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Note

Concerning the effect of periodate oxidation upon the intrinsic viscosity of alginate

INGER-LILL ANDRESEN, TERENCE PAINTER, AND OLAV SMIDSRØD
Institute of Marine Biochemistry, N-7034 Trondheim-NTH (Norway)

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When sodium alginate is oxidised by periodate, there is a dramatic decrease in the intrinsic viscosity[1–4]. This occurs even when oxidation is carried out in the dark[1,2], in the accepted manner[5]. The extent of the decrease is enhanced[1] by phenolic impurities which are known[6,7] to cause oxidative–reductive depolymerisation (ORD)[8,9] in the presence of atmospheric oxygen. It is diminished[1,4] by 1-propanol, which is known[10] to act as a scavenger for hydroxyl radicals and to inhibit the depolymerisation of alginate by Fenton's reagent[10]. These observations strongly indicate that the decrease in viscosity is due, at least in part, to a reaction that is fundamentally similar to ORD, except that periodate is the electron acceptor*.

Despite this seemingly adequate explanation, it has been clearly shown that a large decrease in viscosity still takes place, even when highly purified alginate is oxidised in the presence of 1-propanol[1,2,4]. This situation led Smidsrød and Painter[2] to study the effect of ionic strength upon the intrinsic viscosities of a series of partially oxidised alginates, with the conclusion that oxidation increases the *flexibility* of the polyuronide chains[2]. It was pointed out that this would lead to a contraction of the coiled macromolecules in solution, and hence to a decrease in the radius of gyration (R_G) for a given molecular weight (M). The effect of such a contraction upon the intrinsic viscosity, $[\eta]$, can be readily appreciated from the Flory–Fox relationship for non-drained flexible coils:

$$[\eta] = 6^{1.5}\Phi(R_G)^3/M. \qquad (1)$$

An alternative explanation has been proposed by Scott *et al.*[4], who used light-scattering and ultracentrifugation to supplement the viscosity data. The results seemed to indicate that the decrease in intrinsic viscosity that takes place in the presence of 1-propanol is due to a 20-fold decrease in molecular weight, brought about by some kind of depolymerisation that does not involve free radicals. The authors suggested that selective chain-scission was taking place at atypical sugar units in the alginate molecule[4].

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*All discussion in this paper relates to periodate oxidations that are carried out in the dark. In the light, periodate solutions generate hydroxyl radicals directly[11].

TABLE I

DATA[a] FOR ALGINATE SOLUTIONS IN 0.1M SODIUM CHLORIDE AT 20°

Material	*Identifying letter*	$[\eta]$ *(dl/g)*	$\overline{M}_w . 10^{-3}$	$(\overline{R_G})_z . 10^8$ *(cm)*	$[(\overline{R_G})_z^3/\overline{M}_w] . 10^{24}$	$\Phi . 10^{-21}$ $(dl.g^{-1}.cm^{-3})$	$B . 10^3$ $(mol.cm^3.g^{-2})$
Sodium alginate	A	8.0	300	850	2047	0.6[b]	3.1
A, 26% oxidised	B	2.9	220	450	414	1.0[b]	1.7
B, $NaBH_4$ reduced	C	0.9	70	300[c]	386	0.4[b]	4.3

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[a] Intrinsic viscosities, $[\eta]$, weight-average molecular weights ($\overline{M}_w$), Z-average radii of gyration $(\overline{R_G})_z$, Flory viscosity-constants (Φ), and second virial coefficients (*B*). [b] Calculated from equation *1* with weight-average quantities obtained by assuming $\overline{M}_z:\overline{M}_w:\overline{M}_n = 5:3:1$, and $R_G = K . M^{0.5}$ (see Ref. 14.) [c] The uncertainties in this determination are rather large, because $(\overline{R_G})_z$ is too near to $\lambda/20$, the lower limit for determining R_G by light scattering.

In an attempt to resolve this controversy, we have repeated the light-scattering experiments of Scott *et al.*[4], with the results shown in Table I. Only a 27% decrease in molecular weight was observed when sodium alginate was oxidised to an extent of 26%; the corresponding material studied by Scott *et al.*[4] was 25% oxidised. Even this decrease may not have occurred during the oxidation, as no attempt was made to exclude light or oxygen during the long period of dialysis that was used to remove iodate and equilibrate the solution with 0.1M sodium chloride (see Experimental).

On the other hand, reduction of the oxidised product with sodium borohydride decreased its molecular weight to one-third. This result is in agreement with an earlier finding[12] that considerable depolymerisation of periodate-oxidised alginates can occur under the alkaline conditions of the reduction. The mechanism almost certainly involves a β-elimination that occurs competitively with reduction. The extent of the depolymerisation can be diminished by increasing the concentration of sodium borohydride[12].

The most interesting feature of the present results is the dramatic decrease in $(\overline{R_G})_z^3/\bar{M}_w$ that is brought about by oxidation. This quantity is a kind of reciprocal density that must reflect the ease with which the molecules are able to fold up into a coil. The relatively small change in $(\overline{R_G})_z^3/\bar{M}_w$ that occurs upon reduction is also in agreement with the earlier findings of Smidsrød and Painter[2]. Space-filling molecular models show, fairly convincingly, that the three single bonds adjoining C-4, C-5, O-5, and C-1 of oxidised hexuronic-acid residues must indeed constitute sites of high flexibility in the molecules.

We suggest the following reasons for the apparent disagreement between these results and those of Scott *et al.*[4]. (*a*) The estimate by Scott *et al.*[4] of the molecular weight ($\bar{M}_w$) of their starting material ($\sim 2 \times 10^6$) is too high by a factor of ~ 10. Their reported value (18 dl/g) for the reduced viscosity (η_{sp}/c) at a concentration (c) of 1% w/v in 0.2M sodium chloride corresponds[13] to an intrinsic viscosity of ~ 4, which, in the same solvent, indicates[14] a $\bar{M}_w$ of $\sim 2 \times 10^5$. The reason for the high estimate obtained by light-scattering can be seen from the authors' Zimm diagrams. These indicate low, or even negative, second virial coefficients, which are typical of highly aggregated systems[14,15]. It is normal to obtain such aggregates when alginates are dissolved directly in aqueous salt solutions[15]; in our experience[14,15], the authors' method of clarification (centrifugation for 1 h at 35,000g) is insufficient to remove them. A detailed account of the requirements for obtaining molecularly disperse solutions has been published[15]. (*b*) Recognising that their solutions of 25% oxidised alginate contained aggregates, Scott *et al.*[4] made most of their measurements on borohydride-reduced material, apparently without recognising that reduction would entail depolymerisation. Their estimate of 1.1×10^{-5} for the $\bar{M}_w$ of this material is possibly more accurate than that obtained for the starting material, because the greatly diminished viscosity of the solutions would make them easier to clarify.

EXPERIMENTAL

Sodium alginate was prepared[16] from *Laminaria digitata*, and had an M/G ratio[17] of 1.6. Samples were oxidised to an extent of 26% by mixing aqueous solutions (0.78% w/v; 20 ml) with 1-propanol (4 ml) and 0.25M sodium metaperiodate (0.8 ml), and keeping the mixtures in the dark at 4° for 24 h. Ethylene glycol (1 ml) was then added to reduce any residual periodate, and the solutions were dialysed exhaustively against 0.1M sodium chloride for 4–5 days. Previous work[1,2] had shown that, under these conditions, no oxidised alginate is lost through the membrane. The solutions were diluted as required with 0.1M sodium chloride. Reduced, 26% oxidised samples were prepared by adding sodium borohydride (1 g) instead of ethylene glycol, and neutralising the mixtures with glacial acetic acid prior to dialysis.

Light-scattering experiments were carried out with the equipment and methods described earlier[14,15], except that the solutions were clarified by ultracentrifugation for 2 h at 220,000 *g*, instead of for 4 h at 144,000 *g*. Calculation of molecular parameters was carried out with a computer program[14,15]. Intrinsic viscosities were determined as described elsewhere[18].

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