Formation of Inter-residue Hemiacetals During the Oxidation of Polysaccharides by Periodate Ion

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When sodium alginate,[1] maize-cob xylan,[2] and amylose [3,4] are oxidised in aqueous sodium metaperiodate, the aldehyde groups of oxidised sugar residues spontaneously form six-membered hemiacetal rings with the closest hydroxyl groups on neighbouring, unoxidised residues in the same chain. These hemiacetals exist in a rapid equilibrium with the free or solvated aldehydic forms, and also with any intra-residue hemiacetals or hemialdals that may be formed.[2,3] Oxidation thus takes place in two stages: an initial, rapid stage, producing chains in which every unoxidised unit has at least one oxidised unit in an adjacent position; and a slow, terminal stage, in which the remaining unoxidised units are oxidised at a rate which is diminished to an extent determined by the position of the equilibrium.[1-4]

When, as in alginate and xylan, there is no possibility for the competitive formation of an intra-residue hemiacetal, both aldehyde groups of an oxidised sugar residue show a strong tendency to form inter-residue hemiacetals, and the oxidation of both nearest-neighbouring residues is thereby strongly inhibited. In these cases, the initial, rapid stage of the oxidation is complete after the consumption of about 0.44 mol of periodate for every non-terminal sugar residue.[1,2]

When, as in amylose, there is a possibility for the formation of intra-residue hemiacetals, only one of the aldehyde groups of an oxidised residue shows a strong tendency to form an inter-residue hemiacetal. In this case, the initial, rapid stage of oxidation is complete after the consumption of about 0.64 mol of periodate for every non-terminal residue.[3,4]

A summary is now given of further work, which was carried out to determine to what extent inter-residue hemiacetal formation

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is a general phenomenon in the periodate oxidation of polysaccharides. Unless otherwise stated, oxidations were carried out at 20°, and the concentration of periodate was varied between 5 mM and 25 mM, to give a convenient time-scale. The molar excess of periodate was generally about 2:1. Reference is made to a recent handbook [5] for information or supplementary references about polysaccharides named trivially.

Sodium and methyl pectates, C(6)-oxycellulose, C(6)-oxyamylose, guaran, the water-soluble xylan of *Rhodymenia palmata* (rhodymenan), and the β-1,4-linked galactan of lupin seeds all showed the characteristic behaviour noted earlier [1-4] for polysaccharides containing contiguous, 1,4-linked pyranose rings. With the polyuronides, the terminal stage of Malapradian oxidation was so slow that the only detectable consumption of oxidant could be traced to overoxidation; it was therefore impossible to measure the correct Malapradian oxidation-limit with these materials in the ordinary manner.

With guaran containing residues of galactose and mannose in the usual ratio of 1:2, complete oxidation of the galactose residues, shown by liberation of a theoretical yield (0.33 mol) of formic acid, was accomplished fairly rapidly, but only about half of the mannan backbone was oxidised rapidly. The remaining 50 % of mannose residues were extremely resistant to oxidation, and even prolonged treatment with a 12-fold molar excess of periodate, at a concentration of 60 mM, failed to raise the oxidation-limit significantly above 1.0 mol per hexose unit. This is in agreement with the earliest reports,[6,7] published before methylation [8,9] had established that all the galactose residues were linked to position 6 of mannose residues. It is not yet understood why the later workers [8,9] were able to report the theoretical oxidation-limit of 1.33 mol.

Rhodymenan, which contained 80 % of 1,4-linked xylose residues, behaved very similarly to maize-cob xylan, thus confirming that the behaviour noted earlier [2] for that material was not connected with its limited solubility in water. The galactan from lupin seeds behaved very similarly to amylose.[3,4] Prolonged oxidation of both these polysaccharides ultimately gave the correct oxidation-limit.

With all the above-named polysaccharides, reduction of the hemiacetals with sodium borohydride after completion of the initial, rapid phase of the oxidation permitted the smooth comsumption of further periodate, at a rate close to the initial rate, and the rapid

attainment of the correct, Malapradian oxidation-limit.

Chitosan (N-deacetylated chitin) overoxidises very rapidly,[10] and no satisfactory, Malapradian oxidation-limit has ever been reported for it. However, by performing the oxidation at 0° to depress the rate of overoxidation relative to the rate of Malapradian oxidation,[11] a sharp decrease in rate could be clearly shown to occur after the consumption of 0.65 mol of periodate per non-terminal glucosamine residue. This was the only detectable "oxidation-limit", since further consumption of periodate proceeded continuously up to 2.0 mol and beyond. There were two effects of borohydride reduction of the 65 % oxidised material: it permitted the rapid consumption of a further 0.35 mol of periodate per non-terminal glucosamine residue, and it then prevented any subsequent overoxidation, doubtless because the modified, reducing end-groups could no longer be converted into malondialdehyde derivatives.[12]

Lichenan, which is believed to consist largely of 1,3'-linked repeating-units of cellotriose, and hence of isolated pairs to oxidisable glucose residues, showed a clear, but less extreme deviation from second-order kinetics. It is noteworthy that, on the assumption of random oxidation and unidirectional inhibition of the oxidation of nearest-neighbours,[1,3] only one-half of the oxidisable doublets would be expected to show a deviation from second-order kinetics, following oxidation of the first member.

Nigeran is thought to consist almost entirely of alternating, 1,3- and 1,4-linked α-D-glucose residues, and in agreement with this, oxidation took place in good accordance with second-order kinetics for the first 90 % of the reaction. After this, however, there was a significant decrease in the second-order rate-coefficient, and reduction with borohydride restored it to its initial value. It was concluded that, in the sample of nigeran studied, about 20 % of the 1,4-linked glucose residues were contiguous. Closely similar behaviour was shown by a sample of isolichenan, which was already known[13] to contain a small proportion of contiguous, 1,4-linked glucose residues.

Inulin was oxidised in almost perfect accordance with second-order kinetics throughout, to give the theoretical, Malapradian oxidation-limit. As expected, reduction of the product with borohydride imparted no further susceptibility to periodate. With this polysaccharide, any inter-residue hemiacetals formed during oxidation would have to be seven-membered or higher-membered rings. Thus, the aldehyde group at position 3 of an oxidised fructofuranose residue would be able to form a

seven-membered hemiacetal ring with a neighbouring, unoxidised residue, but this would be in competition with the six-membered hemiacetal that it could form with primary hydroxyl group at position 6 of the same residue. Similarly, the aldehyde group at position 4 of an oxidised residue could form a nine-membered, inter-residue hemiacetal, but only in competition with a six-membered, intra-residue hemialdal.

The β-1,2-linked glucan of Agrobacterium radiobacter[14] was chosen as a more likely substrate for investigating the possibility of seven-membered hemiacetal ring-formation. With this material, the smallest intra-residue hemiacetal that could be formed (discounting four-membered rings) would be between the aldehyde group at position 3 and the primary hydroxyl group at position 6 of an oxidised residue, and it would be seven-membered. The same aldehyde group would also be able to form a seven-membered hemiacetal ring with the hydroxyl group at position 3 of an unoxidised, neighbouring unit, occupying either the preceding or the following position in the chain. It would also be able to form an eight-membered hemiacetal ring with the hydroxyl group at position 4 of either neighbour. A relatively small, but clear deviation from second-order kinetics was observed, with borohydride restoring the rate-coefficient to its initial value as usual. In these experiments, overoxidation was prevented by pre-treating the starting-material with borohydride.[14]

A sugar-beet arabinan, containing about 80 % of oxidisable L-arabinofuranose residues, showed a very clear departure from second-order kinetics, and the usual, rectifying effect of reduction with borohydride. The formation of seven-membered (or eight-membered), inter-residue hemiacetal rings must therefore have occurred, despite the possibility for competitive formation of six-membered, intra-residue hemialdals. Hemialdals thus appear to be less stable than hemiacetals of the same ring-size.

A kinetic analysis of the oxidation of galactocarolose was complicated by its low molecular weight, and consequent end-group effects, including overoxidation (which takes place in this case from the non-reducing end[15]). The reaction curve closely resembled that of chitosan, showing a marked decline in rate after the consumption of about 0.65 mol of periodate per non-terminal galactofuranose residue. Reduction with borohydride then permitted the almost instantaneous consumption of a further 0.15 mol of periodate, probably as a result of the modification of end-groups, but further oxidation then ensued at a clearly enhanced rate, and overoxidation was inhibited.

The unidirectional inhibition of the oxidation of nearest neighbours indicated by this evidence could only have arisen from the formation of seven-membered (or eight-membered), inter-residue hemiacetals. These were probably formed by the aldehyde groups at position 3 of the oxidised residues, because the aldehyde groups at position 2 would have been able to form six-membered hemiacetal rings with the primary hydroxyl groups at position 6 of the corresponding aglycone units. If this is true, the inter-residue hemiacetals would have been formed in competition with six-membered, intra-residue hemialdals, and five-membered, intra-residue hemiacetals.

A bacterial dextran containing 93 % of α-1,6-linkages, and a synthetic dextran [16] containing 100 % of such linkages, were used to study the behaviour of 1,6-linked polysaccharides. The second-order rate-coefficient rapidly decreased with increasing degree of oxidation, and became constant after the consumption of 1.4 mol of periodate for every 1,6-linked glucose residue. By following the liberation of formic acid as well as the total consumption of periodate, it was possible to calculate the fraction of intact glucose residues remaining at any time. It was thus shown that the first 75 % of these were oxidised in close obedience to second-order kinetics, and that only then did the rate-coefficient decline significantly. The initial decrease in the rate-coefficient describing the consumption of periodate must therefore have been due entirely to the formation of six-membered, intra-residue hemiacetals in units that had suffered a single, oxidative attack. Hemiacetals of this kind have previously been studied by Yu and Bishop.[17]

It may be tentatively supposed that the inhibition of the oxidation of the last 25 % of intact glucose residues was associated with the increasing proportion of doubly oxidised glucose residues in the chains, and was caused by the formation of seven-membered hemiacetal rings, in effective competition with the relatively unstable, six-membered hemialdals that would be present in those residues.

Although many questions remain unanswered, it seems reasonable to conclude that inter-residue hemiacetal formation is a general phenomenon in the periodate oxidation of polysaccharides, and that the relative abundance of inter-residue and intra-residue cyclic structures in any instance is fairly predictable from an already familiar hierarchy of stabilities, based upon ring-size and type. Within each such group, smaller differences in free energy can be expected, and should be susceptible to interpretation according to the principles of conformational analysis.

1. Painter, T. and Larsen, B. *Acta Chem. Scand.* **24** (1970) 813.
2. Painter, T. and Larsen, B. *Acta Chem. Scand.* **24** (1970) 2366.
3. Painter, T. and Larsen, B. *Acta Chem. Scand.* **24** (1970) 2724.
4. Smidsrød, O., Larsen, B. and Painter, T. *Acta Chem. Scand.* **24** (1970) 3201.
5. Aspinall, G. O. *Polysaccharides*, Pergamon, Oxford, New York, Toronto, Sydney and Braunschweig 1970.
6. Moe, O. A., Miller, S. E. and Iwen, M. H. *J. Am. Chem. Soc.* **69** (1947) 2621.
7. Whistler, R. L., Li, T. K. and Dvonch, W. *J. Am. Chem. Soc.* **70** (1948) 3144.
8. Ahmed, Z. F. and Whistler, R. L. *J. Am. Chem. Soc.* **72** (1950) 2524.
9. Rafique, C. M. and Smith, F. *J. Am. Chem. Soc.* **72** (1950) 4634.
10. Jeanloz, R. W. and Forchielli, E. *Helv. Chim. Acta* **33** (1950) 1690.
11. Jackson, E. L. and Hudson, C. S. *J. Am. Chem. Soc.* **61** (1939) 959.
12. Cantley, M., Hough, L. and Pittet, A. O. *J. Chem. Soc.* **1963** 2527.
13. Peat, S., Whelan, W. J., Turvey, J. R. and Morgan, K. *J. Chem. Soc.* **1961** 623.
14. Gorin, P. A. J., Spencer, J. F. T. and Westlake, D. W. S. *Can. J. Chem.* **39** (1961) 1067.
15. Halsall, T. G., Hirst, E. L. and Jones, J. K. N. *J. Chem. Soc.* **1947** 1427.
16. Zachoval, J. and Schuerch, C. *J. Am. Chem. Soc.* **91** (1969) 1165.
17. Yu, R. J. and Bishop, C. T. *Can. J. Chem.* **45** (1967) 2195.