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## Note

# A new enzymic method for determination of the number-average degree of polymerization ($\overline{d.p.}_n$) of linear, amylose-type polysaccharides

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The action pattern of the enzyme phosphorylase shows that maltotetraose is the limit-dextrin for both the degradative and synthetic actions. Phosphorylase requires maltotetraose as the lowest primer for the efficient synthesis of larger molecules and it cannot degrade maltotetraose or smaller saccharides[1,2]. Moreover, four D-glucose residues remain in the outer chains after the action of phosphorylase on amylopectin[3]. This action pattern may be used as a basis for the determination of the $\overline{d.p.}_n$ of linear, amylose-type polysaccharides. Banks and Greenwood[4] used a method based on the degradation of amylose chains with beta-amylase. High concentrations of this enzyme produce maltose and D-glucose from chains having even and odd numbers of D-glucose residues, respectively. The $\overline{d.p.}_n$ is then calculated from the concentration of D-glucose and maltose produced, on the assumption that the polysaccharide contains an equal number of molecules having even and odd numbers of D-glucose residues.

The use of phosphorylase instead of beta-amylase eliminates this assumption because phosphorylase will degrade every chain to maltotetraose ($G_4$) in addition to D-glucose 1-phosphate (G-1-P). If the amount of $G_4$ produced can be determined and related to total polysaccharide concentration, then $\overline{d.p.}_n$ is given by $[G_t]/[G_4]$, where $[G_t]$ and $[G_4]$ are the molar concentrations of total D-glucose and maltotetraose, respectively.

TABLE I

$\overline{D.P.}_n$ OF THE LINEAR GLUCANS

| | $G_6$ | $G_7$ | *Amylose samples* | | | | |
|---|---|---|---|---|---|---|---|
| | | | *1* | *2* | *3* | *4* | *5* |
| $\overline{D.p.}_n$ by present method | 6.1 | 7.1 | 165 | 215 | 315 | 586 | 830 |
| $\overline{D.p.}_n$ by Banks and Greenwood method[4] | | [a] | 161 | 218 | 312 | 572 | 818 |

[a]Not determined.

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Table I shows the values of $\overline{d.p.}_n$ determined by the present and previous methods. The present method has two advantages over the method of Banks and Greenwood. First, every chain produces four molecules of D-glucose; therefore, this method is 8 times more sensitive. Second, very small amounts (0.5 mg) of polysaccharide are required for the determination. Moreover, the method may be applied to determine the average chain-length ($\overline{c.l.}_n$) of the branched, amylopectin-type polysaccharides, provided they are completely debranched before degradation with phosphorylase.

## EXPERIMENTAL

Samples of linear amylose were obtained by leaching potato-starch granules[5] followed by degradation with bacterial alpha-amylase. Samples were taken out at intervals and the amylose was precipitated as the amylose–1-butanol complex. The samples were redissolved in water.

Maltohexaose ($G_6$) and maltoheptaose ($G_7$) were prepared by incubating amylose with bacterial alpha-amylase for a longer period, and $G_6$ and $G_7$ were separated chromatographically. The samples were purified by rechromatography and freeze-dried.

Potato phosphorylase (EC 2.4.1.1), prepared as described by Lee[6], was free from alpha-amylase activity. Amyloglucosidase [(1→4)-α-D-glucan glucohydrolase, EC 3.2.1.3], D-glucose oxidase (EC 1.1.3.4), peroxidase (EC 1.11.1.7), bacterial alpha-amylase (EC 3.2.1.1), and beta-amylase (EC 3.2.1.2) were obtained from Calbiochem.

*Preliminary experiments.* — $G_4$ can be determined by treatment with amyloglucosidase and assay of the D-glucose released (D-glucose oxidase method[4]). The total concentration ($G_t$) of the polysaccharide can also be obtained enzymically by the same method after acid hydrolysis of the ester phosphate group. The accuracy of the method depends upon the determination of D-glucose in the presence of large amounts of D-glucose 1-phosphate (G-1-P). There are two possible sources of error: (*1*) G-1-P might be degraded by the amyloglucosidase, (*2*) the determination of D-glucose involves two impure enzymes, D-glucose oxidase and peroxidase, and G-1-P might be hydrolysed by an impurity. The following experiment was designed to check this possibility.

A solution (5 ml) of G-1-P (1 mg/ml) containing 0.1M acetate buffer (pH 4.8, 1 ml) and amyloglucosidase solution (0.1 ml, 1–2 units) was incubated at 37° for 22 h. Assay by the D-glucose oxidase method then revealed that no D-glucose had been formed. Thus, amyloglucosidase can be used for the selective degradation of $G_4$ in the presence of G-1-P. Moreover, the D-glucose oxidase reagent did not interfere in the assay. Also, the assay of D-glucose was not affected by the presence of G-1-P.

*Determination of $\overline{d.p.}_n$.* — (*a*) *Determination of $G_4$.* Digests for $G_6$ contained a solution (0.5 ml) of $G_6$ (1 mg/ml), 0.1M phosphate buffer (pH 6.3, 0.2 ml), and phosphorylase solution (0.2 ml, 350 units/ml)[6]. Similar digests were set up for $G_7$

and amylose. The digests were incubated at 37° for 18 h and the pH was then adjusted to 4.8 by the addition of 0.1M acetate buffer (pH 4.8, 0.5 ml). Amyloglucosidase solution (0.1 ml, 1.2 units)[4] was added, and each digest was diluted to 5 ml with distilled water and incubated at 37° for 4 h. The D-glucose produced was determined by the D-glucose oxidase method.

(*b*) *Determination of* $G_t$. A portion (1 ml) of each of the above digests was hydrolysed with 0.25M $H_2SO_4$ (1 ml) at ~100° for 1.5 h, the acid was then neutralized, and the D-glucose produced was determined as in (*a*).

$\overline{D.p.}_n$ was also determined by degrading the samples with beta-amylase, as described by Banks and Greenwood[4].

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