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D-Mannose-specific interaction between xanthan and D-galacto-D-mannan

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A gelation occurred in a mixed solution of xanthan and locust-bean gum at room temperature; in contrast, gelation did not occur in a solution of xanthan and guar gum. The maximum dynamic modulus was obtained when the mixing ratio of xanthan and locust-bean gum was 1:2 at 0.2% total gums. A mixture of deacetylated xanthan and locust-bean gum showed the highest dynamic modulus, about twice that of the mixture of native xanthan. We concluded that the intermolecular interaction between xanthan and locust-bean gum might occur between the side chains of the former and back-bone of the latter molecules in a lock-and-key arrangement.

Xanthan gum *Synergistic interaction* *Gelation mechanism* *Lock-and-key mechanism*

1. INTRODUCTION

Xanthan is an extensively investigated polysaccharide because of its unique rheological properties and commercial importance [1–4]. Dea et al. [5] have proposed a mode of interaction between the single-stranded helix of the xanthan molecule and the smooth segments of the backbone of the locust-bean gum molecule without taking into consideration the role of the side chains of the xanthan molecule. We have confirmed that the characteristics of the association between the gums depend on the charged trisaccharide side chains of the xanthan molecule [4,6].

We describe here the rheological behavior, with respect to their synergistic interaction, of a mixture of xanthan and locust-bean gum, and guar gum so as to propose a new mode of the interaction as in a lock-and-key mechanism.

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2. MATERIALS AND METHODS

2.1. *Materials*

The xanthan, locust-bean gum and guar gum were identical with those used in [4,6,7] and were obtained from Taiyo Kagaku. A solution of 0.1% xanthan in distilled water was heated at 90°C for 30 min, then cooled to room temperature, centrifuged at 12000 × *g* for 1 h, and filtered through Celite 545. In the presence of 0.5% KCl, ethanol (2 vols) was added to the filtrate and the precipitate was dried in vacuo. A solution of 0.5% locust-bean gum dissolved in hot water (85°C) and guar gum in distilled water was filtered through Celite 545, ethanol (2 vols) added and the precipitate dried in vacuo, respectively. For deacetylation, an aqueous, 0.2% solution of purified xanthan was treated under N_2 with 10 mM KOH in the presence of 1.0% KCl at room temperature for 10 h. The solution was neutralized with 50 mM HCl, and the product isolated as described earlier. Various solutions of xanthan and locust-bean gum, and guar gum having a total concentration of 0.2% were prepared by dissolving

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locust-bean gum or guar gum in distilled water and adding xanthan, respectively.

2.2. *Liquid chromatography*

A solution of locust-bean gum and guar gum (50 mg) in 2 N HCl (25 ml) was heated at 100°C for 3 h. After being cooled in an ice bath, the hydrolyzate was neutralized with Ag_2CO_3 and filtered through Celite 545. The excess Ag^+ was precipitated with H_2S, and the solution concentrated and filtered through Celite 545 into a 10-ml volumetric flask, respectively. Liquid chromatography was performed with a Shimadzu LC-4A chromatograph, equipped with a column of ISA-07/S2504 and a mobile phase of 0.3 M KOH (temperature, 150°C; flow rate, 0.5 cm/min).

2.3. *Specific rotation*

Specific rotation was measured at 589 nm with a DIP-180 automatic digital polarimeter (Japan Spectroscopic) for a solution of 0.5% (w/v) in water.

2.4. M_r

The M_r of locust-bean gum and guar gum was determined by a viscometric method according to the relationship [8] $[\eta] = 3.8 \times 10^{-4} \times M_r^{0.723}$. Intrinsic viscosity $[\eta]$ was determined for each solution having a concentration from 0.05 to ~0.3% by measuring the specific viscosity with an Ostwald-type viscometer at 25°C. The flow rate for water was 42 s.

2.5. *Viscosity and dynamic viscoelasticity measurements*

Viscosity at different shear rates (1.667–66.685 S^{-1}) and dynamic viscoelasticitiy at steady angular velocity (3.768 rad/s) were determined with a rheogoniometer consisting of a coaxial cylinder (1.6 cm diameter) with a rotating outer cylinder (2.2 cm diameter), 6.0 cm long (IR-103, Iwamoto Seisakusho). Shear rate (D), shear stress (S) and apparent viscosity (η) were calculated using the equation of Margules [9]. Dynamic viscosity (η') and elasticity (G') were calculated by a modification of the equation of Markovitz [10].

3. RESULTS AND DISCUSSION

As the intermolecular interaction between xanthan and D-galacto-D-mannan molecules is closely correlated with the degree of substitution of the mannan chain, the degree of substitution of locust-bean gum and guar gum was determined by LC and calculated to be D-mannose to D-galactose, 4.4:1.0 and 2.0:1.0; M_r 263000 and 247000; and $[\alpha]^{20}_{589}$, +22 and +52° (c 0.5, distilled water).

Although neither xanthan nor locust-bean gum gelled alone, a mixture of the gums gave a gel at 0.2% total gums at room temperature, but not for the mixture with guar gum. The flow curves, at 50°C, of a mixture of native and deacetylated xanthan with locust-bean gum solutions at varying ratios of the gum showed plastic behavior in which the S values of the mixtures with deacetylated xanthan were higher than the value of the mixtures with native xanthan.

Fig.1 shows the effect at 25°C, of the ratio of xanthan (native, Na form, and deacetylated) to locust-bean gum in solution on the dynamic viscoelasticity. Maximum gelation was achieved when the mixing ratio of xanthan to locust-bean gum was 1:2. In the case of deacetylated xanthan,

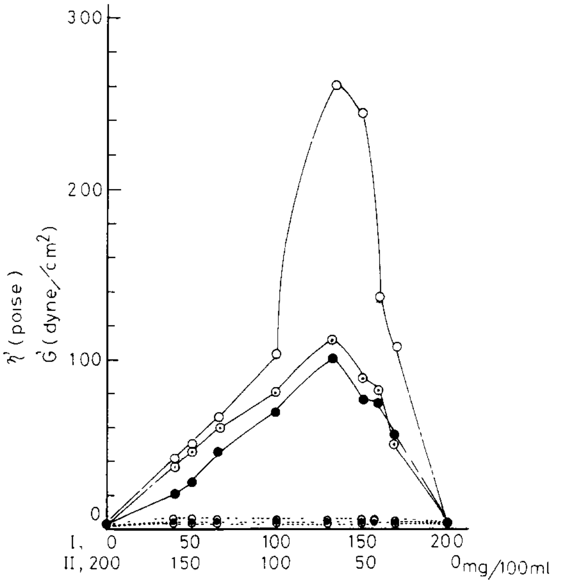

Fig.1. Dynamic viscoelasticity, at 3.768 rad/s and 25°C, of a 0.2% xanthan–locust-bean gum solution as a function of the ratio of components: (●) native xanthan, (⊙) Na-form xanthan, (○) deacetylated xanthan. The broken lines refer to the dynamic viscosity and the full lines to the dynamic modulus. (I) Locust-bean gum, (II) xanthan.

a much stronger gel was observed, about twice as strong as the others, indicating that much more intense intermolecular interactions were produced by deacetylation. The results suggest that there are about twice the number of junction sites available on each xanthan molecule as on the locust-bean gum molecule, and that the molecules become more flexible and can associate with locust-bean gum more easily after deacetylation probably because they are free from its intramolecular association to which the acetyl residues contribute [6].

On the other hand, for a mixture of native xanthan and guar gum, little synergistic increase in dynamic viscoelasticity was observed, as shown in fig.2. However, the synergistic interaction was enhanced in the mixture with deacetylated xanthan.

The dynamic modulus of a mixed solution of xanthan (native and deacetylated) and locust-bean gum increased at a low temperature, and showed the highest value at a xanthan to locust-bean gum ratio of 1:2, which agrees with the results illustrated in fig.1. The dynamic viscosity decreased rapidly with increasing temperature for a xanthan to locust-bean gum ratio of 1:2 to 1:4, but scarcely changed from 2:1 to 4:1, the degree of insensitivity corresponding to that of xanthan alone [4]. Such insensitivity in the dynamic viscosity of xanthan alone resulted from quaternary association [4] and the intramolecular association contributed by acetyl residues [6], indicating that some of the non-association sites on the xanthan molecule remained. The results suggest that the side chains of the xanthan molecule may play a role in the interaction.

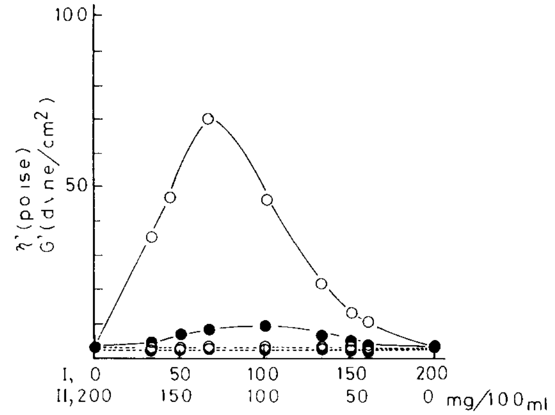

Fig.2. Dynamic viscoelasticity, at 3.768 rad/s and 25°C, of a 0.2% xanthan-guar gum solution as a function of the ratio of components: (●) native xanthan, (○) deacetylated xanthan. (I) Locust-bean gum, (II) xanthan.

In spite of an increase in the dynamic viscoelasticity of xanthan alone by addition of salt [4,6], the dynamic modulus of the mixture with locust-bean gum decreased in the presence of NaCl or $MgCl_2$ even at 0.1%. The dissociation might be brought about by ionic repulsion on charged trisaccharide side chains of the xanthan molecule due to Na^+ or Mg^{2+}.

Thus, we conclude that the intermolecular interaction between xanthan and locust-bean gum may occur between side chains of the former molecule and the backbone of the latter molecules, as in a lock-and-key arrangement (fig.3a). As the tertiary structure of the xanthan molecule may keep a 5-fold, single-stranded helix [11], its side chains are inserted into the adjacent, unsubstituted segments of the backbone of the locust-bean gum molecule. Since the side chains of the native xanthan molecules are somewhat rigid because of the

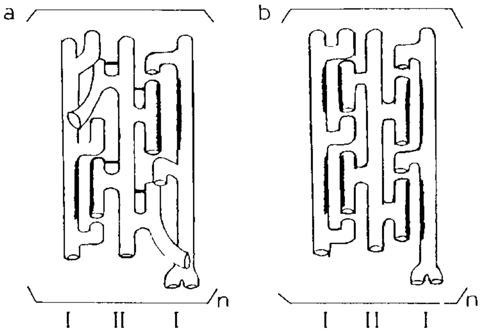

Fig.3. Possible mode of intermolecular interaction between xanthan and locust-bean gum molecule in aqueous solution. The models are viewed perpendicular to the axis. A molecule of xanthan may combine with two or more molecules of locust-bean gum. (a) Incomplete interaction may exist as the side chains of the xanthan molecules are somewhat rigid owing to the intramolecular association between acetyl residues and the backbone. (b) Interaction takes place after deacetylation of xanthan. The bold lines indicate the association sites. (I) Locust-bean gum, (II) xanthan molecule.

intramolecular association, incomplete interaction may exist and greater interaction may result from deacetylation (fig.3b). Although it has been reported that the side chains of the locust-bean gum molecule are distributed in uniform blocks along the backbone of the mannan molecule [12,13], the mode of interaction is independent of the structure, because each junction may take place within three sugar residues, of the xanthan molecule including the pyruvate and acetate residues. The stronger interaction in the mixture with deacetylated xanthan suggests some regular branching units in the locust-bean gum molecule as shown in fig.3. The intermolecular interaction may take place with hydrogen bonding between methyl groups of xanthan and oxygen groups of locust-bean gum, and electrostatic force of attraction between cations of the former and oxygen groups of the latter [4,14–16].

On the other hand, synergistic interaction was not observed in a mixture with guar gum, the structure of which is the same as that of locust-bean gum, except that the side chains are linked to every other unit [12], or two to four units [13]. Thus, the weak interaction may be due to the presence of the side chains of the guar gum molecule. These side chains may prevent the insertion of the charged trisaccharide side chains of the xanthan molecule into the backbone of the guar gum molecules.

Finally, xanthan is a complex exopolysaccharide produced by the plant pathogen *Xanthomonas camperstris* [17]. Surrounding the bacterial cell wall, a variety of carbohydrate polymers of a wide range of structure types exist, whose functions are little understood [18]. The lock-and-key interaction proposed may suggest a part in the host-pathogen relationship and may provide the existence of D-mannose specific binding sites in the other interaction, such as in cell recognition [19].

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