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Low-viscosity α-D-glucan fractions derived from sucrose which are resistant to enzymatic digestion

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Alternan is the name given to the alternating $\alpha(1\rightarrow3)$, $\alpha(1\rightarrow6)$-linked D-glucan produced from sucrose by the extracellular enzyme system of *Leuconostoc mesenteroides* NRRL B-1355. The polysaccharide can be produced fermentatively or by cell-free enzyme preparations. Alternan is resistant to hydrolysis by known endoglucanases, and is a poor substrate for most exoglucanases. Although its viscosity is lower than that of commercial dextran, concentrated aqueous solutions (>12–15% w/v) are still difficult to attain due to high viscosity. Sonication of native alternan lowers its molecular weight from $>10^7$ to $<10^6$. The sonicated material can be dissolved in water to give solutions of 50% (w/v) or greater. These solutions exhibit rheological properties similar to aqueous solutions of gum arabic. A 'limit alternan' produced by hydrolysis with isomaltodextranase has an average molecular weight of approximately 3500, and is rheologically similar to maltodextrins of degree of polymerization (D.P.) approximately 10.

INTRODUCTION

In the early 1950s, Jeanes *et al.* (1954) described dextrans produced by 96 related strains of bacteria. One of these, *Leuconostoc mesenteroides* NRRL B-1355, produces two extracellular α-D-glucans. One of these, fraction L, was found to be a typical dextran. The other, fraction S, was found later to differ from true dextrans in its alternating $\alpha(1\rightarrow3)$, $\alpha(1\rightarrow6)$-linked structure (Misaki *et al.*, 1980). Cote and Robyt (1982) subsequently coined the trivial name 'alternan' for this unusual polysaccharide, and 'alternansucrase' for the enzyme which synthesizes it from sucrose. In their initial studies, Jeanes *et al.* noted the low intrinsic viscosity of alternan, but until recently there has been little interest in low-viscosity polysaccharides; a high viscosity being the most sought-after property. However, with supplies and prices of gum arabic becoming periodically unpredictable, the need has arisen for a highly soluble, relatively low-viscosity gum to replace gum arabic in some applications. In addition, there is now a great demand for reduced-calorie or noncaloric bulking agents and fillers for use in artificially sweetened foods. Some of the products that are presently available for this application include maltodextrins and Polydextrose™.

MATERIALS

Commercial gum arabic samples were purchased from the Sigma Chemical Co. (St Louis, MO) and Serva Fine Biochemicals (Westbury, NY). Maltrin™ maltodextrin products were kindly supplied by Grain Processing Corp. (Muscatine, IA). Polydextrose™ was obtained from Pfizer Chemical Division (New York). Clinical-sized *L. mesenteroides* B-512F dextran fractions and polysaccharide-degrading enzymes were purchased from Sigma. Isomaltodextranase was prepared from *Arthrobacter globiformis* NRRL strain B-4425, and purified according to the method described by Okada *et al.* (1988). Native, high-molecular-weight alternan was produced either fermentatively (Jeanes *et al.*, 1954) or enzymatically (Cote & Robyt, 1982). The author has noted no differences in structure or properties between native polysaccharide produced by either method. Alternan was typically depolymerized by ultrasonication as follows: 30 ml of a 2% (w/v) aqueous solution of

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native alternan was placed in a 50-ml polypropylene centrifuge tube, which was cooled in a bath of ice water. The solution was sonicated for 100 min by use of a 20-kHz 250-W ultrasonic homogenizer (Cole-Parmer 4710 series), set at 40% power in the pulsed mode at 67% duty cycle, fitted with a 0·25 inch semi-microprobe. For larger batches, ~250 ml of an 8% aqueous solution was sonicated for up to 6 h at 80% power by means of a 20-kHz 600-W ultrasonic disruptor (Tekmar model TM600), with an amplitude booster and a 0·75 inch probe. Cooling was provided by a 5 °C water-jacketed reaction vessel. The extent of depolymerization was monitored by high-performance liquid chromatography. Limit alternan was produced by a method similar to that described by Misaki *et al.* (1980), except that the hydrolysis was performed in a dialysis bag, using dialysis tubing with a nominal molecular weight cut-off 6000 (as measured for proteins and peptides). The medium was buffered at pH 5·3 with 20 mM sodium acetate buffer containing 0·01% sodium azide. The product was dialyzed against distilled water, and lyophilized.

ANALYTICAL METHODS

For native and sonicated polysaccharides, viscosity measurements were performed at 20°C, using a Brookfield LVTDV-I digital viscometer, with an SC4-18/13R small sample adapter. Polysaccharide samples were dissolved in distilled, deionized water, and insoluble material was removed by centrifugation at 20 000 × g for 10 min. This centrifugation was necessary to remove dirt and other contaminants from the gum arabic samples, and microscopic metal particles from the sonicated samples. Viscosity measurements were performed on lower viscosity samples (limit alternan, Maltrin™ M100, and Polydextrose™) at 20 °C, using the same Brookfield viscometer and a UL-adapter. Both the small-sample chamber and the UL adapter consist of a rotating cylinder within a cylindrical chamber. Procedures for measuring viscosities and calculating shear rates are described in the instrument manual. Low-angle light scattering measurements were performed on filtered, aqueous solutions (0·22 μm pore size syringe filters) using a Dawn Model B laser photometer (Wyatt Technology Corp.). Zimm plots were constructed from multiple-angle experiments to calculate weight-average molecular weights. High-performance liquid chromatography was carried out using a Spectra-Physics SP8800 ternary gradient pump, and a Millipore-Waters 403 refractive index detector. Chromatography was performed at ambient temperature, with water as the eluent, at a flow rate of 1 ml min^{-1}. Three Shodex gel-permeation columns were used, in series: an S-803/s, an S-805/s and an S-806. The advertised exclusion limits of these columns are 5×10^4, 5×10^6, and 5×10^7, respectively. Various-sized dextran fractions were used for calibration. The total carbohydrate content of solutions was determined by the phenol-H_2SO_4 method (Dubois *et al.*, 1956), and reducing values were measured by the Somogyi-Nelson method (Nelson, 1944); for both measurements, maltose monohydrate was used as the standard.

RESULTS AND DISCUSSION

The $\bar{M}_w$ of native, high-molecular-weight alternan, as described by light scattering, was $(1{\cdot}0 \pm 0{\cdot}1) \times 10^7$. Aqueous solutions exhibited a strong bluish-white opalescence. Upon sonication, this opalescence was lost, and the $\bar{M}_w$ as determined by light scattering fell to $(8{\cdot}6 \pm 0{\cdot}6) \times 10^5$. Figure 1 shows the results of HPLC analysis of native, sonicated, and limit alternan. Native alternan eluted at or near the void volume, indicative of its high molecular weight. Sonicated alternan eluted in a much broader peak, although the molecular weight distribution was uniform about a median of approximately 900 000. This was quite close to the $\bar{M}_w$ obtained by light scattering. The number average molecular weight ($\bar{M}_n$) of sonicated alternan, obtained by comparing the number of reducing ends with the total carbohydrate content, was much lower, at approximately 32 000. This difference indicates a high degree of polydispersity for the sonicated material, as does the broad HPLC elution profile shown in Fig. 1. Even so, sonication liberated little or no glucose or other oligosaccharides. The progress of the depolymerization during sonication was monitored by HPLC analysis. It was found that the molecular weight distribution changed from that of native alternan (peak A. Fig. 1) to that of the completely sonicated alternan (Fig. 1, peak B) by a gradual decrease in the size of peak A, accompanied by a corresponding increase in the size of peak B. No further decrease in molecular size was

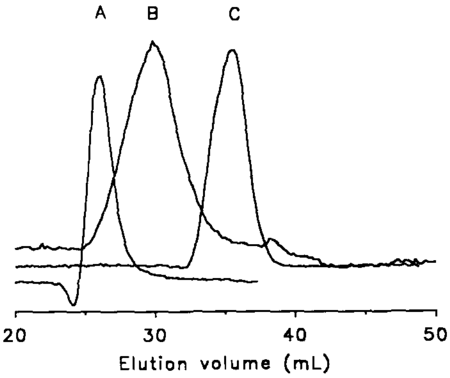

Fig. 1. HPLC Profiles of native, high-molecular-weight alternan (A), sonicated alternan (B), and isomaltodextranase-limit alternan (C). Void volume is ~25 ml; maltohexaose (mol. wt 990) elutes at 38 ml. Chromatographic conditions are described in the text.

observed once all of the alternan was of the size represented by peak B. Thus, the depolymerization by sonication appears to be a discrete change from one molecular weight to another, which is not susceptible to further breakdown.

Isomaltodextranase-limit alternan was estimated by HPLC (Fig. 1) to have a $\bar{M}_w$ of approximately 3000–5000. Its $\bar{M}_n$ was 3500, corresponding to an average degree of polymerization of approximately 22.

^{1}H-Decoupled ^{13}C-NMR spectra of both native and sonicated alternan were identical, and confirmed that the only change incurred upon sonication was in chain length, and not in overall structure of the polymer (data not shown).

Concentrated solutions (>25% w/v) of all the polysaccharides exhibited some non-Newtonian behavior (shear thinning) at shear rates below approximately 10–15 s^{-1}, but the behavior was essentially Newtonian at higher shear rates and/or lower concentrations (data not shown). The concentration dependence of solution viscosity for native and sonicated polymers is shown in Fig. 2. Viscosity values were obtained for each concentration from the portion of the viscosity versus shear rate curve where behavior was Newtonian. It is readily apparent from Fig. 2 that native alternan solutions exhibited significantly lower viscosity than native dextran, but the viscosities of both gums were much higher than that of gum arabic. In contrast, both sonicated dextran and sonicated alternan were similar in viscosity (and solubility) to gum arabic.

Figure 3 shows the relationship between concentration and viscosity for limit alternan, Polydextrose™, and Maltrin™ M100. Polydextrose™ was clearly of lower viscosity than the other two materials, but limit alternan was very similar to Maltrin™ M100 in viscosity behavior. The average D.P. of Maltrin™ M100 is approximately 10, whereas the average D.P. for limit alternan is approximately 20–25.

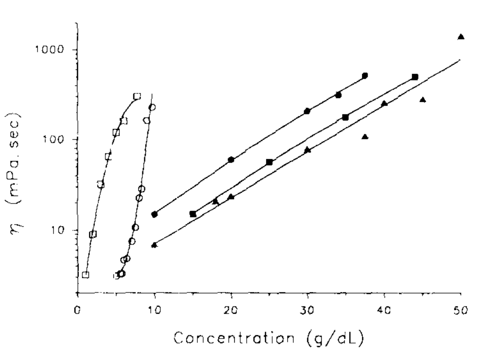

Fig. 2. Relationship between solution viscosity (η) and the concentration for native dextran (□), native alternan (○), sonicated dextran (■), sonicated alternan (●), and gum arabic (△) at 20°C. See text for details.

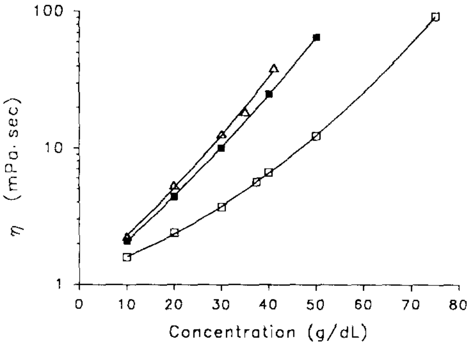

Fig. 3. Relationship between solution viscosity (η) and concentration for Polydextrose™ (□), Maltrin™ M100 (■), and isomaltodextranase-limit alternan (▲) at 20°C. See text for details.

Being an α-D-glucan, one might expect alternan to be readily digested by a number of common enzymes. This proved not to be the case. In fact, the author has been unable to find any endo-hydrolytic enzyme capable of hydrolyzing alternan to any great extent. Misaki *et al.* (1980) reported that endodextranase from a *Penicillium* species hydrolyzed alternan to the extent of 7·3%. The author treated native alternan, B-512F dextran ($\bar{M}_w$ 2×10^6), and soluble starch with a number of commercially available exo- and endo-hydrolytic enzymes, including human salivary α-amylase, porcine pancreatic α-amylase, yeast isomaltase, *Aspergillus niger* glucoamylase, porcine kidney α,α-trehalase, and *Penicillium* endodextranase, and measured the release of reducing sugar in each case. The only enzyme preparation that released any measurable reducing sugar from alternan was *Penicillium* endodextranase. This may reflect actual hydrolysis of alternan (Misaki *et al.*, 1980), or it could be attributable to the presence of a small amount of contaminating dextran in the samples of alternan used (Wilham *et al.*, 1955). The latter may be more likely, as the author has found the hydrolysis of ^{14}C-labeled alternan by *Penicillium* endodextranase to be nearly immeasurable (<3% conversion to small oligosaccharides).

The resistance of alternan to enzymatic hydrolysis can be attributed to its unusual alternating structure, and its limited natural occurrence. In a survey of the action of human colonic bacteria on various dextrans, Sery and Hehre (1956) found that the 'dextran' from *L. mesenteroides* NRRL B-1355 was a very poor substrate for the enzymes from these bacteria. Thus far, only two related exo-hydrolases have been found to hydrolyze alternan to any significant extent. These are the so-called isomaltodextranases from *Arthrobacter globiformis* (Sawai *et al.*, 1978) and from an *Actinomadura* species (Sawai *et al.*, 1981), which release isomaltose units from the non-reducing ends of dextran and alternan, in a manner somewhat analogous to the action of β-amylase on amylopectin. Both isomaltodextranases are more active on dextran than on alternan. The *Actinomadura* enzyme degrades alternan more completely than does the *Arthrobacter* enzyme (Sawai *et al.*, 1981), which leaves a partially degraded limit polysaccharide fragment (Misaki *et al.*, 1980). The results presented here indicate that this 'limit alternan' is much smaller than sonicated alternan, having an average degree of polymerization in the range of 20–25 ($\overline{M}_n = 3500$). According to the data published by Misaki *et al.* (1980), their limit alternan was more highly branched than native alternan.

Sonication and enzymatic hydrolysis were found to be the best methods for producing homogeneous low-molecular-weight alternan in the laboratory. Other methods, including chemical depolymerization (Colegrove & Lindroth, 1989) or acceptor reactions (Tsuchiya *et al.*, 1955) may be more suitable for producing low-molecular-weight material on a commercial scale. Sonication has been proposed for the preparation of clinical-sized dextran (Watson & Wolff, 1955). Other methods of depolymerization, such as partial acid hydrolysis, are notorious for giving rise to a heterogeneous distribution of products, with large amounts of glucose released. Levan has recently been advocated as a low-viscosity gum which can be produced from sucrose (Han, 1990). However, alternan may have certain advantages over levan, including better resistance to hydrolysis, and the added benefit that fructose is the major by-product.

In conclusion, some of the properties of native alternan and low-molecular-weight fractions of alternan have been described, and it has been suggested that these glucan preparations may be of use as non-digestible additives in certain food preparations. Recent results indicate that sonicated alternan, unlike gum arabic, possesses little ability to act as an emulsifier (data not shown), limiting its usefulness as a potential replacement for gum arabic in some applications. However, this difficulty could be overcome by the development of formulations consisting of alternan and an added emulsifier, such as polysorbate or lecithin. If alternan could be made on a cost-competitive basis, then it may have certain advantages over gum arabic and other, similar gums. These advantages include greater control over the synthetic process, higher purity, lack of charged ionic groups, and the reduced dependence on imported gums. In addition, alternan fractions, especially limit alternan, may be useful as noncaloric, carbohydrate-based, soluble fillers and bulking agents in artificially sweetened foods. Alternan is produced from sucrose, a surplus agricultural commodity, and a major by-product of its enzymatic synthesis is fructose, another valuable food carbohydrate. The major by-product of the enzymatic synthesis of limit alternan from alternan is isomaltose, which can be chemically reduced to isomaltitol, one of the two components of the carbohydrate-based sweetener Palatinit (Strater, 1988).

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