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EFFECT OF PERIODATE OXIDATION UPON THE STIFFNESS OF THE ALGINATE MOLECULE IN SOLUTION

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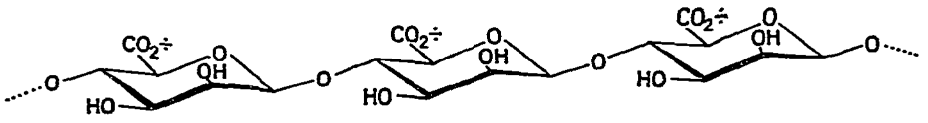
ABSTRACT

Samples of sodium alginate from *Laminaria digitata* were oxidised in aqueous solution to different extents by the addition of calculated amounts of sodium metaperiodate. An empirical measure of the molecular stiffness of the products, both before and after reduction with sodium borohydride, was obtained by studying the sensitivity of their intrinsic viscosities to changes in the ionic strength of the solution. The results showed that, whereas the stiffness decreased very sharply with increasing degree of oxidation, it changed very little upon subsequent reduction of the products. From this, and the known structures of the inter-residue hemiacetals in the unreduced products, it was inferred that the increase in flexibility brought about by oxidation is specifically associated with rotation about the three bonds adjoining C-4, C-5, the original ring-oxygen atom, and C-1 of the oxidised hexuronic-acid residues. This rotation was considerably less-hindered than the rotation around the glycosidic linkage in both the intact alginate and in the partially oxidised and reduced products. The conversion of only a small percentage of the stiff pyranoid rings into these centres of high, local flexibility had a marked effect on the overall flexibility of the chains, showing that the possible presence of such centres should be taken into account in any discussion of the solution properties of heteropolysaccharides or of polysaccharides that may have suffered slight chemical modification.

INTRODUCTION

When sodium alginate is oxidised by periodate, the aldehyde groups of oxidised hexuronic-acid residues spontaneously form six-membered hemiacetal rings with the closest hydroxyl groups on the two adjacent, unoxidised residues in the chains[1,2]. Upon oxidation of a hexuronic-acid residue, the relatively rigid, pyranoid ring is thus converted into a segment of the polymer chain containing three contiguous, single bonds about which rotation may occur. At the same time, rotation about the four single bonds comprising the glycosidic linkages immediately preceding and following this segment is precluded because of their incorporation into cyclic hemiacetal structures[1,2].

Reduction of the partially oxidised alginate with sodium borohydride opens the hemiacetal rings, so that rotation about the original glycosidic linkages again becomes possible, bringing the total number of contiguous, single bonds in the segment up to seven[1,2]. The structures of a triplet of residues of D-mannuronic acid before and after oxidation of the central unit, and again after subsequent reduction, are shown in formulae **1**, **2**, and **3**, respectively.



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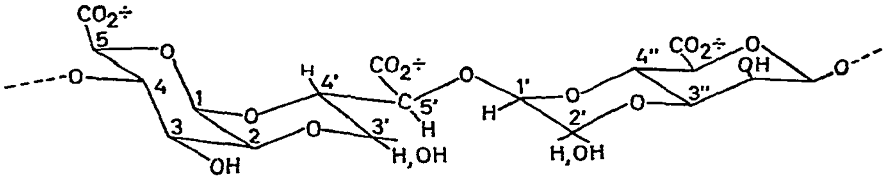

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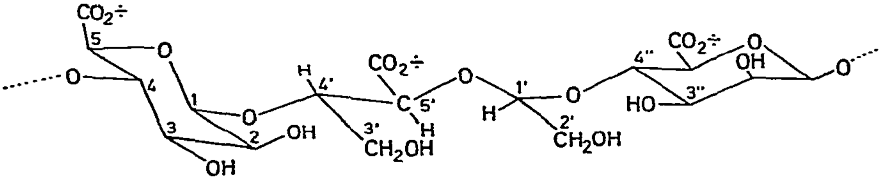

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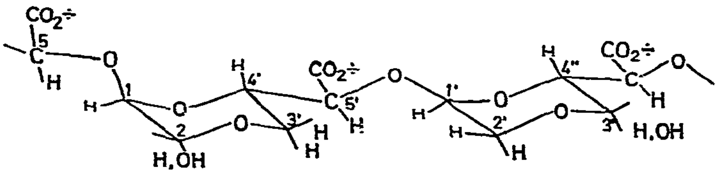
If alginate is treated with an excess of periodate, oxidation ceases when ~44% of the hexuronic-acid residues have been oxidised, and at this stage, every remaining, unoxidised residue has at least one oxidised residue in an adjacent position. Reduction with borohydride and a second treatment with excess of periodate then generates chains in which most (theoretically, 88%) of the original residues of hexuronic acid have been oxidised, and in which most of the original glycosidic linkages have once again become incorporated into cyclic hemiacetal structures of the type shown in **4**. A second reduction with borohydride then again exposes all the original glycosidic linkages, leaving chains containing only 12% of intact hexuronic acid, and 88% of units of the type shown in **5**.



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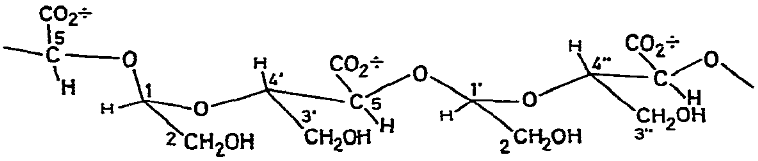

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Previous work in this laboratory has shown that the alginate molecule is very extended in solution[3,4], even at infinite ionic strength or low pH, where the mutual repulsion of the charged carboxyl groups does not contribute to the extension of the chain. It was argued that this is due to a high rigidity inherent in the molecular chain, rather than to solvent-expansion (excluded volume) effects, although the possibility of firmly bound solvent contributing to this rigidity was not excluded.

With regard to rigidity arising from non-bonded interactions between the atoms of adjacent residues of hexuronic acid, it was of interest to determine to what extent this is due to the fact that HO-2 and HO-3 and the carboxyl group attached to C-5 are held in relatively fixed positions because of their attachment to a pyranoid ring. After periodate oxidation and subsequent reduction, the interacting atoms of the oxidised unit are no longer situated on a pyranoid ring. The flexibility arising from rotation around the glycosidic linkage in this case may be measured as an increase in molecular flexibility accompanying the opening of the hemiacetal rings. The sequence of reactions just described therefore offered an opportunity to seek an experimental answer to this question.

THEORY

The estimation of the unperturbed dimensions of polymers, and, hence, the flexibility of their molecular chains, usually entails the measurement of viscosity, radii of gyration, second virial coefficients, and molecular weights in θ-solvents. In the case of polysaccharides, especially charged polysaccharides, it is very difficult to find a θ-solvent, and some theory has to be used to extrapolate to θ-conditions from measurements made in "good" solvents[5]. This procedure is time consuming and is not very sound theoretically. The problem has previously been discussed by Burchard[6], Smidsrød[3], and Banks and Greenwood[7].

The method adopted in the present work was recently developed in this laboratory[4,8], and consisted in measuring the intrinsic viscosity of the sample at a series of different ionic strengths. The slope, S, of the rectilinear plot of the intrinsic viscosity, $[\eta]$, against the reciprocal of the square root of the ionic strength, I, depends upon both the molecular weight of the polyelectrolyte and its unperturbed dimensions.

The value, B, of S corresponding to an intrinsic viscosity of unity at $I = 0.1$ can be empirically correlated with parameters describing the extension of the unperturbed molecule[4].

One such parameter (K_θ) with which B has been correlated (Fig. 4 of ref. 4) is taken from the "equivalent sphere" theory of Flory[5], and is defined by the equation:

$$K_\theta = \Phi\left[\frac{\bar{r}_\theta^2}{M}\right]^{\frac{3}{2}},$$

where Φ is the Flory viscosity-constant, and M the molecular weight. The root-mean-square end-to-end distance of the macromolecule under θ-conditions is given by $(\bar{r}_\theta^2)^{\frac{1}{2}}$. It is thus seen that K_θ, and hence B, are measures of the relative extension of the unperturbed hydrodynamic sphere.

EXPERIMENTAL

Intrinsic viscosities were measured as described previously[3]. Sodium chloride was used throughout to vary the ionic strength. The sodium alginate was prepared from *Laminaria digitata*, harvested at Tarva (August 29th, 1961). It contained 61% of residues of D-mannuronic acid, and 39% of residues of L-guluronic acid, and had an intrinsic viscosity in 0.1M sodium chloride of 10 dl/g, corresponding to a weight-average molecular weight of 5×10^5.

The preparation of alginate oxidised to its first limit (44% oxidation) and to its second limit (88% oxidation), and the method of reduction with sodium borohydride, have been described elsewhere[2]. To prepare samples with lower degrees of oxidation, sodium alginate (8.0 g, dry weight) was dissolved in oxygen-free water (1.3 l) and 1-propanol (200 ml) was added. The solution was divided into four equal parts, to which were added, in water (125 ml), 107, 214, 428, and 642 mg of sodium metaperiodate, respectively. These solutions were kept in the dark at room temperature for 48 h, after which they gave no colour with a solution of starch and potassium iodide, buffered at pH 7.0. They were then concentrated on the rotary evaporator at 30° to 100 ml each, and dialysed against distilled water until the dialysates gave no colour with acidified starch–potassium iodide solution. The salt-free solutions were concentrated, centrifuged for 1 h at 40,000 *g*, and freeze-dried, to give nearly quantitative yields of 5, 10, 20, and 30% oxidised alginates as white solids.

RESULTS

Effect of periodate oxidation upon stiffness

In Fig. 1, the intrinsic viscosities of intact alginate and of six partially oxidised derivatives are plotted as a function of the reciprocal of the square root of the ionic strength. The measured slopes, S, of the straight lines obtained are given in Table I, together with the corresponding values of the intrinsic viscosities, $[\eta]_{0.1}$, at an ionic strength of 0.1. By graphical extrapolation in a plot of log S against log $[\eta]_{0.1}$ as described earlier[4], the value, B, of S corresponding to $[\eta]_{0.1} = 1$ was estimated in each case. The results are also shown in Table I, together with the derived[4] values of the parameter of stiffness, K_θ. In Fig. 2, K_θ is plotted against the degree of oxidation.

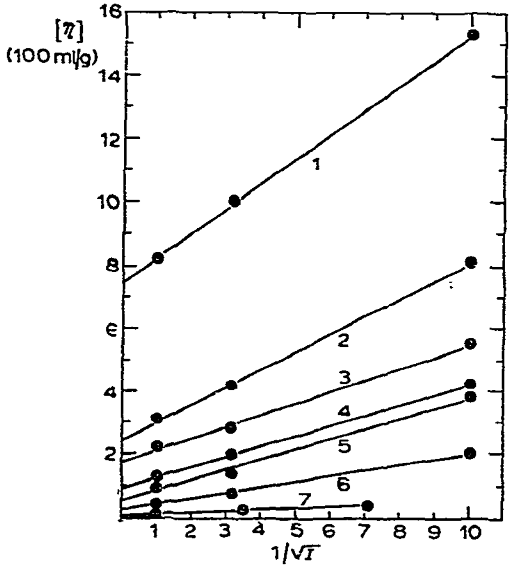

Fig. 1. Intrinsic viscosity as a function of the reciprocal square root of the ionic strength for samples of alginate at different degrees of oxidation (d.o.). Curve 1, d.o. 0%; 2, 5%; 3, 10%; 4, 20%; 5, 30%; 6, 44%; 7, 88%.

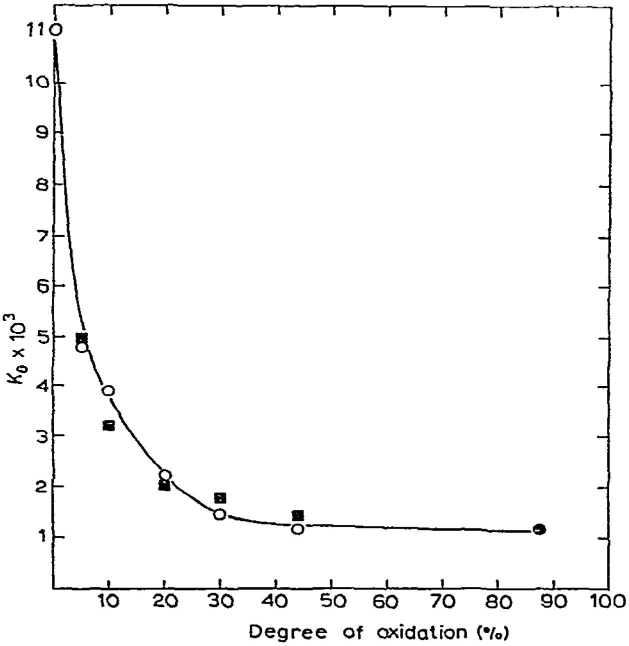

Fig. 2. Values of K_θ at different degrees of oxidation: ○, before reduction; ■, after reduction.

TABLE I

VISCOSITY DATA FOR SAMPLES OF ALGINATE AT DIFFERENT DEGREES OF OXIDATION (D.O.)

D.o. (%)	$[\eta]_{0.1}$ *(100 ml/g)*	S	B	K_θ ($\times 10^3$)
0	9.90	0.79	0.04	11
5	4.15	0.57	0.075	4.8
10	2.90	0.38	0.087	3.9
20	1.90	0.33	0.14	2.2
30	1.50	0.33	0.19	1.45
44	0.8	0.175	0.23	1.15
88	0.180	0.0265	0.23	1.15

It should be emphasised that the decrease in intrinsic viscosity with increasing degree of oxidation (Table I) is not necessarily due entirely to an increase in the flexibility of the chains. It is very probably due in part to depolymerisation of the chains, which is known[2] to take place during periodate oxidation. The parameters *B* and K_θ are, however, independent of molecular weight, and Fig. 2 therefore shows the effect upon flexibility alone.

Effect of reduction upon the stiffness of limit-oxidised alginates.

In Table II are given viscosity results on six partially oxidised and reduced samples. The obtained K_θ-values are plotted in Fig. 2. Quite unexpectedly, the K_θ-values are very nearly the same as before reduction. To test the significance of these results, an estimation of the error involved in measuring the *B*-values had to be made.

TABLE II

VISCOSITY DATA FOR SAMPLES OF ALGINATE WHICH HAVE BEEN REDUCED AFTER PERIODATE OXIDATION

D.o. (%)	$[\eta]_{0.1}$	S	B	K_θ ($\times 10^3$)
5	3.6	0.36	0.073	5.0
10	0.37	0.027	0.105	3.2
20	1.55	0.26	0.15	2.0
30	1.05	0.17	0.16	1.8
44	1.05	0.20	0.19	1.45
88	0.153	0.017	0.23	1.15

An earlier study[2] of the depolymerisation that takes place during the periodate oxidation of alginate had furnished a number of samples of 44% oxidised alginate of widely differing intrinsic viscosities. The data obtained with four such samples are collected in Table III, together with the results for three of the samples after they had been reduced with borohydride.

TABLE III

VISCOSITY DATA FOR DIFFERENT 44% OXIDISED SAMPLES BEFORE AND AFTER REDUCTION

	$[\eta]_{0.1}$	S	B	
Oxidised sample I	0.26	0.036	0.18	Av. 0.21
Oxidised sample II	0.175	0.032	0.21	
Oxidised sample III	1.56	0.327	0.20	
Oxidised sample IV	0.80	0.175	0.23	
Reduced sample II	0.195	0.0255	0.16	Av. 0.19
Reduced sample III	1.05	0.20	0.19	
Reduced sample IV	0.07	0.011	0.22	

The results in Table III indicate that the *B*-values before and after reduction are not significantly different. The limited number of experimental points does not allow the standard deviation to be calculated. It will depend on the difference between the intrinsic viscosity at low and high ionic strength, which again will depend on both the molecular weight and the stiffness of the samples. It seems safe to conclude, however, that differences in *B*-values less than 20% should not in the present case be regarded as significant. The *B*-values of the partially oxidised samples in Table I deviate less than this from the *B*-values of the corresponding reduced samples in Table II.

DISCUSSION

The main feature of the result shown in Fig. 2 is the sharp decrease in unperturbed dimensions accompanying the oxidation. This does not arise from any increase in the number of single bonds in the chains about which rotation may occur. On the contrary, for every unit attacked, two glycosidic linkages containing four single bonds are removed and replaced by only three others (**1** and **2**). In general, the unperturbed dimensions should be dependent upon both the geometry of the chains and the possibility of rotation about single bonds. The most-plausible explanation for the present effect seems to be that rotation about the glycosidic linkages is highly hindered, compared to rotation about the three single bonds with which they are replaced upon oxidation of a residue of hexuronic acid (**2**). The present result therefore supports the conclusion, drawn earlier[3], that rotation about the glycosidic linkages in alginate is highly restricted.

The fact that the unperturbed dimensions do not further decrease to a significant extent when the partially oxidised alginate is reduced (Fig. 2) indicates that rotation about the glycosidic linkages is highly restricted also in this case. The highly restricted rotation around the glycosidic linkage in the intact alginate molecule must therefore be due more to the proximity of the atoms of the interacting groups than to the fact that these groups are situated on a rigid, pyranoid ring. Since alginate contains two monomers, D-mannuronic and L-guluronic acid, a detailed discussion of the hindrance to rotation around the different types of glycosidic linkage requires knowledge of the stiffness of alginates of different chemical composition. Such knowledge has now been obtained and will be presented later[9].

The precipitous drop in unperturbed dimensions accompanying the introduction of only 1 or 2% of oxidised units (Fig. 2) in the chains, which can perhaps be visualised as "universal joints", is an effect of rather general importance, and must be taken into account in any discussion of the solution properties of heteropolysaccharides, or of polysaccharides that may have suffered a slight structural modification during isolation.

The capacity of periodate oxidation to introduce controlled proportions of flexible linkages into alginate and probably also other polysaccharides suggests that such products may be useful for testing physical methods and theories pertaining to the solution properties of polymers, and for studying the effect of molecular stiffness upon such phenomena as counter-ion binding and gel formation.

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