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Note

Evidence for intermolecular binding between xanthan and the glucomannan konjac mannan

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X-Ray diffraction of fibres prepared from oriented gels has become a standard method for producing molecular models for the association between like polysaccharides within the junction zones of gels[1]. Although the preparation of the fibres requires that the gels are stretched, and at least partially dehydrated, studies of single polysaccharide systems have shown, in all but one instance, that the method provides reliable models for the ordered polysaccharide structure present in the hydrated state[1]. Recently, this technique has been applied to study binary polysaccharide gels in order to investigate proposed intermolecular binding between different polysaccharides[2–7]. Such studies of xanthan–galactomannan gels[2,3] have provided evidence for xanthan–galactomannan binding in the binary systems xanthan–carob and xanthan–tara.

The primary structure of xanthan is a (1→4)-linked β-D-glucan backbone (cellulose) substituted through O-3 on alternate glucosyl residues with a charged trisaccharide side-chain[8,9]. The side chains are considered to modify the normal backbone geometry, leading to a helical structure with five-fold symmetry[10,11]. Galactomannans consist of a (1→4)-linked β-D-mannan backbone incompletely substituted at O-6 by galactosyl side-chains[12]. Early workers attributed gelation to an intermolecular binding between the xanthan helix and unsubstituted regions of the galactomannan backbone[12–14]. Recent mixing experiments[2,3] suggest that intermolecular binding (as revealed by X-ray diffraction studies) and gelation occurs only if the two polymers are mixed under conditions which denature the xanthan helix. Such studies have led to the proposal[2,3] that intermolecular binding involves co-crystallisation of sections of the denatured xanthan molecule with segments of the galactomannan chains. Since D-glucose and D-mannose differ only in the orientation of HO-2, an interaction between the cellulosic and mannan backbones provides a stereochemically acceptable basis for xanthan–galactomannan binding. On the basis of the above model, it might be expected that xanthan would gel upon admixture with other polysaccharides whose structures are stereochemically

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compatible with cellulose or mannan.

There is evidence for an affinity between glucomannan structures and cellulose. Chanzy *et al.*[15] reported the growth of glucomannan crystals upon cellulose fibrils and Rydholm[16] has observed reprecipitation of glucomannans on wood pulp during the pulping of softwood. It has been reported[12] that mixtures of xanthan and the glucomannan konjac mannan will gel. Konjac mannan is a linear polymer containing (1→4)-linked β-D-glucosyl and β-D-mannosyl residues. The sequence of residues along the backbone is not known, but the polymer does not contain cellulosic or mannan block-structures[17]. Native konjac mannan is acetylated and does not gel. Gelation of konjac mannan can only be obtained upon de-esterification under alkaline conditions[18]. Xanthan samples do not form true gels[19]. The rheological properties of xanthan samples are complex and sensitive to the conditions of preparation, but are indicative of a medium containing a weak structure which breaks down under shear[19]. Thus, since neither xanthan nor the native konjac mannan alone will gel, the gelation of xanthan–native konjac mannan mixtures is a synergistic interaction that is suggestive of xanthan–glucomannan binding.

The gelation of xanthan–native konjac mannan mixtures was found to be sensitive to the preparation conditions. Samples of xanthan and native konjac mannan were separately dispersed in water at 95° and each sample was cooled to room temperature (25°). Neither sample at 1% or 2% total polymer concentration formed a true strong gel upon cooling. Mixtures (1:1) of xanthan and the native glucomannan, at a total polymer concentration of 1%, were prepared in various ways. Optical rotation was used to monitor the conformation of the xanthan molecules at concentrations, temperatures, and ionic strengths equivalent to those within the mixtures (Fig. 1). Rheological methods were used to distinguish between pourable fluids and true gels (Figs. 2 and 3, Tables I and II). Simple creep compliance tests were used to examine the response of the samples to a stepwise increase in stress (Fig. 2). Xanthan–glucomannan mixtures prepared by mixing at room temperature (25°) with xanthan in the helical form (Fig. 1) did not gel. The creep compliance studies (Fig. 2a) are consistent with a pourable fluid and this is illustrated in Fig. 3 (sample A). Table I shows that the storage modulus (G') is greater than the loss modulus (G'') but that both increase with increasing frequency, indicative of a weakly structured fluid similar to xanthan alone. When these samples were heated to 95° and then cooled to room temperature, they gelled. Mixtures prepared by mixing at 95° under conditions which result in the denaturation of the xanthan helix (Fig. 1) did gel upon cooling to room temperature (Figs. 2b and 3-sample B). Table II demonstrates that these samples are true gels. The storage modulus G' is much greater than G'', and G' is independent of frequency. In the presence of sufficient calcium chloride, the helix–coil transition temperature for xanthan can be raised[20] above 100° (Fig. 1). Mixtures of xanthan and the native glucomannan prepared at 95°, with xanthan in the helical form, remained pourable fluids upon cooling (Figs. 2 and 3-sample C). These data demonstrate that denaturation of the xanthan helix is a crucial step in the gelation process.

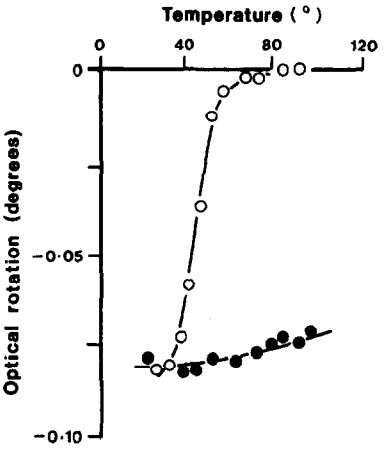

Fig. 1. Optical rotation of a 0.5% sodium xanthan sample at 589 nm; ○, xanthan in water; ●, xanthan in 0.5M $CaCl_2$.

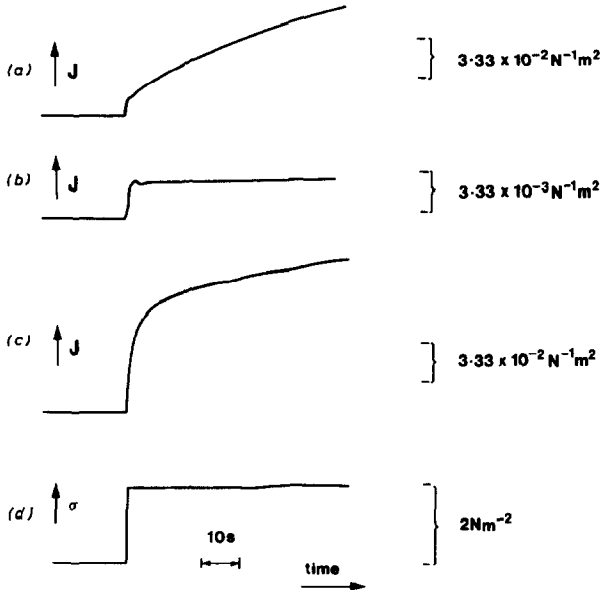

Fig. 2. Creep compliance (J) studies of mixtures of xanthan–native konjac mannan. The total polymer concenoLation is 1% and the mixtures comprise 1:1 xanthan–konjac mannan: (*a*) mixtures prepared at 25°, (*b*) mixtures prepared at 95° and cooled to 25°, (*c*) mixtures containing 0.5M $CaCl_2$ after heating at 95° and cooling to 25°, (*d*) applied stress (σ) profile. The compliance values are calculated from the maximum stress in the parallel plate configuration. Measurements were made using an Instron 3250.

X-Ray fibre diffraction studies have been used to examine whether gelation results from intermolecular binding between xanthan and native konjac mannan. A typical X-ray diffraction pattern obtained from a fibre prepared from native "acetylated" konjac mannan is shown in Fig. 4a. The pattern is crystalline and can be

indexed onto the mannan II lattice (monoclinic: a = 1.88, b = 1.87, c (fibre axis) = 1.02 nm, γ = 57.5°). The weak meridional reflections are characteristic of the absence of a regular repeat-unit in the chemical structure and of the lack of cellulosic or mannan blocks. Since de-esterification under alkaline conditions promotes gelation, it has been suggested[21] that the acetyl groups sterically prevent intermolecular association and crystallisation. The deacetylated form of konjac mannan has also been reported[15] to crystallise in the mannan II lattice. Thus, Fig. 4a demonstrates that the ester groups do not sterically inhibit crystallisation but that de-esterification presumably promotes the tendency to crystallise and gel. Although the position of the ester substituents is unknown, a possible explanation for the decrease in polymer solubility is that de-esterification increases the number of potential intermolecular hydrogen bonds. The X-ray fibre patterns obtained for xanthan gum (Fig. 4b) are consistent with reported data[10,11] which have been interpreted in terms of helices with five-fold symmetry. The X-ray fibre pattern (Fig. 4c) obtained for the xanthan–glucomannan mixed gel provides evidence for intermolecular binding. The pattern corresponds to a new structure present at the junction zones within the gel. A multi-phasic gel structure not involving intermolecular binding would result in patterns corresponding to one of either of the components or a superposition of these patterns. Whereas the patterns obtained for xanthan–galactomannan mixed gels were related to those obtained for the galactomannan, the patterns obtained for

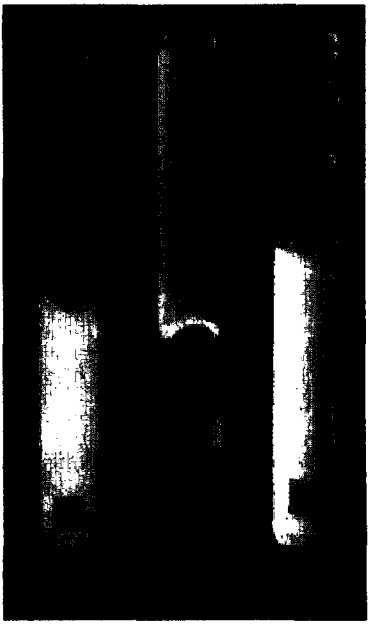

Fig. 3. The effect of inverting a tube containing xanthan–native konjac mannan samples. A, mixture prepared at 25°; B, mixture prepared at 95° (xanthan in coil form) and then cooled to 25°; C, mixture prepared at 95° (xanthan preserved in the helical form by the addition of $CaCl_2$) and then cooled to 25°. Samples A and C are pourable fluids and flow to the bottom of the tube upon inversion. Sample B is a true gel which remains at the top of the tube. Picture taken 48 h after inversion.

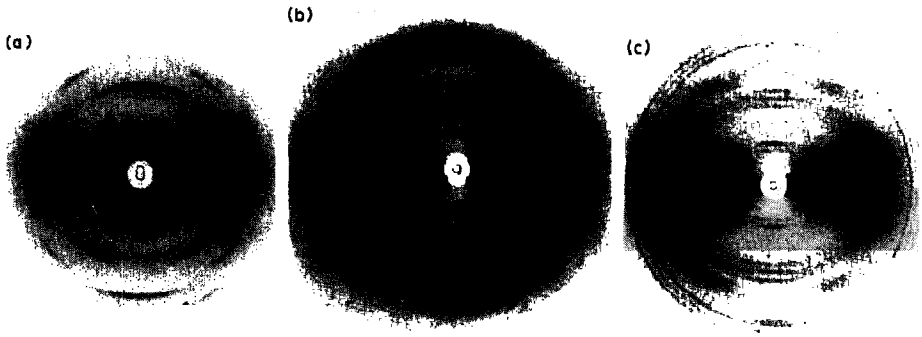

Fig. 4. X-Ray fibre diffraction patterns for (*a*) native konjac mannan, (*b*) xanthan, and (*c*) xanthan–native konjac mannan (1:1) mixed gel. Non-gelling samples were cast onto PTFE and partially dried to produce films. The films or gels (formed from gelling samples) were cut into strips and stretched under controlled relative humidity. The samples stretched 300% (xanthan), 200% (xanthan–native konjac mannan), and 100% (native konjac mannan). Patterns were recorded photographically using a flat plate camera, flushed with helium to reduce air scatter, and maintained at a relative humidity of ~98%. The X-ray wavelength was 0.154 nm.

TABLE I

RHEOLOGICAL DATA[a] OBTAINED FOR A XANTHAN–NATIVE KONJAC MANNAN MIXTURE PREPARED AT 25°

Frequency (Hz)	G′ *(Pa)*	G″ *(Pa)*	ϕ *(degrees)*
0.009	32.5	17.6	28.5
0.028	44.8	22.0	26.1
0.087	59.9	28.1	25.1
0.258	79.7	33.0	22.5
0.768	103.0	37.5	20.0
2.278	130.5	41.1	17.5
6.751	172.0	47.1	15.3
20.000	247.4	41.8	9.6

[a]*G′* is the storage modulus, *G″* the loss modulus, and ϕ the phase angle. Data collected using an Instron 3250.

TABLE II

RHEOLOGICAL DATA[a] OBTAINED FOR A XANTHAN–NATIVE KONJAC MANNAN MIXTURE PREPARED AT 95° AND THEN COOLED TO 25°

Frequency (Hz)	G′ *(Pa)*	G″ *(Pa)*	ϕ *(degrees)*
0.009	366.0	11.5	1.8
0.028	375.4	9.83	1.5
0.087	380.0	6.63	1.0
0.258	384.0	6.70	1.0
0.768	387.7	5.41	0.8
2.278	389.6	5.44	0.8
6.751	402.3	6.32	0.9
20.000	475.0	0.83	0.1

[a]*G′* is the storage modulus, *G″* the loss modulus, and ϕ the phase angle. Data collected using an Instron 3250.

the xanthan–native konjac mannan gel are related to those obtained for xanthan. The most obvious difference is the different layer-line spacing. In the mixed-gel pattern, the layer lines corresponding to l = 1, 2, 3 show a helical pitch of 5.6 nm compared to that of 4.7 nm observed for xanthan alone[10,11]. Analysis of such patterns is complicated by the complex and possibly irregular structure of the glucomannan[17].

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