Carbohydrate Research, 47 (1976) 105–117

ON THE MECHANISM OF SCISSION OF ALGINATE CHAINS BY PERIODATE

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(Received June 23rd, 1975; accepted for publication, July 17th, 1975)

ABSTRACT

The rapid, initial decrease in viscosity of alginate solutions containing periodate has been shown by light scattering and ultracentrifugation to be due to scission of the alginate molecule. This depolymerisation is not affected by •OH scavengers such as 1-propanol or benzoate. 1-Propanol protects alginate against degradation by •OH produced by pulse radiolysis. A slower process of degradation also goes on in periodate solutions, and this is suppressed by 1-propanol. The rapid-phase degradation is suggested to be due to scission of infrequent and specific components of the polymer chain.

INTRODUCTION

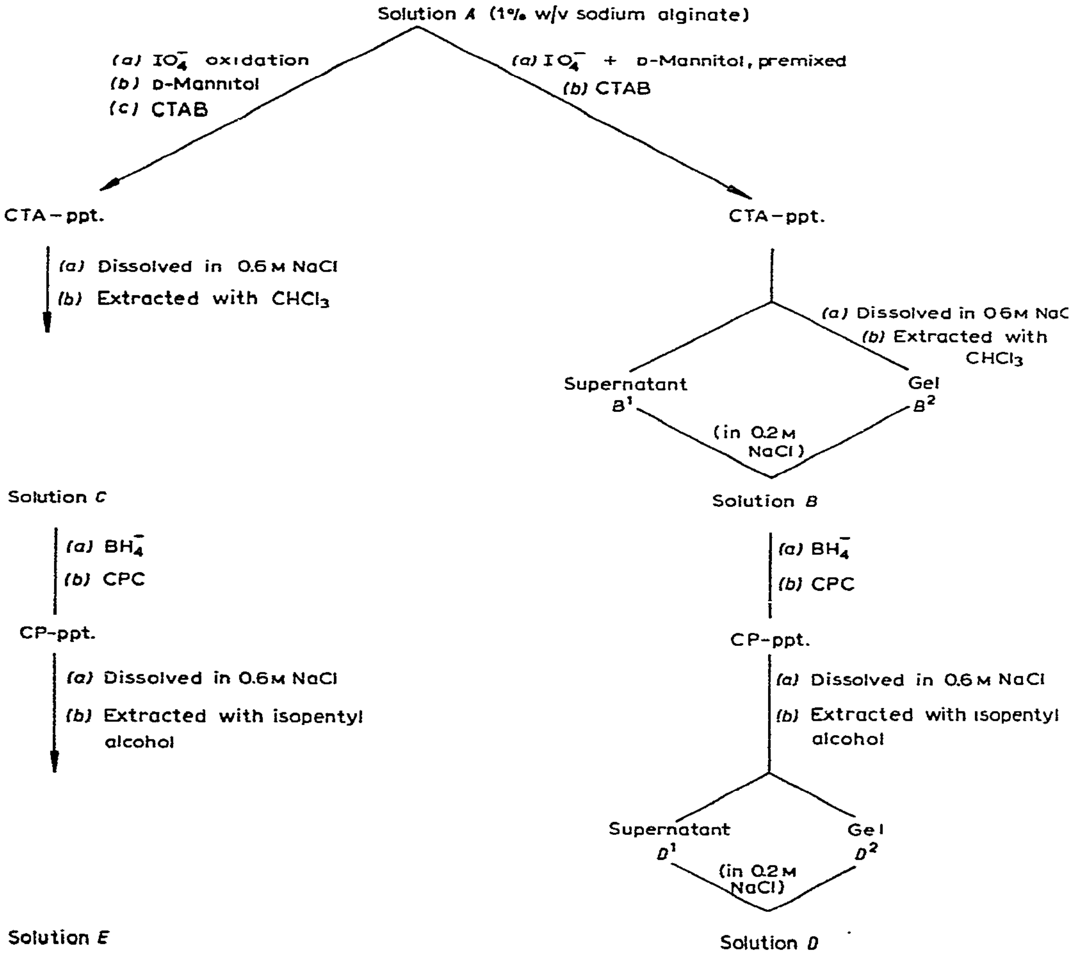
Periodate brings about large (and often rapid) decreases in the viscosity of solutions of polymeric acetals and ethers[1]. This effect seriously limits the application of periodate–Schiff techniques in the histochemistry of glycuronans[2] (*e.g.*, chondroitin sulphate proteoglycans). It should be considered when interpreting the results of periodate treatment of polysaccharides in structural studies.

The dramatic decrease in viscosity of alginate solutions on treatment with periodate stimulated investigations into the underlying mechanism[1,3,4], and three possibilities have been discussed. One postulates that splitting of the C-2–C-3 glycol groups converts a rigid pyranose monomer into a flexible link between unaltered segments of the polymer chain, consequently decreasing total stiffness. Theoretical calculations support this model[3]. The other mechanisms invoke cleavage of the polymer chain, either by periodate-induced free radicals[1,3] or by chemical scission of specific and infrequent bonds in the chain[1].

We now provide evidence that alginate is broken down by periodate by two mechanisms; one rapid, the other slow, and that the mechanism of the rapid breakdown is unlikely to involve •OH radicals.

MATERIALS AND METHODS

Periodate-oxidised alginate (*See Scheme 1*). — Aqueous sodium alginate (Hopkin and Williams; 1% w/v) was centrifuged at 800 *g* for 60 min. The slightly opalescent, supernatant solution from a very small deposit was solution *A* [η (reduced viscosity) = 1800 ml/g in 0.2M NaCl]. Solution *A* (100 ml) plus aqueous 0.2M sodium periodate (12.5 ml) were kept in the dark for 15 min at room temperature. Aqueous D-mannitol (20%, 5 ml) was added, followed by 60 ml of aqueous 5% w/v cetyltrimethylammonium bromide (CTAB, Eastman Kodak) with stirring. The precipitate was centrifuged at 800 *g* for 60 min, washed twice in warm (~50°) distilled water, with stirring, and dissolved in 100 ml of aqueous 0.6M NaCl with stirring at 40°. The cooled, opalescent solution was extracted with chloroform (2 × 100 ml), the upper layer (solution *C*) being recovered after centrifugation for 1 h.



Scheme 1

An aliquot of solution *A* was treated with premixed D-mannitol and sodium periodate, precipitated with CTAB, and recovered as described above (solution *B*), to serve as a control for the oxidation stage.

Borohydride-reduced, periodate-oxidised alginate. — Aqueous sodium borohydride (20 ml, freshly prepared) was added slowly with stirring to 40 ml of solution *C*, and, after 30 min at room temperature, 0.64 ml of glacial acetic acid was added. The pH of 5.25 was adjusted to 6.15 with 1.75 ml of M NaOH. Aqueous cetylpyridinium chloride (CPC, 24 ml) was added, followed by water to a total volume of 365 ml, at a final NaCl concentration of 0.1M. The precipitate was washed twice with warm water and dissolved in 40 ml of 0.6M NaCl, with warming. The cooled solution was extracted twice with 1 vol. of isopentyl alcohol (solution *E*). An aliquot of solution *B* was also treated with sodium borohydride, acetic acid, and CPC, and recovered as described above (solution *D*), to serve as a control for the reduction stage.

Alginate for pulse radiolysis. — Sodium alginate (0.5% w/v, 400 ml) in 0.3M NaCl was centrifuged at 800 *g* for 45 min. The supernatant solution was recovered from the very small, light deposit, and 10 ml of 2M HCl were added, slowly with stirring. The precipitate was centrifuged, and dissolved in 100 ml of aqueous 6% w/v sodium hydrogen carbonate. Ethanol (300 ml) was added, slowly with stirring, and the precipitated alginate was washed successively in aqueous alcohol, alcohol, alcohol–ether, and ether, and then air-dried. An aqueous solution (2% w/v) of the product was dialysed overnight against distilled water. The concentration was checked by CPC titration[5]; $\eta = 740$ ml/g.

Polyethylene oxide. — Polyox WSR N-750 (B.D.H.; mol. wt. ~300,000) was used from stock 1% w/v aqueous solutions ($\eta = 370$ ml/g).

Methods. — Two kinds of viscometry determination were made: (*1*) in a U-tube viscometer ("Ostwald", Baird & Tatlock, type BS/U/M3) at 25° in a water bath, as described previously[1]; and (*2*) less precisely, in a 0.1-ml Warburg pipette, at room temperature. The pipette was clamped vertically, with the tip immersed in the liquid being assayed. The time for the liquid to run from the 0 to the 0.1-ml calibration was measured. Solvent times were 13–14 sec. At least three determinations, with adequate reproducibility (*i.e.*, better than ±1%), were performed on each solution.

Except where stated otherwise, solutions contained 0.2M NaCl.

Spectra were recorded on a Hitachi–Perkin–Elmer recording spectrophotometer, Model 124, in 1-mm or 10-mm stoppered cuvettes.

Concentrations of alginate were determined by titrations with aqueous CPC[5] (0.1% w/v) on solutions containing ~0.5 mg of alginate per ml, in 0.06M NaCl.

Concentrations of periodate were measured by the method of Aspinall and Ferrier[6].

Pulse radiolysis experiments were performed in the Paterson Laboratories (Christie Hospital, Manchester), and we are grateful to Drs. V. Davies and M. Ebert for their help. Nitrous oxide was bubbled through alginate solutions for 30 min, before exposing them in glass vessels to timed pulses of electrons of a nominal

14 meV. The dosages of electrons were calculated from machine parameters[7]. The figures are nominal.

Determinations of critical electrolyte concentrations (c.e.c.) of cetylpyridinium complexes were performed[5] by turbidimetry at 30°.

Light scattering was performed on a Sofica model 42000 photo-gonio-diffusometer (Société Française d'Instruments de Controle et d'Analyses, 78, Le Mesuil-Saint-Denis, France). Stock solutions of 0.5% concentration were in 200 mM NaCl, buffered to pH 7.0 with phosphate. Each solution was dialysed against buffer for 24 h, and centrifuged at 35,000 *g* for 1 h before light-scattering measurements were made.

All the measurements were performed using a cylindrical cell and light of wavelength 546 mm. The apparatus was calibrated with benzene as reference standard; the intensities were measured at scattering angles between 30° and 135°, and corrected for reflections in the cell[8]. A refractive index increment for alginate of 0.16 ml/g was used[9] which yielded an optical constant K of 1.68×10^{-7}.

Analytical ultracentrifugation was performed by using a Beckman Model E centrifuge under standard operating conditions. The maximum ordinate of the Schlieren profile was recorded, and sedimentation coefficients were corrected to water as solvent at 20°. Samples were prepared in 0.1M sodium phosphate buffer (pH 6.0).

RESULTS

Preparative experiment (*Scheme 1*). — During the 15-min oxidation, 45% of the periodate was consumed, corresponding to ~25% oxidation of available glycols. Recovery of the alginate (CPC titration[5]) from the CTA precipitate was ~85% in the 0.6M NaCl (solution *C*). A small amount was present in the pellicle at the interface between the chloroform and aqueous layers. After borohydride reduction of solution *C*, 93% was recovered in 0.6M NaCl (solution *E*) from the CPC precipitate, after extraction with isopentyl alcohol. The overall recovery was 80%, calculated from CPC titrations (Table I).

The behaviour of the control solutions was different. A considerable proportion of the "native", untreated alginate was insoluble in 0.6M NaCl, 50% remained in the supernatant solution, and another 38% could be recovered from the bulky, rather gelatinous precipitate present in solution *E*. Similarly, after borohydride-reduction, 84% of the material present in solution *B* was recovered, 56% of which was insoluble in 0.6M NaCl. The overall recovery was 74%.

The viscosity of the "native" material (solution *A*) fell from 1800 to 68 ml/g (solution *C*) and thence to 55 ml/g (solution *E*) after reduction with borohydride (Table I). Table I lists reduced viscosities (η_{sp}/c) for the control preparations *B* and *D*, both of the soluble-in-0.6M NaCl (supernatant) fractions, and the total (supernatant and insoluble) recovered material, all of which are much higher than those of the periodate-treated alginates. Preparations *B* and *D* were not so viscous as the starting material, probably because of some loss of the more-viscous alginate in the emulsion

TABLE I

Solution	*Recovery (%) CPC titration*	η_{sp}/c *(ml/g)*	$S_{20,w}$ *values (at c = 0)*	*R of G* *(Å)*	*C.e.c. values of cetylpyridinium complexes*
A (1% Na Alginate)	100	1800	11.7	1750	0.28–0.32
B (control for C)			5.9		0.28–0.32
B^1	50	190			
B^1+B^2	88	540			
B (from *A*)	85	68		—	0.38–0.42
D (from *B*)					0.28–0.32
D^1	44	122			
D^1+D^2	74	335			
E (from *C*)	80	55		1200	0.28–0.35

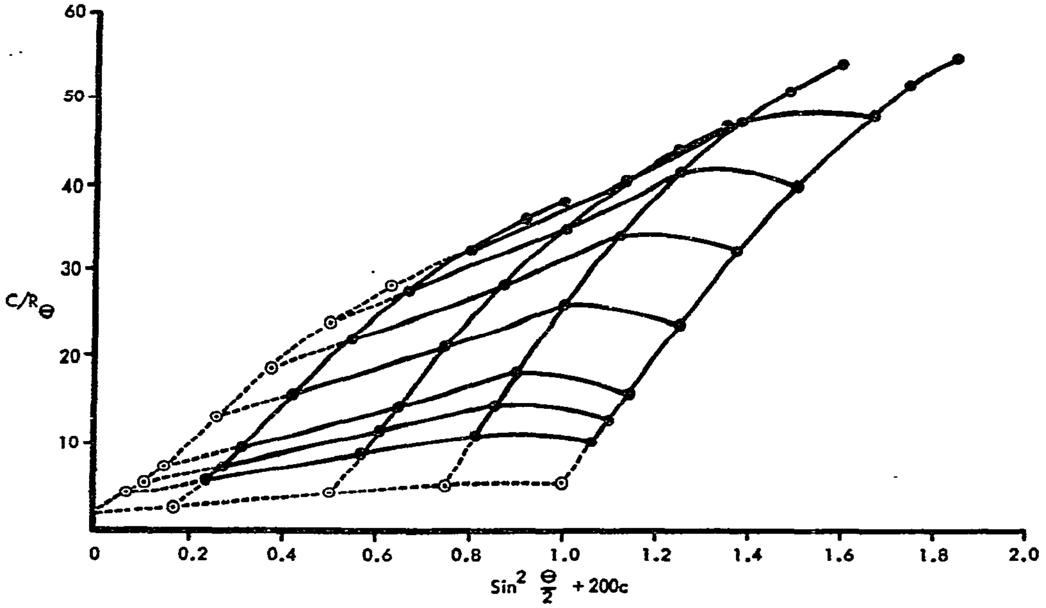


Fig. 1. A Zimm plot of the scattering data from material *A*. Actual data are indicated by full circles; points derived by extrapolation are indicated by a centred circle. Concentrations are in g/ml.

pellicles at the interfaces of the extracting solvents, particularly the chloroform used during the first recovery step (see Table I). Similar pellicles present in solutions *C* and *E* were very small.

Light scattering. — A Zimm plot (Fig. 1) of the scattering data from solution *A* (starting material) shows a zero concentration/zero angle intercept of the ordinate indicating a M_w of the order of 2×10^6*. As can be seen from the limiting slopes of the zero-concentration extrapolates, no meaningful statement can be made of the Z-average radius-of-gyration, since the preparation is polydisperse.

The data from solution *C* showed bizarre effects, both at high and low scattering angles. This combination of polymer and solvent constituted a strongly interacting system. However, the less-perturbed scattering values at $\theta \sim 90°$ indicated a molecular weight comparable with that of solution *E*, namely, $\sim 10^5$.

A Zimm plot of the scattering data from solution *E* (Fig. 2) shows a better behaved system consequent on the reduction of the "aldehyde" groups formed by periodate oxidation. The data yield a $\overline{M}_w$ of 1.1×10^5 and a Z-average radius-of-gyration of 1200 Å.

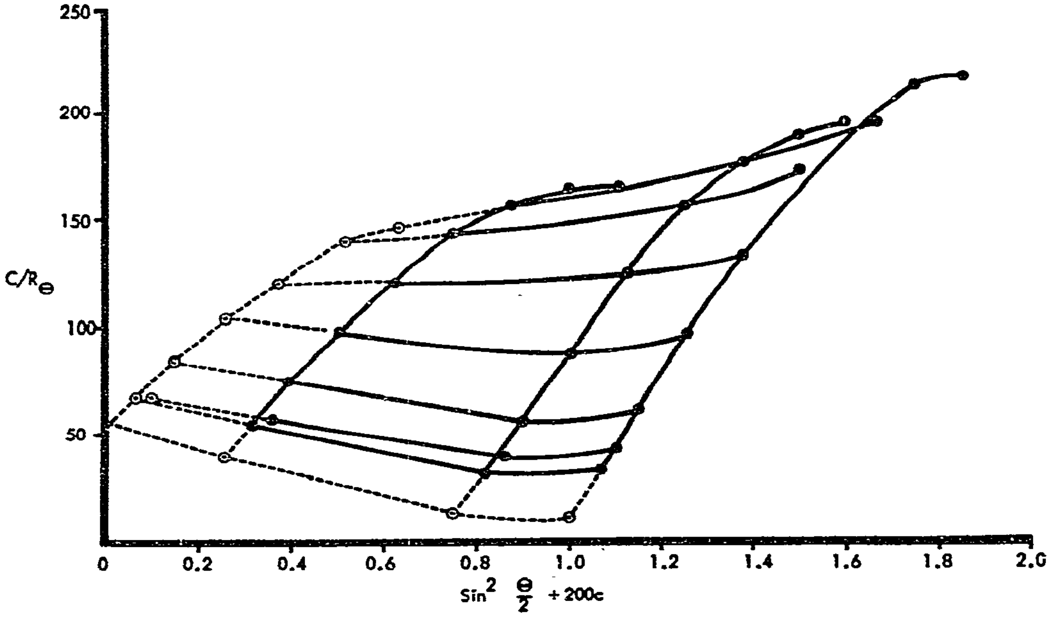


Fig. 2. A Zimm plot of the scattering data from material *E*. Actual data are indicated by full circles; points derived by extrapolation are indicated by a centred circle. Concentrations are in g/ml.

Analytical ultracentrifugation in the sedimentation velocity mode at 1.5 mg/ml of polymer demonstrated little difference between samples. However, at concen-

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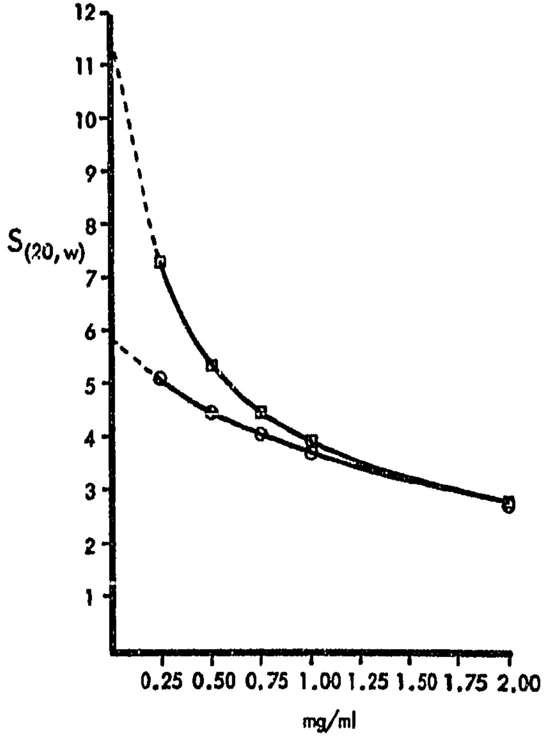
*The relationship between this molecular weight and the observed, reduced viscosity (1800 ml/g, Table I) is in good agreement with that obtained by Smidsrød and Haug[9].



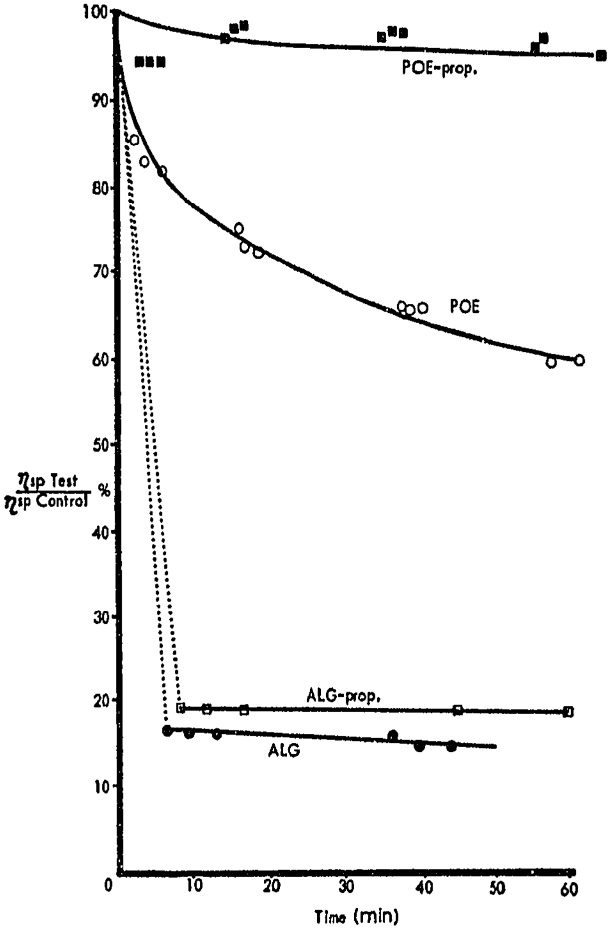


Fig. 3. Concentration dependence of native alginate (—□—) and periodate-reacted alginate (—○—). The buffer was 0.2M NaCl+0.02M sodium phosphate (pH 7.2). The $S_{20,w}$ extrapolates to zero concentration are those obtained from linear plots of 1/S (20, W) *versus* C.

Fig. 4. Change in viscosity with time of polyethylene oxide (POE) and sodium alginate solutions, containing sodium periodate, in the presence or absence of 1-propanol (prop), at 10% v/v. POE was 0.4% w/v, in 0.1M $NaIO_4$, at 37°; —■—, with 1-propanol; —○—, without 1-propanol. Alginate was 0.3% w/v, in 0.1M $NaIO_4$ plus 0.1M $NaClO_4$ at 25°; —□—, with 1-propanol; —●—, without 1-propanol.

trations of polymer below 1 mg/ml, significant differences are seen between the $S_{20,w}$ values of native and borohydride-treated periodate-oxidised material (Fig. 3). Poor discrimination at higher concentrations of polymer is understandable in terms of the large volume-occupancy of these polymers, and only at concentrations less than 1 mg/ml does complete separation of macromolecular chains enable discrete sedimentation to be observed.

These results support the light-scattering data, in showing that a dramatic fall in particle weight is attendant on periodate attack. The data at low concentration are not clear enough to enable diffusion coefficients to be evaluated, but it would appear that the periodate-treated material is not significantly polydisperse, as might result from a mechanism involving random chain-scission.

Pulse radiolysis. — Table II shows the effect of increasing doses of e_{aq}^-, and hence •OH, on the viscosity of alginate solutions. The dosage is calculated (rather than measured) from accelerator parameters (duration and shape of pulse, *etc.*), and is therefore nominal. The viscosity of a 0.47% w/v alginate solution containing 10% of 1-propanol showed no decrease ($\pm 2\%$) on irradiation with 5.2×10^4 rads, whereas that of a control solution, without 1-propanol, decreased by 51%.

TABLE II

Nominal radiation dose (rads)	*Nominal* •*OH conc.* (M)	$\frac{\eta_{sp}\ Test}{\eta_{sp}\ control} \times 100$
0	0	100 (= 780 ml/g)
10^3	5×10^{-6}	77.5
10^4	5×10^{-5}	31.7
10^5	5×10^{-4}	14.2

The effect of free-radical scavengers (1-propanol and benzoate) on the periodate-induced decrease in viscosity of alginate and polyethylene oxide solutions. — Fig. 4 shows the time-course of the drop in viscosity of polyethylene oxide and alginate solutions, expressed as a percentage of the η_{sp} at the outset. 1-Propanol almost completely inhibits the viscosity decrease of polyethylene oxide, and is almost without effect on that of alginate.

In 0.2M benzoate, the decrease in viscosity of a 0.71% w/v alginate solution (η 1330 ml/g) in the presence of 18 mM $NaIO_4$ was 89% in 10 min (and 85% in only 3 min). The decrease in 0.2M sodium acetate solution under the same conditions (room temperature, shielded from light) was identical, to within 1%, and in 0.2M sodium perchlorate was slightly greater (91% in 10 min).

Hydroxylation of benzoate in periodate solutions. — The spectrum of sodium salicylate (2-hydroxybenzoate) shows a marked peak at 295 nm, which is absent from the benzoate spectrum. The spectra of solutions of periodate-treated benzoate, scanned in the range 260–370 nm, showed no detectable change after 18.5 h in the dark, or after 12 h in full daylight on a window ledge. Control solutions containing pre-mixed D-mannitol and periodate, with benzoate and/or salicylate, showed no change in the spectrum of either aromatic acid. Nor did 0.02M IO_4 measurably affect the 295-nm absorption of 10^{-4} M salicylate.

DISCUSSION

Demonstration of chain scission

The previously expressed preference[1] for a chain-breaking mechanism, rather than an increase in polymer flexibility[3], was based on indirect evidence. The results from light scattering and ultracentrifugation reported here demonstrate that the alginate chain is indeed split in the presence of periodate. A decrease in the molecular weight of about an order of magnitude is indicated.

Though it is difficult to quantify the polydispersity of molecular weight produced by periodate attack on alginate, the light-scattering results, taken together with the sedimentation data, hint at homodispersity of the split products. Boundary spreading in low-speed sedimentation velocity experiments was difficult to estimate for the undegraded material, whose inherent polydispersity is therefore unknown. Sedimentation velocity experiments are uninformative above 2 $mg.ml^{-1}$ of solute, and sensitive optical techniques are needed to follow the sedimentation of dilute alginate solutions. Table I includes the data derived from hydrodynamic studies.

The different methods (viscometry, light scattering, ultracentrifuge, and c.e.c.) give estimates of the molecular weight of the oxidation product *C* which at first sight disagree. The c.e.c. of *C* is significantly higher than that of either *A* (the starting material) or *E* (the borohydride-reduced product of *C*). Similarly, when dermatan sulphate was oxidised by periodate, the c.e.c. of the oxidised product was higher than that of the starting material[10]. According to the experience and theoretical background of the c.e.c. phenomenon[11], this might imply a molecular weight of the oxidised product higher than that of the starting material. It might then be assumed[10] that the "aldehydes" produced during oxidation give rise to interchain links, possibly hemiacetal, *etc.*, which would increase the molecular weight and hence the c.e.c. However, this is an oversimplification. In the first place, the relationship between c.e.c. and molecular weight assumes that the chemical composition remains unchanged while the molecular weight is varying[11]. This is clearly not the case in either of the above instances, in which considerable conversion of glycol groups into "dialdehydes" has occurred. The effect on the c.e.c. of this conversion cannot be assessed at present. Secondly, marked increases of c.e.c. with molecular weight occur only in the lower range of molecular weights[11]. At higher molecular weights (and especially for densely charged polymers such as alginate and dermatan sulphate) major changes are accompanied by very minor changes in c.e.c.[11] [the pulse-irradiated, degraded alginates (Table II) have the same c.e.c. values as the starting material]. An incorrect interpretation of the rise in c.e.c. on oxidation to *C* would correspond to a very large increase in molecular weight, impossible to reconcile with the dramatic fall in viscosity and the decreased S value. Nevertheless, the light-scattering results do indicate strongly interacting systems in solution *C*.

The discrepancies may be resolved by assuming that the intermolecular interactions are only strong enough to form aggregates of large size in conditions in which mechanical stress is minimal. Viscometry, as performed here, is associated with considerable shearing stress, and to a smaller extent, this is true of ultracentrifugation. Significantly, in the "equilibrium" conditions of gel chromatography[10], the results show strongly interacting systems.

The "aldehydes" produced on oxidation to *C* are strongly implicated in these phenomena, since (*1*) the borohydride-reduced product *E* from *C* shows only a small increase in c.e.c. over *A* (compare also oxydermatan sulphate with its reduction product[10]) and (*2*) the alginates depolymerised by •OH (and presumably free of such groups) show no increase in c.e.c. The fleeting nature of these interactions is confirmed by the fact that they show best in conditions in which polymer–polymer contact is facilitated, *e.g.*, in salt solution[10], where electrostatic repulsion between molecules is diminished.

It is possible that the situation is one in which the "aldehydes" are able to exchange rapidly between intra- and inter-molecular links. It is well-established that saccharide dialdehydes produced by periodate oxidation can exist as hemialdals or hemiacetals[4,12], while still retaining some aldehyde character (*e.g.*, Schiff reactivity[2], borohydride reducibility, *etc.*). The energy change in going from intra- to intermolecular bridges of similar or identical chemical type would not be large, and given their partial aldehydic character, might, in many cases, be extremely rapid, with intramolecular configurations being preferred.

The product *E* is probably therefore a better material on which to calculate depolymerisation of *A* by periodate.

The mechanism of chain splitting

For several reasons, •OH was thought to be involved in the degradation of alginate[1]. It had been identified in acid solutions of periodate by its ability to hydroxylate benzoate to salicylate[13], and many examples of polymer degradation caused by its action are known. In particular, systems containing hydrogen peroxide–Fe salts degrade alginate by a mechanism probably involving •OH, and •OH scavengers such as 1-propanol were effective inhibitors of the process[14]. Subsequently, 1-propanol was said by Painter and Larsen[4] to protect alginate against the viscosity-reducing action of periodate. However, we were unable to confirm that 1-propanol (at 1.7M) prevented, or even significantly affected, the *initial* rate of depolymerisation of alginate by periodate (Fig. 4). Benzoate (a very effective •OH scavenger[15], $k_2 = 3.3 \times 10^9$ mol^{-1}.sec^{-1}) at 0.2M was without effect on the viscosity decrease, being no better in this respect than acetate or perchlorate at 0.2M. In our experiments, viscosities were monitored continuously, whereas Painter and Larsen[4] examined samples recovered from the reaction mixture.

On a closer examination, their data show that the viscosity of the alginate recovered from a 1-propanol-protected periodate oxidation was 400 ml/g (in 0.01M NaCl) compared with 1240 ml/g (in 0.1M NaCl) at the outset. At least 68% of the original viscosity was therefore lost (on the conservative assumption that the recovered material was not relatively enriched in less-degraded alginate, compared with the whole sample, and without an allowance for the overestimate of the viscosity measured in 0.01M NaCl, compared with that measured in 0.1M NaCl), *i.e.*, the protective effect of 1-propanol is far from complete, in substantial agreement with our data.

It was therefore necessary to confirm that •OH could depolymerise alginate, that 1-propanol could protect against its action, and if confirmed, to find out if periodate solutions contained sufficient •OH to be effective.

The production of •OH by pulse radiolysis of a nitrous oxide-saturated, aqueous solution is substantially unaccompanied by interfering species:

$$e^-_{aq} + N_2O \rightarrow N_2 + {\bullet}OH + OH^-.$$

The rate of reaction of e^-_{aq} with N_2O is 10^2–10^3 times faster[15] ($k_2 = 8.7 \times 10^9\ \text{mol}^{-1}.\text{sec}^{-1}$) than with most polysaccharides[16] (k_2 about $10^7\ \text{mol}^{-1}.\text{sec}^{-1}$). Assuming G_{OH} to be ~5, and that all e^-_{aq} is converted into •OH, 10^4 rads of e^-_{aq} are equivalent to 5×10^{-5} mol of •OH, which was sufficient to cause a 67% loss of viscosity (Table II). 1.7M 1-Propanol was able to protect completely against a radiation dose 5 times as great (Table III), in keeping with its high reactivity[15] toward •OH ($k_2 = 1.5 \times 10^9\ \text{mol}^{-1}.\text{sec}^{-1}$).

The evidence for the presence of •OH in periodate solutions was therefore re-examined, making use of the reaction of •OH with benzoate, which gives rise to hydroxybenzoates[13]. The spectrum of salicylate (2-hydroxybenzoate) shows a peak at 295 nm (ε 3700) clear of the long-wave edge of benzoate absorption (λ_{max} 268, ε 560). 3-Hydroxybenzoate also absorbs at longer wavelengths than benzoate. The absence of change in the spectra of benzoate from 260–360 nm in the presence of IO_4^-, in the dark or in the light, is strong evidence that the production of •OH in 0.02M IO_4^- solution cannot amount to more than ~3 μM (equivalent to a measured E^{1cm} at 295 nm of 0.01) over a period of many hours*.

The amounts of radiation listed in Table II are nominally equivalent to between 6 and 600 μM •OH. The production of so much •OH in the periodate solution would have been very easily demonstrable in the spectroscopic experiment. Even so, the highest level of radiation was not quite as degradative as 0.02M IO_4^-, in spite of the (at least) 200 times higher dose of •OH. Taken together with the inability of the

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*In the experiments[13] referred to earlier, salicylate formation from benzoate could not be demonstrated *in the dark*, in agreement with our results. On irradiation with light from a 125-watt, high-pressure, mercury arc, filtered to remove wavelengths shorter than 3650 Å, salicylate was formed[13] after 2 h. The experiments were carried out at pH 2.0, identification was based solely on the formation of a purple ferric complex, and yields were not recorded. The results are probably not directly relevant to our degradation studies, which were carried out in the dark, at pH ~5 for a much shorter period of time.

TABLE III

Nominal radiation dose (rads)	$\frac{\eta_{sp}\ \mathit{Test}}{\eta_{sp}\ \mathit{control}} \times 100$	
	H_2O	*10% v/w 1-propanol*
0	100 (=321 ml/g)	100 (=288 ml/g)
5.2×10^4	49	100 ±2

efficient free-radical scavengers (benzoate and 1-propanol) to protect against depolymerisation by periodate, the evidence is strongly against the involvement of •OH *in the rapid phase* of degradation.

However, it is probable that •OH is involved in a slower depolymerisation. Although the *initial* degradation of alginate, with or without 1-propanol, is marked and rapid (see Results and Fig. 4), in the longer term, 1-propanol is seen to exert considerable protective action. Thus, alginate exposed for many hours to periodate in the absence of 1-propanol was of far lower viscosity than that similarly treated in the presence of 1-propanol[4] (17 as compared with 400 ml/g). The protection afforded by 1-propanol against •OH-mediated breakdown is so complete (see, *e.g.*, Table III) that a comparison of results obtained in its presence and absence permits an assessment of the role of •OH. For example, 1-propanol almost completely inhibits the degradation of polyethylene oxide by periodate (Fig. 4), which suggests that •OH-mediated breakdown is of prime importance, since its simple structure excludes chemical scission (at least by recognised reactions of periodate) as well as changes in polymer flexibility. The uninhibited rates of depolymerisation are much lower than the *inital* degradation of alginate, and the extent to which 1-propanol is protective is apparent only after quite long periods of time (Fig. 4). The behaviour of polyethylene oxide exemplifies clearly and simply the slow phase of alginate breakdown.

Presumably, a relatively slow reaction of this kind would proceed with rather low concentrations of •OH, which would be compatible with our inability to demonstrate •OH in periodate solution by spectrophotomeric means*.

We conclude that periodate-induced degradation of alginate proceeds by two mechanisms; one fast, not mediated by •OH, and the other slow, probably due to low concentrations of •OH.

The two-fold mechanism of degradation implies that there are infrequent and atypical monomers in the alginate molecule, which can be cleaved by periodate with breakage of the chain (Scheme 2). Whether they are introduced during biosynthesis, or by subsequent *in vivo* or *in vitro* processes[1], remains to be seen. It is worth pointing out that pectate also showed a rapid, initial, degradation phase similar to that of alginate[1].

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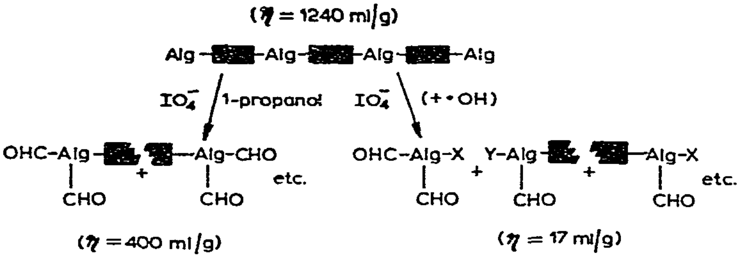
*Hydroxylation of benzoate in sodium periodate solution has now been demonstrated using the much more sensitive spectrofluorimetric assay for salicylate (Scott and Page-Thomas, to be published).



Scheme 2

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