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CONTROL OF DEPOLYMERISATION DURING THE PREPARATION OF REDUCED DIALDEHYDE CELLULOSE*

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ABSTRACT

The severe depolymerisation that occurs when cellulose is oxidised by periodate can be greatly diminished by carrying out the oxidation in steps, with borohydride reduction after each, and by using propan-1-ol as a radical scavenger in the oxidation steps. The reduction steps greatly increase the rate of Malapradian oxidation by eliminating hemiacetal structures and increasing the solubility of the substrate. They also minimise free-radical-mediated depolymerisation, and prevent the over-oxidation (stepwise peeling) that otherwise ensues at the newly liberated reducing end-groups. The use of regenerated cellulose or mercerised cellulose in order to increase accessibility is also advantageous. Depolymerisation by β-elimination in the reduction steps can be minimised by using very concentrated aqueous sodium borohydride. By these methods, a sample of fully oxidised and reduced cellulose with $\overline{M}_n$ = 132,000 was prepared.

INTRODUCTION

Periodate oxycellulose ("dialdehyde cellulose") and its soluble reduction product (RDC), which may be regarded as a stereoregular polymer of 2,3-*O*-(2-hydroxyethylidene)erythritol, are not found on the commercial market. Industrial cellulose chemists are unanimous as to the main reason: it is hard to oxidise cellulose completely with periodate without depolymerising it so severely that the product shows none of the useful properties of polymers. Recent work by Casu's group indicates, however, that RDC and its derivatives have properties that would be potentially very useful in a polymer of high molecular weight[1,2]. Probably because of its stereoregularity, RDC forms molecular aggregates in aqueous solution, and crystallises from aqueous methanol. The derived peracetate behaves similarly in organic solvents[2].

The present paper addresses the problem of depolymerisation, which is essentially one of increasing the rate of Malapradian oxidation (glycol cleavage) *relative* to the rate of non-specific oxidation. An effort is made to identify the factors in-

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fluencing the rates of the two kinds of oxidation, and to show how they may be controlled. The problem of depolymerisation under the alkaline conditions of borohydride reduction of the oxycellulose is also considered.

EXPERIMENTAL

Materials. — The starting materials were cotton wool (surgical grade), Visking cellophane dialysis casings (regenerated cellulose), Avicell PH-101 microcrystalline cellulose, Schleicher and Schüll cellulose powder for column chromatography (purified wood cellulose), and maize cobs. Mercerised cotton was prepared by dispersing cotton wool in aqueous 23% (w/w) sodium hydroxide, stirring the suspension under nitrogen for 12 h at 20°, filtering, and washing with 0.1M acetic acid followed by water. The cellophane was chopped into small squares ($\sim 2 \times 2$ mm) before further treatment. Milled maize cobs were delignified by the chlorite procedure[3], and the resulting holocellulose was extracted exhaustively with 5M potassium hydroxide under nitrogen at 20°; the residual α-cellulose was collected by filtration and washed with 0.1M acetic acid followed by water. All materials were washed with 0.1M ethylenediaminetetra-acetic acid at pH 7, finally extracted with boiling water in a Soxhlet extractor for 24 h, and dried *in vacuo* over phosphoric oxide at 40°. All reagents were of Merck analytical grade. Standard solutions of sodium thiosulphate and sodium hydroxide were prepared from Merck ampoules.

Methods. — Procedural details for both analytical and preparative oxidations are given in previous papers[4-13]. The modifications adopted in the present study were necessitated by the insolubility of the substrate and the protracted time of reaction. The usual procedure of withdrawing samples for analysis from a single reaction vessel was abandoned because (*a*) the ratio of residual substrate to residual oxidant is changed by the sampling, *i.e.*, the samples are not true aliquots; and (*b*) repeated opening of the reaction vessel increases the risk of introducing impurities. The adopted procedure consisted in weighing numerous 50-mg samples of cellulose separately into clean, dry 100-mL Erlenmeyer flasks, all fitted with glass stoppers and covered with aluminium foil to exclude light. Aqueous propan-1-ol (6.25% v/v; 8 mL) was added to each flask, which was then stoppered and clamped into an oscillating platform in a 20° thermostatically controlled water-bath. After equilibration for 12 h, oxidation was started by adding freshly-prepared, 0.25M sodium metaperiodate (2 mL) to each flask. Since all measurements were made in triplicate, these additions were made at 5-min intervals, to allow time for separate titration of each sample after the desired period of reaction. Blanks, consisting of 50mM sodium metaperiodate in aqueous 5% (v/v) propan-1-ol (10 mL), were likewise prepared in triplicate for each data point, to correct for the spontaneous decomposition of periodate.

For measurement of the periodate consumption at any time, an ice-cold mixture of 0.5M sodium phosphate buffer (pH 7; 25 mL) and aqueous 30% potassium iodide (5 mL) was poured quickly, with swirling, into the appropriate flask, and the

liberated iodine was titrated rapidly with 0.02M sodium thiosulphate (starch as indicator). Formic acid was titrated in the usual way, after adding ethane-1,2-diol to reduce the excess of periodate[6,10,11]. The formaldehyde liberated upon re-oxidation of samples after borohydride reduction was determined by the chromotropic acid method[12], after using *myo*-inositol to reduce the excess of periodate, as described earlier[13].

The proportion of unoxidised D-glucose residues in samples of periodate-oxidised and borohydride-reduced cellulose was determined as described for the corresponding products from starch and glycogen[11].

The 20-MHz ^{13}C-n.m.r. spectra were recorded with a Varian CFT-20 spectrometer on solutions in D_2O, as described by Casu *et al.*[2].

RESULTS

Inital rate of Malapradian oxidation of cellulosic chains in solution. — This may be referred to as the "intrinsic rate of oxidation", unmodified by insolubility or crystallinity. Table I presents second-order rate coefficients, k_2, for a number of

TABLE I

INITIAL SECOND-ORDER RATE COEFFICIENTS FOR PERIODATE OXIDATION OF CELLULOSE AND SOLUBLE MODEL SUBSTANCES IN WATER

Material	*Temperature (degrees)*	*State*	k_2 *(L.mol.$^{-1}h^{-1}$)*
trans-Cyclohexane-1,2-diol[14]	25	solution, pH 4	14,800
Amylose[4]	20	solution[a]	225
Methyl 4-*O*-methyl-α-D-gluco-pyranoside[15]	25	solution, pH 6	144
Methyl 4-*O*-methyl-α-D-gluco-pyranosiduronate, sodium salt[15]	25	solution, pH 6	65
methyl ester[15]	25	solution, pH 6	40
Methyl 4-*O*-methyl-β-D-gluco-pyranoside[14]	25	solution, pH 4	67
Lupin-seed (1→4)-β-D-galactan[5-7]	20	solution[a]	47
RDC-55	20	turbid solution[a,b]	14
RDC-71	20	solution[a,b]	32
Maize-cob (1→4)-β-D-xylan[8]	20	turbid solution[a]	10
Rhodymenan (1→3/4)-β-D-xylan[5-7]	20	solution[a]	7.6
Lichenan (1→3/4)-β-D-glucan[5-7]	20	solution[a]	2.1
C-6-oxycellulose[9]	25	solution, 2M NaCl[a]	1.4
Maize-cob α-cellulose[c]	20	suspension[a,b]	0.41
Mercerised cotton	20	suspension[a,b]	0.28
Cellophane	20	film[a,b]	0.27
Wood cellulose[c]	20	suspension[a,b]	0.22
Microcrystalline cellulose[d]	20	suspension[a,b]	0.15[e]
Cotton linters	20	suspension[a,b]	0.13

[a]Unbuffered. [b]In aqueous 5% (v/v) propan-1-ol. [c]Contains small amounts of xylan. [d] $\overline{\text{d.p.}}_n$ = 124. [e]After an initial, rapid consumption of 0.035 mol of periodate per 162 g.

model substances. For the polymeric substrates, they represent initial rates of oxidation, unmodified by inter-residue hemiacetal formation[4-9,11,13]. Unfortunately, some of the rates were measured at 25° and others at 20°. A tentative comparison may nonetheless be made by using a value of ~8 kcal.mole^{-1} for the activation energy[16]; this implies that the rate at 25° is ~25% greater than that at 20°.

Lichenan, which is considered to be a β-(1→3)-linked polymer of cellotriose and cellotetraose units[17,18], is probably the best model for cellulose. Although it is insoluble in cold water[19], a suitably dilute solution (12.5mM) in hot water remains stable for several hours after cooling, thus allowing measurement of the initial rate of oxidation[6,7].

The second-best model is probably the soluble, sodium salt of C-6-oxycellulose, oxidised in 2M sodium chloride in order to minimise the electrostatic exclusion (Donnan equilibrium) effect[7,20]. The carboxylate anion is, however, conformationally larger than the hydroxymethyl group[21-23], and the importance of the size of the substituent at C-5 can be seen by comparing the rates for methyl 4-*O*-methyl-α-D-glucopyranosiduronate and methyl 4-*O*-methyl-α-D-glucopyranoside, and those for lichenan and the nearly homologous xylan, rhodymenan[24].

The best available estimate for the intrinsic rate of oxdation of cellulose is therefore ~2 L.mol.$^{-1}$h^{-1}. The results for amylose and the (1→4)-β-D-galactan from white lupin (*Lupinus albus*) seeds[25] show the effect of changing the substituents at O-1 and O-4, respectively, from equatorial to axial positions. The results for methyl 4-*O*-methyl-β-D-glucopyranoside and *trans*-cyclohexane-1,2-diol illustrate

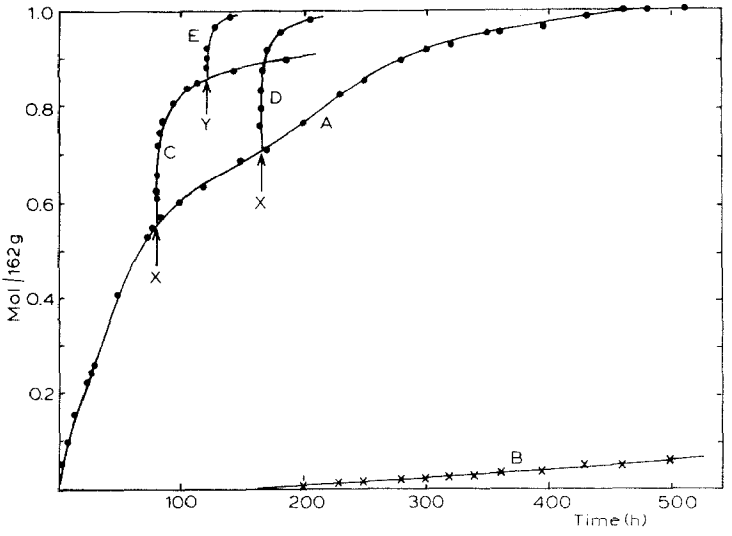

Fig. 1. Oxidation of mercerised cotton cellulose (30.9 mmol.L^{-1}) in 50mM sodium metaperiodate containing 5% (v/v) of propan-1-ol at 20°: A, consumption of periodate; B, release of formic acid; C and D, consumption of periodate after borohydride reduction at points X; E, consumption of periodate after borohydrate reduction at point Y.

the effect of the steric bulk of substituents flanking the *vic*-diol function.

Effect of insolubility and crystallinity upon the initial rate. — Insolubility decreases the initial rate of oxidation by about an order of magnitude (Table I). The Cellulose-II structure of mercerised cotton and of cellulose regenerated by the viscose process (cellophane) is oxidised about twice as fast as the Cellulose-I structure of unmodified cotton. However, the rate found for microcrystalline cellulose is probably higher than that of the microcrystallites in whole cotton, because of a significant contribution from end-groups (*cf.* a cognate study of xylan and xylose-containing oligosaccharides, which can be oxidised without complications due to over-oxidation[8]).

Changes in rate during oxidation. — Fig. 1 (curve A) shows the consumption of periodate by mercerised cotton, up to the expected oxidation-limit of 1.0 mol per "anhydroglucose" unit. The experimental accuracy was such as to justify drawing the curve in the way shown, with points of inflexion at degrees of oxidation (α) of ~ 0.25 and ~ 0.65, respectively. In Fig. 2, these features are further illustrated by plotting the second-order rate coefficient (k_2) against α. The first point of inflexion (at $\alpha \sim 0.25$) was probably associated with a change in the physical state (accessibility) of the cellulosic chains in the solid, while the second ($\alpha \sim 0.65$) was evidently due to the onset of over-oxidation, as judged by the release of formic acid (Fig. 1, curve B). Despite the temporary increases in k_2 brought about by these changes, it decreased from 0.28 L.mol.$^{-1}$h^{-1} at the start of the oxidation to ~ 0.01 L.mol.$^{-1}$h^{-1} at $\alpha = 1.0$.

Effect of borohydride reduction upon the rate. — At the points marked "X" in Fig. 1, samples of 55% and 71% oxidised cellulose were filtered off, washed, and dropped with stirring into aqueous 20% sodium borohydride at room temperature.

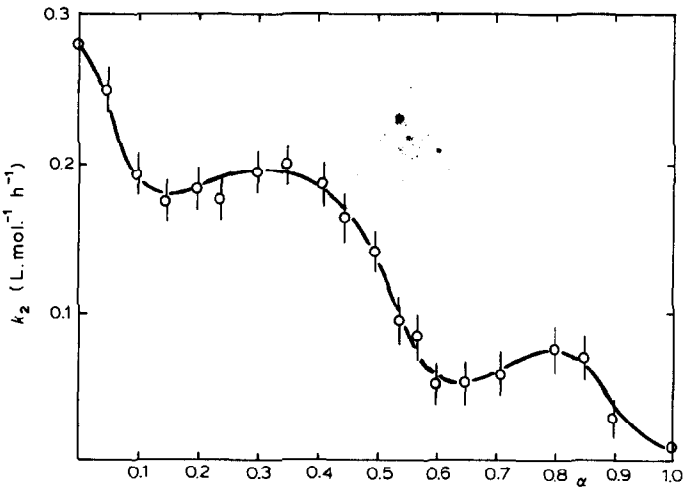

Fig. 2. Data of Fig. 1 (curve A), re-plotted as second-order rate coefficients (k_2) against the apparent degree of oxidation, α (expressed as mol of periodate consumed per 162 g).

The particles first swelled into a jelly, and then dissolved, to give a viscous solution. A 20% solution of borohydride was used because it had the same pH (~9.4 when freshly prepared) as a 2% solution, but reduced aldehydic groups ~9.5 times faster[26]. It was therefore to be expected that depolymerisation by base-catalysed β-elimination, which occurs as a reaction competitive with reduction, would be greatly diminished. This view is consistent with previous work on periodate-oxidised alginates[26,27].

The soluble products (RDC-55 and RDC-71, respectively) were then re-oxidised at 20° in 33 and 28mM periodate, respectively. These concentrations were chosen because they were equal to those remaining in the original reaction mixture (Fig. 1, curve A) at the times when the samples were isolated. The resulting curves (Fig. 1; curves C and D, respectively) therefore give an accurate impresssion of the increase in rate of oxidation brought about by borohydride reduction. In numerical terms, reduction of RDC-55 increased k_2 147-fold, from 0.095 to 14 L.mol.$^{-1}$h^{-1}, while reduction of RDC-71 increased k_2 552-fold, from 0.058 to 32 L.mol.$^{-1}$h^{-1} (see also Table I).

During the re-oxidation of RDC-55, k_2 again decreased, and became constant at ~0.4 L.mol.$^{-1}$h^{-1} at a total degree of oxidation of ~0.75. A second reduction at this point ("Y" in Fig. 1) then allowed the remaining unoxidised D-glucose residues to be re-oxidised at a nearly constant rate of ~35 ± 5 L.mol.$^{-1}$h^{-1} (Fig. 1, curve E). On the other hand, re-oxidation of RDC-71 proceeded smoothly to completion; no attempt was made to estimate the final rate in this case, because the accuracy becomes very low as the reaction nears completion.

Over-oxidation. — Although special precautions were taken to minimise free-radical depolymerisation and the ensuing over-oxidation that takes place from newly exposed, reducing end-groups[28,29] (see Experimental), there was significant release of formic acid during the first oxidation (Fig. 1, curve B). This was first detected after the periodate consumption (α) had reached 0.7 mol. The yield of formic acid had risen to 0.065 mol per "anhydroglucose" unit after 3 weeks, when α was exactly unity.

If this amount of formic acid had been formed entirely by Malapradian oxidation of end-groups (2- mol for every new reducing end-group and 1 mol for every new non-reducing end-group), ~2.2% of the glucopyranosidic bonds would have been cleaved, the $\overline{d.p.}_n$ of the product would have been ~45, and it would have contained ~4.4% of unoxidised D-glucose residues. In fact, the $\overline{d.p.}_n$ of the product (after borohydride reduction) was ~170, and it contained ~8% of unoxidised D-glucose residues (Table II). On the basis of the accepted mechanism for over-oxidation[29], these results indicate that, on an average, about three contiguous D-glucose residues had been eroded for every new reducing end-group that had been exposed by free-radical-mediated depolymerisation up to the time when the reaction was stopped.

There was no significant release of formic acid during the re-oxidation of any of the borohydride-reduced samples.

TABLE II

COMPOSITIONS AND MOLECULAR WEIGHTS OF REDUCED DIALDEHYDE CELLULOSES.

Material	*Unoxidised* D-*glucose residues* (%)	$\overline{M}_n$ (x 10^{-3})
RDC-55	45 ±2	155
RDC-71	30 ±2	143
RDC-100	8 ±2	28
RDC-55-75	24 ±2	110
RDC-55-75-100	<2	99
RDC-71-100	<2	132

Characterisation of reduced dialdehyde celluloses. — Data on composition and molecular weight are presented in Table II. The system of notation in the left-hand column is self-explanatory, but it should be emphasised that the numerals represent apparent degrees of oxidation, based on measurement of periodate consumed. The true degrees of oxidation, based on measurement of the D-glucose liberated upon acid hydrolysis[11], are shown in the second column.

Because of the strong tendency of RDC's to form aggregates in aqueous solution[2], there was no possibility of using a physical method to measure their true molecular weights. This was therefore done simply by measuring the formaldehyde liberated upon periodate oxidation[13], and assuming that 1 mol. of formaldehyde would be liberated by each RDC molecule. This assumption is based upon the much higher rate of oxidation of both reducing and non-reducing end-groups, compared to internal units in the chain[8], and the fact that the rate-determining step in over-oxidation is hydrolysis of the 3-*O*-formyl ester of a 2-*O*-glycosyl-D-erythrose intermediate[28,29]. The importance of using *myo*-inositol to reduce excess of periodate before measuring the liberated formaldehyde should be re-emphasised[13].

The ^{13}C-n.m.r. spectra of RDC-55, RDC-71, and RDC-100 are shown in Fig. 3. The signals due to unoxidised D-glucose residues are easily identified, and could be used as an alternative means of detecting and quantifying these units. Other important characteristics of fully oxidised RDC are reported by Casu *et al.*[2].

DISCUSSION

Ever since Jackson and Hudson published their first, pioneering paper[30], it has been evident that the periodate oxidation of cellulose is complicated to an exceptional degree[30,31] by side-reactions. This is to be expected, because the very low intrinsic rate of oxidation, together with the effects of insolubility, crystallinity, and inter-residue hemiacetal formation, make the side-reactions relatively more significant.

The side-reactions are of two main kinds. The first is the well-known erosion ("overoxidation") of the chains from their reducing ends, entailing hydroxylation of

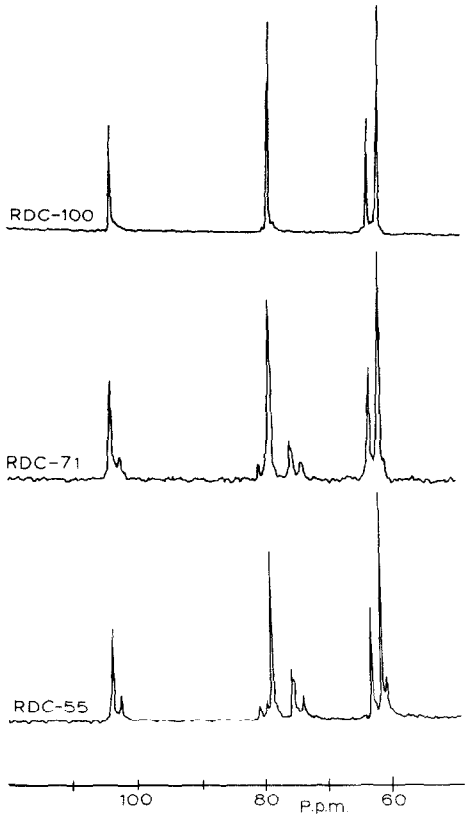

Fig. 3. Proton-decoupled ^{13}C-n.m.r. spectra (20 MHz, D_2O) of reduced dialdehyde celluloses (RDC). Chemical shifts are referred to internal methanol (50.04 p.p.m. from external Me_4Si).

the reactive methine groups of glycosyloxymalonaldehyde intermediates[29]. If this were the only kind of depolymerisation to occur during periodate oxidation, it would not be a serious obstacle to the preparation of high-molecular-weight RDC's from native celluloses, which typically have a $\overline{d.p.}_n$ of 2000 or more.

The second kind of side-reaction consists in the scission of internal linkages in the chains. It is caused by the spontaneous decomposition of periodate in solution, with the formation of hydroxyl radicals[32,33]. This is strongly catalysed by light[32], but it also occurs, very slowly, in the dark[33]. Decomposition in the dark is catalysed by impurities, including Fe^{2+} ions[32], polyphenols (tannins)[34], lignin residues, and protein[35]. Hydroxyl radicals are the principal reactive species in the depolymerisation of polysaccharides by hydrogen peroxide in the presence of Fe^{3+} ions (Fenton's reagent)[36,37], by molecular oxygen in the presence of polyphenols, ascorbic acid, or other autoxidisable reducing compounds[38,39], and by ionising radiation[40,41].

Direct evidence that hydroxyl radicals also cause depolymerisation under the conditions of periodate oxidation has been obtained by showing that propan-1-ol,

which is a good scavenger for hydroxyl radicals[37], inhibits depolymerisation and the ensuing, enhanced rate of over-oxidation, in the periodate oxidation of alginate[27,34,41], starch, and glycogen[11,35]. Periodate also induces viscosity decreases in polyethylene oxide, which should be resistant to Malapradian oxidation[42]. The Schardinger α-dextrin (cyclomaltohexaose) is also over-oxidised, with the release of formaldehyde, despite the initial absence of any reducing end-group from which this could occur[43].

The depolymerisation consists, in the first instance, in the cleavage of glycosidic linkages, with the liberation of new, reducing end-groups[36–41,44]. Subsequently, the latter may be modified to aldonic acid units, which could then undergo Ruff degradation to a lower carbon sugar (D-arabinose from D-glucose)[44]. These secondary reactions would, however, be slow compared to the rate of periodate oxidation of the new end-groups, and in any case they would not prevent over-oxidation.

Depolymerisation by hydroxyl radicals can be minimised (*a*) by retarding their formation by rigorous exclusion of light and use of very pure cellulose, water, and reagents; (*b*) by including a radical scavenger in the reaction mixture; propan-1-ol was preferred in this work, because it does not interfere with the determination of periodate, formic acid, or formaldehyde; a buffer composed of sodium propionate and propionic acid is nearly twice as effective as propan-1-ol (molar basis), but it prevents the titration of liberated formic acid; (*c*) by periodically isolating and washing the insoluble, partially oxidised cellulose, and continuing the oxidation in a freshly prepared periodate solution; or (*d*) by increasing the rate of Malapradian oxidation in any possible way, so that it can be completed before the periodate solution becomes too old.

In the present work, all four strategies were adopted, but (*c*) and (*d*) were accomplished simultaneously by reducing the partially oxidised cellulose with borohydride, before continuing the oxidation in freshly prepared periodate solutions. The acceleratory effect of borohydride reduction is due partly to the solubilisation of the substrate, and partly to the elimination of both intra- and inter-molecular hemiacetal structures[4–11]. Very importantly, reduction of the aldehyde groups to primary alcoholic groups also prevents any subsequent over-oxidation, because it is then no longer possible for glycosyloxymalonaldehyde intermediates to be formed.

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