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Cinnamate of inulin as a vehicle for delivery of colonic drugs

Dorotea López-Molina [a,1], Soledad Chazarra [a,1], Chee Wun How [a], Nikolov Pruidze [b], Enma Navarro-Perán [a], Francisco García-Cánovas [a], Pedro Antonio García-Ruiz [c], Francisco Rojas-Melgarejo [c], José Neptuno Rodríguez-López [a,*]

[a] Departamento de Bioquímica y Biología Molecular A, Facultad de Biología, Universidad de Murcia, 30100 Espinardo, Murcia, Spain
[b] Durmishidze Institute of Biochemistry and Biotechnology of Agrarian University of Georgia, 0131 Tbilisi, Georgia
[c] Grupo de Química de Carbohidratos, Polímeros y Aditivos Industriales, Departamento de Química Orgánica, Facultad de Química, Universidad de Murcia, 30100 Espinardo, Murcia, Spain

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ABSTRACT

Colon diseases are difficult to treat because oral administrated drugs are absorbed at the stomach and intestine levels and they do not reach colon; in addition, intravenous administrated drugs are eliminated from the body before reaching colon. Inulin is a naturally occurring polysaccharide found in many plants. It consists of β 2-1 linked D-fructose molecules having a glucosyl unit at the reducing end. Various inulin and dextran hydrogels have been developed that serve as potential carrier for introduction of drugs into the colon. Because inulin is not absorbed in the stomach or in the small intestine, and inulin is degraded by colonic bacteria, drugs encapsulated in inulin-coated vesicles could be specifically liberated in the colon. Therefore, the use of inulin-coated vesicles could represent an advance for the treatment of colon diseases. Here, we study the use of a cinnamoylated derivative of chicory inulin as a vehicle for the controlled delivery of colonic drugs. The encapsulation of methotrexate in inulin vesicles and its release and activity was studied in colon cancer cells in cultures.

1. Introduction

Inulin is a natural plant-derived carbohydrate with the benefits of soluble dietary fiber (López-Molina et al., 2005). It is not digested or absorbed in the small intestine, but is fermented in the colon by beneficial bacteria. Functioning as a prebiotic, inulin has been associated with enhancing the gastrointestinal system and immune system. In addition, it has been shown to increase the absorption of calcium and magnesium, influence the formation of blood glucose, and reduce the levels of cholesterol and serum lipids (Coudray et al., 1997; Niness, 1999). Inulin is not simply one molecule; it is a polydisperse β-2,1 fructan; the fructose units in this mixture of linear fructose polymers and oligomers are each linked by β-2,1 bonds. A glucose molecule typically resides at the end of each fructose chain and is linked by an α-1,2 bond, as in sucrose. The unique aspect of the structure of inulin is its β-2,1 bonds. These linkages prevent inulin from being digested like a typical carbohydrate and are responsible for its reduced caloric value and dietary fiber effects.

Targeted drug delivery into the colon is highly desirable for local treatment of a variety of bowel diseases such as ulcerative colitis, Crohn's disease, amebiosis, colonic cancer, local treatment of colonic pathologies, and systemic delivery of protein and peptide drugs (Philip and Philip, 2010). The colon specific drug delivery system should be capable of protecting the drug in route to the colon i.e., drug release and absorption should not occur in the stomach as well as the small intestine, and neither the bioactive agent should be degraded in either of the dissolution sites but only released and absorbed once the system reaches the colon (Akala et al., 2003). The fact that inulin is not digested or absorbed in the small intestine made this polymer an attractive carrier for colonic drug delivery (Damian et al., 1999). In addition, inulin may constitute an ideal model for microbially triggered drug delivery to colon (Sinha and Kumria, 2003a,b,b). The microflora of the colon is in the range of 10^{11}–10^{12} CFU/mL, consisting mainly of anaerobic bacteria, e.g., *Bacteroides*,*Bifidobacteria*, *Eubacteria*, *Clostridia*, *Enterococci*, *Enterobacteria* and *Ruminococcus* among others (Vassallo et al., 1992). This vast microflora fulfills its energy needs by fermenting various types of substrates that have been left undigested in the small intestine (Rubinstein, 1990). For this fermentation, the microflora produces a vast number of

Abbreviations: FSCN, cinnamoylated fructose; DHFR, dihydrofolate reductase; INCN, cinnamoylated inulin; MTX, methotrexate; TLC, thin layer chromatography; XTT, 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide.

* Corresponding author. Tel.: +34 868888284.
E-mail address: neptuno@um.es (J.N. Rodríguez-López).

[1] These authors contributed equally to the work.

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biodegradable enzymes, among them is inulinase. Because of the presence of the biodegradable enzymes only in the colon, the use of biodegradable polymers, such inulin, for colon-specific drug delivery seems to be a more site-specific approach as compared to other approaches. These polymers shield the drug from the environments of stomach and small intestine, and are able to deliver the drug to the colon. On reaching the colon, they undergo assimilation by microorganism, degradation by enzyme or break down of the polymer back bone leading to a subsequent reduction in their molecular weight and thereby loss of mechanical strength. They are then unable to hold the drug entity any longer.

Hydrogels are usually formed by the covalent crosslinking of linear hydrophilic polymers to form a network of material capable of absorbing water, yet still remaining insoluble (Chourasia and Jain, 2003). Heterogeneous polymer mixtures may also be used to form hydrogels without the need for covalent crosslinking. Various hydrogels based on the azo polymeric networks have been developed for site-specific delivery of drugs to the colon between them various inulin hydrogels have been developed that serve as potential carrier for introduction of drugs into the colon (Damian et al., 1999). However, some of these inulins were not biodegradable, since no degradation was detected after incubation with *Bifidobacteria* or inulinase from *Aspergillus niger* (Damian et al., 1999). To expand the potential use of inulin as a colonic drug carrier, in this study we optimized the preparation of cinnamoylated derivatives of inulin using methotrexate (MTX) as a model drug. One of the purposes of this study was to investigate whether cinnamoylated inulin can be degraded by inulinase, in order to determine its potential in colonic drug delivery. MTX is used to treat certain types of cancer and also employed to treat rheumatoid arthritis, psoriatic arthritis, and psoriasis (a chronic skin disorder that produces patchy scales) (Cutolo et al., 2001; Khan et al., 2012). When used to treat cancer, MTX works by blocking an enzyme process in cancer cells so they cannot grow. Used for this purpose, MTX is known as an anti-metabolite. For rheumatoid arthritis, it works by reducing inflammation and by suppressing the immune system and under these circumstances, MTX is known as a disease modifying antirheumatic drug. Because MTX can be employed for the treatment of several colonic diseases such as cancer or Crohn's disease, here we validated our colon specific drug delivery system using this useful drug.

2. Materials and methods

2.1. Materials

Standard grade chicory inulin was obtained from Sigma Chemical Co. (Madrid, Spain) and used as provided. Bovine liver DHFR was purchased from Fluka Chemical Co. (Madrid, Spain) and dialyzed exhaustively against distilled water to remove the 1.5 M ammonium sulphate in which it is supplied. The enzyme concentration was determined by MTX titration of enzyme fluorescence (Williams et al., 1979). DHF was obtained from Aldrich Chemical Co. (Madrid, Spain) and NADPH and MTX from Sigma. Inulinase from *A. niger* was from Sigma. The cinnamoylated derivative of D-fructose (FSCN) was obtained as described before (Marín-Zamora et al., 2007) and it was used as a control in TLC experiments. All other chemicals were of analytical grade and purchased from Sigma.

2.2. Apparatus

The activity of DHFR was determined at 25 °C by following the decrease in the absorbance of NADPH and DHF at 340 nm ($\varepsilon = 11800\ M^{-1}\ cm^{-1}$) in a PerkinElmer Lambda-2 spectrophotometer with 1.0 cm light-path cuvettes. The UV–vis spectra were obtained with the same spectrophotometer. MTX concentrations in 0.1 M NaOH were determined at 372 nm ($\varepsilon = 7000\ M^{-1}\ cm^{-1}$). Analysis and measurements were made in quartz cuvettes. Infrared spectra were obtained in a Nicolet Impact 400 spectrophotometer. Cinnamoyl ester of inulin was prepared as thin films on KBr plates. The ^{1}H NMR spectra were obtained in a Bruker 400 MHz UltraShield™ spectrophotometer provided with broad band probe at room temperature, with tetramethylsilane (TMS) as internal standard and using $CDCl_3$ as solvent. The proton decoupled ^{13}C NMR spectra were obtained in the same instrument and probe, operating at 100.82 MHz. Differential thermal analyses was performed on a differential scanning calorimeter (model TGA-DSC 2920). The measurements were carried out over the temperature range of 10–300 °C at a heating rate of 10 °C/min under a flow (5 mL/min) of dry N_2. The size and morphology of the microspheres were determined by electronic microscopy (JSM-6100 Scanning microscope) coupled with a Link Isis software form image capture.

2.3. Preparation of totally cinnamoylated derivative of inulin (INCN)

Preparation followed a modified version of the method of Van Cleve (1963), in which 0.02 mol of inulin was dissolved in 100 mL of pyridine. The mixture was heated at 60 °C for 1 h to ensure complete dissolution. The resulting solution was cooled to room temperature before adding cinnamic acid chloride. The totally cinnamoylated derivative of inulin was obtained by adding 0.07 mol of cinnamic acid chloride to the mixture. The reaction was allowed to proceed at room temperature for 4 h, after which the resulting mixture was poured into vigorously stirred cool water. The precipitate obtained, after decanting and filtering this mixture, was dissolved in chloroform and purified by adding, one drop at a time, to vigorously shaken hexane. The solid obtained was redissolved and reprecipitated before being dried on P_2O_5 at reduced pressure (yield: 85%); mp = 112–114 °C.

2.4. Preparation of INCN microspheres and drug loading

INCN microspheres were obtained by solvent precipitation approach. For this procedure, Tween 20 (5%) was dropping on a solution of INCN (20 mg/mL) dissolved in acetone. Microspheres without drug were obtained by dropping into this solution with distillate water until turbidity. For MTX loading a concentrate solution (10 mg/mL) of this drug in sodium carbonate (pH 8.6) was added drop by drop to the INCN–Tween 20 solution. When a colloid suspension was obtained the precipitate was filtered and extensive washed with sodium carbonate (pH 8.6) to removed unincorporated MTX followed by distillate water to obtain a neutral pH in the precipitate. Microspheres were dried at room temperature and then lyophilised. Samples were stored at room temperature and protected from the light.

2.5. Percentage loading of MTX

Percentage loading of MTX-entrapped INCN-microspheres was assayed by direct methods. About 5 mg of MTX loaded microspheres were dissolved in 10 mL of 2 M sodium hydroxide solution. Aliquots from the solution were filtered through a 045 μm Millipore filter and assayed spectrophotometrically at 372 nm to determine the amount of MTX entrapped in the INCN microspheres.

2.6. Degradation of inulins by inulinase

Inulins (0.1 g) were mixed with 5 mL of acetate buffer (0.1 M; pH 4.0) or with mixtures of acetone and acetate buffer 1:1 (v/v) in the