Carbohydrate Polymers **8** (1988) 99–104

X-Ray Fibre Diffraction Studies on Konjac Mannan–Kappa Carrageenan Mixed Gels

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(Received 3 August 1987; accepted 22 October 1987)

SUMMARY

X-Ray fibre diffraction has been used to probe the molecular structure of kappa carrageenan–konjac mannan mixed gels. The diffraction pattern obtained for the mixed gel is characteristic of those obtained for kappa carrageenan. The results obtained show no evidence for a discrete molecular interaction between the two polymers.

INTRODUCTION

Kappa carrageenan is commonly used in industrial gelling applications, both on its own or in admixture with other polysaccharides to form synergistic mixed gels. The most widely exploited of these synergistic systems is the one containing kappa carrageenan and carob gum. Studies have shown that the carob in the mixture may be replaced by structurally similar galactomannans, such as tara gum (Cairns *et al.*, 1986*b*), or by enzymically modified guar (McCleary & Neukom, 1982). It has also been reported that the glucomannan obtained from the tubers of Amorphophallus konjac shows similar synergistic behaviour to kappa carrageenan as these galactomannans (Dea, 1981). The traditional model for the gelation of kappa carrageenan with galactomannans is shown in Fig. 1. It involves a proposed molecular binding between the carrageenan helix and unsubstituted regions of the galactomannan backbone (Dea *et al.*, 1972; Dea & Morrison, 1975). Galactomannans consist of a $\beta(1\rightarrow4)$ linked mannan backbone solubilised by partial substitution with α-D$(1\rightarrow6)$ galactose residues (Dea & Morrison, 1975). In contrast the structure of glucomannans is a mixture of $\beta(1\rightarrow4)$ linked D-glucose and $\beta(1\rightarrow4)$ D-mannose (Shimihara *et al.*, 1975). The exact

Carbohydrate Polymers 0144-8617/88/$03.50 —

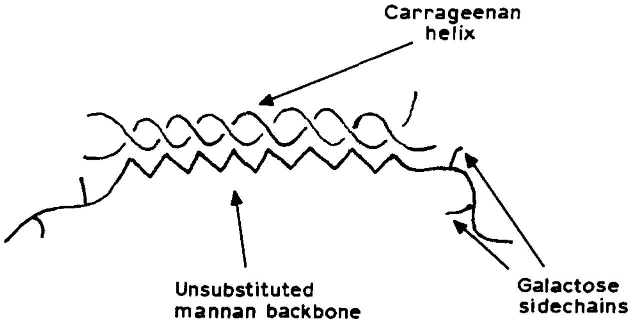

Fig. 1. Proposed model for the intermolecular binding of unsubstituted regions of the galactomannan backbone to the kappa carrageenan helix (Dea *et al.*, 1972; Dea and Morrison, 1975).

sequence is not known but it is believed that there are no cellulosic or mannan blocks. Since D-glucose and D-mannose differ only in the orientation of the hydroxyl substituent at carbon 2 of the sugar ring, it has been argued that the synergistic interaction between glucomannans and kappa carrageenan can be explained by the same process of intermolecular binding as proposed for galactomannan–kappa carrageenan interactions (Dea, 1981).

Recent X-ray fibre diffraction studies of oriented kappa carrageenan–galactomannan mixed gels suggest, however, that no such intermolecular binding occurs (Carroll *et al.*, 1984; Cairns *et al.*, 1986*a*). Such studies, coupled with methods for locating individual polymers in mixed gels, suggest (Cairns *et al.*, 1987) an alternative model for the interaction consisting of a carrageenan network containing the galactomannan as shown in Fig. 2.

This short article reports the use of X-ray diffraction to study aligned fibres prepared from mixed gels in order to test for intermolecular binding in konjac mannan–kappa carrageenan mixed gels. In these studies use is made of the fact that native konjac mannan is acetylated. Acetylation inhibits the independent gelation of konjac mannan.

The sample of kappa carrageenan, extracted from *Eucheuma cottonii*, was purchased from the Sigma Chemical Company (Poole, UK). Konjac mannan was purchased from Senn Chemicals (Dielsdorf, Switzerland). The konjac mannan flour was dispersed in water, heated to 90°C for 5–10 min in order to denature any degrading enzymes present and was then dissolved by stirring at room temperature overnight. Solid impurities were removed by filtering the solution through a sinter funnel. The cleaned solution was then poured onto a PTFE sheet and allowed to dry in order to form a film. Strips were cut from the dried film and stretched

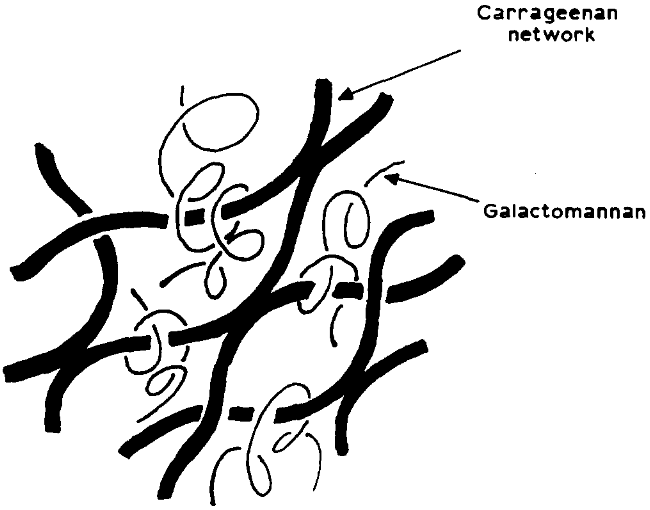

Fig. 2. Alternative model for the carrageenan–galactomannan synergism involving a carrageenan network containing the galactomannan within the gel (Cairns *et al.*, 1987).

up to ≈ 100% elongation by the attachment of weights in a sealed jar at approximately 98% relative humidity, to provide oriented fibres for X-ray fibre diffraction analysis. Konjac mannan-kappa carrageenan mixed gels were prepared by mixing the dried powders in the presence of a small quantity of ethanol (to facilitate dispersion), adding water, heating to 90°C then pouring the hot solutions onto glass substrates and allowing them to cool to room temperature. Strips of gel were cut and stretched up to ≈ 200% elongation under similar conditions as for the pure konjac mannan. Pure kappa carrageenan fibres were prepared using a similar method as for the mixtures. The strips of gel obtained were stretched up to ≈ 50% elongation. X-ray data were recorded photographically. The interior of the camera was maintained at a controlled relative humidity (rh) and continually flushed with helium to reduce air-scatter. CuK_α radiation was used and all fibres were dusted with calcite for calibration.

The X-ray fibre patterns obtained from pure kappa carrageenan and pure konjac mannan are shown in Figs 3(a) and 3(b), respectively. The data obtained for pure kappa carrageenan are identical to those reported by other workers on the polymer (Elloway, 1977). The unit cell dimensions of the acetylated konjac mannan correspond to those reported for mannan II (Frei & Preston, 1968). Only a few weak meridionals can be seen, indicating that konjac mannan does not have a regularly repeating structure. Chanzy *et al.* (1982) reported that deacetylated konjac mannan crystallises in the mannan II lattice. Previous authors have

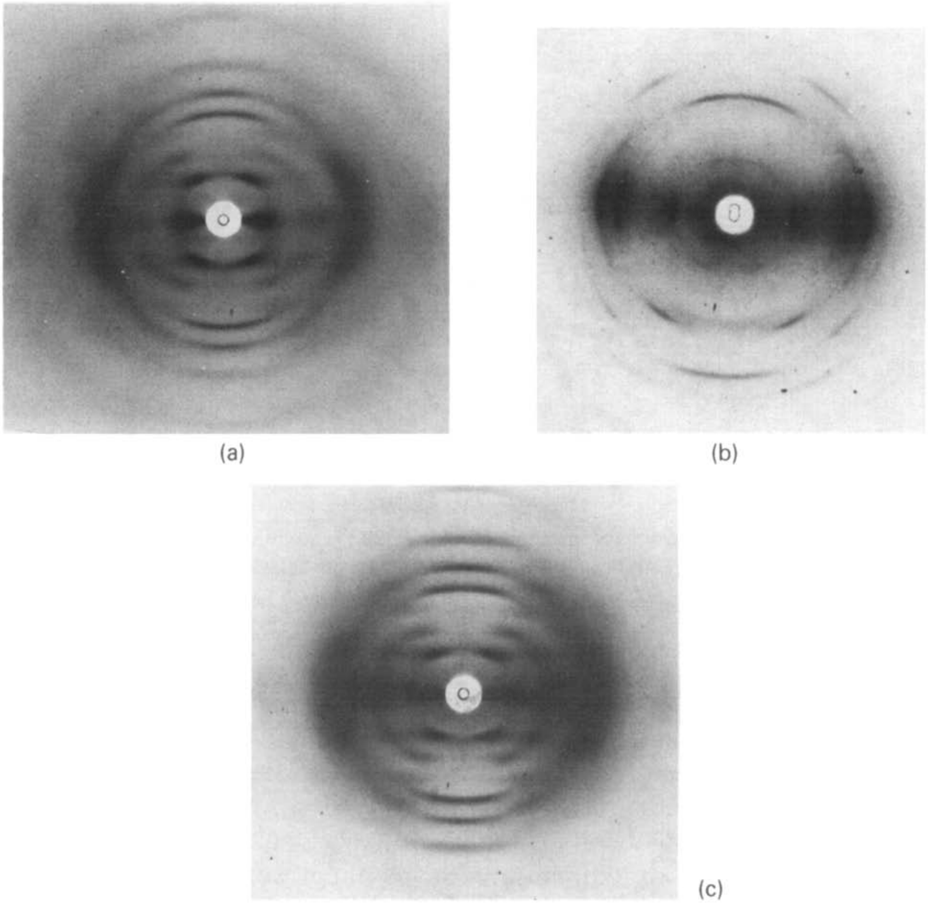

Fig. 3. X-ray fibre diffraction patterns obtained for pure polysaccharides and the mixed gel (wavelength 0·154 nm; rh ≈ 98%). (a) Kappa carrageenan stretched ≈ 50%; (b) konjac mannan stretched ≈ 100%; (c) 1:1 mixture of kappa carrageenan and konjac mannan at 2% total polymer concentration stretched ≈ 200%.

suggested that the gelation of konjac mannan is caused by the removal of acetyl groups from the polymer at alkaline pH (Maekaji, 1974). Steric interference between these groups, when present on the molecule, was thought to prevent intermolecular association and gelation (Torikata, 1952; Dea, 1981). Native konjac mannan, however, yields a highly crystalline diffraction pattern, proving that the presence of acetyl groups on the polymer does not totally suppress association and crystallisation. It may be that the presence of acetyl groups on the glucomannan suppresses intermolecular hydrogen bonding by reducing the number of sites available for such bonding. The removal of the acetyl groups would thus decrease the solubility of the polymer by enhancing intermolecular hydrogen bonding, leading eventually to gelation.

Figure 3(c) shows the X-ray diffraction pattern obtained from a 1:1 mixture of konjac mannan and kappa carrageenan at 2% total polymer concentration. The pattern is found to be characteristic of that obtained from pure kappa carrageenan and the unit cell dimensions are unchanged. No meridional reflections due to the konjac mannan can be seen, which is not surprising considering how weak they are in the pure konjac mannan pattern. If intermolecular binding had occurred between the polymers, in accordance with the traditional model, the mixed junction zones formed would give rise to X-ray diffraction patterns distinct from those obtained from either of the pure polymers. This is not reflected in the results obtained, nor is there any evidence for co-crystallisation of the polymers. The model illustrated in Fig. 1 is thus clearly inadequate to explain the synergism between glucomannans and kappa carrageenan. The results we have obtained suggest that the mixed gel consists of a carrageenan network containing konjac mannan possibly of the type illustrated in Fig. 2.

The synergistic interaction between konjac mannan and kappa carrageenan may have interest beyond establishing the glucomannan as a possible supplement for carob gum in commercial uses of such mixed gels. Konjac mannan may be gelled at alkaline pH and it has been reported that the rheological properties of the resulting gel can be varied depending upon the particular alkaline agent used to induce gelation. Thus the possibility exists for manipulating the gel structure of mixed carrageenan-konjac mannan gels by controlling the polymer ratios and the conditions of pH and temperature under which the gels are formed.

REFERENCES

Cairns, P., Miles, M. J. & Morris, V. J. (1986*a*). *Int. J. Biol. Macromolecules* **8,** 124.

Cairns, P., Morris, V. J., Miles, M. J. & Brownsey, G. J. (1986*b*). *Food Hydrocolloids* **1,** 89.

Cairns, P., Miles, M. J., Morris, V. J. & Brownsey, G. J. (1987). *Carb. Res.* **160,** 411.

Carroll, V., Morris, V. J. & Miles, M. J. (1984). *Macromolecules* **17,** 2443.

Chanzy, H. D., Grosrenaud, A., Joseleau, J. P., Dube, M. & Marchessault, R. H. (1982). *Biopolymers* **21,** 301.

Dea, I. C. M. (1981). *Am. Chem. Soc. Symp. Ser.* **150,** 439.

Dea, I. C. M. & Morrison, A. (1975). *Adv. Carb. Chem. Biochem.* **31,** 241.

Dea, I. C. M., McKinnon, A. A. & Rees, D. A. (1972). *J. Mol. Biol.* **68,** 153.

Elloway, H. F. (1977). PhD Thesis, University of Bristol, UK.

Frei, E. & Preston, R. D. (1968). *Proc. Roy. Soc. London, Ser. B.* **169,** 127.
Maekaji, K. (1974). *Agr. Biol. Chem.* **38,** 315.
McCleary, B. V. & Neukom, H. (1982). *Prog. Food Nutrition Sci.* **6,** 109.
Shimihara, H., Suzuki, H., Sugiyama, N. & Nishizawa, K. (1975). *Agric. Biol. Chem.* **39,** 301.
Torikata, H. (1952). *Nippon Nogeikagaku Kaishi,* **73,** 533; **73,** 535.