accord with the experiments.[3-5]

These results reinforce the conclusion of the previous paper[1] that much of the deviation of experiments on star molecules from the predictions based on preaveraging[6,7] can be attributed to the latter approximation. We would like to note, however, that the deviations are modest, and preaveraging remains a useful tool for approximate calculations because of its relative simplicity.

There seems to be no clear effect of either persistence or excluded volume on g'; such differences as appear are within the precision measures. Thus we would not expect solvent power to have much effect on h and g'. In the experiments a small decrease in g' was observed in going from Θ to good solvents.[4,5] For example, the nonpreaveraged g' values for six-armed stars with no persistence are 0.58 ± 0.04 and 0.56 ± 0.03, respectively, without and with excluded volume; Roovers and Bywater's[4] corresponding experimental ones were 0.63 and 0.57. One would naively expect that excluded volume would cause branched molecules to expand more than linear ones, and hence push g and g' nearer unity, and clearly a decided expansion effect does occur, as the figures in the tables show. However, the distribution of sizes is also narrowed, as the standard deviations in the tables also show, and the character of the distribution is probably changed. These changes in distribution apparently overcome the simple effect of expansion on g'.

Acknowledgment. This work was supported by Grant GM-11916 from the National Institutes of Health.

References and Notes

(1) Zimm, B. H. *Macromolecules* **1984**, *17*, 795.
(2) Fixman, M. *J. Chem. Phys.* **1983**, *78*, 1588.
(3) Berry, G. C. *J. Polym. Sci., Part A-2* **1971**, *9*, 687.
(4) Roovers, J. E. L.; Bywater, S. *Macromolecules* **1972**, *5*, 384; **1974**, *7*, 443.
(5) Roovers, J. E. L.; Hadjichristidis, N.; Fetters, L. J. *Macromolecules* **1983**, *16*, 214.
(6) Stockmayer, W. H.; Fixman, M. *Ann. N.Y. Acad. Sci.* **1953**, *57*, 334.
(7) Zimm, B. H.; Kilb, R. W. *J. Polym. Sci.* **1959**, *37*, 19.

Carob Gum–κ-Carrageenan Mixed Gels: Mechanical Properties and X-ray Fiber Diffraction Studies

MERVYN J. MILES,* VICTOR J. MORRIS, and VINCENT CARROLL

AFRC Food Research Institute, Colney Lane, Norwich, NR4 7UA, U.K. *Received October 21, 1983*

The solution properties of certain polysaccharides may be drastically modified by the addition of certain galactomannans.[1,2] Such additions may lead to an enhancement of the viscosity of the mixed-polymer solution, gelation may occur under conditions for which neither pure polymer would gel, or the mechanical properties and texture of the mixed gel may differ from those of either pure polymer gel.

Synergistic polymer–polymer interactions are attractive commercially and enjoy widespread technological exploitation.[3] Expensive polysaccharides may be replaced by cheaper polysaccharides, leading to savings in cost. Mixtures offer the potential for creating new textures or manipulating the rheology and texture. Synergistic polymer–polymer interactions are believed to mimic natural associations of polymers in complex cellular structures,[4] and certain interactions between the extracellular microbial polysaccharide xanthan and plant glycans have been implicated in biological host–pathogen recognition processes.[5]

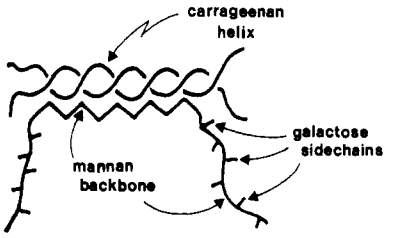

Figure 1. Proposed model[1,2,4] for the interaction between carob gum and κ-carrageenan. Regions of mannan backbone, unsubstituted with galactose side chains, are pictured binding to a carrageenan double helix.

κ-Carrageenan–carob (locust bean) gum mixtures provide a convenient model for studying synergistic interactions. κ-Carrageenan forms brittle transparent thermoreversible gels. Addition of carob gum leads to gelation at total polymer concentrations less than that at which the carrageenan alone will gel.[2] The mixed gels are soft and tough and will sustain substantial tensile plastic deformation.[2] The presently accepted model for gelation is commonly illustrated[1,2,4] as shown in Figure 1 although the stoichiometry and the total number of polymer molecules contributing to a junction zone are vague and unspecified.

The idealized repeat unit[6] for κ-carrageenan (α(1→3)-galactose 4-sulfate–β(1→4)-3,6-anhydrogalactose) is capable of forming threefold right-handed helices.[7,8] Structural regularity is marred by the presence[9] of "kinks" which are believed to restrict helix formation to short segments[10] within the polymer chain. Gelation is claimed to involve an intertwining of two separate chains to form a double-helical region limited by kinking residues, followed by a cation-mediated association of these helices into aggregated or microcrystalline junction zones.[11] At present there is some debate as to whether the helix formation involves an "intermolecular" or "intramolecular" process.[11,12] The binding region of the carrageenan in mixed gels is claimed to be a double-helical segment.[1,2] Carob gum is a galactomannan with a structure based on a β-D-(1→4)-mannan backbone solubilized by substitution with α-D-(1→6)-linked galactose side chains.[2] For carob gum the mannose:galactose (MG) ratio is 3.55, although preparations are likely to be heterogeneous with respect to MG and side-chain distribution.[2] Gelation may occur on standing or be induced by freeze–thaw cycling or the addition of solutes believed to reduce water activity in carob gum solutions.[1,2] Gelation is favored by a high MG, and natural association is believed to occur via unsubstituted regions of the mannan backbone.[1,2] In mixed gels such unsubstituted mannose regions are believed to bind the carrageenan helix[1,2] (Figure 1).

The interactions between polysaccharides and galactomannans are sensitive to the choice of galactomannan and of polysaccharide.[1,2] Carob gum interacts strongly with agar, carrageenan, or xanthan whereas guar gum (MG = 1.63) shows weak interactions.[1,2] Initial X-ray diffraction,[13] chemical,[14] and enzymatic[15] studies implied a uniform distribution of galactose on every second mannose for guar but a block structure for carob gum. Selectivity with respect to galactomannan was attributed to galactose distribution rather than content.[1,2] Recent studies using enzyme,[16] chemical,[17] NMR[18] and X-ray diffraction[19] methods suggest an irregular to random galactose distribution for both guar and carob gum. The suggested model (Figure 1) may still account for selectivity of galactomannan because the higher MG value for carob gum would favor more or longer bare mannan regions. However, the

0024-9297/84/2217-2443$01.50/0

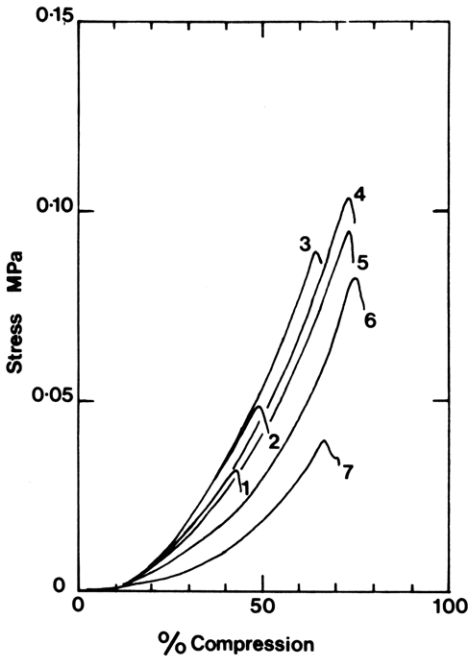

Figure 2. Uniaxial stress–compression plots obtained for a range of polymer compositions at a total polymer concentration of 1.5%: (1) 0%, (2) 12.5%, (3) 25%, (4) 33.3%, (5) 40%, (6) 50%, and (7) 66.7% carob gum.

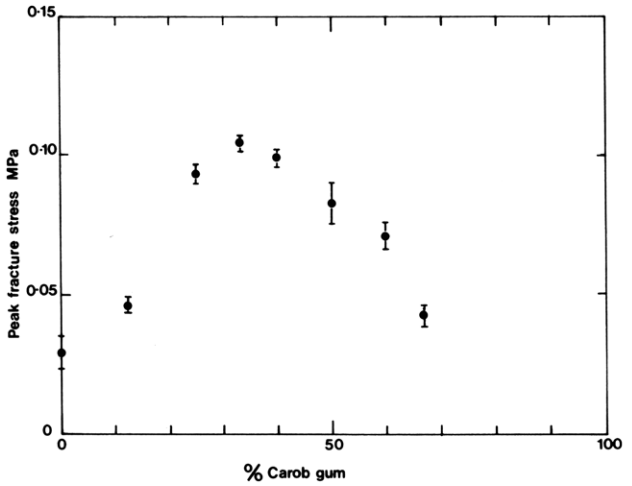

Figure 3. Plot of peak (fracture) stress vs. carob gum concentration. Total polymer concentration 1.5%.

model is unable to explain or predict the selectivity with respect to the choice of polysaccharide.

There is a lack of direct evidence for binding of the galactomannan to the carrageenan. Freeze–thaw studies demonstrate retention of galactomannan within mixed gels[4] but this does not prove intermolecular binding since such treatments are known to favor gelation of galactomannans.[1,2] The addition of carob leads to only minor modifications of the optical rotation changes observed for pure κ-carrageenan.[2,4] The production[4] of isolated helix-forming segments of κ-carrageenan and their subsequent gelation[2,4] on addition of carob gum provide only circumstantial evidence for galactomannan–carrageenan binding.

This note is concerned with an attempt to carry out what is believed to be the first direct test of the model (Figure 1) proposed for this synergistic interaction. X-ray fiber diffraction studies have formed the basis for determining the molecular structure of the junction zones within polysaccharide gels.[20] X-ray fiber diffraction patterns for κ-carrageenan are well documented.[21,22] The model shown in Figure 1 suggests that the fiber diffraction pattern obtained for a mixed gel should be different from the pattern obtained for a pure κ-carrageenan gel. Incorporation of carob gum into the junction zones should lead to a change in either the type of unit cell or the unit cell dimensions. In addition, specific binding of the mannan ribbon to the carrageenan helix should mean that alignment of the junction zones will give rise to alignment of the mannan ribbon and the carrageenan helix. Hence the diffraction pattern should consist of overlapping patterns for the mannan and the carrageenan *or* an entirely new pattern characteristic of the molecular interaction. The mixed system isotactic and syndiotactic poly(methyl methacrylate) (PMMA) is an example of a mixed gel in which the synergism has been probed by X-ray fiber diffraction. The X-ray pattern obtained from the mixed gel differs significantly from the patterns obtained from gels of either pure component.[23,24]

Samples of κ-carrageenan from *Eucheuma cottonii* and carob gum from *Caratonia siliqua* were supplied by Sigma Chemical Co. Ltd. and used without further purification. Aqueous solutions were prepared by heating to 90 °C in sealed tubes under agitation to ensure complete dissolution of the polymers. Mechanical studies were performed on cylindrical gel specimens 10 mm in height and 16 mm in diameter using an Instron 1122 with a 16-mm-diameter plunger. Uniaxial stress–compression plots for a range of compositions at a fixed total polymer concentration are shown in Figure 2. The peak stress prior to failure was found to be dependent on composition. The plots may be divided into low-strain and high-strain regions. Hysteresis studies show that the low-strain regions may be regarded as elastic. The modulus, determined in this region, remained approximately constant up to 40% carob gum and then decreased at higher carob content. The compression at peak stress showed a maximum at 33% carob gum concentration (Figure 3). This agrees with previously reported data.[25] Subsequent studies showed that the optimum carrageenan:carob ratio of 2:1 was independent of total polymer concentration in the range 1–3%.

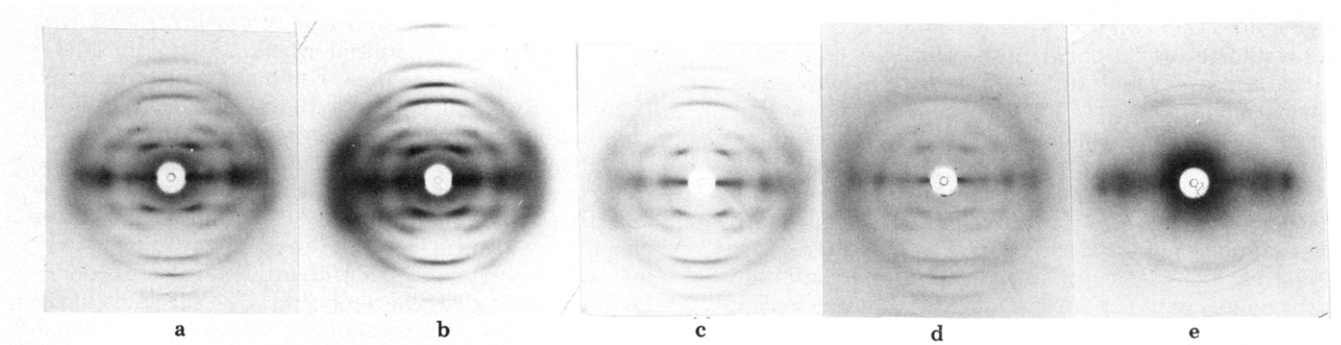

Figure 4. X-ray fiber diffraction data obtained for (a) 25%, (b) 33%, (c) 50%, (d) 67%, and (e) 100% carob gum. The fiber axis is vertical.

Fibers for X-ray diffraction were prepared by stretching thin strips of gel. Elongations of up to 300% were obtained at a relative humidity (RH) of 98%. Under the conditions used to prepare pure κ-carrageenan gels and mixed carrageenan–carob gels, pure carob gum samples did not gel. Carob fibers were prepared by stretching strips cut from a dried film (RH = 98%). The wavelength used was 1.54 Å and fibers were dusted with calcite for calibration. The interior of the diffraction camera was maintained at RH = 98% and flushed continually with helium to reduce scattering. Diffraction patterns were recorded on film. Diffraction patterns obtained at different relative compositions are shown in Figure 4a–d. The diffraction pattern obtained for pure carob gum under nominally identical conditions is shown in Figure 4e. The quality of this pattern can be substantially improved by annealing but Figure 4e is felt to represent the type of pattern that one might expect to observe in a mixed-gel system. For all the compositions studied the X-ray fiber diffraction patterns obtained for mixed gels are, within the limits of experimental accuracy, qualitatively and quantitatively identical with the published photographs[22] obtained for pure κ-carrageenan. Since the nature of the unit cell and its dimensions are unchanged, this implies that the carob gum has not been incorporated into the κ-carrageenan junction zones within the mixed gel. This is in complete contrast to observations on the synergistic interactions leading to mixed gels of isotactic and syndiotactic PMMA where the diffraction pattern obtained from the mixed gel is distinctly different from the patterns obtained from gels of either pure component. Although weak but definite diffraction patterns were obtained for carob gum alone, there was no indication of a carob gum diffraction pattern superimposed on a carrageenan pattern nor was there any detectable modification of the carrageenan pattern for the mixed gels. This would seem to suggest that most of the carob gum molecules have not been oriented by the stretching of the gel network. Thus a specific molecular interaction involving parallel alignment of both the carrageenan and the carob gum within the junction zones of the gel (Figure 1) is unlikely.

Mechanical studies demonstrate the drastic changes induced by adding carob gum to κ-carrageenan. Diffraction studies directed toward testing two predictions implicit in the currently accepted model for the synergistic interaction have failed to reveal any evidence of carob–carrageenan interaction. The model shown in Figure 1 could be modified to accommodate the present results. The mixed gels could be considered to contain large aggregates or microcrystalline carrageenan regions linked by surface attachment of carob molecules. One could further suppose no preferential alignment of surface attached carob molecules or that the mannan attachment regions are small. However, such a picture is more akin to a composite gel structure than a discrete molecular interaction. Further experiments aimed at testing the currently accepted model are urgently required.

Registry No. Carob gum, 9000-20-8; κ-carrageenan, 11114-20-8.

References and Notes

(1) Dea, I. C. M. In "Polysaccharides in Food"; Blanshard, J. M. V., Mitchell, J. R., Eds.; Butterworths: London, 1979; p 229.
(2) Dea, I. C. M.; Morrison, A. *Adv. Carbohydr. Chem. Biochem.* **1975**, *31*, 241.
(3) Glicksman, M. "Gum Technology in the Food Industry"; Academic Press: New York, 1968; p 43.
(4) Dea, I. C. M.; McKinnon, A. A.; Rees, D. A. *J. Mol. Biol.* **1972**, *68*, 153.
(5) Dea, I. C. M.; Morris, E. R.; Rees, D. A.; Welsh, E. J.; Barnes, H. A.; Price, J. *Carbohydr. Res.* **1977**, *57*, 249.
(6) Rees, D. A. *Adv. Carbohydr. Chem. Biochem.* **1969**, *24*, 267.
(7) Arnott, S.; Scott, W. E.; Rees, D. A.; McNab, C. G. A. *J. Mol. Biol.* **1974**, *90*, 253.
(8) Arnott, S.; Fulmer, A.; Scott, W. E.; Dea, I. C. M.; Moorhouse, R.; Rees, D. A. *J. Mol. Biol.* **1974**, *90*, 269.
(9) Anderson, N. S.; Dolan, T. C. S.; Rees, D. A. *J. Chem. Soc., Perkin Trans. 1* **1973**, 2173.
(10) Rees, D. A. *Biochem. J.* **1972**, *126*, 257.
(11) Morris, E. R.; Rees, D. A.; Robinson, G. *J. Mol. Biol.* **1980**, *138*, 349.
(12) Smidsrod, O.; Grasdalen, H. *Carbohydr. Polym.* **1982**, *2*, 270.
(13) Palmer, K. J.; Ballantyne, M. *J. Am. Chem. Soc.* **1950**, *72*, 736.
(14) Baker, C. W.; Whistler, R. L. *Carbohydr. Res.* **1975**, *45*, 237.
(15) Courtois, J. E.; LeDizet, P. *Bull. Soc. Chim. Biol.* **1970**, *52*, 15.
(16) McCleary, B. V. *Carbohydr. Res.* **1979**, *71*, 205.
(17) Hoffman, J.; Lindberg, B.; Painter, T. J. *Acta Chem. Scand., Ser. B* **1975**, *29*, 137.
(18) Grasdalen, H.; Painter, T. J. *Carbohydr. Res.* **1980**, *81*, 59.
(19) Marshessault, R. H.; Buteon, A.; Deslandes, Y.; Goto, T. *J. Colloid Interface Sci.* **1979**, *71*, 375.
(20) Arnott, S. In *Dev. Food Carbohydr.* **1977**, *Ser. 1*, 43.
(21) Anderson, N. S.; Campbell, J. W.; Harding, M. M.; Rees, D. A.; Samuel, J. W. B. *J. Mol. Biol.* **1969**, *45*, 85.
(22) Elloway, H. F. Ph.D. Thesis, University of Bristol, 1977.
(23) Boer, A. Ph.D. Thesis, Rijksuniversiteit te Groningen, 1976.
(24) Atkins, E. D. T., private communication.
(25) Ainsworth, P. A.; Blanshard, J. M. V. *J. Text. Stud.* **1980**, *11*, 149.

α-Helix-to-Random-Coil Transition of Two-Chain, Coiled Coils. Experiments on the Thermal Denaturation of α-Tropomyosin and β-Tropomyosin

LORI L. ISOM, MARILYN EMERSON HOLTZER, and ALFRED HOLTZER*

Department of Chemistry, Washington University, St. Louis, Missouri 63130. *Received February 14, 1984*

Some time ago, an equilibrium statistical mechanical theory was developed for the thermally induced α-helix-to-random-coil transitions of two-chain, α-helical, coiled-coil proteins.[1] This theory was applied, with apparent success, to a 43-residue synthetic polypeptide chain analogue of tropomyosin,[2] to α-tropomyosin itself,[3,4] and to two well-characterized fragments of α-tropomyosin.[5] Realization of the theory requires input information comprising (1) the amino acid sequence of the polypeptide chain under investigation, (2) values of the parameters σ and $s(T)$ that embody the short-range interactions for each amino acid type, and (3) a measurement of helix content vs. temperature, which, with the theory, provides the value of a third parameter (w) that measures the "long-range", i.e., helix–helix, interaction as a function of temperature. In past applications, requirement (1) was satisfied by extant data,[6] (2) by algorithms[2,3] chosen to fit extant data,[7] and (3) by extant or new circular dichroism (CD) measurements.[2-5]

Although these applications seem promising, the case for the theory cannot be considered proved. Moreover, the theory itself has recently been expanded to include the effects of both loop entropy and out-of-register structures,[8-10] both of which were assumed to be absent in the original treatment. For these reasons, we thought it desirable to extend the data base available for testing the expanded theory by making measurements at pH 7.4 of CD vs. temperature over a wide range of protein concentration for β-tropomyosin, a genetic variant of the same chain length as α-rtropomyosin and whose sequence is also known.[6] Furthermore, we have reinvestigated α-tropomyosin over the same large range of protein concentration

0024-9297/84/2217-2445$01.50/0