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EFFECT OF GALACTOSE CONTENT ON THE SOLUTION AND INTERACTION PROPERTIES OF GUAR AND CAROB GALACTOMANNANS*

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ABSTRACT

Guar galactomannan has been modified by treatment with an α-D-galactosidase A preparation from lucerne seeds. This enzyme was purified by affinity chromatography on *N*-ε-aminocaproyl-α-D-galactopyranosylamine linked to Sepharose 4B, had a high activity towards galactomannans, and was completely devoid of β-D-mannanase. On incubation for 2 h, this enzyme removed >75% of the galactose from guar galactomannan with no concurrent decrease in viscosity. Eventual decrease in viscosity was associated with the formation of insoluble, mannan-type precipitates. This phenomenon, although directly related to the galactose content of the galactomannan, was also time-dependent. The limiting viscosity numbers calculated for the "mannan backbones" of α-D-galactosidase-treated, guar galactomannan having galactose–mannose ratios of 38:62 to 15:85 were the same. Modified, guar galactomannan (at 0.4% w/v) having a galactose–mannose ratio of 20:80, or less, forms a gel on storage at 4° over several weeks. Also, gel particles form when solutions of these galactomannans are passed through a freeze–thaw cycle. Samples containing <10% of galactose rapidly precipitate from solution even at 30°. The interaction of guar galactomannan with xanthan is greatly increased by removal of galactose residues. Samples having galactose–mannose ratios of ~19:81 interact with xanthan to essentially the same degree as carob galactomannan (Gal/Man = 23:77).

INTRODUCTION

Galactomannans interact with several polysaccharides, resulting in a substantial viscosity increase or gel formation[1]. Carob galactomannan gives a substantial increase in the firmness of agar and carrageenan gels, and causes gel-formation in the

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presence of xanthan, a non-gelling polysaccharide. However, guar galactomannan interacts with each of these polysaccharides to a much lesser extent and no gel is formed on mixing guar galactomannan and xanthan[1].

Both carob and guar galactomannans consist of a (1→4)-linked β-D-mannan backbone to which single (1→6)-linked α-D-galactosyl groups are attached[2]. These polymers have similar limiting-viscosity numbers[3] and in solution each appears to exist in a fully extended, ribbon-like conformation[3]. Consequently, the difference in their interaction with xanthan must be related to either their galactose content or the distribution of this galactose along the mannan chain, or both. A "block-type" distribution of galactose along the mannan chain of carob galactomannan[4,5] and a uniform distribution of galactose in guar galactomannan[6] have been proposed. On the basis of these different patterns of galactose distribution, Rees and his co-workers proposed that the sections of galactomannan which interact with xanthan, agar and carrageenans are those unsubstituted with galactose[1,7-9]. However, more recent information on the fine-structures of carob and guar galactomannans, obtained by enzymic techniques[10,11], chemical procedures[12], and n.m.r. spectroscopy[13], indicates that the galactose distribution in both these galactomannans is irregular to random. Evidence has also been presented which suggests that there is not an absolute requirement for long sections of contiguous, unsubstituted mannose residues at the "junction-zones" for interaction of xanthan with galactomannan, but rather that xanthan may also interact with sections of the galactomannan where galactosyl groups are positioned on only one side of the mannan backbone[11].

If the galactose distributions in guar and carob galactomannans are irregular to random, then it must be concluded that the difference in the degree of interaction of these two polymers with xanthan and other polysaccharides is essentially due to their different contents of galactose. To investigate this question more fully and to study the effect of galactose content on the solution characteristics of galactomannans, the properties of α-D-galactosidase-treated guar and carob galactomannans have been studied in detail.

EXPERIMENTAL

General. — D-Galacto-D-mannans were extracted from commercial carob- and guar-seed endosperm-flours (Sigma) as previously described[14].

T.l.c. was performed as previously described[11]. For determination of carbohydrate components, D-galacto-D-mannan samples were hydrolysed with acid, the acid was neutralised with barium carbonate[3], and the products were converted into the aldononitrile acetates for g.l.c.[15]. The galactose–mannose ratios were also determined by a method employing D-galactose dehydrogenase for D-galactose[16] and anthrone for total carbohydrate[3,17].

Enzyme activity. — One nkat of α-D-galactosidase activity is defined as the amount of enzyme which will release 1 nmol/s of galactose reducing-sugar equivalent from the substrate used in the particular experiment under the incubation conditions

defined for that experiment. One nkat of β-D-mannanase is the amount of enzyme which will release 1 nmol of mannose reducing-sugar equivalent from 0.2% carob galactomannan at pH 4.5 and 40° in 1 s.

β-D-Mannanase was routinely assayed with RBB-carob galactomannan as substrate[18]. It was also assayed viscometrically[19] and by the *p*-hydroxybenzohydrazide reducing-sugar method[18], but with carob galactomannan (0.2%, 0.5 mL) as substrate.

α-D-Galactosidase was routinely assayed[14] with *p*-nitrophenyl α-D-galactopyranoside (10mM) as substrate. Activity on galactomannan was routinely assayed by incubating the enzyme preparation (0.1 mL) with guar or carob galactomannan (0.5 mL, 0.1%) in 0.1M acetate buffer (pH 5) for 5–10 min at 40°. The reaction was terminated by addition of *p*-hydroxybenzohydrazide solution (5 mL), and the color was developed[18,20] by incubating the tubes for 6 min at 100°.

Degrees of hydrolysis were determined as previously described[11].

Purification of enzymes. — β-D-Mannanase was purified from commercial Driselase preparation (Kyowa, Hakko Kogyo Co. Ltd., Japan) as previously described[21].

α-D-Galactosidases A and C from germinated lucerne-seeds were purified by a modification of a previously published technique[14]. Lucerne seed (500 g, *Medicago sativa* var. Hunter river) was surface-sterilised by soaking in 0.5% NaOCl for 10 min and then washed. The seeds were soaked in distilled water for 6 h to allow imbibition, washed, and germinated on filter paper for 2 days at room temperature (~22°). After maceration in 0.1M acetate buffer (pH 5), the homogenate was incubated at 40° for 1 h, to allow breakdown of residual galactomannan. The homogenate was filtered through muslin and centrifuged (8,000g, 20 min, 2°), $(NH_4)_2SO_4$ was added to 80% saturation, and the precipitate was dialysed for 2 × 24 h against distilled water. After centrifugation, phosphate buffer (M, pH 6.5) was added to a final concentration of 10mM. The preparation was then chromatographed on DEAE-cellulose (phosphate form; bed volume, 4 × 17 cm), using a 0–0.3M KCl gradient (total volume of 2 L) in 10mM phosphate buffer (pH 6.5). α-D-Galactosidases A and C were concentrated separately by $(NH_4)_2SO_4$ precipitation and were dialysed against 0.5M KCl in 0.1M NaOAc (pH 4.5) for 8 h. These enzymes were then applied separately to a column (2.5 × 35 cm) of *N*-ε-aminocaproyl-α-D-galactopyranosylamine(*N*-6-aminohexanoyl-α-D-galactopyranosylamine)–Sepharose 4B[22] at 4° and eluted with 0.5M KCl plus 20% of ethylene glycol in 0.1M NaOAc (pH 4.5) at 4°. α-D-Galactosidase was eluted without the addition of galactose, well behind the peak of non-retarded protein. The eluted enzyme was concentrated by dialysis against poly(ethylene glycol) 4,000, dialysed against distilled water for 20 h, and stored frozen in polypropylene containers.

Effect of removal of galactose on the intrinsic viscosity of solutions of guar galactomannan. — Guar galactomannan (160 mL, 0.092% w/v) in 0.5M KCl (pH 5, unbuffered) was incubated with lucerne α-D-galactosidase A (0.6 mL, 9.9 nkat) at 40°. After 0, 1, 3, and 6 h, aliquots (25 mL) were removed and treated with mM phenylmercuric chloride solution (0.1 mL), to inhibit α-D-galactosidase activity.

Separate aliquots (1 mL) were removed and incubated at 100° to inactivate α-D-galactosidase, and sub-samples (0.2 mL) were assayed for galactose by a reducing-sugar method[20] and total carbohydrate by the anthrone method[17]. An aliquot (15 mL) of the phenylmercuric chloride-treated sample was added to an Ubbelohde suspended-level viscometer which allowed *in situ* dilution, and limiting viscosity numbers were determined at 25°. The remaining sample (10 mL) was dialysed, concentrated, hydrolysed with H_2SO_4, and analysed for total carbohydrate by the anthrone method[17] and for D-galactose by using D-galactose dehydrogenase[16]. The concentration of galactomannan or "mannan-backbone" in solution was calculated from the original carbohydrate concentration by using the determined galactose–mannose ratios of the α-D-galactosidase-treated samples.

Action of β-D-mannanase on carob galactomannan/xanthan mixtures. — Aliquots of carob-galactomannan solution (0.1%, 0.2 mL) in 0.05M NaOAc (pH 4.5) were added to tubes containing either xanthan solution (0.1%, 0.2 mL) or water (0.2 mL) and equilibrated to either 25 or 80°. All tubes were vigorously mixed on a vortex stirrer and those pre-incubated at 80° were chilled to 2°. The tubes were then all added to a water bath equilibrated at 15°. After 10 min, an aliquot (0.05 mL) of Driselase β-D-mannanase[11] (0.16 nkat on 0.05% carob galactomannan at 15°) was added to each tube. Reaction was stopped after various times by heating the tubes in a steam bath for 2 min. The amount of mannose reducing-sugar equivalent was measured by the *p*-hydroxybenzohydrazide method[20].

Degree of hydration. — A low-temperature n.m.r. method[23–25] was used to determine the degree of hydration of the galactomannan samples. This method is valid when applied to polysaccharides[24]. A value of 0.22 ±0.07 g/g was obtained for native guar galactomannan (Gal/Man = 38:62) and for α-D-galactosidase-treated guar galactomannan (Gal/Man = 17:83).

Partial specific volume. — A solution of native guar galactomannan was made up in 0.5M KCl and dialysed against this solvent. The concentration of carbohydrate was determined by the anthrone method[17] and the density was determined by using a Digital Precision Density Meter DMA 02C (Anton Paar, K.G., Austria). The partial specific volume of native guar galactomannan in 0.5M KCl was 0.65931 mL/g.

Preparation of α-D-galactosidase-modified guar galactomannans. — To aliquots (1 L) of guar galactomannan solution (0.4% w/v) in 50mM sodium acetate buffer (pH 4.5) was added lucerne α-D-galactosidase A (0–60 nkat on this substrate). The solutions were overlaid with a few drops of toluene, sealed, and incubated at 35° for 40 h. Reaction was then terminated, and free galactose was removed by precipitation of the polymer with ethanol (2 vol.). The galactomannans were washed with alcohol and acetone and dried *in vacuo*

RESULTS AND DISCUSSION

Purification of α-D-galactosidase. — The α-D-galactosidase used in the current studies was α-D-galactosidase A from germinated lucerne seeds[14]. This was separated

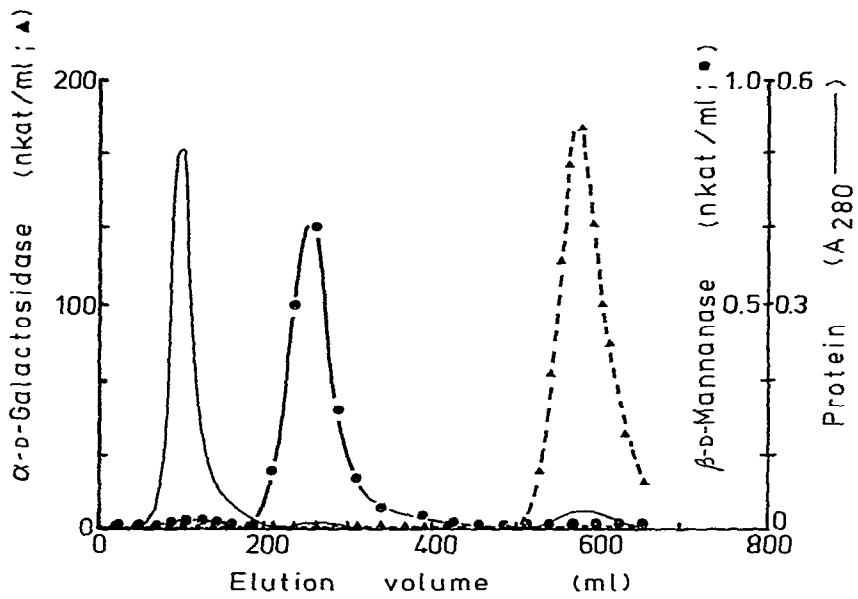

Fig. 1. Chromatography of α-D-galactosidase A from lucerne on a column of *N*-ε-aminocaproyl-α-D-galactopyranosylamine–Sepharose 4B conjugate (2.5 × 35 cm). α-D-Galactosidase (5 mL, 8 μkat) in 0.5M KCl was applied, and the column washed with 0.5M KCl plus 20% of ethylene glycol in 0.1M NaOAc (pH 4.5). α-D-Galactosidase was eluted without the addition of galactose, well-behind the major peak of protein.

from another form of α-D-galactosidase, termed C, by chromatography on DEAE-cellulose. Both enzymes were then purified by chromatography on *N*-ε-aminocaproyl-α-D-galactopyranosylamine–Sepharose 4B. α-D-Galactosidase C was obtained as a single protein band on isoelectric focusing (pI = 4.6). α-D-Galactosidase A consisted of several protein bands having isoelectric points in the range 5.7 to 6.6; however, each protein band had α-D-galactosidase activity. The chromatography of lucerne α-D-galactosidase A on *N*-ε-aminocaproyl-α-D-galactopyranosylamine–Sepharose 4B is shown in Fig. 1. Under the conditions described, α-D-galactosidase was highly retarded, but could be eluted without the addition of D-galactose. Addition of 0.1M D-galactose to the eluting solvent resulted in immediate elution of the enzyme. β-D-Mannanase was also slightly retarded on this affinity column (Fig. 1). The nature of the binding was considered to be hydrophobic, as it was partially overcome by including 20% of ethylene glycol in the elution solvent. However, β-D-mannanase was not retarded on either octyl–Sepharose or aminohexane–Sepharose under the elution conditions used in these studies. Elution was not affected by the addition of D-galactose to the eluting solvent. The specific activity of purified α-D-galactosidase C (1.28 μkat/mg protein) was very similar to that previously reported for the same enzyme purified by conventional chromatographic procedures[14]. However, these values are almost two orders of magnitude lower than that reported by Itoh *et al.*[26]. This difference and the properties of other α-D-galactosidases will be discussed elsewhere.

TABLE I

SEPARATION AND PURIFICATION OF LUCERNE α-D-GALACTOSIDASES A AND C

Stage of purification	*Total protein (mg)*	*Total activity (μkat)*	*Specific activity (μkat/mg of protein)*	*Recovery*	
				Per step (%)	*Overall (%)*
Crude extract	45,240	52.0	0.0011	—	—
0–80% $(NH_4)_2SO_4$	4,314	51.8	0.012	99	99
DEAE-cellulose					
Galactosidase A	816	20.2	0.025	39	39
Galactosidase C	251	24.0	0.096	46	46
Affinity column					
Galactosidase A	13	17.7	1.36	85	33
Galactosidase C	16	20.6	1.28	86	40

The weak, but significant, binding of β-D-mannanase to the affinity column made the preparation of α-D-galactosidase completely devoid of β-D-mannanase more difficult. For this reason, lucerne α-D-galactosidase A, which could be more readily obtained devoid of β-D-mannanase, was used in the current studies. A scheme for the purification of this enzyme is shown in Table I.

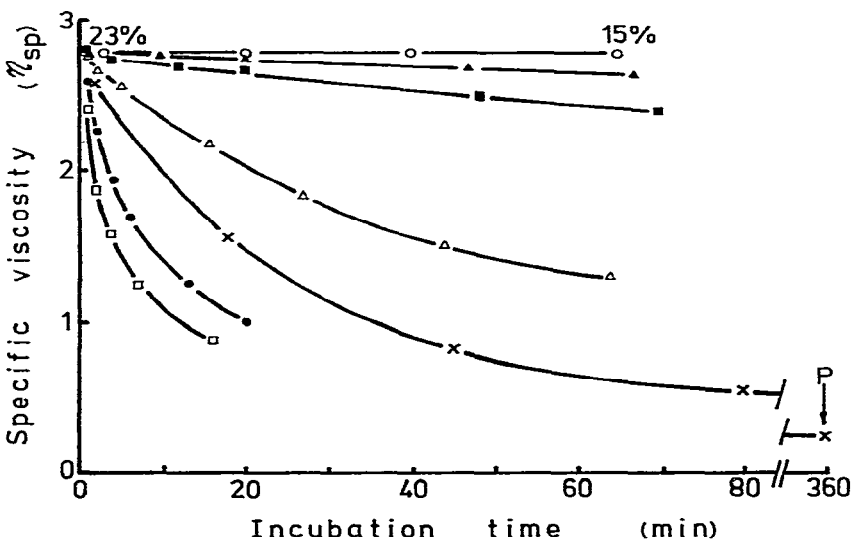

Fig. 2. Effect of α-D-galactosidase and β-D-mannanase on the solution viscosity of carob galactomannan. Carob galactomannan (15 mL, 0.1%) in 0.1M NaOAc (pH 4.5) was incubated at 30° in an Ubbelohde suspended-level viscometer with either α-D-galactosidase (○) 3.3 nkat (on this substrate); or β-mannanase (□) 0.4 nkat, (●) 0.2 nkat, (△) 0.04 nkat, (■) 0.004 nkat, or (▲) 0.0004 nkat; or with a mixture (×) of α-D-galactosidase (3.3 nkat) plus β-D-mannanase (0.12 nkat). During 65-min incubation, the galactose content of carob galactomannan was diminished from 23 to 15% by 3.3 nkat of α-D-galactosidase. At point P, a mannan precipitate had formed.

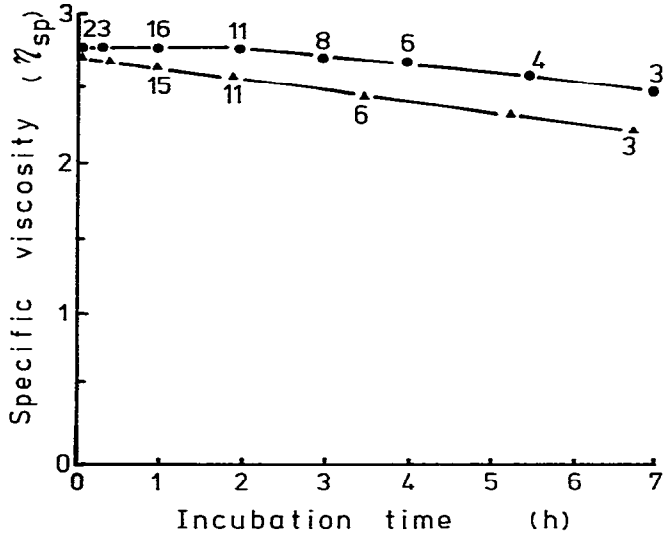

Fig. 3. Effect of pure, lucerne α-D-galactosidase A and commercial, coffee-bean α-D-galactosidase on the solution viscosity of carob galactomannan. Carob galactomannan (17 mL, 0.1%) in 0.1M NaOAc (pH 4.5) was incubated with lucerne (●) or coffee-bean (▲) α-D-galactosidase (3.3 nkat) in an Ubbelohde suspended-level viscometer at 30°. Viscosity was measured after various time intervals, and aliquots were removed for determination of released galactose by the *p*-hydroxybenzohydrazide method. The galactose:mannose ratio of the remaining polysaccharide was determined by difference. Numbers represent the galactose content of the remaining polysaccharide.

Effect of α-D-galactosidase and β-D-mannanase on the solution properties of guar and carob galactomannans. — To study the effect of D-galactose content on the solubility and interaction of galactomannans, it is essential to ensure that the α-D-galactosidase employed is absolutely devoid of the endo-depolymerase β-D-mannanase; *i.e.*, that the mannan chain remains unaltered during D-galactose removal.

The presence of traces of β-D-mannanase in α-D-galactosidase preparations can be sensitively detected by viscometry, employing carob galactomannan as substrate. The effect of pure α-D-galactosidase A and β-D-mannanase on the solution viscosity of carob galactomannan is shown in Fig. 2. α-D-Galactosidase (3.3 nkat on this substrate) removed 35% of the D-galactosyl residues from carob galactomannan without causing a decrease in viscosity. However, even 0.0004 nkat of β-D-mannanase caused a significant decrease in viscosity over the same incubation period. On extended incubation (~6 h) of carob galactomannan with pure α-D-galactosidase (3.3 nkat), an insoluble, mannan-type precipitate formed. A similar precipitate formed even if the α-D-galactosidase was contaminated to an extent of 3.6% with β-D-mannanase (*i.e.*, 0.12 nkat under these incubation conditions). Consequently, contrary to the suggestions of other workers[27], the formation of a mannan precipitate on incubation of galactomannan with α-D-galactosidase is not a sensitive index for the purity of this enzyme. In fact, contamination of α-D-galactosidase with β-D-mannanase to an extent of only 0.01% is sufficient to complicate an interpretation

of the effect of D-galactose removal on the solution and interaction properties of the galactomannans. Viscometric studies indicate that the commercially available, coffee-bean α-D-galactosidase (Boehringer Mannheim) contains a β-D-mannanase contamination of ~0.01% (Fig. 3).

Hydrolysis of guar, carob, and *L. leucocephala* galactomannans by α-D-galactosidase A is shown in Fig. 4. After incubation for 2 h, essentially all of the D-galactosyl residues were removed, but with only a slight decrease in viscosity. Viscosity decrease was due to alignment of β-D-mannan-type polymers, which eventually (after 4–5 h) formed particles large enough to be visible. The solution became distinctly turbid and, after a further 1 h, a β-D-mannan precipitate formed and settled from solution. The precipitation of β-D-mannan polymers from solution, as well as being time-dependent, was also dependent on the concentration of the galactomannan in solution (see Fig. 5). Carob galactomannan at three different concentrations was incubated with α-D-galactosidase such that the ratio of enzyme to galactomannan was constant, and thus the degree of hydrolysis at any time was essentially the same for each sample. However, an insoluble precipitate formed in the 0.4%

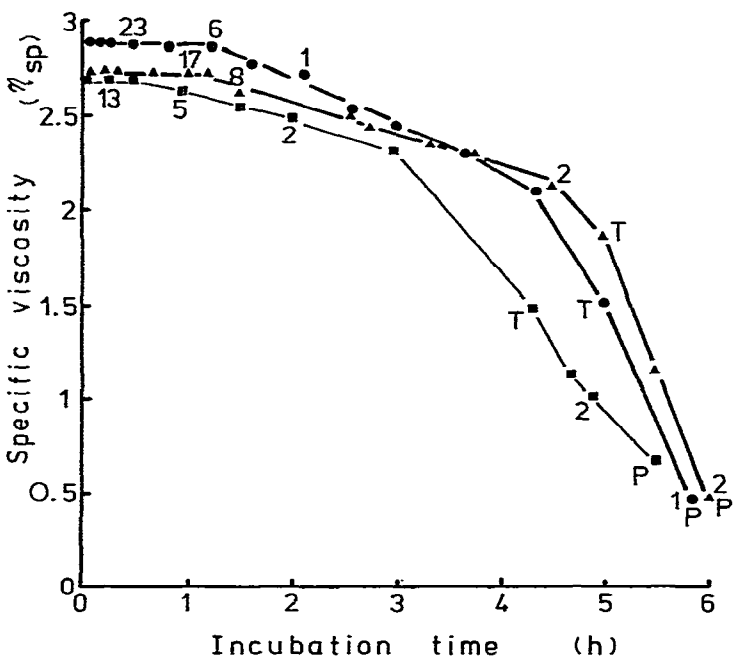

Fig. 4. Effect of galactose removal on the solution viscosity and solubility of galactomannans from guar (●), *Leucaena leucocephala* (▲), and carob (■). Galactomannan solution (17 mL, 0.1%) in 0.1M NaOAc (pH 4.5) was incubated with lucerne α-D-galactosidase A (6.2 nkat) in an Ubbelohde suspended-level viscometer at 40°. Samples were removed for determination of released galactose by the *p*-hydroxybenzohydrazide reducing-sugar method. Numbers represent the galactose content of the remaining polysaccharide. At point T, the solution was very turbid; at point P, a precipitate had formed.

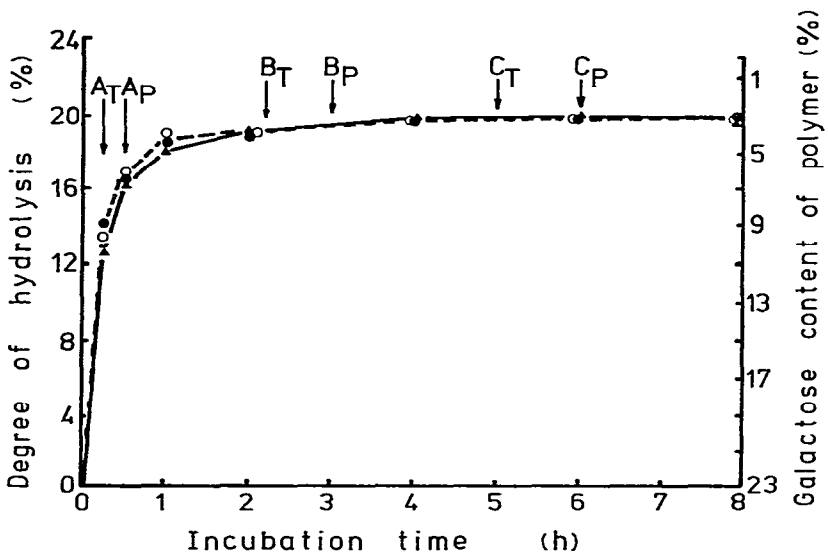

Fig. 5. Effect of substrate concentration on the time required for precipitate formation during hydrolysis of carob galactomannan by lucerne α-D-galactosidase A. Carob galactomannan (GM, 0.1 mL) in 0.1M NaOAc (pH 4.5) was incubated with α-D-galactosidase (10 μL) under the following conditions; A (●) 0.4% GM + 0.35 nkat of α-D-galactosidase; B (○) 0.2% GM + 0.17 nkat of α-D-galactosidase; and C (▲) 0.1% GM + 0.08 nkat of α-D-galactosidase. After various time-intervals, the tubes were examined for turbidity or precipitate formation, the reaction was stopped by heating, and the degree of hydrolysis was determined as described in the Experimental section. A_T is the point at which the solution in tube A became turbid, and A_P is the point at which a precipitate was observed.

carob galactomannan solution, after only 30 min. At a galactomannan concentration of 0.1%, mannan-precipitate formation took up to 6 h.

The effect of concentration of α-D-galactosidase on D-galactose removal, solution viscosity, and precipitate formation is shown in Fig. 6. Independent of the enzyme concentration used, there was no detectable decrease in viscosity until the D-galactose content of the guar galactomannan was reduced from 38% to <11%. In each case, the D-galactose content of the precipitated, mannan-type material was ~1%. After removal of essentially all of the D-galactose from guar galactomannan, there was a much shorter time-lag for precipitate formation when higher levels of enzyme were used. The reason for this is not clear. An identical phenomenon was experienced with carob galactomannan as substrate (Fig. 7). This was intially thought to be due to an interaction between enzyme protein and β-D-mannan polymers, such that more rapid precipitation would occur at higher enzyme-protein concentrations. However, when bovine serum albumin was added to the reaction mixture in a 100-fold excess over possible enzyme-protein concentrations, the rate of precipitate formation was not altered.

The viscosity curves and limiting viscosity numbers of α-D-galactosidase-treated guar galactomannan samples are shown in Fig. 8. Removal of galactose from

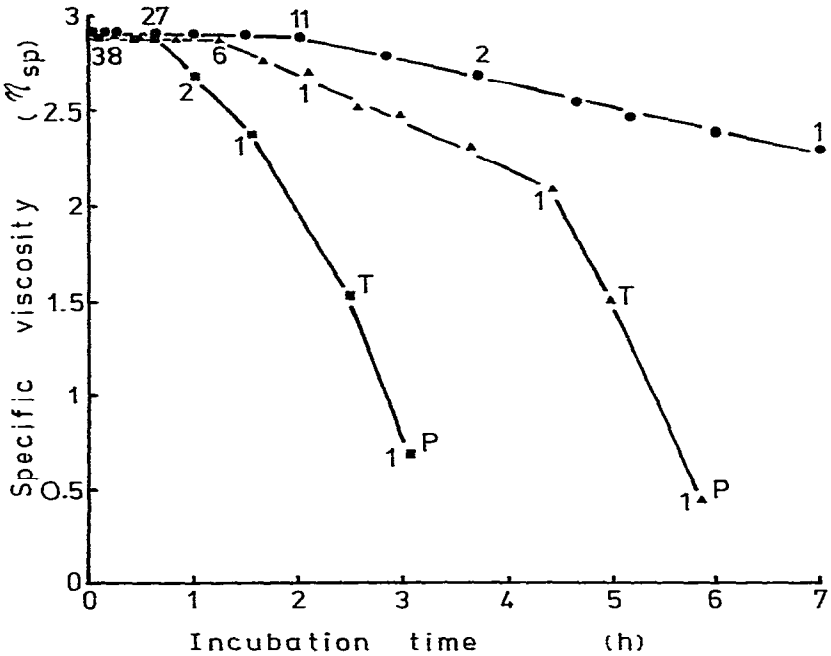

Fig. 6. Effect of lucerne α-D-galactosidase A on the solution viscosity of guar galactomannan. Guar galactomannan (17 mL, 0.1%) in 0.1M NaOAc (pH 4.5) was incubated with 3.1 (●), 6.2 (▲), or 12.4 nkat (■) of lucerne α-D-galactosidase A in an Ubbelohde suspended-level viscometer at 40°. Numbers represent the galactose content of the remaining polysaccharide. At point T, the solution became turbid, and a precipitate had formed at point P.

guar galactomannan results in galactomannans which have increased limiting-viscosity numbers and steeper viscosity curves. However, if the viscosity curves are plotted against the concentration of the "mannan-backbone" in these polymers, a single curve is obtained independent of the galactose content. This finding indicates that the solution viscosity of such galactomannans is totally dependent on the nature of the mannan backbone. The galactose side-chains play a very important role in determining the ease with which galactomannans can be dissolved, but they do not affect the degree of interaction between galactomannan molecules in dilute solution (assuming, of course, that the galactose content is sufficient to maintain solubility), nor do they have any apparent effect on the conformation of galactomannan molecules in solution. Similar results have been obtained for galactomannans in the solid state. Computer, model-building calculations on data obtained from X-ray diffraction patterns of guar and *Gleditsia amorphoides* galactomannans indicate that these molecules exist in an extended, ribbon-like conformation in the solid state, and that the D-galactose side-chains may not necessarily affect the conformation of the main chain[1].

From gel-filtration data for guar and carob galactomannans, it has been suggested that these polymers exist in solution in a highly extended conformation.

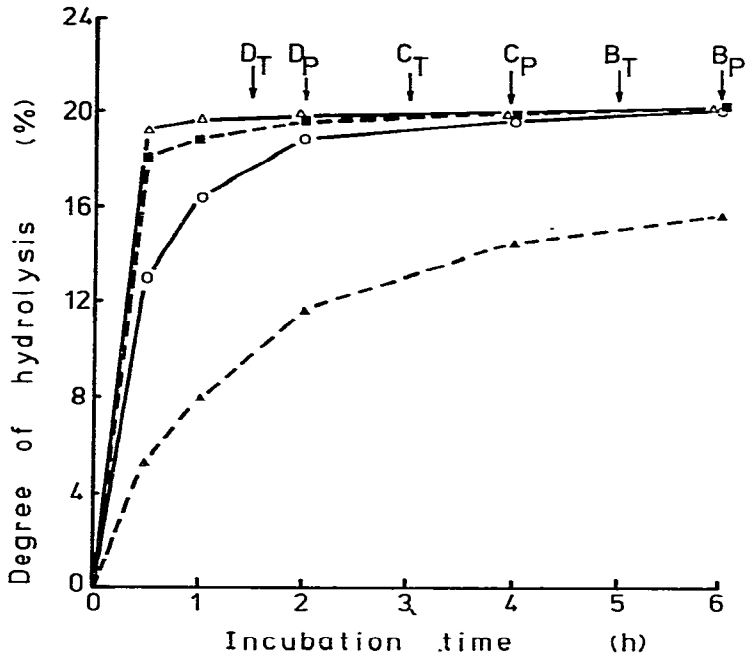

Fig. 7. Hydrolysis of carob galactomannan by lucerne α-D-galactosidase A. Carob galactomannan (2 mL, 0.1%) in 0.1M NaOAc (pH 4.5) was incubated with lucerne α-D-galactosidase A; A (▲) 0.44 nkat; B (○) 1.10 nkat; C (■) 2.20 nkat; or D (△) 4.40 nkat; at 40°. Aliquots (0.1 mL) were removed at various times for determination of reducing sugar.

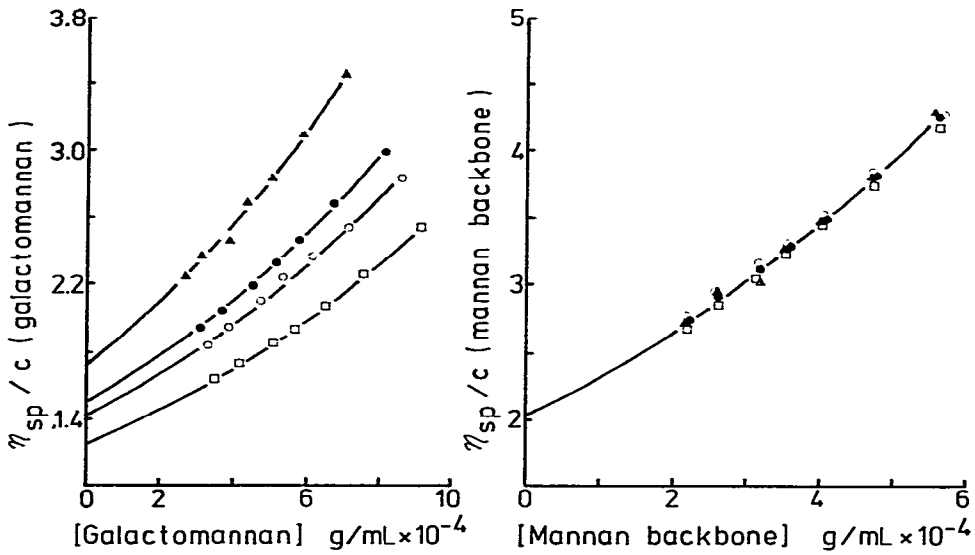

Fig. 8. Effect of galactose removal from guar galactomannan on limiting viscosity number. The galactose:mannose ratios of the polysaccharides are 38:62 (□), 32:68 (○), 27:73 (●), and 15:85 (▲).

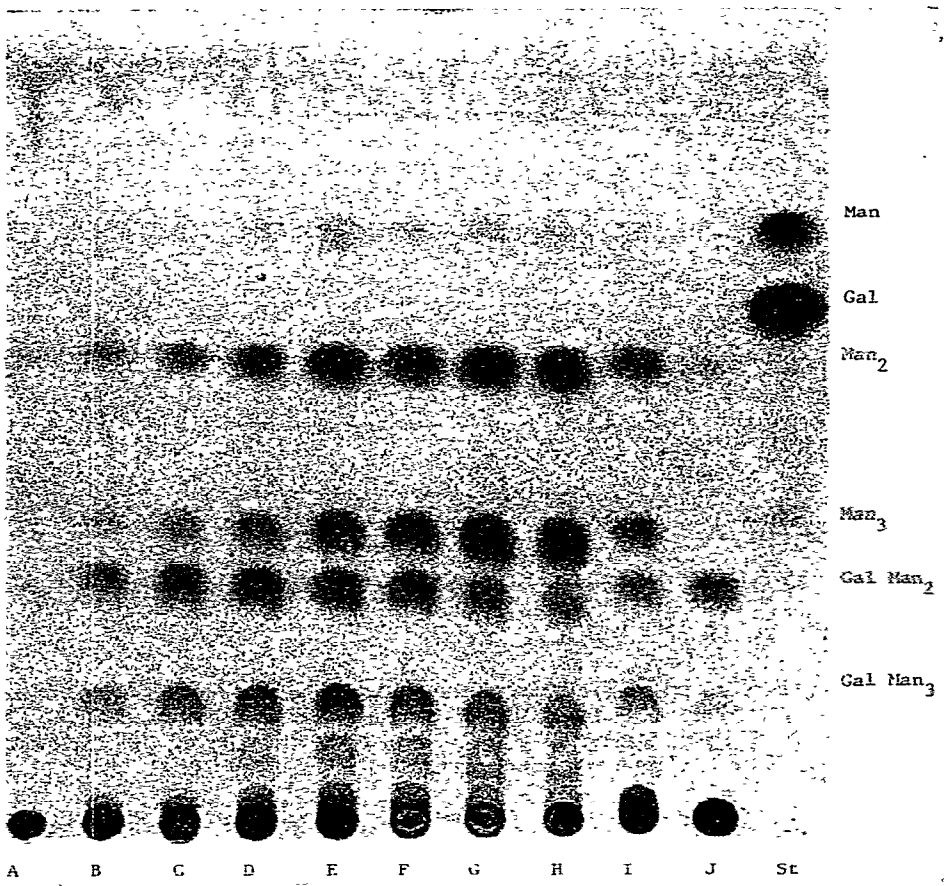

Fig. 9. T.l.c. of oligosaccharides produced on hydrolysis of guar galactomannan (pre-hydrolysed with lucerne α-D-galactosidase A) by Driselase β-D-mannanase. Galactomannan (10 mL, 0.4%) was incubated with Driselase β-D-mannanase (40 nkat on carob galactomannan) for 4 h at 40°, and aliquots were removed for chromatography. A, Native guar galactomannan; B–H, pre-treated with lucerne α-D-galactosidase A, to galactose:mannose ratios of B, 33:67; C, 29:71; D, 25:75; E, 19:81; F, 15:85; G, 13:87; and H, 10:90; I, native carob galactomannan; J, native *L. leucocephala* galactomannan; St, standard sugars.

The currently calculated, Simha shape-factor[28,29] (1365) and axial ratio (a/b = 158) for guar galactomannan in solution support this proposal. A similar conclusion was reached for the conformation of arabinoxylans in solution[25]. However, removal of D-galactosyl branch-units from galactomannans did not cause a steady decrease in viscosity, as was found when arabinosyl groups were removed from arabinoxylans. The removal of arabinosyl groups from arabinoxylans was considered to cause a conformational change which decreased the effective hydrodynamic radius and thus the solution viscosity[25].

The effect of D-gal- ·es· content on solution properties and interaction of galactomannans wa· ·tu·ied using samples of α-D-galactosidase-modified guar

TABLE II

EFFECT OF GALACTOSE CONTENT ON THE SOLUTION PROPERTIES OF α-D-GALACTOSIDASE-TREATED GUAR GALACTOMANNAN[a]

Galactose:mannose ratio	*Storage time (days)*	*Storage temperature*		
		30°	*4°*	*Freezing & thawing*
38:62 to 25:75	1	S	S	S
20:80 to 15:85	1	S	S	G
	15	S	G	G
	60	—	G/P	G
Less than 10:90	1	P	P	P

[a]Galactomannan solutions (0.4%, salt free) were stored under the described conditions and defined as totally soluble (S) if there was no evidence of gel formation (G) or precipitate formation (P).

galactomannan. To obtain information on the distribution of the D-galactosyl residues along the mannan chain, samples were treated with Driselase β-D-mannanase[11], and the resulting oligosaccharides were separated chromatographically (Fig. 9). As the D-galactose content of the treated galactomannan sample decreased, the degree of hydrolysis and the quantities of oligosaccharides having low d.p. increased. The hydrolysates contained significant quantities of mannobiose, mannotriose, and the mixed oligosaccharides $GalMan_2$ (galactose:mannose = 1:2), $GalMan_3$ (galactose:mannose = 1:3), and others. This indicated that the D-galactose is distributed in an irregular to random fashion in these galactomannans.

The solution properties of the α-D-galactosidase-modified galactomannans are summarised in Table II. At concentrations of 0.4% w/v, solutions of galactomannans with D-galactose contents ranging from 25 to 38% showed no tendency towards gel formation or retrogradation on storage at 4°. However, those containing between 15 and 20% of D-galactose formed gels on storage at 4° for 15 days. On storage for a further 45 days, the gels began to retrograde and became quite opaque. Solutions of galactomannans which contain <10% of D-galactose are quite unstable at 30° or 4°, and an insoluble mannan-type precipitate rapidly forms. Gel formation and retrogradation are due to ordered, non-covalent associations between sections of the galactomannans which are essentially unsubstituted with D-galactose. The extent of this interaction is dependent on the D-galactose content of the galactomannans, such that those samples containing 25–38% of D-galactose have essentially no regions which can enter into these associations, whereas those with 15–20% of D-galactose interact to form a three-dimensional gel network. Gel formation is due to an aggregation of the essentially unsubstituted mannan regions in a regular, ribbon-like conformation, with the non-interacting "galactose-rich" regions serving to solubilise the network[1]. Galactomannans with <10% of D-galactose have a large proportion of the mannan chain available for interaction, but have insufficient

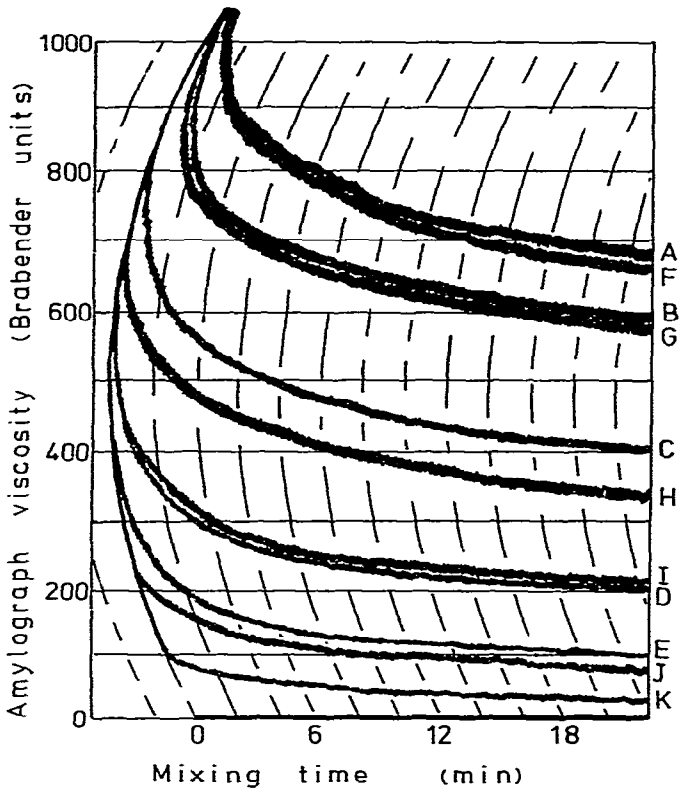

Fig. 10. Gelling interactions of galactomannans with xanthan as determined with a Brabender (Duisburg) Amylograph. Samples A–E are xanthan (0.1% w/v) plus carob galactomannan at concentrations of A, 0.1%; B, 0.07%; C, 0.05%; D, 0.03%; and E, 0.01% w/v. Samples F–J are xanthan (0.1% w/v) plus α-D-galactosidase-treated guar galactomannan (0.1% w/v) with galactose: mannose ratios of F, 19:81; G, 25:75; H, 29:71; I, 34:66; and J, 38:62 (native). Sample K is xanthan (0.1% w/v) plus guar galactomannan (0.05% w/v).

"galactose-rich" regions to solubilise the network; thus, an insoluble precipitate forms.

Interchain associations are induced by a freeze–thaw treatment of galactomannan solutions. The formation of ice crystals progressively raises the effective concentration of polymer in the residual, unfrozen solution and thus promotes association[30]. Thus, although solutions of treated guar galactomannan of D-galactose content of 15–20% are quite stable on storage at 4° for 24 h, a freeze–thaw cycle results in gel-particle formation. Carob galactomannan (23% of galactose) behaves in a very similar way[8].

Galactomannans interact to various degrees with such polysaccharides as agar, carrageenans, and xanthan. Carob galactomannan interacts to a much greater extent than does guar galactomannan. It has been proposed that this is due to the quite different distribution patterns of D-galactose in these two polymers, *i.e.*, a "block-type" distribution of D-galactosyl residues has been proposed for carob

galactomannan[4,5], whereas, in guar galactomannan, a uniform distribution of D-galactose was favoured[6]. However, a re-investigation of the fine structures of carob, guar, and other galactomannans by enzymic techniques[11] indicates that most galactomannans, including guar and carob galactomannans, have an irregular to random distribution of D-galactose. The effect of D-galactose removal from guar galactomannan on its interaction with xanthan is shown in Fig. 10. As the D-galactose content decreases, the degree of interaction increases. This supports the proposal[8] that the sites of interaction between galactomannan and xanthan are the less-substituted regions. Treated guar galactomannan having a D-galactose content of 19% interacts with xanthan to essentially the same degree as carob galactomannan (23% of D-galactose). The patterns of amounts of oligosaccharides produced on hydrolysis of these two galactomannans by β-D-mannanase is similar and, moreover, suggests an irregular to random distribution of galactose (Fig. 9). Lucerne α-D-galactosidase A has been shown to remove galactose from galactomannans in an apparently random fashion. Consequently, it would appear that the profound difference in the interaction of native guar and carob galactomannans with xanthan can be explained simply in terms of their different galactose : mannose ratios. A major difference in the "fine-structures" of these two galactomannans, as proposed by other workers[4-6], is not necessary to explain this phenomenon.

Some information about the nature of the junction-zones between galactomannan and xanthan has been obtained from studies of the effect of xanthan on the susceptibility of carob galactomannan to β-D-mannanase hydrolysis. Results of such studies are shown in Fig. 11. The rates and degree of hydrolysis of carob galactomannan in the presence or absence of xanthan were essentially the same if the solutions were simply mixed at 25° before equilibration to the hydrolysis temperature

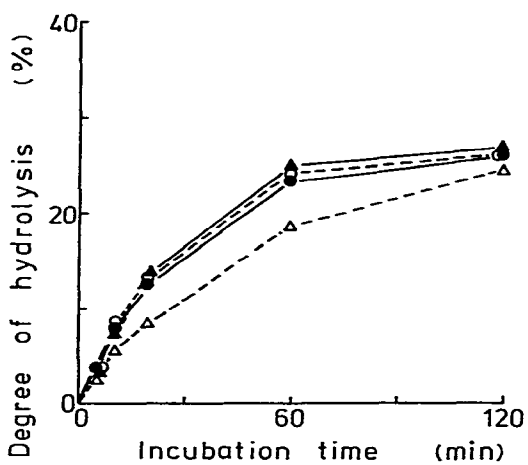

Fig. 11. Effect of xanthan on the hydrolysis of carob galactomannan by β-D-mannanase. Samples of carob galactomannan (0.2 mL, 0.1%) were mixed with xanthan (0.2 mL, 0.1%) or water at 25 or 80° (followed by chilling) before equilibration to 15° and addition of β-D-mannanase. Mixed at 25°: plus water (●); plus xanthan (○). Mixed at 80°: plus water (▲); plus xanthan (△).

(15°). However, if the carob galactomannan and xanthan were mixed at 80°, and chilled to 2° before equilibration to the hydrolysis temperature (15°), the presence of xanthan retarded the rate of hydrolysis of carob galactomannan. It is suggested that heating to 80° and chilling allows a greater degree of interaction between the two polymers which hinders β-D-mannanase action. However, although the hydrolysis studies were performed at a temperature favourable for interaction of the two polysaccharides, the presence of xanthan does not significantly affect the overall degree of hydrolysis. This suggests either that only a very small proportion of the available unsubstituted sections of carob galactomannan are involved in interaction with xanthan at the "junction-zones" or, alternatively, that the interaction is dynamic, such that the "galactose-poor" regions of carob galactomannan are susceptible to β-D-mannanase attack once the polysaccharide molecules separate.

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