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Synthesis and preliminary characterisation of new esters of the bacterial polysaccharide gellan

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ABSTRACT

Under the appropriate experimental conditions, ethyl, propyl, and methylprednisolon-21-yl esters of gellan can be obtained without significant degradation. At low degrees of esterification (de), depending on the ester moiety, the products are water-soluble, which allows the influence of hydrophilicity and charge density on their ability to assume an ordered conformation in dilute aqueous solution to be studied.

With high de, the products were soluble only in organic solvents (e.g., methyl sulphoxide) with good film-forming capacity. The methylprednisolon-21-yl esters have been characterised in a preliminary manner in terms of drug-release kinetics.

INTRODUCTION

The controlled esterification or etherification of hydroxyl groups of polysaccharides can give products of industrial importance. The amino groups in *N*-deacetylated chitin (chitosan) can be substituted selectively. Alkyl or aryl esters of polyuronates are less frequently encountered, but do have potential commercial value[1]. Work[1,2] with alginates and hyaluronic acid showed that when conditions were optimised so as to obtain high yields, control the extent of reaction, and largely avoid undesirable side processes and/or chain scission, novel alkyl or alkylaryl esters of polyuronates can be obtained that have a number of applications, notably in the biomedical sector[1,2].

We now report preliminary results on the synthesis and characterisation of esters[3] of the bacterial polysaccharide gellan (**1**). The purposes of this work were (*a*) to study the influence of ester alkyl (ethyl or propyl) side-chains in the water-soluble products (degree of esterification, de, $< 40\%$) on the conformational

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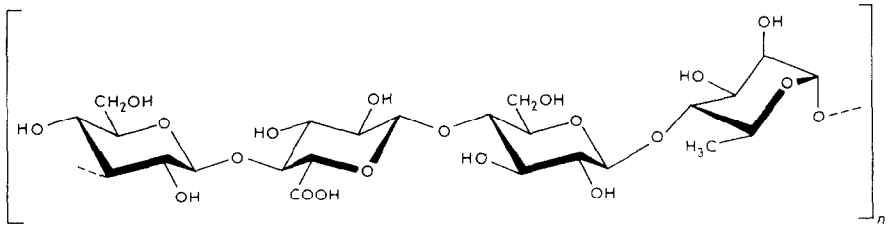

→3-β-D-Glcp-(1→4)-β-D-GlcpA-(1→4)-β-D-Glcp-(1→4)-α-L-Rhap-(1→

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properties of the polysaccharidic backbone in dilute aqueous media, and (*b*) to explore the possible use of the gellan esters in systems for the controlled release of drugs. In the latter context, derivatives obtained by partial esterification of gellan with methylprednisolone 21-bromide have been studied.

RESULTS AND DISCUSSION

Ethyl and propyl esters of gellan.—The polysaccharide chains of aqueous 0.1% solutions of deacetylated gellan at room temperature are essentially disordered[4]. However, the addition of univalent salts to these solutions can induce a conformational disorder → order transition (isothermal transition).

Light-scattering and other experimental data indicate that the ordered state of gellan in 0.1 M tetramethylammonium chloride at 25° involves a double helix, the stability of which mainly depends on the nature of the counterions and whether H_2O or D_2O is used[4d]. This finding is in qualitative agreement with evidence obtained by X-ray fibre-diffraction studies[5].

"Melting" of the ordered chain state of gellan in dilute solution in the presence of univalent electrolytes (isoionic transitions) is characterised by complete reversibility and absence of hysteresis. As expected, the "melting temperatures" of gellan increase with increasing concentration of salt, the relative increase being strongly dependent on the nature of counterions[4f]. With higher concentrations of gellan and added electrolyte, gelation eventually takes place, the phenomenon being strongly favoured by salts of Mg^{2+}, Ca^{2+}, or Pb^{2+}.

Thus, and as found for other ionic biopolymers, the extent of solvation (i.e., chain hydrophilicity) and effective charge density markedly influence the ability of the stereoregular gellan chains to assume characteristic, minimum-energy helical forms in aqueous media.

The hydrophilicity and charge density of gellan molecules can be decreased by esterification of the carboxyl groups with simple alkyl halides (see Experimental). Thus, two ethyl esters (de 0.08 and 0.17) and four propyl esters (de 0.02, 0.07, 0.17,

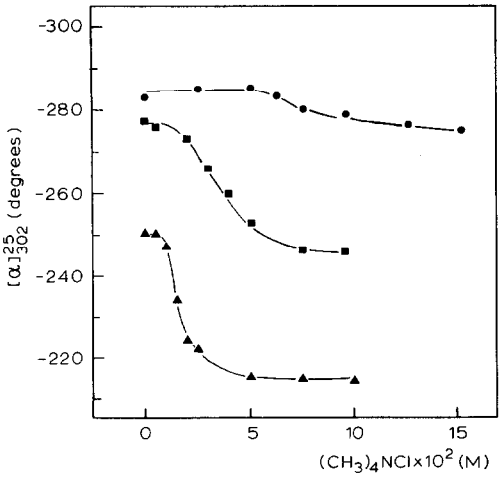

Fig. 1. Dependence of $[\alpha]^{25}_{302}$ of 0.1% gellan ethyl esters on the concentration of $Me_4N^+Cl^-$: ●, de 0.17; ■, de 0.08; ▲, de 0 (gellan).

and 0.37) have been prepared and partially characterised as the tetramethylammonium salts.

The dependencies of the $[\alpha]^{25}_{302}$ of the ethyl esters on ionic strength and of $[\alpha]_{302}$ on temperature in 0.1 M tetramethylammonium chloride, are shown in Figs. 1 and 2, respectively. Fig. 1 shows that, with increasing de, higher ionic strengths are necessary in order to induce the conformational changes, the phenomenon becoming barely observable for the sample with de 0.17. Fig. 2 indicates that the thermal stability of the ordered conformation is reduced with increasing de. Similar data for the propyl esters are shown in Figs. 3 and 4.

For each ester, the thermal profile was reversible and there was no hysteresis. This finding demonstrates that, for the experimental conditions adopted (pH 6, 0.1 M tetramethylammonium chloride), no significant hydrolysis of the gellan esters occurred during the thermal cycles (15 → 50 → 15° in < 6 h). With a de of 0.17, no conformational changes could be detected, and the optical activity was nearly independent of ionic strength at 25° and decreased linearly with increase in temperature in 0.1 M tetramethylammonium chloride.

Whereas for the sample with de 0.37, similar behaviour was observed, the $[\alpha]^{25}_{302}$ value was quite different from those found for the other esters studied. For the latter esters, an increase in de was characterised by larger, negative $[\alpha]^{25}_{302}$ values.

One conclusion from the above data is that, even at relatively modest de values (e.g., 0.1), the presence of a few ethyl or propyl groups along the gellan chains markedly lowers the stability of the ordered conformation.

The results in Figs. 3 and 4 suggest that the propyl esters with de > 0.17 would be unable to attain a gellan-like ordered state in aqueous solution under the

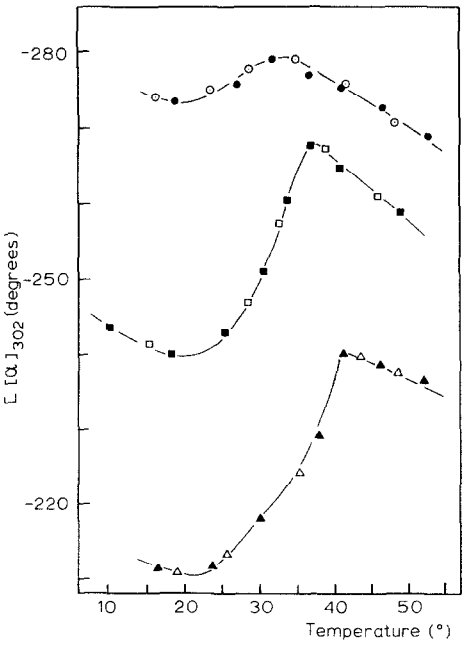

Fig. 2. Temperature dependence of $[\alpha]_{302}$ of 0.1% gellan ethyl esters in 0.1 M $Me_4N^+Cl^-$: ●, de 0.17; ■, de 0.08; ▲, de 0 (gellan); full symbols, heating; open symbols, cooling.

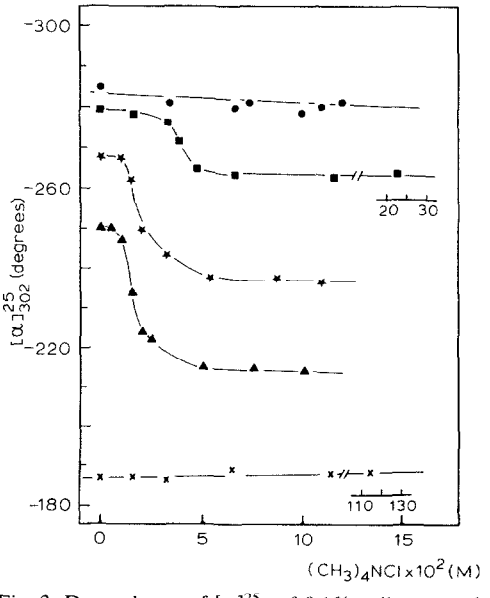

Fig. 3. Dependence of $[\alpha]^{25}_{302}$ of 0.1% gellan propyl esters on the concentration of $Me_4N^+Cl^-$: ●, de 0.17; ■, de 0.07; ★, de 0.02; ▲, de 0 (gellan); ×, de 0.37.

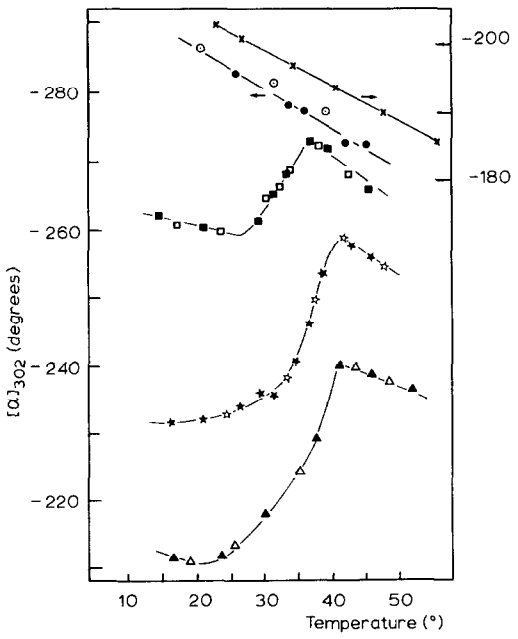

Fig. 4. Temperature dependence of $[\alpha]_{302}$ of 0.1% gellan propyl esters in 0.1 M $Me_4N^+Cl^-$: ×, de 0.37 in water; ●, de 0.17; ■, de 0.07; ★, de 0.02; ▲, de 0 (gellan); full symbols, heating; open symbols, cooling.

conditions used. For the ethyl esters, it is estimated that a similar limiting de value should be ~ 0.2 (see above comments on Fig. 1). If the ordered conformation is always a double helix, then *at least* four consecutive unesterified repeating units may be required. In fact, if the distribution of the ester groups is random, as seems probable, more than four consecutive unesterified units will be necessary.

These inferences are in agreement with the findings of X-ray fibre-diffraction studies that point out the essential role played by the carboxylate functions (and associated water molecules and counterions) in stabilising the double-helical structure of gellan chains[5].

Preliminary observations indicated that only gellan esters with de *lower* than ~ 0.2 can form aqueous gels in the presence of excess of tetramethylammonium chloride. In fact, the propyl ester with de 0.37 did not gel, even in the presence of excess of magnesium perchlorate.

All of these data clearly reinforce our view that gellan esters with the stated de values are unable to attain, in aqueous media, a gellan-like ordered state that should be a prerequisite to gel formation for all species considered.

Methylprednisolon-21-yl esters of gellan.—Methylprednisolon-21-yl esters with de values 0.36 and 0.49 were studied. For the experiments on the time course of the drug release, a stirred suspension of each powdered sample in 0.1 M phosphate buffer (pH 7.2) was kept at 37°. As shown in Fig. 5, for each sample, the amount of

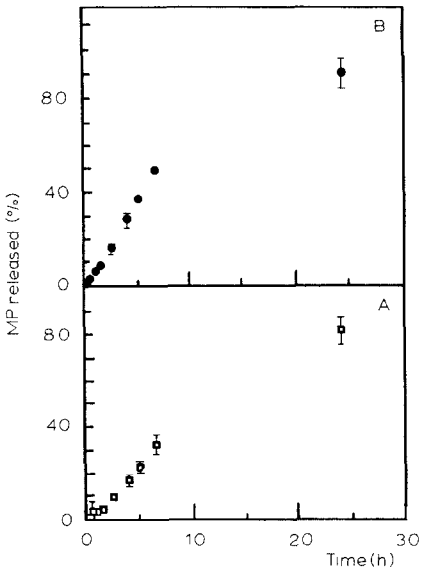

Fig. 5. Plot of the amount of the drug (methylprednisolone, MP) released as a function of time from gellan methylprednisolon-21-yl esters suspended in phosphate buffer (0.1 M, pH 7.2) at 37°: A, de 0.36; B, de 0.49.

drug released (see Experimental) first increased linearly with time (within ~ 5 h), thus indicating apparent zero-order release kinetics, and then almost ceased. This phenomenon is difficult to explain. In fact, there was a progression from the powder suspension to an aggregated and partially gelled system. Moreover, methylprednisolone partially decomposes on storage in aqueous solution, which makes the data collected after ~ 35 h unreliable. However, despite the relatively crude experimental approach, the results suggest that gellan chains with drug molecules attached may constitute a novel and efficient type of controlled-release system. The synthesis procedure allows the preparation of samples in which the amount of drug chemically attached to the polysaccharidic backbone can be varied within wide limits.

EXPERIMENTAL

Gellan esters.—(a) Synthesis. A sample of "gelrite" (completely deacylated gellan, sodium salt) kindly provided by Kelco–Merck (San Diego, CA) was purified as described[4a], and then converted into the tetrabutylammonium salt, using Dowex 50-X8 resin.

The freeze-dried salt was solubilised in anhydrous Me_2SO at room temperature during ~ 10 h to give a solution of 12–13 mg/mL. To this solution, protected from moisture, butylhydroxyanisole (radical scavenger) was added to 1%, and the solution was purged with pure N_2 for 1 h. The temperature was then lowered to 4°

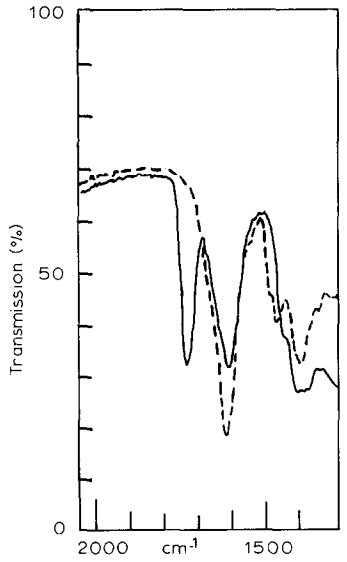

Fig. 6. IR spectra of films of the tetramethylammonium salts of gellan (- - - - -) and gellan propyl ester (de 0.37) (———).

and the alkyl halide (i.e., ethyl iodide, propyl iodide, or methylprednisolon-21-bromide) was added dropwise. The amount of alkyl halide added was approximately twice that stoichiometrically necessary to obtain the desired final de. The temperature was raised gradually to room temperature, and the reaction was allowed to proceed for 24 h and completed by stirring the mixture at 37° for 12 h. The resulting gellan derivative was precipitated by adding cold acetone (5 vol) dropwise with stirring. The white powdery precipitate was collected on a G-3 glass filter, washed three times with acetone, and dried under vacuum at room temperature (24 h).

Water-soluble gellan derivatives were purified by dissolution in 0.01 M tetramethylammonium chloride and prolonged dialysis of the solution against distilled and double-distilled water.

Water-insoluble gellan esters were purified by dissolution to 12–13 mg/mL in Me_2SO followed by precipitation with acetone as described above. The treatment was repeated twice for each sample.

The sample of methylprednisolone-21-bromide was prepared and purified as described[6].

(b) Characterisation. IR spectra were recorded with a Hitachi 270-30 spectrophotometer, using films on an Irtran-2 support, after equilibration in D_2O and drying at 40°. Fig. 6 shows the IR spectrum for the propyl ester with de 0.37; the strong band at $\sim 1740\ cm^{-1}$ is associated with ester carbonyl groups.

The ^1H-NMR spectra were recorded at 85° with a Varian XL 300-FT instrument on solutions in D_2O or Me_2SO-d_6. The de values of the propyl esters could

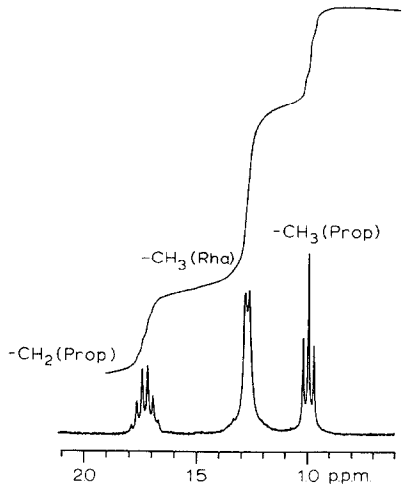

Fig. 7. ^{1}H-NMR spectrum of gellan propyl ester (de 0.58) in Me_2SO-d_6.

be determined from the ^{1}H-NMR spectra. Thus, for the sample with the spectrum shown in Fig. 7, integration and comparison of the signals at 1.3 (d, Me-5 of the rhamnose residues) and 1.0 ppm (t, Me of Pr) gave a de of 0.58.

The de values of the ethyl and propyl esters were determined also by GLC of the EtOH or propanol released by saponification (0.1 M NaOH, 65°, 1 h), using a Carlo Erba instrument with Carbowax 1500 on Chromosorb-W at 50°, and 2-butanol as internal standard.

The de values of the methylprednisolon-21-yl esters were determined by HPLC of the drug released after complete hydrolysis at pH 10 and 37°.

The drug-release kinetics were determined by HPLC of the drug released from the esters with de 0.36 and 0.49, each suspended in powder form in aq phosphate buffer (0.1 M, pH 7.2) at 37° under continuous stirring. HPLC was performed with a Waters instrument, using a Nova-Pak C-18 column (3.9 × 150 mm), a UV detector (243 nm), and water–acetonitrile–2-propanol (65 : 25 : 10) at 1 mL/min.

These experiments also confirmed the partial decomposition on storage of a solution of methylprednisolone in aq. phosphate buffer (pH 7.2) for 35 h.

Optical activity and viscosity measurements were carried out as described[4a].

The intrinsic viscosities of the tetramethylammonium salts in 0.1 M tetramethylammonium chloride at 25° were 14 mL/g for purified gelrite and 12 mL/g for the ethyl ester with de 0.08. Thus, chain degradation is concluded to be a minor event in the above derivatisation procedure.

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