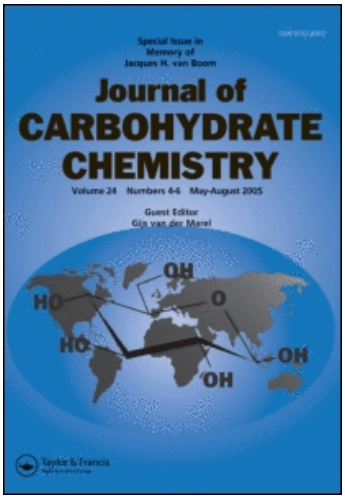

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SYNERGISTIC INTERACTION BETWEEN DEACYLATED XANTHAN AND GALACTOMANNAN[1]

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ABSTRACT

The dynamic modulus and optical rotation of a mixed solution of denatured xanthan (depyruvated and deacylated) and galactomannan (locust-bean gum and guar gum) were measured with a rheogoniometer and a polarimeter. Gelation occurred in a mixture of native xanthan with locust-bean gum at a concentration of 0.2% total gums at room temperature, but not with guar gum. A mixture of deacylated xanthan and locust-bean gum showed the highest dynamic modulus, about three times as strong as that of a mixture with depyruvated xanthan. The dynamic modulus of a mixture of deacylated xanthan and locust-bean gum stayed at very small value in the presence of $CaCl_2$ (6.8 mM) and urea (4.0 M). Possible binding sites between deacylated xanthan and locust-bean gum molecules are proposed.

INTRODUCTION

The bacterial polysaccharide xanthan produced by plant pathogen Xanthomonas campestris is of interest not only because of its unique rheological properties,[2-4] but also for its formation of a mixed gel with plant galactomannan[5-7] in aqueous solution. The xanthan has a repeat unit based upon a cellulose backbone with alternate glucose residues O-3-substituted with a charged trisaccharide side chain. The internal mannose of the side chain is substituted at O-6 with an acetyl group. About one-half or two-thirds of the terminal mannose residues bear a pyruvic acid.[10] Galactomannans have a mannan backbone

to which are attached varing amounts of single galactose residues.[11,12] Such galactomannans have attracted interest in relation to their role in polysaccharide interaction with κ-carrageenan,[5,13] agarose,[14,15] and xanthan.[5,6]

The trend to form gels of xanthan-galactomannan increase with decreasing content of galactose side chain in galactomannan.[16] Early workers[6,7] attributed the gelation to an intermolecular binding between the xanthan helix and the unsubstituted region of the galactomannan backbone. Recently, an alternate association mechanism between the cellulose backbone of the xanthan and the mannan backbone has been proposed.[17,18] We have demonstrated a new mode of intermolecular interaction involving the side chains of the xanthan and backbone of the galactomannan (locust-bean gum).[19-21] The interaction proposed is a model for host-pathogen recognition, adhesion of Xanthomonas bacteria within plant vascular systems, and mannose-specific binding sites in other interactions, such as in cell recognition.[6,21]

In order to discuss the relationship between association characteristics and rheological properties in more detail, we describe herein the dynamic modulus and optical rotation of a mixture of denatured xanthan (depyruvated and deacylated) and galactomannan (locust-bean gum and guar gum).

RESULTS

By a spectrophotometric estimate, native xanthan had pyruvic and acetic acid contents of 5.7 and 5.4% (W/W), which meant that 67 and 98% of the terminal and inner mannosyl groups bore pyruvic and acetic acid moieties, respectively. The pyruvate groups were removed by heating a solution of native xanthan in 1 mM oxalic acid at 95 °C for 2 h. Under these conditions, about 84% of the pyruvate groups were removed, so that 1/10 of the terminal mannosyl groups bore a pyruvate moiety, but the acetyl groups were unaltered (5.2%). This product was labelled as depyruvated xanthan. The acetyl free xanthan was prepared by dissolving the native or depyruvated xanthan in distilled water making the solution 10 mM in KOH and maintaining this reaction mixture at room temperature for 10 h. This product was labelled as deacetylated or deacylated xanthan. The deacetylation of the samples was confirmed by loss of infrared absorption at 1730 cm^{-1}.

As the intermolecular interaction between xanthan and galactomannan 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 liquid chromatography and the calculated mannose to galactose ratios were 4.1:1.0 and 2.0:1.0; mol. wt. 263,000 and 247,000, respectively.

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. Figure 1 shows the dynamic modulus vs. relative amount of locust-bean gum and xanthan (native, deacetylated, depyruvated, and deacylated) at 0.2% total gums and 25 °C. Maximum dynamic modulus was achieved when the mixing ratio of xanthan to locust-bean gum was 1:2. In the case of deacylated xanthan, a much stronger gel was observed, about three times as strong as the mixture with native or with depyruvated xanthan, indicating that much more intense intermolecular interaction was produced by deacetylation. In spite of an increase in dynamic modulus of xanthan alone[22-25] by addition of salt, a very small dynamic modulus was observed in a mixture with locust-bean gum on addition of $CaCl_2$ (6.8 mM), indicating that carboxyl groups of the glucuronic acid residue of the intermediate side-chain of xanthan may take part in the interaction with locust-bean gum molecule. For the mixtures of guar gum with native and depyruvated xanthan, little synergistic increase in dynamic modulus was observed at room temperature (not cited in the Figure). This may be due to the presence of side-chains on the guar gum molecule.[11,26,27] However, the synergistic interaction was enhanced in the mixture with deacylated xanthan, indicating that the xanthan molecule had become more flexible and could associate with guar gum molecule more easily due to freedom from the intramolecular association contributed by the acetyl groups.[25]

Effect of pH, at 25 °C, on the dynamic modulus of mixed solutions of locust-bean gum with native, deacetylated, depyruvated, and deacylated xanthan in a combination ratio of 2:1 at 0.2% total gums is shown in Fig. 2. The large dynamic modulus of the mixed solutions was nearly independent of pH change between pH 5-10 and was controlled by addition of 100 mM HCl or KOH. However, the dynamic modulus decreased rapidly with decreases in pH from 5-3.5 for mixtures with native, deacetylated, depyruvated, and deacylated xanthan, respectively. At basic pH, a

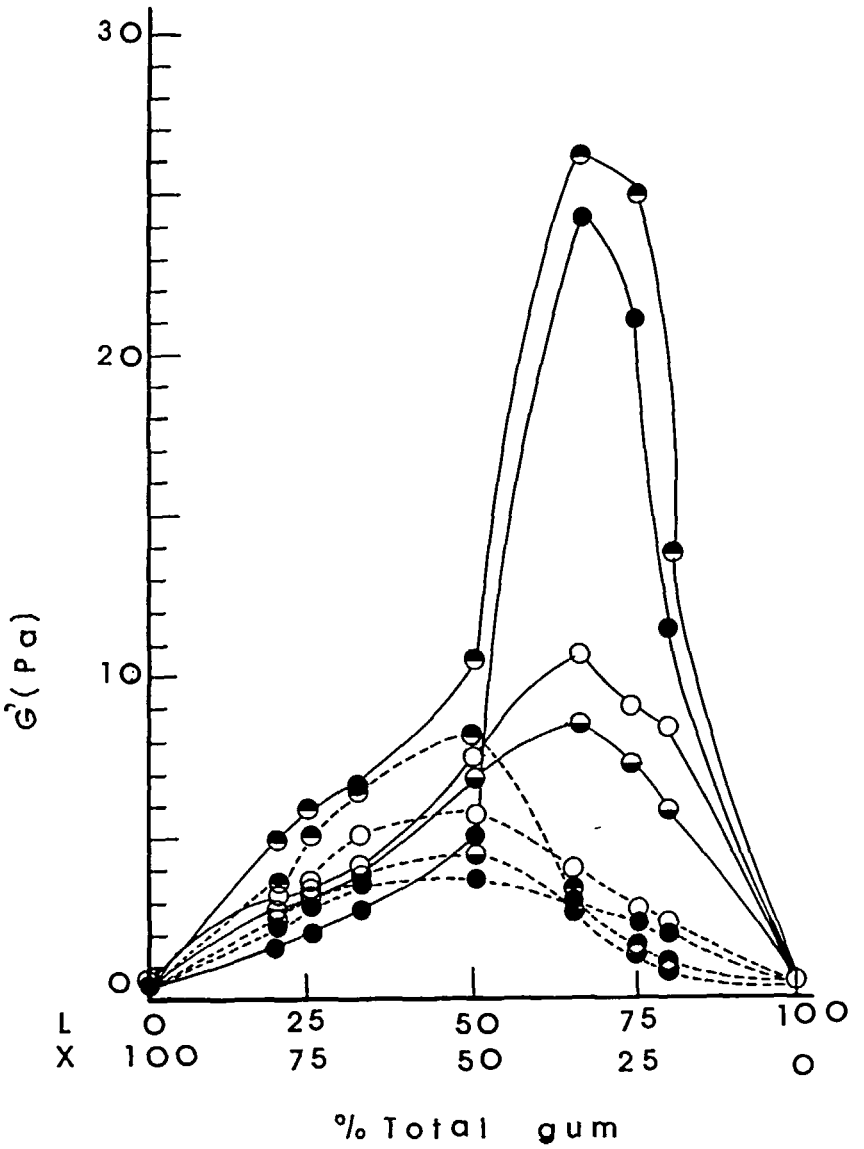


Fig. 1. Dynamic modulus vs. relative amount of locust-bean gum and xanthan at 0.2% total gum and 25°C. The full lines refer to the mixture of polysaccharides alone and the broken lines to addition of $CaCl_2$ (6.8 mM).
Symbols: (○) native; (◐) deacetylated; (◒) depyruvated; (●) deacylated xanthan. (L), Locust-bean gum and (X), xanthan.

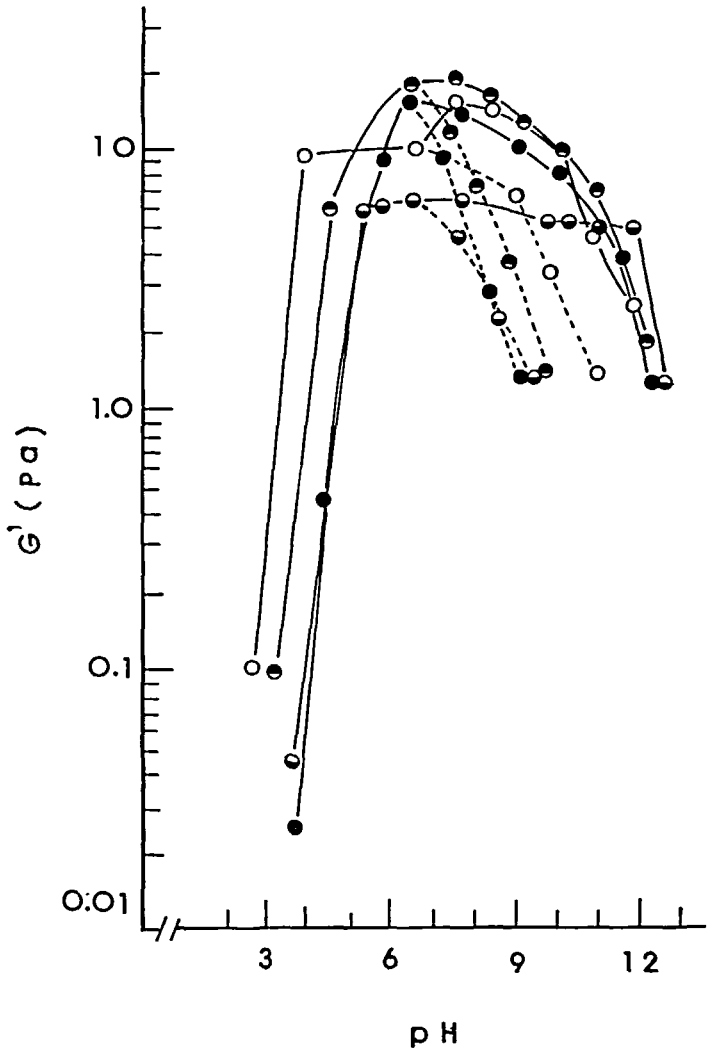


Fig. 2. Effect of pH on the dynamic modulus, at 3.77 rad/sec and 25°C, for a 0.2% mixed solution of xanthan and locust-bean gum at the mixing ratio of 1:2. The full lines refer to addition of 100 mM HCl and KOH, and the dotted lines to addition of $Ca(OH)_2$. The symbols are the same as those of Fig. 1.

little decrease of the dynamic modulus was observed by addition of KOH, but a large decrease by addition of $Ca(OH)_2$. The rapid decrease of the dynamic modulus may be due to the formation of self-association

within xanthan molecules via Ca^{2+} with ionic bonding on the carboxyl groups of glucuronic acid residues on different molecules.[23]

Figure 3 shows the effect of temperature on the dynamic modulus of mixed solutions of locust-bean gum with native, deacetylated, depyruvated, and deacylated xanthan in a ratio of 2:1 at 0.2% total gums. Though the dynamic modulus of a mixture with deacylated xanthan showed a very large value at a temperature of 20 °C, a mixture with depyruvated xanthan had a lower value at this temperature. The dynamic modulus of all the mixed solutions decreased rapidly with increasing temperature. The dynamic modulus of the mixture with deacylated xanthan, however, had a small value in the presence of urea (4.0 M) even at a temperature of 20 °C, suggesting that hydrogen bonding may have a dominant role in the interaction, since urea is known as a hydrogen bond breaker.

Figure 4 (A) shows the optical rotation-temperature profile of locust-bean gum and xanthan alone, and (B) of a gelling mixture of the two. The optical rotation of locust-bean gum (A) stayed at a constant value (+0.01°) with decreasing temperature until 25 °C, then it increased with further decrease of the temperature, the increase perhaps due to self-association[16] within locust-bean gum molecules at low temperature. The optical rotation of depyruvated and deacylated xanthan (-0.098 and -0.104°, respectively), at a temperature of 60 °C was observed to increase with decreasing temperature until 25 °C, where it remained constant. For a solution of native and deacetylated xanthan, the optical rotation (-0.062 and -0.066°, respectively) at 60 °C was also observed to increase with decreasing temperature until 25 °C, and then become constant. Though the temperature profile of optical rotation in the mixed solution (0.2% total gums) of locust-bean gum with native, deacetylated, depyruvated, and deacylated xanthan almost agreed with that of xanthan alone (A) until 25 °C, as shown in Fig. 4(B), it increased with further decrease of temperature up to 20 °C. Such an increase of the optical rotation may be due to increase not only of self-association within locust-bean gum molecules, but also of intermolecular association with xanthan molecule.

DISCUSSION

The synergistic interaction between xanthan and locust-bean gum has been confirmed from evaluation of a mixed solution of deacylated xan-

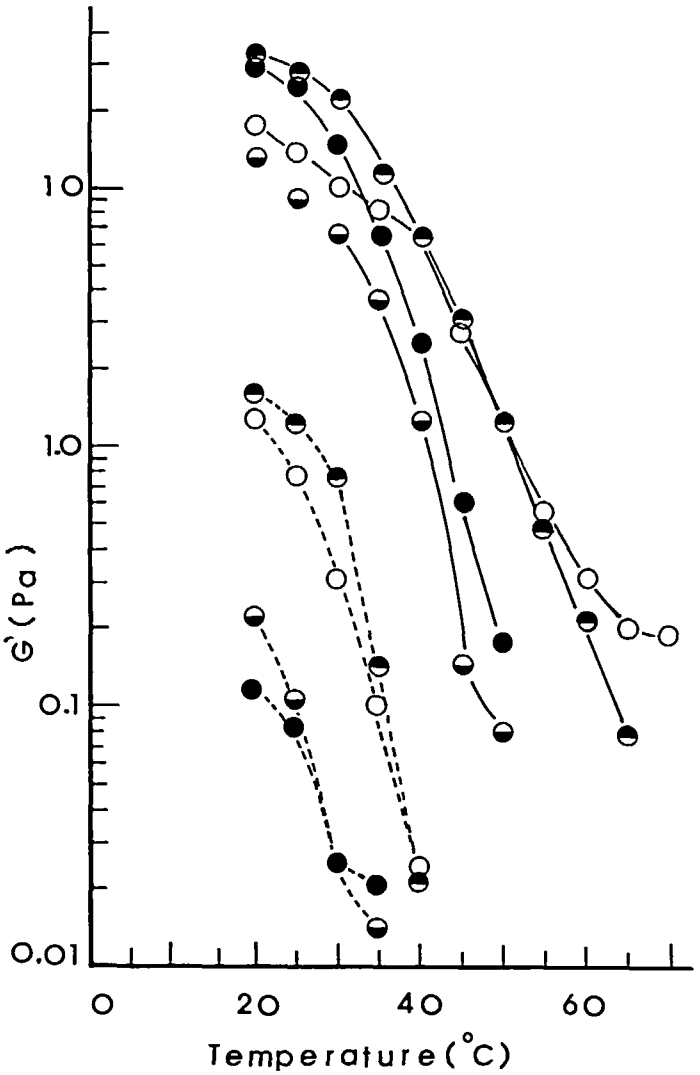


Fig. 3. Effect of temperature on the dynamic modulus, at 3.77 rad/sec, for a 0.2% mixed solution of xanthan and locust-bean gum at a mixing ratio of 1:2. The full lines refer to the mixture of polysaccharides alone and the dotted lines to addition of urea (4.0 M). The symbols are the same as those of Fig. 1.

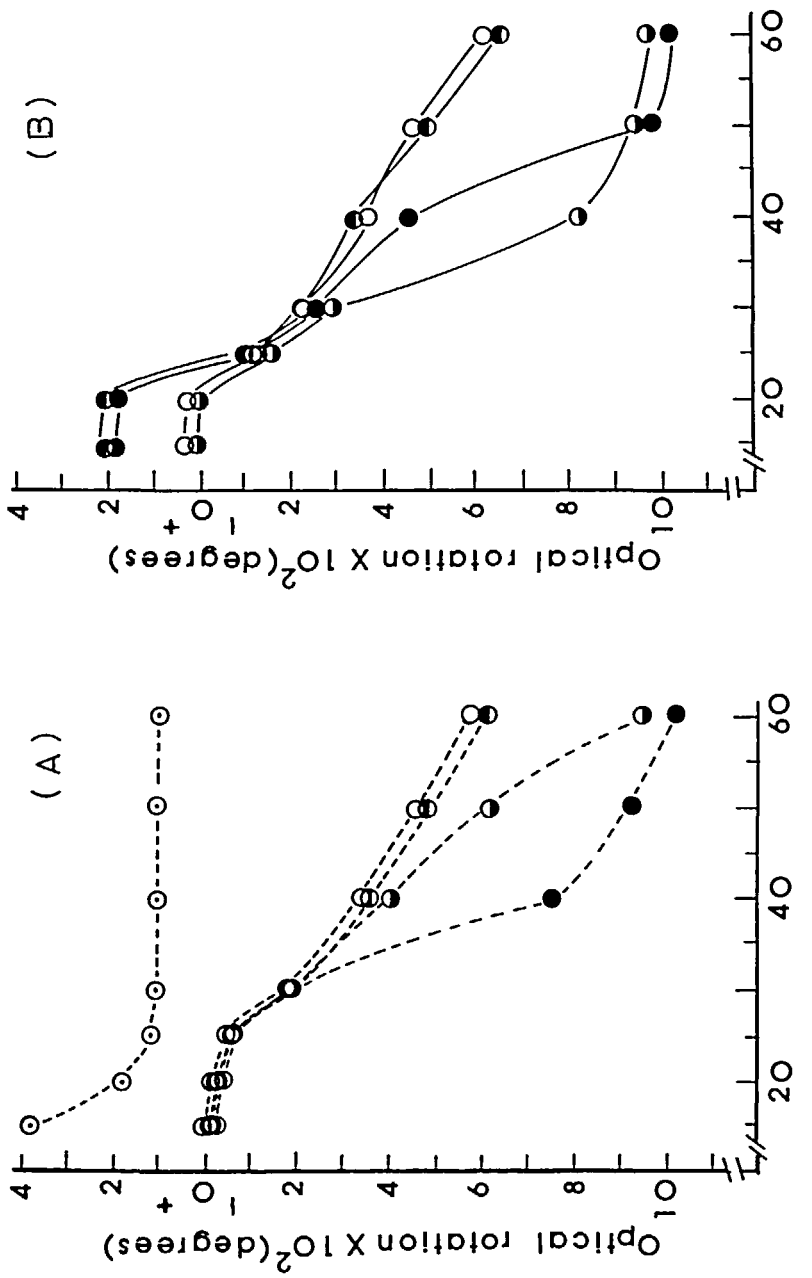
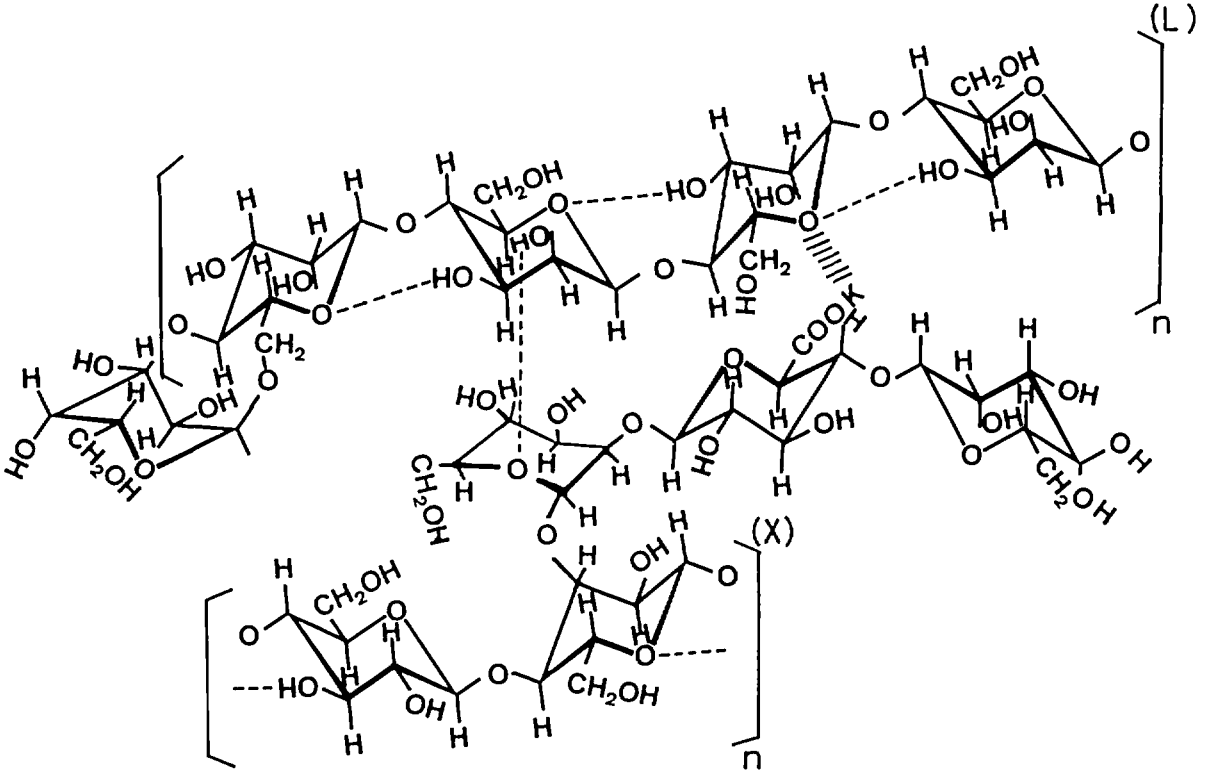


Fig. 4. Optical rotation-temperature profile for a mixed solution of xanthan and locust-bean gum at 589 nm. The dotted lines refer to the xanthan (0.07%) and locust-bean gum (0.13%; ⊙) alone (A) and full lines to a mixed solution of the two gums in the combination ratio of 1:2 at 0.2% total gum (B). The symbols of xanthan are the same as those of Fig. 1.

than and locust-bean gum. Free from substitution of pyruvic and acetic acid groups on the terminal and inner mannosyl side chains of xanthan molecules, very strong gelation was observed, as in a mixture with deacetylated xanthan[19,21] at room temperature. The deacetylation of xanthan molecules marginally improved its gelation behavior even after depyruvation with locust-bean gum molecules. The breakdown of the gel by addition of $CaCl_2$, $Ca(OH)_2$, or HCl suggests that the univalent cation (K^+) on the carboxyl groups of the glucuronic acid residues of the side chains of xanthan seem to take part in the interaction by electrostatic forces of attraction as in the self-association of κ-carrageenan molecules.[28] Furthermore, a very small dynamic modulus in the mixture of deacylated xanthan and locust-bean gum in the presence of urea (4.0 M) suggests that another association site seem to involve a hydrogen bond.

Galactomannans have a characteristic of self-association in aqueous solution, the strength of which increase with decreasing content of the galactose side-chains.[16] These results suggest that mannose residues of the backbone of galactomannan molecules take part in the self-association, and also interact with mannose residues of the side chains of xanthan molecules. For example, a hydroxyl group at C-2, which has an axial orientation in the anhydro-α-L-galactose residue, seems to take part in intermolecular hydrogen bonding with the ring oxygen atom of the adjacent anhydro-α-L-galactose residue with different molecules in agarose solutions.[29] Such analogous counter association may also have a dominant role in the interaction between deacylated xanthan and locust-bean gum molecules. The conformation of xanthan, in which the terminal mannose side chain is absent, agreed with that of native xanthan, both polymers adopting a five-fold, single stranded helix.[30,31] This suggests that any interactions involving the terminal mannose unit of the side-chains of xanthan appear not to be essential for stability of the structure.

The results and discussion presented here lead us to believe that the oxygen atom of the inner mannosyl residue of side-chain of xanthan interacts with the adjacent mannosyl OH-2 of the backbone of locust-bean gum by hydrogen bonding, as illustrated in Scheme 1. The univalent cation (K^+) which is ionically associated with the carboxyl oxygen atom on the glucuronic acid residue of the side-chain of xanthan may also interact with an adjacent hemiacetal oxygen atom of



Scheme 1. Possible binding sites for D-mannose-specific interaction between deacylated xanthan and locust-bean gum in aqueous solution. The dotted lines refer to hydrogen bonding and broken line to electrostatic force of attraction. (L), Locust-bean gum and (X) xanthan.

the mannan backbone of locust-bean gum by an electrostatic interaction. As the mannan backbone of the locust-bean gum molecule has a rigidity owing to intramolecular hydrogen bonding, O(5)----HO(3'),[32,33] the side chains of the xanthan molecule are inserted into unsubstituted segments of the mannan backbone.

Although it has been reported that the side-chains of the locust-bean gum molecules are distributed in uniform blocks along the backbone of the mannan molecules,[12,27] the mode of interaction (Scheme 1) is independent of the structure, because each junction may take place within three sugar residues of the xanthan molecule side-chains. The analogous counter-association between mannose residues of the xanthan and locust-bean gum molecules may play a dominant role in the interaction.

The interaction between the extracellular bacterial polysaccharide, xanthan, and typical galactomannan components of the plant cell wall may suggest a part in the host-pathogen relationship, since Xanthomonas campestris is a plant pathogen bacterium. Furthermore, the mode of interaction may provide carbohydrate-carbohydrate binding sites for mannose-specific binding in several cell-recognition processes as suggested for galactose-specific interactions.[34,35]

EXPERIMENTAL

Polysaccharides. Xanthan, locust-bean gum from Ceratonia siliqua, and guar gum from Cyamopsis tetragonolobus were identical those used in our preceding studies[19-21] and were obtained from Taiyo Kagaku Co., Ltd. A solution of 0.1% xanthan in distilled water was heated at 90 °C for 30 min, and then cooled to room temperature and filtered through Celite 545 (which had been treated with boiling 3 M HCl for 30 min and washed with distilled water until pH 6.5). In the presence of 0.1% KCl, ethanol (2 vols.) was added to the filtrate, and the precipitate was dried in vacuo. Purified xanthan was redissolved in water, deionized by passing through a column of Amberlite IR 120 (H^+), and neutralized with 50 mM KOH. Ethanol (2 vols.) was added in the presence of 0.1% KCl, and the precipitate was dried in vacuo.

The pyruvate groups were removed by heating a solution of the K salt of xanthan (1g/L in 1mM oxalic acid, 0.1 M KCl, pH 3.0) at 95 °C for 2 h.[36] After being neutralized with 50 mM KOH, the product was isolated in the same manner as described above.

An aqueous 0.2% solution of native or depyruvated xanthan was treated under a nitrogen atmosphere with 10 mM KOH in the presence of 0.1% KCl at room temperature for 10 h to accomplish deacetylation.[37] The solution was neutralized with 50 mM HCl, and then the product was isolated in the same manner as described above.

Solutions of 0.5% locust-bean gum in hot water (90 °C) and 0.5% guar gum in distilled water were filtered through Celite 545, ethanol (2 vols.) was added to these and thus obtained precipitates were dried in vacuo.

Various mixed solutions of xanthan and locust-bean gum, and guar gum having a total concentration of 0.2% were prepared by dissolving locust-bean gum in hot water (85 °C) or guar gum in distilled water and adding xanthan (native, deacetylated, depyruvated, and deacylated).

Pyruvic acid and acetic acid measurements. Pyruvic and acetic acid were measured colorimetrically as the 2,4-dinitrophenylhydrazone,[38] and as the hydroxamic acid,[39] respectively.

Liquid chromatography. A solution of locust-bean gum and guar gum (50 mg) in 2 M HCl (20 mL) was heated at 100 °C for 3 h, respectively. 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 was concentrated and filtered through Celite 545 into a 10-mL volumetric flask. Liquid chromatography was performed with a Shimadzu LC-4A chromatograph, equipped with a column of ISA-7/S2504 using a mobile phase of 0.3 boric acid (temperature 150 °C; flow rate 0.5 cm/min).

Infrared spectroscopy. Deacetylated xanthan was identified by infrared spectroscopy. Spectra were recorded with an infrared spectrophotometer IR-440 (Shimadzu Seisakusho Co., Ltd.) for samples dispersed in KBr discs.

Molecular weights. The molecular weights of locust-bean gum and guar gum were determined by a viscometric method according to the relationship[40] $[\eta]=3.8\times10^{-4}\cdot Mr.^{0.723}$ Intrinsic viscosity $[\eta]$ was determined by measuring the specific viscosity with an Ostwald-type viscometer at 25 °C. The flow time for water was 42 s.

Optical rotations. Optical rotations were measured at 589 nm with an automatic digital polarimeter DIP-180 (Japan Spectroscopic C., Ltd.) in a 0.07% and 0.13% (W/V) solution of xanthan and locust-

bean gum, respectively, and in a 0.2% mixed solution in a combination ratio of the former to the latter of 1:2. The optical rotation was determined after dissolving the sample at 85 °C, and cooling to temperature from 60 to 15 °C.

Dynamic modulus measurements. Dynamic modulus at a steady frequency (3.77 rad/sec) was determined with a rheogoniometer consisting of a coaxial cylinder (1.8 cm diam.) and rotating outer cylinder (2.2 cm diam.), 6.0 cm long (IR-103, Iwamoto Seisakusho Co., Ltd.). The temperature of the sample was controlled by circulating oil from 20 to 70 °C and raised at a rate of 1 °C/min. The dynamic modulus (G') was calculated by a modification of Markovitz's equation.[41]

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