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COMPARATIVE BEHAVIOR OF THE BACTERIAL POLYSACCHARIDES XANTHAN AND SUCCINOGLYCAN

GEORGES GRAVANIS, MICHEL MILAS, MARGUERITE RINAUDO, AND BERNARD TINLAND
Centre de Recherches sur les Macromolécules Végétales, CNRS-Grenoble, B P 68, 38402 – Saint Martin d'Hères Cedex (France)

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ABSTRACT

The bacterial polysaccharides xanthan and succinoglycan, each in a series of samples covering a range of molecular weights, have been compared. Their rheological properties show many similarities, although the extension of the succinoglycan in its ordered conformation is larger than that of xanthan. An irreversible decrease in viscosity observed for both polymers following heating above their respective conformational transition-temperatures, T_m, suggests the presence in the backbone of covalent breaks that are masked by strong sidechain–backbone interactions in both polymers. The principal differences in the behavior of the two polymers are found in the sharpness of their conformational transitions and the rheology of their solutions at temperatures above T_m. Succinoglycan displays the more abrupt, temperature-driven, conformational transition, and, above T_m, it is a more randomly coiling molecule than xanthan, which loses its stiffness only gradually on passing through T_m.

INTRODUCTION

It has been known for more than twenty years that extracellular, microbial polysaccharides can be produced in good yield in controlled fermentations[1,2]. Among these materials, xanthan has become a commercially important, water-soluble polymer, useful especially for its large viscosity increment in the presence of low-molecular-weight salts[3]. The molecular basis of this behavior is still a matter of active discussion[4]. Recently we have investigated the aqueous-solution properties of some succinoglycans produced by *Pseudomonas* and *Rhizobium*[5,6]. Their general behavior is similar to, but in some ways very different from, that of xanthan.

Herein, some solution properties of xanthan and succinoglycan are compared. The chemical structures of the two polymers are shown in Fig. 1.

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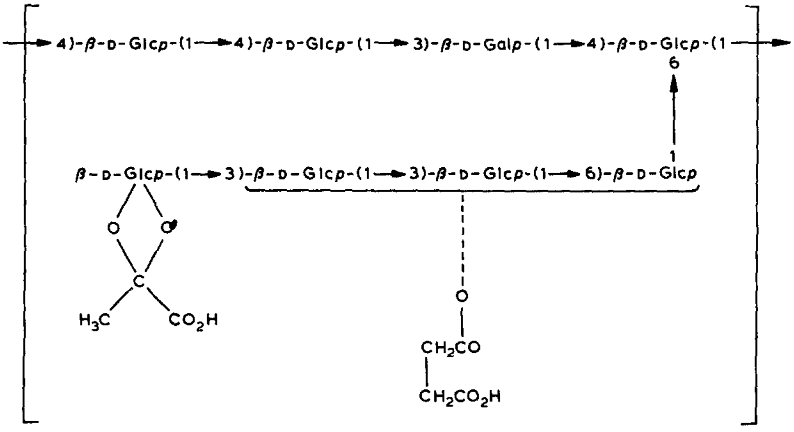
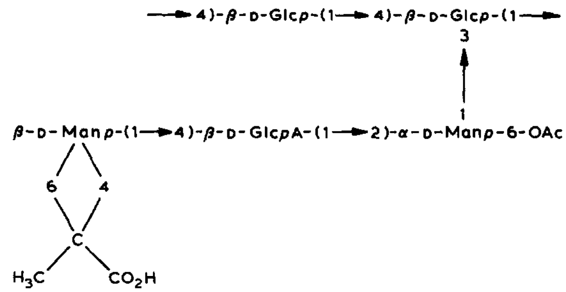


Succinoglycan



Xanthan

Fig 1 Chemical structure of succinoglycan and xanthan.

EXPERIMENTAL

The native xanthan and succinoglycan were kindly supplied by Shell (Sittingbourne, UK). Purification and isolation of the experimental samples as their sodium salts have been described previously[7]. The weight-average molecular weights, $\overline{M}_w$, of the native polymers were 4.2×10^6 and 7.0×10^6 for succinoglycan and xanthan, respectively, and the polydispersity index was ~1.2 for each. The acetyl and pyruvic acetal contents in xanthan were 0.75 and 0.4, respectively, expressed as mole fraction per repeating unit; the succinate and pyruvic acetal in the succinoglycan were each 1.0. Viscosities were determined as a function of shear rate, in a Contraves Low Shear 30 viscometer thermostated at the desired temperature. Optical rotation was measured at 300 nm in a thermostated cell of 5-cm path-length. Samples of lower molecular weight were obtained by sonication of the native polymers with a Branson apparatus (Model B12, 150 W, 20 kHz) equipped with a standard, 3-mm microprobe[8].

A series of succinoglycan samples free from succinate groups was obtained by hydrolysis for 12 h at 25° in 0.05M NaOH without change in the degree of polymerization[5].

Certain xanthan samples were subjected to partial hydrolysis for 2 h at 90° in 1mM oxalic acid containing 0.1M NaCl. After neutralization of the acids, these samples were precipitated as usual, and then dissolved in 0.01M NaCl for viscosity and optical-rotation measurements. Under these conditions, the ordered conformation is preserved.

RESULTS AND DISCUSSION

A. Analogies in the rheological behavior

Relative viscosity versus *shear rate.* — Fig. 2 shows the dependence of relative viscosity η_{rel} on shear rate, $\mathring{\gamma}$, for aqueous solutions containing polymer at 1 g/L and 0.1M NaCl, at 25°. Under these conditions, both polymers are in their ordered form, as disclosed by measurements of the optical rotation[5,9].

It is clear that η_{rel} is larger for succinoglycan than for xanthan at a given $\overline{M}_w$. This comparison is complicated, however, by the difference in chemical structure of the repeating units; but, even if the relative viscosities are compared at a given number of chemical repeating units, or at a given number of mainchain-backbone sugar residues, the comparison is unchanged. Departures from Newtonian behavior are, however, more pronounced for xanthan; the curves bend downward at lower $\mathring{\gamma}$ for xanthan than for succinoglycan at comparable values of η_{rel}. Otherwise, the two ordered polymers show very similar behavior.

Relationship between specific viscosity and overlap parameter. — Recently, the rheology of xanthan in dilute and semi-dilute aqueous solution has been re-

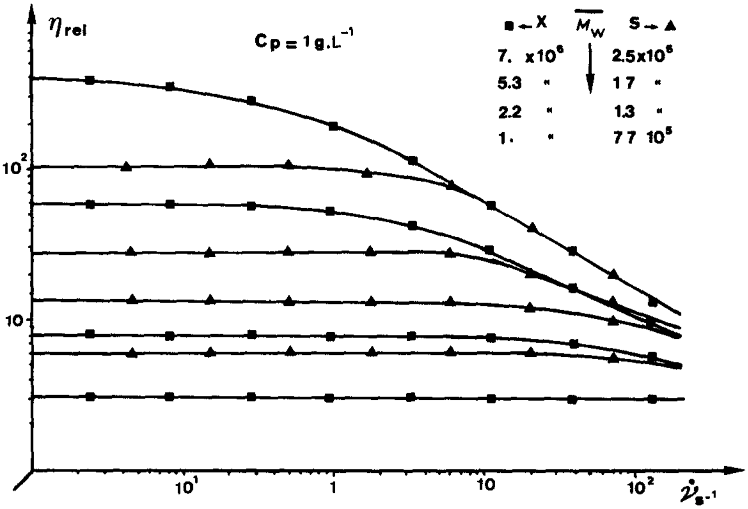


Fig 2. Relative viscosity *versus* shear rate for xanthan (X, ■) and succinoglycan (S, ▲) for samples with different molecular weights Solvent, 0.1M NaCl, temperature, 25°.

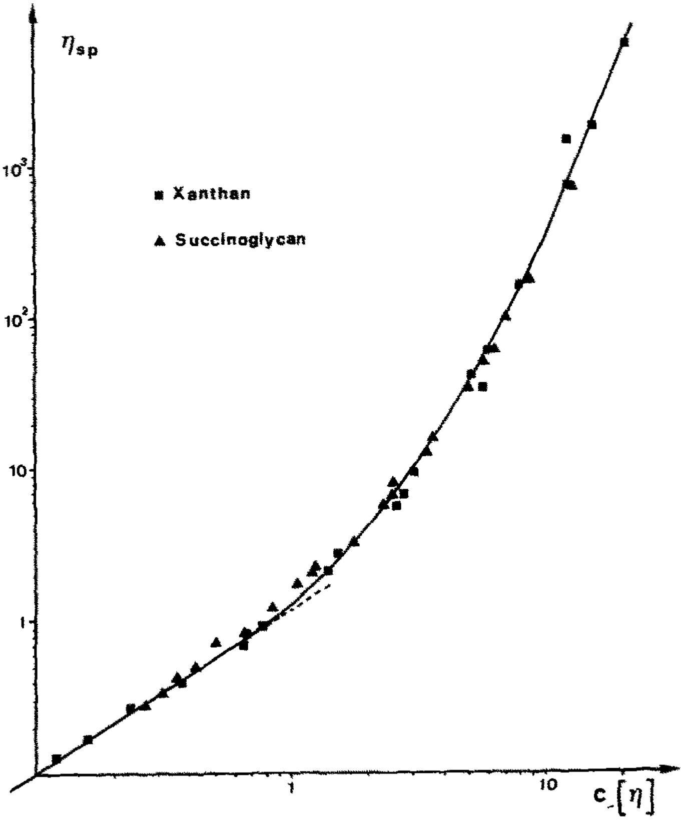


Fig 3. Specific viscosity as a function of the overlap parameter $C[\eta]$, $[\eta]$ is the intrinsic viscosity at $\mathring{\gamma} = 0$ and C is the polymer concentration

ported[10]. Here, as may be seen in Fig. 3, where specific viscosity, η_{sp}, is plotted *versus* the dimensionless overlap parameter, $c[\eta]$, we combine the earlier results for ordered xanthan[10] with similar data for the ordered succinoglycan[5]. All data were gathered for aqueous solutions containing 0.1M NaCl at 25°. In Fig. 3, η_{sp} always refers to measurements in the Newtonian regime, C is the polymer concentration, and $[\eta]$ is the intrinsic viscosity at $\mathring{\gamma} = 0$, expressed in units of inverse polymer-concentration. The data for xanthan and succinoglycan are evidently represented by a single curve in Fig. 3. The change in slope near $C^* [\eta] \simeq 0.8$ signifies the concentration, C^*, above which the aqueous-solution rheology of a polymer sample having intrinsic viscosity $[\eta]$ is dominated by intermolecular contacts with other polymer molecules.

Mark–Houwink relation. — Fig. 4 displays $[\eta]$ ($\mathring{\gamma} = 0$) as a function of $\overline{M}_w$ for ordered xanthan and succinoglycan in aqueous, 0.1M NaCl at 25°. These data confirm the greater viscosity-increment of the succinoglycan, especially in the absence of the succinoyl substituent groups. The Mark–Houwink coefficients (slopes of the lines in Fig. 4) are: xanthan, 1.14; succinoglycan without succinate,

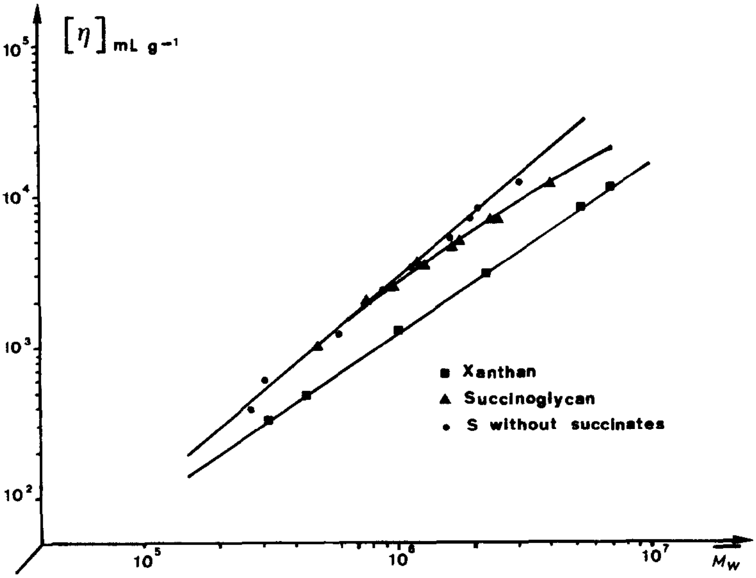


Fig 4 Mark–Houwink relationship

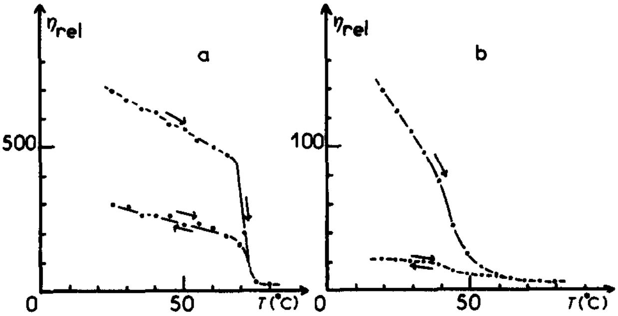


Fig 5 Relative viscosity as a function of the temperature. Polymer concentration 1 g/L, 0 1M and 0 01M NaCl, respectively, for (a) succinoglycan and (b) xanthan

1.4; and succinoglycan, 1.07 to 1.3. The larger value associated with the succinate-free succinoglycan suggests that this is the stiffest of the three polymer species compared here.

In the linear range, the Mark–Houwink relations are then, for succinoglycan ($\overline{M}_{\mathrm{w}} < 10^6$), $[\eta] = 6.5 \times 10^{-6}\ \overline{M}_{\mathrm{w}}^{1.4}$, and for xanthan[10], $[\eta] = 1.7 \times 10^{-4}\ \overline{M}_{\mathrm{w}}^{1.14}$. Nevertheless, it must be pointed out that the parameters are sensitive to the origin of the polysaccharides.

Existence of covalent breaks in the backbone of the native polymer. — When native succinoglycan is heated in neutral, aqueous 0.1M NaCl solution above T_{m} (see Fig. 5a), its relative viscosity, when returned to room temperature, is, by a factor of ½ (or more), smaller than before the thermally induced, conformational transition; this change in reduced viscosity is accompanied[5] by a corresponding reduction in $\overline{M}_w$. Because the chain stiffness, charge density, and chemical structure of succinoglycan remain unchanged after the heating and cooling cycle, it has been proposed[5] that covalent breaks exist in the backbone of the native polymer. These are thought to be masked by the presence of strong interactions between the backbone and the long, flexible side-chains which are presumed to be folded to lie parallel with the main chain.

Recently, very similar behavior has been observed for xanthan (see Fig. 5b) which has been subjected to partial depolymerization in the ordered conformation by acid, sonication, or a hydrolytic enzyme[11,12]. Certain native-xanthan samples have also exhibited this property without prior depolymerization, suggesting that covalent breaks may even exist in the backbone of some native xanthans.

B. Differences in the conformational transition

As shown in Fig. 5a, η_{rel} decreases sharply for succinoglycan at a temperature which coincides with the T_m measured by optical rotation (see Fig. 6); the rheological properties of the polymer are[5] Newtonian above T_m for $\mathring{\gamma}$ up to 1270 s^{-1}. The large increase in chain flexibility displayed by succinoglycan at T_m is not[11] matched by analogous behavior in xanthan at low $\mathring{\gamma}$, presumably because the inherent stiffness of the xanthan backbone (*i.e.*, in the absence of sidechain–backbone interaction) is significantly greater than that in succinoglycan.

The conformational transition, as detected by optical rotation, is also different for the two polymers (see Fig. 6). For succinoglycan, the transition is very sharp and is completed in a temperature range of some 2°. The chiroptically detected transition in xanthan, on the other hand, usually covers a range some 10 times as large. This difference is certainly consistent with the fact that the calorimetric ΔH for transition per monomer unit of the main chain is much larger for succinoglycan[5] ($\Delta H = 39.5$ kJ.mol^{-1}) than for xanthan[13] ($\Delta H = 3.55$ kJ.mol^{-1}), but there may also be contributions to the difference in transition breadth due to (*a*) the difference in

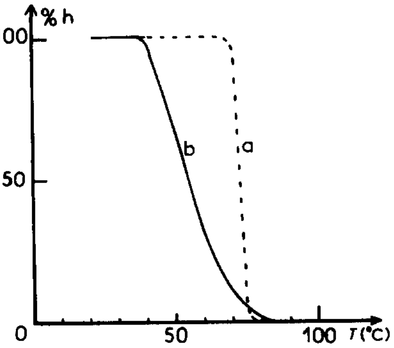


Fig 6 Degree of order of both polymers as a function of the temperature Polymer concentration 1 g/L, solvent, 0 1M and 0 01M NaCl, respectively, for (a) succinoglycan and (b) xanthan.

the chemical homogeneity of xanthan and succinoglycan, and (*b*) differences in the cooperativity of the transition for the two polymers. Moreover, the screening of the charge by external salts that stabilize the ordered conformation is much larger for xanthan than for succinoglycan; for example, for an increase of salt concentration by a factor of 10, T_m increases 44° for xanthan, but only 12° for succinoglycan.

CONCLUSION

Analogies exist in the rheological behavior of the bacterial polysaccharides xanthan and succinoglycan. They behave as stiff molecules: in the ordered conformation, the succinoglycan is shown to be stiffer than the xanthan, but, over T_m the reverse is observed. The main conclusion from the comparison presented concerns the role of the side chains, which is much more important for stabilization of the succinoglycan in the ordered conformation; as a consequence, a very sharp transition-region in viscosity and optical rotation is observed.

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