ELSEVIER

0144-8617(95)00091-7

Carbohydrate Polymers, Vol. 29, No. 1, pp 1–5, 1996

0144-8617/96 $15.00 + .00

Adsorption of galactomannans onto agarose

Christer Viebke* & Lennart Piculell

Physical Chemistry 1, Lund University Chemical Center, Box 124 S-221 00 Lund, Sweden

(Received 6 December 1994; revised version received 26 April 1995; accepted 5 May 1995)

New direct evidence for a mixed association between aggregated agarose and aqueous galactomannan is presented. Gel permeation chromatography of two galactomannans, locust bean gum (lbg) and guar gum (gg), on a column packed with unmodified and highly aggregated agarose showed that the eluted amount of lbg was greatly reduced compared to the injected amount, whereas the loss of gg was smaller. The loss of material was significantly smaller, especially for lbg, with sodium iodide (rather than sodium nitrate) in the eluent salt solution. No loss of material for either galactomannan under any salt condition was found on a column packed with a hydroxylated polymethacrylate gel. The results strongly suggest that galactomannans adsorb onto aggregated agarose, and that the adsorption depends on the chemical nature of both the adsorbent and the substrate. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

It is well known that solution properties of certain biopolymer systems may be modified by adding a second (different) polymer to the solution (Dea *et al.*, 1979; Dea & Morrison, 1975; Morris, 1990). A large enhancement in viscosity or even a gelation, below the concentration which this takes place for either of the pure polymer systems, may be found. The non-additive (synergistic) effect may be ascribed to either an effective attraction or a repulsion between unlike polymers, resulting in mixed aggregate formation or phase separation. Usually, a phase separation in aqueous polymer mixtures is segregative, but a combination of aggregation and phase separation is also possible, where the polymer aggregation is followed by a phase separation (Morris, 1990; Piculell *et al.*, 1994*a*). In gelling mixtures, an infinite three-dimensional network has to be formed, including junction zones and branching points. Different types of gel structure are possible in mixed systems. Cairns *et al.* (1987) distinguish between separate polymer networks, which interlace and form an interpenetrating network, a phase separated network and a network where one of the polysaccharides binds to the other and forms a coupled network. In these three structures both polymers take part in the network(s). A fourth structure involves a network of one of the polymers, in which the other polymer is dissolved.

One type of mixture where synergistic effects are well-known to occur is that involving certain galactomannans and certain helix-forming galactans from the agar/carrageenan family. Galactomannans consist of linear chains of β-(1→4)-linked D-mannopyranosyl residues, to which side chains of α-(1→6)-linked galactopyranosyl are attached (Fig. 1a). They are normally non-gelling polysaccharides used as food additives. Various types of galactomannans differ in the degree and pattern of side chain substitution (Morris, 1990). The agars and the helix-forming carrageenans are similar in backbone structure (Rees *et al.*, 1982), but differ in the degree of sulfation per repeating disaccharide unit in the order agarose (O) < furcellaran (0.6) < kappa-carrageenan (1) < iota-carrageenan (2). The structure of agarose (alternating units of (1→4)-3,6-anhydro-α-L-galactose and (1→3)-β-D-galactose) is shown in Fig. 1b. It is widely accepted that the gelling of agarose and carrageenan involves a coil-to-helix transition of the galactan molecule.

The extent of synergism observed in the galactan/galactomannan mixtures varies from none, in certain cases, to very large in others. Generally, conditions enhancing the tendency of galactan to self-aggregate (lower sulfate content, presence of certain salts) also enhances the synergistic effects (Morris, 1990). As regards the galactomannans, the synergistic effects depend on both the degree and pattern of side chain substitution. A large, synergistic effect is seen for locust bean gum (lbg), which typically has a mannose:galactose ratio of 3.5, whereas little or no effect is seen for guar gum (gg), where the mannose:galactose ratio is 1.55 (Morris, 1990). Since the molecular

*Author to whom correspondence should be addressed.

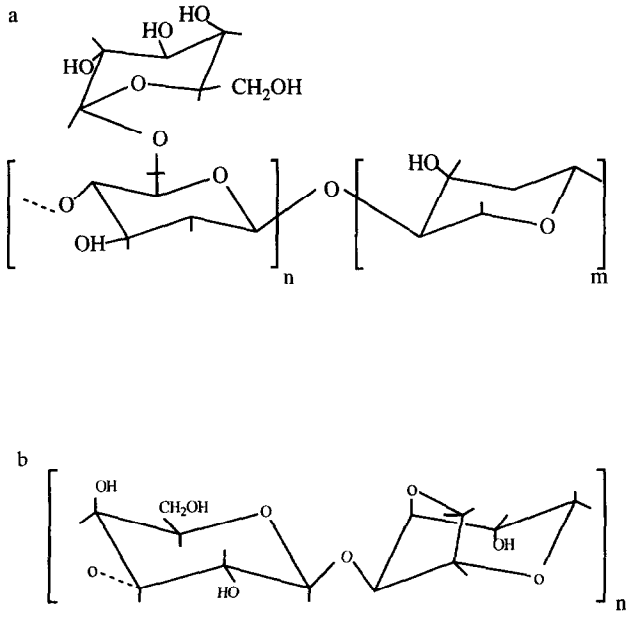

Fig. 1. Repeating units of (a) galactomannan: and (b) agarose. For galactomannans, the lengths of the *n* and *m* blocks vary within and between samples.

structures and all features of the non-additivity (when observed) are quite similar for all mixtures of galactan/galactomannan gels, there seems to be general agreement that the mechanism behind the synergism is always the same in these mixtures (Morris, 1990, 1992). The close analogy also holds for mixtures with konjac-glucomannan, rather than galactomannan. This makes it possible to compare, for example, experiments on agarose/galactomannan with those on kappa-carrageenan/galactomannan, as long as consideration is given to the specific ion effects for kappa-carrageenan.

The first mechanism of synergistic gelation that was suggested for galactomannan/galactan mixtures was a binding of the bare mannan backbone of the galactomannan to the double-helix of the galactan (Rees, 1972*a*, *b*; Dea *et al.*, 1972). This model was based on experiments of agarose and kappa-carrageenan mixed with various galactomannans. It was shown that, by adding galactomannan, gelling could be induced below the normal gelling concentration for the galactans. Furthermore, it was seen that low molecular weight galactans that normally did not form a gel, gelled or precipitated when galactomannan was added to the solution. The synergistic interaction was highest for the least substituted galactomannan polymers, which was taken as evidence that the mannan backbone was involved in gelation. The intermolecular binding between the mannan backbone and the galactan double-helix was later challenged by Cairns *et al.* (1987). They studied the X-ray diffraction patterns for kappa-carrageenan and furcellaran, alone and in mixtures with galactomannans, and found no differences between the pure and the mixed systems. On the other hand, the authors were equally unable to find evidence of a segregative phase separation, since sulfur and potassium mapping showed the mixed gels to be homogeneous at a resolution of 1 μm. In the absence of evidence of either a coupled or a phase separated network, the authors favoured a gel structure where the galactomannan chains were simply contained in the galactan network. Since then, however, spectroscopic evidence has been presented, showing that both galactomannan (Rochas *et al.*, 1990) and glucomannan (Williams *et al.*, 1993) chains are involved in some association in the corresponding mixed gels. Very recent evidence has also shown that association of the galactan helices is affected in the mixed gels (Piculell *et al.*, 1994*a*, *b*).

To reconcile new and old observations, one of us has recently suggested (Williams *et al.*, 1993; Piculell *et al.*, 1994*a*) that the mixed gels indeed contain mixed galactan/mannan aggregates, but that these mixed aggregates consist of disordered galactomannan chains, adsorbed onto bundles of self-aggregated galactan helices, rather than some more ordered molecular complex of a well-defined stoichiometry. Although this model seems to satisfactorily account for available data, there is still a lack of direct evidence for a surface adsorption of galactomannans onto mannan aggregates. Dea *et al.* (1972) found that the liquid released from a mixed agarose/galactomannan gel after freeze–thaw treatment was significantly depleted in galactomannan, and argued that this was due to binding of galactomannan to the agarose network. However, this result could also be explained by an interpenetrating network structure, since galactomannans are known to self-associate on freeze–thawing. In this paper, we make a simple and direct study of the galactomannan/agarose interaction, by gel permeation chromatography (GPC) of lbg and gg both on an agarose column and on a column with a different packing material.

MATERIALS AND METHODS

Materials

Locust bean gum and guar gum (gifts from Sanofi Bio-Industries) were used without further purification. The galactomannans were dissolved in the appropriate solvent (0.1 M sodium nitrate or 0.05 M sodium iodide, used as eluents) by stirring overnight at room temperature, followed by heating in a water bath at 80°C for approximately 1 h. Clear viscous solutions were obtained for both lbg and gg.

Methods

The GPC system consisted of a Pharmacia pump P-500 and an autoinjector ACT100, all controlled by liquid

chromatography controller LCC-500. The agarose GPC column was packed with Sepharose 4B (Pharmacia, Uppsala, Sweden). This material consisted of unmodified agarose gel with a separation range of 3×10^4–5×10^6, according to the supplier. Reference measurements were made on a set consisting of two ultrahydrogel columns ('linear' and '250') supplied by Waters, a division of Millipore. The ultrahydrogel consisted of a hydroxylated polymethacrylate with residual carboxyl groups. A LKB 2142 differential refractometer, working at 950 nm, was used both for sample detection and for measurements of the refractive index increment. Concentrations used in the experiments were 0.4% w/w for the agarose column and 0.2% w/w for the ultrahydrogel columns. All measurements were made at ambient temperature. The injected volume on the columns was 50 μl.

RESULTS AND DISCUSSION

Figure 2 shows chromatograms for lbg and gg obtained on the agarose column. The chromatograms have the shape expected for a GPC experiment. This indicates that the agarose column is homogeneously packed and that separation occurs according to molecular size. The results in Fig. 2a, with aqueous sodium nitrate as eluent, clearly indicate that the amount of material that passes the column is much less for lbg than for gg. This conclusion is confirmed by Fig. 3, which shows that the refractometer response (i.e. the refractive index increment) is very similar for the two polymers. There was no sign of lbg being eluted at higher elution times, even longer than the dead volume of the column. This does not necessarily mean that the adsorption is irreversible, but if the adsorbed lbg finally passes through the column, it is too dilute to be detected by the refractometer. No sign of any large aggregates, which might have indicated self-association, was seen at the beginning of the elution peak.

Figure 2b shows the same experiment as Fig. 2a, but with aqueous sodium iodide as the eluent. Again, the amount of material that passes the column is smaller for lbg than for gg, but both peaks are significantly larger than in Fig. 2a. The increase is, however, most important for lbg. This indicates that iodide to some extent inhibits the adsorption of lbg. It has previously been observed that iodide removes synergistic effects in kappa-carrageenan/glucomannan mixtures (Piculell *et al.*, 1994*a*), but the reason for this is unclear, since iodide also prevents self-aggregation of the kappa-carrageenan double-helices, and thereby gel formation (Grasdalen & Smidsrød, 1981). Thus, it might be argued that the inhibition by iodide of the kappa-carrageenan/mannan association is an indirect effect, due to the decrease in aggregate size. (See the correlation of synergistic effects with

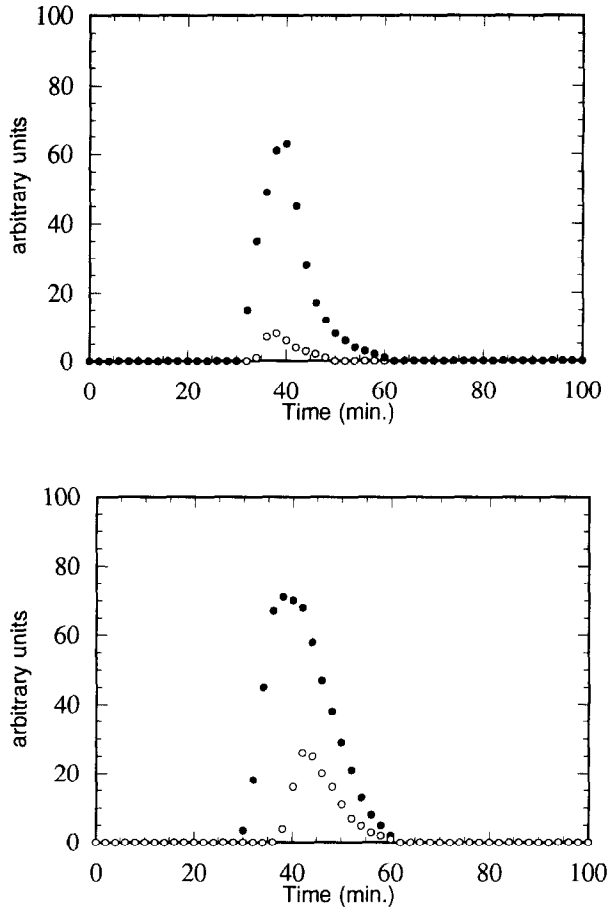

Fig. 2. Chromatograms on the Sepharose 4B column for locust bean gum (open circles) and guar gum (filled circles) in (a: top) 100 mM $NaNO_3$; and (b: bottom) 50 mM NaI.

the extent of galactan self-aggregation referred to in the Introduction.) The present results indicate, however, that it is the interaction of iodide with one or both of the polysaccharides that affects the galactan/galactomannan association, rather than the size of the galactan aggregates. Although it is known that high concentrations of iodide ions destabilise agarose gels (Piculell & Nilsson, 1989), the effect on the aggregate size in the column packing material should be marginal in the present concentration range (0.05 M).

It might be argued that the loss of material seen in Fig. 2 could be due to some self-association (gelation) of the poorly soluble lbg in the column, induced by the GPC procedure. To test this possibility, we also ran GPC experiments on lbg and gg on an ultrahydrogel column set, with the chemically different hydroxylated polymethacrylate as packing material. We have previously used this set extensively to study carrageenans (Viebke *et al.*, 1995), and it has showed good stability under different electrolyte and temperature conditions. On this column (Fig. 4), no difference between lbg and gg was observed, regardless of the eluent salt solution (sodium nitrate or iodide),

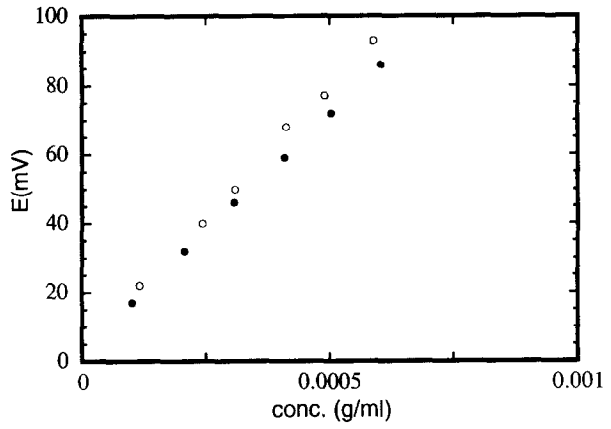

Fig. 3. Refractometer response for locust bean gum (open circles) and guar gum (filled circles).

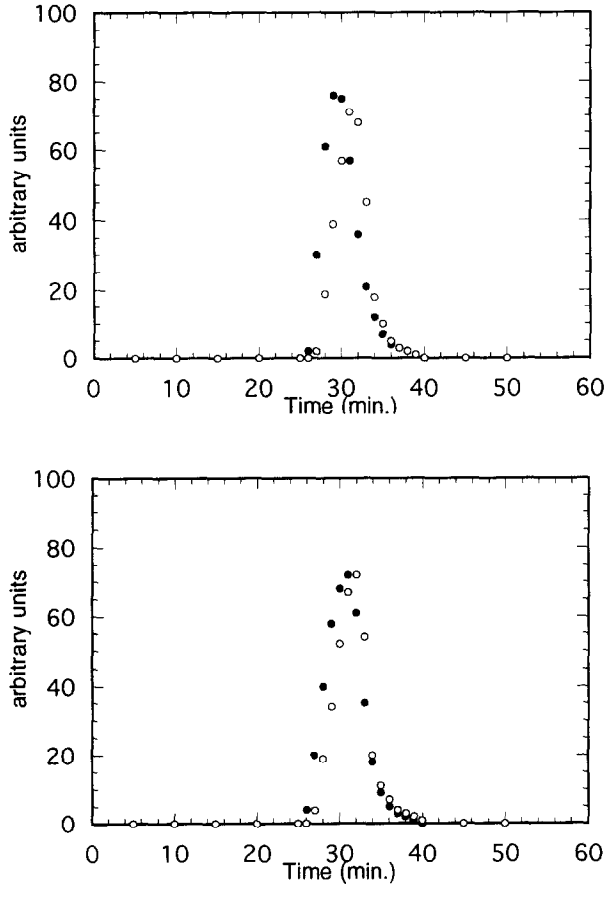

Fig. 4. Chromatograms on the ultrahydrogel column for locust bean gum (open circles) and guar gum (filled circles) in (a: top) 100 mM $NaNO_3$; and (b: bottom) 50 mM NaI.

and there were no signs of significant adsorption of either of the polysaccharides.

CONCLUSIONS

The results of the above simple GPC experiment, comparing different chromatography gels and different galactomannans, clearly indicate a mixed association at room temperature between the agarose aggregates and the galactomannan with a low degree of galactose side chains. The association is specific in the sense that it depends on the chemical nature of both the adsorbent (lbg or gg) and the substrate (agarose or hydroxylated polymethacrylate). The fact that some of the lbg is eluted, while some of the gg is retained on the agarose column, probably reflects the structural heterogeneity of the galactomannans. A test of the difference in galactomannan structure before and after chromatography would be interesting, but is beyond the scope of this study.

After this study was initiated, we learned about a similarly simple study by Parker *et al.* (1995), who could demonstrate binding of a range of galactomannans, except guar gum, to solvent-swollen kappa-carrageenan particles equilibrated at room temperature with solutions of the galactomannans. Together, our study and that conducted by Parker *et al.* provide rather conclusive evidence of a heterotypic association for those galactomannan/galactan couples that also show synergistic gelation. The correlation between association and synergism is further strengthened by the finding of Parker *et al.* that lbg did not bind to swollen particles of iota-carrageenan. These results clearly support the recent proposal that the synergism in mixed gels is due to the adsorption of disordered galactomannan chains onto bundles of self-aggregated galactan helices (Williams *et al.*, 1993; Piculell *et al.*, 1994*a*). Still, our experiments provide little information on the nature and driving force of the association. This is still poorly understood. The synergism decreases in the series agarose, furcellaran, kappa-carrageenan, and no association is seen for iota-carrageenan, which does not self-aggregate. The fact that self-aggregation of the galactans promotes the synergistic effect might suggest that the size of the galactan aggregates has an important role in the interaction. On the other hand, it may well be that the heterotypic association and the self-association in these cases are controlled by the same effects. Possible mechanisms that may provide both homotypic and heterotypic repulsion are the entropy of the dissociated counterions (Piculell *et al.*, 1994*a*), and the competitive binding of low molecular additives, such as iodide, to the polysaccharides.

Another important factor for the association is the structure of the galactomannans. Previous studies, summarised in the review by Morris (1990), have indicated that the occurrence of long stretches of unsubstituted mannan, or, indeed, regions that in the preferred two-fold conformation of the mannan chain are substituted only on one side of the backbone, are important for the association with galactans. These findings are entirely consistent with the notion of an adsorption of galactomannans onto galactan aggregates. As long as the side chains themselves do not

have a particular tendency to stick to the surface — which is unexpected in the present case — they should prevent adsorption by the so-called steric effect: side chains trapped between the polymer backbone and the surface lose configurational entropy on adsorption.

ACKNOWLEDGEMENTS

We are grateful to Alan Parker for sharing with us his results on the adsorption of galactomannans to kappa-carrageenan, prior to publication. This work was supported by grants from the Swedish Natural Science Council and from the Swedish Board for Industrial and Technical Development.

REFERENCES

Cairns, P., Miles, M.J., Morris, V.J. & Brownsey, G.J. (1987). X-ray fibre diffraction studies of synergistic, binary polysaccharide gels. *Carbohydr. Res.*, **160,** 411–423.

Dea, I.C.M., Blanshard, J.M.V. & Mitchell, J.R. (1979). In *Polysaccharides in Food.* Butterworths, London, pp. 229–247.

Dea, I.C.M., MacKinnon, A.A. & Rees, D.A. (1972). Tertiary and quaternary structure in aqueous polysaccharide systems which model cell wall cohesion: reversible changes in conformation and association of agarose, carrageenan and galactomannan. *J. Mol. Biol.*, **68,** 153–172.

Dea, I.C.M. & Morrison, A. (1975). Chemistry and interactions of seed galactomannans. *Adv. Carbohydr. Chem. Biochem.*, **31,** 241–312.

Grasdalen, H. & Smidsrød, O. (1981). Iodide-specific formation of kappa-carrageenan single helices. *Macromolecules*, **14,** 1842–1845.

Morris, E.R. (1990). Mixed polymer gels. In *Food Gels*, ed. P. Harris. Elsevier Applied Science, London, pp. 291–359.

Morris, V.J. (1992). Designing polysaccharides for synergistic interactions. In *Gums and Stabilisers for the Food Industry* **6**, eds G.O. Phillips, P. Williams & D.J. Wedlock, IRL Press, Oxford, pp. 161–171.

Parker, A., Lelimousin, D., Miniou, C. and Boulenguer, P. (1995). Binding of galactomannans to kappa-carrageenan after cold mixing. *Carbohydr. Res.*, submitted.

Piculell, L. & Nilsson, S. (1989). Anion-specific salt effects in aqueous agarose systems 1. Effects on the coil–helix transition and gelation of agarose. *J. Phys. Chem.*, **93,** 5596–5601.

Piculell, L., Iliopoulos, I., Linse, P., Nilsson, S., Turquois, T., Viebke, C. & Zhang, W. (1994*a*). Association and segregation in ternary polymer solutions and gels. In *Gums and Stabilisers for the Food Industry* 7, eds G.O. Phillips, P. Williams & D.J. Wedlock, IRL Press, Oxford, pp. 309–322.

Piculell, L., Zhang, W., Turquois, T., Rochas, C., Taravel, F.-R. & Williams, P.A. (1994*b*). Effects of added galacto- and gluco-mannans on the NMR spectra of ^{133}Cs ions in kappa-carrageenan gels. *Carbohydr. Res.*, **265**, 251–290.

Rees, D.A. (1972*a*). Shapely polysaccharides. *Biochem. J.*, **126,** 257–273.

Rees, D.A. (1972*b*). Polysaccharide gels. A molecular view. *Chem. Ind.*, 630–636.

Rees, D.A., Morris, E.R., Thom, D. & Madden, J.K. (1982). Shapes and interactions of carbohydrate chains. In *The Polysaccharides* **1**, ed. G.O. Aspinall. Academic Press, New York, pp. 195–290.

Rochas, C., Taravel, F-R. & Turquois, T. (1990). N.M.R. studies of synergistic kappa carrageenan–carob galactomannan gels. *Int. J. Biol Macromol.*, **12,** 353–358.

Viebke, C., Borgstrom, J. & Piculell, L. (1995). Characterisation of kappa- and iota-carrageenan coils and helices by MALLS/GPC. *Carbohydr. Polym.*, **27**, 145–154.

Williams, P.A., Clegg, S.M., Langdon, M.J., Nishinari, K. & Piculell, L. (1993). Investigation of the gelation mechanism in κ-carrageenan/konjac mannan mixtures using differential scanning calorimetry and electron spin resonance spectroscopy. *Macromolecules*, **26,** 5441–5446.