The Mechanism of Development of Viscosity in Yam (*Dioscorea opposita* THUNB) Mannan

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This study investigated the mechanism of development of viscosity in mannan, the primary component of yam mucilage. The viscosity of the viscous polysaccharide fractions was lower for mannan obtained from the Nagaimo variety, characterized by the large number of side chains, than for the Tsukuneimo and Ichioimo varieties. Mannan aggregates due to a hydrophobic interaction which in turn results in viscosity. The number of side chains in mannan molecules has a major effect on aggregation and viscosity. A mutual relationship exists between viscosity of viscous polysaccharides of Tsukuneimo, Ichioimo and Nagaimo varieties and mannan structure.

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The authors^{1),2)} previously fractionated yam mucilage by gel filtration, and were the first to show that yam mucilage consists of a mixture of polysaccharide and glycoprotein. A numbers of studies have been conducted on yam polysaccharides. TAKAHASHI³⁾ separated mannan from Tsukuneimo, and MISAKI *et al.*⁴⁾ has reported that Tsukuneimo mannan incorporates a straight chain with a molecular weight of approximately 20,000. In the present study, the authors investigated the polysaccharide structure of Tsukuneimo, Ichioimo and Nagaimo. All three varieties were found to contain the straight chain β -(1,4)-link, itself containing β -(1,3)-linked 1-mannose residue with a molecular weight of approximately 20,000 with 6 side chains per molecule for Tsukuneimo, 7 for Ichioimo, and 10 for Nagaimo¹⁾. The low viscosity Nagaimo mucilage was shown to contain more mannan side chains than that of Tsukuneimo and Ichioimo. The study proved instrumental in clarifying differences in mucilage viscosity. The molecular weight of mannan in yam mucilage is approximately 20,000, however, when the

authors fractionated yam mucilage by gel filtration, a product having a molecular weight in excess of 2,000,000 was obtained. TOMODA *et al.*⁵⁾ have reported the separation of yam mucilage in ultracentrifuge, the molecular weight of the product obtained being in excess of 2,000,000. On this basis, an investigation was conducted to determine the reason for the considerable difference in molecular weight of mannan when separated by chemical and physical methods. The investigation showed that the mannan molecules aggregated due to a hydrophobic interaction, creating a product with a molecular weight in excess of 2,000,000. This results an increase in viscosity and the intermolecular bond, of which that for Nagaimo mannan is the weakest.

Materials and methods

1. Materials

The three varieties of yam used in this study were Tsukuneimo, obtained from Kyoto, Ichioimo obtained from Chiba and Nagaimo from Hokkaido.

2. Preparation of mucilage and viscous polysaccharide fractions

Each material was peeled and ground, then 10 g of ground yam was stirred with 90ml of water at room temperature for 1 hour. The solid matter was then removed by centrifugation (18,000×G, 10°C, 20 min.), the supernatants were dialyzed at 10°C for 6 hours using Spectra/Por 3 (Spectrum Laboratories Inc.), and made up to 200ml with distilled water. These afforded the mucilage solution used in this study.

3. HPLC analysis of polysaccharide fractions

Polysaccharide fraction (0.5ml, 100 µg/ml of sugar contents for Tsukuneimo and Ichioimo, 250 µg/ml for Nagaimo) were analyzed by HPLC on a GMPWx1 column (7.8 mm I.D. × 30cm, Toso) using distilled water as a eluent (0.5ml/min.). The eluent solution was fractionated down to 0.5ml, and sugars were determined by the phenol-sulfuric acid method^{(6),(7)}.

4. Measurement of viscosity

The viscosity of 1ml samples of polysaccharide fractions with the same concentration (150 µg/ml) were measured using an E-type viscometer (Tokyo Keiki DVM-E, rotor size: ϕ 2.4cm, 100 rpm) at 20°C. A stable reading of viscosity was obtained after 1 minute of measurement.

5. Hydrophobicity of polysaccharide fractions

Hydrophobicity of polysaccharide fractions were determined by the method of HAYAKAWA *et al.*⁽⁸⁾. First, 100 µl of 10 mM phosphate buffer (pH 7.6) containing 0.8 mM 8-anilino-1-naphthalene sulfonic acid was added to 2 ml of polysaccharide fraction (5.0 mg/ml) and let stand in a dark place at 20°C for 2 hours. The ultraviolet absorption spectra of each fraction was measured at an excitation wavelength of 380 nm and fluorescent wavelength of 480 nm. The fluorescence intensity was used to obtain hydrophobicity.

Results and discussion

1. Polysaccharide viscosity and HPLC pattern

The polysaccharide fractions prepared from the mucilage of the three varieties of yam were adjusted to the same concentration (150 µg/ml) and viscosity measured. Viscosity for Tsukuneimo and Ichioimo was

Fig. 1 Gel filtration pattern of polysaccharide fraction from yam mucilage by GMPWxl

3.0 mPa/s respectively, whereas that for Nagaimo was 2.4 mPa/s, a difference of 20%. The viscous polysaccharide fractions consisted of mannan, the Nagaimo mannan having the greatest number of side chains with the lowest viscosity. YAMADA⁽⁹⁾ has reported that viscosity increases with increasing linearity of chains, and that viscosity is reduced as the number of side chains increases, even for the same molecular weight. The same result was obtained from yam mannan in this study.

As shown in Fig.1, the molecular weight of the viscous polysaccharide fractions obtained by HPLC was more than 2,000,000 in all cases. As the mannan viscous polysaccharide has a molecular weight of approximately 20,000, it was apparent that the mannan was aggregated in units of more than 100 molecules. The peak elution time was 12 minutes for Ichioimo, 13 minutes for Tsukuneimo, and 14 minutes for Nagaimo. This illustrates the relationship between viscosity of the polysaccharide fraction and elution position, the assumption being that the low-viscosity Nagaimo has fewer aggregations of mannan molecules than Tsukuneimo and Ichioimo.

2. Effects of DMSO on viscosity of viscous polysaccharide fractions and molecular weight

The non-proton polar solvent DMSO (dimethyl sulfoxide) was added to the viscous polysaccharide fraction of each of the three varieties of yam, and viscosity was measured using an E-type viscometer. As shown in Fig.2, the viscosity of the polysaccharide

fraction varied with DMSO concentration, the extent of this variation differing with the variety of yam. The effect was greatest for the Nagaimo fraction, with no viscosity shown at 20% DMSO. Viscosity of the Ichoimo fraction was reduced to approximately 60% at 20~40% DMSO, with no viscosity shown at 50% DMSO. The reduction in viscosity of the Tsukuneimo fraction with DMSO concentration was less than for the Nagaimo and Ichoimo fractions, with approximately 80% of the initial viscosity being maintained at 50% DMSO.

The relationship between reduction in viscosity of the viscous polysaccharide fractions with DMSO concentration, and the elution pattern of HPLC were also investigated. As shown in Fig.3, the elution pattern of each yam polysaccharide fraction when processed with 20% DMSO differs considerably from that shown in Fig.1. The peak in the molecular weight

above 2,000,000 is smaller, with the majority being obtained within the retention times of 23 minutes for Tsukuneimo, 25 minutes for Ichoimo, and 26 minutes for Nagaimo. The experiments showed that reduction in viscosity of the viscous polysaccharide fraction with DMSO concentration was due to a breaking of the intermolecular forces and a reduction in the proportion of high-molecular weight aggregates. As the elution position of the low-molecular weight fractions differed between yam varieties at the same DMSO concentration, it is assumed that the intermolecular forces differ between mannan in the different varieties of yam.

Measurements of hydrophobicity of each viscous polysaccharide fraction using fluorescent light wavelengths produced values of 191.6 for Tsukuneimo, 136.1 for Ichoimo, and 91.5 for Nagaimo, the variety with a high number of side chains. As hydrophobicity of polysaccharide molecules is increased by side chains, it is considered that this indicates the hydrophobicity of Nagaimo, the variety with a high number of side chains. The more viscous Tsukuneimo and Ichoimo varieties have few hydrophilic side chains, and a higher level of hydrophobicity, showing clearly that the mannan molecules in the viscous polysaccharide fraction aggregate primarily due to an interactive hydrophobic action. As the Nagaimo mannan contains large numbers of side chains it is hydrophilic, with weak intermolecular hydrophobic action. Its aggregates have a lower molecular weight, and are significantly affected by DMSO concentration.

Based on the above results, the mannan forming the bases of yam mucilage forms aggregates with a molecular weight in excess of 2,000,000 due primarily to hydrophobic interaction, and resulting in the characteristic viscosity. The side chains of the mannan molecules contribute to the hydrophobic interaction between the molecules, and greatly affect the formation of the aggregates, their strength and viscosity.

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Fig. 2 Effect of DMSO on viscosity of mannan from yam mucilage

Fig. 3 Effect of 20% DMSO soln. on elution pattern of polysaccharide from yam mucilage

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ヤマイモマンナンの粘性発現の機序について

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3種類のヤマイモ(ツクネイモ, イチョウイモ, ナガイモ)を用い, 粘質物の構成成分であるマンナンの粘性発現機序を明らかにすることを目的として検討を行ったところ, ヤマイモ粘質物を構成するマンナンの粘度は, ツクネイモ, イチョウイモに比べ, 構造上側鎖数の多いナガイモが低かった。また, マンナンは主に疎水性相互作用により, 分子量200万以上の会合体を形成して粘性を発現することが明らかとなった。さらに, マンナンの側鎖は, マンナン分子間の疎水性相互作用に関与し, 会合体の形成とその強度ならびに粘度に大きな影響を及ぼし, 粘度と構造との相関を明らかにした。

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お詫び

本研究ノートは3号に掲載いたしましたが, 編集上の手違いがありましたので再掲載いたします。
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