Carbohydrate Research, 108 (1982) 323–327
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

Note

The periodate-oxidation limit of sodium and methyl pectates: conformational aspects of hemiacetal stability

TERENCE J. PAINTER
Institute of Marine Biochemistry, N-7034 Trondheim-NTH (Norway)
(Received March 15th, 1982; accepted for publication, March 26th, 1982)

Although it has been recognised for some time that pectates behave abnormally upon oxidation with periodate[1,2], the precise value of the oxidation limit of a pure, α-(1→4)-linked D-galacturonan has never been measured. The principal difficulties are the presence of neutral sugar residues in most native pectins[3,4] and a tendency for rapid overoxidation[5–8]. However, an unusually pure D-galacturonan has been obtained by fractionation of sunflower pectin[9,10], and a method of correcting for overoxidation has been described, based upon measurement of the formic acid liberated in excess of that originating from end-groups and calculation of the excess of periodate consumed in non-Malapradian oxidation[11]. We now report on the periodate-oxidation limit of the sodium salt and methyl ester of an almost pure D-galacturonan, and on additional experiments leading to an interpretation of the results in terms of inter-residual hemiacetal formation[11–13].

Sunflower heads were extracted, and the extracts fractionated, essentially as described by Zitko and Bishop[10]. The purest fraction contained 95% of D-galacturonic acid (Dische carbazole assay[14]). An aqueous solution (2% w/v) was kept at 20° for 3 h at pH 12, and then made 0.1M with respect to hydrochloric acid. The degraded pectic acid precipitated in this way was an essentially pure galacturonan, having an equivalent weight of 178 and $[\alpha]_D^{20}$ +286° (Na^+ salt; *c* 1, water). The methyl ester was prepared[15] by freeze-drying the sodium salt, and suspending the solid in methanolic 0.5M sulphuric acid; after 2 weeks at 2°, the solid contained 16% of OMe (calc. for methyl polygalacturonate: OMe, 16.3%).

Periodate oxidation was carried out as described earlier for C-6-oxycellulose[11]. Fig. 1 shows the consumption of periodate (*P*) and the liberation of formic acid (*F*) by the sodium salt. Extrapolation of *F* to zero time indicated the rapid liberation of 0.017 mol. from end-groups, which corresponds to a $\overline{d.p.}_n$ of 176. This would entail the consumption of (5/3 × 0.017) = 0.028 mol. of periodate by end-groups, and the curve, P_{corr}, showing the periodate consumed in the Malapradian manner was

0008-6215/82/0000–0000/S 02.75,

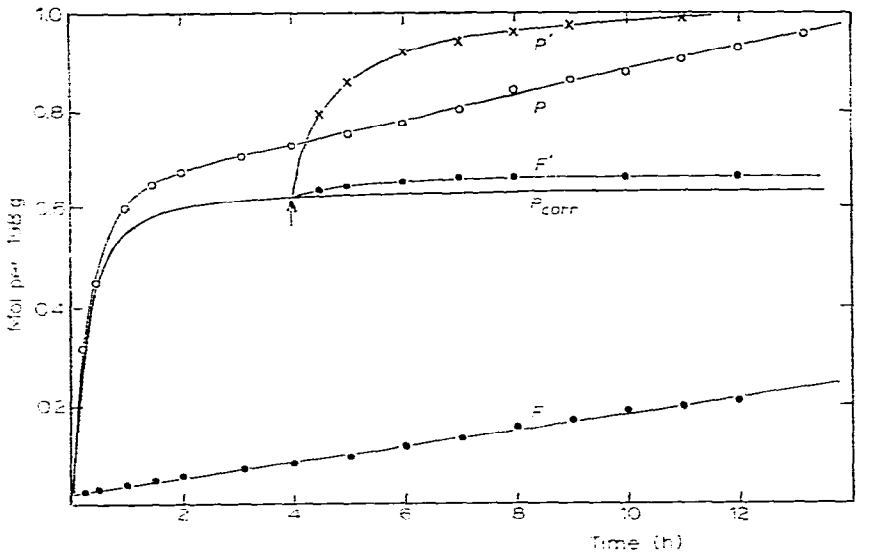

Fig. 1. Consumption of periodate (P) and release of formic acid (F) by 10mM sodium pectate in 25mM sodium metaperiodate at 25°. The mixture was 2.5M with respect to sodium chloride. P_{corr} was calculated from the formula $(P - 0.028) - 1.34\,(F - 0.017)$. At the point marked with an arrow, a sample of partly oxidised material was isolated, reduced with sodium borohydride, and oxidised again under the same conditions, with measurement of periodate consumed (P') and formic acid released (F'). Overoxidation is not expected in the second oxidation, and F' probably originated from C-1 of non-reducing end-groups that had already consumed 3 mol. of periodate in the first oxidation[8].

calculated from the formula* $P_{corr} = (P - 0.028) - 1.34\,(F - 0.017)$. A clear, anomalous limit of 0.64 $\pm$0.01 mol. of periodate consumed per non-terminal, D-galacturonic acid residue is indicated. A similar result was obtained with the methyl ester, and there was no loss of methoxyl upon oxidation.

The special theoretical significance of this numerical result has been discussed in detail[12]. It is the limit of reaction when any linear homopolymer of d.p. >15 undergoes random modification of its units, with unidirectional protection, from subsequent modification, of unreacted units adjacent to reacted ones. This phenomenon has previously been observed in the oxidation of amylose and carboxyl-reduced pectin by lead tetra-acetate in dimethyl sulphoxide[2], and in the oxidation of C-6-oxycellulose by aqueous periodate[11].

The same result could, of course, be obtained fortuitously for other reasons, such as the presence of 36% of (1→3) linkages in the chains. A sample of the sodium salt was therefore isolated after oxidation for 4 h under the conditions of Fig. 1. It had $[\alpha]_D^{20}$ $-70°$, which increased to $+49°$ after reduction with sodium borohydride (*cf.* limit-oxidised alginate[12]). The borohydride-reduced material contained 34%

*The derivation of this formula has been described[11]. It is based upon accepted mechanisms of over-oxidation, including the kind which, in glycuronans, takes place from the non-reducing ends of the chains[8].

of galacturonic acid, and it rapidly consumed 0.35 mol. of periodate (calculated from the weight of the original galacturonan), thus bringing the total Malapradian consumption to the expected value of 1.0 mol. (Fig. 1).

With alginate, the protection of unoxidised units by oxidised neighbours is bidirectional, the oxidation limit is lower (0.44 mol.), and the product, which does not react with Schiff's reagent, can be methylated without severe depolymerisation[12]. By comparing the results of methylation analysis before and after reduction with borohydride, it was easily established that the protective mechanism was inter-residual hemiacetal formation[12]. In contrast, limit-oxidised pectate reacted strongly with Schiff's reagent, and the molecule was split into small fragments upon methylation, doubtless because of β-elimination reactions occurring under the basic conditions of the reaction. Therefore, the "direction" of the protection, that is, the identity of the hydroxyl group on an unoxidised unit that becomes blocked, could not be determined by methylation analysis.

The limit-oxidised sodium pectate was therefore oxidised in 50mM aqueous bromine at pH 5 and 20° for 3 h. The amount of rapidly consumed bromine (determined iodometrically[16]) corresponded to the oxidation of ~75% of the aldehyde groups, and the equivalent weight (122) of the sodium salt of the product (determined by titration with cetylpyridinium chloride[17]) showed that they had been quantitatively converted into free carboxylic acid groups (as opposed to lactones[16]).

The bromine-oxidised product did not react with Schiff's reagent, but Barry degradation[18] readily yielded glyoxal bisphenylhydrazone (m.p. and mixture m.p. 178°), which was extracted from the reaction mixture with diethyl ether and recrystallised from benzene–hexane. This was the only phenylosazone of low molecular weight in the reaction mixture that was detectable on thin-layer chromatograms (silica gel: 9:1 toluene–ethanol, for small fragments; or 2:1:3 ethyl acetate–acetic acid–water, for oligosaccharide phenylosazones).

The aldehyde groups that were unreactive towards Schiff's reagent and bromine therefore originated from C-2 of oxidised D-galacturonic acid residues, and their proportion in the limit-oxidised pectate was identical with that of the periodate-resistant residues. Their protective action must have involved hemiacetal formation with HO-2 or HO-3 of intact residues of D-galacturonic acid lying to their right, when the chain is drawn in the usual way. Of these two possibilities, the first can be ruled out, because O-2 and O-4 on the intact residue are *trans*, and too far apart to allow ring closure. The hemiacetal must therefore be formed with HO-3, to give a six-membered ring.

These findings can be easily rationalised in terms of conformational principles. Fig. 2 shows an unoxidised D-galacturonic acid residue in the expected $^4C_1$ conformation, with part of an oxidised residue in both neighbouring positions. The two, different, possible hemiacetal-rings are shown, with the hemiacetal hydroxyl-groups arbitrarily assigned to equatorial positions, to avoid *syn*-axial interactions with C-2 and C-3, respectively, in the intact pyranose ring.

The hemiacetal on the left would be stabilised by the axial orientation of O-5

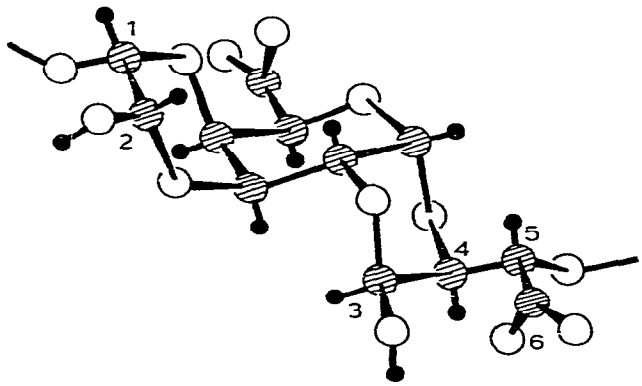

Fig. 2. Model of an intact D-galacturonic acid residue in sodium pectate, showing the hemiacetals that can be formed with the closest aldehyde groups in periodate-oxidised residues in both neighbouring positions. The numerals refer to carbon atoms originating from the oxidised residues.

of the oxidised unit (anomeric effect), and destabilised by only one *gauche* interaction, namely, that between C-2 of both rings. Such an instability factor does not prevent the formation of stable hemiacetals in periodate-oxidised alginate[11,12]. On the other hand, the hemiacetal on the right is destabilised not only by a similar *gauche* interaction, but also by the axial orientation of C-5 of the oxidised unit, and a *syn*-axial interaction between the hemiacetal oxygen and the bulky carboxylate anion at C-6 of the oxidised unit. Of these instability factors, the second alone has been recognised as substantially preventing ring-closure in periodate-oxidised C-6-oxycellulose in aqueous solution[11]. Therefore, the periodate oxidation of pectate may be a model example of a purely unidirectional, auto-inhibitory reaction.

The resistance of the stable hemiacetal to bromine oxidation in its ring-closed form, to give a lactone, is also predictable, from the principles enunciated by Barker *et al.*[3] for reducing pyranose sugars. These consist in recognising that, in the intermediate hypobromite ester, the bulky bromine atom has to become antiperiplanar with the anomeric hydrogen atom before HBr can be eliminated. In the left-hand hemiacetal in Fig. 2, this would be opposed by a large *syn*-axial interaction with O-5 of the oxidised unit.

ACKNOWLEDGMENTS

Sunflower heads were kindly sent from Bratislava by Professor R. Kohn, and the initial extractions were carried out by Eric Lindeberg[20]. Skilful technical assistance was provided by Kjersti Andresen.

REFERENCES

1 J. Szejtli, *Acta Chim. Acad. Sci. Hung.*, 49 (1966) 205–216.
2 V. Zitko and C. T. Bishop, *Can. J. Chem.*, 44 (1966) 1749–1756.
3 G. O. Aspinall, *Polysaccharides*, Pergamon Press, Oxford, 1970, pp. 119–129.

4 G. O. ASPINALL, in W. PIGMAN AND D. HORTON (Eds.), *The Carbohydrates*, 2nd edn., Vol. IIB, Academic Press, New York, 1970, pp. 515–521.
5 D. B. SPRINSON AND E. CHARGAFF, *J. Biol. Chem.*, 164 (1940) 433–449.
6 T. G. HALSALL, E. L. HIRST, AND J. K. N. JONES, *J. Chem. Soc.*, (1947) 1427–1432.
7 R. A. EDINGTON, E. L. HIRST, AND E. E. PERCIVAL, *J. Chem. Soc.*, (1955) 2281–2288.
8 M. CANTLEY, L. HOUGH, AND A. O. PITTET, *J. Chem. Soc.*, (1963) 2527–2535.
9 C. T. BISHOP, *Can. J. Chem.*, 33 (1955) 1521–1529.
10 V. ZITKO AND C. T. BISHOP, *Can. J. Chem.*, 43 (1965) 3206–3214.
11 T. J. PAINTER, *Carbohydr. Res.*, 55 (1977) 95–103.
12 T. J. PAINTER AND B. LARSEN, *Acta Chem. Scand.*, 24 (1970) 813–833, 2366–2378, 2724–2736.
13 M. F. ISHAK AND T. J. PAINTER, *Acta Chem. Scand.*, 25 (1971) 3875–3877.
14 T. BITTER AND H. M. MUIR, *Anal. Biochem.*, 4 (1962) 330–334.
15 W. J. HERI, H. NEUKOM, AND H. DEUEL, *Helv. Chim. Acta*, 44 (1961) 1939–1945.
16 M. F. ISHAK AND T. J. PAINTER, *Acta Chem. Scand.*, 27 (1973) 1321–1327.
17 J. E. SCOTT, *Methods Biochem. Anal.*, 8 (1960) 145–197.
18 P. S. O'COLLA, *Methods Carbohydr. Chem.*, 5 (1965) 382–392.
19 I. R. L. BARKER, W. G. OVEREND, AND C. W. REES, *J. Chem. Soc.*, (1964) 3254–3262.
20 E. LINDEBERG, Diploma thesis, Institute of Technical Biochemistry, Technical University of Norway, Trondheim, 1971.