Biophysical Chemistry 41 (1991) 61–71
Elsevier Science Publishers B.V., Amsterdam

BIOCHE 01605

Solution and gelling properties of polysaccharide polyelectrolytes

V. Crescenzi *, M. Dentini and T. Coviello

Department of Chemistry, University La Sapienza, Piazzale Aldo Moro 5, 00185 Rome (Italy)

Abstract

Attention is focussed on the special class of polysaccharide polyelectrolytes belonging to the family of the microbial polysaccharides. But a few exceptions, these are high molecular weight water- soluble polycarboxylates with complex, regular structures. Complexity and regularity in primary structure — two features normally not shared by other polysaccharides of either vegetal or animal origin — quite naturally entail unique conformational propensities, special physico-chemical properties in bulk and in solution and, as a consequence, make these biopolymers of particular interest from both a basic research and an industrial standpoint. What is outlined in this presentation should demonstrate that for many exocellular microbial polycarboxylates the solution properties are, as expected, dictated by the conformation assumed by the polyelectrolytic chains which, in turn, are governed by several free energy terms in particular stemming from specific solvent–chain interactions, among which the coulombic contribution may play a minor role.

Keywords: Polysaccharide polyelectrolytes; Microbial polysaccharides; Solution and gelling properties

1. Introduction

Polysaccharide polyelectrolytes, either directly obtained from different renewable resources or semisynthetic, are quite numerous and many have been studied in detail because of their peculiar solution and gelling properties.

We shall limit attention to the special class of polysaccharide polyelectrolytes belonging to the family of the microbial polysaccharides. But a few exceptions, these are high molecular weight water soluble polycarboxylates with complex, regular structures.

Complexity and regularity in primary structure — two features normally not shared by other polysaccharides of either vegetal of animal origin — quite naturally entail unique conformational propensities, special physico-chemical properties in bulk and in solution and, as a consequence, make these biopolymers of particular interest from both a basic research and an industrial standpoint. In fact, fundamental and applied studies on microbial polysaccharides have flourished in the last decades, attention having been focussed primarely on species of industrial potential as polymeric specialties. A list of industrially relevant microbial polysaccharides, including non-ionic species, and of the microbial sources normally employed for their production are reported in Table 1.

In terms of primary structure — in order to facilitate a rapid and schematic presentation — one can distinguish among microbial polycarboxylates three main categories: (1) strictly linear chains, (2) chains having side-groups com-

* To whom correspondence should be addressed.

0301-4622/91/$03.50

posed of one or two neutral sugar residues, and (3) chains with bulky and charged side-groups. Actual chains belonging to categories (1), (2), and (3) are exemplified in a purely pictorial fashion in Fig. 1.

1.1 Type 1 chains

Sialic acid homopolymers are, to our knowledge, the only *ionic* microbial polymers whose *linear* chains are built up exclusively by one type of sugar. Normally they have low molecular weights, in the 10–30 kDa range, and, depending on the nature of the interresidue glycosidic linkage, can be strongly antigenic.

Bacterial alginates have structures similar to the algal ones, i.e. they are non-regular copolymers of L-guluronic and D-mannuronic acids: however, L-guluronate content and monomers sequence along

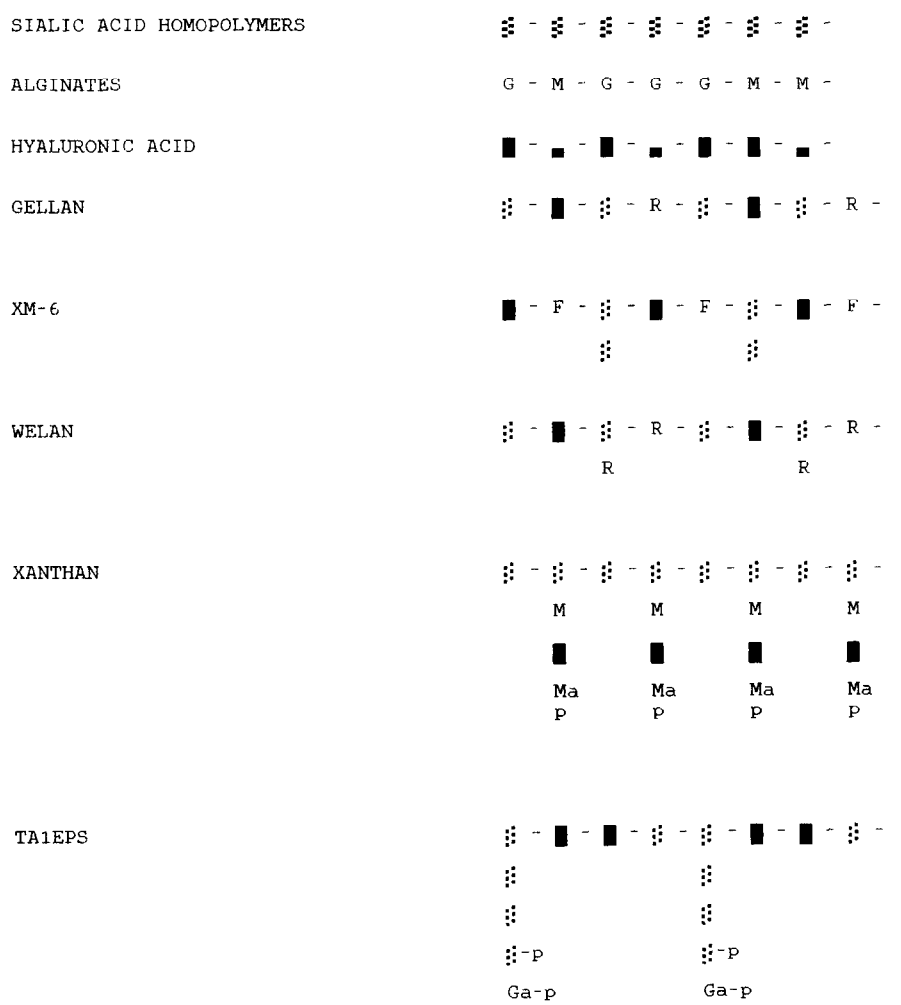


Fig. 1. Schematic representation of chain sections of different microbial polysaccharide polyelectrolytes. G = L-guluronate; M = D-mannuronate; (■) = D-glucuronate; (▬) = D-glucosamine; (⁝⁝) = D-glucose; R = L-rhamnose; F = L-fucose; Ma = D-mannose; Ga = D-galactose; −p = pyruvyl residue.

Table 1

Polysaccharides of industrial interest obtainable exclusively or also (*) from microbial sources

Polysaccharide	Microbial	Applications
hyaluronic acid *	*Streptococcus* spp.	E
alginates *	*Azotobacter vinilandii*	C, F
xanthan	*Xanthomonas campestris*	A, C, D
succinoglycan	*Agrobacterium* spp.	
	Pseudomonas spp.	A, B, H
gellan	*Pseudomonas elodea*	F
welan	*Alcaligenes* spp.	B
rhamsan	*Alcaligenes* spp.	H
emulsan	*Acin. calcoaceticus*	I
dextran	*Leuconostoc* spp.	E, F
scleroglucan	*Sclerotium* spp.	A, B
curdlan	*Agrobacterium* spp.	F
pullulan	*Auerobas. pullulans.*	G

[a] (A) tertiary oil recovery; (B) drilling fluids; (C) food; (D) textiles; (E) pharmaceuticals; (F) absorbents, gels, chromat.; (G) films; (H) suspensions; (I) emulsions.

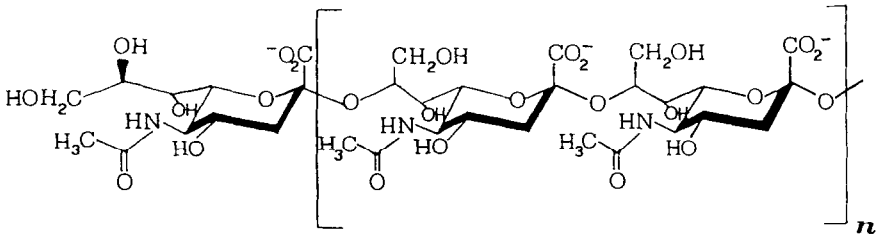
the chains are different and the bacterial species can contain important amounts of acetyl groups linked to the mannuronate residues.

As a result, native bacterial alginates are poorer gelling agents than the algal ones.

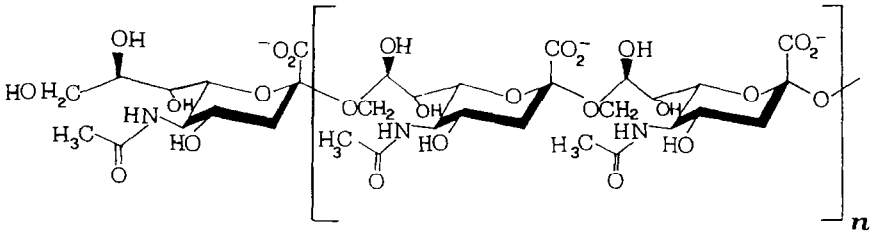
Bacterial hyaluronic acid is identical to the mammalian biopolymer and results from the regular enchainment of two monomers only.

Four sugar residues constitute the repeating unit of gellan, the industrially most successful bacterial polysaccharide — in terms of amount produced and sold, now also for food applications — after xanthan and dextran.

As the trade name is meant to emphasize, gellan is a powerful gelling agent yielding thermoreversible aqueous gels even at relatively low polymer concentrations (e.g. 1% w/v).



–> 8) – α – NeupNAc – (2–>8) – α – NeupNAc –>



–> 9) – α – NeupNAc – (2–>9) – α – NeupNAc –>

Fig. 2. Chain repeating units of Type B and Type C homopolymers of sialic acid.

1.2 Type 2 chains

The polysaccharides welan and rhamsan both have a skeleton identical to that of gellan but exhibit neutral side-groups, as schematically shown in Fig. 1 for welan.

Also in the case of the XM-6 polysaccharide the side-chain in each repeating unit is a single, neutral sugar residue.

1.3 Type 3 chains

Typical examples are the well known industrial polymers xanthan and succinoglycan bearing fixed charges on the side chains only. They are extensively used in view of their unique rheological properties ideally coupled with high thermal and hydrolytic stability.

Soil bacteria, for example bacteria of the genus *Rhizobium*, can also produce large amounts of structurally even more complex polysaccharides exhibiting uronic acid residues in the main chains and long side-groups also bearing fixed charges.

Only one example is schematically presented in Fig. 1, viz. the exocellular biopolymer secreted by *Rhizobium trifolii* strain TA-1.

Microbial sources from which many of the polysaccharides mentioned above can be obtained are listed in Table 1.

The panoramic outlined above clearly shows that the structural complexity (and stereoregularity) of microbial polysaccharides can reach levels unattainable by carbohydrate polymers of different origins. Quite naturally, going from one polysaccharide to another in the list of Fig. 1 the solution and gelling properties change markedly,

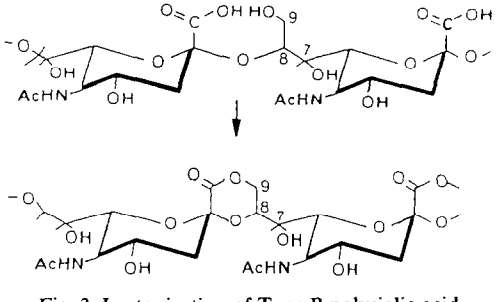


Fig. 3. Lactonisation of Type B polysialic acid.

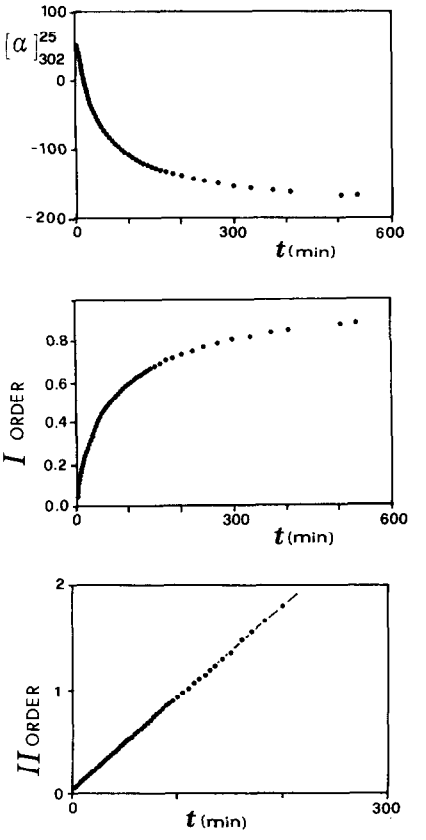


Fig. 4. Kinetics of colominic acid lactonisation in dilute aqueous solution (25 °C), pH 4.15, polymer concentration 0.9% (w/v). If longer times are considered, the first-order plot shows large deviations from linearity. The apparent second order rate of reaction results 0.124 L/mol·s.

often in a very peculiar, unexpected fashion. One important feature, however, is common to all species considered: high configurational regularity allows the chains to assume *ordered conformations also in dilute aqueous media*. The present paper will be centered on this aspect and is based on original, experimental information recently obtained in our laboratory for some of the polysaccharides listed in Fig. 1. A few experimental facts will be presented affording evidence on how *stability, nature* and *rate of attainment* of ordered conformational states depend on polysaccharide primary structures. The special subject concerning the "reactivity" of a sialic acid homopolymer will also be briefly mentioned.

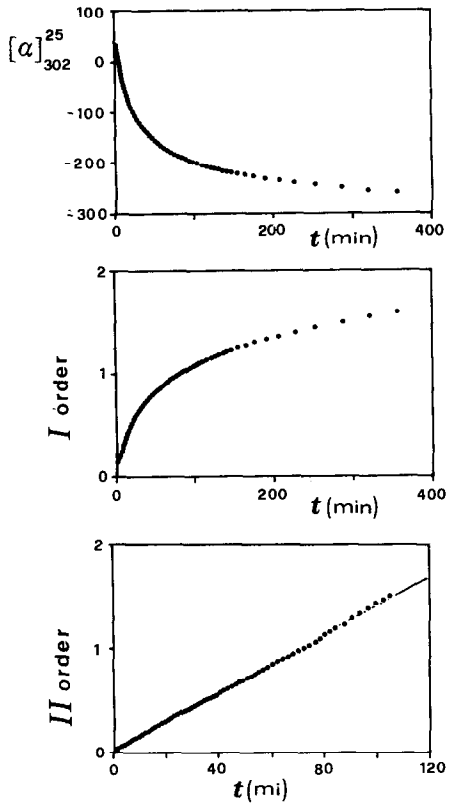


Fig. 5. Kinetics of colominic acid lactonisation in dilute aqueous solution (25 °C, pH 2.79, polymer concentration 0.9% (w/v). If longer reaction times are considered, the first-order plot shows large deviations from linearity. The apparent second order rate of reaction results 0.244 L/mol·s.

A thorough discussion of these fundamental aspects would entail detailed consideration of numerous factors, especially: nature *and* sequence of sugar residues in the backbone and in the side-chains; nature *and* sequence of glycosidic linkages; amount and location along the polysaccharide chains of the so called "minor substituents" (e.g. acyl groups). What follows is therefore far from being a comprehensive account and we suggest that additional, important results obtained by many different authors — with viewpoints possibly divergent from ours — should be looked for in the recent literature.

2. Experimental information and discussion

For the sake of brevity, only dilute polysaccharide solutions in water and/or aqueous media containing 1 : 1-valent salts will be discussed.

Considering first the case of the bacterial sialic acid homopolymers, three main types are known in which the sialic acid residues are linked α-(2 → 8) (Type B), or α-(2 → 9) (Type C), or alternating α-(2 → 8)/α-(2 → 9), respectively (Fig. 2). Interestingly, because of these linkage differences the immunological properties of these polysaccharides are very different. While Type C polysaccharide is highly immunogenic, and is currently used as a constituent of a vaccine against meningococcal meningitis in humans, Type B polysaccharide is only poorly immunogenic [1,2]. For the polymer containing alternating α-(2 → 8) and α-(2 → 9)

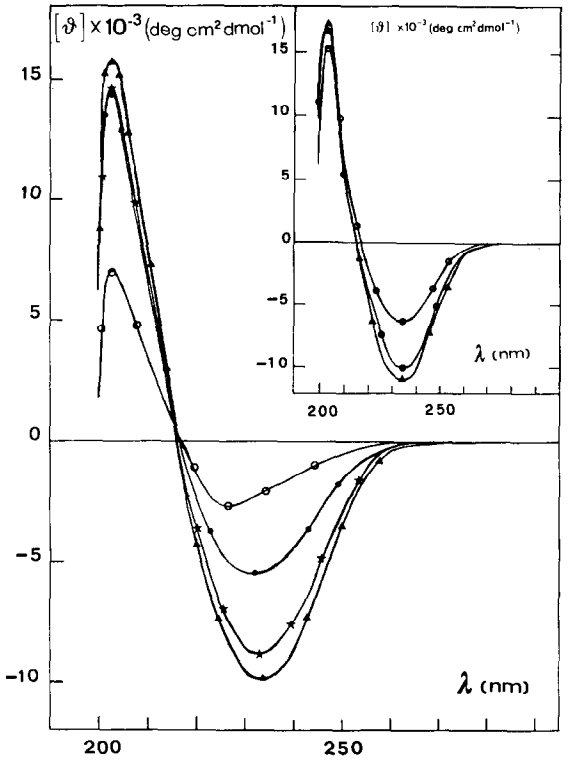


Fig. 6. Equilibrium (25 °C) circular dichroism spectra of colominic acid at different pH values in 100 m*M* NaCl – (▲) pH 2.63; (★) pH 2.92; (●) pH 3.80; (○) pH 4.50 – and in water (the insert) – (▲) pH 2.62; (●) pH 3.04; (○) pH 4.39)

linkages, only antibodies with a specificity for the latter linkage have been detected.

It has been proposed that the poor antigenicity of Type B polysaccharide is the result of interactions between the C(1)-COOH and C(9)-OH groups of adjacent residues leading in aqueous solution to lactonisation through condensation (Fig. 3) with the loss of conformational flexibility and the exclusion of immunogenic determinant conformations [2]. Indeed, lactone formation has been demonstrated *in vivo* and *in vitro* also for different gangliosides [3] in which disialosyl residues contain α-(2 → 8) glycosidic linkages.

We have very recently started a comparative analysis of the solution properties of sialic acid homopolymers, including a study of the process of lactone formation in dilute aqueous solutions of "colominic acid" (secreted by *E. coli* strains and identical in structure to the B polysaccharide from *Neisseria meningitidis*). A few results of our polarimetric pH-jump experiments (Figs. 4 and 5) show that a pH lower than 5 the chirooptical properties of colominic acid exhibit a characteristic time dependence traceable to a progressive increase in lactonic ring formation along the chains (as ascertained by IR spectral determinations). Data shown in Figs. 4 and 5 demonstrate, as expected, that lactonisation is catalysed by protons and follows simple second order rate kinetics (irreversible) at least during the early stages of the process. The second order law may be tentatively interpreted by assuming that at the two pH values considered (4.15 and 2.79, respectively) colominic acid is 50% and 100% protonated, approximately: $-COOH$ groups themselves would catalyse lactonisation and their concentration thus appears twice in the empirical reaction velocity expression. This is in qualitative agreement with the finding that the apparent second order rate constant doubles when going from pH 4.15 to pH 2.79.

For longer reaction times, however, the reaction kinetics becomes more complex. This may be due to the onset of a backward reaction and, probably, to the fact that extensive lactonisation by increasing polymer chains stiffness increases the apparent activation energy of the process which finally attains an equilibrium state. Equilibrium circular dichroism spectra collected in Fig. 6 are a manifestation of the gradual increase in the extent of lactone formation with lowering the pH. The pro-

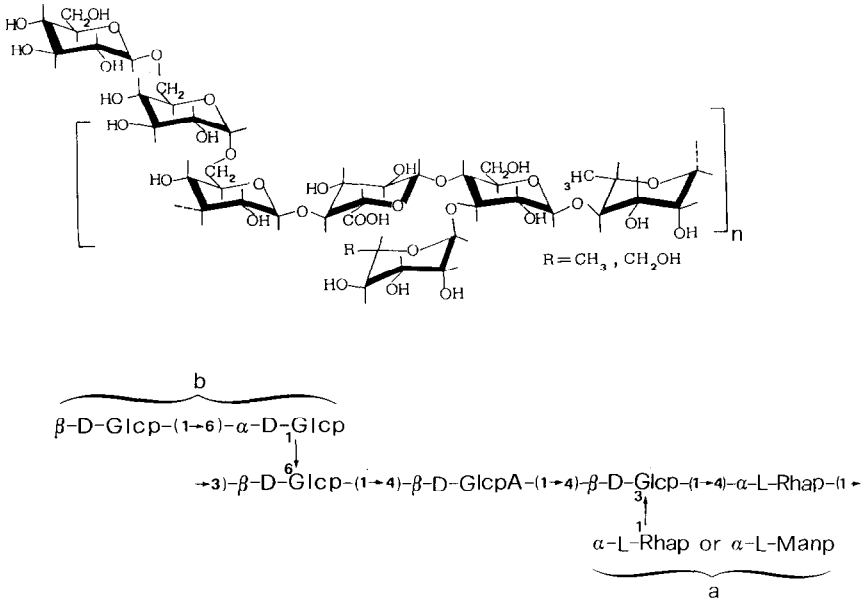
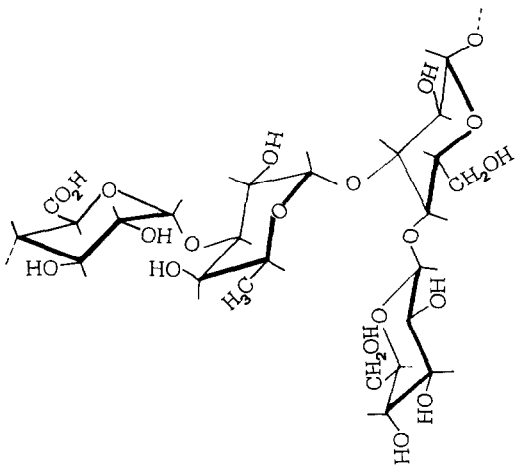


Fig. 7. Structure of the repeating units of gellan, welan and rhamsan. Gellan chains are devoid of substituents, rhamsan has substituent 'a' only, and welan has substituent 'b' only.



->4) α-D-GlcpA (1->3) α-L-Fucp (1->3) β-D-Glcp (1->

4

↑

β-D-Glcp

Fig. 8. Repeating unit of XM-6 polysaccharide.

cess of intrachain, localised ring closure, to our knowledge exhibited to such a notable extent only by sialic acid homopolymers, is certainly an interesting subject in the field of "polyelectrolytes reactivity" worth further, more detailed studies.

Passing to relatively more simple cases for which only the conformation of polysaccharide chains may change with change in ionic strength or pH of the aqueous solvent, the first point we find worth attention concerns a comparison of the behaviour of gellan and XM-6 with that typical of welan and rhamsan (Figs. 7 and 8). In brief, summarizing a vast amount of experimental information, it is now clear that while gellan and XM-6 give rise to salt-induced disorder → order conformational transitions at around room temperature (polymer concentration about 0.1% w/v). welan and rhamsan solution properties appear nearly insensitive to added salt concentration. Moreover, solution properties of the last polymers are scarcely influenced by temperature up to 100 °C, while the ordered conformational states of both gellan and XM-6 "melt" — with very cooperative order → disorder processes — in easily accessible temperature ranges (depending of course on salt concentration).

For gellan, both the isothermal and thermal transitions are very fast processes with no hysteresis. Only at higher polymer and/or added salt concentrations gel formation takes place for gellan as well as for XM-6, the sol → gel transition being accompanied, as usual, by a marked hysteresis.

Interesting enough, solution properties exhibited by gellan — including main features of the conformational changes — markedly depend on the counterionic nature and, in addition, change in a peculiar fashion going from water to heavy water [4,5]. It has been proposed on the basis of X-ray fiber diffraction data that gellan, welan, and rhamsan assume very similar double helical conformations [6].

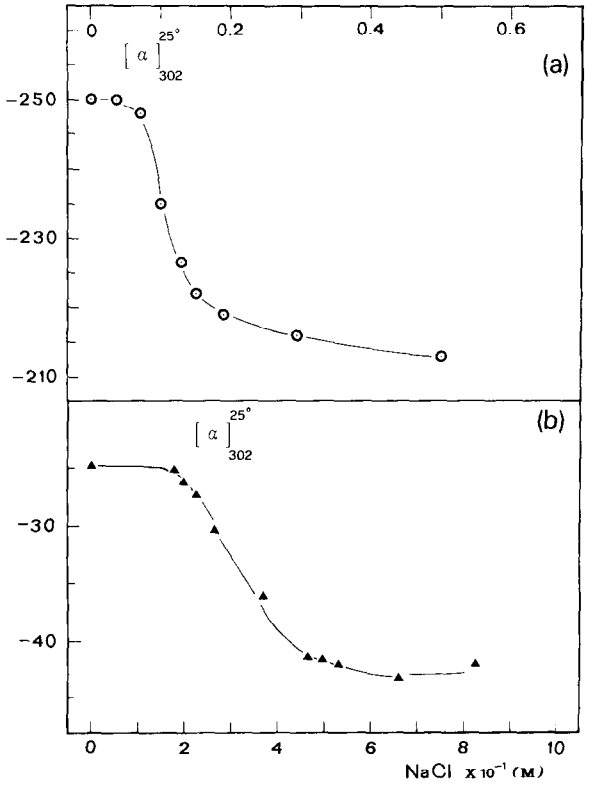


Fig. 9. Isothermal (25 °C), salt-induced conformational transitions of gellan (a) and XM-6 (b) in dilute aqueous solution. Polymer concentration 0.1% (w/v).

From all what schematically has been said above, the conclusion has been drawn — not without dispute — that gellan and XM-6 chains in dilute solution in water are in an essentially disordered state while, on the contrary, welan and rhamsan would always be in their ordered, double helical state (i.e. up to about 100 °C). Evidently, this is a striking manifestation of the importance of the nature *and* geometrical location in the repeating unit of a simple, neutral side-group in determining the solution behaviour of polysaccharides mentioned above. In particular, as directly shown by a number of experimental data, the side-groups along welan and rhamsan chains are capable of interacting with the backbone screening the fixed charges on the glucuronic acid residues [4]. Therefore, for welan and rhamsan the side-groups would play an unexpected major role in *stabilising* the ordered, double helical conformation in dilute aqueous solution. Moreover, both polymers are unable to gel.

In the case of XM-6 the situation is different inasmuch as the glucose side-group is not in a steric position favouring interactions with the main chain, leaving the carboxylate groups essentially unscreened: as a consequence, XM-6 physico-

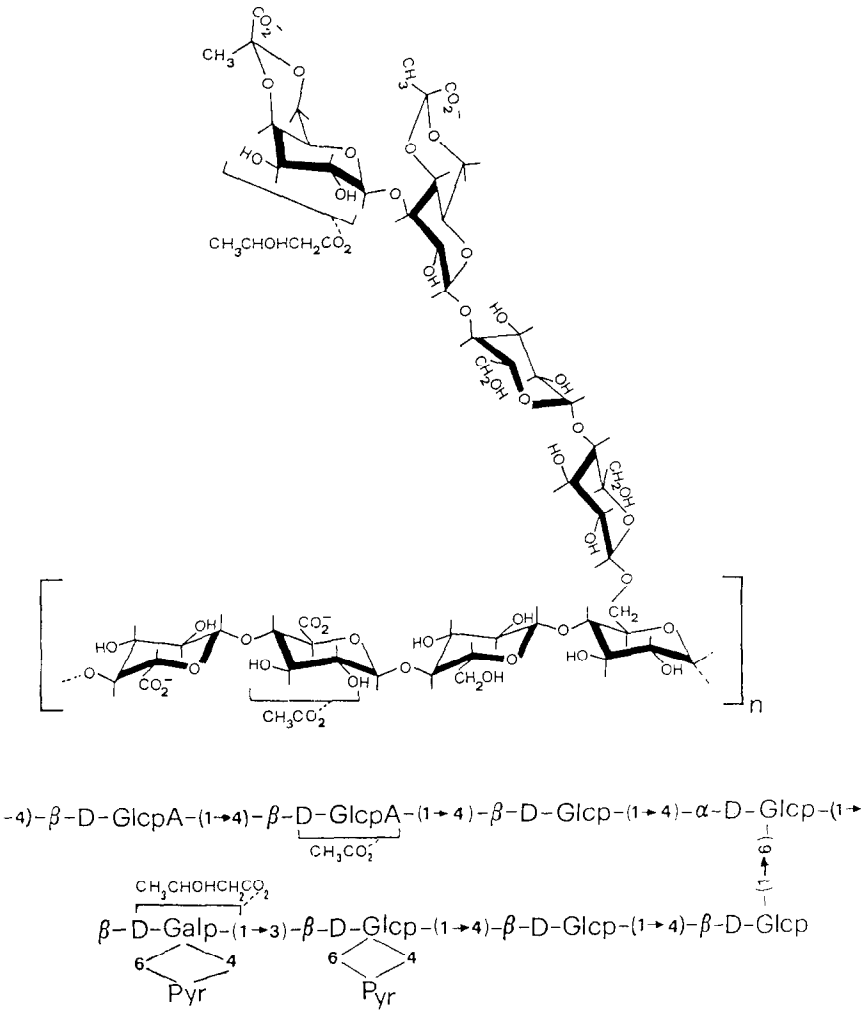


Fig. 10. Repeating unit of TA1EPS polysaccharide (see text).

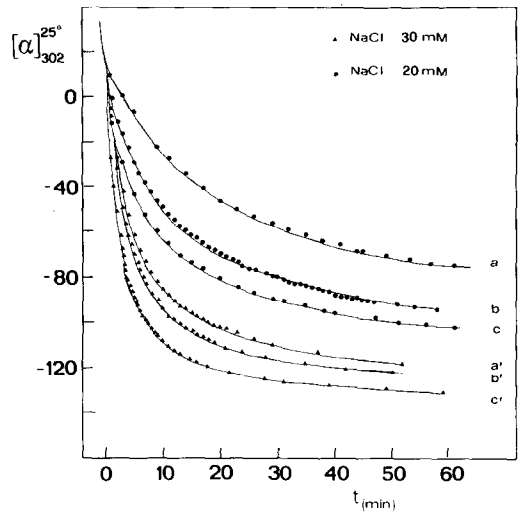


Fig. 11. Salt-jump experiments with TA1EPS. Time dependence of polysaccharide optical activity at 302 nm after mixing with aqueous NaCl (25°C). (●) 20 m*M* NaCl, (▲) 30 m*M* NaCl Polymer concentration (w/v): a = a′ = 0.06%; b = b′ = 0.12%; c = c′ = 0.22%.

chemical properties are sensitive to changes in ionic strength, though less than experienced with gellan (Fig. 9). In this context, it is interesting to point out that, from our experience, the mid-points in the isothermal, salt-induced conformational changes as detected by polarimetric measurements for different polysaccharide polyelectrolytes depend also on polymer molecular weight: therefore, differences between the plots of Fig. 8 for gellan and MX-6 might well be traced to an influence of molecular weight (besides charge density).

For chains with complex, charged side-groups the situation becomes, as easily anticipated, more complicated. This is exemplified by the case of the polysaccharide from *Rhizobium trifolii*, strain TA-1 (TA1EPS: Fig. 10). In brief, TA1EPS chains undergo a salt-induced disorder → order conformational change leading, eventually, to gelation at high salt concentrations [7,8]. However, different from what was found with gellan (and indeed with all other polysaccharides so far investigated), the

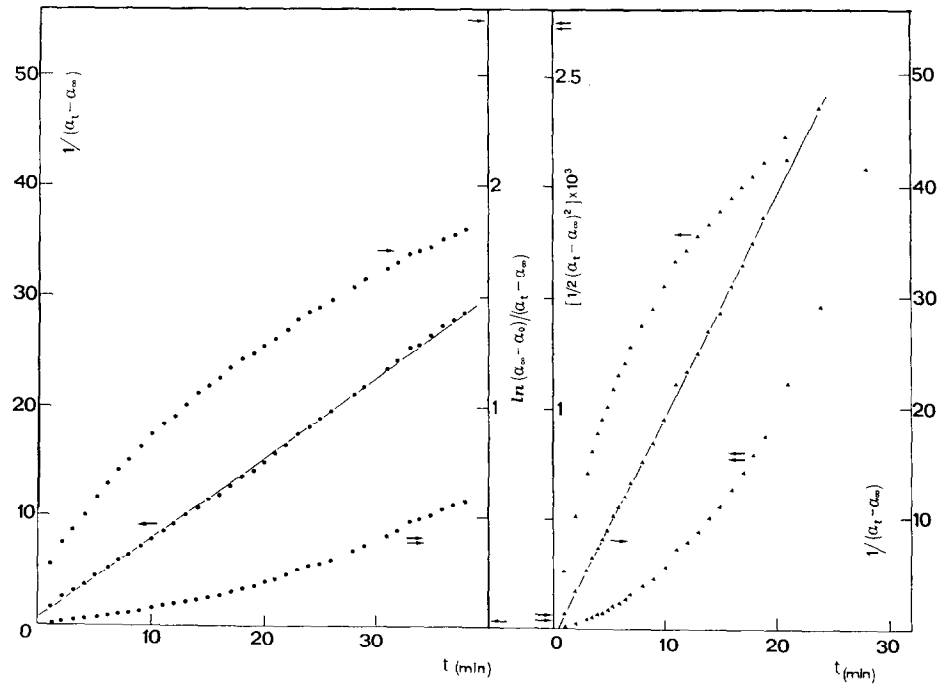


Fig. 12. First, second and third order kinetic plots of the data of Fig. 11 (same symbols). The second order rate constant (L/mol·s) results are: 2.4 (20 m*M* NaCl: left) and 8.3 (30 m*M* NaCl: right).

rate of conformational change of TA1EPS at low ionic strength (NaCl, pH 7) is sufficiently slow to be readily monitored by conventional polarimetry. The results of polarimetric salt-jump experiments, performed at 25 °C using three difference polymer concentrations in 20 m*M* and 30 m*M* NaCl, respectively, are shown in the Figs. 11 and 12. Main features stemming from these data and from results collected at higher NaCl concentrations using stopped-flow polarimetry [9] are, in summary: (1) the reaction "half-lives" of the conformational ordering processes decrease with polymer concentration and strongly decrease with ionic strength; and (2) in 20–30 m*M* NaCl the conformational ordering process is second order (conventional analysis of the polarimetric data collected up to 30 minutes after the isothermal salt-jump: Fig. 11) and can thus be depicted as:

$$C + C \rightarrow H(2)$$

where two randomly coiled chains, C, unite to form, possibly, a double helix, H (step 1).

More detailed and elaborate computerised analysis of a wider set of data (including data collected using a stopped-flow apparatus [8]), shows that, if long "reaction" times are considered, step 1 would be followed by another step in which a third chain joins the basic two-chain structure. This is in agreement with laser light scattering data for TA1EPS in 100 m*M* NaCl according to which the equilibrium conformational state of the polysaccharide is multistranded with a mass-per-unit length compatible with a three-chain, average structure [7]. In this context, it is relevant to mention that TA1EPS ordered state in solution can be heat "denatured": upon cooling, "renaturation" is characterised by a marked hysteresis, the rate of attainment of the pristine ordered state at 25 °C depending on ionic strength (Fig. 13). At the roots of the peculiar behaviour of TA1EPS there would be, on one hand, the tendency of the stereoregular polysaccharide backbone to assume an ordered conformation (a typically cooperative, fast process) and, on the other hand, the steric hindrance of the side-arms opposing, at least kinetically, the ordering process. The charged side-chains, moreover, might establish intra- and interchain interactions, includ-

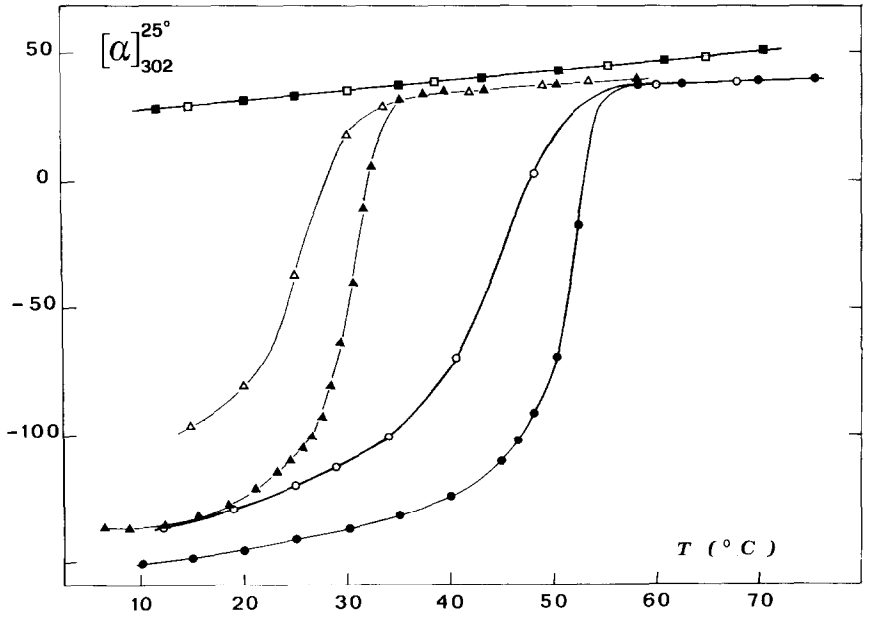


Fig. 13. TA1EPS polysaccharide optical activity at 302 nm as a function of temperature in water (squares), 20 m*M* NaCl (triangles), and in 100 m*M* NaCl (circles). Full symbols: heating, and open symbols: cooling. Polymer concentration: 0.12% (w/v).

ing counterions-mediated interactions, leading to the alleged TA1EPS three-stranded structure.

Features mentioned above also depend on polysaccharide molecular weight. For instance, we have found that the amount of NaCl necessary for the onset of the 25 °C conformational change steadily increases with decreasing TA1EPS average molecular weight. At equilibrium, however, the same three-stranded average structure would prevail [10].

Microbial exocellular polysaccharides discussed above and others schematically depicted in Fig. 1 are polyelectrolytes of low or relatively low "charge density". For species belonging to categories (1) and (2) of Fig. 1, the charge density can be expressed via a characteristic value of the well known ξ parameter, as concise and significant as for other synthetic or natural polyelectrolytes. For said species, the ξ values range approximately between 1.6 (e.g.: alginates) and 0.4 (e.g.: gellan in the single chain state). Indeed, Manning's theory has been applied to discuss equilibrium and transport properties of such polysaccharides [11]. Still, the dependence of solution properties of, for instance, gellan on the nature of monovalent counterions is so marked as to make questionable the use of a purely electrostatic line-charge model with, if required, "condensed" counterions. For polysaccharides of category (3) of Fig. 1, even the estimate of a significant ξ value clearly seems rather questionable.

In conclusion what has been outlined in this presentation should demonstrate that for many exocellular microbial polycarboxylates the solution properties are, as expected, dictated by the conformation assumed by the polyelectrolytic chains which, in turn, are governed by several free energy terms — in particular stemming from specific solvent-chain interactions — among which the coulombic contribution may play a minor role.

Acknowledgement

Work summarized in this paper has been carried out with financial support from the Italian Ministry of Science and Education, Rome.

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