as well as co-precipitated mixture were