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Preliminary communication

Possibilities for selective Smith-degradation

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The Smith degradation[1] continues to find extensive application in the structural elucidation of complex heteropolysaccharides and glycoconjugates[2,3]. The initial, periodate-oxidation step is normally carried out under conditions that are expected to cleave all the vicinal diol groups in the material. Recently, however, Ebisu *et al.*[4] found that, after a limited period of oxidation, the β-D-galactopyranosyl side-groups in the *Pneumococcus* S-14 polysaccharide could be selectively removed by Smith degradation, despite the presence of 1,4-linked β-D-glucopyranose residues in the main chain. With a much longer period of oxidation, the latter residues could also be cleaved, thus permitting a controlled, stepwise degradation of the molecule[4]. Selective periodate oxidation of 1,4-linked β-D-galactopyranose residues in the presence of similarly linked β-D-glucopyranose residues has also been observed for two other bacterial polysaccharides[5,6].

In recent years, we have used kinetic methods to study the hemiacetals that are formed during the periodate oxidation of polysaccharides[7–19] and simple glycopyranosides[20], and have collected relevant information about the initial rates of oxidation of a considerable number of model substances. So far, numerical values for the second-order rate coefficients have been published only for maize-cob xylan[8], amylose[9], and dextran[17]. We are therefore prompted to publish all the rate coefficients that we have measured, and these are now collected in Tables I and II.

Unless otherwise stated, the results refer to oxidation in unbuffered, aqueous sodium metaperiodate at 20°. The estimated limits of error vary considerably, and reflect such practical difficulties as overoxidation[21,22] and other end-group effects in the shorter-chained polysaccharides, and the presence of ~6–8% of neutral sugar residues in the pectates.

For dextran (Table I) and the methyl glycopyranosides (Table II), the presence of two adjacent, oxidisable sites in the pyranoid rings implies that the kinetics are of the competitive, consecutive, second-order type[23], and are therefore rather complex[17,23]. For this reason, the liberation of formic acid (*F*) was measured as well as the consumption of periodate (*P*). This permitted calculation of the mole fraction ($1 - P + F$) of unreacted

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TABLE I

INITIAL, SECOND-ORDER RATE COEFFICIENTS FOR PERIODATE OXIDATION OF MODEL POLYSACCHARIDES

Material	k ($L mol^{-1}.min^{-1}$)	*References*
Amylose	3.75 ±0.3	9,10,11
Nigeran	4.50 ±0.2	12,18
Isolichenan	4.50 ±0.2	12
Maize-cob xylan	0.166 ±0.015	8
Rhodymenan	0.127 ±0.008	12
Lichenan	0.035 ±0.005	12
C-6-Oxycellulose,		
in H_2O at 25°	0.0055 ±0.0005	12,16
in 2M NaCl at 25°	0.023 ±0.005	12,16
Chitosan, pH 5 at 0°	2.75 ±0.08	12
Chitosan, pH 4 at 0°	1.15 ±0.08	12
Chitosan, pH 3 at 0°	0.063 ±0.006	12
Mannuronan[a],		
in H_2O	0.86 ±0.15	7,14
in 40mM $MgCl_2$	7.0 ±2.0	7,14
Guluronan[a],		
in H_2O	1.25 ±0.15	7,14
in 40mM $MgCl_2$	10.3 ±2.0	7,14
Sodium pectate,		
in 2M NaCl at 25°	0.9 ±0.2	18
Methyl pectate,		
in H_2O at 25°	4.2 ±0.5	18
Guaran,		
branched mannose residues	7.8 ±0.4	13,19
unbranched mannose residues	11.4 ±0.4	13,19
Lupin-seed galactan	0.78 ±0.05	12
Inulin	1.13 ±0.05	12
Sugar-beet arabinan	2.05 ±0.20	12
Galactocarolose	1.5 ±0.20	12
Dextran	1.17 ±0.02	12,17

[a]Isolated from alginic acid[36].

glycoside remaining at any time and, hence, the second-order rate coefficient that describes the decay of the intact pyranoid rings[17]. These are the quantities reported for dextran and the methyl glycopyranosides, and they allow direct comparison with the other data.

The following, main conclusions are drawn. (*a*) For uronic acid-containing polysaccharides, there should be considerable scope for introducing versatility into periodate oxidations by varying the ionic strength of the reaction mixture. This is because inorganic salts diminish the mutual electrostatic repulsion between the uronate anion and the attacking periodate ion[24]. The phenomenon can be formally regarded as a Donnan effect[25], or, more generally, as a primary salt effect, which is always large for reactions between two ionic species, even when both are monomeric[26]. We have used sodium chloride and

TABLE II

SECOND-ORDER RATE COEFFICIENTS FOR PERIODATE OXIDATION OF METHYL GLYCOPYRANOSIDES[20]

Glycoside	k ($l.mol^{-1}.min^{-1}$)
Methyl α-D-glucopyranoside	1.34
Methyl β-D-glucopyranoside	0.79
Methyl α-D-mannopyranoside	6.22
Methyl β-D-mannopyranoside	10.1
Methyl α-D-galactopyranoside	9.79
Methyl β-D-galactopyranoside	12.3
Methyl α-L-rhamnopyranoside	5.86
Methyl α-D-arabinopyranoside	13.0
Methyl β-L-arabinopyranoside	12.2
Methyl α-D-xylopyranoside	1.00
Methyl β-D-xylopyranoside	0.95

magnesium chloride to vary the ionic strength. For a given ionic strength, magnesium chloride is considerably more effective in increasing the rate, possibly because, by analogy with other systems[26], there is formation of a complex, or ion-triplet, between uronate, periodate, and magnesium ions. In theory, salt effects should also be important for polysaccharides containing sulphate or phosphate groups. For uronic acid-containing polysaccharides such as gums, which are soluble in their free-acid forms, a decrease in pH should have a similar effect to an increase in ionic strength[24] (*cf.* methyl pectate in Table I).

(*b*) For *N*-deacetylated or *N*-desulphated glycosaminoglycans, the salt effects should operate in reverse. In addition, there is a profound effect of pH, as demonstrated for chitosan, which is due to the fact that protonated amino-groups do not react[27].

(*c*) In the presence of β-D-glucopyranose or β-D-glucopyranuronic acid residues, linked through positions 1 and 4, it should be possible to bring about a highly selective oxidation of many other kinds of oxidisable units. There are also numerous possibilities for selective oxidation in the presence of 1,4-linked β-D-xylopyranose residues. The low reactivities of these residues must be due to steric hindrance. An interesting parallel can be found in the work of Smith and his co-workers[28], who showed that phenyl β-D-glucopyranoside was oxidised selectively between HO-3 and HO-4, whereas methyl 6-*O*-trityl-α-D-glucopyranoside was oxidised selectively between HO-2 and HO-3. On the other hand, phenyl α-D-glucopyranoside did not appear to be oxidised selectively[28]. In the oxidation of a *trans*-(*e*,*e*)-1,2-diol, it has been recognised[29] that formation of the cyclic-ester intermediate[30,31] would entail severely increased puckering of the ring at the site of reaction (*cf.* the formation of the corresponding *O*-isopropylidene derivative[32]). This would bring bulky, equatorial substituents flanking the diol system closer to the periodate ion, while axial groups would move away.

(*d*) More generally, the selectivities are less extreme, but Smith degradation after partial oxidation with periodate could still provide a useful alternative to partial hydrolysis with acid or enzymes, since the fragmentation pattern will usually be different. No exception has been found to the general rule[31,33–35] that *cis*-1,2-diols are oxidised faster than the corresponding *trans*-isomers, which therefore remains a useful guide. This phenome-

non has been explained[29] by recognising that the oxygen atoms of a *cis*-1,2-diol can take up the eclipsed (or near-eclipsed) conformation that is required for cyclic-ester formation[30,31] by a comparatively minor flattening of the ring, similar to that which occurs upon *O*-isopropylidenation[32].

It should perhaps be noted that the methyl glycopyranosides (Table II) are likely to be valid models for non-reducing end-groups or side-groups, only when these are linked to the rest of the chain *via* primary hydroxyl groups. When they are linked to secondary hydroxyl groups, special steric effects are likely to intervene, as discussed above. These studies will therefore be extended to oligosaccharides and other model substances.

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