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Polysaccharide structures and interactions in a lithium chloride/urea/water solvent

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ABSTRACT

The molten salt hydrate, lithium chloride (LiCl)/urea/water has previously been shown to swell cellulose, but there has so far been no work done to explore its effect on other polysaccharides. In this paper we have investigated the solvent effects of LiCl/urea/water on four natural polysaccharides. Fenugreek gum and xyloglucan, which are both highly branched, were found to increase in viscosity in LiCl/urea/water relative to water, possibly due to the breakage of all intra-molecular associations whereas the viscosity of konjac glucomannan which is predominantly unbranched did not change. Locust bean gum (LBG) had a lower viscosity in LiCl/urea/water compared to water due to the disruption of aggregates. Confocal microscopy showed that fenugreek gum and LBG are able to bind to cellulose in water, however, the conformational change of fenugreek gum in these solvent conditions inhibited it from binding to cellulose in LiCl/urea/water whereas conformational change allowed xyloglucan to bind to cellulose in LiCl/urea/water whilst it was unable to bind in water. Konjac glucomannan did not bind to cellulose in either solvent system. These results provide new insights into the impact of polysaccharide fine structure on conformational change in different solvent environments.

1. Introduction

Hofmeister (1888) was the first to recognise that electrolytes have differing effects on proteins by either increasing their solubility (salting in) or increasing precipitation (salting out). Heydweiller (1910) later discovered that salts dissolved in water increased the surface tension of the solution-air interface where anions were the major influencer. The variation in surface tension followed the Hofmeister series where anions are arranged in order of increasing electrostatic surface potential difference:

$$CO_3^{2-} > SO_4^{2-} > IO_3^- > F^- > BrO_3^- > Cl^- > NO_3^- > Br^- > ClO_3^- > I^- > ClO_4^-$$

The order of some of the cations in the Hofmeister series are:

$$NH_4^+ > K^+ > Na^+ > Li^+ > Mg^{2+} > Ca^{2+}$$

The ions to the left of the series decrease the solubility of nonpoar molecules (salting out) and are referred to as chaotropes as they exhibit weaker interactions with water than water itself and so do not interfere to a great degree with hydrogen bonding whereas the ions with a high charge density, to the right of the series, are refered to as kosmotropes as they exhibit stronger interactions with water molecules than water itself and so are able to break water–water hydrogen bonds.

Kosmotropes are usually small, strongly hydrated ions and are able to structure water, while chaotropes are generally large and poorly hydrated so break the structure of water. A simple method of assessing the nature of an electrolyte is to measure its effect on the viscosity of water. As salt concentrations increase, kosmotropes will increase the viscosity of water whilst chaotropes will decrease it (Wiggins, 2002).

Chloride ions are weakly chaotropic but the behaviour of a halide salt will normally be determined by the stronger metal ion. Therefore, the overall power of a LiCl solution will be kosmotropic. Urea is a chaotrope but acts as a kosmotrope at high concentrations and is able to denature proteins at concentrations of 4–5 M (Russo, 2008). Urea is commonly refered to as a hydrogen bond breaker (Mcgrane, Mainwaring, Cornell, & Rix, 2004). It has been found to increase the intrinsic viscosity of chitosan by breaking intramolecular hydrogen bonds allowing the molecules to exist in a more extended form (Tsaih & Chen, 1997). The concentration of urea required to dis-

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rupt the intramolecular hydrogen bonds increased with increasing molecular weight (Chen & Tsaih, 2000).

Urea, as a chaotrope, acts as a co-solvent by promoting a better solvating interaction between the solute and water (Breslow & Guo, 1990). It breaks the structure of water in the bulk and disrupts the hydrophobic parts of non-ionic surfactants (Deguchi & Meguro, 1975). The unfolding process of ribonuclease by urea and LiCl have been compared (Ahmad, 1983). Urea is able to cause complete denaturation where the unfolded molecule acts as a linear random coil whereas the addition of LiCl leads to incomplete unfolding. When low concentrations of LiCl (i.e. below the concentration it is able to denature ribonulcease alone) were added to urea solutions, the salt actually stabilised the protein against urea denaturation (Ahmad, 1984). This may be due to the ability of the carbonyl oxygen of the urea molecules to form strong complexes with the lithium ions.

LiCl/urea/water is a novel molten salt hydrate that has been found to swell cellulose (Tatarova, Manian, Siroka, & Bechtold, 2010) but there has so far been no work done to explore its effect on other natural polysaccharides. Four different polysaccharides, fenugreek gum (FG), locust bean gum (LBG), konjac glucomannan (KGM) and xyloglucan (XG) have been chosen to identify any solvent effects of the LiCl/urea/water solution. The binding of the polysaccharides to cellulose in the different solvent environments has also been investigated.

2. Materials and methods

2.1. Materials

The polysaccharides used were; Konjac glucomannan from the tubers of *Amorphophallus Konjac, K. Koch*: Propol RS (Shimizu Chemical Corporation, Japan), Xyloglucan from tamarind seed (Dainippon Pharmaceutical Company, Japan), Fenugreek Gum Powder T (Air Green Co., Ltd., Japan) and Locust bean gum (Danisco, Norway), all received as kind donations.

The celluloses used were cellulose fibre (Solka 900FCC, International Fibre Corporation, USA) and Avicel MCC type PH-101 Ph Eur (Sigma Aldrich, UK).

LiCl ≥99%, urea, fluorescein isothiocyanate (FTIC) and rhodamine B were purchased from Sigma Aldrich (UK). Dimethyl sulfoxide (99.8%), toluene (99.8%), pyridine (99.5%) were purchased from Acros Organics (UK) and dibutyltin dilaurate (95%) was purchased from Alfa Aesar (UK).

2.2. Sugar analysis

The sugar analysis was carried out by classical methanolysis of polysaccharide followed by trimethylsilyated-derivatization of the released methyl glycosides using the method described by Nagy et al. (2012). Analysis was with Gas Chromatography with a Flame Ionisation Detector (GC-FID).

All samples and standards were dried over phosphorous pentoxide under vacuum. Approximately 5 mg of each sample was weighed out, with the exact weight recorded. Then, to each sample 333 μl of 300 μg/ml sorbitol in anhydrous methanol was added. Following the addition of 666 μl of 3 M methanolic-HCl, the samples were incubated for 5 h at 100 °C. The samples were then dried over a stream of nitrogen and stored in a desiccator under vacuum for 1 h. Subsequently, the samples were mixed with 75 μl pyridine, 75 μl hexamethyldisilazane and 35 μl chlorotrimethylsilane and incubated for 2–3 h and then evaporated to dryness under nitrogen. Finally, 0.6 ml of hexane was added the samples transferred to Eppendorf tubes and centrifuged for 10 min. The clear supernatant was transferred to a GC vial.

GC set-up was as follows; an Agilent HP-1 capillary column (25 m × 0.2 mm × 11 μm) with flame ionisation detection, inlet temperature: 260 °C, FID temp 300 °C, oven gradient 100–150 °C at 10 °C per min – hold 1 min, 150–190 °C at 4 °C per min – hold 5 min, 190–290 °C at 12 °C per min. Helium was used as carrier gas at 0.7 ml/min. Results expressed as grams polysaccharide per 100 g dry weight sample.

2.3. Molecular weight measurement

Molecular weight analysis was carried out with size-exclusion chromatography (SEC)-refractive index detection via the traditional 'peak-position' method, as described by Rieder et al. (2015). Pullulan molar mass standards were used to construct a calibration curve. From this analysis a pullulan relative weight average molar mass was calculated for the entire molar mass distribution.

Approximately 3 mg of each sample was accurately weighed out into 2 ml screw cap microtubes with an O-ring seal, and then wetted with 25 μl 96% v/v ethanol for about 1 h. 2 ml 0.02% sodium azide was added to each tube and the dissolution of the polysaccharide was aided by shaking in a Precellys 24 homogenizer for 3 × 20 s cycles at 5550 rpm. The tubes where then placed in a boiling water bath for 30 min until the polysaccharide had dissolved. Each sample was then filtered through a 0.8 μm syringe filter (Millipore). The HPLC system consisted of two pumps (Dionex P680), a Spectraphysics AS3500 auto injector, a guard-column (Tosoh PWXL), two serially connected columns (Tosoh TSK-gel G6000 PWXL followed by G5000 PWXL, maintained at 40 °C) and a fluorescence detector (Shimadzu RF-10A, Shimadzu, Germany) or a refractive index detector (Shimadzu RID- 6A). The eluent (50 mM Na_2SO_4) was delivered at a flow rate of 0.5 ml/min. Raw data was collected by Chromeleon software v.6.8 (Dionex, USA).

2.4. LiCl/urea/water solution preparation

The swelling solution was prepared with 0.28:0.11:0.61 mol fractions of LiCl, urea and water respectively (Tatarova et al., 2010). The water was added to the dry powders and stirred over heat until the solution turned clear. Any water lost as vapour when solutions were heated was replenished after the solutions were cooled. The final solution had a pH of 6.3.

2.5. Hemicellulose purification and polymer solution preparation

Initially, hemicellulose powders were added to the LiCl/urea/water solution but there was great difficulty in dissolving the hemicelluloses. This may have been due to the small amounts of insoluble impurities present in the samples. The following purification step was then employed:

Hemicellulose stock solutions were prepared by stirring the powders in deionised water and heating to 80 °C for 30 min. The solutions were then left on a roller bed overnight at room temperature. The hemicellulose solutions were then centrifuged at 2000*g* for 40 min at 25 °C. The stock solutions were then diluted to the desired concentration for rheological testing.

Alternatively, the supernatant from the centrifuged polymer stock solutions was freeze dried. The freeze dried material was then dissolved in LiCl/urea/water solution, heated to 80 °C for 30 min and then left on a roller bed overnight at room temperature. The polymer solutions were then diluted to the desired concentration for rheological testing.

2.6. Rapid Visco Analyser (RVA)

Dispersions of cellulose and the polymers in water or LiCl/urea/water were prepared using a Rapid Visco Analyzer (RVA)