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Note

Molecular weight analysis of starch polysaccharides using cross-linked allyl-dextran gels

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Native starch granules are composed mainly of two polysaccharides, amylose and amylopectin. The proportions of these two components depend on the origin of the plant species and its variety. The heterogeneity of the molecular weight range and the kind of branched structures in the polymers are also determined by their origin. Cereal starches, *e.g.*, maize or wheat differ quite a lot in the molecular weight distribution of their amyloses when compared with tuber or root amyloses as found in potatoes or tapioka[1,2].

The determination of molecular weight distributions is frequently carried out by gel permeation chromatography (GPC). Commonly used gel types are dextran or agarose gels or porous glass[3–11]. Recent articles have described the use of high-performance gel chromatography for the determination of starches and related polysaccharides[12–17].

This article describes the application of a new type of gel for the separation and determination of native starches.

MATERIALS AND METHODS

Native starches from maize and potato were isolated from fresh corn and potato tubers[18]. Preparative fractionation of amylose and amylopectin was carried out with *n*-butanol in the usual way[19,20]. For the separation of potato starch, a system consisting of two columns, Sephacryl gels S-500 and S-1000 (Pharmacia), each 90 × 1.6 cm I.D., was used. For maize starch a system of three columns containing Sephacryl gel S-400 (60 × 1.6 cm I.D.), S-500 (67 × 1.6 cm I.D.) and S-1000 (135 × 1.6 cm I.D.) was used.

All columns were operated with adaptors to minimize the void volume. Chromatographic separations were performed at room temperature with 0.005 *M* sodium hydroxide containing 0.002% sodium azide as eluent, flow-rate 25 ml/h. A peristaltic pump P-1 (Pharmacia), a Waters refractive index (RI) detector 403 and an Omniscribe recorder (Houston Instruments) were employed. The eluate was sampled in 3-ml fractions on an Ultrorac 700 fraction collector (LKB). The total carbohydrate content was determined with the anthrone method[21], amylose and amylopectin being distinguished by iodine staining[8,22].

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Void volume (V_0) was determined with a modified, high-molecular-weight amylopectin[22]; total volume (V_t) was determined with glucose.

Starch samples (30 mg) were swelled in 1 ml dimethyl sulphoxide (DMSO) and diluted in eluent to 3 ml, centrifuged and 1 ml of the supernatant was injected into the column system with a loop valve.

Calibration

The column systems were calibrated with synthetic amyloses having a defined, narrow distribution in the low-molecular-weight range[2]. For calibration of the high-molecular-weight range, a broader distributed amylose was synthesized by a modified procedure[22]. Its molecular mass-distribution function was determined by preparative fractionation of the amylose-tricarbanilate[23], and by viscosimetric determination with a structure viscosimeter modified according to Schurz[24,25]. The viscosimetric integral mass-distribution was then correlated with the integral mass-distribution function obtained by GPC. Statistical evaluation of the chromatograms was performed with the method of Schulz[26,27]. Fig. 1 shows the calibration curve for the S-400/S-500/S-1000 column system obtained as described. The S-500/S-1000 system shows a similar calibration curve, but compressed in the low-molecular-weight range.

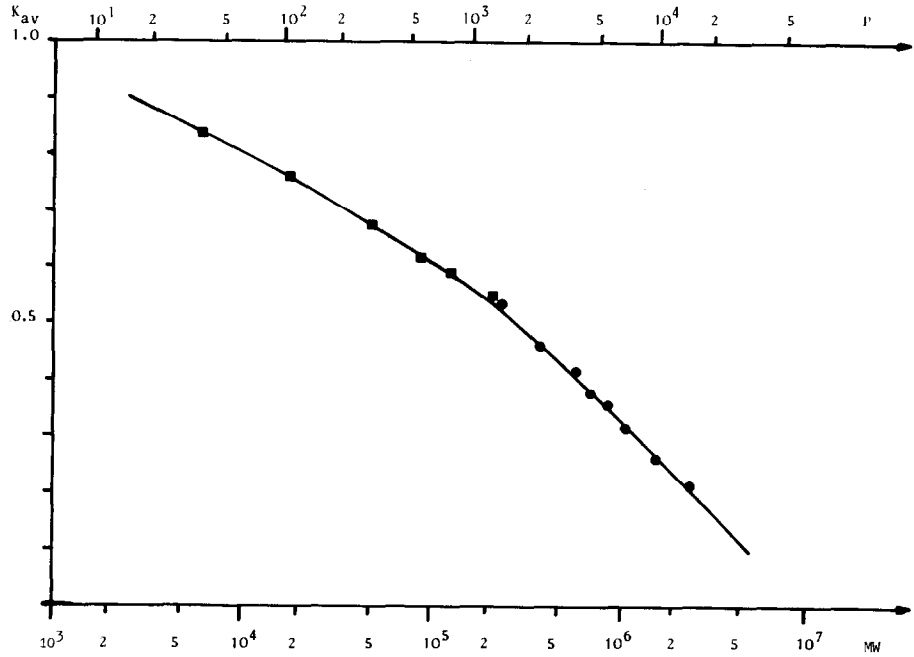

Fig. 1. Calibration curve for the Sephacryl gel S-400/S-500/S-1000 column system: relationship between the distribution coefficient, K_{av}, the molecular weight, MW, and the degree of polymerization, P. ■, Synthetic molecular-homogeneous amyloses; ●, molecular weights obtained from the viscosimetrically determined integral mass-distribution function of a high-molecular-weight synthetic amylose.

RESULTS AND DISCUSSION

The special qualities of the Sephacryl gels, *i.e.* mechanical stability even at high flow-rates and good separation characteristics, allow exactly reproducible separations of high-molecular-weight viscous polysaccharides[27]. Using the Sephacryl S-1000 column, a total separation of amylose and partially of amylopectin polymers is possible.

Fig. 2 shows the GPC separation of native potato starch. There is considerable overlapping of the amylose and amylopectin components, which can be visualized by iodine staining[8,22]. For a more efficient evaluation of the molecular weight distribution of the amylose polysaccharides a prefractionation with *n*-butanol is advantageous. The chromatogram of the prefractionated amylose, Fig. 2b, still shows amylopectin as revealed by iodine staining. After mathematical correction for the amylopectin portion, the molecular weight distribution of amylose is sufficiently precise. Because of the high-molecular-weight distribution of potato amylose, the Sephacryl S-500/S-1000 column system is suitable.

The native starch of maize can be separated totally by GPC (Fig. 3b). It appears that the amylopectin is mostly excluded whereas the lower-molecular-weight amylose is optimally separated on the Sephacryl S-400/S-500/S-1000 system. Besides the determination of the molecular weight distribution, a quantitative statement on the content of amylose and amylopectin is also possible. For a more detailed exam-

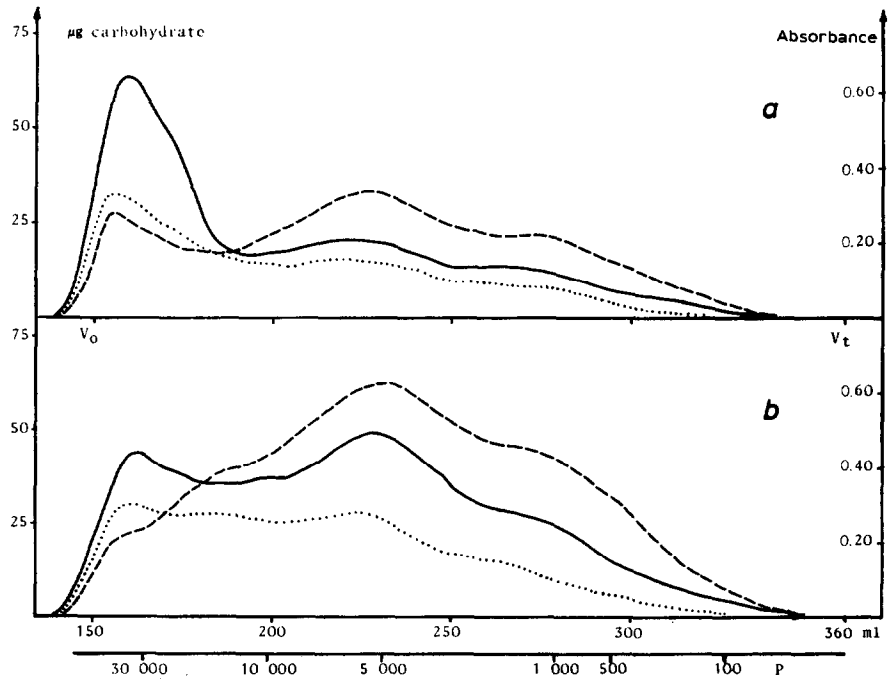

Fig. 2. GPC separation of potato starch on the Sephacryl gel S-500/S-1000 column system. For chromatographic conditions see Materials and methods. (a) Native starch; (b) *n*-butanol-precipitated amylose. ——, µg carbohydrates/ml; . . . . . , iodine colouring absorbance at 525 nm; – – –, iodine colouring absorbance at 640 nm.

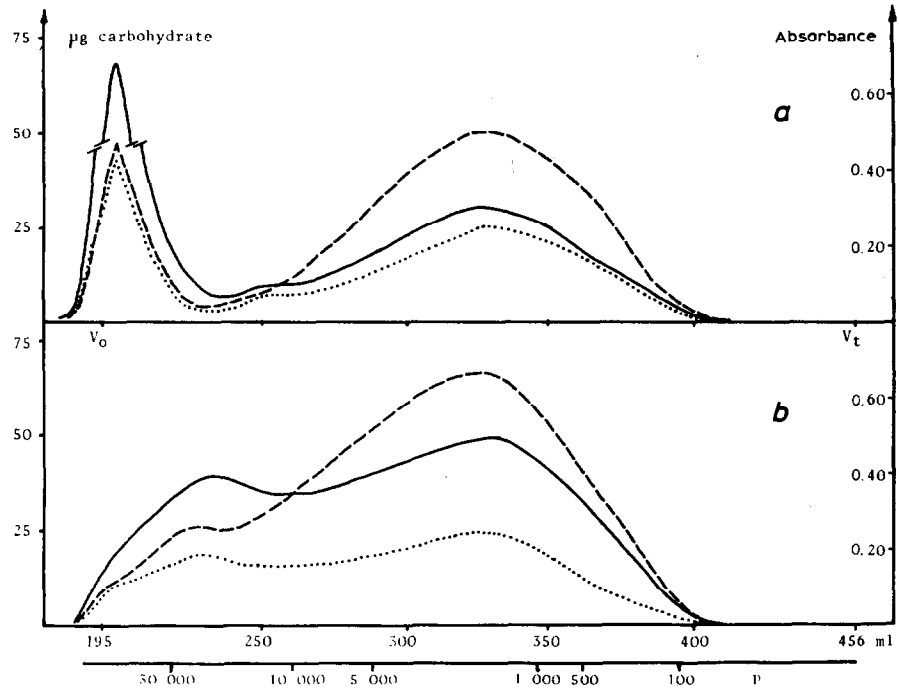

Fig. 3. GPC separation of maize starch on the Sephacryl gel S-400/S-500/S-1000 column system. For chromatographic conditions see Materials and methods. Other details as in Fig. 2.

TABLE I

WEIGHT-AVERAGE ($\overline{MW}_w$) AND NUMBER-AVERAGE ($\overline{MW}_n$) MOLECULAR WEIGHTS, WEIGHT-AVERAGE ($\bar{P}_w$) AND NUMBER-AVERAGE ($\bar{P}_n$) DEGREES OF POLYMERIZATION AND DISPERSITY FACTORS ($\bar{P}_w/\bar{P}_n$) OF POTATO AND MAIZE STARCH

	$\overline{MW}_w$	$\overline{MW}_n$	$\bar{P}_w$	$\bar{P}_n$	$\bar{P}_w/\bar{P}_n$
Potato amylose	$1.05 \cdot 10^6$	$0.32 \cdot 10^6$	6480	1970	3.3
Maize amylose	$0.63 \cdot 10^6$	$0.27 \cdot 10^6$	3850	1700	2.3

ination of maize amylose, the main portion of amylopectin is removed by *n*-butanol precipitation (Fig. 3b) of the amylose as usual. From the molecular weight distribution the number- and weight-average molecular weights and the dispersity factor can be calculated. The molecular parameters for both samples are given in Table I.

Since the Sephacryl gels, particularly S-1000, have excellent separation qualities in the high-molecular-weight range and long-term stability they can also be used for the preparative purification of amylose and amylopectin. These pure polysaccharides can then be used for structural, enzymatic studies leading to a better characterization of the starch components[9,20,22]. This seems to be a major advantage compared with merely analytical HPLC methods.

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