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Diffusion and viscosity in arabinoxylan solutions: Implications for nutrition

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ABSTRACT

Non-starch polysaccharides such as arabinoxylans have important roles in the human diet, resulting in potential benefits such as increased microbial fermentation, promotion of beneficial microflora, prevention of re-absorption of bile acids leading to lower plasma cholesterol, and retardation of starch digestion. The latter two beneficial effects may arise from viscosity and/or diffusion phenomena in the gastrointestinal tract. To study this, measurements of the viscosity and diffusion coefficients of a polymer probe similar in size to both bile salt micelles and alpha-amylase were carried out for water solutions of three arabinoxylans with differing viscosities. Diffusion coefficients were obtained using fluorescence recovery after photobleaching (FRAP). The concentration dependence of both viscosity and diffusion coefficients followed the usual behaviour of polymers for each of three arabinoxylan samples. However, at a given concentration, the sample with the highest viscosity also had the highest probe diffusion coefficient: the reverse of what would be expected for homogeneous solutions. This apparent anomaly is ascribed to differences in polymer structure between the three samples giving rise to varying levels of local polymer aggregation and consequent microvoids. These differences are verified using characterisation with multiple-detection size-exclusion chromatography. Deviations from simple Stokes–Einstein behaviour are ascribed to the existence of aggregates in solution. The results show that studies of the role of arabinoxylans in human nutrition cannot assume that the diffusion coefficients of species with sizes in the range important for digestive processes in a series of samples will increase with decreasing viscosity at a given concentration: diffusion coefficient and viscosity must be measured independently.

1. Introduction

Cereal grains, important for human nutrition, mainly consist of starch and protein together with non-starch polysaccharides (NSPs). These NSPs are major component of cell walls, particularly in the starchy endosperm (Saulnier, Guillon, Sado, & Rouau, 2007, Saulnier, Sado, Branlard, Charmet, & Guillon, 2007). Although NSPs typically represent only about 3–8% of the total of the grain, they have major effects on the technological use and nutritional value of cereal grains due to their hydration properties, viscosity in aqueous solution, and consequent role as dietary fibre. Arabinoxylan (AX) is the major NSP of wheat endosperm cell walls and shows quantitative differences in structural features depending on tissue location as well as grain species (Saulnier, Guillon, et al., 2007; Saulnier, Sado, et al., 2007). AX is a copolymer with a linear backbone of (1–4)-linked β-D-xylopyranosyl units; these β-D-xylopyranosyl units are substituted at some O_2 and/or O_3 positions with α-L-arabinofuranose. Some of these arabinofuranose units are esterified with ferulic acid, with the consequent potential for oxidative cross-linking. Diverse physicochemical properties, particularly solubility and network formation, can result from variation in the level and pattern of arabinose substitution and the extent of cross-linking via ferulic acid substituents (Saulnier, Guillon, et al., 2007; Saulnier, Sado, et al., 2007).

The structure of AX, its physicochemical properties, and its role in food quality and beneficial health effects, have received some attention in the literature (Hoffmann, Kamerling, & Vliegenthart, 1992; Izydorczyk & Biliaderis, 1992, 1995; Knudsen & Jorgensen, 2007; Pitkanen, Virkki, Tenkanen, & Tuomainen, 2009; Saulnier, Guillon, et al., 2007; Saulnier, Sado, et al., 2007; Warrand et al., 2005). AX is a recognised dietary fibre, and has been reported to lower cholesterol absorption, to improve metabolic control in type 2 diabetes subjects, and to increase microbial fermentation and promote microbial flora in the large intestine (Hanai et al., 1997; Lopez et al., 1999; Lu, Walker, Muir, & Dea, 2004). Despite this importance, AXs are considerably less studied than other NSPs such as (1,3;1,4)-β-D-glucan ('β-glucan') and guar galactomannan. Blackburn and Johnson (1981) and Jenkins et al. (1978) measured changes in the

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viscosity of gastrointestinal contents of rats in the presence of guar galactomannan and inferred that the presence of this soluble dietary fibre reduces absorption of glucose from the small intestine because it causes viscosity to increase. The principal hypotheses for the reduced glycemic response in the presence of soluble fibres are delayed gastric emptying, a lower rate of diffusion of alpha-amylase to starch substrates and of released hydolysed starch fragments from the intestinal lumen to the absorptive epithelial cells that line the digestive tract and/or increased intestinal mobility resulting in faster intestinal passage (Dikeman & Fahey, 2006; Jenkins et al., 1978; Lu et al., 2004). These mechanisms are considered to be the result of either the highly viscous nature of soluble fibres in aqueous solution and/or the effect on the diffusion of digestive enzymes in the presence of viscous dietary fibres in the gut. Although the presence of soluble dietary fibre in food is likely to alter the physical state of intestinal contents, particularly increasing the viscosity, it is not yet established whether there is a simple relationship between bulk viscosity and the diffusion of probe molecules of the size of bile salt micelles and alpha-amylase for solutions of soluble dietary fibres such as AX.

The present investigation describes the rheological and diffusion behaviour of solutions of three AX samples from wheat and rye sources. Both viscosity and diffusion effects are measured as a function of AX concentration. The diffusion coefficients of an enzyme-sized probe (FITC–dextran) in AX solutions are measured using fluorescence recovery after photobleaching (FRAP) (Axelrod, Koppel, Schlessinger, Elson, & Webb, 1976; Bryers & Drummond, 1998; Burke, Park, Srinivasarao, & Khan, 2000; Perry, Fitzgerald, & Gilbert, 2006), an optical-microscopy technique. The rheological behaviour of the AXs is characterised in water and in water–dextran solution. A series of experiments using both steady and oscillatory shear in Couette geometry are performed. In addition, the molecular size and weight distributions in dimethyl sulfoxide solution, and the average size of AX molecules/aggregates in aqueous solution, are obtained using size-exclusion chromatography (SEC; also known as gel-permeation chromatography, GPC) and dynamic light scattering, respectively. The objective of the size distribution analysis is to see if there are significant differences between the structure and/or chemical heterogeneity in three samples of AX, and that of the dynamic light scattering is to see if the average size depends on AX concentration, which would indicate aggregation.

2. Materials and methods

2.1. Materials

Three AXs, with kinematic viscosity at 1% (w/v) concentration reported by the manufacturer to be 24, 29 and 33 cSt (corresponding to dynamic viscosities of 24, 29 and 33 mPa s; 1 cP = 1 mPa s for a fluid of density 1 g cm^{-3}) were procured from Megazyme International Ireland Ltd. We denote these here as AX24 (WAX-MV, lot 20401), AX29 (WAX-MV, lot 40301) and AX33 (RAX-HV, lot 20601). AX24 and AX29 are from wheat and AX33 is from rye. These materials have recently been characterised (Pitkanen et al., 2009), including chemical composition analysis by NMR spectroscopy and molecular size analysis by size-exclusion chromatography (SEC) in 0.1 M $NaNO_3$ in water, and 0.01 M LiBr in dimethyl sulfoxide. Fluorescein isothiocyanate conjugate tagged dextrans (FITC–dextran) of weight-average molecular weight 7.0×10^4, denoted FD70S, was obtained from Sigma Chemical Co. and used as received. This probe was chosen due to size similarities with digestive enzymes such as α-amylase (crystallographic radius of ~3 nm, Payan et al., 1980) as well as bile salt micelles (typically ~10 nm diameter, Carey & Small, 1970). FD70S contained 0.003–0.02 mol of FITC per mol of glucose and had a stated dispersity (this term replacing what used to be denoted by terms such as 'polydispersity' or 'polydispersity index', Gilbert et al., 2009) ($\bar{M}_w/\bar{M}_n$) < 1.30. The hydrodynamic radius of the probe was 6 nm (Burke et al., 2000; Perry et al., 2006; Pluen, Netti, Jain, & Berk, 1999).

2.2. Measurement of diffusion coefficients using FRAP

The method used was similar to that used for the measurement of diffusion coefficient of FITC–dextran in starch solutions by Perry et al. (2006) 0.7% (w/v) solution of FITC–dextran in deionised (DI) water was prepared by dissolving FITC–dextran in DI water at ambient temperature. The sample for FRAP was prepared by dissolving AX (at concentrations ranging from 0.025% to 1%, w/v) in FITC–dextran/DI water solution at 40 °C, using 300 rpm over 1–2 h in a thermomixer (Eppendorf AG 22331, Hamburg, Germany). The sample was introduced into the cavity of a microscope glass slide using a micropipette (38–40 μL) and the glass slide was covered with a cover slip using liquid nail polish. FRAP experiments were carried out with a ZEISS LSM 510 META Confocal Microscope using a 10× objective lens. The uniform bleach disc radius was ~25 μm and samples were bleached using a 10 mW argon ion laser. FRAP image acquisition was performed using frame size 256 × 256 with maximum scan speed. The bleaching of the sample was ~40–50% after the first two scans. The numerical aperture and depth range were the same as described by Perry et al. (2006); the temperature was ambient (~23 °C). Data were fitted using the model of Braeckmans for the normalised recovery curve at time t, $F_{\mathrm{tot}}(t)/F_0$ (Braeckmans, Peeters, Sanders, De Smedt, & Demeester, 2003):

$$\frac{F_{\mathrm{tot}}(t)}{F_0} = 1 + [1 - e^{-2y}(I_0(2y) + I_1(2y))] \sum_{n=0}^{\infty} \left[\frac{(-K_0)^n}{n!} \frac{1}{\sqrt{1+n}} \right] \qquad (1)$$

where $y = \omega^2/(4Dt)$, D is the two-dimensional lateral diffusion coefficient, ω is the e^{-2} radius of the Gaussian bleach spot, I_0 and I_1 are the modified Bessel functions of the zero and first order, respectively, and K_0 an instrumental parameter. The fit to the data involves least-squares minimisation with respect to the quantities D and K_0.

2.3. Rheological characteristics of arabinoxylan

Rheological studies were performed with an Advanced Rheometric Expansion System (ARES; TA Instruments). Solutions at different concentrations, ranging from 0.2% (w/v) to 10% (w/v), were prepared by dissolving AX29 and AX33 in DI water at 80 °C for 2 h; rheology was not performed on AX24 because of limited sample availability. Further samples were prepared by dissolving AXs in a 0.7% (w/v) dextran/water solution. Steady-shear and oscillatory measurements were carried out over a range of frequencies using strain control with both Couette and parallel plate geometries. All the samples were first tested in oscillatory mode to determine their linear viscoelastic region by performing a strain sweep experiment at a frequency of 10 rad s^{-1}. This then allowed for a suitable choice of strain amplitude to be employed for frequency sweep experiments from 0.1 to 100 rad s^{-1}. The steady-shear experiments were performed for a range of frequencies from 0.5 to 100 s^{-1}. All the samples were freshly prepared for each measurement and all the measurements were performed in duplicate at 23 °C.

2.4. SEC

The three different AX samples were injected into an Agilent 1100 Series SEC system (PSS GmbH. Mainz, Germany) using a GRAM preColumn and two 100 columns (PSS GmbH. Mainz, Germany) in series, in a column oven at 80 °C. The flow rate was 0.3 mL min^{-1}. The system was equipped with multi-angle laser light scattering