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Binding of galactomannans to kappa-carrageenan after cold mixing

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Abstract

The room temperature interaction between swollen carrageenan particles and solutions of carob, cassia, guar, and tara galactomannans has been studied. Analysis using gel permeation chromatography of the supernatant solution above the carrageenan particles demonstrated binding of all the galactomannans, except guar, to kappa-carrageenan. It was also demonstrated that carob galactomannan did not bind to iota-carrageenan. Binding after cold mixing saturates at high kappa-carrageenan/carob ratios, of the order of 80, unlike the interaction in mixtures after heating, which saturates at ratios close to 1. The existence of binding shows that the interaction between these two species is attractive, and thus suggests that the synergistic rheological interaction which occurs after heating is due to the formation of mixed junction zones.

Keywords: Carob galactomannan; Galactomannan; Kappa-carrageenan; Carrageenan; Interaction

1. Introduction

The mechanism of the interaction between carob galactomannan and kappa-carrageenan has been a subject of debate since its discovery [1]. Essentially three different structures have been proposed for mixed kappa-carrageenan/carob gels. Originally, Dea et al. [1] proposed that the unsubstituted mannan regions of the carob bind directly to the kappa-carrageenan in helical form, forming a coupled network with mixed junction zones, by implication crystalline. However, X-ray diffraction measurements of films of mixed gels by Cairns et al. [2] failed to show any diffraction spots which could not be

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attributed to either kappa-carrageenan or carob. These authors suggested that this is strong evidence that no direct interaction occurs. Instead, they proposed a second model in which the kappa-carrageenan gels alone and is surrounded by dissolved carob chains. Despite preferring this second model, Cairns et al. [2] pointed out that their results do not eliminate the possibility of mixed junction zones, only the possibility that they are co-crystallised. Fernandes et al. [3] have recently supported a third model, also discussed by Cairns et al. [2], in which the kappa-carrageenan and carob gel separate to form a phase-separated network. Cairns et al. [2] rejected this model because they could find no evidence of phase separation at the micrometre scale. Despite this past controversy, several very recent publications [4–6] conclude that the correct model for the interaction is the adsorption of disordered galactomannan chains to aggregated kappa-carrageenan helices.

In this work, contrary to all previous studies, the behaviour of unheated mixtures of carrageenan with galactomannans has been investigated, in the presence of sufficient electrolyte to ensure that the former is in the form of swollen particles. This method allows straightforward determination of the composition of the molecules in solution.

2. Experimental

Materials.—Carob galactomannan was obtained from the seeds of the carob tree, *Ceratonia siliqua*, cultivated in Morocco. Guar galactomannan was obtained from *Cyamopsis tetragonolobus*. Tara galactomannan was obtained from *Caesalpinia spinosa* and that of cassia from several *Cassia* species. Kappa-carrageenan and iota-carrageenan were obtained from the red seaweeds *Kappaphycus alvarezii* and *Eucheuma denticulatum*, respectively, both cultivated in the Phillipines.

The pure galactomannans were prepared by first dissolving the ground seeds in deionised water at 90°C and filtering while still hot. The clear solutions were precipitated in an excess of 2-propanol, and the products dried and ground. Carob galactomannan was an experimental sample prepared at S.B.I.'s pilot plant at Baupte, France. The molecular weights of the carob, tara, and guar galactomannans were of the order of 1 million, whilst that of the cassia was about 2 million, estimated by column calibration using poly(ethylene oxide) standards. The mannose/galactose ratio was determined by anion-exchange chromatography at high pH with amperometric detection [7] after acid hydrolysis. The ratios for cassia, carob, tara, and guar galactomannans were 5.0, 3.9, 3.2, and 1.5, respectively. Kappa- and iota-carrageenan were commercial samples manufactured by S.B.I. Both have been shown (samples $\kappa 1$ and $\iota 3$ in ref. [8] to be at least 98% pure by ^{13}C NMR. They were not ion-exchanged before use. As determined previously [8], 70 mol% of the counter-ions were potassium and 30 mol% were sodium. The ground samples were fractionated by sieving and only the fractions between sieve meshes 70 and 120 were used. The mannanase used was "Gamanase L" obtained from Novo Industri AS, Copenhagen, Denmark.

Methods.—Solutions of carob, cassia, and tara were prepared by heating in a water bath at 90°C for 30 min. Solutions of guar were prepared by stirring at 40°C. Galactomannan or aqueous solutions contained 20 g L^{-1} KCl when mixed with

kappa-carrageenan and 100 g L^{-1} when mixed with iota-carrageenan. These electrolyte concentrations ensured equal swelling of kappa- and iota-carrageenan particles. Samples were prepared by dispersing ground carrageenan (0.5 g) in galactomannan or aqueous solution (100 mL). After inverting several times to disperse the particles, the samples were left to stand for ca. 24 h at 20 to 22°C, before removing a small volume of the supernatant solution for gel permeation chromatography (GPC) analysis.

Gel permeation chromatography was carried out using a high-pressure GPC system fitted with two Varian TSK PW columns (one 6000 and one 5000) and refractometric detection. The columns were thermostated at 60°C. The eluent was 0.1 M $LiNO_3$. Samples (400 μL) were filtered through a 0.22-μm pore-size filter immediately before injection, to avoid the injection of aggregates. Calibration curves for galactomannan determination were established at concentrations between 10 and 250 mg L^{-1}.

Since the conclusions drawn here concern the binding of galactomannans, we must be certain that no loss of material occurs during GPC. We have two reasons to believe that this is the case. Firstly, the concentrations calculated from the peak area and the refractive index increment were equal to the actual solution concentrations, to within experimental error. Secondly, the galactomannan calibration curves were linear and passed through the origin.

3. Results and discussion

Figure 1 shows the GPC trace of the supernatant solution of the kappa-carrageenan alone compared with a typical trace of a supernatant solution containing carob before and after treatment with mannanase. A small amount of carrageenan was present in solution, representing about 5% of the total mass. The galactomannans had a much higher molecular weight than the carrageenan in the supernatant solution, so that they

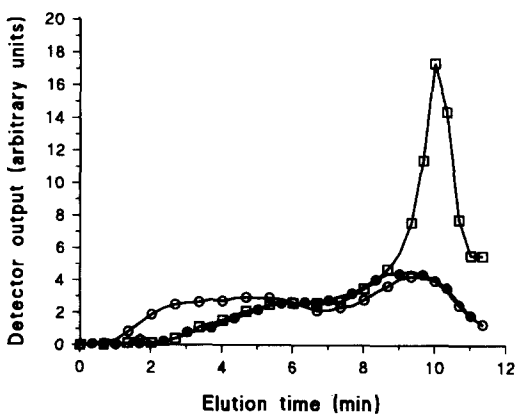

Fig. 1. Gel permeation chromatography traces from refractive index detector, arbitrary units. Supernatant solution above kappa-carrageenan alone (●), above kappa-carrageenan in the presence of carob (○), above kappa-carrageenan in the presence of carob after treatment with mannanase (□).

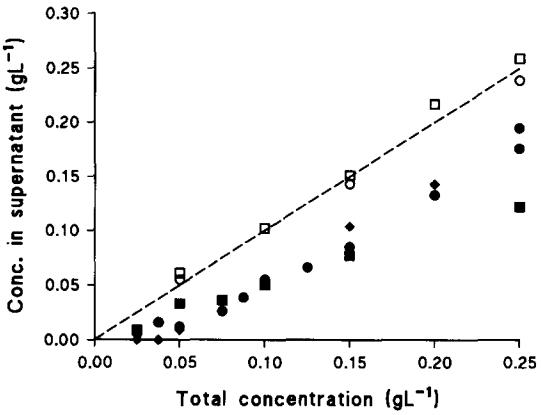

Fig. 2. Concentration of galactomannans found in supernatant solutions as a function of the total concentration in the presence of swollen carrageenan particles. The dashed line shows the expected concentration of galactomannan in the supernatant solution, if no binding occurs. Guar (□), carob (●), tara (♦), and cassia (■) galactomannans with kappa-carrageenan. Carob + iota-carrageenan (○).

produced a shoulder on the carrageenan peak at short elution times. This shoulder disappeared after treatment with mannanase and the original peak due to kappa-carrageenan reappeared, verifying the identities of the peaks. The additional peak at long elution times for the enzyme-treated sample is due to the mannanase and degraded fragments of galactomannan.

The concentration of galactomannan in the supernatant solutions was calculated as the integral of the peak obtained after subtracting that obtained from the supernatant solution of kappa-carrageenan alone. Figure 2 shows the concentration of galactomannan found in the supernatant solution as a function of the total concentration present in the sample. The dashed line indicates the result expected if all of the galactomannan added were present in the supernatant solution. The results for guar plus kappa-carrageenan and carob plus iota-carrageenan follow this line, showing that the galactomannan solution concentrations were unaffected by the presence of carrageenan in these two cases. This result is not unexpected, since the interaction between guar and kappa-carrageenan after heating is, at best, weak [5] and that between carob and iota-carrageenan non-existent [9]. However, the solution concentrations of the other three galactomannans were reduced by the presence of kappa-carrageenan. These data provide the first direct evidence for the binding of galactomannans to kappa-carrageenan. Despite the fact that the strengths of the synergistic rheological interaction of these galactomannans with kappa-carrageenan after heating differ [9], they do not show significantly different binding behaviour after cold mixing.

Figure 3 shows the data for carob galactomannan from Fig. 2 replotted as an adsorption isotherm, that is showing the amount of carob bound as a function of the equilibrium solution concentration, as opposed to the total amount added, which has no thermodynamic significance. The rounded shape of the isotherm is not necessarily indicative of weak binding; it is typical of the shape found for the strong adsorption of a polydisperse polymer to a solid surface [10].

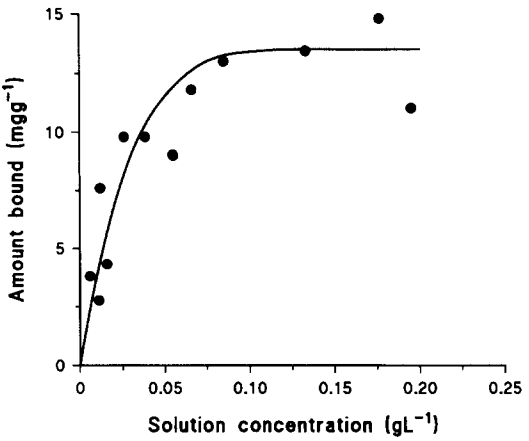

Fig. 3. Adsorption isotherm of carob galactomannan onto kappa-carrageenan after cold mixing: amount of carob bound as a function of its equilibrium concentration in solution. The line is to guide the eye.

The data in Fig. 3 show that the amount of carob bound reaches a plateau at about 13 mg g^{-1}, that is at a kappa-carrageenan/carob ratio close to 80. It is striking that the binding of carob to kappa-carrageenan after cold mixing saturates at much higher kappa-carrageenan/carob ratios than it does on cooling a mixture. For instance, the ^{133}Cs NMR data of Piculell et al. [6] show that the slowing down of caesium ion exchange with kappa-carrageenan, caused by the presence of carob, saturates at kappa-carrageenan/carob ratios close to 1 for heated mixtures. A possible explanation for this difference is that only a fraction of the binding sites available after heating are accessible on cold mixing. This seems quite reasonable if we suppose that, on cold mixing, the carob only has access to the exterior of fully formed aggregates of double helices, whereas when the two species are cooled together, adsorption can occur as soon as the aggregates are large enough. The latter case will naturally lead to higher adsorption of galactomannan per unit mass of kappa-carrageenan, and so to saturation at lower kappa-carrageenan/carob ratios, due to the larger surface area available.

Nature of the interaction between kappa-carrageenan and galactomannans.—Intuitively, it can be seen that the three models for mixed galactomannan/kappa-carrageenan gels described in the Introduction imply different interactions between the two polysaccharides: (*a*) the formation of mixed junction zones requires attraction; (*b*) the preferred model of Cairns et al. [2], with free carob chains surrounding a kappa-carrageenan gel, requires that the interaction between the two species be close to zero; (*c*) segregative phase separation [11] of the two species requires that carob and kappa-carrageenan repel each other, which is the usual case for mixtures of polymers [12].

The fact that carob, tara, and cassia galactomannans bind to kappa-carrageenan shows conclusively that there is an attraction between the two species, under the conditions used here. If there were no interaction between the two, the presence of kappa-carrageenan would not be expected to affect the galactomannan concentration, as is the case for guar. Finally, if there were a repulsion, which is necessary if the phase separation model is correct, then the concentration of galactomannan should be greater than

expected, due to its exclusion from the regions close to the kappa-carrageenan. If it is assumed that the same intermolecular forces are at work in the interaction after cold mixing and after heating, our results suggest that the synergistic rheological interaction between the two species is due to the formation of mixed junction zones. Piculell and co-workers [4,5] have come to the same conclusion very recently, on the basis of differential scanning calorimetry, NMR, and electron spin resonance results. They conclude that the interaction is best described by: "self-aggregated carrageenan helices with disordered mannan chains adsorbed to the surface" [5]. The results reported here are in agreement with this model.

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