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Profiling of modified citrus pectin oligosaccharide transport across Caco-2 cell monolayers

Fraser L. Courts *

HONEI, Centre for Cardiovascular and Metabolic Research, Hull York Medical School, Hertford Building, University of Hull, Cottingham, Hull HU6 7RX, UK

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ABSTRACT

Modified citrus pectin (MCP) is a commercially-available dietary supplement produced by the hydrolysis of plant pectins, producing a mixture of galacturonic acid-, galactose- and arabinose-rich oligosaccharides. Evidence from clinical studies suggest a role for oral MCP as an exciting dietary therapy in cancer and acute renal injury, supported by *in vitro* data showing involvement of neutral oligosaccharides from MCP in the blockade of galectin-3, a signalling protein implicated in tumour spread in cancer and inflammatory fibrosis following organ failure. The relationship between the oligosaccharide profile of MCP, *in vitro* structure-function data and clinical observations is unclear however, as the orally bioavailable MCP oligosaccharide profile is currently unknown.

The present study therefore aimed to characterise the profile of bioavailable MCP oligosaccharides using a two-compartment transwell Caco-2 cell monolayer system as a pharmacologically-predictive model of the small intestinal epithelium. Preferential transport of short-chain galactans and arabinogalactans, but not galacturonic acid polymers from MCP across Caco-2 cell monolayers is demonstrated by a combination of FITC-labelling and high performance anion-exchange chromatography (HPAEC), and the structures of transported oligosaccharides partially elucidated by graphitised-carbon LC–IT-MS/MS, suggesting that these species are capable of traversing the small intestinal epithelium.

1. Introduction

Pectins are highly-branched, heterogeneous carbohydrates found in the cell wall of the middle lamella of all higher terrestrial plants and commercially extracted for use in the food and pharmaceutical industries. Sequential alkali and acidic hydrolytic modification of pectin extracted from citrus fruit produces modified citrus pectin (MCP), a preparation that is attracting increasing attention as a dietary supplement with putative pharmacological properties, principally due to a rapidly-growing body of evidence surrounding a role for MCP in inhibiting the activity of the mammalian cell-signalling protein, galectin-3 (Gal3) [1]. Of the numerous known roles of Gal3 *in vivo*, its participation in the promotion of tumour spread and metastasis in cancer, and involvement in the elevation of adverse remodelling and fibrosis in the liver, kidney and heart are of particular interest to those attempting to predict and ameliorate disease phenotypes associated with these processes [2–6].

The Gal3 structure contains a single C-terminal carbohydrate recognition domain (CRD) responsible for lectin-type activity, allowing interaction with endogenous carbohydrate-bearing ligands such as cell surface glycoproteins [7], and which may be pharmacologically targeted for blockade by competitive binding of an alternative, exogenous ligand in order to ablate activity. The utility of an oral xenobiotic in this approach is of particular interest where Gal3 overexpression exists to produce deleterious effects such as those previously outlined. MCP has been suggested for this purpose and shows some early promise, however the use of this preparation in its crude form poses several challenges to those attempting to elucidate its true pharmacological value. Whilst studies describing Gal3-ligand interactions of particular MCP components at the CRD exist [8], the carbohydrate structure and composition of this hydrosylate is yet to be fully elucidated.

Several partially-defined pectic regions occur *in planta*, giving some predictive indication of the carbohydrate profile of MCP. The

Abbreviations: 2-AP, 2-aminopyridine; Ap, apical; Ara, arabinose; ASF, asialofetuin; ASGP-R 1, asialoglycoprotein receptor 1; Bl, basal; CRD, carbohydrate recognition domain; DMEM, Dulbecco's modified Eagle's medium; EIC, extracted ion chromatogram; EVOM, epithelial volt-ohm meter; Gal, galactose; Gal3, galectin-3; GalA, galacturonic acid; HBSS, Hank's balanced salt solution; HGA, homogalacturonan; HPAEC, high performance anion-exchange chromatography; MCP, modified citrus pectin; Rha, rhamnose; RGI, rhamnogalacturonan I; TEER, transepithelial electrical resistance; tR, retention time.

* Tel.: +44 01482 305 207; fax: +44 01482 305 206.
E-mail address: f.courts@hull.ac.uk.

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predicted oligosaccharide fragments of two specific pectic regions are of particular interest, both due to relative abundance in the macro structure of pectin and potential biological activities [8]. These oligosaccharides are summarised in Fig. 1. MCP is known to be rich in linear galacturonic acid (GalA) oligomers (α-D-Gal*p*A-[(1-4)-α-D-Gal*p*A]$_n$-(1-4)-α-D-Gal*p*A) with variable degrees of polymerisation (DP), produced from alkali hydrolysis of polygalacturonic acid backbones from the pectic homogalacturonan (HGA) region. The more complex and structurally variable rhamnogalacturonan I (RGI) regions comprise a chain of alternating Gal*p*A and rhamnose moieties ([(-4)-α-D-Gal*p*A-(1-2)-α-L-Rha*p*-(1-]$_n$) branched with repeating galactose ([-4)-β-D-Gal*p*-1-]$_n$) and arabinose ([-5)-α-L-Ara*f*-1-]$_n$) residues *in planta*. Following extraction, this structure is extensively hydrolysed during the production of MCP by acid treatment, releasing neutral oligosaccharides of variable DP, although the limited monosaccharide composition is likely to restrict structural variability. The decrease in DP has been touted to improve intestinal absorption, however oral bioavailability and therefore the biological relevance of MCP is entirely undefined [1]. The present study therefore aimed to profile small intestinal transport of both the major acidic and neutral oligosaccharide fragments from a representative MCP preparation, PectaSol-C (EcoNugenics Inc., CA, USA), using a two-compartment transwell Caco-2 cell monolayer system as a pharmacologically-predictive *in vitro* model of the small intestinal epithelium.

2. Materials and methods

2.1. Materials

Unless otherwise stated, all chemicals and reagents were purchased from Sigma–Aldrich Ltd. (Poole, Dorset, UK). Sodium hydroxide, sulphuric acid, MS-grade acetonitrile, isopropanol and formic acid, and HPLC-grade sodium acetate and acetic acid were purchased from Fisher Scientific (Loughborough, Leicestershire, UK). Research-grade CO_2 and N_2 was purchased from Energas (Hull, East Yorkshire, UK).

2.2. FITC labelling of neutral carbohydrates from MCP

Neutral sugar residues of MCP carbohydrate oligomers were covalently linked with FITC at hydroxymethyl groups by a modification to a reaction originally described for labelling of dextran by de Belder and Granath [9]. Briefly, 1 g MCP (PectaSol-C,

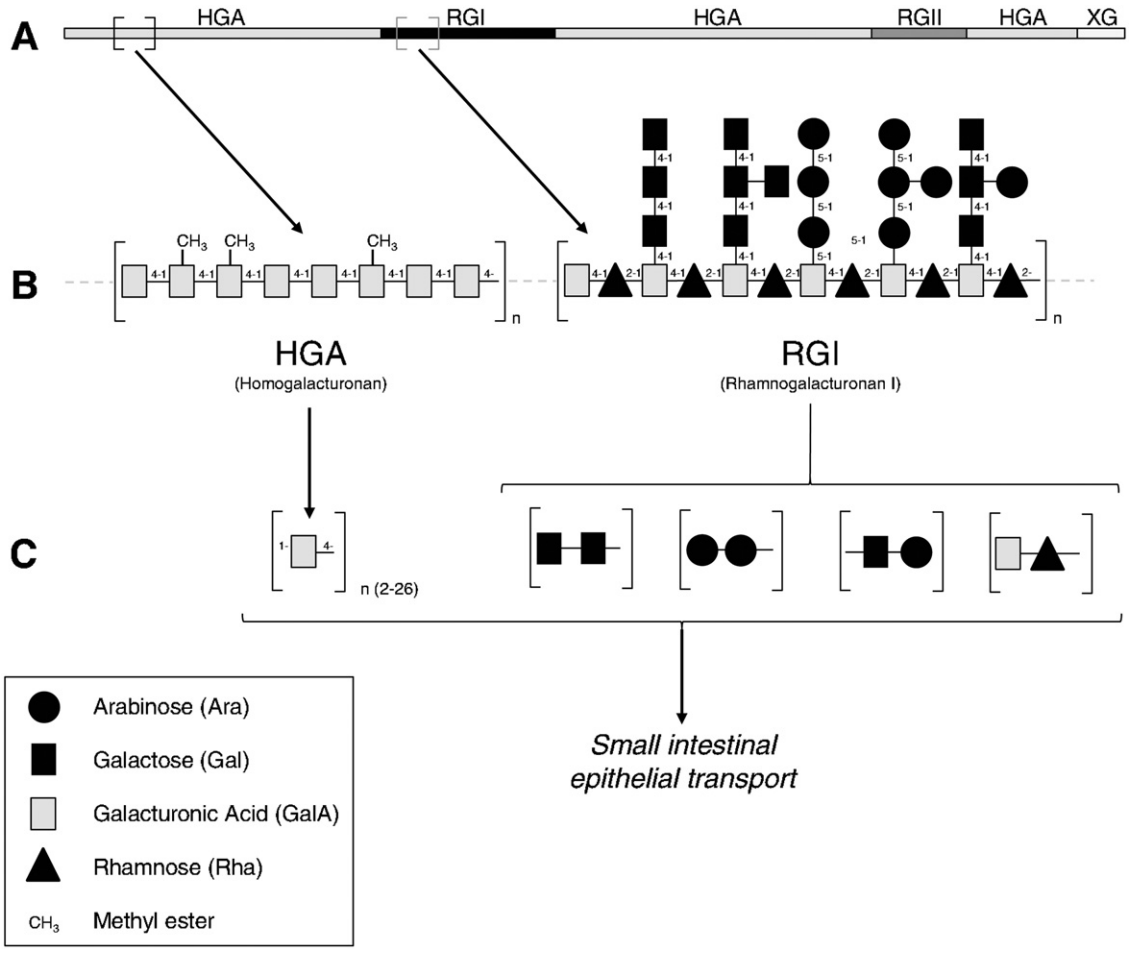

Fig. 1. Schematic diagram showing the macro pectin structure, containing the ubiquitous linear homogalacturonan regions (HGA) linked between the less-frequently occurring rhamnogalacturonan I (RGI), and the occasionally-occurring rhamnogalacturonan II (RGII) and xylogalacturonan (XG) regions (A). Representative structures of the two major pectic carbohydrate regions of interest in this study are expanded below (B). The HGA region is a polymer of linear and frequently-methylated 1-4 *O*-linked galacturonic acid (α-D-Gal*p*A-[(1-4)-α-D-Gal*p*A]$_n$-(1-4)-α-D-Gal*p*A) with a high DP, whereas RGI regions comprise a backbone of alternating galacturonic acid and rhamnose moieties ([(-4)-α-D-Gal*p*A-(1-2)-α-L-Rha*p*-(1-]$_n$) branched and sub-branched with galactose ([-4)-β-D-Gal*p*-1-]$_n$) and arabinose ([-5)-α-L-Ara*f*-1-]$_n$) oligomers. The acid and alkali treatment used to manufacture MCP results in the loss of GalA methylation and significant carbohydrate depolymerisation, producing a complex mixture rich in galacturonic acid oligomers (DP 2–26) and uncharacterised fragments of RGI, represented in (C). The transport of these acidic and poorly-characterised neutral oligomers from MCP across Caco-2 cell monolayers as a model for the small intestinal epithelium is the subject of this study.