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The cation-controlled and hydrogen bond-mediated shear-thickening behaviour of a tree-fern isolated polysaccharide

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ABSTRACT

The shear-thickening rheological behaviour (between 5 and 20 s^{-1}) of a 5% (w/w) viscoelastic gum extracted from the fronds of the native New Zealand black tree fern or *mamaku* in Māori was further explored by manipulating the salt content. The freeze-dried mamaku gum contained a high mineral content and sugars which upon removal *via* dialysis, resulted in the loss of shear thickening. However, this loss was reversible by the addition of salts to the dialysed dispersion. The mechanism of shear-thickening behaviour was therefore hypothesised to be due to shear-induced transition of intra- to intermolecular hydrogen bonding, promoted by the screening effect of cations. Mono-, di- and trivalent salts, *i.e.* Na^+, K^+, $N(CH_3)_4{}^+$, Ca^{2+}, Mg^{2+}, Al^{3+} and La^{3+} at concentrations between 0.001 and 1.0 M were tested to support the hypothesis as well as to demonstrate the sensitivity of the biopolymer to cation valency and concentrations. The cation valency and concentration were crucial factors in determining: (i) zero-shear viscosity, (ii) critical shear rate, $\dot{\gamma}_c$ (or shear rate at the onset of shear-thickening) and (iii) the extent of shear-thickening of the solution. For mono- and divalent cations these parameters were similar at equivalent ionic strengths and fairly independent of the cation type. Trivalent cations (La^{3+}) however caused precipitation of the gum in the concentration range of 0.005–0.05 M but clear dispersions were obtained above 0.05 M.

1. Introduction

The black tree fern, also known as '*mamaku*' in Māori, is a native and endemic tree fern of New Zealand. The pith from the fronds exudes a slimy mucilage when cut, which is traditionally applied to wounds and burns or consumed by the Māori as food or for treating diarrhoea (Foster, 2008). This mucilage has been found to exhibit unusual rheological behaviours including shear-thickening and the Weissenberg effect (Goh, Matia-Merino, Hall, Moughan, & Singh, 2007), which holds great potential as an ingredient in food, pharmaceutical or cosmetic applications. Our recent structural characterisation of the purified mamaku polysaccharide molecule indicated that it is a glucuronomannan backbone (methylesterified 4-GlcpA with 2,3- and 2,3,4-linked Manp) with branched sugar side-chains of galactose, arabinose, non-methylesterified glucuronic acid and other simple sugars at the O-3 and O-4 of the mannose residues. The total uronic acid content of the polysaccharide is about 50 mol% (Wee, Matia-Merino, Carnachan, Sims, & Goh, 2014).

The freeze-dried native mucilage consists of 11% (w/w) non-starch polysaccharide, 10.3% (w/w) starch, 49% (w/w) sugars, 2.2% (w/w) crude protein and 18% (w/w) ash and 0.2% (w/w) fat. The ash is made up of potassium (6.6%, w/w), sodium (1.5%, w/w), calcium (0.20%, w/w), magnesium (0.12%, w/w), aluminium (0.12%, w/w) and trace amounts of other minerals (Goh et al., 2007). The high mineral content of this mucilage seems to serve a purpose in nature; one of the reasons could be the result of a defence mechanism in plants where toxic minerals are adsorbed within the mucilage matrix by carboxylic groups of plant cell wall polysaccharides to prevent further transport into the plant system (Kinraide, Parker, & Zobel, 2005). Furthermore, cations may also be absorbed by the mucilage for storage of essential mineral nutrients, facilitating ion transport to other parts of the plant, and regulating ion nutrition (Edmond-Ghanem et al., 2010).

Biopolymers with shear-thickening properties are rarely encountered in nature, except those which are chemically modified. Some examples of shear-thickening biopolymers include pectin at low shear rates (Kjøniksen, Hiorth, & Nyström, 2005; Kjøniksen, Hiorth, Roots, & Nyström, 2003), hydrophobically modified HM-alginate (Burckbuchler et al., 2006), waxy maize starch (Kim, Willett, Carriere, & Felker, 2002), 1-deoxylactit-1-yl

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chitosan (Yalpani, Hall, Tung, & Brooks, 1983), and enzymatically-synthesised dextran (Sabatié, Choplin, Paul, & Monsan, 1986). For their synthetic counterparts the most common of such shear-thickening polymers studied are *e.g.* poly(ethylene) oxide (Ma & Cooper, 2001; Rivero, Gouveia, Mueller, & Saez, 2012), hydrophobic alkali-soluble associative polymers (HASE) solutions (Tan, Tam, & Jenkins, 2001), hydrophobically modified ethoxylated urethane (HEUR) (Barmar, Kaffashi, & Barikani, 2005; Suzuki, Uneyama, Inoue, & Watanabe, 2012; Tam, Jenkins, Winnik, & Bassett, 1998; Tripathi, Tam, & McKinley, 2006). The shear-thickening effect in these synthetic polymers may be better explained than in the biopolymers due to their known and controlled chemical structures.

Currently, the widely accepted mechanisms for shear-thickening are: (i) chain stretching into the non-Gaussian regime under shear (Marrucci, Bhargava, & Cooper, 1993) and (ii) shear-induced transition from intra- to intermolecular association for associating polymers (Leibler, Rubinstein, & Colby, 1991; Witten & Cohen, 1985). The second mechanism may be of greater relevance to (ionic) biopolymers such as polysaccharides and proteins which are made up of numerous functional groups capable of intermolecular associations *via* electrostatic attraction (—COO^-, $\text{—SO}_3{}^-$), hydrogen-bonding (—OH, —NH_2, —C=O) and/or hydrophobic associations (—CH_3, —COOCH_3). Studies done on various classes of materials such as micelles (Vasudevan, Shen, Khomami, & Sureshkumar, 2008) (cetyl-trimethylammonium bromide/sodium salicylate solution), polysaccharides (pectin) (Kjøniksen et al., 2003) and hydrophobic polymers (Feng, Grassl, Billon, Khoukh, & François, 2002; Tan et al., 2001) have attributed intermolecular associations formed during shear to the shear-thickening phenomenon. Salt concentration and valency have been found to be key factors in influencing these associations and the subsequent shear-thickening due to the ionic nature of these materials.

Ionic polysaccharides generally exhibit more complex behaviours than neutral polysaccharides. The ability of charged groups to bind and interact with other charged species gives rise to functional properties such as gelation, complexation with cations (Rendleman, 1978a, 1978b), and formation of protein-polysaccharide complexes (de Kruif, Weinbreck, & de Vries, 2004). Metal-polysaccharide interactions are governed by key parameters of both polysaccharides and cations: (i) polysaccharide concentration, (ii) linear charge density of the polysaccharide backbone, (iii) distribution of ionic sites along the chain, (iv) ionic charge, (v) cation-specificity and (vi) ionic radius. By comparing the effect of a few selective cations, the overall reactivity of the polysaccharide to cations can be described, as we will in this paper.

The sensitivity of charged polysaccharides to salts is manifested in their rheology. The concentration of salts can alter the chain conformation and their interactions. In the absence of salts, electrostatic repulsion between similarly charged groups along the backbone expands the chain and results in an extended conformation contributing to a higher intrinsic viscosity. When salts are present in solution, the counterions screen the electrostatic repulsion between charged groups therefore shrinking the conformation of the chain and lowering its viscosity. In some acidic polysaccharides such as pectin and alginate, the intermolecular network formed with cations can lead to gelation. Apart from the well-known viscosity reduction of polyelectrolytes in the dilute and semi-dilute concentration regime, charged polysaccharides like xanthan, carrageenan and welan exhibit an increase in viscosity with cations in the electrostatic blob overlapping concentration (C_D) (Dobrynin, Colby, & Rubinstein, 1995; Wyatt, Gunther, & Liberatore, 2011; Wyatt & Liberatore, 2010). This phenomenon is attributed to the formation of hydrogen bonds when the electrostatic repulsions are screened (Wyatt & Liberatore, 2010).

The shear-thickening mechanism of the mamaku mucilage has yet to be fully understood. Our previous study (Matia-Merino, Goh, & Singh, 2012) reported the effect of salt (mono- and divalent cations), temperature and pH on the native extract. This study showed that salts (NaCl, KCl, $CaCl_2$, $MgCl_2$) at 0.5 M concentration did not have a significant effect on the shear-thickening behaviour of the native fraction. The total salt concentration within the native extract (16.07% ash, Goh et al., 2007) was sufficiently high. Hence, further addition of salt showed insignificant effect on the polysaccharide viscosity. However, the lowered viscosity and slightly suppressed shear-thickening at low pH (being the opposite for high pH) could suggest the involvement of anionic groups. From the earlier study it was also clear that the shear-thickening effect was lost at high temperatures (≥50 °C), suggesting that hydrogen-bonds were involved in the shear-thickening behaviour.

In the present study, as a result of the loss of shear-thickening exhibited by the dialysed mamaku samples, we decided to test the effect of salt on the dialysed mamaku mucilage (*i.e.* >90% endogenous salt removed). The aim of this study was to determine the effects of the total ionic strength, cation-specificity and ion valency (mono-, di- and trivalent) on the rheological properties of the dialysed mamaku mucilage. Based on the data obtained from this study and in addition to the recent structural data analysis, we propose a possible mechanism of shear-thickening based on the nature of interactions occurring under shear.

2. Materials and methods

2.1. Mamaku extraction

The mamaku fronds (~40 kg) were harvested from the grounds of Massey University, Palmerston North in June 2011. The extraction procedure was carried out as described in the paper by Goh et al. (2007). Briefly, the mamaku tree fronds were washed and cut into slices approximately 5 mm thick using a slicer. The mucilage was extracted using warm water (~50 °C) by blending in a wet disintegrator for 5 min. The crude water-soluble extract was separated from the disintegrated fronds using muslin cloth and then centrifuged at 13,600 × *g* for 30 min at 20 °C to remove any insoluble materials. The supernatant was then freeze-dried. This extraction method yields approximately 1% (w/w) of freeze-dried material. The weight-average molecular weight of this polysaccharide extract is approximately 3×10^6 Da (in 0.1 M NaCl) with a root-mean square radius of 144 nm (Goh, Matia-Merino, Pinder, Saavedra, & Singh, 2011). The coil overlap concentration (c^*) of the extract is ~2.5%, and shear-thickening is observed only at concentrations above c^* *i.e.* semi-dilute and concentrated regimes (Goh et al., 2007).

2.2. Sample preparation

2.2.1. Dialysed samples

The freeze-dried extract (50 g) was first dispersed (6.5%, w/w) and hydrated in water overnight under continuous stirring (native extract). The hydrated native extract was subjected to starch hydrolysis with thermostable α-amylase (100 U/ml in 100 mM sodium acetate buffer, pH 5.0; 1 ml) at 100 °C for 6 min and then with amyloglucosidase (Megazyme: 330 U/ml; 0.1 ml) at 50 °C for 30 min. Rheological properties of the extract did not change after starch hydrolysis. The starch-hydrolysed native extract was dialysed stepwise in reducing concentrations of sodium chloride (NaCl) solutions. The native extract had a high salt concentration (~20%, w/w), therefore it was dialysed against 0.01 M NaCl solution, followed by 0.001 M NaCl and lastly against milli-Q water. This was to prevent 'bloating' of the dialysis tubing, whereby water molecules