Carbohydrate Polymers **12** (1990) 315–321

X-Ray Fiber Diffraction Studies of a Variant of Xanthan Gum in which the Sidechain Terminal Mannose Unit is Absent

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(Received 3 March 1989; revised version received and accepted 19 May 1989)

ABSTRACT

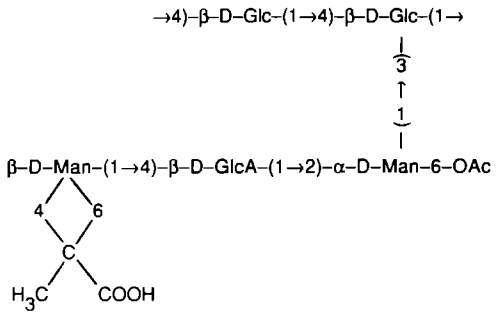
A variant of xanthan gum produced by a genetically engineered mutant of Xanthomonas campestris, *in which the sidechain terminal mannose unit is absent, has been trapped in an ordered conformation in oriented fibers. X-ray diffraction patterns from these fibers show that the variant polymer has a molecular repeat of 47·5 Å and five-fold helix symmetry, almost identical to that of xanthan. They also suggest that the effective molecular diameter is approximately 3 Å less than that of xanthan. These results indicate that the conformation of the variant polymer is similar to that of xanthan, and show that removal of the sidechain terminal mannose unit does not critically affect the stability of the xanthan ordered structure.*

INTRODUCTION

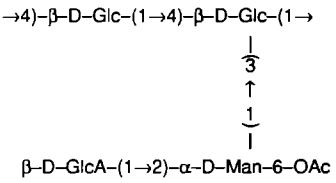
Xanthan gum is an anionic extracellular polysaccharide produced by the bacterium *Xanthomonas campestris*. It has a wide variety of industrial applications because of its high viscosity and pseudoplasticity, and the stability of its rheological properties over wide ranges of temperature and pH (Sandford & Baird, 1983). It is commonly used as a thickening agent in food products, for mobility control in secondary and tertiary oil recovery, in petroleum drilling fluids, and in the paint, pharmaceutical and cosmetic industries.

The primary structure of xanthan is a $\beta(1\rightarrow4)$ linked D-glucose (cellulosic) main chain with a trisaccharide sidechain attached to the 3-position of every second glucose, giving a pentasaccharide repeating unit (Fig. 1(a)). The sidechain is β-D-Man-$(1\rightarrow4)$-β-D-GlcA-$(1\rightarrow2)$-α-

Carbohydrate Polymers 0144-8617/90/$03.50



a



b

Fig. 1. Primary structures of (a) xanthan and (b) the polytetramer.

D-Man(1→ and the α- and β-linked mannose units are specifically and variably acetylated and pyruvylated respectively (Jansson *et al.*, 1975; Melton *et al.*, 1976). In aqueous solution, xanthan undergoes a thermally induced cooperative, conformational transition (Rees, 1972) which has been monitored by a number of physical techniques including optical rotation, viscometry, NMR, calorimetry and potentiometric titration (Rees, 1972; Morris *et al.*, 1977; Milas & Rinaudo, 1979). Although xanthan has been the subject of many physicochemical studies, the details of the ordered structure, particularly the number of chains involved, are still the subject of lively debate (Norton *et al.*, 1984; Milas & Rinaudo, 1986; Liu & Norisuye, 1988), and will not be discussed here.

X-ray diffraction studies of xanthan show that it forms a helical structure with five-fold screw symmetry and a molecular repeat distance of 47·0 Å in hydrated fibers (Moorhouse *et al.*, 1977*a*; Okuyama *et al.*, 1980; Millane *et al.*, 1989). The diffraction data are insufficient however to define the molecular structure precisely, or the number of chains involved.

Recent studies of xanthan biosynthesis utilizing an in-vitro system have allowed characterization of mutant strains of *Xanthomonas* that are

defective in normal gum biosynthesis (Betlach *et al.*, 1987; Vanderslice *et al.*, 1989). Mutant strains have been identified that produce variant gums that are polymers of truncated versions of the normal repeating unit of xanthan. Some of these variant gums have rheological properties similar, but different, to xanthan (Betlach *et al.*, 1987) which may make them useful in specific applications. One of these, referred to as 'polytetramer', has a repeating unit identical to xanthan except that the sidechain terminal mannose unit is absent (Fig. 1(b)). Structural studies of variant xanthan polymers may provide information on the molecular basis of their physical properties. They may also shed light on the details of the ordered conformation of xanthan and the sidechain functionality, which at present are poorly understood.

In this paper X-ray fiber diffraction studies on the polytetramer that allow comparisons to be made between its ordered structure and that of xanthan are described.

METHODS

A sample of the polytetramer (supplied by the Synergen-Texaco Joint Venture, Boulder, Colorado) was passed through an Amberlite IR-120 column (Rohm and Hass Co. Philadelphia Pennsylvania) and converted to the lithium salt by dialysis against 0·4 M LiCl. Excess salt and any impurities were removed by additional dialysis against a large volume of distilled water, and the sample lyophilized. Samples were dry spun using standard methods, and X-ray diffraction patterns obtained at 75% relative humidity using a flat film pinhole camera and Ni filtered CuKα radiation. Spacings were calibrated by dusting the specimens with calcite. The optical densities on the diffraction pattern were digitized using an Optronics Photoscan P-1000 rotating drum microdensitometer. The center and orientation of the diffraction pattern, fiber tilt to the X-ray beam, and c-repeat were determined using standard methods (Millane & Arnott, 1985).

Fibers were prepared and diffraction patterns similarly recorded from the sodium salt of wild-type xanthan (courtesy of M. Milas, CERMAV-CNRS, France) at 92% relative humidity, and spacings measured as for the polytetramer.

RESULTS

A diffraction pattern obtained from the polytetramer is shown in Fig. 2 where it is compared to that from wild-type xanthan gum. The diffrac-

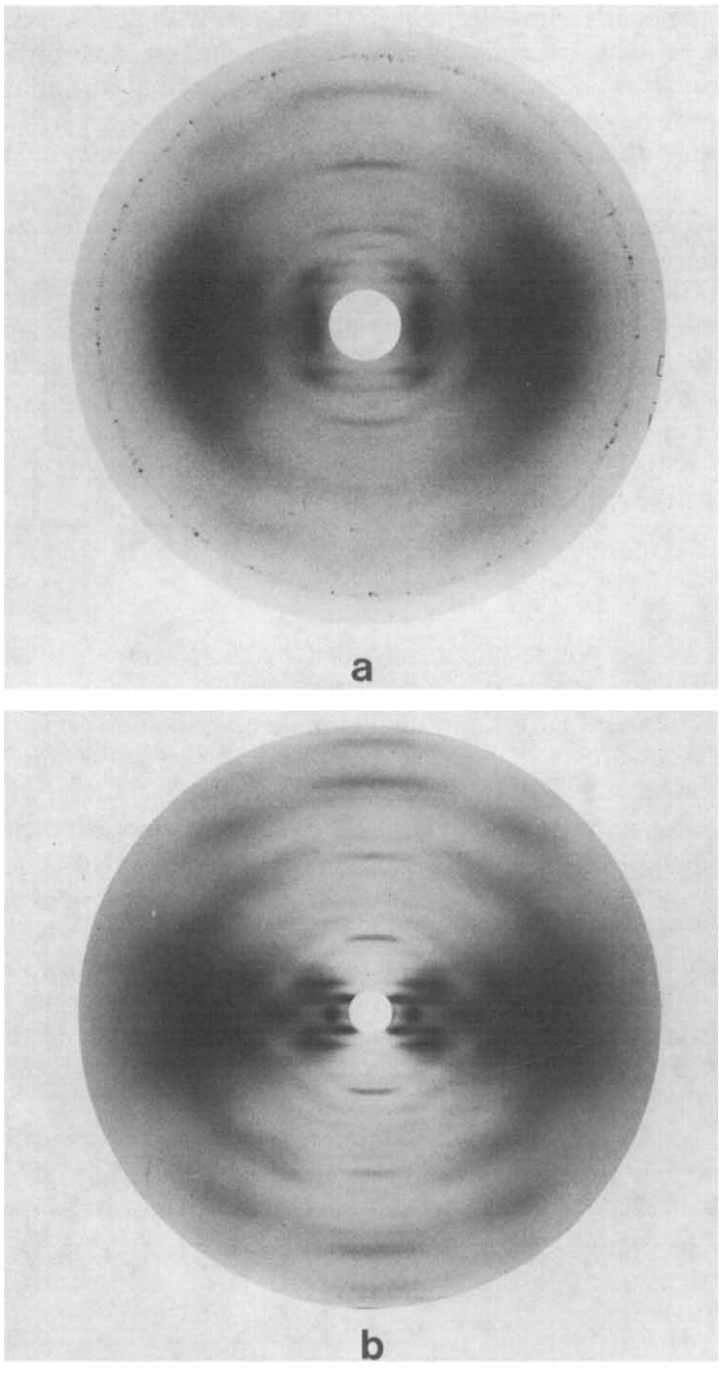


Fig. 2. X-ray fiber diffraction patterns from (a) Li^+ polytetramer (fiber tilted by 10° to the normal to the incident X-ray beam) and (b) Na^+ xanthan.

tion pattern from xanthan is better resolved than that from the polytetramer, indicating that the molecules in the xanthan fiber are better oriented than in the polytetramer fiber. The polytetramer pattern is however sufficiently well resolved to determine the c-repeat and molecular symmetry unequivocally. The layer line spacings correspond to a molecular repeat distance of 47·5 (±0·3) Å. The spacings on the xanthan pattern give a molecular repeat of 47·2 (±0·3) Å, consistent with previous measurements (Moorhouse *et al.*, 1977*a*; Okuyama *et al.*, 1980). The axial repeats of the two molecules are therefore identical within experimental error. The polytetramer diffraction pattern has meridional reflections on the 5th and 10th layer lines indicating that the molecule has five-fold helix symmetry, as is the case with xanthan. The polytetramer diffraction pattern has quite strong diffraction on the sixth layer line close to (but not on) the meridian, whereas the xanthan pattern contains little diffracted intensity in this region. The first diffraction maximum on the equator of the polytetramer pattern corresponds to a lateral spacing of 14·4 (±0·3) Å. This spacing reduces to 13·4 Å when the relative humidity is reduced to 15%. The first maximum on the equator of the xanthan pattern is at a spacing of 18·0 (±0·2) Å. Somewhat different lateral spacings were seen on some other xanthan patterns however. Although there are some differences in the distribution of intensities, the two diffraction patterns are rather similar overall.

DISCUSSION

The diffraction pattern from the polytetramer indicates an ordered molecular conformation in which the molecules are aligned approximately parallel but are not organized laterally. Conservation of the c-repeat and helix symmetry with respect to xanthan, suggest that the conformations of the two molecules are similar. The identical c-repeats suggest rather strongly that the backbone conformations of the two molecules are identical, as it is extremely unlikely that significant changes in these would not be accompanied by a change in the c-repeat. The sidechain probably interacts with the mainchain as is observed with other branched polysaccharides (Moorhouse *et al.*, 1977*b*), so that any significant changes in the sidechain conformations (and therefore their interactions) would probably induce changes in the mainchain, thereby altering the c-repeat. The sidechain conformations of the polytetramer are therefore likely (but not necessarily) to be similar to those of xanthan also. The overall similarity between the two diffraction patterns supports these conclusions. The differences, particularly on the 6th layer line, are

probably due to the absence of the terminal mannose unit which, together with the attached pyruvate group, represents a strong X-ray scatterer.

The first diffraction maxima on the equator of both diffraction patterns are rather sharp and therefore indicate some limited lateral packing of the molecules. The 1·0 Å decrease in this lateral spacing on the polytetramer pattern upon reducing the humidity, is probably due to the removal of water molecules between the chains, allowing them to pack more closely together. Note that the axial repeat however, is unchanged upon drying. The lateral spacing for xanthan is somewhat greater, which (aside from possible solvent effects) could be due, at least in part, to the effective diameter of the xanthan molecule being about 3·0 Å greater than for the polytetramer. This indicates that the outer 1·5 Å of the xanthan structure is occupied by the terminal mannose unit, which is not surprising given the bulk of this group (including the attached pyruvate) and its terminal position.

The similarity between the ordered structures of the polytetramer and xanthan indicates that this conformation is quite robust. Any interactions involving the terminal mannose unit appear, therefore, not to be essential for the stability of the structure. The stability of this conformation is consistent with the xanthan structure (represented by the diffraction pattern in Fig. 1(b)) being the only one trapped (so far) in oriented fibers, even at different humidities, different types of salt, and different pyruvate contents.

ACKNOWLEDGMENTS

The Authors are grateful to the Joint Venture between Synergen Inc. and Texaco Inc. for providing us with the polytetramer samples, Dr M. Milas for provision of the xanthan samples, the US National Science Foundation for support (DMB-8606942 to RPM), Deb Zerth for word processing and Robert Werberig for photography.

REFERENCES

Betlach, M. R., Capage, M. A., Doherty, D. H., Hassler, R. A., Henderson, N. M., Vanderslice, R. W., Marrelli, J. D. & Ward, M. B. (1987). In *Industrial Polysaccharides: Genetic Engineering, Structure/Property Relations and Applications*, ed. M. Yalpani. Elsevier Science Publishers, Amsterdam, pp. 35–50.

Jansson, P. E., Kenne, L. & Lindberg, B. (1975). *Carbohydr. Res.*, **45**, 275–82.

Liu, W. & Norisuye, T. (1988). *Int. J. Biol. Macromol.*, **10**, 44–50.

Melton, L. D., Mindt, L., Rees, D. A. & Sanderson, G. R. (1976). *Carbohydr. Res.*, **46,** 245–57.
Milas, M. & Rinaudo, M. (1979). *Carbohydr. Res.*, **76,** 189–96.
Milas, M. & Rinaudo, M. (1986). *Carbohydr. Res.*, **158,** 191–204.
Millane, R. P. & Arnott, S. (1985). *J. Macromol. Sci.—Phys.*, **B24,** 193–227.
Millane, R. P., Narasaiah, T. V. & Arnott, S. (1989). In *Recent Developments in Industrial Polysaccharides: Biomedical and Biotechnological Advances*, ed. S. S. Stivala, V. Crescenzi & I. C. M. Dea. Gordon and Breach, New York, 1989, in press.
Moorhouse, R., Walkinshaw, M. D. & Arnott, S. (1977*a*). *ACS Symp. Ser., vol. 45*, Am. Chem. Soc., Washington, DC, pp. 90–102.
Moorhouse, R., Winter, W. T., Arnott, S. & Bayer, M. E. (1977*b*). *J. Mol. Biol.*, **109,** 373–91.
Morris, E. R., Rees, D. A., Young, G., Walkinshaw, M. D. & Darke, A. (1977). *J. Mol. Biol.*, **110,** 1–16.
Norton, I. T., Goodall, O. M., Frangou, S. A., Morris, E. R. & Rees, D. A. (1984). *J. Mol. Biol.*, **175,** 371–94.
Okuyama, K., Arnott, S., Moorhouse, R., Walkinshaw, M. D., Atkins, E. D. T. & Wolf-Ullish, C. (1980). *ACS Symp. Ser., vol. 141*, Am. Chem. Soc., Washington, DC, pp. 411–27.
Rees, D. A. (1972). *Biochem. J.*, **126,** 257–73.
Sandford, P. A. & Baird, J. (1983). In *The Polysaccharides*, Vol. 2, ed. G. O. Aspinall. Academic Press, New York, pp. 411–90.
Vanderslice, R. W., Doherty, D. H., Capage, M. A., Betlach, M. R., Hassler, R. A., Henderson, N. M., Ryan-Graniero, J. & Tecklenburg, M. (1989). In *Recent Developments in Industrial Polysaccharides: Biomedical and Biotechnological Advances*, ed. S. S. Stivala, V. Crescenzi & I. C. M. Dea. Gordon and Breach, New York, 1989, in press.