ELSEVIER

Carbohydrate Polymers **23** (1994) 137–138

0144-8617/94/$07.00

Technical Note

Periodate oxidation: a source of error in the assay of formaldehyde with chromotropic acid

Terence J. Painter

Institute of Biotechnology, University of Trondheim, N-7034 Trondheim-NTH, Norway

(Received 22 April 1993; accepted 13 July 1993)

Bisulphite is an unsuitable reagent for removing the excess of periodate from solution prior to the determination of formaldehyde, because the reaction is too slow. Serious errors may arise, but they can be eliminated by using an excess of *myo*-inositol to reduce the periodate.

The chromotropic-acid method (MacFadyen, 1945), as modified by Frisell *et al.* (1954), is usually used to measure the formaldehyde liberated by periodate from the glycitols of simple mono- and oligo-saccharides. This application does not demand high accuracy, because it is usually necessary only to determine whether 0, 1 or 2 moles of formaldehyde are liberated per mole of glycitol. With glycan derivatives, however, applications such as pendant-group analysis (Ishak & Painter, 1973) or molecular-weight determination (Painter, 1988) demand a higher accuracy, and for these purposes the method in its original form is unsatisfactory.

A sample of Dextran 2000 (Pharmacia, Uppsala, Sweden) was used as a model substance. It contained 93% of oxidisable α-D-glucopyranosyl residues, all with a 2,3,4-triol function, while the remaining 7% were resistant to periodate (Ishak & Painter, 1978). Portions (85 mg) were oxidised in unbuffered 12·5 mM sodium metaperiodate (200 ml) at 20°C, and the consumption of periodate (P) and the liberation of formic acid (F), both expressed as moles per D-glucosyl residue, were measured accurately by titrimetric methods (Ishak & Painter, 1978; Aalmo & Painter, 1981). The mole fraction of D-glucosyl residues that had suffered a single oxidative attack at any time was therefore given by $(P - 2F)$.

At intervals during the reaction, samples of partially oxidised dextran were isolated, reduced with sodium borohydride, and then oxidised again, with measurement of the formaldehyde released (A). Since P and F were known at any time, it was possible to calculate the average formula weights of the monomer units in the isolated samples, and to express the values of A as moles of formaldehyde liberated for every D-glucosyl residue in the original dextran. These should therefore be identical with the values of $(P - 2F)$, expressed in the same way.

The formaldehyde was assayed by two different procedures (**1** and **2**). In the first (**1**), 20 mg of borohydride-reduced, partially periodate-oxidised dextran was oxidised for 20 min at 20°C in 12·5 mM sodium metaperiodate (50 ml). A portion (25 ml) of the reaction mixture was then mixed with aqueous sodium metabisulphite (2·6% w/v; 12·5 ml), and diluted with water to 50 ml. Erythritol (5 mg) was treated similarly to give a standard, while the blank was prepared from 12·5 mM periodate alone. Portions (0·5 ml) of the three solutions were then taken in quadruplicate for heating with the chromotropic acid reagent in the usual way (Frisell *et al.*, 1954).

The second procedure (**2**) was the same, except that the portion (25 ml) of reaction mixture was first mixed with aqueous *myo*-inositol (2·5% w/v; 10 ml), and kept at 20°C for 1 h before addition of sodium metabisulphite and dilution to 50 ml as before.

The results (Table 1) show that the values of A were much too high when measured by procedure **1**, but correct when measured by procedure **2**. Prior to the development of procedure **2**, many experiments were performed in which the concentration and molar excess of bisulphite were increased, and in which the time allowed for reduction was varied, but in no case was a completely satisfactory result obtained. This belief that bisulphite resembles iodide in reducing periodate rapidly to iodate near neutral pH is therefore incorrect. In strongly acidic solutions, periodate and iodate are both smoothly reduced to iodide and iodine (Moody, 1991), but the reaction is not instantaneous, as is evident from its use in a well-known 'clock reaction' (Henderson & McCulloch, 1939; Conway, 1940).

After addition of the strongly acidic chromotropic acid reagent, hydrolysis of the dextran derivatives must occur, liberating erythritol, glycerol, D-glyceraldehyde and glycolaldehyde. These would then compete with bisulphite for reaction with the residual periodate, liberating additional formaldehyde. Almost all of the error comes from D-glyceraldehyde and glycolaldehyde. This was shown by adding the pure compounds to the ice-cold chromotropic-acid reagent immediately before this was added to the reagent blank, consisting of previously-mixed periodate and bisulphite. Hence, these α-hydroxyaldehydes must react faster with the residual periodate than do erythritol and glycerol. This is consistent with other evidence that aldoses in general are oxidised much faster between positions 1 and 2 than at other sites in the molecule (Warsi & Whelan, 1958).

An alternative explanation is that bisulphite does reduce all of the unreacted periodate, but that periodate is formed, *de novo*, during subsequent heating with the chromotropic-acid reagent. This, however, was opposed by the finding that mixtures of sodium iodate and D-glyceraldehyde or glycolaldehyde yielded no formaldehyde when analysed by procedure **1**.

These findings seem to challenge the results of numerous investigations in which the procedure of Frisell *et al.* has been used. It should be noted, however, that exceptionally large amounts of D-glyceraldehyde and glycolaldehyde would have been liberated from the dextran derivatives used in the present work, and that periodate-oxidised glycans are usually more sensitive to acid-hydrolysis after they have been reduced with borohydride. It should also be noted that there is no problem with the method of O'Dea and Gibbons (1953), in which lead dithionate is used to remove the excess of both periodate and iodate.

Table 1. Oxidation of dextran (5 mM) in 12·5 mM sodium metaperiodate at 20°C

Time (h)	P^a	F^b	$(P - 2F)$	A^c, by procedure	
				1	**2**
1	0·735	0·216	0·303	0·59	0·29
2	1·036	0·320	0·396	0·82	0·40
3	1·201	0·391	0·419	0·91	0·42
4	1·308	0·450	0·408	0·93	0·41
5	1·387	0·496	0·395	0·96	0·38
6	1·430	0·532	0·366	1·01	0·36

[a]Periodate consumed.
[b]Formic acid released.
[c]Formaldehyde released after reduction with sodium borohydride, followed by re-oxidation with periodate under the same conditions; all in moles per D-glucosyl residue.

REFERENCES

Aalmo, K.M. & Painter, T.J. (1981). *Carbohydr. Res.*, **89**, 73–82.

Conway, W.J. (1940). *J. Chem. Educ.*, **17**, 398.

Frisell, W.R., Meech, L.A. & Mackensie, C.G. (1954). *J. Biol. Chem.*, **207**, 709–16.

Henderson, A. & McCulloch, W.P. (1939). *J. Chem. Soc.*, 675–6.

Ishak, M.F. & Painter, T.J. (1973). *Acta Chem. Scand.*, **27**, 1268–76.

Ishak, M.F. & Painter, T.J. (1978). *Carbohydr. Res.*, **64**, 189–97.

MacFadyen, D.A. (1945). *J. Biol. Chem.*, **158**, 107–33.

Moody, B. (1991). *Comparative Inorganic Chemistry*. Edward Arnold, London, p. 442.

O'Dea, J.F. & Gibbons, R.A. (1953). *Biochem. J.*, **55**, 580–6.

Painter, T.J. (1988). *Carbohydr. Res.*, **179**, 259–68.

Warsi, S.A. & Whelan, W.J. (1958). *Chem. & Ind.* (London), 71.