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Calcium pectinate gel beads obtained from callus cultures pectins as promising systems for colon-targeted drug delivery

Elena A. Günter*, Oxana V. Popeyko

Institute of Physiology, Komi Science Centre, The Urals Branch of the Russian Academy of Sciences, 50, Pervomaiskaya str., 167982 Syktyvkar, Russia

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ABSTRACT

Low methyl-esterified pectins obtained from the cell walls of the campion (SV, SV > 300), tansy (TV, TV > 300) and duckweed (LM, LM > 300) callus cultures and apple pectin (AP, Classic AU 701) were used as the carriers for colon delivery of prednisolone. The pectins with molecular weight more than 300 kDa (SV > 300, TV > 300, LM > 300) formed gels which exhibited the higher gel strength. The higher gel strength of these gels appeared to be related to the higher Mw and the lower degree of methylesterification (DE) of these pectins. Release aspects of prednisolone in the simulated gastric (pH 1.25), intestinal (pH 7.0) and colonic (pH 7.0 + pectinase) media were investigated. The LM-5%, AP-3% and AP-5% beads destroyed in simulated intestinal medium probably due to the higher DE of the LM and AP pectins. The SV > 300-3% and TV > 300-3% prednisolone loaded bead systems showed a high stability at pH 1.25 and pH 7.0. Prednisolone release occurred in a larger extent in colonic medium due to the enzymatic erosion of the beads. The SV > 300-3% and TV > 300-3% particles showed a more controlled release that appeared to be related to the lower DE, rhamnogalacturonan content, rhamnogalacturonan I branching and the higher linearity and Mw of the TV > 300 and SV > 300 pectins, as well as to the higher gel strength. This in vitro study suggests that calcium pectinate gel beads obtained from callus cultures pectins can be proposed as potential systems for colon-targeted drug delivery.

1. Introduction

Colonic drug delivery is intended for the local treatment of ulcerative colitis, irritable bowel syndrome and can potentially be used for colon cancer or the systemic administration of drugs that are adversely affected by the upper gastrointestinal tract (Yang, Chu, & Fix, 2002). The advantages of local treatment in the colon have been described: reduced incidence of systemic side effects, administration of lower doses of drug, and maintenance of the drug in its intact form as close as possible to the target site (Chambin, Dupuis, Champion, Voilley, & Pourcelot, 2006).

Prednisolone, a typical glucocorticoid, has been widely used for the treatment of inflammatory bowel disease (Rosenberg, Ireland, & Jewell, 1990). However, when prednisolone is administered orally, a large amount of the drug is absorbed from the upper gastrointestinal tract and enters into the systemic circulation. This deteriorates the therapeutic efficacy of prednisolone and causes systemic side effects, such as adrenosuppression, hypertension and osteoporosis, etc. (Stephen & Joseph, 1988). Therefore, it is preferable for treatment of inflammatory bowel disease to deliver the drug site-specifically to colon. Several methods of drug delivery to the colon have been developed: coating with biodegradable polymers, coating with pH-sensitive polymers, gastrointestinal pressure controlled release and prodrug approach (Yano, Hirayama, Kamada, Arima, & Uekama, 2002).

Natural polysaccharides are now extensively used for the development of solid dosage forms for delivery of drug to the colon (i.e. pectin, chitosan, cyclodextrin, dextran) (Chambin et al., 2006). Pectic formulations are easily tailored into gels, 3-D matrices, films, micro- or nano-particles. Low methyl-esterified pectin with a DE less than 50% can form rigid gels via the action of calcium ions or multivalent cations that cross-link the galacturonic acid chains. Calcium pectinate hydrogels are stable in low pH solutions and have been investigated as a carrier material in different controlled release systems (Sriamornsak & Kennedy, 2010). Various drugs can be incorporated into pectic formulations with high loading efficiency using simple procedures. However, the commercial potential pectin in drug delivery system technology has yet to be realised. This is partially because of the lack of reproducible performance of pectic formulations. The obstacles include

* Corresponding author.
E-mail addresses: gunter-ea@mail.ru, gunter@physiol.komisc.ru (E.A. Günter).

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the large diversity in pectin's molecular characteristics, which generates difficulty in quality control and quality assurance during the intermediate preparation of pectic derivatives and in the final products. The solutions to resolve these problems fall into two categories: the development of new technologies for pectin isolation, purification, and characterisation and the modification of pectic macromolecules (Liu, Fishman, & Hicks, 2007). One obstacle in applying pectic gels for colon-specific drug delivery is their high swelling behaviour in physiological environments. To overcome this problem, pectin has been used in combination with polyacrylate derivatives or with hydroxypropylmethyl cellulose and chitosan (Liu, Fishman, Hicks, Kende, & Ruthel, 2005; Park, Lee, Cho, Baek, & Lee, 2002). These strategies enhance the water resistance of the resultant pectic complexes to some degree. However, improved pectin-based drug delivery systems are still desired.

The characteristics of the final gel and its functional properties depend on many factors, especially the chemical fine structure of pectin and the system composition (e.g., pH, soluble solids, and pectin content) (Fraeye, Duvetter, Doungla, Loey, & Hendrickx, 2010). In this connection, the controllable swellability and degradability of the gel may be realised through alteration of the sugar composition, DE, molecular weight of pectin. Previously, we demonstrated that the campion cell culture conditions affect the chemical characteristics of pectin and subsequently, the pectins with different characteristics result in different calcium pectinate gel (CaPG) bead properties (Günter et al., 2014). An applied strategy altered the swelling behaviour of the CaPG beads and enhanced the acid and water resistance of the resultant pectinate hydrogels in physiological environments. Callus cultures are considered to be useful as an alternative raw material source for obtaining new valuable pectins and calcium pectinate hydrogels.

The aims of this research were to investigate the swelling properties and morphology of the CaPG beads made from pectins of different callus cultures that exhibited gastric and small intestinal resistance to the release of prednisolone in vitro and the rapid liberation of drug in colonic medium as well as evaluate the influence of the chemical characteristics of the pectins upon in vitro release of prednisolone beads.

2. Materials and methods

2.1. Materials

Low methyl-esterified pectins with a DE of 6–22% were obtained from the cell walls of *Silene vulgaris* (Moench) Garcke (*Oberna behen* (L.) I.), *Tanacetum vulgare* L. and *Lemna minor* L. callus cells. Commercial apple pectin (AP) (Classic AU 701) with a DE of 36–44% was purchased from Herbstreith & Fox, Germany. Prednisolone was purchased from Richter Gedeon, Hungary. All other chemicals were of analytical grade.

2.2. Callus cultures

Callus cultures of the *S. vulgaris* (Moench) Garcke (*O. behen* (L.) I.), *T. vulgare* L. and *L. minor* L. were maintained on the agarized Murashige and Skoog medium (Murashige & Skoog, 1962) that contained 15 g/l sucrose, 15 g/l glucose, 8 g/l agar, 0.5 mg/l 6-benzylaminopurine, and 1.0–1.5 mg/l 2,4-dichlorophenoxyacetic acid. The *S. vulgaris*, *T. vulgare* and *L. minor* callus cultures were subcultured at 24 °C in the darkness for 21, 28 and 28 days, respectively.

2.3. General methods

Total amounts (% of total amount) of glycuronic acids in polysaccharide fractions were estimated using a reaction with 3,5-dimethylphenol in the presence of concentrated sulfuric acid (Usov, Bilan, & Klochkova, 1995). Total protein content was determined according to the Lowry method (Lowry, Rosebrough, Farr, & Randall, 1951). The degree of methylesterification (DE) was calculated using the method described earlier (Wood & Siddiqui, 1971). The absolute configuration of D-galacturonic acid was evaluated using specific optical rotation determined using a Polartronic MHZ polarimeter (Germany). Spectrophotometric measurements were made with an Ultrospec 3000 instrument (UK). GLC was performed with a Hewlett-Packard 4890A chromatograph (USA) fitted with an RTX-1 (0.25 mm × 30 m, Restek, USA) capillary column with argon as a carrier gas, using a flame-ionisation detector and HP 3395A integrator. Molecular masses of polysaccharides were estimated using high performance liquid chromatography. The polysaccharide sample (3 mg) was dissolved in 0.15 M NaCl (1 ml) prepared using bidistilled water, and the solution was filtered and subjected to analysis using the following chromatographic system: a LS-20AD pump (Shimadzu, Japan), a Shodex OHpak SB-804HQ column (8.0 mm × 30 cm; Shimadzu) with a Shodex GS-26 7B pre-column (7.6 mm × 5 cm; Shimadzu), CTO-10AS thermostat (Shimadzu), and RID-10A refractometer (Shimadzu). Elution was carried out with 0.15 M NaCl at 40 °C with an effluent rate of 0.4 ml/min. The pullulan samples with molecular masses in ranges 1.3, 6, 12, 22, 50, 110, 200, 400 and 800 kDa (Sigma, USA) were employed for column calibration. Molecular masses (Mw, Mn and Mw/Mn) of polysaccharides were analyzed using the LC solution program (Version 1.22 SP1), which provides the definition of retention time, peak area, processing, construction of the curve of the molecular weight distribution, printing and storing the output curves. Molecular masses of polysaccharides were measured in triplicate.

2.4. Isolation of polysaccharides

Isolation of the SV, TV and LM low methyl-esterified pectins from the cell walls of the *S. vulgaris*, *T. vulgare* and *L. minor* callus cultures, respectively, was performed as described earlier (Günter & Ovodov, 2007). The callus was extracted with water at 50 °C for 2 h. The residual material was centrifuged and then treated with dilute HCl (up to pH 4) at 50 °C for 3 h, then the mixture was filtered, and the plant material was extracted with 0.7% aqueous ammonium oxalate at 68 °C for 2 h. A crude polysaccharide fraction was precipitated with 2 volumes of 96% ethanol. The precipitate was dissolved in distilled water followed by dialysis against distilled water and lyophilization.

The polysaccharide fractions of SV > 300, LM > 300 and TV > 300 were obtained from the SV, TV and LM pectins, respectively, using ultrafiltration membranes. Each fraction of SV, TV and LM (300 mg) was dissolved in distilled water (500 ml) and separated using ultrafiltration membrane polyethersulfone 300 kDa (filter code: PBMK, diameter 63.5 mm, NMWL: 300,000, Millipore, USA). The fractions were concentrated and lyophilized to furnish the purified SV > 300, LM > 300 and TV > 300 with Mw more than 300 kDa. The polysaccharide fractions with Mw more than 300 kDa were selected because according to our preliminary data, the CaPG beads made from the pectic fractions with Mw more than 300 kDa exhibited gastric and small intestinal resistance to the release of prednisolone in vitro in comparison with those made from the pectic fractions with Mw lower than 300 kDa.

2.5. Complete acidic hydrolysis

Polysaccharide samples (2 mg) were hydrolyzed with 2 M TFA (0.5 ml) at 100 °C for 3–4 h in sealed tubes. The acid was removed by repeated co-evaporation with methanol. The neutral sugars (% of total amount) were quantified by GLC as the corresponding alditol acetates using *myo*-inositol as the internal standard (York, Darvill,