Carbohydrate Research, 63 (1978) 261–264

Note

A rapid Smith-degradation for the determination of non-reducing, terminal residues of (1→4)-α-D-glucans

SUSUMU HIZUKURI AND SHIGEMITSU OSAKI
Department of Agricultural Chemistry, Kagoshima University, Kagoshima 890 (Japan)
(Received June 2nd, 1977; accepted for publication June 16th, 1977)

The Smith degradation[1] (oxidation with periodate, reduction with sodium borohydride, and hydrolysis with acid) produces glycerol from the non-reducing, terminal groups of glucans of the glycogen and amylopectin type. The glycerol is usually assayed by g.l.c.[2,3] or by using glycerol kinase (EC 2.7.1.30) and glycerol 3-phosphate dehydrogenase[4] (EC 1.1.1.8). The glycerol can be assayed rapidly, but the Smith-degradation procedure is time-consuming. We now describe a rapid Smith-degradation procedure that is suitable for the determination of non-reducing, terminal groups of (1 → 4)-α-D-glucans and involves an enzymic assay of glycerol.

Periodate oxidation is usually effected below room temperature, in order to minimize over-oxidation which degrades the glucans stepwise from their reducing terminals and gives formic acid and formaldehyde. However, over-oxidation does not interfere with the assay of glycerol, provided that it does not proceed to non-reducing, terminal glucose residues. Under these conditions, the oxidation can be carried out rapidly at temperatures higher than usual.

The data in Table I show that maximal release of glycerol from maltotriose by Smith degradations at 16° and 50° occurred after at least 8 h and 30 min, respectively. However, oxidation for 5 h at 50° decreased the yield of glycerol, possibly due to over-oxidation. Maltose, which was the most sensitive to over-oxidation in this series of oligosaccharides, also yielded the theoretical amount of glycerol by oxidation at 50° for 30–120 min. These results indicate that it is possible for the oxidation in this analysis to be carried out at 50°.

The polyaldehyde produced by periodate oxidation was completely reduced with borohydride during 1 h at room temperature. The enzymic assay of glycerol was not significantly inhibited at the concentration of sodium borohydride used in the standard conditions, but was appreciably inhibited at twice the concentration, so that the assay took more than 2 h. The optimal conditions for the hydrolysis, evaluated using maltotriose and the standard Smith-degradation conditions, involved treatment at 100° for 20 min.

Table II summarizes the data for the release of glycerol from glycogen and

TABLE I

RELEASE OF GLYCEROL BY PERIODATE OXIDATION, BOROHYDRIDE REDUCTION, AND ACID HYDROLYSIS

Maltotriose (3.03mM) at 16°							
Time (h)	1.0	2.0	4.5	8.0	13.5	26	50
Periodate consumed (mol/mol)	3.45	3.78	4.35	4.68	4.86	5.13	5.40
Glycerol released (mol/mol)	0.81	0.93	0.97	0.99	0.98	0.98	0.95
Maltotriose (2.83mM) at 50°							
Time (min)	10	30	40	60	180	300	480
Periodate consumed (mol/mol)	3.64	4.34	4.48	4.69	5.42	6.15	7.30
Glycerol released (mol/mol)	0.87	0.98	1.00	0.98	1.00	0.96	0.92
Maltose (1.54mM) at 50°							
Time (min)	20	30	60	90	120	150	180
Glycerol released (mol/mol)	0.97	0.99	0.99	0.98	1.00	0.95	0.88

TABLE II

DETERMINATION OF UNIT CHAIN-LENGTHS BY THE STANDARD SMITH-DEGRADATION

Oyster glycogen[a] *(15.78mM as glucose)*								
Oxidation time (min)	15	30	40	60	120	180	275	330
Periodate consumed (mol/mol of glucose)	0.68	0.76	0.80	0.81	0.91	0.97	1.03	1.03
Unit chain-length	11.1	10.9	10.7	10.7	10.5	10.6	10.6	10.7
Potato amylopectin[b] *(21.65mM as glucose)*								
Oxidation time (min)	30	60	120	180	300	420		
Unit chain-length	25.6	24.5	24.2	24.0	24.2	23.2		

[a]An average chain-length of 10.7 was determined by the reducing value after debranching with *Pseudomonas* isoamylase[7]. [b]An average chain-length of 23.3 was determined by the reducing value after debranching with *Pseudomonas* isoamylase[7].

TABLE III

SMITH DEGRADATION AT 16° OF 3mM METHYL α-D-GLUCOPYRANOSIDE

Time (h)	2	4	8	23.4	49	99
Periodate consumed (mol/mol)	1.38	1.52	1.82	1.94	1.99	2.00
Glycerol released (mol/mol)	0.92	0.97	0.98	0.98	0.98	0.97

amylopectin by the standard Smith-degradation, and the average chain-lengths. The yield of glycerol by oxidation for 40 min–5 h was constant. A small increase in the yield of glycerol observed when amylopectin was oxidized for 7 h was possibly caused by hydrolysis of the glucan. The average chain-lengths (glycogen, 10.6; amylopectin, 24.1) agree well with the values determined by the method using isoamylase[7].

The release of glycerol from glycogen and amylopectin was linearly proportional to the concentration below 5 mg/ml.

Maximal formation of glycerol from maltotriose (Table I), and from glycogen and amylopectin (Table II), preceded completion of periodate uptake, suggesting that cleavage of the C-3–C-4 bond of the non-reducing, terminal D-glucose residue takes precedence over that of the C-2–C-3 bond. Methyl α-D-glucopyranoside gave 1 mol. of glycerol before oxidant consumption was complete (Table III), indicating the preferential cleavage of the C-3–C-4 bond. The complete oxidation of the whole glucan is not always necessary, but that of the C-3–C-4 bond is required for the determination of non-reducing terminals by this procedure.

From the foregoing data, we conclude that oxidation at 50° for 1 h is suitable for the standard conditions of a routine assay. The whole procedure takes only a few hours and requires only a few mg of material.

EXPERIMENTAL

Materials. — Glycerol kinase and glycerol 3-phosphate dehydrogenase were purchased from Boehringer Mannheim Yamanouchi K.K. Potato amylopectin was prepared by the method of Schoch[6], purified *Pseudomonas* isoamylase was a gift from Professor T. Harada (Osaka University), and glycogen (oyster) was purchased from Katayama Kagaku K.K.

Standard Smith-degradation procedure. — To an aqueous glucan solution (0.5 ml; 0.2–5 mg/ml for oligosaccharides, 1–10 mg/ml for amylopectin and glycogen), 60mM sodium periodate (0.5 ml) was added, and the mixture was incubated at 50° for 1 h. After ethylene glycol (10 μl) was added, the mixture was left for 10 min at room temperature and then treated with 0.2M sodium borohydride–0.01M sodium hydroxide (1 ml, prepared daily) for 1 h at room temperature. To the resulting polyalcohol solution, M sulphuric acid (0.5 ml) was added slowly with shaking. The mixture was heated in a boiling water bath for 20 min and then cooled to room temperature, the pH was adjusted to 9.5–10.5 with 2M sodium hydroxide, and the volume was made up to 5 ml with distilled water.

Determination of glycerol. — To portions (75 μl) of the above-treated samples were added hydrazine buffer (M hydrazine hydrate, 0.2M glycine, and 2mM $MgCl_2$; pH 9.8; 250 μl), glycerol 3-phosphate dehydrogenase (400 U/ml, 5 μl), ATP (50mM, 5 μl), and NAD (20mM, 10 μl). The reaction was started by the addition of glycerol kinase (85 U/ml, 10 μl). The total volume was 355 μl. Absorbances were measured with a Hitachi Perkin–Elmer spectrophotometer 139 at 340 nm until no further

change was observed (20–40 min). The extinction coefficient of NADH at 340 nm was taken as 6.22×10^6 cm^2/mol.

Determination of periodate. — The method of Avigad[7], involving 2,4,6-tri-2-pyridyl-*s*-triazine, was used.

ACKNOWLEDGMENTS

We thank Dr. T. Harada for the gift of *Pseudomonas* isoamylase, and Hayashibara Seikagaku Kenkyu-sho for that of maltotriose.

REFERENCES

1 J. K. Hamilton and F. Smith, *J. Am. Chem. Soc.*, 78 (1956) 5907–5909.
2 H. Yamaguchi, T. Ikenaka, and Y. Matsushima, *J. Biochem. (Tokyo)*, 63 (1968) 553–554.
3 G. G. S. Dutton, K. B. Gibney, G. D. Jensen, and P. E. Reid, *J. Chromatogr.*, 36 (1968) 152–162.
4 D. W. Noble and R. J. Sturgeon, *Carbohydr. Res.*, 12 (1970) 448–452.
5 H. Akai, K. Yokobayashi, A. Misaki, and T. Harada, *Biochim. Biophys. Acta*, 237 (1971) 422–429.
6 S. Lansky, M. Kooi, and T. J. Schoch, *J. Am. Chem. Soc.*, 71 (1949) 4066–4075.
7 G. Avigad, *Carbohydr. Res.*, 11 (1969) 119–123.