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# On the solution properties of bacterial polysaccharides of the gellan family

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## ABSTRACT

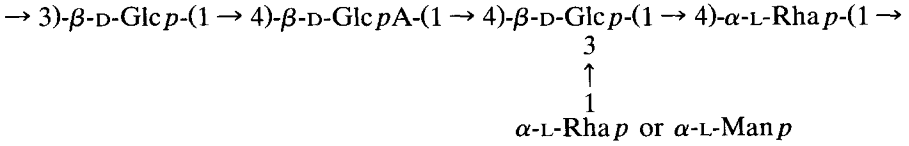
The influence of side chains and substituents on the polyelectrolyte behaviour of aqueous solutions of polysaccharides of the gellan family has been studied. The results of conductimetric and potentiometric titrations suggest that each of these polysaccharides adopts a double-helix conformation. Deacetylation destabilises the helix of rhamsan and gellan, and optical rotation data confirm these results. The side chain in rhamsan is more flexible and is remote from the carboxylate groups, and the behavior of this polysaccharide is similar to that of gellan. Gelation of deacetylated rhamsan occurs in the presence of calcium ions. The intrinsic viscosity as a function of ionic strength depends on the structure of the polysaccharide and on the presence of acetyl groups.

## INTRODUCTION

The microbial polysaccharides gellan, welan, rhamsan, and S657 have similar repeating units in their backbone[1–6] (the structures **1**–**4** are depicted without substituents).

$$\rightarrow 3)\text{-}\beta\text{-D-Glc}p\text{-}(1\rightarrow 4)\text{-}\beta\text{-D-Glc}p\text{A-}(1\rightarrow 4)\text{-}\beta\text{-D-Glc}p\text{-}(1\rightarrow 4)\text{-}\alpha\text{-L-Rha}p\text{-}(1\rightarrow$$

**1** (gellan)[1,2]



$$\rightarrow 3)\text{-}\beta\text{-D-Glc}p\text{-}(1\rightarrow 4)\text{-}\beta\text{-D-Glc}p\text{A-}(1\rightarrow 4)\text{-}\beta\text{-D-}\underset{\substack{3\\ \uparrow\\ 1\\ \alpha\text{-L-Rha}p\text{ or }\alpha\text{-L-Man}p}}{\text{Glc}p}\text{-}(1\rightarrow 4)\text{-}\alpha\text{-L-Rha}p\text{-}(1\rightarrow$$

**2** (welan)[4,5]

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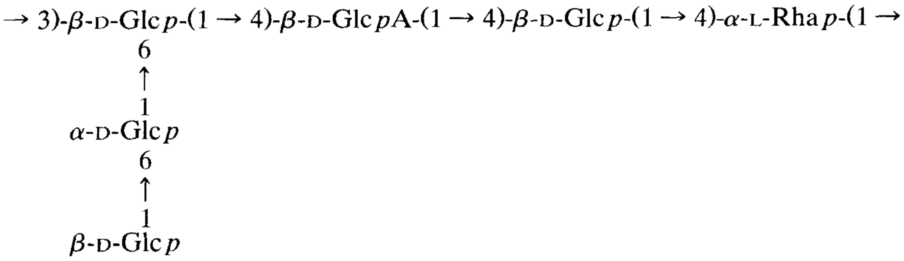
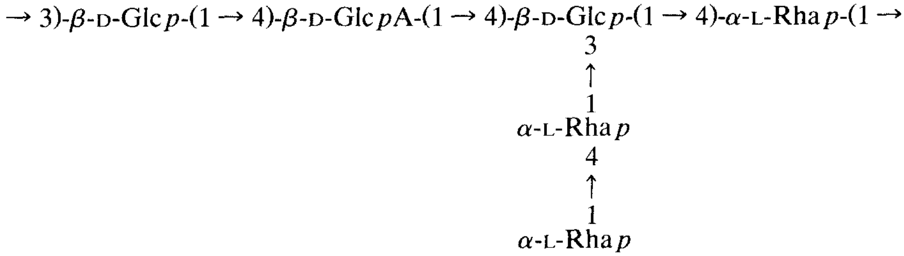


$$\begin{array}{l}\rightarrow 3)\text{-}\beta\text{-D-Glc}p\text{-}(1\rightarrow 4)\text{-}\beta\text{-D-Glc}p\text{A-}(1\rightarrow 4)\text{-}\beta\text{-D-Glc}p\text{-}(1\rightarrow 4)\text{-}\alpha\text{-L-Rha}p\text{-}(1\rightarrow \\ \qquad\qquad 6 \\ \qquad\qquad \uparrow \\ \qquad\qquad 1 \\ \qquad \alpha\text{-D-Glc}p \\ \qquad\qquad 6 \\ \qquad\qquad \uparrow \\ \qquad\qquad 1 \\ \qquad \beta\text{-D-Glc}p\end{array}$$

**3** (rhamsan)[3]



$$\begin{array}{l}\rightarrow 3)\text{-}\beta\text{-D-Glc}p\text{-}(1\rightarrow 4)\text{-}\beta\text{-D-Glc}p\text{A-}(1\rightarrow 4)\text{-}\beta\text{-D-Glc}p\text{-}(1\rightarrow 4)\text{-}\alpha\text{-L-Rha}p\text{-}(1\rightarrow \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad 3 \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad \uparrow \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad 1 \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad \alpha\text{-L-Rha}p \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad 4 \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad \uparrow \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad 1 \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad \alpha\text{-L-Rha}p\end{array}$$

**4** (S657)[6]

In the native state, gellan and rhamsan contain one *O*-acetyl group per repeating unit and welan has one *O*-acetyl group per two repeating units[3,7]. Gellan also has L-glycerate[7] and poly($\beta$-hydroxybutyrate)[8] substituents.

Gellan forms gels, the strength and stability of which depend on the type and concentration of cation present in solution and on the degree of acetylation[9–11]. Welan, rhamsan, and S657 are non-gelling polysaccharides, but their aqueous solutions exhibit marked low shear-rate viscosity and good thermal stability in comparison with xanthan gum[12].

Crescenzi et al.[9,13] concluded that the side chains in welan and rhamsan prevent conformational ordering. Urbani and Brant[14] confirmed the absence of conformational transition under the usual conditions and a weak-polyelectrolyte character for welan and S657, whose dilute aqueous solutions display similar behaviour. Campana et al.[15] claim that native and deacetylated welan adopt double-helical conformations irrespective of the pH and ionic strength of their solutions. The polyelectrolyte character of this polysaccharide is confirmed by the decrease in intrinsic viscosity with increase in the ionic strength and no experimental conditions were found to produce the conformational transition to a disordered coil. From the application of Smidsrod and Haug's treatment[16] to the data for intrinsic viscosity versus ionic strength, it was concluded that the stiffness of the welan chain is in the same range as that of DNA. The viscosity of aqueous solutions of welan as a function of shear rate and polymer concentration indicates that it is similar to xanthan[15].

We now report on the solution properties of native and deacetylated rhamsan and S657.

## EXPERIMENTAL

The samples of rhamsan and S657, kindly provided by Kelco, were purified from the unpasteurised broth and isolated as sodium salts, as with other bacterial polysaccharides[15]. This procedure preserves the native conformation and the acetyl groups. *O*-Deacetylation was effected by treatment of a solution of each polysaccharide (5 g/L) in 5:1 $Me_2SO$–$H_2O$ with 0.1 M NaOH at 25° for 2 h. The products were purified as described above. The IR spectra showed the absence of acetate groups. S657 required two successive treatments with base.

The acid forms of rhamsan and S657 were obtained by passing a solution of each sodium salt ($\sim$ 1 g/L) through Amberlite IR-120 ($H^+$) resin.

Conductimetric and potentiometric measurements were performed with a CD 78 conductimeter (conductivity cells from Tacussel) and a Minisis 6000 millivoltmeter associated with a specific $H^+$ electrode (Tacussel).

Viscosity measurements were performed in a Low Shear 30 from Contraves ($10^{-2}$ $s^{-1} < \dot{\gamma} < 128$ $s^{-1}$). The concentrations of polysaccharides used were below the overlap concentration, and the viscosity measurements corresponded to the Newtonian plateau.

Optical rotations ($[\alpha]_{300}$ values) were determined with a Spectropol 1b Fica (5-cm path length).

All measurements were performed at 25° except when temperature dependence was considered.

## RESULTS AND DISCUSSION

Rhamsan (**3**) and S657 (**4**) are polyelectrolytes due to the presence of D-glucuronic acid residues in their backbones. The characteristic charge parameter $\lambda$ can be estimated as $\lambda_c \sim 0.37$ or $\lambda_h \sim 0.75$ for stretched-coil and double-helix conformations, respectively, by reference to gellan[17,18]. These values allow prediction of the thermodynamic properties of rhamsan and S657 in dilute solution, and comparison with experimental results gives an estimate of the conformation as proposed for gellan[17,18] and welan[15].

*Polyelectrolytic behavior.—(a)* $pK_0$ *measurements.* By titration of the acid form of each native and deacetylated polysaccharide, the mass per equivalent repeating unit ($m_0$) was determined as 1250 ± 100 and 1115 ± 100 g/equiv for native and modified rhamsan, respectively, and 1340 ± 100 and 1195 ± 100 g/equiv for native and deacetylated S657, respectively. As found with welan, the values do not correspond to those predicted from the structures depicted in **1–4**. Values of 992 and 960 g/equiv are found for rhamsan (**3**) and S657 (**4**), respectively, if the acetyl contents are ignored. The differences do not correspond to one acetyl group per repeating unit. This result is similar to that obtained with welan[14,15] and suggests that substituents other than acetyl groups must be present.

The values of $pK_0$ for native and deacetylated polysaccharides were deduced from their respective titration curves, taking into account the degree of autodissociation of the carboxylic groups ($\alpha_H$) and the degree of neutralisation ($\alpha_N$) as proposed by Nagasawa[19]:

$$pK_a = pH + \log([1 - \alpha_t]/\alpha_t), \qquad (1)$$

where $\alpha_t = \alpha_H + \alpha_N$.

From the experimental data, the values of $pK_0$ (value of $pK_a$ extrapolated to $\alpha_t = 0$) for both forms of rhamsan and S657 are ~ 3.0 ± 0.1 and ~ 3.6 ± 0.1, respectively. For welan, a $pK_0$ value of 2.6 ± 0.1 was found for both native and modified structures[15]. The usual values of $pK_0$ for carboxylic polysaccharides are in the range[20] 2.9–3.35 and that of gellan[17,18] is 3.0. The differences observed for S657 and welan might be attributed to hydrogen-bond interactions. The lower $pK_0$ found for welan suggests that both oxygen atoms of carboxylate groups can act as acceptors in hydrogen-bond interactions of the side and main chains.

On the other hand, the higher value found for S657 suggests that the protonated oxygen atoms of the carboxyl groups serve as hydrogen-bond donors. These possibilities have been discussed for welan and S657 in the solid state, on the basis of X-ray and computer modeling studies, by Lee and Chandrasekaran[21]. In rhamsan, these authors found a more flexible side chain than in welan and S657, which is more remote from the carboxylate groups in the double helix. Thus, on this assumption, it is normal to find a $pK_0$ value of 3.0, which is similar to that determined in absence of the side chain (gellan)[17,18].

*(b) Interaction with counterions.* The transport coefficients of monovalent ($f^+$) and divalent ($f^{2+}$) counterions, determined[17,22] by conductivity titrations, were compared with those predicted from Manning's theory[22] and with the results for

TABLE I

Transport coefficients and equivalent conductivities ($\lambda_p$) compared to theoretical values

| Polysaccharide | $f^+$ | $f^{2+}$ | $\lambda_p$ [a] |
|---|---|---|---|
| S657 | 0.910 | 0.520 | 27.5 |
| Deacetylated S657 | 0.890 | 0.510 | 25.1 |
| Rhamsan | 0.933 | 0.604 | 23.3 |
| Deacetylated rhamsan | 0.973 | 0.804 | 17.8 |
| Welan [b] | 0.940 | 0.550 | 24.7 |
| Deacetylated welan [b] | 0.931 | 0.539 | 24.3 |
| Gellan (double helix) [c] | 0.920 | – | 27.2 |
| Gellan (random coil) [c] | 0.975 | – | 21.2 |
| Double helix [d] | 0.930 | 0.580 | |
| Random coil [d] | 0.981 | 0.922 | |

[a] Values in $\Omega^{-1}$. cm$^2$. equiv$^{-1}$. [b] Experimental data from Campana et al.[15]. [c] Experimental data from Milas et al.[18]. [d] Theoretical values from Manning's theory[22], using charge parameters defined for gellan[18].

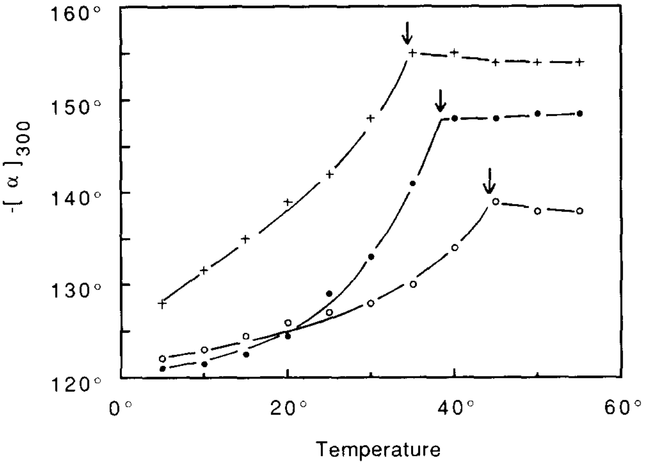


Fig. 1. Optical rotary power, $[\alpha]_{300}$ as a function of temperature for modified rhamsan in aqueous solutions ($C_p = 0.5$ g/L) for 0.1 (+), 0.3 (●), and 1 M NaCl (○). The arrows indicate $T_m$.

gellan and welan in Table I. This comparison shows that the experimental values for $f^+$ and the equivalent conductivities ($\lambda_p$) for these samples (except modified rhamsan) are similar to those obtained for gellan in the double-helix conformation. Moreover, the values for $f^{2+}$ of native welan, rhamsan, and S657, and that of modified welan and S657, are consistent with the predictions of Manning's theory for double-helical conformations. These results are in agreement with the results of X-ray and computer modeling studies of these polysaccharides[21].

The results obtained with modified rhamsan are in fairly good agreement with those for gellan as a random coil and with the predictions of Manning's theory for randomly coiled chains. For modified rhamsan, the experimental values for $f^{2+}$ were lower than that predicted from Manning's theory, which may be attributed to chain aggregation caused by the presence of $Ca^{2+}$ ions.

The difference between the values of the equivalent conductivities of random-coil and double-helix gellan[18] ($[\lambda_p]_C - [\lambda_p]_H$) is close to that observed with modified and native rhamsan, supporting the occurrence of a double-helix → random-coil conformational transition for deacetylated rhamsan.

*Conformational transition.*—The $[\alpha]_{300}$ values of native rhamsan and of native and modified S657, as functions of the temperature and ionic strength, do not show any significant changes. These results are similar to those obtained with native and modified welan. However, effects were observed with modified rhamsan as shown in Fig. 1. The effects of temperature and ionic strength are as has been demonstrated for the conformational transition observed with gellan[18]. An ordered conformation is proposed to exist at low temperature and/or high ionic strength and, as observed with gellan, the transition is asymmetric.

The melting temperature of the ordered conformation ($T_m$) is usually determined from the point of half transition. However, due to the difficulty imposed by the asymmetry of the curves, the procedure employed by Milas et al.[18] was used and $T_m$ was taken for 100% of the coil conformation. This procedure allows a direct comparison between these results and those for gellan[18].

As usually observed, the values of $T_m$ change with the total ionic concentration as shown in Fig. 1, and the slope of the curve ln $C_T$ versus $1/T_m$ is related directly to the change in enthalpy involved in the melting process[23]:

$$\Delta H_{trans} \sim d(\ln C_T)/d(1/T_m), \quad (2)$$

where $\Delta H_{trans}$ is the change in enthalpy associated with the melting of the ordered transition, and $C_T$, the total ionic concentration of free counterions, is $C_s + \gamma C_p$.

The results in Fig. 1 give a slope of −16000 (cf. −10610 for gellan[10]). Hence, the change in enthalpy associated with the melting of the ordered conformation is higher for deacetylated rhamsan than for deacetylated gellan. Thus, the ordered conformation is more stable for the former polysaccharide, since the charge parameters of the ordered and disordered structures are similar. The specific behavior observed for rhamsan, compared to that of welan and S657, might be attributed to the greater flexibility of the side chain for the former polysaccharide as described by Lee and Chandrasekaran[21].

*Effect of the ionic strength on the intrinsic viscosities.*—The dependence of intrinsic viscosity of solutions of native and modified rhamsan and S657 on the ionic strength was studied with the sodium salts in the range 0.03 equiv/L < [NaCl] < 0.3 equiv/L.

Native rhamsan shows the behavior expected for a polyelectrolyte. In the range of salt concentration studied, the following relation is found:

$$|\eta| = 1850 + 172\mu^{-1/2}, \quad (3)$$

but the values of the Huggin's constants are high ($1.20 < k_H < 1.50$) and reflect the poor quality (aggregation) of the solutions.

The measurements of intrinsic viscosity of solutions of modified rhamsan as a function of the ionic strength, which cannot be determined accurately due to aggregations, give $|\eta|$ values which are smaller and less sensitive to the variation of the ionic strength. These problems may be associated with the occurrence of the conformational change $T_m \sim 25°$ in ~0.02 M NaCl and chain aggregation, and prevents comparative analysis of the two forms of this polysaccharide. The aggregation was confirmed by the addition of > 0.01 M calcium chloride which causes a sol → gel transition. The formation of a gel with deacetylated rhamsan and not with welan and S657 agrees with the predictions of Lee and Chandrasekaran[21]. The side chain is more flexible in rhamsan and remote from the carboxylate groups in the double helix, which allows gel formation by interaction of the carboxylate groups and cations. On lowering the temperature, gel formation occurs at $T_m$ and confirms the conformational transition for the deacetylated rhamsan. For welan and S657, Lee and Chandrasekaran[21] suggested intramolecular interactions that involve the side chains and glucuronic acid residues, and reduce the ability to form gels through intermolecular aggregation via carboxylate groups and cations. For

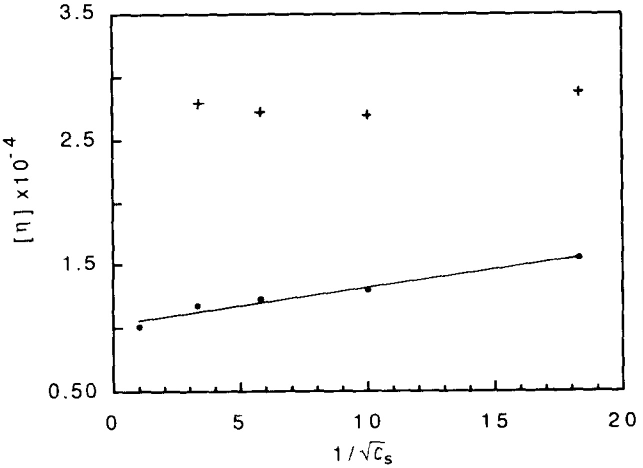


Fig. 2. Intrinsic viscosity as a function of the reciprocal of the square root of the salt concentration (in mol/L) for native (+) and modified (●) S657.

both of these polysaccharides, in agreement with this assumption, gel formation did not occur in the presence of calcium ions.

Compared with rhamsan, opposite effects of ionic strength on intrinsic viscosities were found for S657 (Fig. 2). Polyelectrolyte behavior was observed for the deacetylated form but not for the native structure. This observation is consistent with the conclusions of Crescenzi et al.[9] and Urbani and Brant[14], who found a weak polyelectrolyte character for the native forms of welan and S657. The precise role of the acetyl substituents on the properties of these polysaccharides cannot be determined because of the presence of aggregates and other substituents such as poly($\beta$-hydroxybutyrate)[8].

Thus, it is concluded that native rhamsan and S657, like welan and gellan, adopt ordered conformations (double helix) in solution, and the similar structure of the backbones of the native polysaccharides is the determining factor. The same conclusions have been deduced for the solid state[21].

In the deacetylated polysaccharides, the side chains (position, length, and structure) have a marked influence on the solution properties.

Native and deacetylated gellan and deacetylated rhamsan, in pure water, have the physicochemical parameters expected for random coils, and conformational transition was observed in the presence of NaCl, whereas native rhamsan, native and modified welan, and S657 have those predicted for the double helix and no conformational transition was observed (Table II).

These observations suggest that the position or the structure of the side chains is more important than their length in determining the conformations in solution. Glycosyl substituents stabilise the ordered conformation and are more effective in welan and S657, involving intrachain hydrogen bonds. The fact that soft-gel formation occurs in dilute solutions of modified rhamsan in the presence of $Ca^{2+}$

TABLE II
Possible structures in solution [a]

| | Gellan | Welan | Rhamsan | S657 |
|---|---|---|---|---|
| Native | $(DH, SC)_x^*$ | DH | DH | DH |
| Deacetylated | DH, SC* | DH | DH, SC* | DH |

[a] SC, single coil; DH, double helix; *, gel formation under certain conditions; $x$, aggregation prevents determination of structure.

ions, whereas modified gellan forms rigid gels under the same conditions, shows that the glycosyl substituents play an important role in gel cohesion and/or in the intermolecular interactions (Table II).

The $pK_0$ values for native and deacetylated welan and S657 differ from those for gellan and rhamsan, and suggest the existence of hydrogen bonds involving the carboxylic group of the glucuronic acid residue in the main chain and the side chain. The different behavior of these polysaccharides in solution can also be explained from the results of Lee and Chandrasekaran[21] for the solid state using X-ray and computer modeling studies.

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