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EFFECT OF ACETYLATION ON SOME SOLUTION AND GELLING PROPERTIES OF ALGINATES

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ABSTRACT

Whereas acetylation does not severely affect the molecular weight and polydispersity index of alginate, it greatly enhances the swelling ability of calcium gels made from these polymers. The affinity of alginate for calcium ions is diminished by acetylation as shown by the effect on the c.d. spectra and by the decrease in gel strength. Viscosity data suggest that a modest degree of acetylation (d.a. up to ~11%) causes an expansion of the molecular chain, whereas a higher d.a. generates more flexible polymers.

INTRODUCTION

A novel procedure has been reported[1] for the graded acetylation of alginate by using calcium alginate gel beads. Initially, acetylation occurred selectively on the mannuronic acid residues, but eventually a small proportion of the guluronic acid residues was acetylated[1]. The results may reflect the selective binding of calcium ions by groups of two or more contiguous guluronic acid residues since non-contiguous guluronic acid residues were acetylated preferentially. Small proportions of acetyl groups can have a marked effect on the macromolecular properties of both highly viscous[3,4] and gelling polysaccharides[5,6].

We now report on the influence of acetylation on some macromolecular properties of alginate.

EXPERIMENTAL

Materials. — The alginate samples were those prepared[1] from *Laminaria*

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hyperborea (containing 68% of guluronic acid and with $\bar{N}_{G>1} = 14$). Twice-distilled water was used throughout the work.

Determination of the distribution of molecular weights. — Mol. wt. distribution curves were determined by gel-permeation chromatography, involving a JASCO BIP-1 pumping system with a Rheodyne Mod. 7125 injector, a Model TSK G 5000 PW column (LKB), and a concentration detector (Waters Refractive Index Detector Model 410) coupled to a low-angle laser light-scattering detector (LDC-Chromatix CMX-100 system). Values for dn/dc were taken from the literature[7]. Data were processed[8] with an LDC computer program (PCLALLS) implemented by an IBM XT PC.

Viscosity measurements. — Aqueous solutions of sodium chloride were used at 25° with Ubbelohde suspended capillary viscometers and an automatic dilution viscosity system (Schott–Geräte).

Isothermal microcalorimetry. — Heats of dilution were measured[9] at 25° with an LKB 10700-2 batch type microcalorimeter.

C.d. measurements. — A JASCO J-500A dichrograph was used[9], and measurements were made at 22°.

Preparation of gels and measurements of gel strength. — 2% (w/w) Solutions of sodium alginate in 0.2M sodium chloride were introduced into plastic cylinders (diameter, 1.4 cm; height, 1.9 cm) and capped with a cellophane dialysis membrane at each end. The cylinders were immersed in 0.1M calcium chloride (400 mL) containing 0.2M sodium chloride and dialysed for 72 h. The dialysate was changed every 12 h. The excess of sodium ions was present to ensure the formation of more homogeneous gels[10]. The weight of each acetylated sample was corrected for by multiplying with M_0(NaAlg)/M_0(NaAlg-Ac) in order to obtain the same concentration of uronate residues in all solutions. The modulus of rigidity of each plug of gel was determined at room temperature (22 ±2°) by compressing the gel plugs by 2 mm at a constant rate of 0.2 mm/s in a Stevens Texture Analyser. The moduli were calculated as described by Smidsrød *et al.*[11].

Measurements of water binding. — Spherical calcium alginate beads, prepared[1] from native and acetylated alginates, were washed with water, aqueous 50% ethanol to remove excess of salt, and aqueous 96% ethanol, and then air-dried in a rotary shaker. The dry beads were suspended in distilled water and their increase in diameter was monitored by light microscopy.

RESULTS AND DISCUSSION

Molecular weights of alginate of various degrees of acetylation. — The weight-average molecular weight ($\bar{M}_w$), the number-average molecular weight ($\bar{M}_n$), and the polydispersity index ($\bar{M}_w/\bar{M}_n$) of the alginate samples with various degrees of acetylation (d.a.), reported in Table I, indicate that no severe degradation of the polymer chains had occurred on acetylation. The changes in $\bar{M}_w$ may be ascribed to losses during purification.

TABLE I

MACROMOLECULAR PARAMETERS OF ACETYLATED ALGINATES

D.a.	0	0.053	0.103	0.476	1.17
$\overline{M}_w$ ($\times 10^{-3}$)	211.7	172.0	162.2	308.8	247.1
$\overline{M}_n$ ($\times 10^{-3}$)	80.2	53.7	101.9	168.4	92.0
$\overline{M}_w/\overline{M}_n$	2.6	3.2	1.6	1.8	2.7

Conformation of acetylated alginate. — The results of the viscosity measurements at 25° as a function of polymer concentration were analysed in terms of the usual Huggins and Kræmer equations, and the parameters obtained are given in Table II. All data were obtained for solutions in 0.1M sodium chloride except for the sample with d.a. 1.17, for which the values reported were obtained by extrapolation from those determined in 0.05, 0.06, 0.07, 0.08, and 0.09M sodium chloride (the same values were used for a series of determinations also for the sample with d.a. 0.105). This extrapolation was necessary because solutions of the extensively acetylated polymer in 0.1M NaCl were slightly opalescent, indicating the onset of salting out. No turbidity or any anomaly in viscosity was detectable for solutions in 0.09M sodium chloride.

The values of the intrinsic viscosity increased rapidly as the d.a. increased from 0 to 0.105, and then remained practically constant. The values of the Huggins constant (k') were largely independent of d.a., suggesting that this parameter is governed more by the electrostatic interactions than by the structure of the chains.

In order to assess the effect of acetylation on the rigidity of alginate molecules, the intrinsic viscosities were also determined at ionic strengths in the range 0.05–0.09M, for the samples having d.a. 0.105 and 1.17. The $[\eta]$ data used to calculate the flexibility parameter B, as defined by Smidsrød and Haug[12], gave values of 0.051 ±0.009 and 0.105 ±0.027, respectively. The value[12] of B for unsubstituted alginate of the same uronic acid composition is 0.03. By comparison of the values of $\overline{M}_w$, $[\eta]$, and B, it may be inferred that an increase in chain extension at an ionic strength of 0.1M occurs on acetylation, accompanied by an increase in chain flexibility in the unperturbed state as deduced from the increase in the values of B. Some tentative conclusions may be drawn.

TABLE II

VISCOSITY PARAMETERS OF ACETYLATED ALGINATES, SODIUM SALTS

D.a.	0	0.053	0.105	0.476	1.17
$[\eta]$	5.82	6.26	7.21	7.04	7.43[a]
Huggins constant (k')	0.49	0.42	0.41	0.48	0.46[a]
Kræmer constant (k'')	0.10	0.12	0.12	0.09	0.09[a]
$k' + k''$	0.59	0.54	0.53	0.57	0.55[a]

[a]Obtained by extrapolation to 0.1M NaCl.

On increasing d.a. from 0 to 0.10, M_w decreases by ~20% (Table I), indicating that the parallel increase of $[\eta]$ (Table II) must stem from a chain expansion caused by even a small proportion of acetyl groups. Such a change in polymer dimensions is not parallelled by an increase in stiffness, probably because it is almost impossible to increase the rigidity of a backbone which is already very rigid.

The hypothesis that an order–disorder conformational transition takes place with some degree of co-operativity was used to explain the striking effect of 8% of acetyl groups on the enthalpy of dilution of a bacterial alginate[2]. The enthalpy of dilution curves (Fig. 1) for samples at d.a. 0.105 and 1.17 were always exothermic as expected for linear polyelectrolytes devoid of co-operative conformational phenomena. However, non-abrupt changes in conformation may be reflected by the curvature observed for the sample with d.a. 1.17. Even a small proportion of acetyl groups on the mannuronic acid (ManA) residues may play a very critical role in determining the conformation of the polymer. At low d.a. values, practically all of the acetyl groups are localised on the ManA residues and, to a lesser extent, on the guluronic acid (GulA) residues embedded in an alternating sequence[1]. It is possible that the introduction of acetyl groups shifts the position of the minimum of the conformational energy without increasing the accessible surface of the molecules. Further acetylation eventually produces a rather flexible polymer with a corresponding increase in the number of conformations. The induced flexibility may be associated with a loss of stability of the states of the GulA–GulA dimers which have a minimum energy at low d.a. values.

Interaction with calcium ions and the formation of gels. — The addition of calcium ions to a solution of sodium alginate perturbs the c.d. spectrum of the COO^- group in the far u.v. Such an effect has been correlated with a co-operative disorder–order conformational transition of the GulA sequences, which are the molecular basis of the so-called "egg-box" junctions in the gel structure[13]. The c.d. spectra of the alginate derivatives were also perturbed upon addition of aqueous calcium perchlorate (see insert to Fig. 2), but the effect decreased with increasing

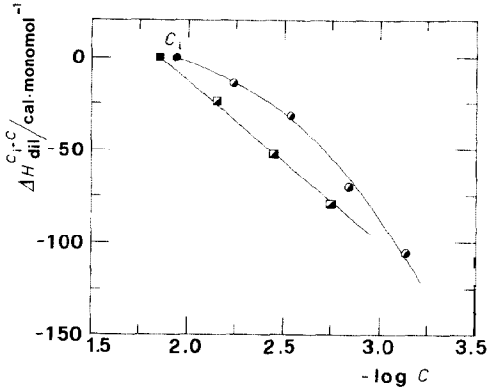

Fig. 1. Plot of the log of the polymer concentration (C) against the enthalpy change of dilution of acetylated alginates (as sodium salts) in water at 25°: ●,◕ d.a. = 1.17; ■,◪ d.a. = 0.10; ●,■ C_i (initial).

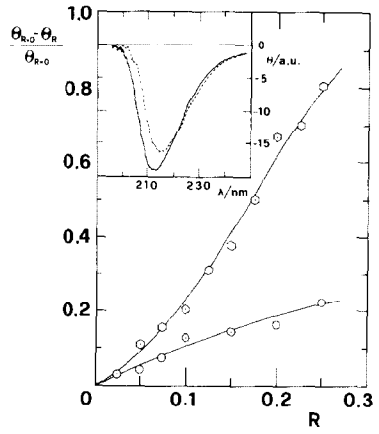

Fig. 2. Dependence on R of the relative excess molar ellipticity (at 213 nm) of solutions of various alginate derivatives with respect to the calcium-free solution. R is the molar ratio of added calcium to polymer repeating-unit; polymer concentration, 10mM; pH 7; ⊙ , d.a. = 0; ○, d.a. = 1.17. Polymers with intermediate d.a. values showed intermediate curves (data not shown). Inset: c.d. spectra (arbitrary units) of 10mM solutions of acetylated alginate (d.a. = 1.17) at R = 0 (——) and R = 0.25 (-----).

acetyl content (Fig. 2). Hence, the ability of calcium ions to induce a conformational ordering of the alginate chain is severely diminished as the proportion of acetyl groups increases. In parallel with this effect was a marked decrease in the strength of calcium gels of acetylated alginates, as reported by Schweiger[14].

Fig. 3 shows that the modulus of rigidity (G) of the gels decreased rapidly with increasing d.a. even at low values and indicates that the mechanism of gel formation in alginates is complex. Indeed, in order to form a gel with good mechan-

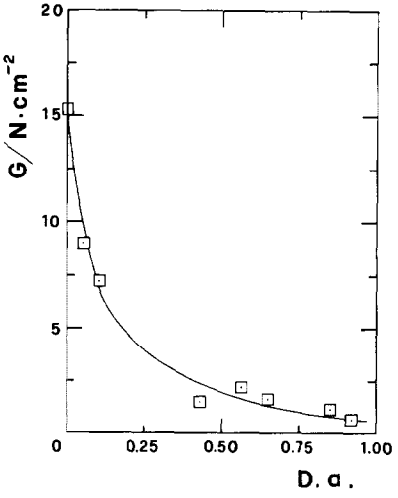

Fig. 3. Dependence of the modulus of rigidity (G) of calcium gels of alginate derivatives on the degree of acetylation (d.a.).

ical properties, the GulA residues must be present not only in substantial proportions and in long homopolymeric sequences[15,16], but also be unsubstituted. Even more surprising is that the ManA monomers also need to be free of acetyl groups for maximal gel strength. Thus, it may be inferred that an elongation of sequences of acetylated ManA residues is not compatible with the severe topological constraints of the 3D arrangements of the gel "cross-links".

Swelling of beads and binding of water. — Spherical gel beads of Ca alginate gel, acetylated to various extents, were formed by calcium cross-linking[1]. When the beads were dried and re-swollen in water, light microscopy showed that they retained their original spherical form. The relative increase in volume (V/V_0) as a function of time is shown in Fig. 4. Re-swelling occurred during several hours, there was an induction period, and the duration of swelling was proportional to the d.a. There were striking differences in the equilibrium volumes of the various samples. Equilibrium was reached after ~24 h. The degree of swelling increased 500-fold on passing from d.a. 0.0 to 0.65 (see Fig. 5).

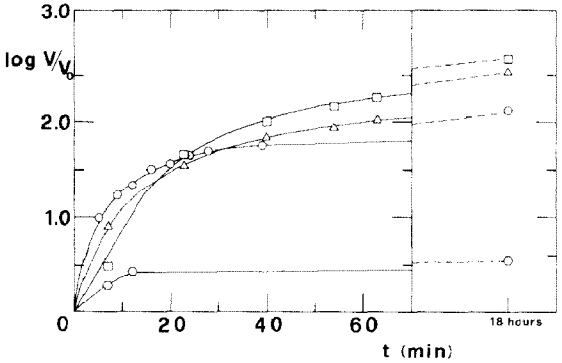

Fig. 4. Time dependence of the log of the ratio of the volume (V) of swelling beads to the volume (V_0) of the dry bead for acetylated alginates at room temperature. Symbols: ⊙, d.a. = 0; ○, d.a. = 0.40; △, d.a. = 0.56; □, d.a. = 0.65.

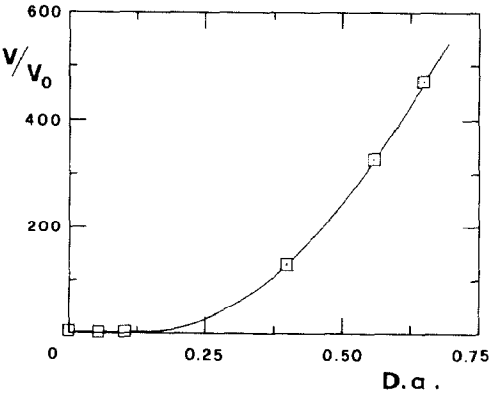

Fig. 5. Dependence on the d.a. of the ratio of the equilibrium swelling volume (V) to the volume (V_0) of the dry bead for acetylated alginates at room temperature.

Generally, the volume of an ionic gel will be governed mainly by a positive osmotic pressure, in this case due mainly to the positive entropy of mixing of the counterions with water, which is counterbalanced at equilibrium by a negative pressure due to elasticity of the network[17]. For calcium alginate gels (which are enthalpic rather than entropic[18]), the elasticity depends upon the number and the strength of the cross-links.

Since the introduction of acetyl groups impairs the co-operative binding of calcium ions, the number of dissociated counterions per polymer chain will increase with increasing d.a. and hence enhance the positive osmotic pressure. Simultaneously, it will weaken the forces holding the network together. Reduction in the co-operative binding of calcium ions will probably reduce both the strength and the number of cross-links in the network and thus contribute positively to the swelling.

A decreased polymer–water interaction due to the increased hydrophobicity of the acetylated chains should lead to increased polymer–polymer interaction and decreased swelling. This effect is much smaller at equilibrium than those considered above. However, it may be more important for understanding the kinetics of the swelling reaction. The increased length of the induction period in the swelling with increased d.a. (Fig. 4) occurs in spite of the increased surface area of the dry beads[19] (the average diameter of the dry beads increases from 0.72 mm at d.a. 0.0, to 0.85 mm at d.a. 0.105, and to 0.90 mm at d.a. 0.40). This effect may be due to hydrophobic polymer–polymer interactions, which will diminish the wettability and form a kinetic barrier to swelling in the early phase of the process.

Further work is necessary in order to elucidate the mechanism of swelling.

Concluding remarks. — By an appropriate choice of the acetylation procedure, one can obtain gel-forming, and therefore bead-forming, alginate derivatives with reduced calcium affinity, but with exceptional swelling properties. The potential biomedical and biotechnological applications of these tailor-made derivatives are of interest, especially in view of their increased compatibility with non-aqueous solvents. In spite of the fact that acetyl substitution of the synthetic derivatives is not confined to the ManA residues, they resemble very closely bacterial alginates of the *Azotobacter vinelandii* strains[20,21] both in composition and in the amount of acetylation. Some of the observations made for the present compounds may therefore help to explain some of the biological functions of the acetyl groups in native alginates, for instance in the modulation of their rheology and in the re-swelling of the microcyst[22].

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