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Sodium alginate decreases the permeability of intestinal mucus

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ABSTRACT

In the small intestine the nature of the environment leads to a highly heterogeneous mucus layer primarily composed of the MUC2 mucin. We set out to investigate whether the soluble dietary fibre sodium alginate could alter the permeability of the mucus layer. The alginate was shown to freely diffuse into the mucus and to have minimal effect on the bulk rheology when added at concentrations below 0.1%. Despite this lack of interaction between the mucin and alginate, the addition of alginate had a marked effect on the diffusion of 500 nm probe particles, which decreased as a function of increasing alginate concentration. Finally, we passed a protein stabilised emulsion through a simulation of oral, gastric and small intestinal digestion. We subsequently showed that the addition of 0.1% alginate to porcine intestinal mucus decreased the diffusion of fluorescently labelled lipid present in the emulsion digesta. This reduction may be sufficient to reduce problems associated with high rates of lipid absorption such as hyperlipidaemia.

1. Introduction

The putative health benefits of dietary fibre (DF) have been investigated over a number of years and have been shown to be many and varied (Brownlee, 2011; European Food Safety, 2010; Gunness & Gidley, 2010). By definition DF is not digested in the upper GI tract therefore early investigations into health benefits focused on the colon and the use of DF as a nutrient source for intestinal microbiota (Gibson & Roberfroid, 1995). However, more recently attention has also focused on interactions in the upper GI tract. In particular, the ability of DF to increase both gastric and intestinal viscosity has been investigated by a number of groups but a key early work showed that dietary fibre could be used to increase the viscosity of digesta and reduce the rate of absorption of glucose (Jenkins et al., 1978). More recent studies have confirmed the health benefits of a wide range of dietary fibre (Anderson et al., 2009; Frassetto, Schloetter, Mietus-Synder, Morris, & Sebastian, 2009; Nilsson, Ostman, Holst, & Bjorck, 2008). These include decreasing risk factors for CVD, lowering energy intake and glycaemic index, which have an impact on risk factors for type 2 diabetes. However, the mechanisms by which such health benefits are gained is still poorly understood (Lattimer & Haub, 2010). The DF sodium alginate has also been shown to reduce energy intake. In a study where participants consumed a preload sodium alginate formulation, daily preprandial ingestion of the sodium alginate formulation produced a significant 134.8 kcal (7%) reduction in mean daily energy intake (Paxman, Richardson, Dettmar, & Corfe, 2008).

Alginates are linear polysaccharides produced by marine brown algae (Phaeophyceae). They are used extensively in the food and chemical industries as thickening and stabilising agents. Alginate (E401) is a linear polysaccharide polymer with homopolymeric blocks of (1-4)-linked β-D-mannuronate (M) and α-L-guluronate (G) residues covalently linked together in different blocks. The monomers can appear in homopolymeric G-blocks, consecutive M-blocks or alternating MG-blocks. The ratio of M to G has a marked effect on the properties of the polymer. In a study by Jensen et al. (Jensen, Knudsen, Viereck, Kristensen, & Astrup, 2012) alginate solutions with a low M:G ratio (0.8) exhibited higher gel strength than solutions with higher M:G ratios (1.3 and 2.5). The gelation of alginate (sol/gel transition) is almost independent of temperature but is

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induced by the presence of divalent cations such as Ca^{2+}, which is associated with the G-blocks. Indeed there is a strong link between the strength of calcium alginate gels and the average length of the G-blocks (Smidsrod & Draget, 1997).

Digestion in the upper GI tract releases nutrients, which then diffuse through the unstirred mucus layer to the intestinal enterocytes where they are absorbed. However, many nutrients such as lipids and some vitamins are not water soluble and so spontaneously form self-assembled structures such as micelles and liposomes (Mu & Hoy, 2004) before diffusing away from the intestinal lumen. Both small particles and soluble hydrocolloids are free to diffuse from the lumen but have the potential to be trapped by the intestinal mucus layer. The ability of dietary fibre to increase luminal viscosity is known to be an important factor affecting both transit times and rates of hydrolysis. Thus fibre may trap the products of digestion such as mixed micelles and consequently slow absorption. It has also been suggested that soluble dietary fibre may be trapped on the surface of the mucus and that this in turn prevents the diffusion of other hydrocolloids or mixed micelles (Gunness & Gidley, 2010; Theuwissen & Mensink, 2008). Such a mechanism has been used as a possible explanation of the cholesterol lowering ability of DF (Othman, Moghadasian, & Jones, 2011) as it would also interrupt the recycling of bile salts leading to the conversion of cholesterol into bile in the liver. In the absence of an attractive interaction between the DF and mucus the trapping of the DF by the mucus is dependent on the relative sizes of the DF polymers and the pores in the mucus network. Recent work has shown that the pore size of the mucus varies quite widely from 25 to 200 nm (Round et al., 2012). This compares to the size of a typical DF such as β-glucan, molecular weight 100–1000 kDa (150–250 nm) (Shelat, Vilaplana, Nicholson, Gidley, & Gilbert, 2011) or alginate of ~190 KDa and ~100 nm in diameter (Strand, Boe, Dalberg, Sikkeland, & Smidsrod, 1982).

Examples of alginate/mucin interaction with biological relevance have been shown previously (Taylor, Pearson, Draget, Dettmar, & Smidsrod, 2005). Indeed, the authors of the article have a patent for using oligo-guluronates alone (polyguluronic acid) to disrupt airway mucus in cystic fibrosis patients (Taylor, Draget, & Smidsrod, 2009). In addition to the function of the oligo-guluronates, the mixed block polymers have been suggested to be mucin secretagogs (Shimotoyodome, Meguro, Hase, Tokimitsu, & Sakata, 2001). This appears to be a general affect for high viscosity fibre (Ito et al., 2009) and this is partly supported by the fact that the use of low molecular weight sodium alginate in rats showed no activity after feeding for 10 days (Hino et al., 2013). Thus it appears that while G-block alginate polymers disrupt mucus structure, other alginates enhance the mucus layer.

In the work described here we have studied the ability of two different types of alginate with differing M:G ratios to diffuse into intestinal mucus and subsequently change its permeability to a range of particles. We have used 500 nm carboxylated latex beads as a probe particle in order to determine the micro-viscosity of the mucus alginate mixture and fluorescently labelled chyme resulting from simulated digestion of a fat containing meal in order to determine changes in mucus permeability to the self-assembled products of lipid digestion.

2. Materials and methods

2.1. Mucus

Porcine mucus was prepared as described previously (Macierzanka et al., 2011). Briefly, fresh porcine small intestine was obtained from a local abattoir and stored on crushed ice for transport to the laboratory. The gut was rinsed through with ice cold phosphate buffer (10 mM phosphate pH 6.5, 5 mM EDTA) followed by a further rinse with the same buffer containing a protease inhibitor (0.5 mM Pefabloc (AEBSF)). The gut was then opened out flat and mucus was collected by gently scraping the jejunal surface. Samples were frozen and stored at −20 °C for further use. MUC2 mucin was prepared for DPI measurements using the methods described elsewhere (Macierzanka et al., 2011).

2.2. Alginate

Alginates with a range of mannuronic/guluronic (M/G) ratios were kindly donated by Danisco. Their composition, molecular weight and the viscosity of a 1% (w/w) solution are as follows: Sample D1 had an M/G ratio of 60/40, a molecular weight of 350 kDa. The D4 sample had an M/G ratio of 35/65, a quoted molecular weight of 315 kDa. Subsequent measurement of the molecular weight yielded a weight average of 280 kDa and the size distribution given in Fig. 1. Samples of both forms of alginate were fluorescently labelled using DTAF (5-(4,6-dichlorotriazinyl) aminofluorescein) (Life Technologies Ltd, Paisley, UK). Polysaccharide was dissolved at 10 mg/mL in 50 mM sodium bicarbonate adjusted to pH 9.0 with 1.0 M NaOH. This was mixed overnight at 1:0.4 v/v with a solution of DTAF (10 mg/mL in DMSO) at room temperature. The reaction mixture was dialysed in 10 kDa cut-off dialysis tubing against PBS until no residual DTAF could be detected in the dialysate by UV absorbance at 490 nm. The labelling density of the resulting alginate was an average of 2.5 fluorophore molecules per alginate molecule. Although the labelling involved prolonged exposure of the alginate to pH 9, this is unlikely to have degraded the alginate by β-elimination and certainly no evidence of this was seen.

2.3. Alginate heterogeneity and molecular weight characterisation

Sedimentation velocity was performed on a solution of alginate. A very low concentration (~0.03 mg/mL) and high ionic strength solvent (0.3 M NaCl) were used so as to render negligible the effects of non-ideality. A high ionic strength was chosen to minimise non-ideality effects an Optima XL-I (Beckman Instruments, Palo Alto, USA) equipped with Rayleigh interference optics was used. Sample solution (400 μl) and reference buffer (400 μl) were injected into

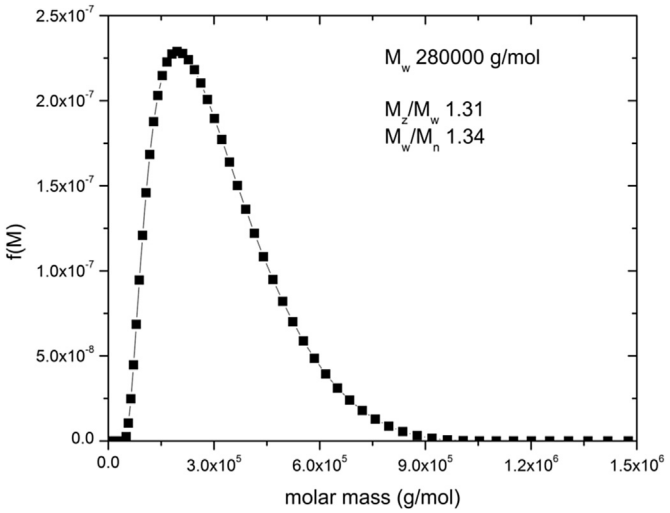

Fig. 1. Molecular weight distribution for D4 alginate in 0.3 NaCl obtained from transformation of the sedimentation coefficient distribution at concentration (0.03 mg/mL), using the Extended Fujita method (Harding et al., 2011).