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Effect of pH and added salt on the synergistic interaction between xyloglucan and xanthan

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A new synergistic interaction between xyloglucan and xanthan aqueous solutions was recently found. The effects of pH and added salt on the synergistic interaction were studied using dynamic viscoelasticity and differential scanning calorimetry (DSC). No salt and pH effects were found in the aqueous solution of xyloglucan alone. pH and salt effects, even for xanthan alone, and both values of G' and G'' at pH 5.4 or in the absence of NaCl are larger than those at pH 2.7 or in the presence of salt. It is considered as the result of the change of interaction between xanthan molecules due to screening of electrostatic repulsion among charged side chains. At low temperatures, the storage modulus, G', of the mixture of xyloglucan and xanthan at low pH or in the presence of salt were lower than those at pH 5.4 or in the absence of salt, and the results of the mixture at pH 2.7 or in the presence of 15mM NaCl are similar to that of xanthan alone under the same pH or salt condition.

Key word: Xyloglucan, Xanthan, Synergistic interaction, Salt effect, pH

1. INTRODUCTION

Polysaccharides have been widely used as food additives for years, as a thickener and a gelling agent. Combinations of polysaccharides have been studied to develop a new different texture, and to make better products, when only one polysaccharide cannot achieve[1].

Xyloglucan extracted from Tamarind seed is a storage polysaccharide[2]. Even though xyloglucan alone does not form a gel, it was reported that xyloglucan forms a gel by addition of a kind of polyphenol, such as epigallocatechin gallate[3], or gellan gum[4]. The chemical structure of xyloglucan backbone is β-(1-4) linked D-glucan, which is the same as that of cellulose. Xyloglucan polymer consists of three different repeating units with different substitution patterns of galactose, heptasaccharide (Glu4 Xyl3), octasaccharide (Glu4 Xyl3 Gal), and nonasaccharide (Glu4 Xyl3 Ga2)[5].

Xanthan is a polysaccharide produced by a bacterium *Xanthomonas campestris*, and has been widely used as a food additive. Its structure consists of a β-D-(1,4)-linked glucose backbone and a charged trisaccharide side-chain, composed of a glucuronic acid residue between two mannose units. The aqueous solutions of xanthan exhibit a temperature induced conformational transition, thermo-reversible coil-helix transition[6]. The transition temperature depends on the contents of acetyl and pyruvate groups at the inner mannose or the terminal mannose, on the ionic strength, and on pH, respectively[6-8]. It was reported that the mixture of xanthan and several polysaccharides, such as galactomannan[9,10] or glucomannan[11], exhibits synergistic gelation. We recently demonstrated that xyloglucan also associate with xanthan[12]. The mixture of xyloglucan and xanthan showed a significant increase of G' at low temperatures, which is a kind of a new synergistic interaction.

Physicochemical properties of polysaccharide aqueous solution are affected by various chemicals, for instance, salt, acid, sugar, etc. Salt and pH do not have an essential effect on xyloglucan because it is a neutral polysaccharide[13]. In contrast, xanthan is affected by the conditions of salt concentration and pH[6,14], because it carries a charge, and carboxyl group. It was reported that the synergistic interaction between xanthan and galactomannan is affected by salt and pH conditions, and these investigations are useful to examine the mechanism of synergistic interaction[15]. The aim of this study is to examine the effects of pH and ionic strength on this synergistic interaction, xyloglucan/xanthan mixture, and clarify the mechanism of the interaction.

2. EXPERIMENTS

2.1 Sample preparation

Xyloglucan extracted from Tamarind seed was provided from Dainippon Pharmaceutical Co., Ltd (Osaka, Japan). Xanthan was supplied by CP Kelco (U.S.A). The total concentration of the mixture solution is fixed at 1.0wt%, and the weight ratio of each polysaccharide in the mixture solution of xyloglucan and xanthan is 1:1. Acetic acid and a sodium chloride used in the present study were extra fine grade reagents(Wako Pure Chemical Industries, Ltd. Osaka, Japan).

2.2 Measurements

Dynamic viscoelastic measurements were carried out using a Rheostress 600 (Haake, Thermo Electron, Germany) with a cone plate geometry(diameter is 59.997mm, gap, 0.103mm, cone angle, 1.995°) at a constant frequency of 1.0 rad/s. Differential Scanning Calorimetry (DSC) measurements were carried out by using a high sensitivity calorimeter, Micro DSC III

(Setaram, France). DSC and the rheological measurements were examined at a rate of 0.5°C/min.

3. RESULTS AND DISCUSSION

Figure 1 shows the temperature dependence of storage modulus, G', and loss modulus, G'', for 0.5wt% xanthan, 0.5wt% xyloglucan, and 1.0wt% mixture solution of xanthan and xyloglucan. Although G' and G'' for the solutions of 0.5wt% xanthan and 0.5wt% xyloglucan alone increased monotonically with decreasing temperature, 1.0wt% mixture solution of xyloglucan and xanthan exhibits a synergistic interaction. That is, G' and G'' of xyloglucan/xanthan mixture are much larger than those of xyloglucan and xanthan alone in the entire temperature range, and it was found that G' and G'' steeply increased around 20°C on cooling. On the subsequent heating, both values, G' and G'', showed a steep decrease at the same temperature. There is almost no thermal hysteresis. In the present study, we investigated effects of acid and salt on the synergistic interaction of xyloglucan/xanthan mixture.

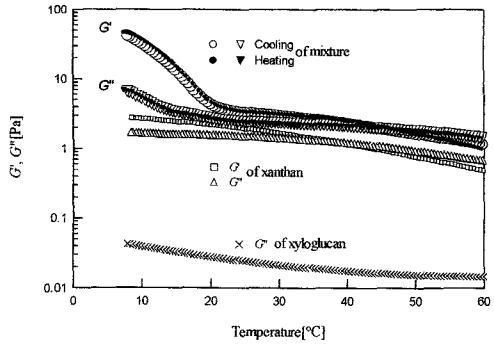
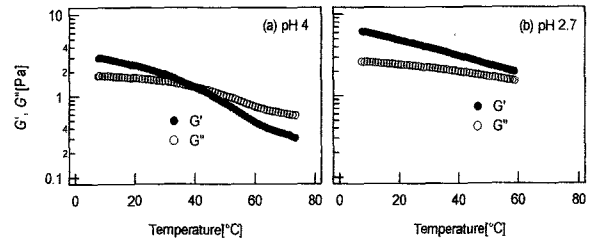


Figure 1 Temperature dependence of G′ and G″ for 0.5wt% xyloglucan, 0.5wt% xanthan, and 1.0 wt% xyloglucan/xanthan(weight ratio=1:1, at pH 5.4) mixture. Scanning rate is 0.5°C/min; angular frequency, 1.0 rad/s.

Figures 2 and 3 show the temperature dependence of G' and G'' indicating the effect of pH for 0.5wt% xanthan alone and the synergistic interaction of 1.0wt% xyloglucan/xanthan mixture. Viscoelasticity of xyloglucan is unaffected by the addition of acid (data not shown). For the solution of xanthan alone, G' and G'' increased as pH decreased (from pH 5.4 of Figure 1 to pH 2.7), and a crossover temperature of G' and G'' shifts to higher temperature on cooling. However, for 1.0wt% xyloglucan/xanthan mixture it showed the opposite tendency. G' and G'' at low temperature decreased with decreasing pH. The temperature at which G' and G'' steeply increased also decreased with decreasing pH. At pH 2.7, no transition temperature at which G' and G'' steeply increase was found. In addition, G' and G'' of xanthan alone at pH 2.7 is almost the same as those of xyloglucan/xanthan mixture at the same pH 2.7(Figure 2(b) and Figure 3(c)). The protonation of the carboxyl group of xanthan side chain occurs by lowering pH, which results in the decrease of the effective charge density along the polymer chain. This induces the screening of electrostatic repulsion among the charged side chain in xanthan. This phenomenon can be expected both for xanthan alone and the synergistic interaction of xyloglucan/xanthan mixture.



Figrue 2 Temperature dependence of G' and G'' for 0.5wt% xanthan alone at (a) pH 4 and (b) pH 2.7. Cooling rate is 0.5°C/min; angular frequency, 1.0 rad/s.

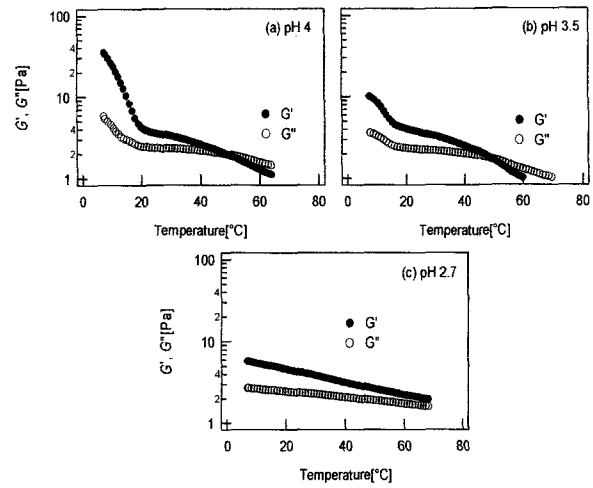


Figure 3 Temperature dependence of G' and G'' for 1.0wt% xyloglucan/xanthan mixture at (a) pH 4, (b)pH 3.5, and (c) pH 2.7. Cooling rate is 0.5°C/min; angular frequency, 1.0 rad/s.

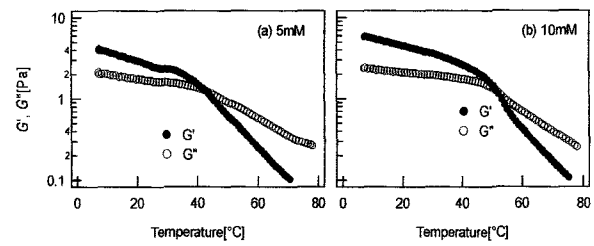


Figure 4 Temperature dependence of G' and G'' for 0.5wt% xanthan alone in the presence of salt, (a) 5 and (b) 10mM NaCl. Cooling rate is 0.5°C/min; angular frequency, 1.0 rad/s.

Figure 4 shows the temperature dependence of G' and G'' for 0.5wt% xanthan alone in the presence of 5mM and 10mM NaCl observed on cooling. With increasing

NaCl concentration, G' and G'' at low temperatures increased, and the crossover temperature of G' and G'' also shifted to a higher temperature compared with that for 0.5wt% xanthan in the absence of salt, as shown in Figure 1. It was reported that conformational (coil-helix) transition temperature of xanthan, negatively charged polysaccharide, is dominated by the total activity coefficient of cation (the sum of counterion and salt), which is interpreted as the screening effect of the salt for the electrostatic repulsion among charges along the polymer chain[16,17].

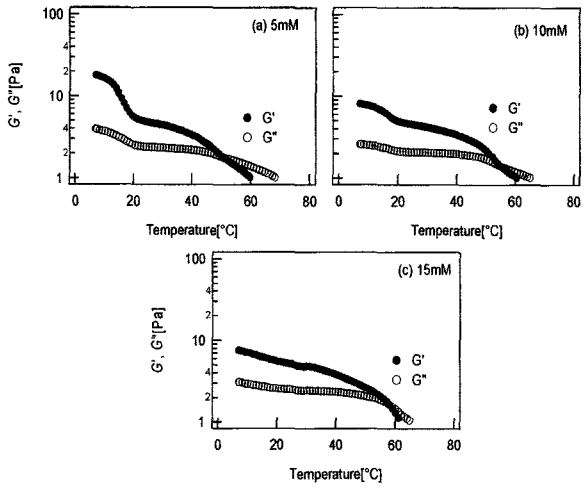


Figure 5 Temperature dependence of G' and G'' for 1.0wt% xyloglucan/xanthan mixture in the presence of salt, (a)5, (b)10, and (c)15mM NaCl, respectively. Cooling rate is 0.5°C/min; angular frequency, 1.0 rad/s.

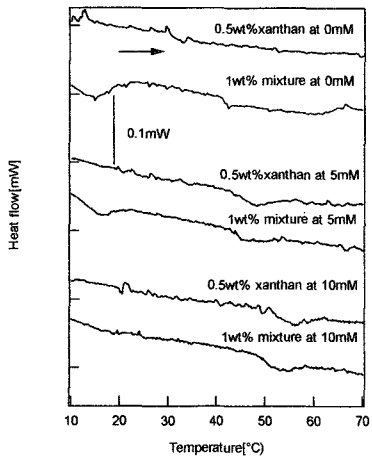


Figure 6 DSC heating curves for 0.5wt% xanthan alone and 1.0wt% xyloglucan/xanthan mixture in the absence or presence of 0, 5, and 10mM NaCl, respectively. Heating rate is 0.5°C/min.

Figure 5 and Figure 6 show the temperature dependence of G' and G'', and DSC heating curves for 1.0wt% xyloglucan/xanthan mixture in the absence and presence of salt, 0, 5, 10, and 15mM NaCl, respectively. The temperatures at which G' and G'' steeply increased, at about 20°C, are almost the same as those in the absence or presence of NaCl. While only one step-like increase was observed in the absence of salt as shown in Figure 1, in mixtures in the presence of 5mM and 10mM, the step-like increase was observed twice at around 20°C and 40~60°C, respectively. In the presence of salt, one step-like increase appeared at 40~60°C, which became steeper with increasing NaCl concentration. That is, a more remarkable change in G' and G'' was observed in the presence 10mM NaCl than in the presence of 5mM NaCl. The other step-like increase appeared at lower temperature, and can be attributed to the synergistic interaction for mixture of xyloglucan and xanthan, as illustrated in Figure 1, because the onset temperature at which G' and G'' start to increase steeply is not affected by the addition of salt, and is the same as that in the absence of salt. However, the values of G' and G'' at 5°C, resulted in the synergistic interaction, decreased with increasing NaCl concentration, and in 15mM NaCl, the step-like increase of G' and G'' around 20°C could not be observed.

Essentially the same tendency was observed from DSC measurements on heating, as shown in Figure 6. No DSC peak was observed for 0.5wt% xyloglucan (data not shown) and 0.5wt% xanthan alone in the absence of salt. Although a peak for 0.5wt% xanthan in the absence of NaCl could not be detected due to sensitivity limit, a peak appeared by adding NaCl. The DSC transition enthalpy estimated from the DSC peak area increased with increasing NaCl concentration, which agreed with the reported results for xanthan[16] or gellan gum[18]. The DSC peak temperature of xanthan alone shifts to a higher temperature, and this temperature agreed with the temperature at which G' and G'' steeply increased. It was reported that xanthan aqueous solution undergoes the coil-helix transition, and endothermic peak appears on heating, we can assign the characteristic rheological change at higher temperatures shown in Figure 4 to the coil-helix transition of xanthan.

For the DSC heating curves of 1.0wt% xyloglucan/xanthan mixture in the absence of NaCl, an endothermic peak was observed at around 17°C. At the same temperature, both G' and G'' steeply decreased on heating as shown in Figure 1, indicating that the DSC peak at lower temperature can be attributed to the synergistic interaction of xyloglucan/xanthan mixture. The apparent ΔH of the synergistic interaction, around at 17°C, decreased with increasing NaCl concentration as shown in Figure 6.

By addition of salt, a new DSC peak appeared at high temperature (around 40~60°C). The lower temperature DSC peak (around 17°C) appeared at the same temperature as in the absence of salt. The temperature at which junction zones (the lower temperature peak) are formed between xanthan and xyloglucan is independent on the ionic strength. In the presence of 10mM NaCl, the difference between DSC peak temperature of the conformational transition of xanthan and the synergistic interaction is greater than that at 5mM NaCl. The same effects were reported for the different systems showing synergistic gelation, xanthan/galactomannan or xanthan/glucomannan mixtures. For example, Bresolin et al. reported that the coil-helix transition temperature of xanthan is affected by

the salt concentration, but the DSC peak temperature attributed to the synergistic interaction is not affected by the addition of salt[15]. They suggested that galactomannan can interact only with xanthan in the coil conformation, and cannot with the helix part of xanthan. Another explanation is that the galactomannan interacts with xanthan in the third conformation (not coil and not helix state). The chemical structure of galactomannan and glucomannan is different from that of xyloglucan, and the mechanism of the interaction for xanthan/xyloglucan systems may be different from that for xanthan/galactomannan systems. However, the result is essentially the same, and above models can also explain the result even for this system. To clarify the detail, further study is required.

CONCLUSIONS

The effects of addition of salt, NaCl, on the synergistic interaction of xyloglucan and xanthan are similar to that of lowering pH. In the presence of large amount of salt or at low pH, the synergistic interaction between xanthan and xyloglucan disappeared. With increasing NaCl concentration, the conformational transition temperature of xanthan shifts to higher temperatures due to shielding of electrostatic repulsion in a xanthan molecule. Essentially the same phenomenon can be expected at low pH, because the protonation of a carboxyl group at the side chain in xanthan occurs as low pH.

Thus, both the addition of salt and the lowering pH increase the thermal stability of helix conformation. The disappearance of the synergistic interaction may suggest that xyloglucan can interact only with the coil conformation of xanthan or the interaction among xanthan, such as self-association of xanthan, is more favorable compared with the formation of the heterotypic junction zones, as proposed for xanthan/galactomannan [15] or xanthan/glucomannan mixtures[19].

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