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XM-6: A New Gel-forming Bacterial Polysaccharide

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SUMMARY

A new extracellular microbial polysaccharide, XM-6, has been isolated from cultures of an Enterobacter *species and shows unusual gelation properties of potential technological significance. The polysaccharide contains* D*-glucose,* L*-fucose and* D*-glucuronate in the approximate molar ratio 3:1:1. No significant amounts of acetate or pyruvate were detected.* D*-Glucuronate and some* D*-glucose are destroyed on periodate oxidation, but* L*-fucose and some* D*-glucose may be recovered intact, indicating the presence of some 1,3 linkages in the primary structure. The major oligosaccharide isolated from autohydrolysates was an aldobiuronic acid containing equal amounts of* D*-glucose and* L*-fucose.*

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Thermally-reversible gels are formed on addition of salt to solutions of the polysaccharide. A preliminary investigation of the mechanism of gelation by optical rotation, circular dichroism, high resolution n.m.r. and mechanical spectroscopy suggests interchain association through conformationally ordered 'junction zones', with specific incorporation of site-bound cations within the ordered structures. In the sol state the polysaccharide shows the shear-rate and temperature dependence of viscosity typical of a disordered ('random coil') polymer solution. Divalent cations are, in general, more effective than monovalent cations in promoting gelation of XM-6, while trivalent cations normally cause precipitation. Within Groups I and II, optimum gelation is achieved with Na^+ *and* Ca^{2+} *(ionic radius* $\simeq$ *0·1 nm), with larger and smaller ions becoming progressively less effective. Both gel strength and melting temperature increase with increasing salt concentration.*

XM-6 forms gels of reasonable strength at unusually low concentrations of the polysaccharide. For example, gels comparable to those required for normal industrial or food applications may be obtained using 0·3% w/v XM-6 and 1% w/v NaCl. Gel strength increases with increasing polymer concentration but there is no systematic variation in melting point. The sol-gel transition of XM-6 is unusually sharp and, by suitable adjustment of salt concentration, can be made to occur just below body temperature (e.g. 30–35°C), with obvious implications for biomedical or food applications.

INTRODUCTION

Although an increasing number of microbial polysaccharides have been examined for potentially useful physical properties, relatively few appear to be capable of gel formation. One exception is curdlan, a 1,3 linked β-D-glucan synthesised by *Agrobacterium* spp. and by *Rhizobium meliloti* (Harada, 1979; Harada and Amemura, 1981). Another gel-forming polymer is an acetylated heteropolysaccharide from *Pseudomonas elodea* containing glucose, rhamnose and uronic acid in the approximate ratio 2:3:1 (Cottrell, 1980; Moorhouse *et al.*, 1981; Kang *et al.*, 1982). This material was unusual in that aqueous solutions, after heating and cooling, formed a weak and very elastic gel. Following deacetylation at pH 10·0, the polysaccharide formed firm non-elastic brittle gels with a melting point of 90°C.

We now report the unusual and potentially valuable gelation behaviour of a new microbial polysaccharide, XM-6, isolated as an extracellular product of an *Enterobacter* species (deposited as NCIB 11870 with the National Collection of Industrial Bacteria, Torry, Aberdeen).

METHODS

Polysaccharide XM-6 was isolated from culture and purified, using standard procedures. Sugar ratios in the native and periodate-oxidised polymer were determined by alditol acetate analysis on a Pye 104 chromatograph, using columns of 3% SP-2340 on 100/120 Supelcoport, or OV225. Uronic acid was determined by the method of Blumenkrantz & Asboe-Hansen (1973). Gels were prepared by addition of an appropriate salt solution to an aqueous solution of the polymer, heating to ~45°C to melt the initial gel and recooling. Solutions for optical studies were clarified by filtration through a 0·45 μm Millipore membrane.

The gel strength (yield stress) was determined using a simple penetrometer constructed for the purpose while the rigidity modulus was measured on a Rheometrics Mechanical spectrometer (RMS-605) using the standard transducer with a 50 mm diameter cone and plate of cone angle 0·04 rad. Solution viscosity measurements were made on a Ferranti cone and plate viscometer. The optical rotation was measured at 436 nm on a Perkin Elmer 241 polarimeter, and circular dichroism spectra were recorded on a Jobin-Yvon Dichrograph Mk V. In both cases, the temperature was controlled by a circulating water bath and monitored by a thermocouple within the cell, but out of the light path. High resolution ^{1}H-n.m.r. measurements were made at 300 MHz on a Bruker CXP 300 Fourier transform spectrometer. Peak areas were quantified relative to a standard pyrazine solution contained in a 2 mm-diameter capillary located centrally within the n.m.r. tube.

COMPOSITION

Hydrolysates of the polysaccharide (1 M total fatty acid (TFA) at 100°C for 16 h) revealed material chromatographically identical with glucose, fucose and glucuronic acid. Preparation of alditol acetates confirmed the identity of the neutral sugars and the colour development in the procedure of Blumenkrantz & Asboe-Hansen (1973) confirmed the presence of uronic acid. The ratio of sugar components

TABLE 1

Composition of Polysaccharide XM-6

Polymer	*Molar ratios*				
	L-fucose		*D-glucose*		*D-glucuronate*
Native polysaccharide	1	:	3·3	:	1·06
Oxidised polymer	1	:	1·96	:	–

before and after periodate oxidation of the polysaccharide is shown in Table 1. The D-configuration of the glucose residues was confirmed by glucose oxidase. No significant amounts of *O*-acetyl groups or pyruvate ketal were detected.

Attempts to obtain oligosaccharides by partial acid hydrolysis were largely unsuccessful. However, autohydrolysis of a 0·5% (w/v) solution of the acid form of the polymer yielded one major oligosaccharide product. This proved to be an aldobiuronic acid which on hydrolysis yielded equal quantities of fucose and glucuronic acid. Other oligosaccharides, obtained in lesser yield, were a trisaccharide containing equimolar amounts of fucose, glucuronic acid and glucose and a tetrasaccharide in which the same three monosaccharides were present in a molar ratio of 1:1:2. One neutral disaccharide containing D-glucose only was obtained. Detailed structural studies will be published elsewhere.

GELATION BEHAVIOUR

Gelation of XM-6 polysaccharide may be induced by addition of salt to an aqueous solution of the polymer. The resulting gels are thermally reversible and show sharp melting and setting behaviour. More homogeneous and cohesive gels may be obtained by melting the gel formed initially at room temperature and allowing it to reform on cooling, or by mixing polymer and salt solutions at a temperature above the melting point of the final gel.

The ionic concentration required varies widely for different salts and, in common with other gelling polymers (Richardson & Ross-Murphy, 1981), a minimum critical concentration of the polysaccharide is required for gel formation. In general, gels of comparable strength to those normally used for typical industrial or food applications (Whistler, 1959; Glicksman, 1969) may be obtained at a polymer concentration of ~0·3%, i.e. somewhat higher than for agar, but appreciably lower than for carrageenan, alginate, gelatin or starch.

The strength (yield stress) of XM-6 gels formed at this polymer concentration is shown in Fig. 1 for different concentrations of various cations (chloride salt form). In all cases, gel strength increases with increasing salt concentration. Divalent cations are in general more effective than monovalent in promoting gelation of XM-6, while trivalent cations normally cause the polymer to precipitate. Cohesive gels were, however, obtained with Fe^{3+} ions over the concentration range 0·30–0·34 mM.

There is also considerable selectivity between different cations of the same valency. Figure 2 shows the molar concentrations of Group I and

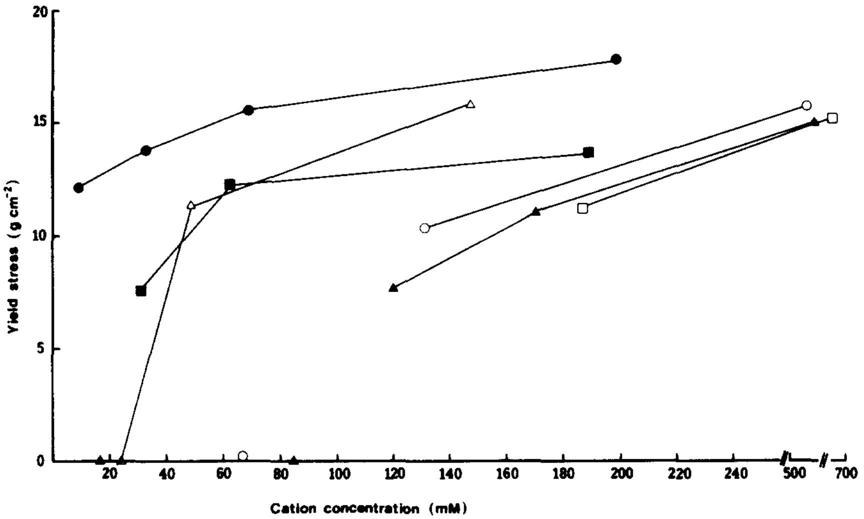

Fig. 1. Variation in gel strength (yield stress; 25°C) of polysaccharide XM-6 (0·3% w/v) with cation concentration. The cations shown (chloride salt form) are Na$^+$ (▲), K$^+$ (○), NH$_4^+$ (□), Mg^{2+} (△), Ca^{2+} (●) and Sr^{2+} (■).

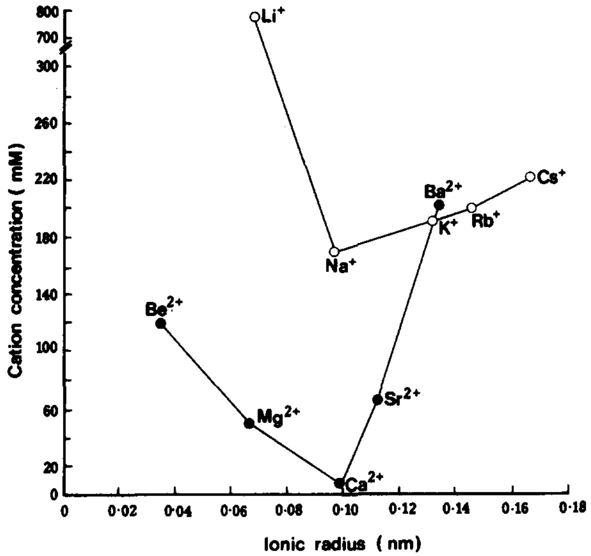

Fig. 2. Molar concentrations of different cations (chloride salt form) required to produce XM-6 gels (0·3% w/v; 25°C) of the same strength (yield stress = 10·9 g cm^{-2}).

Group II cations required to produce gels of the same strength, using a fixed concentration (0·3% w/v) of polymer. In both cases, optimum gelation is obtained at an ionic radius of ~0·1 nm (Na^+ and Ca^{2+} respectively), with higher concentrations being required for both larger and smaller cations.

The minimum critical concentration of polymer necessary for gelation also varies with the cation used (Table 2). For example, at the lowest polysaccharide concentration tested (0·05% w/v) a gel was formed only with Ca^{2+}. At concentrations above the minimum required for gelation, gel strength increases with increasing polymer concentration as illustrated in Fig. 3.

The melting temperature of XM-6 polysaccharide gels shows little, if any, systematic variation with polymer concentration, but increases appreciably with increasing ionic strength (Table 2). Similar behaviour is well-known for other charged polysaccharides (Milas & Rinaudo, 1979; Morris *et al.*, 1980), and may be attributed to suppression of electrostatic repulsion, thus facilitating interchain association and

TABLE 2
Properties of XM-6 Gels

Polymer %	*Salt*	*mM*	*Melting temperature (°C)*	*Gel strength (g cm^{-2})*
0·1	NaCl	170	30	–
0·1	NaCl	509	40	1·3
0·2	NaCl	170	33	2·8
0·2	NaCl	509	45	7·7
0·3	NaCl	170	30	11·3
0·3	NaCl	509	45	15·1
0·1	KCl	133	33	1·1
0·1	KCl	400	40	2·85
0·2	KCl	133	30	–
0·2	KCl	400	45	7·3
0·3	KCl	133	30	6·4
0·3	KCl	400	45	16·3
0·1	CsCl	178	30	–
0·2	CsCl	178	33	6·4
0·3	CsCl	178	33	5·6
0·1	LiCl	235	30	–
0·1	LiCl	706	30	1·4
0·2	LiCl	235	30	1·4
0·2	LiCl	706	40	4·8
0·3	LiCl	706	45	10·2
0·1	NH_4Cl	190	30	0·6
0·1	NH_4Cl	571	33	0·95
0·2	NH_4Cl	190	37	2·3
0·2	NH_4Cl	571	45	5·2
0·3	NH_4Cl	190	30	10·7
0·3	NH_4Cl	571	45	15·5
0·1	$MgCl_2$	49	30	0·7
0·1	$MgCl_2$	148	30	1·7
0·2	$MgCl_2$	49	33	3·4
0·2	$MgCl_2$	148	33	5·7
0·3	$MgCl_2$	49	33	11·2
0·3	$MgCl_2$	148	45	13·9

TABLE 2 (*Continued*)

Polymer %	*Salt*	*mM*	*Melting temperature (°C)*	*Gel strength (g cm^{-2})*
0·3	$SrCl_2$	31	33	4·6
0·3	$SrCl_2$	63	40	11·6
0·3	$SrCl_2$	188	45	10·6
0·05	$CaCl_2$	202	33	0·5
0·1	$CaCl_2$	67	40	1·3
0·1	$CaCl_2$	202	45	3·5
0·2	$CaCl_2$	67	40	7·0
0·2	$CaCl_2$	202	45	8·0
0·3	$CaCl_2$	67	33	12·1
0·3	$CaCl_2$	335	40	13·9
0·3	$CaCl_2$	67	45	15·6
0·3	$CaCl_2$	202	45	16·6

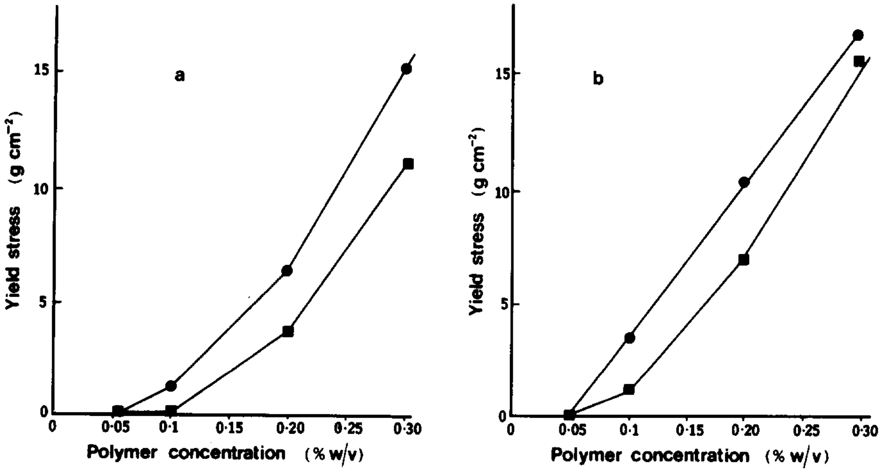

Fig. 3. Effect of polymer concentration on XM-6 gel strength (yield stress; 25°C). (a) Na^+ concentrations of 170 (■) and 500 (●) nM (1% and 3% NaCl, respectively). (b) Ca^{2+} concentrations of 67 (■) and 200 (●) mM (1% and 3% $CaCl_2.2H_2O$, respectively).

network formation. In the following section, we report some preliminary characterisation of the mechanism of gelation, by a variety of physical techniques.

CONFORMATIONAL STUDIES

The thermally-induced gel–sol transition of polysaccharide XM-6 is accompanied by a sharp change in optical rotation, with a closely similar temperature course (Fig. 4) to the loss of gel structure, as monitored by rigidity modulus (G'). Polysaccharide optical rotation is sensitive to chain geometry (Rees, 1970) and an abrupt change of this

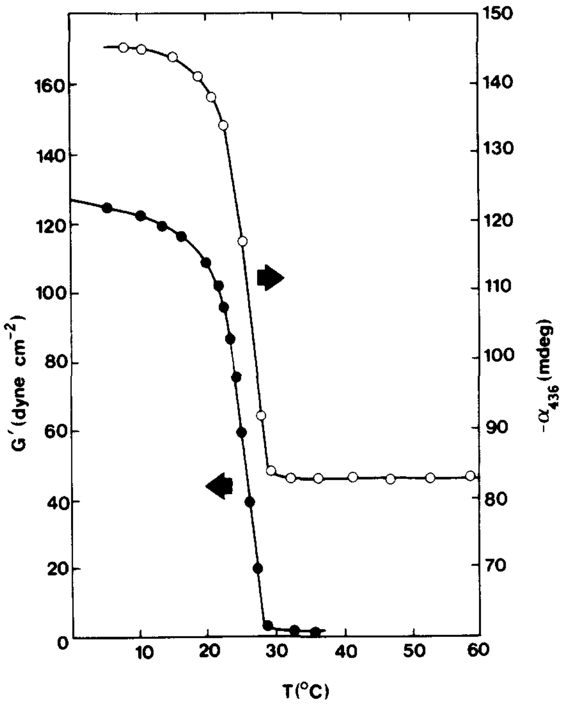

Fig. 4. Gel–sol transition of XM-6 (0·3% w/v; 0·2 M NaCl), monitored by the temperature (T) dependence of rigidity modulus, G' (●) and optical rotation (o). G' was measured at 10 rad s^{-1} and 10% strain, using a heating rate of 1°C min^{-1}; optical rotation was measured at 436 nm in a 10 cm-pathlength cell.

type is indicative of a cooperative conformational transition (Bryce *et al.*, 1974). For example, the thermally-induced order–disorder transitions of agarose (Arnott *et al.*, 1974), carrageenan (McKinnon *et al.*, 1969; Morris *et al.*, 1980) and xanthan (Rees, 1972; Holzwarth, 1976; Morris *et al.*, 1977; Milas & Rinaudo, 1979) all give optical rotation–temperature profiles similar to that shown in Fig. 4. In comparison to these other systems, however, the XM-6 transition is unusually sharp, suggesting a highly cooperative process (Zimm & Bragg, 1959).

Figure 5 shows the high resolution ^{1}H-n.m.r. spectrum of XM-6 recorded at 300 MHz in the high temperature (sol) state. The spectrum includes a complex resonance at $\delta = 1{\cdot}5$, which we tentatively assign to overlapping methyl doublets from fucose residues in non-equivalent molecular environments. This band is well-resolved from other peaks in the spectrum and may, therefore, be used in quantitative characterisa-

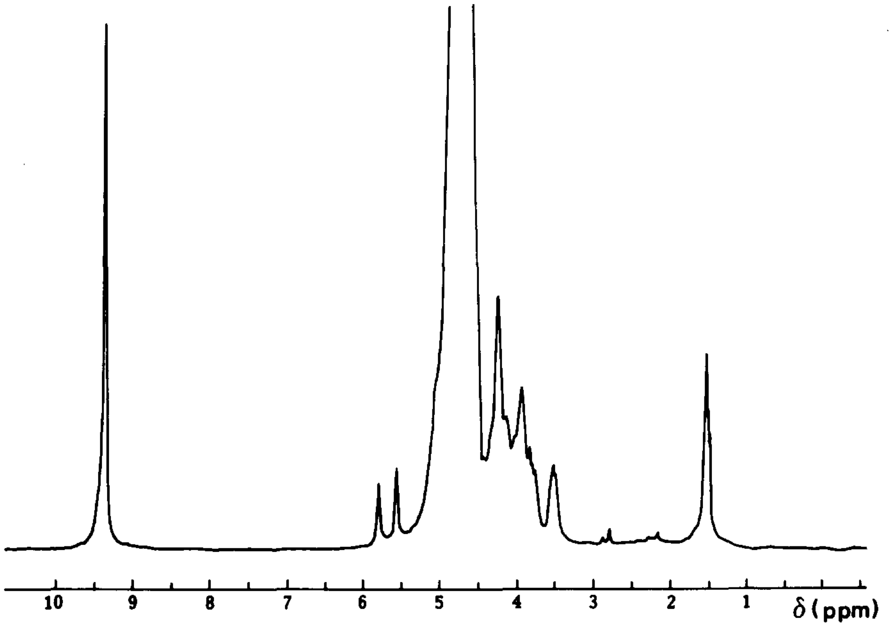

Fig. 5. High resolution ^{1}H-n.m.r. spectrum of XM-6 recorded at 300 MHz in the disordered (sol) state at 60°C. The band at $\delta \simeq 1{\cdot}5$ was used to quantify the residual visible signal at lower temperatures, relative to a pyrazine standard at $\delta \simeq 9{\cdot}4$.

tion of the mechanism of gelation. As shown in Fig. 6, the conformation transition indicated by optical rotation is accompanied by a progressive loss of detectable high resolution n.m.r. signal on cooling, as expected when a conformationally rigid, ordered structure is adopted (Bryce *et al.*, 1974). The changes in both optical rotation and n.m.r. are fully reversible (Fig. 6), with no detectable thermal hysteresis.

At temperatures above the gel-point, solutions of XM-6 show the pseudoplastic viscosity behaviour (Fig. 7) typical of polymer solutions (Ferry, 1980). Viscosity decreases with increasing temperature, as expected for a disordered ('random coil') polysaccharide, rather than a conformationally rigid structure such as that of xanthan (Morris *et al.*, 1977), which shows little temperature dependence (Rock, 1971). The

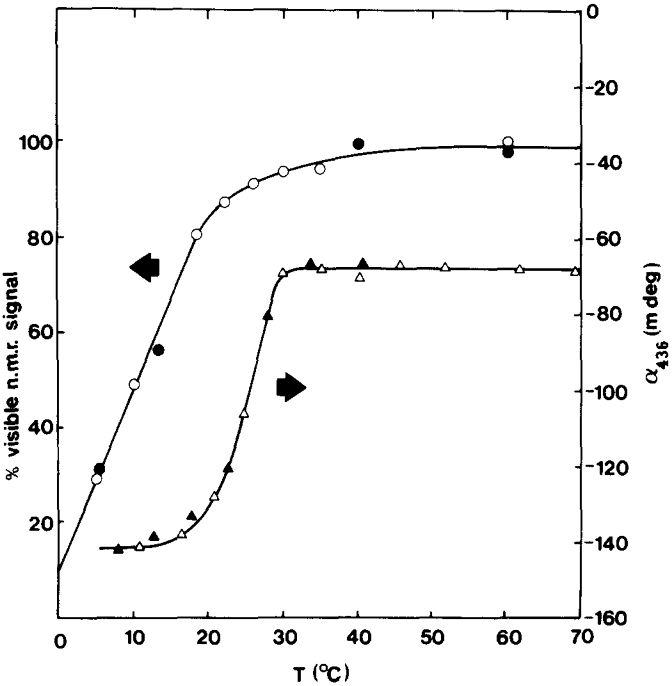

Fig. 6. Order-disorder transition of XM-6 (0·3% w/v in D_2O; 0·2 M NaCl), monitored by loss of detectable high-resolution n.m.r signal (circles) and by optical rotation (triangles). Filled symbols show results obtained on heating and open symbols results obtained on cooling. Optical rotation was measured at 436 nm in a 10 cm-pathlength cell.

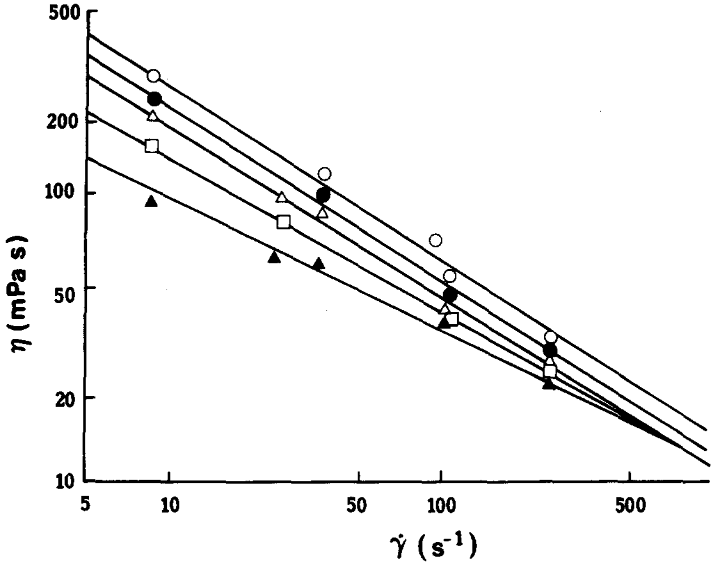

Fig. 7. Shear rate (γ̇) dependence of viscosity (η) for an aqueous solution of XM-6 polysaccharide (0·3% w/v) at 70°C (▲), 60°C (□), 50°C (△), 40°C (●) and 25°C (○).

evidence presented so far therefore indicates that the gelation of XM-6 involves a cooperative transition from a disordered chain conformation to a conformationally ordered structure.

Figure 8 shows the circular dichroism (c.d.) of XM-6 over a range of temperatures spanning the sol–gel transition. The spectra are similar to those reported (Morris *et al.*, 1975) for the carboxyl $n \rightarrow \pi^*$ transition of uronic acid glycosides, consistent with the presence of glucuronosyl residues in the primary structure of XM-6. The negative sign of the observed c.d. band confirms the D configuration of these residues. Since neutral sugars such as glucose and fucose have no electronic transitions over the wavelength range shown in Fig. 8 (Morris & Frangou, 1981) and acetate and/or pyruvate substituents are present in negligible amounts, if at all, observed c.d. intensities may be expressed as molar ellipticity, $[\theta]$, per glucuronate residue (Fig. 8, right-hand axis) using the composition data from Table 1.

The sol–gel transition of XM-6 is accompanied by an appreciable enhancement of c.d. intensity which follows the same temperature course as the change in optical rotation at higher wavelengths (Fig. 9).

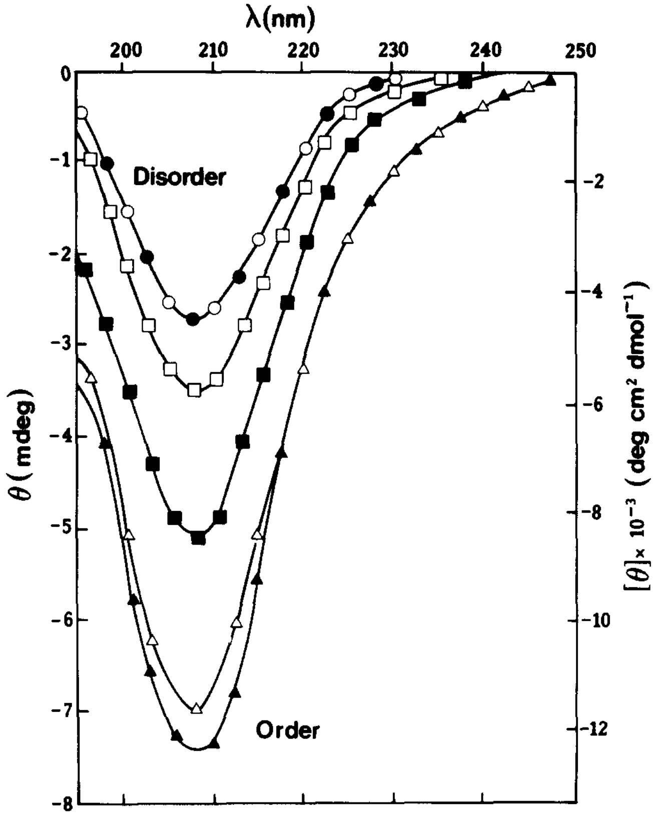

Fig. 8. Circular dichroism (θ) of XM-6 polysaccharide (0·3% w/v; 0·2 M NaCl; 0·2 mm pathlength) at 43°C (○), 31°C (●), 28°C (□), 25°C (■), 22°C (△) and 13°C (▲). Molar ellipticity values per glucuronate residue ([θ]) are also shown.

As shown in Fig. 10, the c.d. change between the sol and gel states is similar in form to the spectral changes observed on Ca^{2+}-induced gelation of alginate or pectate (Morris *et al.*, 1978; Thom *et al.*, 1982) and centred at the same wavelength (≃ 208 nm), although of somewhat greater magnitude. The direction of c.d. change (to more negative values) for the D-glucuronate residues of XM-6 is the same as that for D-galacturonate in pectin, and opposite to that observed for L-guluronate residues of alginate (as expected for near mirror-image molecules experiencing the same perturbation). These close similarities in c.d. behaviour suggest that the underlying molecular process causing the c.d.

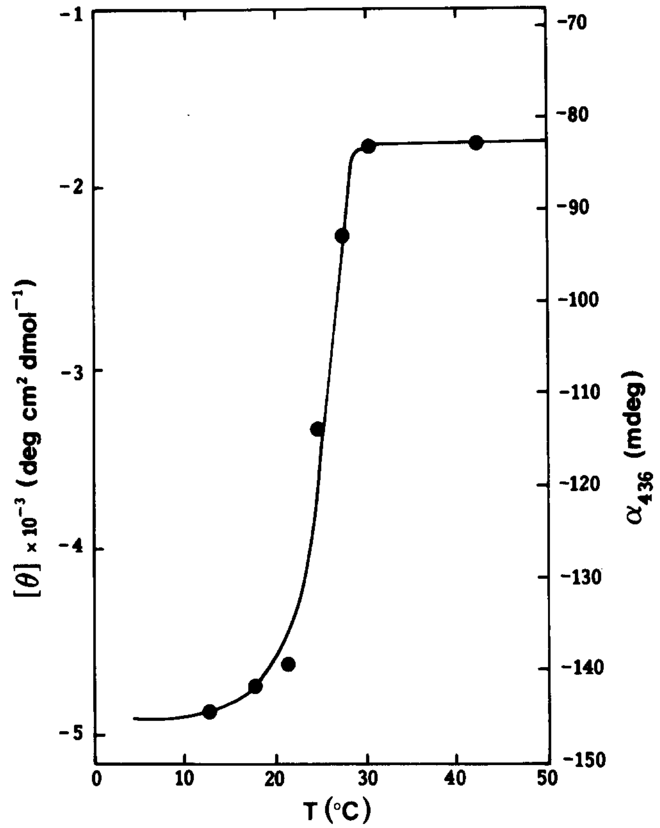

Fig. 9. Comparison of the temperature (T) dependence of circular dichroism (●) and optical rotation (——) of XM-6 polysaccharide (0·3% w/v; 0·2 M NaCl) throughout the sol-gel transition.

change may also be similar in all three cases, i.e. specific site-binding of cations in close proximity to the carboxyl chromophores.

Figure 11 shows the temperature course of optical rotation change for two different concentrations of XM-6 under identical high salt conditions (1 M NaCl). Although for experimental reasons the concentration range studied is small (two-fold increase), there is no evidence of any change in transition midpoint temperature (T_m) with polymer concentration. This is consistent with the gel melting points recorded in Table 2, which also show no systematic variation with polymer

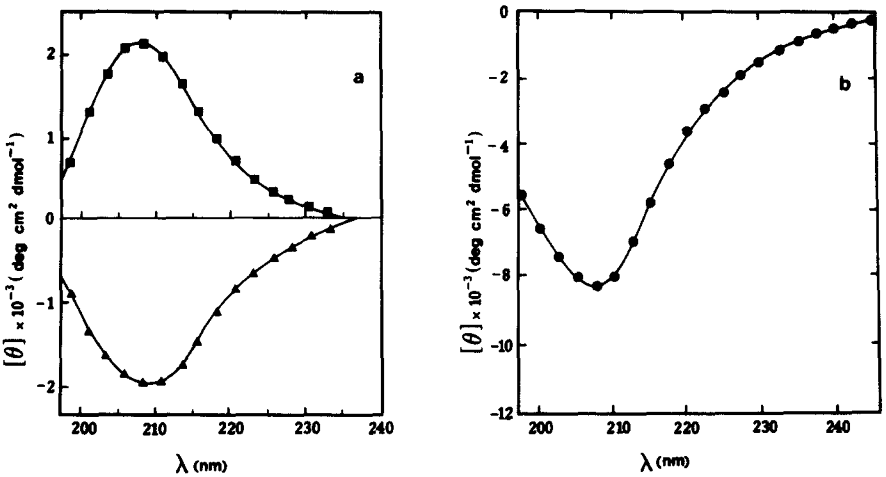

Fig. 10. Circular dichroism changes accompanying cation-induced gelation. (a) Ca^{2+}-induced gelation of alginate poly-L-guluronate sequences (■) and poly-D-galacturonate sequences from pectin (▲). (b) Na^{+}-induced gelation of XM-6 (●) (0·3% w/v; 0·2 M NaCl; 0·2 mm pathlength). (With permission, from Morris *et al.* (1982), © Academic Press (London) Ltd.)

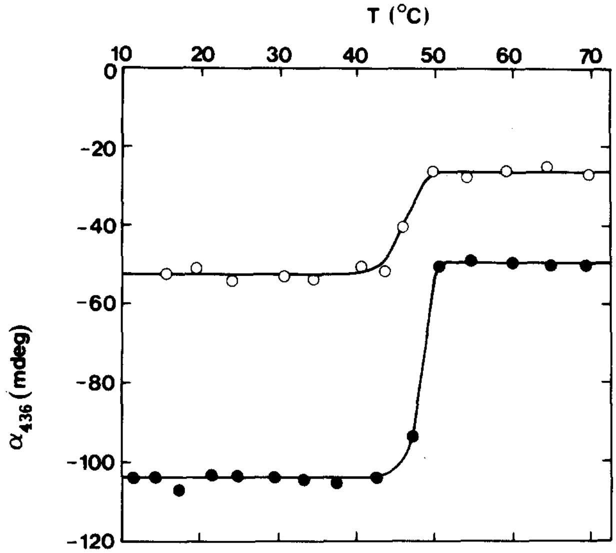

Fig. 11. Temperature dependence of optical rotation (436 nm; 5 cm pathlength), for XM-6 polysaccharide in 1·0 M NaCl, at polymer concentrations of 0·5 (○) and 1·0 (●) mg ml^{-1}.

concentration. The observed value of T_m does, however, increase significantly with increasing salt concentration (compare Figs 4 and 11), which is again consistent with the melting-point data.

GENERAL DISCUSSION AND CONCLUSIONS

The gelation properties of XM-6 are quite different from those of other known microbial polysaccharides and of all current commercial hydrocolloids and may be of potential technological value. In particular, gel melting occurs over a very narrow temperature range which, using a suitable salt concentration, may be conveniently adjusted to fall just below body temperature. The ability of gelatin gels to 'melt in the mouth' is one of their most valuable attributes, but has not so far been duplicated satisfactorily in any other gelling system.

The concentration of XM-6 required to give reasonable gel strength is unusually low, matched only by the extremely expensive polysaccharide, agar. The new polysaccharide combines the cation-induced gelation behaviour of alginate and pectate at ambient temperature with the thermal melting displayed by agar, carrageenan and gelatin gels and is in this respect unique.

Our spectroscopic studies indicate that, as in the case of other gelling polysaccharides (Rees *et al.*, 1982), gelation of XM-6 occurs by formation of extended, cooperative junction zones in which the participating chains are locked in a regular, ordered conformation, while on thermal melting the polymer reverts to the disordered ('random coil') state. The order–disorder transition is unusually sharp, indicating a high degree of cooperativity. The absence of any detectable thermal hysteresis further suggests that interchain association is limited to the formation of ordered junctions involving a small, finite number of chains, rather than large aggregates as in the case of, for example, agarose (Liang *et al.*, 1979; Morris & Norton, 1983).

The marked selectivity for cations of a particular ionic radius (Fig. 2) indicates specific incorporation of counterions within the ordered structure (as in the 'egg-box' binding of divalent cations in alginate and pectate gels (Grant *et al.*, 1973)) rather than non-specific 'condensation' of ions along the polymer chain (Manning, 1972). The large, characteristic changes in carboxyl c.d. which accompany the sol–gel transition (Fig. 10) lend further support to this interpretation. These changes follow the same temperature course as the disorder–order transition of the polymer chain, indicating that cation binding is an integral part of the ordering process, rather than occurring as a separate event.

In summary, the gelation of XM-6 shows features in common with both the cation-induced gelation of alginate and pectate and the thermal gel–sol transition of the double-helix forming polysaccharides agar and carrageenan. In contrast to alginate and pectate, however, the cation-induced gels of XM-6 are thermally reversible and occur at a substantially lower concentration, while in contrast to carrageenan and agar polysaccharides, cations appear to be a necessary, integral part of the ordered structure, rather than simply promoting subsequent association of such ordered structures once formed (cf. Morris *et al.*, 1980).

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REFERENCES

Arnott, S., Fulmer, A., Scott, W. E., Dea, I. C. M., Moorhouse, R. & Rees, D. A. (1974). *J. Mol. Biol.* **90**, 269.

Blumenkrantz, N. & Asboe-Hansen, G. (1973). *Anal. Biochem.* **54**, 484.

Bryce, T. A., McKinnon, A. A., Morris, E. R., Rees, D. A. & Thom, E. (1974). *Faraday Discuss. Chem. Soc.* **57**, 221.

Cottrell, I. W. (1980). *ACS Symp. Ser.* **126**, 251.

Ferry, J. D. (1980). *Viscoelastic Properties of Polymers*, 3rd edn, Wiley, New York.

Glicksman, M. (1969). *Gum Technology in the Food Industry*, Academic Press, New York.

Grant, G. T., Morris, E. R., Rees, D. A., Smith, P. J. C. & Thom, D. (1973). *FEBS Letts* **32**, 1.

Harada, T. (1979). In: *Polysaccharides in Food*, eds J. M. V. Blanshard and J. M. Mitchell, Butterworths, London, p. 283.

Harada, T. & Amemura, A. (1981). *Mem. Inst. Sci. Ind. Res. Osaka Univ.* **38**, 37.

Holzwarth, G. (1976). *Biochemistry* **15**, 4333.

Kang, K. S., Veeder, G. T., Mirrasoul, P. J., Kaneko, T. & Cottrell, I. W. (1982). *Appl. Env. Microbiol.* **43**, 1086.

Liang, J. N., Stevens, E. S., Morris, E. R. & Rees, D. A. (1979). *Biopolymers* **18**, 327.

McKinnon, A. A., Rees, D. A. & Williamson, F. B. (1969). *Chem. Commun.*, 701.

Manning, G. S. (1972). *Ann. Rev. Phys. Chem.* **23**, 117.

Milas, M. & Rinaudo, M. (1979). *Carbohydr. Res.* **76**, 189.

Moorhouse, R. *et al.* (1981). *ACS Symp. Ser.* **150**, 111.

Morris, E. R. & Frangou, S. A. (1981). *Techniques in Carbohydrate Metabolism*, Elsevier, Amsterdam, B308.

Morris, E. R. & Norton, I. T. (1983). In *Aggregation Processes in Solution*, eds E. Wyn Jones and J. Gormally, Elsevier, Amsterdam.

Morris, E. R., Rees, D. A., Sanderson, G. R. & Thom, D. (1975). *J. Chem. Soc. Trans. Perkin II*, 1418.

Morris, E. R., Rees, D. A., Young, G., Walkinshaw, M. D. & Darke, A. (1977). *J. Mol. Biol.* **110**, 1.

Morris, E. R., Rees, D. A., Thom, D. & Boyd, J. (1978). *Carbohydr. Res.* **66**, 145.

Morris, E. R., Rees, D. A. & Robinson, G. (1980). *J. Mol. Biol.* **138**, 349.

Morris, E. R., Powell, D. A., Gidley, M. J. & Rees, D. A. (1982). *J. Mol. Biol.* **155**, 507.

Rees, D. A. (1970). *J. Chem. Soc. B*. 469.

Rees, D. A. (1972). *Biochem. J.* **126**, 257.

Rees, D. A., Morris, E. R., Thom, D. & Madden, J. K. (1982). In *The Polysaccharides*, vol. 1, ed. G. O. Aspinall, Academic Press, New York, p. 195.

Richardson, R. K. & Ross-Murphy, S. B. (1981). *Int. J. Biol. Macromol.* **3**, 315.

Rock, J. K. (1971). *Food Technology* **25**, 476.

Stipanovich, A. J. & Stevens, E. S. (1981). *ACS Symp. Ser.* **150**, 303.

Thom, D., Grant, G. T., Morris, E. R. & Rees, D. A. (1982). *Carbohydr. Res.* **100**, 29.

Whistler, R. L. (1959). *Industrial Gums*, Academic Press, New York.

Zimm, B. H. & Bragg, J. K. (1959). *J. Chem. Phys.* **31**, 526.