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Physicochemical properties of 'Exogel' exocellular β(1-4)-D-glucuronan from *Rhizobium meliloti* strain M5N1 C.S. (NCIMB 40472)

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The *Rhizobium meliloti* mutant strain M5N1 C.S. (NCIMB 40472) produces a polyuronic acid as $\beta(1 \rightarrow 4)$-D-glucuronan with molecular weight between $1{\cdot}5 \times 10^5$ and 3×10^5. With a pK_0 of about 3 and $L_p \simeq 110$ Å, this polyelectrolyte is comparable with alginates and pectins. In solution, acetyl content and high external salt concentrations favour aggregation. At high concentrations the polysaccharide forms thermoreversible or thermally stable gels according to the nature of the counterion, the polymer concentration and the ionic strength.

INTRODUCTION

Polysaccharides are macromolecules having large-scale technological applications. This industrial interest results from a number of peculiar physico-chemical properties which depend upon the structure of the polysaccharide. In the course of our studies on the structure properties of polysaccharides in aqueous solutions, attention has been drawn to the exopolysaccharides synthesized by bacteria of the *Rhizobium* family (Courtois *et al.*, 1986). Among them, succinoglycan produced by *Rhizobium meliloti* M5N1 strain has attracted our interest (Heyraud *et al.*, 1986). In order to obtain modified succinoglycans, mutagenesis was performed on the M5N1 strain and a mutant producing a new polysaccharide named 'Exogel' (Courtois *et al.*, 1992) was selected. 'Exogel' is the code name for the extracellular polysaccharide produced by the mutant strain M5N1 C.S. (NCIMB 40472). This polymer is a linear β(1-4)-D-glucuronan, partially acetylated (Courtois *et al.*, 1993; Heyraud *et al.*, 1993), whose structure appears to be the first example of homoglucuronic acid secreted by bacteria. According to the interest of our laboratory in ionic polysaccharides such as xanthan, gellan, κ-carrageenan and especially alginates and pectins, two well-known polyuronates, physico-chemical properties of $\beta(1 \rightarrow 4)$-D-glucuronan have been investigated and preliminary results on solution and gel-state properties have been obtained and are presented in this paper.

EXPERIMENTAL

Preparation of the exopolysaccharide

The mutant strain *R. meliloti* M5N1 C.S. (NCIMB 40472), obtained by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) treatment of *R. meliloti* M5N1 strain, was cultivated on RC medium supplemented with sucrose (1%). The excretion was controlled according to a method previously described (Courtois *et al.*, 1990). This polysaccharide (EPS) was isolated from the broth medium by microfiltration (pore size 0·2 μm) and purified by ultrafiltration on a 100 000 NMWCO membrane.

Isolation and ionic form modification of the polysaccharide

The Na form was obtained by precipitation of the polysaccharide in purified broth (as described previously) by 1·5 vol. of 2-propanol in the presence of NaCl (1 M). The precipitate was washed with an

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increasing concentration of propanol (75–99%) and dried for 48 h in vacuum at room temperature. Its homoionic acidic form, for titrations, was obtained by passing polysaccharide sodium salt at 1 g/litre in pure water through an acidic cation-exchange resin (IR 120 H).

Deacetylation of glucuronans

The *O*-acetyl group content depends upon the biosynthesis conditions. The samples used for this study are listed in Table 1. For each sample, the *O*-acetyl group percentage expressed in wt% was estimated by ^{1}H NMR (Courtois *et al.*, 1993).

For deacetylation, a sodium salt solution of glucuronan (1 g/litre) was kept in alkaline medium (pH $\simeq$ 10) overnight. After neutralization by hydrochloric acid, the polysaccharide was recovered by 2-propanol precipitation according to the method described above.

Viscosity measurements

The equipment used to determine the viscosity was a low-shear viscometer (Contraves LS 30) with temperature control of ±0·1°C which allows viscosity measurements in the shear rate range between 10^{-2} and $128\,s^{-1}$.

Light-scattering experiments

The molecular weights, $\overline{Mw}$, were obtained from low-angle laser light-scattering (LALLS) of a 0·01% (w/v) polysaccharide solution in 0·1 M NaCl with a Chromatix KMX 6 photometer. The solutions were filtered through 0·2 μm membranes before measurement. The d*n*/d*c* value, which is about 0·141 ml/g, was determined with a Brice Phoenix apparatus in 0·1 M NaCl solutions without dialysis.

Characterization of EPS by Size Exclusion Chromatography (SEC)

SEC experiments were performed at room temperature using a modified Waters 150C apparatus with multidetection: a differential refractometer for concentration, a capillary viscometer for intrinsic viscosity [η] determination and a multiangle laser light-scattering detector (MALLS) (DAWN F from Wyatt Technology) for molecular weight and radius of gyration determinations (Tinland *et al.*, 1988; Malovikova *et al.*, 1993; Rinaudo *et al.*, 1993). Two aqueous gel filtration columns, B-804 and B-805 from Shodex, were used in series with 0·1 M NH_4NO_3 as eluent. The samples were filtered through 0·22 μm cellulose nitrate membranes before injection. The injected volumes were 200 μl (polymer concentration 1 g/litre and the flow rate was 1 ml/min.

Table 1. List of different β(1-4)-D-glucuronans used; $\overline{m_0}$ is the average mass of the monomer

EPS	Preparation method	$\overline{Mw}$ ($\times 10^{-5}$)	% *O*-Acetyl	$\overline{m_0}$
EPS-0[a]		—	0	198
EPS-1	Erlenmeyer	1·63	3	204
EPS-2	Erlenmeyer	1·67	10	218
EPS-3	Fermentor	1·70	18	233
EPS-4	Fermentor	1·90	22·8	243

[a] After deacetylation of EPS-1 by NaOH treatment.

Elastic modulus measurements

A solution of 10 g/litre sodium polyglucuronate heated at 60°C was poured into dialysis tubing (diameter = 14 mm) and immersed in 0·34 M solutions of different salts at 60°C for 5 h and kept overnight at room temperature. The gel formed was introduced into a cylindrical mould, cut into small cylinders (height = 17 mm) and immersed in a 0·34 M solution of the same salt used in the dialysis. The gel volumes have been determined by weighing, assuming a density of 1 g/cm^3, and by calculation after diameter and height measurements. The mechanical properties of different gels were obtained by compression between parallel plates with an Instrom 4301 instrument, at constant deformation rate (25 mm min^{-1}) at room temperature. The elastic moduli were calculated from the linear region of the stress–strain diagram (length of deformation < 1 mm).

pH-metry and conductimetry measurements

pH-metry and conductimetry measurements were performed at 25°C in the absence of external salt using a pH combined electrode TC 100 489 189 with a Minisis 6000 pH meter and a conductivity cell CM 01/G with conductimeter CD78 from Tacussel (France).

RESULTS AND DISCUSSION

Using static light-scattering measurements, a weight-average molecular weight of between $1{\cdot}5 \times 10^5$ and 3×10^5 is obtained (Table 1). One can observe that these values are much lower than that of succinoglycan ($\overline{Mw} \simeq 10^6$), the polymer produced by *R. meliloti* M5N1 strain before the chemical mutagenesis (Heyraud *et al.*, 1986).

Polyelectrolyte behaviour

pH-metry

The pH-metry titrations of polyglucuronic acid in pure water were performed with KOH and $Ca(OH)_2$ on the EPS-0 sample. The apparent dissociation coefficient

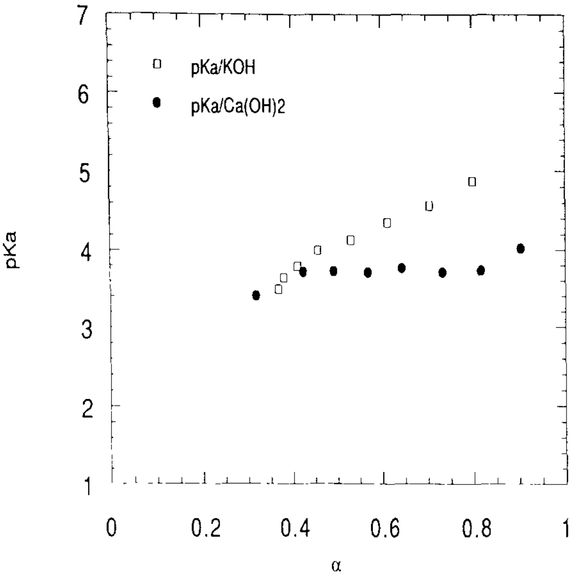

Fig. 1. Dependence of the apparent pK_a on α for polyglucuronic acid aqueous solution titrated with KOH and $Ca(OH)_2$ (EPS-0; $C_p = 2{\cdot}1 \times 10^{-3}$ eq. litre^{-1}).

pK_a as a function of the total degree of dissociation $\alpha(\alpha_N + \alpha_H)$ has been calculated from titration curves (Fig. 1), using the well-known equation

$$pK_a = \mathrm{pH} + \log\frac{1-\alpha}{\alpha} \qquad (1)$$

where α_N is the degree of neutralization and α_H the autodissociation, $\alpha_H = [H^+]/C_p$.

The experimental variations of pK_a *versus* α for mono and divalent counterions are extrapolated to $\alpha = 0$ to obtain the intrinsic pK, pK_o, associated with the removal of a proton from the uncharged chain. The pK_o found, 3·0, is in agreement with the literature values for carboxylic polysaccharides (Rinaudo & Milas, 1974). No increase of pK_a at low α values is observed, which means the absence of large aggregates in pure water solutions.

Conductimetry

The specific conductivity of polyelectrolyte solutions in the absence of external salt can be represented by the following relationship (Manning, 1974):

$$\tau = 10^{-3} \cdot C_p f(\lambda_x + \lambda_p) \qquad (2)$$

where C_p is the polyelectrolyte concentration in eq. litre^{-1}, f is the transport coefficient, λ_x and λ_p the equivalent conductance of the counterions X and polyion, respectively.

Taking into account the fact that f is the same for counterions of the same valence, we measured τ for Li^+ and K^+ salt forms of the polyelectrolyte, and the difference in their solution conductivity gives

$$\Delta\tau_{K\,Li} = 10^{-3} C_p f(\lambda_K - \lambda_{Li}) \qquad (3)$$

where τ_{Li} and τ_K were obtained from conductimetric neutralization curves (Fig. 2a). They correspond to $\alpha = 1$ and allow us to calculate f, knowing the values of λ_K and λ_{Li} (Robinson & Stokes, 1968).

The equivalent conductance of the polyion, independent of counterion, can be deduced from eqn (2) and the f value deduced previously for monovalent counterions:

$$\lambda_p = (\tau - 10^{-3} f C_p \lambda_x)/10^{-3} f C_p \qquad (4)$$

Then we can calculate f_{Ca} with τ_{Ca}, the conductivity of the calcium polysaccharide solution ($\alpha = 1$ in Fig. 2b):

$$f_{Ca} = \frac{\tau_{Ca}}{10^{-3} C_p(\lambda_p + \lambda_{Ca})} \qquad (5)$$

The values of the transport coefficients are related to the charge parameter of the polyelectrolyte chain from Manning's theory (Manning, 1974):

$$\begin{aligned} f &= 0{\cdot}87\,\lambda^{-1} \text{ for monovalent ions } [\lambda > 1] \\ f' &= 0{\cdot}435\,\lambda^{-1} \text{ for divalent ions } [\lambda > 0{\cdot}5] \end{aligned} \qquad (6)$$

where λ, the charge parameter, is given by the relationship

$$\lambda = \frac{\nu}{b}\frac{\varepsilon^2}{DKT} \qquad (7)$$

In this relationship ν is the number of ionic charges per

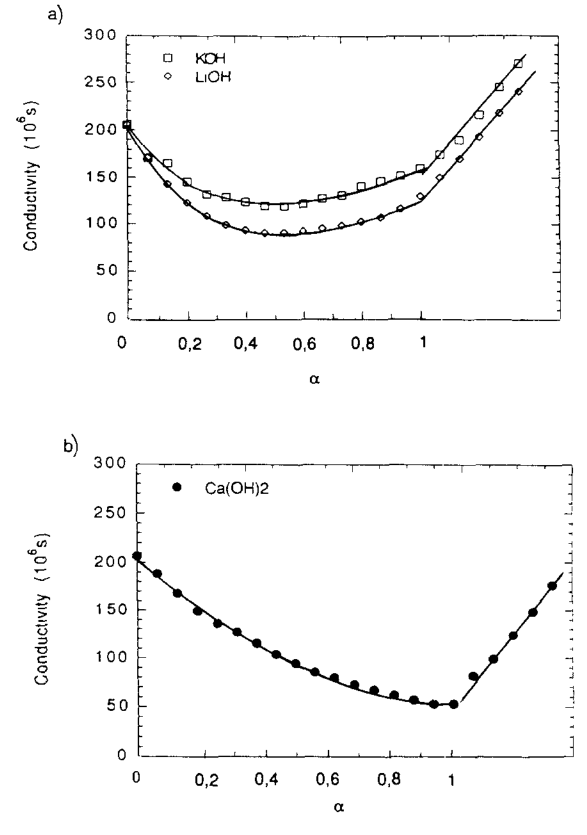

Fig. 2. Conductimetric titrations of β(1 → 4)polyglucuronic acid (EPS-0; $C_p = 2{\cdot}1 \times 10^{-3}$ eq. litre^{-1}); (a) with KOH and LiOH, (b) with $Ca(OH)_2$.

monomeric unit of length b, ε the electronic charge, D the dielectric constant and KT the Boltzmann term. For polyglucuronic acid we obtained $\lambda = 1{\cdot}36$, if we consider a single stretched chain and $\lambda = 2{\cdot}72$ for a double-stranded stretched chain. The experimental and theoretical values of the transport coefficient are compared in Table 2. One can see that the experimental values are compatible with the theoretical ones using the single stretched chain model. The lower experimental value found with divalent counterions is probably due to aggregation.

Viscosity

The reduced viscosities *versus* polysaccharide concentration are plotted as a function of NaCl concentrations in Fig. 3. One obtains the classic behaviour of polyelectrolyte solution with an increase in η_{sp}/C in pure water at low polymer concentration and a decrease as the salt content increases. In the presence of salt and in the dilute regime the reduced viscosity is given by the Huggins law:

$$\eta_{sp}/C = [\eta] + k'[\eta]^2 C \qquad (8)$$

where k' is the Huggins constant. The values of k' calculated from the different curves are listed in Table 3.

Table 2. Experimental and theoretical values of the transport coefficient for β(1 – 4)-D-glucuronan EPS-0

	f^+	f^{2+}
Experimental value	0·63	0·25
Theoretical value $\lambda = 1{\cdot}36$ (single chain)	0·64	0·32
Theoretical value $\lambda = 2{\cdot}72$ (double stranded)	0·32	0·16

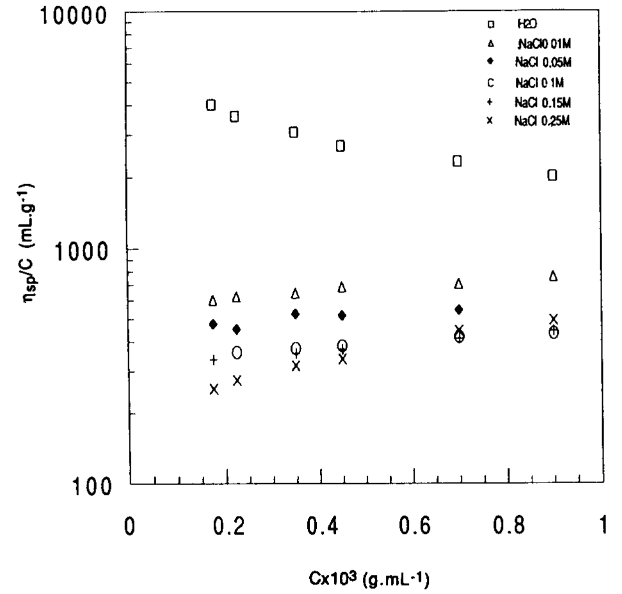

Fig. 3. Reduced viscosity as a function of polymer concentration at different external salt concentrations (EPS-4; *O*-acetyl % = 22·8).

Table 3. Influence of external salt concentration *I*, on Huggins constant k' and intrinsic viscosity $[\eta]$ for EPS-4 (22·8% *O*-acetyl group content)

I	$[\eta]$ (ml g^{-1})	k'
NaCl 0·01 M	570	0·61
NaCl 0·05 M	450	0·66
NaCl 0·10 M	340	0·96
NaCl 0·15 M	300	1·73
NaCl 0·25 M	240	8

Their values are higher than 0·4 and they increase with external salt concentration particularly above 0·1 M. This behaviour is probably due to the presence of intermolecular interactions with aggregate formation when the NaCl concentration increases above 0·1 M.

In Table 4 the results obtained with two samples, EPS-1 and EPS-4, (3% and 22·8% of *O*-acetyl group content, respectively) are reported. At 0·1 M NaCl external salt concentration, $[\eta]$ is much higher for the less acetylated sample, but on the other hand k' is lower for this sample. This suggests that the less acetylated sample is the more soluble. The presence of a large quantity of *O*-acetyl groups promotes hydrophobic interchain associations with the formation of aggregates. The influence of acetyl groups on interchain interactions and physico-chemical features of polysaccharides (Dentini *et al.*, 1984) are well known.

Hydrodynamic parameters

In order to compare the samples with different acetyl group contents, determination of hydrodynamic parameters by SEC has been performed in 0·1 M NH_4NO_3. From this equipment, we have been able to determine the parameters K and a of the Mark–Houwink relationship:

$$[\eta] = KM^a \qquad (9)$$

Three samples were compared: EPS-1, EPS-2 and EPS-3. Each solution of the three samples was divided into two fractions: one fraction heated (80°C; 10 min) and filtered (membrane 0·2 μm) and the other just filtered through the same membranes. The characteristics obtained for these six solutions are given in Table 5. The values of the molecular weights of heated samples showed that there were no significant variations concerning the method of production and precipitation of these samples. The difference noted between the two

Table 4. Influence of acetyl groups on $[\eta]$ and k' in NaCl 0·1 M, 25°C

% of acetyl groups in weight (w/w)	$[\eta]$ (ml g^{-1})	k'
3·0% (EPS-1)	820	0·59
22·8% (EPS-4)	340	0·96

Table 5. Results obtained by SEC coupled with a multidetection system

Samples	EPS-1		EPS-2		EPS-3	
	H	NH	H	NH	H	NH
$10^{-5}\overline{Mw}$	1·63	1·60	1·67	2·40	1·70	1·72
$[\eta]$ (ml/g)	796	786	701	776	451	511

EPS-1. 3% Acet.; EPS-2· 10% Acet.; EPS-3: 18% Acet., H —Heated, NH—Not heated

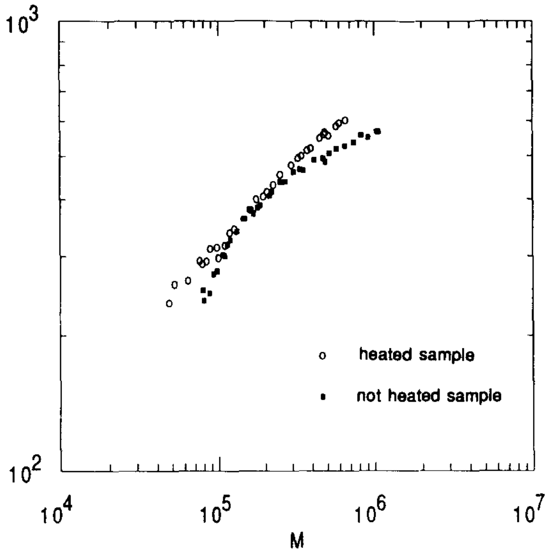

Fig. 5. R_g of sodium polyglucuronate EPS-2 as a function of molecular weight in 0·1 M NH_4NO_3 at 25°C.

solutions of EPS (heated and not heated) seems to depend upon the existence of aggregates before heating. These results confirm that the intrinsic viscosity is largely influenced by the presence of *O*-acetyl groups as previously found (Table 4). For equivalent molecular weights, a higher acetyl group content leads to a large decrease in $[\eta]$ (> 30%) which is due to contraction of the coil or aggregation.

Additional information which can be obtained from SEC if MALLS is used as a detector is the radius of gyration as a function of molecular weight. For a gaussian chain, in θ conditions the radius of gyration R_g, scales with the molecular weight M, according to the following relationship: $R_g \sim M^{\nu}$ with $\nu = 0{\cdot}5$ (Flory, 1953). In a good solvent, the effects of excluded volume and the polymer–solvent preferential interactions lead to coil expansion. In this case ν may increase up to 0·6. Figures 4–6 show the $R_g(M)$ variation for the three samples studied.

After heating of the solutions, the behaviour is quite different and depends upon the acetyl content. For the lower content (EPS-1) log R_g *versus* log M is linear for both heated and non-heated solutions. We notice only a small increase in the ν exponent on heating which can be attributed to an expansion of the chain knowing that the average molecular weight remains the same and $[\eta]$ increases slightly (Table 5). For intermediate acetyl content (EPS-2), heating leads to a linear relationship between log R_g and log M which can be explained by a disaggregation process and a decrease in the average molecular weight and intrinsic viscosity (Table 5). The sample with the highest acetyl content (EPS-3) shows no difference after heating. The R_g *versus* M plot (Fig. 6) corresponds to the presence of aggregates which are stable with temperature. This explains the low intrinsic viscosity value found for the most acetylated samples (Tables 4 and 5).

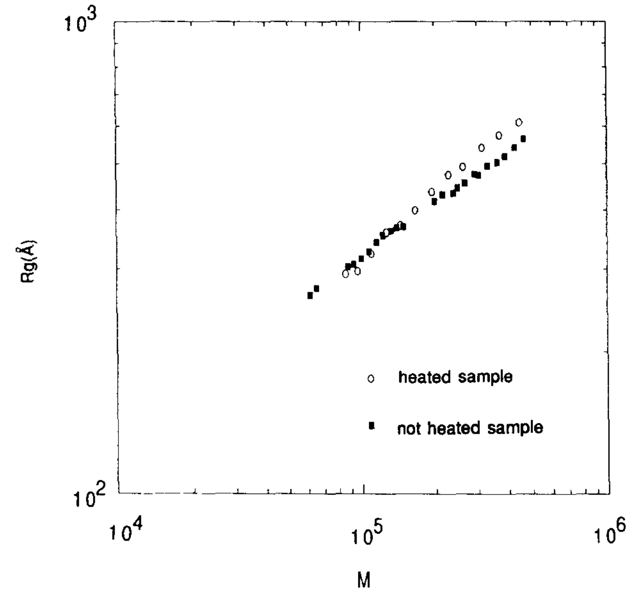

Fig. 4. R_g of sodium polyglucuronate EPS-1 as a function of molecular weight in 0·1 M NH_4NO_3 at 25°C.

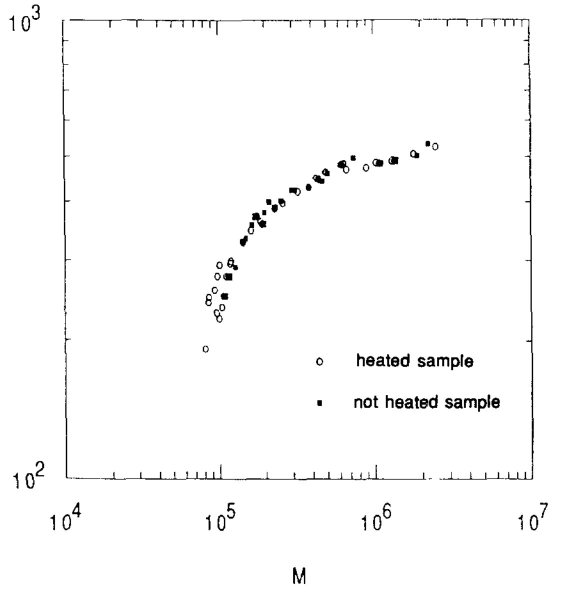

Fig. 6. R_g of sodium polyglucuronate EPS-3 as a function of molecular weight in 0·1 M NH_4NO_3 at 25°C.

Considering these facts, we decided to calculate the persistence length, L_p, for the results obtained with the heated samples in the lower molecular weight range corresponding to values of v near 0·5. No calculation has been performed on the aggregated high-acetyl-content sample.

Assuming that our polymer behaves as a worm-like chain (Yamakawa, 1971; Odijk, 1977) and by fitting experimental data with the Benoit–Doty relationship (Benoit & Doty, 1953):

$$<R_g^2>=\frac{1}{3}L_T L_p - L_T^2 + 2L_T^3 L_p^{-1} - 2L_T^4 L_p^{-2}\left[1-\exp\left(-\frac{L_p}{L_T}\right)\right] \quad (10)$$

(with total chain length $L_T = (M/m_0)d$; $d = 5{\cdot}15$ Å monomer unit length, m_0: monomer unit molecular weight) (see Table 1), the values of L_p which give the best fit are listed in Table 6 together with the Mark–Houwink parameters. The values of about 110 Å for L_p and $a = 0{\cdot}8$ are in agreement with the values found for similar polyuronates in the same range of ionic strength (Malovikova *et al.*, 1993). Then the 'Exogel' polysaccharide behaves as a semi-rigid polymer with low aggregate content at least for the lower acetyl degree and ionic strength according to a and L_p values.

Gel of $\beta(1 \rightarrow 4)$ glucuronan

The polyglucuronan can gel in the presence of mono, di or trivalent cations depending on polymer concentration and ionic strength. For a molecular weight of about $\overline{Mw} = 1{\cdot}5 \times 10^5$, polyglucuronan solution (5 g/litre) forms thermoreversible gel in the presence of 1 M NaCl. By increasing the ionic strength the gel stability increases and a gel formed in 2 M NaCl does not melt even when it is heated to 100°C, and the Young's modulus remains too low to be measured in our apparatus.

In $CaCl_2$ solutions, the polyglucuronan forms a thermally stable gel when the $CaCl_2$ concentration is equal to or higher than 10^{-2} M. The Young's modulus (E) of gels formed in the presence of divalent cations (Ca^{2+}) were compared to those of an alginate ($\overline{Mw} = 2{\cdot}3 \times 10^5$ and $M/G \sim 0{\cdot}28$). Two samples of sodium polyglucuronate with different O-acetyl group contents were used (EPS-2 and EPS-3 with 10% and 18% of O-acetyl group), respectively. Comparison of the curves (Fig. 7) shows that the Young's modulus of polyglucuronate is higher for the lowest content (EPS-2), the presence of O-acetyl group decreasing the gel resistance as usually observed for other polysaccharides (Dea *et al.*, 1977; Tako *et al.*, 1984). O-acetyl groups may inhibit perfect junction zone formation and this explains the less compact tridimensional network obtained.

Gels of EPS-3 with different divalent cations were prepared according to the process described in the experimental section by using different 0·34 M salt solutions.

Young's modulus values for the gels were compared: Table 7 shows no significant difference in the Young's modulus values and of the gel volume (V) for the different cations used in gel preparation.

Finally, we have studied the retention of calcium ions in $\beta(1 \rightarrow 4)$ polyglucuronate gels in the presence of sodium ions. The gels were obtained from 15 g/litre polymer concentration following the method described in the experimental section, using a 0·34 M $CaCl_2$ solution. Their Young's moduli were initially measured, then the samples were divided into groups and put in stirred NaCl solutions at different concentrations: 0·1 M, 1 M and 4 M for five days, renewing the solutions every day. The modulus of each group was measured and the gels were returned to a 0·34 M $CaCl_2$ solution. The moduli were again measured five days later. By microanalysis of the dry gels at each stage, the C, Cl, Ca and Na contents can be measured. Gel cylinder volume measurements (see experimental section) before and after each change in the ionic strength of the medium have shown a variation that induces a modification of the effective polymer concentration on the gels and mostly explains the modulus change, for example after the second stabilization in 0·34 M $CaCl_2$ (Table 8).

Analysis of the values shown in Table 8, reveals that more than 50% of calcium ions present in the initial gels are removed by sodium ions when the gels are submerged in a high ionic strength solution (4 M), without a decrease in gel cohesion. This behaviour is different from that of polysaccharides such as polygalacturonates (pectins) and polyguluronates (alginates), which give very stable calcium gels where calcium ions are very difficult to displace (Bouffar-Roupe, 1989).

Table 6. Persistence lengths and Mark–Houwink parameters corresponding to molecular weight range: $7 \times 10^4 < Mw < 2 \times 10^5$

	EPS-1	EPS-2
L_p (Å)	114	103
a	0·80	0·83
$10^3 K$	51·7	35

Table 7. Young's Modulus values of polyglucuronate gels obtained from different cations (EPS-3; C_p = 10 g/litre; C_s = 0·34 M)

Salt	$10^{-4} E$ (Pa)	V (ml)
$BaCl_2$	1·54	2·65
$CaCl_2$	1·70	2·66
$CuCl_2$	1·56	2·52

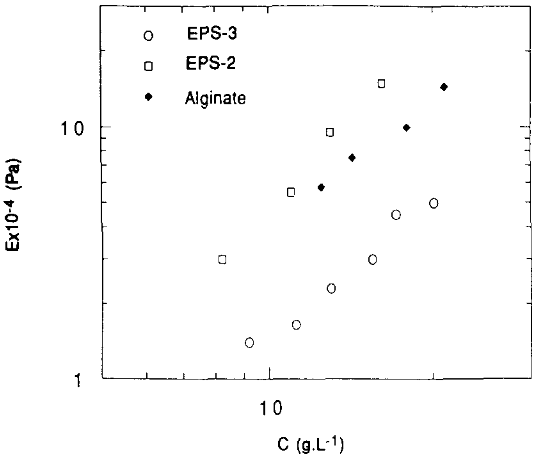

Fig. 7. Variation of Young's modulus (E) with polymer concentration Comparison with alginate ($M/G = 0{\cdot}28$; $\overline{M_w} = 2{\cdot}3 \times 10^5$).

Table 8. Values of Young's modulus of Ca^{++} gels of $\beta(1 \rightarrow 4)$ polyglucuronate which suffered ionic changes with NaCl (EPS-3; $C_p = 15$ g/litre)

Salt	$10^{-4}E$ (Pa)	V (ml)	Ca% eq. Ca^{2+}/eq. COO^-
$CaCl_2$ 0·34 M	8·1	2·12	100
NaCl 4 M	8·8	2·03	45
NaCl 0·1 M	2·2	2·66	97
NaCl 4 M → $CaCl_2$ 0·34 M	9·7	2·04	100
NaCl 1 M → $CaCl_2$ 0·34 M	8·1	2·10	100
NaCl 0·1 M → $CaCl_2$ 0·34 M	6·7	2·35	100

On the other hand, gels stored in solutions with lower ionic strengths lose their cohesion very quickly and their modulus decreases with increase in volume. We do not recover the initial Young's moduli and gel volumes after the second stabilization in 0·34 M $CaCl_2$. Some irreversible rearrangements occurred during stabilization in the different solutions of NaCl, but it seems that the change in gel modulus depends mainly upon the volume change (polymer concentration), which is a consequence of the counterion and ionic strength.

CONCLUSIONS

The new polyuronide, $\beta(1 \rightarrow 4)$-D-glucuronan, produced by a mutant strain of *Rhizobium meliloti* M5N1 behaves like a single chain in dilute solutions of its monovalent salt form. A $pK_0 \simeq 3$ has been found as for the other well-known polycarboxylic acid, alginates and pectins.

The presence of substituents such as an *O*-acetyl group related to biosynthesis conditions can modify the solution properties due to aggregate formation, which is easier as the *O*-acetyl content increases. Aggregation is also favoured when the external salt concentration is greater than 0·1 M. Nevertheless, a persistence length of around 110 Å is deduced and is in agreement with the value found for other polyuronates.

This polysaccharide forms thermoreversible gels with monovalent salts and thermally stable gels with divalent salts. Even if the moduli are comparable with those of alginates rich in guluronate units, the formation process seems to be different, with no peculiar selectivity and a gel modulus depending mainly upon the ionic strength.

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REFERENCES

Benoit, H. & Doty, P. (1953). *J Phys. Chem.*, **57**, 958–63.

Bouffar-Roupe, C. (1989). Thesis, Grenoble University, France.

Courtois, B., Heyraud, A. & Rinaudo, M. (1986). *J. Gen Appl. Microbiol.*, **32**, 527–34.

Courtois, J., Pheulpin, P., Heyraud, A & Courtois, B. (1990). *J. Gen. Microbiol.*, **36**, 215–20.

Courtois, J., Courtois, B., Heyraud, A., Colin-Morel, Ph. & Rinaudo, M. (1992). *Fr. Pat.* 9 202 510.

Courtois, J., Seguin, J.P., Declomesnil, S., Heyraud, A, Colin-Morel, Ph., Dantas, L., Barbotin, J.N. & Courtois, B. (1993). *J Carbohydr. Chem.*, **12**, 441–8.

Dea, I C.M., Morris, E.R., Rees, D.A., Welsh, E.J., Barnes, H.A. & Prince, J. (1977). *Carbohydr Res.*, **57**, 249–72.

Dentini, M., Crescenzi, V. & Blasi, D. (1984). *Int. J Biol. Macromol.*, **6**, 93–8.

Flory, P.J. (1953). *Principles of Polymer Chemistry*. Cornell University Press, Ithaca, New York, USA

Heyraud, A, Rinaudo, M. & Courtois, B (1986). *Int. J. Biol. Macromol.*, **8**, 85–8.

Heyraud, A., Courtois, J., Dantas, L., Colin-Morel, Ph. & Courtois, B. (1993). *Carbohydr. Res.*, **240**, 71–8.

Malovikova, A., Rinaudo, M. & Milas, M. (1993). *Carbohydr. Polym.*, **22**, 87–92.

Manning, G.S (1974). In *Polyelectrolytes I*, ed E. Sélégny. Reidel, Dordrecht, The Netherlands, pp. 9 37.

Odijk, T. (1977). *J. Polym. Sci.*, **15**, 477 83.

Rinaudo, M. & Milas, M. (1974) *J. Polym. Sci.*, **12**, 2073–81.

Rinaudo, M., Milas, M. & Le Dung, P. (1993). *Int. J. Biol. Macromol.*, **15**, 281–5.

Robinson, R.A. & Stokes, R.H. (1968). *Electrolyte Solutions*. Butterworths, London, UK.

Tako, M., Asato, A. & Nakamura, S (1984). *Agric. Biol. Chem.*, **48**, 2995–3000.

Tinland, B., Mazet, J. & Rinaudo, M. (1988). *Macromol. Chem. Rapid Commun.*, **9**, 69–73.

Yamakawa, H. (1971). In *Modern Theory of Polymer Solutions*. Harper and Row, New York