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X-Ray Fibre Diffraction Studies of Members of the Gellan Family of Polysaccharides

P. Cairns, M. J. Miles & V. J. Morris

AFRC Institute of Food Research, Norwich Laboratory, Colney Lane, Norwich NR4 7UA, UK

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ABSTRACT

X-Ray fibre diffraction studies are reported for gellan gum and the family of related polysaccharides S-130, S-198, S-88 and S-194. Whereas the linear gellan molecules yield highly crystalline patterns, the branched polysaccharides yield well-aligned but poorly crystalline patterns. These patterns are consistent with the proposed 3-fold double helical structure of gellan with small changes in pitch dependent upon type and position of branches.

Recent structural studies (Jansson *et al.*, 1983, 1985, 1986*a*, *b*; O'Neill *et al.*, 1983, 1986; Chowdhury *et al.*, 1987*a*, *b*) have revealed a family of extracellular bacterial polysaccharides (Fig. 1) whose structures are related to the structure of the linear polysaccharide gellan gum (Fig. 1a). Modifications of the gellan structure (Figs 1b–f) include partial replacement of the 4-linked α-L-rhamnosyl residue(s) in the main chain with 4-linked α-L-mannosyl residues and/or the addition of mono- or di-glycosyl side-chains.

Despite the similarity in the chemical structures, the rheology of aqueous dispersions of the polysaccharides are very different (Baird *et al.*, 1983; Colegrave, 1983; Kang *et al.*, 1983; Sandford *et al.*, 1984). Gellan gum is an effective gelling agent (Sandford *et al.*, 1984). The native polysaccharide is esterified on the 3-linked β-D-glucosyl residue with partial L-glyceric ester substitution at C2 and 50% acetic ester substitution at C6 (Kuo *et al.*, 1986). Esterification does not prevent gelation but does alter the texture of the gels (Sandford *et al.*, 1984) by inhibiting intermolecular association and crystallisation (Carroll *et al.*,

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(a) 3)βDGlcp(1→4)βDGlcpA(1→4)βDGlp(1→4)αLRhap(1→

(b) 3)βDGlcp(1→4)βDGlcpA(1→4)βDGlcp(1→4)αLRhap(1→
3
↑
1
αLRhap **or** αLManp

(c) 3)βDGlcp(1→4)βDGlcpA(1→4)βDGlcp(1→4)αL $\begin{matrix}\text{Man}p\\ \text{Rha}p\end{matrix}$(1→
3
↑
1
αLRhap

(d) 3)βDGlcp(1→4)βDGlcpA(1→4)βDGlcp(1→4)αLRhap(1→
3
↑
1
αLRhap(1→4)αLRhap

(e) 3)βDGlcp(1→4)βDGlcpA(1→4)βDGlcp(1→4)αLRhap(1→
6
↑
1
βDGlcp(1→6)αDGlcp

(f) 3)βDGlcp(1→4)βDGlcpA(1→4)βDGlcp(1→4)αL $\begin{matrix}\text{Rha}p\\ \text{Man}p\end{matrix}$(1→
4
↑
1
αLRhap

Fig. 1. Family of chemical structures based upon the structure of gellan gum. Bacterial polysaccharide structures reported for (a) gellan gum produced by *Pseudomonas elodea*, (b) S-130 produced by *Alcaligenes* ATCC 31555, (c) S-88 produced by *Alcaligenes* ATCC 31554, (d) S-657 produced by *Xanthomonas* ATCC 53159, (e) S-194 produced by *Alcaligenes* ATCC 31961, and (f) S-198 produced by *Alcaligenes* ATCC 31853.

1982, 1983). The branched polymers exhibit good thermal stability and thixotropy but do not gel (Baird *et al.*, 1983; Colegrave, 1983; Kang *et al.*, 1983; Sandford *et al.*, 1984). In an attempt to account for these differences, early comparative X-ray fibre diffraction studies of gellan and S-130 (Attwool *et al.*, 1986) have been extended to include other members of this family of polysaccharides.

Figure 2 shows X-ray fibre diffraction patterns obtained for the native branched polysaccharides S-130, S-198, S-194 and S-88. All of these branched polysaccharides yielded patterns indicating good molecular alignment but poor intermolecular packing or crystallisation. Two

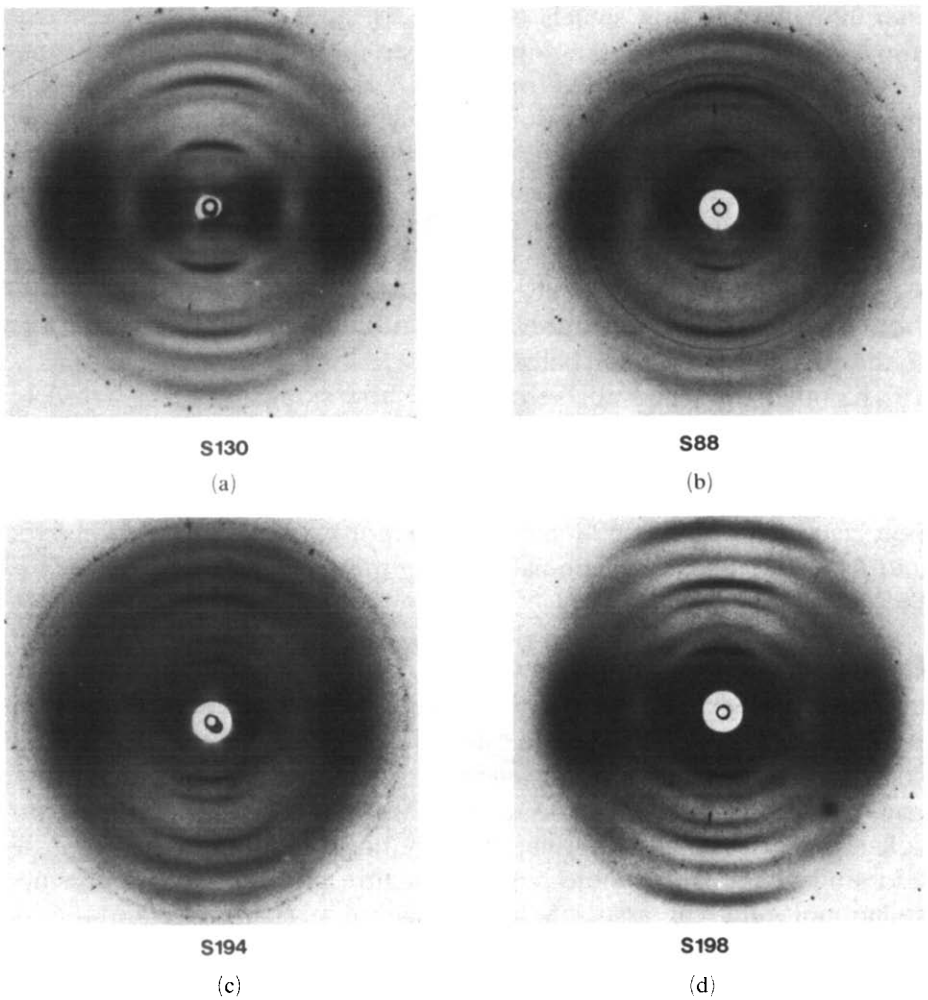

Fig. 2. X-ray fibre diffraction patterns obtained for (a) aged S-130, (b) S-88, (c) S-194 and (d) S-198. The fibre axis is vertical. Cast films of the branched polysaccharides were oriented by stretching, with typical extensions of 100–150%. The X-ray wavelength was 0·154 nm and the flat plate camera was flushed with helium. Data similar to (a) and (d) has been obtained independently by Atkins and Attwool (Attwool, 1987). Samples were examined as mixed cation forms.

different types of X-ray fibre diffraction patterns were obtained for S-130. The first type of pattern has been reported previously (Attwool *et al.*, 1986) and consists of meridional diffraction signals as orders of a spacing of 1·83 nm. This value correlates with the expected extended length (~ 2 nm) for the chemical repeat units. The second type of pattern obtained for S-130 is shown in Fig. 2a. This is characterised by strong

meridional diffraction signals as orders of a spacing of 0·92 nm and apparently occurring on the third and sixth layer lines. These reflections are similar to those observed for gellan (Attwool *et al.*, 1986) suggesting that S-130 may adopt a three-fold gellan-like helix. The apparent halving of the meridional spacing in the second type of pattern is suggestive of a double helical structure. The latest modelling studies of gellan (Chandrasekaran *et al.*, 1988*a*, *b*) suggest that the polysaccharide forms a three-fold left-handed double helix with an axial advance per repeat of 0·94 nm. The change in pitch observed for S-130 presumably results from stereochemical restrictions imposed by the addition of the branch to the outside of the 'gellan helix'.

The other branched polysaccharides show similar patterns to S-130. The patterns obtained for S-194, S-198 and S-88 all yield meridional diffraction signals as orders of spacings of 0·92 nm, 0·92 nm and 0·93 nm, respectively. Thus it would seem that, for this entire family of polysaccharides, the backbone conformation is dominant and the type and nature of the side-chain causes only minor changes in the pitch of the 'gellan helix'.

Partial substitution of the gellan helix by ester substituents has been shown (Carroll *et al.*, 1982, 1983; Attwool *et al.*, 1986) to restrict intermolecular association and crystallisation. This presumably results from the effect of the large L-glyceric ester substituent. Figure 2 suggests that complete substitution of the gellan helix with branched residues completely prevents crystallisation. Thus the solution properties of gellan and its branched derivatives may have a common origin in the adoption of the stiff double helical structure but only the unbranched gellan molecules can associate and crystallise, permitting gelation in the presence of gel-promoting cations. Further analysis of the X-ray data, or of solution properties is complicated by the fact that the partial substitution of L-mannosyl residues for L-rhamnosyl residues indicated in Fig. 1 may be indicative of the presence of a mixture of polysaccharides. In this context it is interesting to note that a recently discovered gelling polysaccharide (NW-11) is believed to be structurally similar to gellan and to differ only in the replacement of L-rhamnose by L-mannose (O'Neill *et al.*, 1990).

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