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KINETIC EVIDENCE FOR HEMIACETAL FORMATION DURING THE OXIDATION OF DEXTRAN IN AQUEOUS PERIODATE

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ABSTRACT

A kinetic analysis is described of the periodate oxidation of a dextran in which all the 93% of oxidisable D-glucose residues contained a 2,3,4-triol system. Measurements were made of the periodate consumed and the formic acid liberated by the dextran, the periodate consumed and the formaldehyde liberated by samples that had been partially oxidised and then reduced with sodium borohydride, and the glycerol and erythritol released from these samples by acid hydrolysis. Initially, the oxidisable D-glucose residues decayed according to second-order kinetics. After the first oxidative attack, ~40% of the singly oxidised residues very rapidly consumed a second mole of periodate, while the remainder consumed further periodate at about one-seventh of the rate of an intact D-glucose residue. Residues cleaved between positions 3 and 4 were generated 7.5 times faster than residues cleaved between positions 2 and 3, but the two kinds of singly oxidised residue subsequently decayed at similar rates. Towards the end of their reaction, the rate of decay of intact, oxidisable D-glucose residues declined in a way that was simply correlated with the proportion of doubly oxidised residues in the chains. A simple scheme is presented that explains these facts in terms of intra-residual hemiacetal formation by singly oxidised residues, and inter-residual hemiacetal formation between doubly oxidised residues and intact D-glucose residues adjacent to them in the chains.

INTRODUCTION

Yu and Bishop[1] observed that, when dextran was oxidised with periodic acid in methyl sulphoxide, it consumed only one mole of oxidant for every 1,6-linked D-glucose residue. After reduction of the product with sodium borohydride, acid hydrolysis yielded both glycerol and erythritol, and a similar oxidation of methyl β-L-arabinopyranoside afforded the hemiacetal **1**, identified as its crystalline acetate. These observations indicated that initial attack on the *trans-trans*-2,3,4-triol system in dextran was non-specific, and that a second attack was inhibited by spontaneous formation of the intra-residual hemiacetals **2** and **3**.

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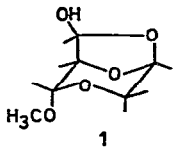

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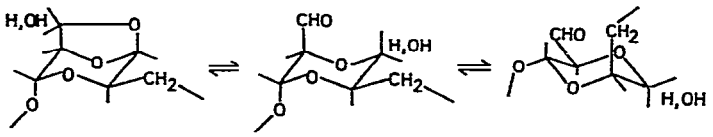

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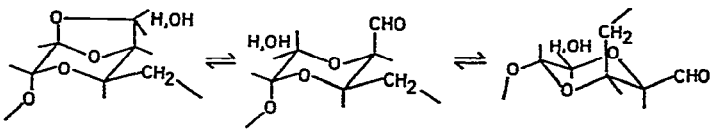

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We have investigated the possible formation of these and other hemiacetals in aqueous periodate, because of the importance of achieving complete oxidation in conventional, analytical oxidations of dextrans and other (1→6)-linked polysaccharides. One instance of a spuriously low oxidation-limit has already been reported for a dextran of very high molecular weight[2].

In principle, the required information could be obtained by n.m.r. spectroscopy of partially oxidised dextran in D_2O, but the number of different possible hemiacetal and hemialdal structures is formidably large, and it would be expected to vary with the degree of oxidation. The kinetic analysis now reported helps to simplify the problem, and provides a background for further work with n.m.r. and other methods.

EXPERIMENTAL

"Dextran 2000", having a weight-average molecular weight of $\sim 2 \times 10^6$, was supplied by Pharmacia Fine Chemicals AB, Uppsala, Sweden. It contained 0.42% of ash, which was corrected for, and was dried over phosphorus pentaoxide, *in vacuo* at 80°, before use. All reagents were of Merck analytical quality. Standard solutions were purchased in ampoules, and accepted as primary standards. The sodium metaperiodate was consistently ~99% pure by this criterion.

The analytical methods, and the method for preparing and reducing partially oxidised dextrans, were essentially as described for an earlier study of guaran[3], except that the volume of samples removed for titration of formic acid was increased to 25 ml. Analytical oxidations were carried out on 85-mg samples of dry dextran or reduced, partially oxidised dextran in 12.5mM sodium metaperiodate (200 ml) in the dark at 20.2°. The full course of the oxidation of dextran was studied by carrying

out a series of such oxidations in relays. Preparative oxidations were carried out under the same conditions as the analytical ones.

Samples (100 mg) of reduced, partially oxidised dextran were hydrolysed, in sealed tubes, in 0.25M sulphuric acid (2 ml) at 100° for 6 h. This was followed by neutralisation with barium carbonate, filtration, evaporation to dryness, and acetylation with acetic anhydride (2 ml) and dry pyridine (1 ml) at 80° for 1 h. In control experiments, artificial mixtures of erythritol and glycerol were treated similarly to convert chromatographic peak-area ratios into molar ratios. The inclusion of glycolaldehyde in these mixtures did not change the results.

The gas chromatograph was a Perkin–Elmer Model F11, coupled with a Model 165 recorder. Separation was effected on a stainless-steel column (2 m × 3 mm) filled with 1.5% Silicone XF-1150 and 1.5% poly(diethyleneglycol succinate) on acid-washed Chromosorb W (100–120 mesh). The flow-rate of nitrogen was 40 ml/min. A constant temperature of 110° was applied until glycerol triacetate was eluted, after which a linear gradient of 3.0°/min, up to 210°, was applied to elute erythritol tetra-acetate and α- and β-D-glucose penta-acetates. Samples were injected as solutions (1% w/v) in chloroform (1 μl). Peak areas were determined by weighing the peaks, excised from the paper.

RESULTS

The initial stages of the consumption of periodate (P_t) and the liberation of formic acid (F_t) by the dextran are shown in Fig. 1. Because the last part of the reaction was very slow, it is convenient to present the results for this part in tabular form, and this is done in Table I. The final consumption of periodate was 1.86 mol per D-glucose residue, and this yielded 0.93 mol of formic acid. All of the oxidisable residues therefore contained 2,3,4-triol systems.

Experiments were next carried out to determine whether the observed oxidation-limit was genuine, or spuriously low because of inter-residual hemiacetal formation[3,4]. Six samples of partially oxidised dextran were isolated after different periods of oxidation, reduced with sodium borohydride, and oxidised again. The results were corrected for the change in weight brought about by the release of formic acid in the first oxidation, and calculated on the basis of the intact D-glucose residues in the original dextran. They are shown, in part, in Fig. 1; in every case, a final oxidation-limit of 1.84 ±0.02 mol was indicated, in close agreement with the result obtained in the first oxidation.

The results for the second oxidations (Fig. 1) suggest that there was virtually instantaneous oxidation of D-glucose residues that had already suffered a single oxidative attack, and that this was followed by a much slower oxidation of the D-glucose residues that still remained intact in the samples. This view was confirmed by showing that the yield of formaldehyde in the second oxidation corresponded closely to the amount of rapidly consumed periodate (Table II). In addition, the

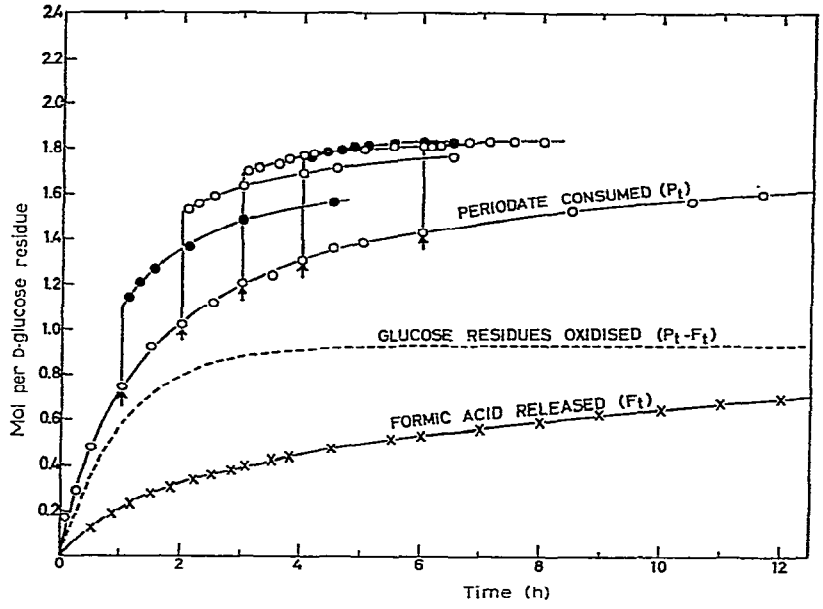

Fig. 1. Oxidation of dextran (5mM) in 12.5mM sodium metaperiodate at 20°. P_t and F_t are, respectively, the periodate consumed and the formic acid liberated at time t. At the points indicated by arrows, samples of partially oxidised dextran were isolated, reduced with borohydride, and oxidised again. The appended curves show periodate consumed by the samples.

initial slopes of the slow parts of the curves indicated a rate of oxidation similar to that of the original dextran.

Portions of the partially oxidised, borohydride-reduced samples were also hydrolysed with acid, and the products were acetylated and analysed for glycerol triacetate and erythritol tetra-acetate by g.l.c. The molar ratios (R) of the glycerol to the erythritol were too large for accurate measurement from the peak areas, but approximate values are given in Table II.

TABLE I

TERMINAL STAGES OF THE PERIODATE OXIDATION OF DEXTRAN[a]

t (h)	P_t	t (h)	F_t
14.67	1.67	14.00	0.733
16.67	1.69	16.00	0.766
18.67	1.71	26.00	0.844
19.67	1.72	36.00	0.930
21.67	1.73	38.30	0.930
23.67	1.76		
36.00	1.84		
38.30	1.85		
48.30	1.86		

[a]The experimental conditions and symbols are the same as for Fig. 1.

TABLE II

ANALYSIS[a] OF PARTIALLY OXIDISED DEXTRANS AFTER REDUCTION WITH BOROHYDRIDE

Time of first oxidation (h)	$(P_t - 2F_t)$ *in first oxidation*	IO_4^- *rapidly consumed*	*HCHO released*	*Molar ratio (R) Glyc/Ery*	R_{corr}[b]
1	0.306	0.338	0.356	13.4	7.4
2	0.425	0.435	0.427	15.2	8.0
3	0.448	0.460	0.420	17.1	8.4
4	0.425	0.415	0.410	16.4	7.3
5	0.392	0.390	0.380	17.8	7.3
6	0.371	0.370	0.370	18.6	7.1

[a]All quantities are calculated as mol per D-glucose residue in the original sample of unoxidised dextran. [b]Calculated from the formula $R_{corr} = [(P_t - 2F_t)R - F_t]/(P_t - F_t)$.

DISCUSSION

The numerical data provide a complete analysis of the composition of the reaction mixture at any time. Thus, P_t gives the concentration of periodate, F_t the mole fraction of doubly oxidised D-glucose residues, and $(P_t - 2F_t)$ the mole fraction of singly oxidised residues. The sum, $(P_t - F_t)$, which is the total fraction of D-glucose residues that have been oxidised at any time, is plotted in Fig. 1. An independent measure of the fraction of singly oxidised D-glucose residues is provided by the formaldehyde assays and the estimates of rapidly consumed periodate in the second oxidations, and the agreement with calculated values of $(P_t - 2F_t)$ is very good (Table II).

After correction for the glycerol originating from doubly oxidised D-glucose residues, the molar ratios of glycerol to erythritol (R_{corr} in Table II) indicate that residues cleaved between HO-3 and HO-4, and residues cleaved between HO-2 and HO-3, are generated in a ratio of $\sim 7.5:1$, respectively, and that they then undergo further oxidation at similar rates.

For the present purpose, the two most important quantities are P_t and $(P_t - F_t)$. By drawing tangents to the curve for P_t, and dividing their slopes by the concentration of residual periodate and the mole fraction of residual *vic*-diol groups $(2 - P_t)$, second-order rate-coefficients (k_p) for the consumption of periodate were calculated, and plotted against the degree of oxidation (Fig. 2, curve A). Similarly, slopes of tangents to the curve for $(P_t - F_t)$ were measured, and divided by the concentration of residual periodate and by $2[1 - (P_t - F_t)]$, to give second-order rate-coefficients (k_G) describing the decay of intact, oxidisable D-glucose residues. These are also plotted in Fig. 2 (curve B).

Despite the considerable loss of accuracy that is involved in drawing tangents, the steady decline in k_G with increasing degree of oxidation appeared to be significant, and an attempt was therefore made to correlate it with some other quantity that had been measured. It was found that the equation

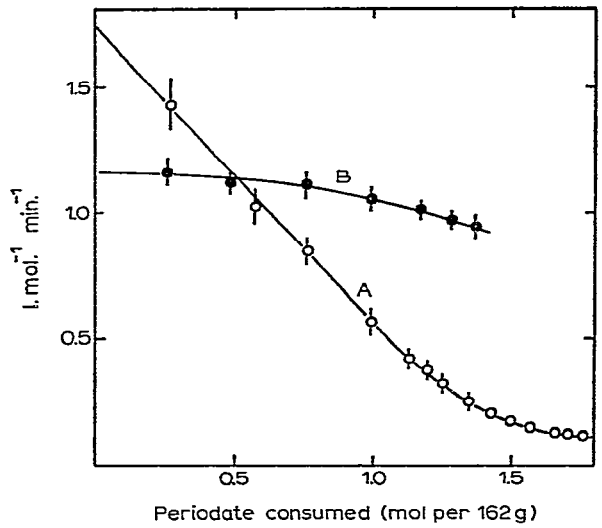

Fig. 2. Data from Fig. 1, re-plotted as second-order rate coefficients against the degree of oxidation. Curve A is the rate of consumption of periodate (k_P), and curve B the rate of decay of intact D-glucose residues (k_G).

$$k_G = 1.17(1 - 0.4\,F_t)\ \text{l.mol}^{-1}.\text{min}^{-1}$$

accounted reasonably well for the changes in k_G.

The dramatic decrease in k_p, during the initial period when k_G is changing very little (Fig. 2), clearly implies formation of the intra-residual hemiacetals **2** and **3**, provided one can assume that the singly oxidised residues, in their acyclic forms, are oxidised very rapidly, as expected from their behaviour after reduction (Fig. 1). Two facts must be noted: (*i*) the initial rate of consumption of periodate is ~40% higher than the initial rate of decay of intact D-glucose residues (Fig. 2); and (*ii*) the curve (F_t) for the liberation of formic acid (Fig. 1) does not show an induction period.

From a consideration of the theory of consecutive reactions[5], it is possible to appreciate that this situation can only come about when the rate of a second step is vastly greater than that of the first. We accordingly suggest that it is only possible to explain all of the facts in terms of the general reaction scheme shown in Fig. 3. The essential feature of this scheme is that, after the first oxidative attack, a singly oxidised residue subsequently reacts by one of two competing pathways, both of which are very fast.

(*a*) Ring-closure to give an unoxidisable, intra-residual hemiacetal. The possibility that a periodate ion may be involved in an unreactive complex with this hemiacetal should perhaps not be overlooked. The hemiacetal eventually reaches a state of equilibrium with the rapidly oxidisable, acyclic form, and attainment of the correct, Malapradian oxidation-limit is only possible because a minute amount of this form is always present at equilibrium.

(*b*) Consumption of a second mole of periodate before the equilibrium condition is reached.

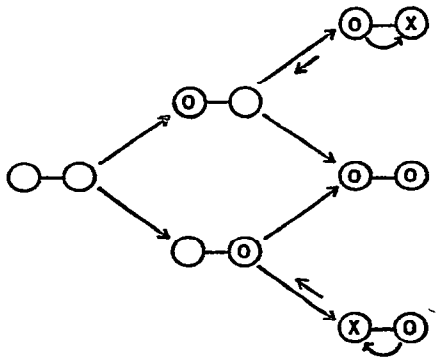

Fig. 3. Schematic representation of the periodate oxidation of an alicyclic *vic*-triol. Pairs of circles represent the two adjacent, oxidisable sites; "O" signifies that a site has been oxidised; "X" represents a site that is protected from oxidation by hemiacetal formation; and curved arrows represent hemiacetal rings.

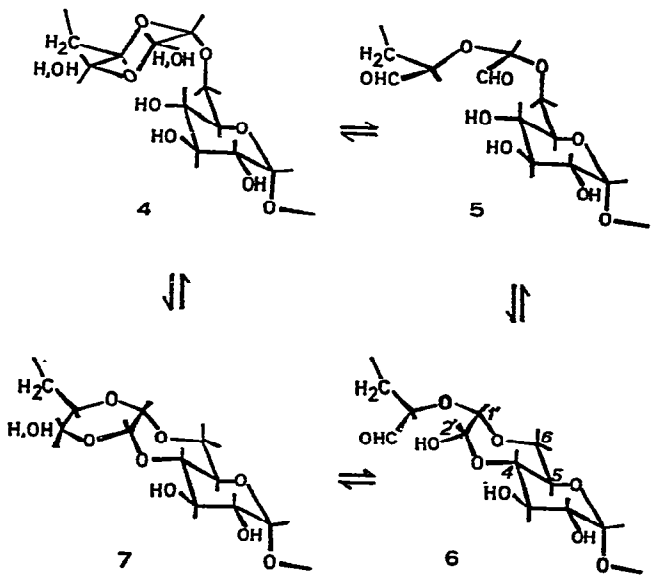

Fig. 4. Suggested explanation for the correlation between the rate of decay of intact D-glucose residues (k_G) and the proportion of doubly oxidised D-glucose residues (F_t) in the chains. It is postulated that the two kinds of residue have to be adjacent, as in **5**, and that oxidation between HO-3 and HO-4 of the intact residue is inhibited by formation of **6** and **7**. The dioxepane ring in **6** and **7** is shown in the $^{5,4}TC_{O,6}$ conformation, with substituents at C'-2 isoclinal to the reference plane.

Our interpretation (Fig. 4) of the decline in k_G as the fraction of doubly oxidised residues increases is necessarily more tentative, since it does not rest upon the firm identification of a model hemiacetal corresponding to **1**. We have, however, reported other evidence[4] to show that seven-membered hemiacetal rings exist to a significant extent in aqueous solution when there is no possibility for the competitive

formation of a six-membered hemiacetal by the same aldehyde group*. On the other hand, when there is a possibility for forming a six-membered hemialdal, such as **4**, in apparent competition with a seven-membered hemiacetal, the latter is still detectable[4]. This may be because hemialdals are fundamentally unstable in water (*cf.* Ref. 8), but it should be noted that both rings could be freely incorporated into a composite structure such as **7**.

Fig. 4 accordingly shows formation of a seven-membered, inter-residual hemiacetal between the aldehyde group derived from C-2 of a doubly oxidised D-glucose residue, and HO-4 of an intact D-glucose residue adjacent to it in the chain. This would block the more reactive of the latter's two oxidisable sites. The proposed structure is conformationally plausible, with the 1,4-dioxepane ring as a twist-chair, and bulky substituents either equatorial or isoclinal. Formation of a similar hemiacetal between the aldehyde group derived from C-4 of a doubly oxidised residue and HO-2 of an intact one is less likely, because the two rings would be *cis*-fused, and encounter a severe "H-inside" interaction.

None of the hemiacetals considered here is sufficiently stable to give rise to an absolutely anomalous periodate-oxidation limit. The different results reported by Yu and Bishop[1] for oxidation in methyl sulphoxide must reflect the inability of this aprotic solvent to stabilise the oxidisable, acyclic forms of the singly oxidised residues by solvation of the free aldehydic groups (*cf.* Ref. 8). The same effect must also enhance the rate of cyclisation, relative to the rate of oxidation of the acyclic forms, in order to give the observed oxidation-limit of 1.0 mol of periodate consumed[1].

The present results do not help to explain the spuriously low limit reported by Leonard and Richards[2] for oxidation, in water, of a dextran of very high molecular weight. These authors associated the phenomenon with an observed tendency for the dextran to exist in solution as aggregates. Such a tendency might not only modify the reactivity of the D-glucose residues, but would also introduce the possibility of intermolecular hemiacetal formation.

ACKNOWLEDGMENT

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