Supramolecular structure of microbial polysaccharides in solution: from chain conformation to rheological properties*

Attilio Cesàro†‡§, Amelia Gamini‡ and Luciano Navarini§
‡Dipartimento di Biochimica, Biofisica e Chimica delle Macromolecole, Università di Trieste, 34127 Trieste, Italy
§Laboratorio di Biopolimeri Tecnologici, Centro Ricerche Polybiòs, Area di Ricerca, Padriciano 99, 34012 Trieste, Italy
(*Received 20 January 1992; accepted 3 June 1992*)

Literature data and unpublished results have been used to correlate the primary and secondary structures of gellan, welan, rhamsan, succinoglycan and galactoglucan in solution with their rheological properties. In dilute solution the chain conformation ranges from that of the random coil of galactoglucan to that of the stiffer double helix of gellan, and possibly a similar conformation exists for welan and rhamsan. In semidilute solution the rheological behaviour of the above microbial polysaccharides is mainly determined by their conformation in solution. Ordered regions along the polysaccharide chains seem to be the prerequisite to establish intermolecular interactions which lead to non-transient polymer networks. The lack of ordered secondary structure leads to entangled networks in which the topological constraints govern the rheological behaviour. Experimental evidence is reported to support the hypothesis that the chain conformation and rigidity can be qualitatively predicted on the basis of rheological measurements.

(Keywords: microbial polysaccharides; solution properties; conformation; rheological properties)

INTRODUCTION

The actual and commercial potential of many microbial polysaccharides has been well recognized. These polysaccharides play important biological roles, such as physical (mechanical) and chemical (solvation) protection of bacterial cells against environmental changes.

The solution properties of polysaccharides are of considerable interest for a number of practical commercial applications, such as thickening, suspending and gelling agents[1]. The wide range of rheological behaviour shown by polysaccharides in aqueous solution is due to the variety of conformations and chain flexibility, spanning from the coil to the rigid rod limit which is peculiar for this class of polymers. The variability of chain flexibility arises from both the large number of naturally available sugar residues and of linkage patterns and from the possible occurrence of intra- and intermolecular interactions. The former controls the overall chain geometry and the latter governs the pattern of shapes and dimensions characterizing the polymer in solution. Under given conditions the above constraints may induce regular helical conformations to some extent.

The aim of this paper is to present some new data and relevant literature results on the solution conformation and rheological properties of some microbial polysaccharides and to provide a molecular (conformational) interpretation of the macroscopic properties. The rationale for this interpretation is that the rheological properties are a unique function of the three-dimensional degree of hydrodynamic correlation between interacting centres and that the supramolecular structures with various *persistence times* are dynamically formed among polysaccharide chains.

Polysaccharides offer a wide spectrum of rheological properties which in general could contribute towards the understanding of the molecular origin of viscoelastic phenomena. However, with the exception of xanthan and guar, only a few detailed rheological studies have been reported so far.

In order to correlate the chemical structure and the conformational features of the chains with the rheological behaviour displayed, five different high molecular weight polysaccharides have been considered. In particular, gellan from *Pseudomonas elodea*, and rhamsan and welan from two strains of *Alcaligenes* (structure I) share the same backbone repeating unit, but while gellan is unbranched, welan and rhamsan display a comb-like branching. Among these three structurally related polysaccharides only gellan shows a remarkable ability to form gels, the strength of which is modulated by the non-saccharidic content (i.e. acyl substitution) and type of added cations. Welan and rhamsan greatly enhance the viscosity of aqueous media, leading with increasing polymer concentration to thermally stable solutions with peculiar rheological properties.

A highly branched structure is also present in the succinoglycan (structure II), produced by several strains of *Alcaligenes*, *Pseudomonas*, *Agrobacterium* and *Rhizobium*[2], in comparison with the simple linear chain of the galactoglucan (structure III), produced by *Rhizobium*

---

* Presented at 'Speciality Polymers 91', 30 September–2 October 1991, Mainz, Germany

† To whom correspondence should be addressed

0032-3861/92/194001-08

spp. Indeed, some *Rhizobium* strains produce mixtures of succinoglycan and galactoglucan in different ratios depending on the fermentation conditions, which suggests that the production of these two polysaccharides occurs following an identical biosynthetic pathway[3]. On increasing polymer concentration at high ionic strength and low temperature succinoglycan gives highly viscous solutions, while galactoglucan exhibits the rheological behaviour of an entangled system which is generally observed for flexible synthetic polymers.

STRUCTURE AND CONFORMATION

Chemical structure

The primary structure of gellan consists of a regular sequence of tetrasaccharide (A-B-C-D) repeating units (structure I), as shown in *Figure 1*. The presence of an *O*-acetyl group every two[4] or four[5] repeating units linked to C6 of the 3-linked β-D-glucose residue has been found, in addition to one L-glycerate per repeating unit located at the C2 of the same sugar residue. The tetrasaccharide backbone of rhamsan and welan is identical (A-B-C-D) to that of gellan. In addition, two-thirds of the welan repeat unit contains terminal α-L-rhamnopyranosyl groups 1,3-linked to the residue C, while the remaining third contains α-L-mannopyranosyl groups. In addition, ~50% of the units contain an *O*-acetyl substituent[6]. The rhamsan repeat unit is characterized by the presence of a β-D-Glcp(1 — > 6)-α-D-Glcp-(1 — > 6)-disaccharide side chain linked to the A glucose residue of the main chain and by the presence of one *O*-acetyl group distributed over secondary positions[7].

The tetrasaccharide backbone of succinoglycan (structure II) bears at position 6 of one glucose residue a tetrasaccharide side chain with a pyruvic ketal linked at position O-4, O-6 on the terminal side-chain glucose. An *O*-acetyl group located on the O-6 of some of the D-glucose residues and a succinyl substituent are also present[8,9]. The molar ratios of the acyl/pyruvic and succinyl/acetyl residues are 1.05 and 1.4, respectively[9].

The galactoglucan exopolysaccharide (structure III) is composed of a disaccharide repeat unit, having galactose and glucose with acetyl and pyruvyl substituents (molar ratio 1:1:1:1)[10]. This structure is undoubtedly very simple and suitable for both molecular modelling and quantitative structure–property correlations. It is of interest to note that the presence of galactoglucan in the culture broths of bacteria mixed with succinoglycan[11] modulates the rheological properties of the broth.

Ordered helical conformations

X-ray diffraction patterns have been obtained from oriented fibres of gellan (Li^+ salt and K^+ salt)[12,13]. A three-fold left-handed double helix with a pitch of 5.63 nm has been deduced. The double helical chains are organized in parallel fashion in an intertwined duplex, one chain shifted half a pitch with respect to the other. The trigonal cell ($a = b = 1.575$ nm, $c = 2.815$ nm for the K^+ salt) allocates two duplexes in an antiparallel orientation. Oriented fibres of deacylated gellan samples, which had higher crystallinity than that of the native polymer, have been used in the X-ray diffraction studies.

Stabilization arises from the intrachain hydrogen bond between the D-glucuronate and the 1,4-linked D-glucose and the binding action of embedded cations and water molecules surrounding the polysaccharide structure. Carboxylate groups are important not only for the stability of the gellan double helix but play an essential role in conjunction with water-mediated crosslinks for the association of the double helices which eventually leads to gelation[12].

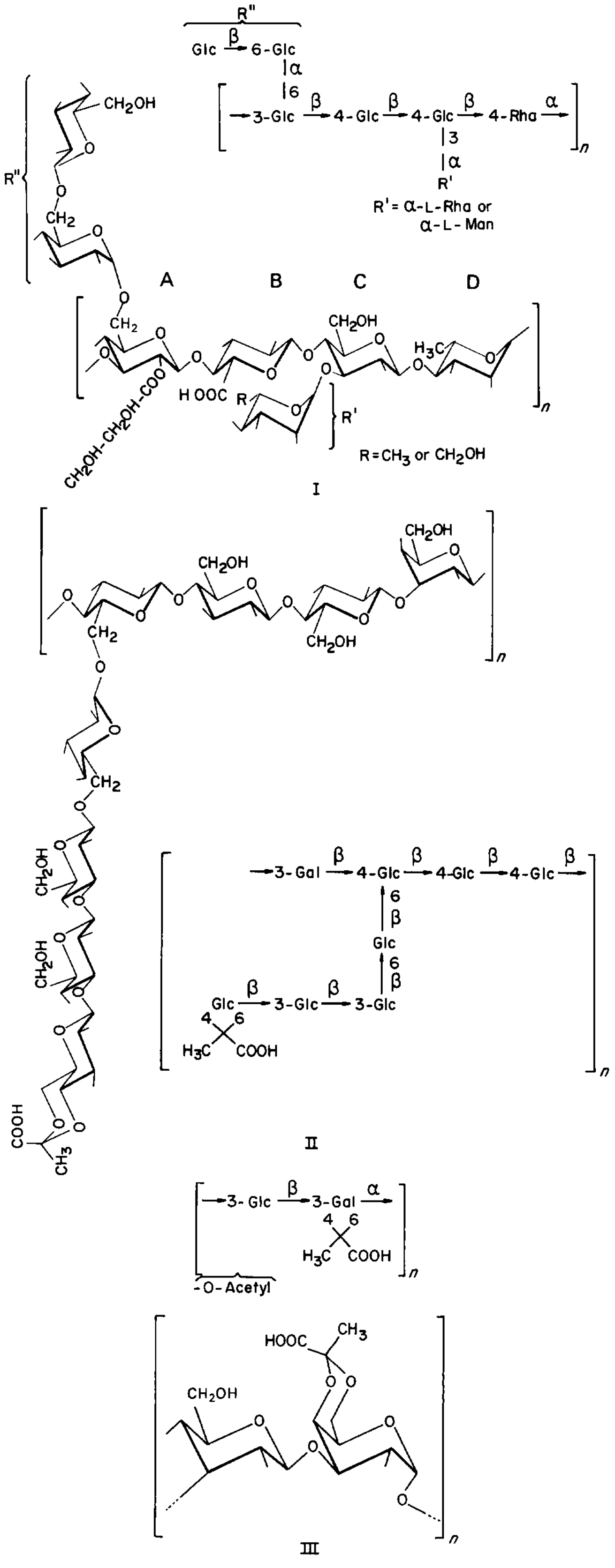

Figure 1 Structure of the repeating unit of gellan, welan and rhamsan (structure I), of succinoglycan (structure II) and of galactoglucan (structure III)

Acyl groups probably disturb the double helices packing of the native gellan, which forms only weak gels. In particular, the amount and crucial location of L-glycerate groups is a serious perturbation for the intermolecular interactions of double helices. The shielding effect on the carboxylate group also results in a decreased carboxyl-mediated association of the double helices[14].

X-ray fibre diffraction studies and computer modelling of the Na^+ form of welan and rhamsan lead to the conclusion that in the solid state they adopt the same half-staggered double helix of that of the unbranched deacylated gellan[15]. However, in the case of welan hydrogen bonding between the side chain and carboxylate group and the carboxylate shielding of the side chain reduce the ability of double helices to associate more effectively than that of the L-glycerate and acetate substituents in native gellan.

In rhamsan, X-ray and computer modelling studies showed that hydrogen bonds between the side chain and main chain cannot occur within the double helix. Although in this case the carboxylate groups are unscreened and available for chain association, the presence of the bulky and flexible side chain prevents the association of the double helices via carboxylate–cation–carboxylate interactions.

Conclusive X-ray studies on succinoglycan and galactoglucan have not yet been published. However, the form has been quoted[16] to be an extended single helical structure characterized by a repeat length of 1.92 nm, while from preliminary X-ray fibre diffraction studies[17], galactoglucan assumes a two-fold single helix having a pitch of ~1.6 nm.

Various levels of conformation may be present in solution, with respect to that derived from diffraction studies on oriented fibres. In the solid state a number of factors (e.g. orientational constraints, presence of water molecules) are taken into account to be consistent with the conformation theoretically evaluated from the minimization of the internal energy and with the symmetry and stereochemical requirements for the efficient packing of chains.

In contrast, in solution and in the absence of specific ordering constraints one often has to deal with statistical conformations. There is a lot of experimental evidence for these disordered conformations, as well as, under given circumstances, for an ordering process promoted by decreasing temperature or screening electrostatic repulsions. However, it is still difficult to take into account all the energetic contributions and to correctly predict the thermodynamic stability of the system. As an example, the results of molecular mechanics studies on the conformation of gellan, welan and rhamsan led Talashek and Brant to conclude that intramolecular van der Waals interactions alone are not able to justify the physicochemical differences of this family of polysaccharides[18].

DILUTE SOLUTION PROPERTIES

Gellan

Elucidation of the secondary structure of gellan in dilute solution is the prerequisite for understanding the gelation mechanism. In fact, the ordered conformations adopted by biopolymers in some solution conditions are responsible for promoting the favourable molecular interactions that lead to gel formation.

Due to the strong aggregation ability, strategies to prevent aggregate formation have been developed to study gellan dilute solution properties. Dimethylsulphoxide inhibits gelation and shear-thinning behaviour of aqueous solutions of gellan at room temperature[19]. It has also been found[20] that purified gellan samples in the Me_4N^+ salt form yield stable aqueous solutions with very little aggregation for polymer concentrations of $< \sim 0.1\%$. Chiro-optical, calorimetric and viscometric data have been obtained with this counterion (Me_4N^+) over a wide range of ionic strength, I, and as a function of temperature. The results shown in *Figure 2* indicate that the addition of Me_4NCl to a dilute aqueous gellan solution promotes a disorder-to-order transition at low temperature. In non-gelling conditions (i.e. low polymer concentration), both the extent and co-operativity of the conformational transition depend on the type of counterions ($Na^+ \approx Cs^+ > Li^+ > Me_4N^+ > C_4H_9N^+$)[21].

The salt-induced optical activity change is accompanied by a change in the enthalpy of mixing and by a rather abrupt increase in the intrinsic viscosity by a factor 3 in aqueous Me_4NCl (see *Figure 2*). The intrinsic viscosity is characterized by the presence of three distinct zones: the two extremes are due to the screening effect of electrostatic intrachain interactions, while the inter-

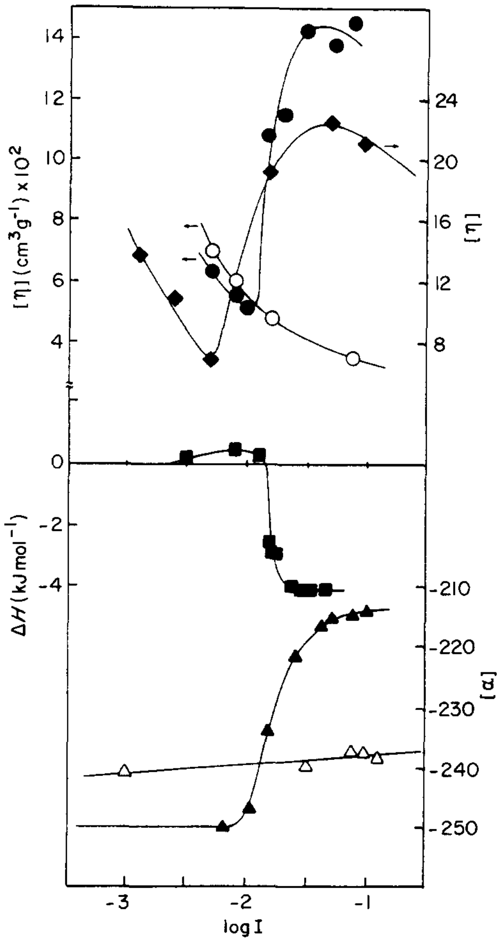

Figure 2 Experimental evidence of a conformational transition of gellan at 25°C induced by increasing ionic strength I (Me_4NCl): optical activity [α] (▲), enthalpy of mixing ΔH (■) and intrinsic viscosity [η], at 25°C (●) and at 20°C (◆). Above the transition temperature, at 45°C both optical activity (△) and intrinsic viscosity (○) change maintaining the disordered conformation. Data taken from references 21–23

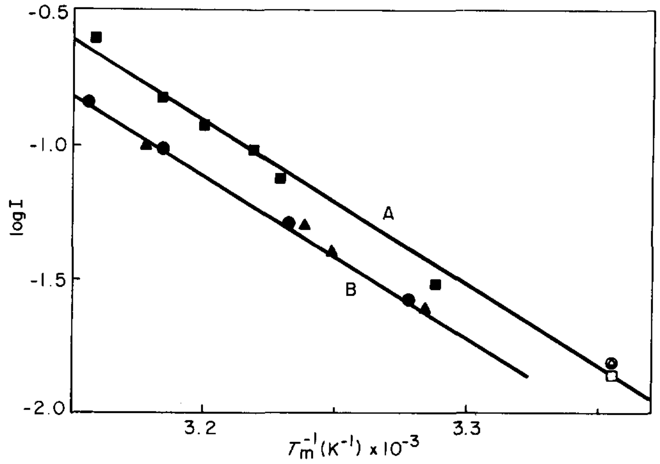

Figure 3 'Phase diagram' of the Me_4N^+ (A) and of the Na^+ (B) salt form of gellan. Data from references 20–23 and 27 refer to thermal transitions monitored by optical activity (■, □), viscometry (▲, △) and d.s.c. (●, ○)

mediate zone corresponds to the conformational 'chain-ordering zone'[22,23]. In contrast, the intrinsic viscosity is shown to decrease linearly on increasing I at a temperature above the mid-point transition temperature, T_m (45°C), following a behaviour generally ascribed to disordered polyelectrolytes. As evidenced by viscosity measurements, the gellan average chain dimensions at low salt concentration and/or high temperature are smaller than those prevailing at high I and/or low temperature. The disordered conformational state of gellan in solution prevailing at 45°C is characterized by a chain flexibility, in terms of Smidsrød and Haug parameters[24], intermediate between that of *O*-carboxymethylamylose and that of *O*-carboxymethylcellulose.

In the range of ionic strength examined, the conformational transition is thermally reversible without detectable hysteresis. The T_m increases moderately and linearly with increasing salt concentration when plotted as $-\log I$ *versus* T_m^{-1} (*Figure 3*). For co-operative conformational changes of linear polyelectrolytes, the slope of this plot is theoretically related to changes of the non-ionic enthalpy contribution and of the linear charge density[25]. The data in *Figure 3* show that the enthalpic contribution to the process is constant for both Na^+ and Me_4N^+ salt systems, being the different stability of entropic origin. However, the energetics of the process evaluated in this way, on the assumption of a transition from a double helix to a single strand coil, is not in agreement with direct calorimetric measurements. By measuring the heat of mixing of gellan with both NaCl and Me_4NCl, a value of $\sim -4.5\,\mathrm{kJ\,mol^{-1}}$ of repeat unit has been estimated[21] for the enthalpy of the disorder-to-order salt-induced transition, compared with a value of $-4\,\mathrm{kJ\,mol^{-1}}$ obtained by d.s.c. measurements[23].

In order to elucidate the structure of the salt-promoted conformation, light scattering investigations[26,27] have been carried out on aqueous salt (Me_4NCl) solutions of gellan both below and above the T_m. At low temperature the experimentally obtained mass per unit length of $720\,\mathrm{nm^{-1}\,g\,mol^{-1}}$, in comparison with the value of $383\,\mathrm{nm^{-1}\,g\,mol^{-1}}$ expected for a single helix, indicated that the gellan macromolecule is built up of $\sim$1.85 strands. In addition, high elongation of the gellan chains in the ordered state is evidenced by the value of the radius of gyration. These experimental findings suggest that the gellan double helix observed in the solid state[15] is probably preserved in aqueous salt solution at low temperature.

Welan and rhamsan

The solution properties of welan and rhamsan are similar as far as the I dependence of both optical activity and molar ellipticity is concerned. Preliminary physicochemical investigations[20] outlined the different behaviour of welan and rhamsan aqueous solutions from that shown by the closely related gellan. In particular, the optical activity and the molar ellipticity were independent of both added salt concentration and temperature.

Moreover, the intrinsic viscosity of welan is nearly independent of I. This behaviour is quite unusual for a polyelectrolyte solution, while it is normal for uncharged polymer solutions. In addition, only a slight decrease of the specific reduced viscosity of a 0.04% welan solution on increasing temperature was observed in 60 mM Me_4NCl. In contrast to welan, a linear dependence of the intrinsic viscosity on $I^{-0.5}$ and a pronounced decrease of viscosity on increasing temperature was observed for rhamsan.

In more recent investigations, which confirmed these preliminary experimental observations, the empirical Smidsrød stiffness parameter B obtained for rhamsan[28] was 0.003 and ranged from $\sim$0.001 to $\sim$0.002 for welan[28,29]. The low B values observed and the little effect produced by I and temperature on the viscosity suggest that in aqueous solution these polysaccharides adopt a highly stiff and stable conformation. In the case of welan this hypothesis is supported by the large mean chain extension per backbone sugar residue found by the radius of gyration and by the estimated value of 1.41 for the Mark–Houwink exponent[29]. This value is very close to that obtained for very stiff and multistrand microbial polysaccharides like xanthan and schizophyllan with relatively low molecular weights ($M_w \leqslant 5 \times 10^5$). However, whether the welan and rhamsan secondary structure in solution is of single or multistrand type is yet to be verified.

Succinoglycan

Succinoglycan in aqueous salt solution exhibits a temperature induced order-to-disorder conformational transition, detected by a sharp decrease of relative viscosity and of optical activity upon heating. Succinoglycan samples produced by *Rhizobium meliloti* M5N1 in different fermentation conditions show very different intrinsic viscosity in 0.1 M NaCl; those produced by resting cells have a viscosity about five times as high as that of the polymer excreted in growing medium. Subsequent heating and cooling cycles result in an irreversible loss of viscosity[30]. This decrease of viscosity has also been observed for succinoglycans from other micro-organisms[31–34], and has been interpreted in terms of cleavage of the polymer backbone[30] and of irreversible disruption of aggregates upon heating[31]. The latter hypothesis is supported by the observation that in most cases after the first thermal cycle subsequent heating treatments do not further reduce the intrinsic viscosity.

As detected by optical activity, the temperature-induced order-to-disorder transition of succinoglycan in aqueous solution shows upon cooling, a considerable hysteresis in the typical sigmoidal trend of optical activity

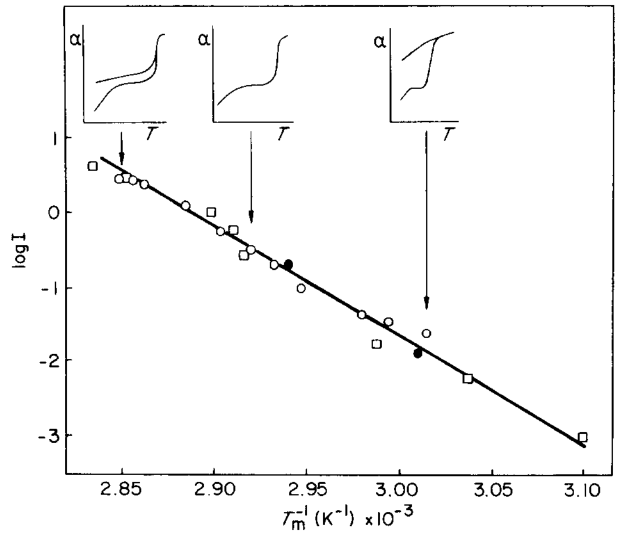

Figure 4 'Phase diagram' of the Na$^+$ salt form of succinoglycan. Data from references 30–32 and 34 refer to thermal transitions monitored by optical activity (□, ○) and d.s.c. (●)

as a function of temperature[31,35]. The hysteresis depends on I and on the charged substituents. In fact, it is absent in the 0.1–1 M salt concentration range and disappears completely in the case of pyruvate-succinate-free samples in water[34]. In all cases the original optical activity and molar ellipticity values are recovered after a single thermal cycle and a suitable cooling time of the solutions.

Like in gellan polyelectrolyte, the T_m increases by increasing I. *Figure 4* plots log$I$ *versus* T_m^{-1} using data obtained by several authors using different techniques. Most of the points fall on a straight line, to a reasonable approximation, in the whole range of I investigated.

On the assumption of a transition from single helix to stretched coil and using the experimental ΔH values[31,34], the slope obtained by the least-square fit led to a linear charge density of 0.77 for the ordered conformation, in satisfactory agreement with the expected value[31] of 0.74. Accordingly, data from light scattering and viscosity experiments[31,36] suggest that the native polysaccharide chain in aqueous solution adopts a rather stiff single helix with an estimated Kuhn length[31] of 285 nm.

Once stability and the ordered helical structure are assessed, the peculiar irreversibility of the temperature-dependent behaviour of succinoglycan can be further discussed. The tendency to form aggregates is clearly deduced from the value of the mass per unit length obtained by light scattering experiments[31]; approximately one-third of each chain seems involved in pair-wise intermolecular associations, probably disrupted by heating. On cooling, the presence of simple electrolytes may prevent lateral chain reassociation by inducing the side chain to assume a conformational state which is not favourable for the renewal of the intermolecular association. The crucial role played by the side chain in the aggregation process results from solution properties of succinoglycan after the removal of pyruvate and/or succinate groups. In fact, while the removal of the solely succinate group does not prevent the aggregate formation of succinoglycan, the association process is completely inhibited in the case of pyruvate-free and pyruvate-succinate-free samples[34].

Galactoglucan

Much of the work carried out on the solution properties and conformation of galactoglucan has given results which can be consistently interpreted in terms of a randomly disordered chain conformation.

The galactoglucan sample, which contained a small amount of impurities of succinoglycan, had a molecular weight of 7.8×10^5. It has been characterized by means of chiro-optical, viscometric and calorimetric measurements. The behaviour of the polymer does not show abrupt changes which is typical of conformational transitions as a function of pH, temperature or ionic strength[37]. On a more quantitative basis, the dependence of the intrinsic viscosity on the ionic strength gives a B value of 0.07, characteristic of a moderately hindered chain and comparable to that of carboxymethylcellulose or alginate. Given the overall random coil behaviour with moderate stiffness, the data of heat of dilution were comparable with the theoretical prediction of the polyelectrolyte theory, on the basis of the assumed charge density parameter of 0.98. These results are consistent with the statistical mechanical calculation of average chain properties by using the methods of molecular mechanics. The disaccharides (Glcp-Galp) and (Galp-Glcp) have been studied to obtain the conformational energy surface as a function of the rotations about the glycosidic bonds. Dimensions of the unperturbed chain have been evaluated to be more similar to the cognate homogalactan than to the related homoglucan. The strictly alternating sequence of the two monomers confers to the polymer a random coil behaviour but stiffer than that expected on the basis of mere qualitative analysis.

RHEOLOGICAL PROPERTIES

In order to interpret the rheological behaviour of polysaccharide solutions in terms of polymer conformation, a wide range of rheological experiments, including small deformation oscillatory, steady and transient shear, have to be performed under well defined experimental conditions. Little attention has been paid to the rheological properties of polysaccharide solutions with respect to synthetic polymers and it is difficult to find comprehensive studies in the literature.

The influence of polymer conformation and rigidity on rheological properties is especially evident in the semi-dilute concentration regime; for non-gelling systems two limiting rheological behaviours can be traced from comparative analysis of the few reported studies, mainly on xanthan and guar solutions[38,39]. The rheological behaviour, obtained by small deformation oscillatory experiments, of flexible linear polysaccharides in good solvent is that of an entangled network of physically interacting chains, similar to that observed for solutions of synthetic polymers like polystyrene in toluene.

The viscoelastic spectrum at low frequencies shows a predominantly liquid-like behaviour with $G'' > G'$ and the dependence on frequency ($G'' \propto \omega$ and $G' \propto \omega^2$) expected for a liquid system, where G' is the storage modulus, G'' is the loss modulus and ω is the frequency. At higher frequency a region appears in which $G'' < G'$ so that the curves cross each other; the cross-over frequency (for $G'' = G'$) decreases by increasing concentration or molecular weight. The complex viscosity, η^*, is nearly constant at low frequencies and shows a power law decrease as the frequency increases. From steady shear measurements, the flow curves in the semidilute regime are characterized by the Newtonian plateau at low shear rate, $\dot{\gamma}$, and by 'shear thinning' behaviour in the region of high shear rates. The exponent of the power law in the non-linear viscosity region is ~ 0.74. The superposition of the steady shear viscosity, η, *versus* $\dot{\gamma}$ and η^* *versus* ω curves (Cox–Merz rule) is obeyed except at very low polymer concentration. The entangled network formed by linear flexible polysaccharides has been observed to be less strain-dependent than that formed by stiff worm-like (or rigid rod) molecules. Moreover, the recovery of non-linear properties after steady shearing flow (transient experiments) has been observed to be dependent upon the chain flexibility; the time scale of the re-establishment of entangled network is several orders of magnitude faster for flexible than for rigid molecules.

In the same concentration regime and under conditions of an ordered conformation, worm-like polysaccharides with low flexibility (i.e. with persistence length $\geqslant 60\,\mathrm{nm}$) show a remarkable deviation from the behaviour described for flexible molecules. In particular, the viscoelastic spectrum resembles that of a typical gel system with both G'' and G' nearly frequency independent and with $G' > G''$ for the whole range of frequency accessible with conventional instrumentation. The flow curves give an indication of the existence of an apparent yield stress (apparent infinite viscosity), at low shear rate, without a Newtonian plateau. The remarkable shear thinning behaviour can be quantified in terms of the higher slope (~ 0.9) in the power-law region. The Cox–Merz superposition fails and η^* appears higher than η in the range of $\dot{\gamma}$ and ω investigated.

This behaviour, typically of so-called 'weak gel', likely reflects the occurrence in the polymer network of weak non-covalent intermolecular forces, in addition to the topological constraints.

The viscoelastic spectra of weak gels are similar to those of true gels indicating that non-transient supramolecular structures also occur in the former systems. However, under shear the former flows while the latter ruptures suggesting a different strength and/or extent of the interactions between the ordered chains. For true polysaccharidic gels the rheological techniques mentioned above give little information on the conformation, although the ability to form gel is often evidence of the existence of ordered interacting segments along the chains.

These rheological behaviours suggest that the polysaccharide conformation in solution can be predicted on the basis of the rheological properties of semidilute solutions. In order to support this hypothesis, the available rheological data for the selected microbial polysaccharides are discussed and critically compared. The polysaccharides of the gellan family, especially, provide the possibility for correlating the conformational and structural features with the rheological properties.

As described above, in dilute solution and in non-gelling conditions, gellan undergoes a co-operative conformational transition from an ordered state (high I and/or low T) to a disordered state (low I and/or high T). Although a detailed characterization has not yet been made, the viscoelastic spectrum of gellan in aqueous solution in the presence of large organic cations at 30°C (i.e. above T_m) is strikingly different from that observed at lower temperature[28]. In fact, these samples flow like normal solutions both below and above the transition temperature. At low temperature the viscoelastic spectrum typical of weak gel systems is obtained, while at 30°C the viscoelastic behaviour is that of an entangled network of flexible chains. These findings, on the one hand clearly provide further evidence for the two limiting rheological behaviours exhibited by polysaccharides in semidilute solutions in relation to their conformational states, and on the other hand strongly support the hypothesis that under appropriate conditions, the gellan secondary structure observed in the solid state is preserved in solution and that its capability to establish intermolecular interactions mainly governs the rheological response. The intermolecular interactions (carboxylate–cation–carboxylate) shown to be crucial for gelation in the presence of K^+ ions are weakened by large organic cations and as a consequence a weak gel behaviour results. The same intermolecular interactions are weakened by non-saccharide substituents in the native gellan and also in this case preliminary rheological measurements given indications of a weak gel behaviour[19].

The presence of side chains in welan and rhamsan has a similar influence, in principle, to that of the large organic cations and of non-saccharidic substituents on the solution properties of the deacylated and native gellan, respectively. In welan, as suggested by computer modelling as well as crystal structure, side-chain–carboxylate interactions are responsible for promoting the stability of the double helix and at the same time for weakening the intermolecular interactions between double helices in which carboxylate groups are involved. In rhamsan the carboxylate groups are not screened but because of the flexible and bulky side chains, rhamsan double helices cannot aggregate via carboxylate–cation–carboxylate interactions as in gellan. Although for welan and rhamsan detailed rheological investigations have not yet been carried out, it has been quoted that the viscoelastic behaviour of their semidilute solutions is that of a typical weak gel system and that the weak gel behaviour is not affected by I[28]. These experimental results suggest that both welan and rhamsan may be conformationally ordered in solution and that the side chain plays an important role not only in preventing the aggregation of the double helices but also in the polyelectrolytic behaviour of both polymers. Unfortunately, the rheological investigations carried out so far do not provide evidence for the thermal stability of the welan and rhamsan conformations. However, if the stable ordered structure is unaffected by heating, weak gel behaviour should then be expected at high temperature.

On the basis of our conformational–rheological relationship assumption indicated previously, succinoglycan, under conditions promoting the ordered conformation, is expected to exhibit weak gel behaviour in the semidilute regime. Flow curves below and above the T_m (polymer concentration up to $0.3\,\mathrm{g\,l^{-1}}$) show differences which have been ascribed to the different conformation adopted by succinoglycan in solution[32]. However, in the absence of rheological data on succinoglycan at high concentration, it is not possible to verify the relationship between the conformation and the rheological properties.

Quantitative data have been obtained on the mixture of succinoglycan and galactoglucan polysaccharides produced by *Rhizobium meliloti* strain YE-2(S1) under different osmolarity of the medium. Viscoelastic spectra are reported in *Figure 5* for succinoglycan-rich (85%)

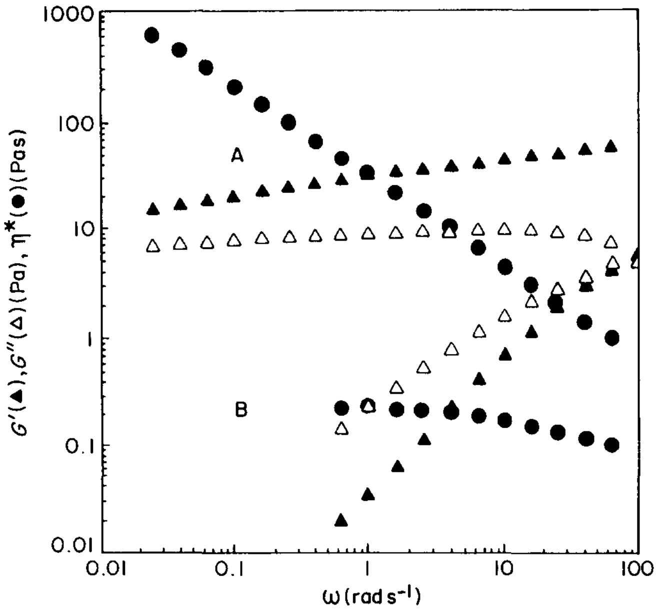

Figure 5 Viscoelastic spectra of the native mixtures of succinoglycan-galactoglucan in aqueous 0.1 M NaCl at 25°C (polymer concentration 1% w/v); (A) succinoglycan-rich (85%) mixture; (B) galactoglucan-rich (80%) mixture

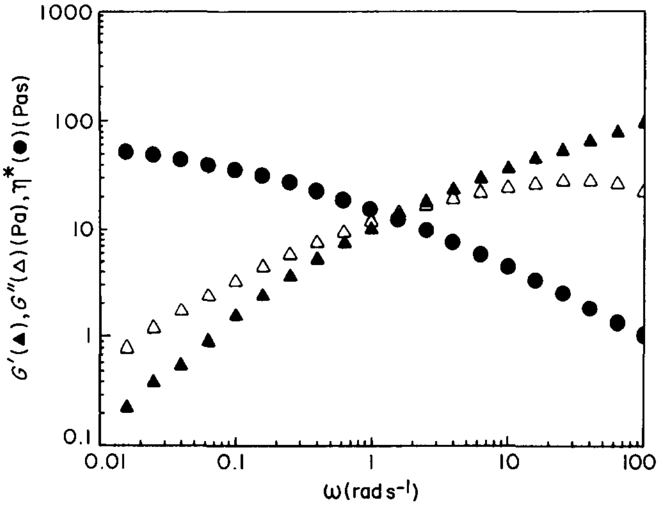

Figure 6 Viscoelastic spectra of the native galactoglucan aqueous solution at 25°C (polymer concentration 2% w/v). The measurements were carried out using a Rheometrics mechanical spectrometer RMS-605 with cone-and-plate geometry (50 mm diameter, 0.4 radian cone angle)

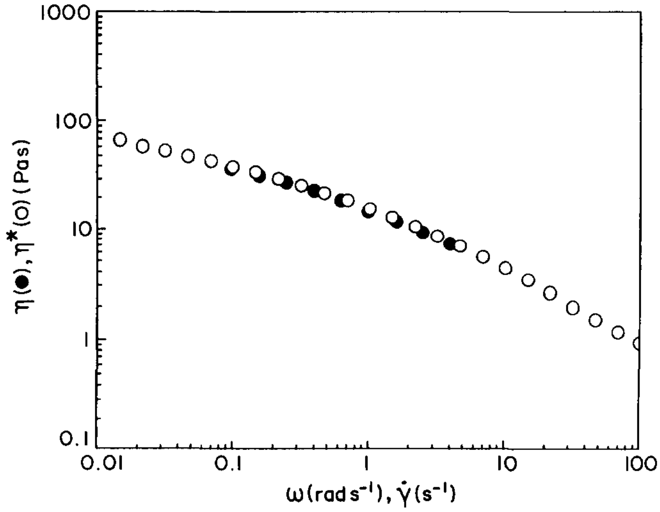

Figure 7 Cox–Merz plot for the galactoglucan aqueous solution at 25°C (polymer concentration 2%). Same experimental set-up as for *Figure 6*

and galactoglucan-rich (80%) mixtures. The spectra show a weak gel behaviour for the polysaccharide mixture at high weight fraction of succinoglycan, while the viscoelastic spectrum of the mixture with a high galactoglucan content is the typical response of an entangled network formed by conformationally disordered polysaccharides[40].

As shown in *Figure 6*, the rheological behaviour of the native galactoglucan semidilute solutions (polymer concentration up to 2% w/v) is close to that of the galactoglucan-rich mixture; in agreement with the experimental data obtained in dilute solution as well as with the theoretical results of the conformational analysis, it reflects the random coil conformation adopted by the polymer. Furthermore, the Cox–Merz superposition (*Figure 7*) is obeyed independently of salt concentration, and transient experiments (stress growth upon inception of a steady shear rate) show that the supramolecular structure is almost completely re-established in the typical time-scale of conformationally disordered polysaccharides.

CONCLUSIONS

The data presented here for five microbial polysaccharides have been interpreted on the basis that in some cases the ordered helical structure in the solid state can persist in the dilute solution.

Solution properties of disordered polysaccharides can be modelled within a reasonable degree of accuracy and the rheological behaviour is that of a typical network of flexible chains. Whenever ordered conformations are stable in solution, both solution properties and rheological behaviour display features that arise not only from the stiffening of the chains in the ordered conformation, but from the likely interchain interactions between the ordered 'array(s)' of chains. The strength of these interactions is reflected in the behaviour of the gel (weak to true gel). A detailed molecular picture of the supramolecular structures is still elusive, although a reasonable understanding of the molecular transitions and of their thermodynamics has been achieved.

ACKNOWLEDGEMENTS

We wish to thank Professor Crescenzi for beneficial discussions and his co-workers, who have contributed to some of the original results quoted in this paper. 'Progetto Finalizzato del CNR, Chimica Fine' and the National Research Council (CNR), Rome, are acknowledged for financial support.

REFERENCES

1 Yalpani, M. and Sandford, P. A. in 'Industrial Polysaccharides' (Ed. M. Yalpani), Elsevier, Amsterdam, 1987, p. 311
2 Glazebrook, J., Reed, J. W., Reuber, T. L. and Walker, G. C. *Int. J. Biol. Macromol.* 1990, **12**, 67
3 Zevenhuizen, L. P. T. M. in 'Biomedical and Biotechnological Advances in Industrial Polysaccharides' (Eds. V. Crescenzi, I. C. M. Dea, S. Paoletti, S. S. Stivala and I. W. Sutherland), Gordan & Breach, Amsterdam, 1989, p. 301
4 Kuo, M. S., Mort, A. J. and Dell, A. *Carbohydr. Res.* 1986, **156**, 173
5 Jansson, P. J., Lindberg, B. and Sandford, P. A. *Carbohydr. Res.* 1983, **124**, 135
6 Jansson, P. E., Lindberg, B., Widmalm, G. and Sandford, P. A. *Carbohydr. Res.* 1985, **139**, 217
7 Jansson, P. E., Lindberg, B., Lindberg, J., Maekawa, E. and Sandford, P. A. *Carbohydr. Res.* 1986, **156**, 157

8 Aman, P., McNeil, M., Franzen, L. E., Darvill, A. G. and Albersheim, P. *Carbohydr. Res.* 1981, **95**, 263

9 Harada, R., Anemura, A., Jansson, P. E. and Lindberg, B. *Carbohydr. Res.* 1979, **77**, 285

10 Her, G. R., Glazebrook, J., Walker, G. C. and Reinhold, V. N. *Carbohydr. Res.* 1990, **198**, 305

11 Zevenhuizen, L. P. T. M. and Faleschini, P. *Carbohydr. Res.* 1991, **209**, 203

12 Chandrasekaran, R., Millane. R. P., Arnott, S. and Atkins, E. D. T. *Carbohydr. Res.* 1988, **175**, 1

13 Chandrasekaran, R., Puigjaner, L. C., Joice, K. L. and Arnott, S. *Carbohydr. Res.* 1988, **181**, 23

14 Chandrasekaran, R. and Thailambal, V. G. *Carbohydr. Polym.* 1990, **12**, 431

15 Lee, E. J. and Chandrasekaran, R. *Carbohydr. Res.* 1991, **214**, 11

16 Gravanis, G. *PhD Thesis* University of Grenoble, 1985

17 Chandrasekaran, R. personal communication

18 Talashek, T. A. and Brant, D. A. *Carbohydr. Res.* 1987, **160**, 303

19 Carroll, V., Chilvers, G. R., Franklin, D., Miles, M. J., Morris, V. J. and Ring, S. G. *Carbohydr. Res.* 1983, **114**, 181

20 Crescenzi, V., Dentini, M., Coviello, T. and Rizzo, R. *Carbohydr. Res.* 1986, **149**, 425

21 Crescenzi, V., Dentini, M., Coviello, T., Paoletti, S., Cesàro, A. and Delben, F. *Gazz. Chim. Ital.* 1987, **117**, 611

22 Grasdalen, H. and Smidsrød, O. *Carbohydr. Polym.* 1987, **7**, 371

23 Crescenzi, V., Dentini, M. and Dea, I. C. M. *Carbohydr. Res.* 1987, **160**, 283

24 Smidsrød, O. and Haug, A. *Biopolymers* 1971, **10**, 1213

25 Paoletti, S., Smidsrød, O. and Grasdalen, H. *Biopolymers* 1984, **23**, 1771

26 Dentini, M., Coviello, T., Burchard, W. and Crescenzi, V. *Macromolecules* 1988, **21**, 3312

27 Milas, M., Shi, X. and Rinaudo, M. *Biopolymers* 1990, **30**, 451

28 Robinson, G., Manning, C. E. and Morris, E. R. in 'Food Polymers, Gels and Colloids' (Ed. E. Dickinson), Royal Society of Chemistry, Cambridge, 1991, p. 22

29 Urbani, R. and Brant, D. A. *Carbohydr. Polym.* 1989, **11**, 169

30 Heyraud, A., Rinaudo, M. and Courtois, B. *Int. J. Biol. Macromol.* 1986, **8**, 85

31 Dentini, M., Crescenzi, V., Fidanza, M. and Coviello, T. *Macromolecules* 1989, **22**, 954

32 Gravanis, G., Milas, M., Rinaudo, M. and Clarke-Sturman, A. J. *Int. J. Biol. Macromol.* 1990, **12**, 201

33 Simovà, E., Klekner, V. and Ricica, J. *Kvasny Prumysl* 1986, **32**, 218

34 Fidanza, M., Dentini, M., Crescenzi, V. and Del Vecchio, P. *Int. J. Biol. Macromol.* 1989, **11**, 372

35 Gravanis, G., Milas, M., Rinaudo, M. and Tinland, B. *Carbohydr. Res.* 1987, **160**, 259

36 Gravanis, G., Milas, M., Rinaudo, M. and Clarke-Sturman, A. J. *Int. J. Biol. Macromol.* 1990, **12**, 195

37 Cesàro, A., Tomasi, G., Gamini, A., Vidotto, S. and Navarini, L. *Carbohydr. Res.* in press

38 Richardson, R. K. and Ross-Murphy, S. B. *Int. J. Biol. Macromol.* 1987, **9**, 257

39 Richardson, R. K. and Ross-Murphy, S. B. *Int. J. Biol. Macromol.* 1987, **9**, 249

40 Navarini, L., Cesàro, A. and Ross-Murphy, S. B. *Carbohydr. Res.* 1992, **223**, 227