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Details of the fine structure of nigeran revealed by the kinetics of its oxidation by periodate

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ABSTRACT

A theoretical analysis of the kinetics of periodate oxidation of nigeran from *Aspergillus niger* revealed that, whereas 78% of the 4-linked D-glucopyranosyl residues were present as isolated singlets (with 3-linked residues in both neighbouring positions), the remaining 22% were present as isolated doublets. Sequences of more than two contiguous 4-linked residues were not detected.

INTRODUCTION

Whereas the Barry[1] and Smith[2] degradations can be used to determine the sequence-length distribution of periodate-resistant (3-linked) D-glucopyranosyl residues in such polysaccharides as nigeran [3,4], they provide no direct information about the sequence-length distribution of the periodate-oxidisable (4-linked) residues. The missing information can be obtained by studying the *shape* of the periodate-oxidation curve in addition to the final oxidation-limit usually measured.

In unbuffered 5mM sodium metaperiodate at 20°, oxidation of any D-glucopyranosyl residue in amylose diminishes the reactivity of one of its unoxidised neighbours to 9% and the reactivity of the other to 37%, of its initial value (k), where k is a second-order rate coefficient. Such residues are referred to as "singly inhibited", and their rates of oxidation may be defined as $A_L k$ and $A_R k$, respectively, where A_L (0.09) and A_R (0.37) are "reactivity coefficients". When an unoxidised residue is flanked on both sides by oxidised residues, it becomes "doubly inhibited", and its reactivity is then $A_L \times A_R$, *i.e.*, 3.33%, of its initial value (k)[5-7].

The phenomenon is due to inter-residual formation of hemiacetals, which has also been observed with many other polysaccharides[8-15]. The effect manifests itself as an apparent, extreme deviation from second-order kinetics, which are invariably obeyed by simple monomeric *vic*-diols[16], and also by amylose after it has been oxidised until only isolated (doubly inhibited) unoxidised residues remain[5].

Thus, the *shapes* of the reaction curves for such polysaccharides as nigeran should be profoundly sensitive to the sequential arrangement of the oxidisable, 4-linked D-glucopyranosyl residues. Isolated, oxidisable residues ("singlets") should consume periodate in strict accordance with second-order kinetics; isolated doublets should give rise only to singly inhibited, oxidisable residues, and hence to an apparent, moderate

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deviation from second-order kinetics; and multiplets larger than doublets should give rise to doubly as well as singly inhibited, oxidisable residues, and hence to an apparent, extreme deviation from second-order kinetics, similar to that observed for amylose[5].

EXPERIMENTAL

Material. — Nigeran (1 g; Koch–Light, from *Aspergillus niger* strain 152[3]) was dispersed in boiling water (300 mL) for 10 min. The solution was cooled to 80°, and stirred thereat whilst sodium chlorite (3 g) was added, followed by glacial acetic acid (3 mL), dropwise, during 10 min. After 2 h, sodium hydrogen sulphite (5 g) was added, and the mixture was cooled, dialysed exhaustively against distilled water, frozen, and thawed. The precipitated nigeran was collected by centrifugation, washed in sequence with ice-cold water, ethanol, and ether, then air-dried. The product was free from nitrogen and ash.

Methods. — The experimental methods have been described[5]. The number-average d.p. of the nigeran was determined by titration[11-15] of the formic acid liberated from end-groups after 12.5 h of oxidation, and by determination of the formaldehyde liberated by periodate from end-groups after reduction with sodium borohydride[11,13,15]. Values of 235 and 242 were obtained by the two methods, respectively.

RESULTS

Fig. 1 shows the consumption of periodate by nigeran (0.426 mol per "anhydroglucose" unit after 12.5 h and 0.427 mol after 24 h). The latter value was taken as the oxidation limit and used in a plot of $[1/(a-b)]\ln[b(a-x)/a(b-x)]$ against time. The results (Fig. 2) show that the second-order rate coefficient started at 265 $\pm$20

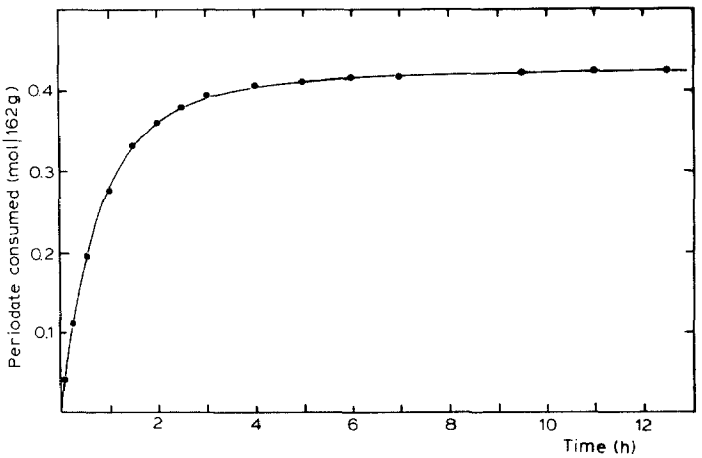


Fig. 1. Oxidation of nigeran (3mM) in 5mM sodium metaperiodate at 20°. The curve drawn through the data points is the best fit provided by equation *1*.

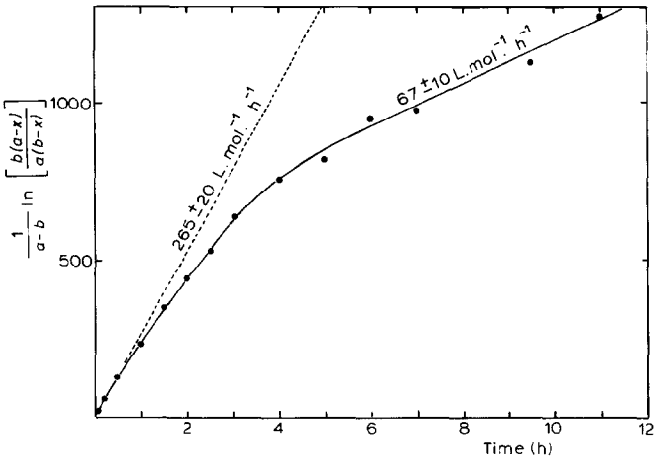


Fig. 2. Data of Fig. 1, re-plotted according to second-order kinetics.

$L.mol^{-1}.h^{-1}$, and decreased steadily, becoming constant at 67 ± 10 $L.mol^{-1}.h^{-1}$ after 5 h.

This result implies that, after 5 h, all of the remaining reactive sites in the molecule were identical and were oxidised at approximately 25% of the initial rate. This finding is in contrast to the behaviour of amylose, for which the final rate was <4% of the initial rate[5], and it may be inferred that the nigeran did not contain groups of three or more consecutive, oxidisable D-glucopyranosyl residues. Therefore, it should be possible to match the curve in Fig. 1 with a theoretical model which assumes the presence of oxidisable "singlets" and "doublets" only.

THEORY

Let the nigeran contain a fraction n_1 of oxidisable singlets and a fraction n_2 of oxidisable doublets. The total fraction of oxidisable units is then $n_1 + 2n_2 = 0.427$. Let the singlets be oxidised at a rate k_1. These units will be 4-substituted and linked to an unoxidisable D-glucopyranosyl residue at position 3. In the doublets, one of the units will also be linked in this way, and therefore should be oxidised also at the rate k_1. The other unit will be linked in the same way as the units in amylose, and is assumed to be oxidised at a different rate, k. Let the periodate concentration at any time be P, and let unreacted and reacted units be denoted by ○ and ⊕ respectively.

Then for singlets $$\frac{d}{dt} n_1(\bigcirc) = -k_1 P n_1(\bigcirc)$$

and for doublets $$\frac{d}{dt} n_2(\bigcirc\bigcirc) = -(k + k_1) P n_2(\bigcirc\bigcirc)$$

$$\frac{d}{dt}n_2(\oplus\bigcirc) = kPn_2(\bigcirc\bigcirc) - k_1PA_Rn_2(\oplus\bigcirc)$$

$$\frac{d}{dt}n_2(\bigcirc\oplus) = k_1Pn_2(\bigcirc\bigcirc) - kPA_Ln_2(\bigcirc\oplus)$$

$$\frac{d}{dt}n_2(\oplus\oplus) = k_1PA_Rn_2(\oplus\bigcirc) + kPA_Ln_2(\bigcirc\oplus)$$

The solutions to these equations are

$$n_1(\bigcirc) = n_1[\exp - k_1Pt];\ n_1(\oplus) = n_1[1 - \exp(-k_1Pt)]$$

$$n_2(\bigcirc\bigcirc) = n_2[\exp - (k + k_1)Pt]$$

$$n_2(\oplus\bigcirc) = \frac{n_2k}{k_1 - A_Rk_1 + k}\left[[\exp - A_Rk_1Pt] - [\exp - (k + k_1)Pt]\right]$$

$$n_2(\bigcirc\oplus) = \frac{n_2k_1}{k_1 - A_Lk + k}\left[[\exp - A_LkPt] - [\exp - (k + k_1)Pt]\right]$$

$$n_2(\oplus\oplus) = n_2 - \frac{n_2k}{k_1 - A_Rk_1 + k}[\exp - A_Rk_1Pt] - \frac{n_2k_1}{k_1 - A_Lk + k}[\exp - A_LkPt]$$
$$+ n_2\left[\frac{k}{k_1 - A_Rk_1 + k} + \frac{k_1}{k_1 - A_Lk + k} - 1\right][\exp - (k + k_1)Pt]$$

The degree of oxidation (Q), therefore, is given by

$$Q = n_1(\oplus) + n_2(\oplus\bigcirc) + n_2(\bigcirc\oplus) + 2n_2(\oplus\oplus)$$
$$= n_1 + 2n_2 - n_1[\exp - k_1Pt]$$
$$- \frac{n_2k}{k_1 - A_Rk_1 + k}[\exp - A_Rk_1Pt] - \frac{n_2k_1}{k_1 - A_Lk + k}[\exp - A_LkPt]$$
$$+ n_2\left[\frac{k}{k_1 - A_Rk_1 + k} + \frac{k_1}{k_1 - A_Lk + k} - 2\right][\exp - (k + k_1)Pt] \qquad (1)$$

In the experiment, the concentration of periodate (P) was initially 5mM and finally 5mM − (0.427 × 3mM) = 3.72 mM. This change was taken into account by computing the increments in Q corresponding to successive, small increments in time, and adjusting the value of P [= (5 − 3Q)mM] after each. The procedure has been described[7].

When matching equation *1* to the experimental curve, time and effort were saved by starting out tentatively from the following premises, which proved to be accurate: (*a*) the value of k should be the same as for amylose[5-7], namely 225 ± 12 L.mol^{-1}.h^{-1}; (*b*) since most of the oxidisable D-glucose residues in nigeran are isolated singlets[3,4] (*i.e.*, since $n_1 \gg n_2$), k_1 should be only slightly greater than the initial rate of oxidation of the whole

molecule, namely, 265 $\pm$20 L.mol^{-1}.h^{-1} (Fig. 2); (*c*) since the formation of inter-residue hemiacetals occurs (in solution) between nearest neighbours only[5-15], and since the immediate environment of one of the D-glucopyranosyl residues in each oxidisable doublet is identical with that of a D-glucopyranosyl residue in an amylosic chain, one of the reactivity coefficients (A_L or A_R) should be the same as for amylose (0.09 or 0.37); (*d*) the *smaller* of the two reactivity coefficients should be approximately equal to the ratio of the final to the initial rates, namely 0.25 $\pm$0.05 (Fig. 2).

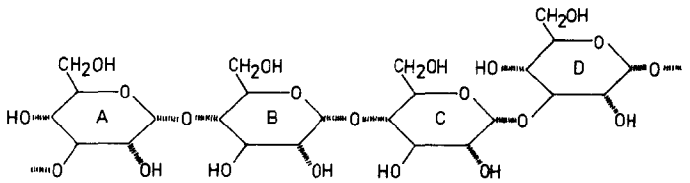
The curve drawn through the experimental points in Fig. 1 was obtained by substituting the following values for the parameters in equation *1*:

n_1	= 0.335	n_2	= 0.046
k_1	= 270 L.mol^{-1}.h^{-1}	k	= 225 L.mol^{-1}.h^{-1}
A_L	= 0.30	A_R	= 0.37

The proportion of oxidisable units occurring in doublets is therefore $2n_2/(n_1 + 2n_2)$ = 21.5%, which corresponds to 9.2% of all the D-glucose residues in the nigeran.

DISCUSSION

In addition to the detection and determination of oxidisable doublets, the present analysis provides an insight into the preferred sites for the formation of hemiacetals. When the structure is drawn in the usual way (**1**), the unit B to the left of an oxidisable doublet is the one that resembles a unit in amylose. In each polysaccharide, the aldehyde group originating from C-3 of unit B can form either a five-membered hemiacetal ring with HO-6 of the same unit (to give a derivative of D-erythrofuranose), or a six-membered one with HO-2 of the unoxidised unit A. Likewise, the aldehyde group originating from C-2 of unit B can form a six-membered hemiacetal ring, either with HO-6 of the same unit, or with HO-3 of the unoxidised unit C. It must be this last hemiacetal that gives rise to the same reactivity coefficient, A_R, in both nigeran and amylose.



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An explanation for the higher value of A_L found for nigeran (0.30) than for amylose[5-7] (0.09) must be sought in the (1→3) linkage between units C and D. The aldehyde group originating from C-2 of unit C can, as in amylose, still form a six-membered hemiacetal ring with HO-6 of the same unit, but it can also form six-

membered hemiacetal rings with HO-2 and HO-4 of unit D. This possibility of forming two, instead of one, inter-residue hemiacetals probably shifts the position of the equilibrium away from the intra-residue hemiacetal. As a result, the aldehyde group originating from C-3 of unit C should spend more of its time linked to HO-6 of the same unit, and less of its time linked to HO-2 of the unoxidised unit B. Hence, A_L should increase.

On the basis of this rationale, it can now be specified that the subscripts in the symbols A_L and A_R signify "left" and "right", respectively, and that they refer to the direction of the nearest-neighbour, auto-inhibitory effect along the chain.

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