Comparison of the Methods for Determining Degree of Polymerization of Water-Insoluble β-1, 3-Glucans

Bull. Univ. Osaka Pref., Ser. B, Vol. 35,
大阪府立大学紀要, 農学・生物学, 1983

Comparison of the Methods for Determining Degree of Polymerization of Water-Insoluble β-1, 3-Glucans

Kazutaka MIYATAKE and Shozaburo KITAOKA

Nutrition Laboratory, College of Agriculture

(Received October 30, 1982)

Abstract

Some representative methods, basing on periodate oxidation, arsenomolybdate colorimetry and enzymatic oxidation and resin separation of the glucitol formed by reduction of glucans, for the determination of number-average degree of polymerization of β-1, 3-glucans were applied to paramylon, pachyman and curdlan and the results compared with discussion of the characteristic feature of each technique. It was advised to perform a few different methods in parallel, but when a single method should be selected the microchemical technique by Yamaguchi and Makino is the most simple and reliable.

It is well known that determination of degree of polymerization of polysaccharides, especially water-insoluble, β-linked glucans which resist periodate oxidation is very difficult. The present paper compares some representative methods to seek the most reliable one for routine estimation of number-average degree of polymerization of β-1, 3-glucans.

Materials and Methods

1. *Chemicals*

All reagents were analytical grade. Pachyman, from *Poria cocos* Walf was kindly supplied by Dr. H. Yamaguchi, this University, and curdlan (Lot N-20) from *Alkaligenes faecalis* var. *myxogenes* 10C3 by Dr. H. Kimura, Takeda Chemical Industries, Osaka. Paramylon was isolated and purified from *Euglena gracilis* z.[1)]

2. *Methods*

The number-average degree of polymerization of the β-1, 3-glucans was determined by the periodate oxidation method by Hay *et al.*[2)], its modified methods,[3,4)] the modified Nelson-Somogyi method,[5)] the enzymatic method by Manners *et al.*,[6)] and the microchemical method by Yamaguchi and Makino.[7)]

Results and Discussion

Table 1 shows the number-average degrees of polymerization of paramylon, pachyman and curdlan obtained by different methods together with the values reported by some other methods. The data show that the microchemical method and the modified Nelson-Somogyi method are in good agreement with each other. The former method bases on quantitative separation of a trace amount of alditol derived from endo groups of a glucan be reduction with sodium borohydride from the mixture with a large amount

Table 1. The Number-average Degree of Polymerization of Water Insoluble β-1, 3-glucans

Analytical Methods	Glucans		
	Paramylon	Pachyman*	Curdian*
Microchemical method	691	897	2381
Modified Nelson-Somogi method	700	870	2300
Enzymatic method	620 (663)**	801 (889)**	1899 (2240)**
Periodate oxidation methods			
A[2,3]	225	287	580
B[4]	432	735	1379
Physicochemical methods			
Osmometry[8]	–	690	–
Cadoxen[9]	–	–	2500 – 4900

*; able to form a gel on heating, **; calculated values after correction.
All values are average of 5 determinations.

Table 2. Change in Extinction by 5μg of D-glucitol in the Presence and Absence of Different Amounts of D-glucose in Sorbitol Dehydrogenase Assay System

Presence of D-glucose (weight ratios)	Relative extinction (%)	
	Exp. 1	Exp. 2
0	100.0	100.0
100	100.0	100.0
200	104.8	105.2
500	107.0	108.3
1000	110.0	109.5
2000	114.0	115.4

5μg of D-glucitol and absence of D-glucose as a control (100%. Sorbitol dehydrogenase (from Sheep liver, Lot. No. 109312) was purchased from Boehringer Mannheim.

of glucose after hydrolysis by using a strongly basic ion-exchange resin and gas-chromatographic determination of the alditol in acetate derivative.

In the method of Manners *et al.* a glucan, after reduction with borohydride, is hydrolyzed with an acid, and D-glucitol there formed is estimated by means of an enzymatic oxidation with sorbitol dehydrogenase. The D-glucose content in the hydrolyzate, on the other hand, is determined by a reductometric method or by the use of D-glucose oxidase, and the number-average degree of polymerization is calculated from the relative amount of D-glucitol. This enzymatic method has limitation depending on the specificity of the enzyme and the polymerization degree of D-glucose or D-xylose.

The degrees of polymerization of the three β-glucans as determined by the enzymatic method of Manners *et al.* are slightly smaller than those by the microchemical and modified Nelson-Somogyi methods (Table 1). Manners *et al.* have reported that the presence of D-glucose in an excess ranging from 100- to 1000-folds has no effect on the assay of D-glucitol.[6] However, in our experiments the presence of up to 2000-folds of glucose caused changes in extinction in assaying 5μg of D-glucitol, and the effect was in proportion to the amount of D-glucose in the mixture as shown in Table 2. A correction,

therefore, should be applied in assaying a polysaccharide with a relatively high molecular weight by this method. After correction, the results by this method is in good accordance with those by the microchemical and the modified Nelson-Somogyi methods. It is noted, in addition, that mannitol is also formed from the reducing end of α-1, 4- and α-1, 6-glucans beside glucitol during the borohydride treatment.[5] Mannitol escapes detection by the Manners' method, causing errors in assaying the degree of polymerization of these polysaccharides by this method. A similar situation is suspected to occur in the assay of β-1, 3-glucans.

In the analyses involving periodate oxidation, water-insoluble β-1, 3-glucans which are resistant to this reagent tend to give much smaller values of degree of polymerization than those by other methods. When periodate oxidation is applied after reduction of the reducing end of glucans, the terminal hexitol residues in the 1, 2-, 1, 5- and 1, 6-linked glucans should give one molecular proportion of formaldehyde while the residues in the 1, 3- and 1, 4-linked glucans yield two molecular proportions. In practice cyclization is liable to occur in the latter group of glucans in the periodate oxidation with the result of causing uncertainity in quantitation.[7] The experimental procedures are rather complicated requiring removal of polysaccharide and periodate and iodate ions prior to determination of formaldehyde. Some overoxidation may take place in the periodate oxidation with additional production of formic acid and it also constitutes the factors contributing unreliable results of analysis. The much small values of degree of polymerization with the three β-1, 3-glucans in the present experiments should be due to various factors discussed above.

We suggest that it is advisable to carry out some different methods in determining number-average degree of polymerization of glucans with good knowledge of characteristic feature of the methods to obtain a reliable value after comparison. However, in case of selecting a single method it appears that the microchemical technique by Yamaguchi and Makino is most simple in procedure and reliable in result.

We have found that paramylon from *E. gracilis* is not able to form a gel upon heating while curdlan and pachyman is able.[1] We suggested that relatively low degree of polymerization of paramylon is the cause of its inability of gel formation. The present data support the reasoning. Curdlan and pachyman lose the ability of gel formation after treatment with an endo-type β-1, 3-glucanase,[1] and this should also be due to lowering of the degree of polymerization.

Reference

1) MIYATAKE, K., FUJII, S. and KITAOKA, S. (1977). Physicochemical Properties of Paramylon, a Storage Polysaccharide of *Euglena gracilis z. NOKASHI*, **51**, N. 14 (in Japanese).
2) HAY, G. W., LEWIS, B. A., SMITH, F., and UNRAU, A. M. (1965). Determination of Reducing End-groups. By Periodate Oxidation. in "Methods in Carbohydrate Chemistry" (WHISTLER, R. L., BEMILLER, J. N. and WOLFROM, M. L., eds) Vol. **5**, p. 251 – 252, Academic Press, New York.
3) HANAHAN, D. J. and OLLEY, J. N. (1958). Chemical Nature of Monophosphoinositides. *J. Biol. Chem.*, **231**, 813–828.
4) DURAND, G., FEGER, J., COINGNOUX, M., ANGERAY, J., and PAY, M. (1974). Rapid Estimation of Small Amounts of Formaldehyde Liberated During Periodate Oxidation of a Sialoglycoprotein. *Anal. Biochem.*, **61**, 232–236.

5) YAMAGUCHI, H., INAMURA, S. and MAKINO, K. (1976). Studies on a Microchemical Method for the Determination of the Degree of Polymerization of Neutral Oligo- and Polysaccharides. I. Quantitative Separation of Trace Amounts of Alditol from Mixture with a Large Excess of Monosaccharides. *J. Biochem.*, **79**, 299–303.
6) MANNERS, D. J., MASSON, A. J. and STURGEON, R. J. (1971). An Enzymic Method for the Determination of the Degree of Polymerisation of Glucans. *Carbohyd. Res.*, **17**, 109–114.
7) YAMAGUCHI, H. and MAKINO, K. (1977). Studies on a Microchemical Method for the Determination of the Degree of Polymerization of Neutral Oligo- and Polysaccharides. II. Application to Oligosaccharides and Glucans. *J. Biochem.*, **81**, 563–569.
8) HOFFMAN, G. C., SIMSON, B. W. and TIMELL, T. E. (1971). Structure and Molecular size of Pachyman. *Carbohyd. Res.*, **20**, 185–188.
9) HIRANO, I., EINAGA, Y. and FUJITA, H. (1979). Curdlan (Bacterial β-1, 3-Glucan) in a Cadoxen-Water Mixture. *Polymer J.*, **11**, 901–904.