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Roles for dietary fibre in the upper GI tract: The importance of viscosity

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ABSTRACT

Dietary fibre has long been recognised as healthy because of its prebiotic quality and a number of dietary fibres, especially beta glucan have been shown to lower levels of circulating LDL cholesterol. However, although EFSA allow health claims to be made for this, there is no fundamental understanding of the detailed mechanism involved. More recently dietary fibre has been shown to have a range of functionality in the upper GI tract. The presence of fibre can alter gastric emptying thus affecting fullness and satiety. These alterations are a result of differences in viscosity, nutrient release and nutrient sensing in the duodenum. The current proposed mechanisms for the cholesterol lowering effects involve disruption of the normal recycling of bile possibly by sequestering bile salts and fatty acids or by significantly decreasing the rate of absorption as a result of entanglement with intestinal mucus.

The use of quantitative confocal microscopy methods such as fluorescence recovery after photobleaching (FRAP) and multiple particle tracking has provided evidence that dietary fibre can combine with intestinal mucus and produce a layer that significantly delays the transport of lipid digestion products. We have also used similar methods in conjunction with more conventional rheology to show that DNA from the gut epithelium can contribute significantly to the barrier properties of the intestinal mucus layer.

The delay in the transport of nutrients to the gut epithelium has implications for the control of gastric emptying and through secretion of GI hormones such as CCK and thus for the satiating ability of foods. It may also have implications for the reabsorption of bile.

1. Introduction

Fibre has long been seen as part of a healthy diet that can reduce the risk of developing a wide range of disorders including cardiovascular disease and diabetes (Anderson et al., 2009). The definition of dietary fibre as food that is not digested in the upper GI tract has inevitably led to much of the research on fibre being concentrated on fermentation in the colon and only recently have the benefits to the gut more generally been discussed (Brownlee, 2011; Gidley, 2013). There have now been a number studies that show the health benefits of fibre in the diet and this fact has become sufficiently accepted to allow health claims to be made about specific types of fibre such as beta glucan (EFSA-NDA, 2011). However, despite the fact that health claims can be made based on clinical data, the detailed mechanism leading to the outcome may not be well understood (Lattimer & Haub, 2010). Such mechanisms are thought to include the increase in viscosity caused by the high molecular weight polymers in soluble fibre and the ability of the fibre to bind endogenous compounds such as enzymes or bile acids. In particular the increase in viscosity has been linked to lower calorie intake. For example, sodium alginate has been shown to reduce energy intake in a study where participants consumed a preload sodium alginate formulation, the daily premeal consumption of the polymer produced a significant 134.8 kcal (7%) reduction in mean daily energy intake (Paxman, Richardson, Dettmar, & Corfe, 2008).

Alginates are linear polysaccharides produced from marine algae. They are widely used in the food industry as a thickener (E401) and in the pharmaceutical industry for encapsulation. The linear alginate polymer is made from two types of saccharide building blocks. These are β-D-mannuronate and α-L-guluronate, known as M and G residues respectively. The M and G residues are 1–4 linked together in different blocks that can be comprised of G only (G-block), M only (M-block) or a mixture of M and G (MG-blocks). The ratio of M and G in the polymer has an impact on the rheological properties of the gels formed. Alginate solutions with a low M:G ratio (0.8) have been shown to have a higher gel strength than solutions with higher M:G ratios (1.3 and 2.5) (Jensen, Knudsen, Viereck, Kristensen, & Astrup, 2012). The gelation of alginate is almost independent of temperature but is induced by the presence of divalent cations such as Ca^{2+}, which is associated with the G-blocks and by reduction in pH.

Mucins are widely found in nature and provide a protective layer to a wide range of epithelial surfaces. Mucins are broadly separated into two categories, those that are membrane bound and those that are secreted. The viscoelastic properties of the secreted mucins are key to their functionality. In particular, gastrointestinal and pulmonary secreted mucins provide both lubrication and a barrier function to particulates while allowing the passage of small molecules (Corfield, 2015). In the small intestine on mammals, the secreted mucins such as MUC2 form

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a complex structure. After secretion from the goblet cells of the intestinal epithelium, the mucin granules unfold to form a tightly adherent mucus layer that comprises a hexagonal network with a mean pore size around 100 nm (Johansson, Sjövall, & Hansson, 2013; Round et al., 2012). This layer varies in thickness along the intestine, being thinnest in the proximal regions and thicker more distally. On the top of the tightly adherent layer is a loosely adherent layer that can be much more heterogeneous, especially in the small intestine.

There is already evidence that certain types of alginate can have a significant effect on the rheological properties of mucus (Taylor-Nordgard, Nonstad, Olderoy, Espevik, & Draget, 2014). Taylor-Norgard et al. showed that the addition of low molecular weight G-block oligomers were able to decrease the rheological properties of sputum from cystic fibrosis patients. The same group has also shown the ability of high molecular weight alginate to increase the rheological properties of gastric mucus (Taylor, Pearson, Draget, Dettmar, & Smidsrod, 2005). In this study we have attempted to build on this earlier work by showing that alginate may decrease the permeability of intestinal mucus by increasing the local viscosity. Thus, we hypothesise that soluble dietary fibre, in this case sodium alginate interacts with intestinal mucus in a way that decreases its permeability.

2. Materials and methods

2.1. Materials

Alginates with a range of mannuronic/guluronic (M/G) ratios were kindly donated by Danisco (DK-8220 Brabrand). The sample chosen had an M/G ratio of 35/65 and a quoted molecular weight of 315 kDa. Subsequent measurement of the molecular weight yielded a weight average of 280 kDa. The ex vivo porcine mucus was prepared as described previously (Macierzanka et al., 2011). Briefly, fresh porcine small intestine was obtained from a local abattoir. The gut was rinsed through with ice cold phosphate buffer (10 mM phosphate pH 6.5, 5 mM EDTA, 0.5 mM Pefabloc (AEBSF)). The gut was then opened out flat and mucus was collected by gently scraping. Samples were frozen and stored at −20 °C for further use.

2.2. Fluorescent labelling

Samples of alginate were fluorescently labelled using DTAF (5-(4,6-dichlorotriazinyl) aminofluorescein (Life Technologies Ltd., Paisley, UK). The alginate was dissolved at 10 mg/ml in 50 mM sodium bicarbonate and 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.

2.3. Rheology

Rheological properties of the ex vivo mucus containing increasing concentrations of sodium alginate were investigated using a controlled strain AR2000 rheometer (TA Instruments, Crawley, West Sussex, UK) equipped with a cone and plate geometry (aluminium cone; 6°/20 mm cone angle/diameter, truncation gap 12 μm). A viscosity ramp test was conducted using a shear rate range from 0.01 to 500 s^{-1} over 15 min and the temperature was held at 37 °C. All experiments were done in triplicate and the data shown as mean values.

2.4. Emulsion and digestion

A protein stabilised emulsion was produced using the method described in Mackie et al. (Mackie, Macierzanka, Aarak, Ridout, & Bajka, 2015). Briefly, 3.0 mg/mL sodium caseinate solution in 150 mm NaCl pH 6.5 stabilised emulsion containing 18% triglyceride (sunflower oil) was prepared by passing a premix of oil and Na-Cas for a total of 6 times at 20,000 psi through a Microfluidiser (Microfluidics, Massachusetts, USA). The emulsion was digested using the standardised in vitro digestion protocol recommended by the Infogest COST Action (Minekus et al., 2014), involving 2 h gastric followed by 2 h of small intestinal simulation. The mean size ($D_{3,2}$) of the original emulsion was 1.0 μm. The mean size of the emulsion after digestion was 725 nm and the size distributions can be seen in a supplementary figure (Figure S1) to (Mackie et al., 2015).

2.5. Diffusion

The diffusion coefficients were determined using three different methods. The ability of the sodium alginate to penetrate into porcine mucus was determined using time-lapse microscopy. Ex vivo porcine mucus and fluorescein-labelled alginate were layered in a 9 mm diameter by 0.9 mm depth perfusion well (CoverWellTM, Sigma, Poole. UK). Laser scanning confocal microscopy was used to follow the diffusion of the alginate into the mucus for 90 min using a Leica SP1 with a 20× objective (Leica Microsystems, Mannheim, Germany). Linear fluorescent intensity profiles were generated from the time-lapse images and the diffusion coefficient was calculated. The diffusion of alginate in mucus was also assessed using fluorescence recovery after photobleaching (FRAP). Briefly, fluorescein-labelled alginate was mixed into mucus samples at concentrations between 0 and 0.1%. Samples were gently mixed and then loaded onto glass slides using 9 mm × 120 μm SecureSeal spacers (Sigma, Poole, UK). The FRAP measurements were made using a Leica SP5 (II) Laser scanning confocal microscope. A bleach spot of 50 μm diameter was used with an initial post bleach of 3.7 s at 37 ms/frame, followed by 25 s at 250 ms/frame. Diffusion coefficients were calculated from the fluorescence signal using nonlinear least-square fitting as described by (Ladha et al., 1996).

The final method for determining diffusion coefficients of probe particles was particle tracking, again using a confocal microscope. The Stokes viscosity was calculated from ensemble data from particle tracking of 500 nm latex beads. The mean square displacement (MSD) of 500 nm latex beads was determined over 50 frames at 2 frames/s using multiple particle tracking as outlined in more detail previously (Macierzanka et al., 2011). The diffusion coefficient (D) was calculated from the MSD using the relation D = MSD/4 t, where t is the timescale over which the displacement has occurred. The viscosity was then calculated from the diffusion coefficient via the Stokes Einstein relation.

3. Results and discussion

In this article we describe a set of experiments to assess the ability of sodium alginate to decrease the diffusion of the products of digestion from the intestinal lumen to the site of absorption. As outlined above, one of the primary effects of soluble fibre in the small intestine is thought to be its ability to increase the viscosity of luminal contents and thus decrease the diffusion of nutrients from the site of hydrolysis to the intestinal enterocytes. Thus our first measurement was to determine the minimum concentration required to increase viscosity under conditions simulating the small intestinal environment. In particular, we determined the viscosity of sodium alginate as a function of concentration over a shear rate range between 10 to 500 s^{-1}. Over the specified range there was no significant difference in viscosity as a function of shear rate, therefore the mean value was used. The results are shown in Fig. 1 and indicate that in the intestinal environment, local concentrations of the alginate would need to be greater than 0.1% in order to increase the viscosity much above that of water and that concentrations close to 1% would be required to have a really significant effect on viscosity. This data is consistent with previous assessment of the critical overlap concentration (c*) for alginate of 0.2–0.4% wt/wt depending on conditions (Nickerson & Paulson, 2004). Although this study showed that c* decreases as the ionic strength increases, it is