Carbohydrate Research, 28 (1973) 53–59

PERIODATE-INDUCED VISCOSITY DECREASES IN AQUEOUS SOLUTIONS OF ACETAL- AND ETHER-LINKED POLYMERS

J. E. SCOTT AND M. J. TIGWELL

M.R.C. Rheumatism Unit, Canadian Red Cross Memorial Hospital, Taplow, Maidenhead, Berkshire (Great Britain)

(Received October 30th, 1972; accepted for publication, November 16th, 1972)

ABSTRACT

Alginate, polygalacturonate, and methylcellulose (polyacetals), and polyethylene oxide (polyether) rapidly lost viscosity in periodate solution. Three other polymers, namely DNA, polyacrylate, and gelatine, which are not polyethers or polyacetals, were almost unaffected in similar conditions. A mechanism is proposed, based on a known disproportionation of ether-type free-radicals, which is induced by hydroxyl radicals in periodate solution. Scission of the polymer chain could occur solely by ether-type disproportionation, or by glycol cleavage following ring opening caused by disproportionation involving the ring oxygen atom. The susceptibility of glycuronans to periodate degradation might, in part, be due to the known ease of formation of free radicals from α-hydroxy acids by abstraction of H-5, followed by ring opening and glycol fission by periodate. The relevance of these findings and interpretations to other free-radical-induced degradations of polysaccharides is discussed.

INTRODUCTION

Solutions of proteoglycans and glycosaminoglycuronans containing periodate decrease rapidly in viscosity[1]. Free radicals were thought to be responsible. Other polymers have now been examined to see whether the presumed depolymerising action of periodate is general, or whether only certain types of polymer can be involved. An abstract of part of this work has appeared[2].

MATERIALS AND METHODS

The polymers used were methylcellulose 20 (BPC), DNA (sodium salt, *ex* calf thymus, Koch–Light), sodium polyacrylate (prepared by neutralising Versicol E13, a gift from Allied Colloids Ltd, with sodium hydroxide), gelatine 246 (from acid-processed pig-skin, a gift from the British Leather Research Association), polyvinyl alcohol (Mowiol N 70–98, visc. 20, Hoechst), sodium polygalacturonate (from polygalacturonic acid 85%, Koch–Light), polyethylene oxide (Polyox WSR N-750, mol. wt. ~300,000, B.D.H.), and sodium alginate (B.D.H.).

Viscometry was carried out in aqueous solutions of sodium perchlorate, as described previously[1]. Polymers were exposed to 0.1M sodium periodate (except for gelatine which was in 94mM sodium periodate). Sufficient sodium perchlorate was included to bring the total Na^+ molarity to 0.5 (except for alginate and polygalacturonate, which were at 0.2M; Table I).

TABLE I

VISCOSITY DATA FOR PERIODATE-TREATED POLYMERS

Polymer	*Polymer concentration* (% *w/v*)	*Total* Na^+ *concentration* (M)	*Decrease in* η_{sp} *Test*/η_{sp} *Control* (%, *in 1 h*)
Polyvinyl alcohol	1.0	0.5	78
Gelatine	0.5	0.5	10
Polygalacturonate	0.4	0.2[a]	70
Methylcellulose	0.3	0.5	28
Polyethylene oxide	0.3	0.5	55
Alginate	0.3	0.2[a]	85
Polyacrylate	0.3	0.5	0
DNA	0.075	0.5	13

[a]Instead of 0.5M, at which concentration polymer was precipitated.

Periodate consumption by polyethene oxide, methyl cellulose, polyacrylate, and polyvinyl alcohol was estimated at 222.5 nm, as described previously[1], in sodium periodate–sodium perchlorate solutions at room temperature in the dark (Table II). Corrections for iodate absorption were made[3]. Spectra were measured with a Perkin–Elmer–Hitachi recording spectrophotometer, Model 124.

TABLE II

DATA FOR TREATMENT OF POLYMERS WITH PERIODATE

Polymer	*Methylcellulose*[a]	*Polyethylene oxide*	*Polyacrylate*	*Polyvinyl alcohol*
Polymer concn. (% w/v)	0.95	0.95	4.0	1.0[b]
$NaIO_4$ concn. (mM)	9.5	0.95	10.0	10.0
$NaClO_4$ concn. (M)	0.475	0.475	0.50	0.50
Consumption of periodate (mole %) in 2 h	1.3	1.02	2.95	1.06[c]

[a]Mol. wt. of average monomer residue, 177. [b]Nominal; not all of the material was soluble. [c]*Cf.* 1.23–1.95, ref. 5.

RESULTS

Solutions of polymers held together by ether and acetal (*i.e.* glycosidic) links (particularly polygalacturonate, alginate, and polyethylene oxide; Fig. 1), and also polyvinyl alcohol, showed marked decreases in viscosity in the presence of periodate,

but not in control solutions containing sodium perchlorate instead. Gelatine, polyacrylate, and DNA solutions lost little viscosity (Fig. 2), compared with the controls. The gelatine *control* solution slowly decreased in viscosity, and results are therefore

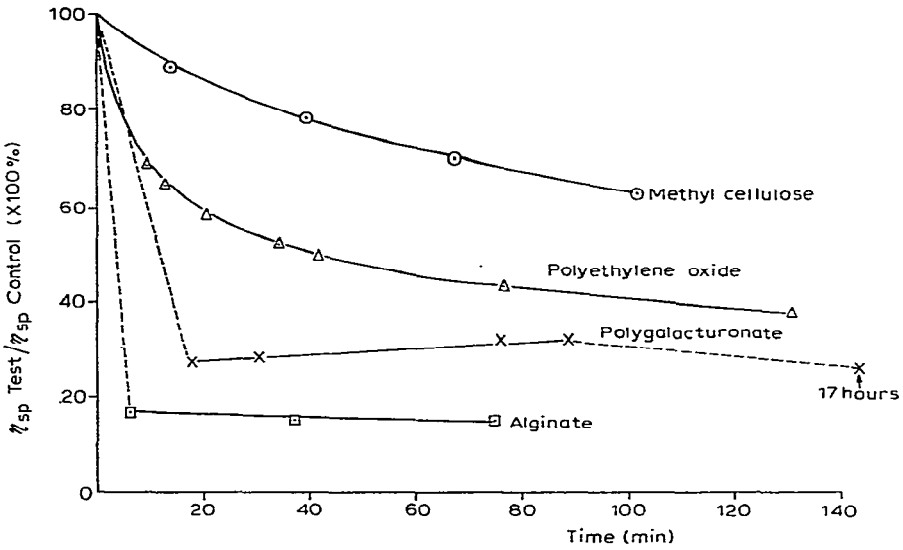


Fig. 1. Change with time of viscosity of solutions of polyacetals and a polyether in 0.1M sodium metaperiodate. Almost all of the observed drop in the viscosity of alginate and polygalacturonate solutions occurred in the time taken to make the first measurement.

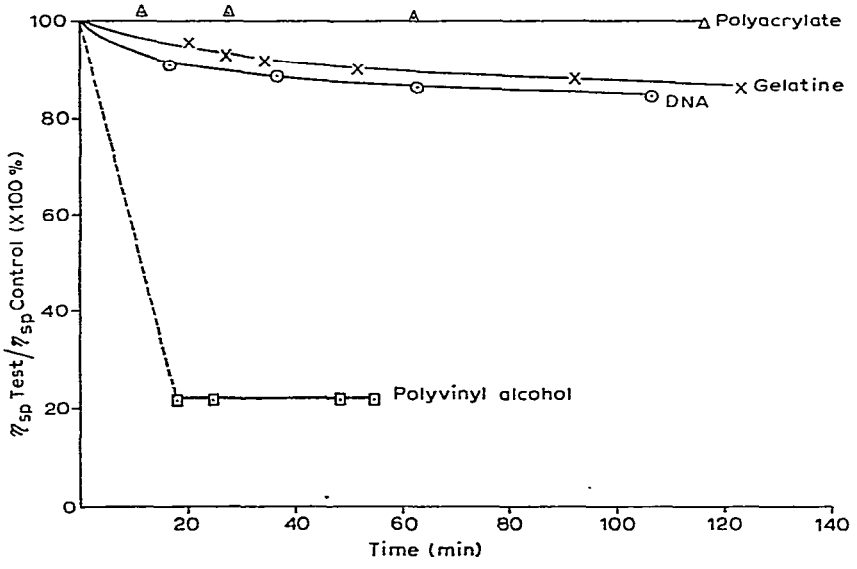


Fig. 2. Change with time of viscosity of solutions of polymers (not polyethers) in 0.1M sodium metaperiodate (except for gelatine, which was 94mM). The entire observed drop in the viscosity of polyvinyl alcohol solution occurred before the first measurement was made.

expressed as percentages of the control viscosity at comparable times following mixing of the gelatine solution with salt ($NaIO_4$ and/or $NaClO_4$) solution.

Periodate "consumption" by polyethylene oxide was low, whereas polyacrylate "consumed" somewhat more oxidant. The figures given in Table II are maxima, since the samples of polymer were probably not completely pure. The polyacrylate was free of anti-oxidants, but could have contained traces (<0.05%) of monomer and thiol compounds (Allied Colloids, personal communication). The actual polymer concentrations would be lower than assumed, and impurities might have consumed relatively large amounts of periodate. In addition, it may be inaccurate to attribute the change in absorption at 222.5 nm *solely* to oxidation by periodate with concomitant production of iodate.

DISCUSSION

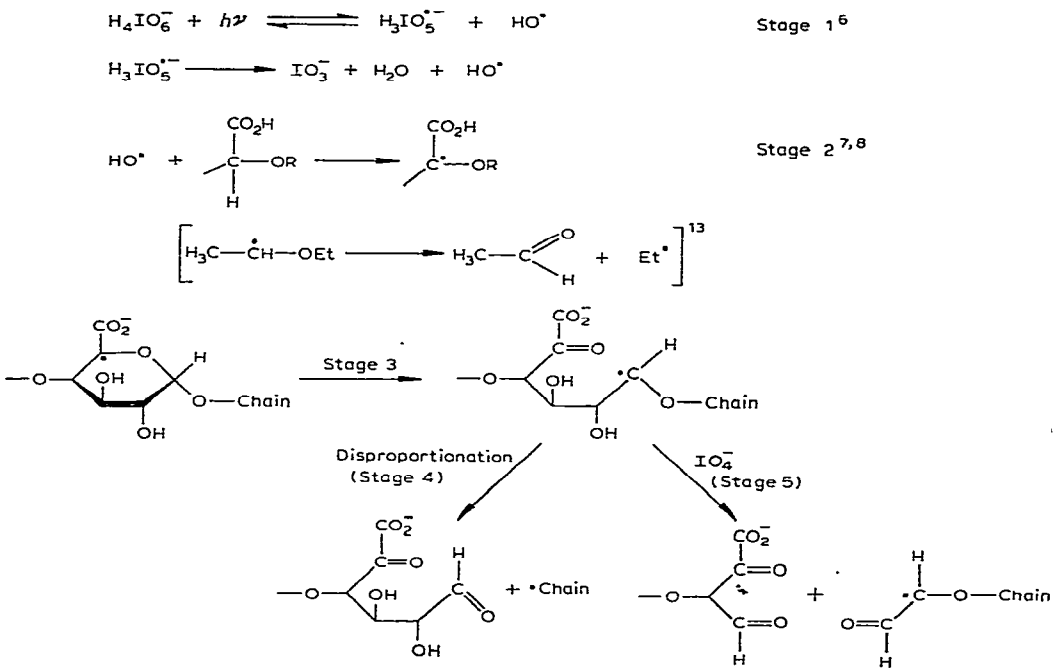
Glycol cleavage in polysaccharides has been suggested to increase polymer flexibility, decreasing hydrodynamic interactions and therefore lowering the viscosity[4] in solution. The very limited glycol-cleavage in the very short time in which great decreases in viscosity occur (see Fig. 1, also ref. 1) is against this being a major factor in these experiments. Moreover, the decreased viscosity of polyethylene oxide solutions could not be due to this effect, and methylcellulose would probably undergo very few glycol cleavages.

Of the eleven polymers in this and the previous investigation, three (polyacrylate, DNA, and gelatine) were not susceptible to the viscosity-reducing action of periodate in solution The remainder, except polyvinyl alcohol, contain monomers linked wholly or mainly by –C–O–C– bonds and are polyethers or polyacetals. The anomaly of polyvinyl alcohol is apparent, rather than real, since occasional head-to-head additions in the polymerisation of vinyl monomer[5] result in glycol groups which are very rapidly split by periodate, producing material of low molecular weight and low viscosity[5]. Our results quantitatively confirm these findings, which place polyvinyl alcohol in a special category, in that some of the polymer links are *directly* split by periodate.

Thus, the seven remaining susceptible polymers are polyethers or polyacetals, whereas none of the resistant polymers are in this category. It is significant that polyethylene oxide is "degraded" by periodate in view of the simplicity of its structure.

From literature sources, a detailed picture can be assembled of a mechanism for the degradation of polyethers brought about by hydroxyl radicals produced in periodate solution (Scheme 1). The evidence for each step is direct. The key reaction (stage 3, ref. 12) produced a high yield of products from ethyl ether, which is formally almost identical with any dimer unit in polyethylene oxide.

Certain conclusions follow. The work of Dixon and Norman[7] indicates that the formation of the free-radical intermediate $^{-}O_2C–\dot{C}(I)–OH$ (Stage 2, Scheme 1) is easy. This type of radical has been detected in the uronic acid of γ-irradiated hyaluronate[8].



Scheme 1. Stages 1 and 2 establish the feasibility of the route to the glycuronan free-radical. The disproportionation of the ethyl ether free-radical (in parenthesis) is the prototype reaction of those shown in Stages 3 and 4, which could, in principle, be undergone by any polysaccharide. Stage 5 demonstrates the possibility of chain scission, specific to periodate and glycol-containing polysaccharides, secondary to disproportionation involving the ring oxygen atom.

Hence, uronic acids should be susceptible to attack by HO·, and the especially rapid depolymerisation of alginate and polygalacturonate (Fig. 1) would follow.

Polyacrylate could (less readily, *cf.* ref. 7) give rise to $^{-}O_2C–\dot{C}(H)–C–$, but there is no disproportionation reaction comparable to that of stage 3 which would disrupt the polymer, and decarboxylation, recombination, and termination steps might be expected to intervene.

Opening of the pyranoid ring in the first disproportionation step allows a new possibility, namely cleavage of the polymer chain (Scheme 1) due to glycol splitting by periodate, which would be expected to be rapid, as in the case of polyvinyl alcohol. Thus, although the first disproportionation step could be caused by all agents that produce HO·, subsequent depolymerisation in periodate solution would be faster if a glycol group were present in monomers in which the pyranoid ring had been opened. The relatively low rate of degradation of methylcellulose compared with alginate might be attributed to the absence of glycol groups after opening of the pyranoid ring. By analogy, the contribution of glycol splitting to chain scission, as compared with free-radical ether disproportionation, could be assessed by comparing, for example, *O*-substituted alginates with alginate.

Vink[9] observed that degradation of polymers in aqueous solution catalysed by Cr^{3+} and Fe^{3+} was limited to polymers [including polyethylene oxide and (2-hydroxyethyl)cellulose] containing C–O–C groups in the backbone. Polymers with –C–C– backbones (including polyvinyl alcohol and polymethacrylic acid) were unaffected. Free radicals were not specifically mentioned, but the similarity of Vink's experiments to other oxidation–reduction depolymerisation systems is very clear, and his results lend strong support to the mechanism now proposed.

The potential importance in carbohydrate chemistry of the Evans disproportionation reaction of ethereal free-radicals seems not to have been realised. The juxtaposition of ring and glycosidic acetal links in polysaccharides gives rise to many possible sequences of reactions. Particularly interesting would be products from a free radical originating at the glycosidic link, which, if formed at C-1, could lead to an ester link in place of the original glycosidic bond, following disproportionation. Alternatively, a lactone could be formed, with scission of the chain. This may be relevant to the frequent observations that acid is produced in the course of oxidation–reduction depolymerisation[10,11].

It is, of course, possible that ring-opened structures analogous to those in stage 3 (Scheme 1) were present in the alginate and polygalacturonate preparations before addition of periodate, as a result of prior encounters with free radicals. This would account in part for the extremely rapid initial decrease in viscosity in these experiments, so similar to that of polyvinyl alcohol.

It has been reported that another $HO^{\cdot}$-producing system (ferrous ion) degrades hyaluronic acid to an unsaturated disaccharide[10], which can be oxidised by periodate to produce a β-formylpyruvate chromogen in the Warren–Aminoff assay. This pathway would be an alternative in our systems to that proposed above; we failed to demonstrate the formation of a β-formylpyruvate chromogen from periodate-treated alginate or hyaluronate. However, we were unable to detect this chromogen in hyaluronate treated with ferrous ion, either exactly as described by Kennedy and Cho Tun[10] or when using ten times their stated concentrations of hyaluronate. This would be expected, since the figures published by Warren for the sensitivity of his assay[12] (which we confirmed) would not permit detection of the maximum amounts expected in the experiments[10] described*.

Degradation to smaller fragments caused by periodate may seriously affect conclusions based on recovered material, if it were assumed that the polymer were intact. Dialysis, precipitation, and other techniques based on polymer properties might best be avoided, or carefully checked for losses, which could be specific to certain parts of the molecule, *e.g.* those containing uronic acids.

The results presented here reinforce the earlier, tentative conclusion[1] that the protein core of proteoglycans was probably not the primary target for degradation by periodate, which was instead to be found in the polysaccharide side-chains.

*We have since heard (Dr. J. F. Kennedy, personal communication) that an essential concentration step prior to measurement was accidentally omitted from the published account.

REFERENCES

1 J. E. SCOTT, M. J. TIGWELL, AND S. W. SAJDERA, *Histochem. J.*, 4 (1972) 155.
2 J. E. SCOTT AND M. J. TIGWELL, *Scand. J. Clin. Lab. Invest., Suppl.* 29 (1972) 28.
3 G. O. ASPINALL AND R. J. FERRIER, *Chem. Ind. (London)*, (1957) 1216.
4 A. HAUG AND T. J. PAINTER, personal communication.
5 P. J. FLORY AND F. S. LEUTNER, *J. Polym. Sci.*, 3 (1948) 880.
6 M. C. R. SYMONS, *J. Chem. Soc.*, (1955) 2794.
7 W. T. DIXON, R. O. C. NORMAN, AND A. L. BULEY, *J. Chem. Soc.*, (1964) 3625.
8 E. A. BALAZS, J. V. DAVIES, G. O. PHILLIPS, AND M. D. YOUNG, *Radiation Res.*, 31 (1967) 243.
9 H. VINK, *Makromol. Chem.*, 67 (1963) 105.
10 J. F. KENNEDY AND H. CHO TUN, *Carbohyd. Res.*, 22 (1972) 43.
11 B. SKANSE AND L. SUNDBLAD, *Acta Physiol. Scand.*, 6 (1943) 37.
12 L. WARREN, *J. Biol. Chem.*, 234 (1959) 1971.
13 W. V. EVANS, R. PEARSON, AND D. BRAITHWAITE, *J. Amer. Chem. Soc.*, 63 (1941) 2574.