

Structure of amylopectin

IV.* The α -amylase dextrans and the structure of amylopectin of sorghum starch

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Dedicated to Dr. J. Tamchyna, in honour of his 85th birthday

α -Amylase dextrans were prepared from the β -limit dextrin isolated from sorghum starch. The title compounds separated by gel chromatography on Sephadex G-25 were characterized by estimating their relative molecular masses and formic acid arising in periodate oxidation. The enzyme susceptibility of the original starch was measured; it was found that linear sections of uniform length and a small occurrence of sites with double branching were present in the amylopectin molecule of sorghum starch.

Our preceding papers concerned the investigation of potato [1], wheat and maize [2] and barley [3] starches. We described the distribution pattern of internal chains in the amylopectin structure of the above-mentioned starches; these are rich in regions with a relatively high branching density and also contain longer linear sections forming amylopectin structure, the model of which has generally been denominated as cluster [4, 5]. Connection between the structure determined and the mechanism of biosynthesis of the starch components was presented [3].

This paper deals with the sorghum starch, interesting from several aspects [6—8]. Four laboratory samples (see Ref. [6]) were similar as for the composition (content of amylose in the range 16.42—17.97 mass %) and the course of amylolyses. In all cases their fractionation was accompanied with some problems and therefore, the classic approach [9] for separation of amylose and amylopectin was modified. The sorghum starch was found to peptidize with some relative difficulty, since a several-day action of alkali was needed and solvation of crude components in the purification process had to be carried out in dimethyl sulfoxide. This solvent accelerated considerably the preparation of solutions prior to centrifugation and transformation of components in solid form which were isolated in high purity.

* For Part III see Ref. [3].

The β -limit dextrin of relative molecular mass 330 000 was isolated in 55 % yield by β -amylolysis of amylopectin of sorghum starch. The α -limit dextrans, prepared from the β -limit dextrin by the action of α -amylase, were separated by column chromatography on Sephadex G-25 (Table 1) into a higher-molecular dextrin *I*, a lower-molecular dextrin *II* and by-products (maltose, a little amount of maltotriose, and traces of D-glucose).

Table 1

Characteristics of α -amylase dextrans

Sample	M_r	$\frac{\text{Yield}}{\%}$	DP	$\frac{n(\text{HCOOH})}{n(\text{GU})}$	$N(\text{NGU})$	ICL
Dextrin <i>I</i>	3730	5	23.0	0.30	4.9	1.5
Dextrin <i>II</i>	1840	8	11.5	0.40	2.6	1.5

The periodate oxidation was employed for characterization of dextrans prepared. The preceding studies on mechanism of periodate oxidation together with comparison of methods for determination of formic acid [10, 11] stimulated us to use again the periodate method enabling us to determine both the free formic acid and its bonded part [12]. The amount of formic acid being formed and the calculated characteristics of dextrans branching are listed in Table 1. Results and the determined relative molecular masses indicated a deep enzymic degradation of the β -limit dextrin up to limit α -amylase dextrans. These represent double branching points with such a short internal chain as not to be further enzyme-hydrolyzed.

Increase of the reduction power of the solution by α - and β -amylases action proceeded slower at the determination of enzyme susceptibility of sorghum starch when compared with that of potato starch (Fig. 1). Though the hydrolysis proceeded even in the later stages it did not reach such high values during a longer time of partial amylolysis as with the standard.

The course of β -amylolysis and the yield of β -limit dextrin at the determined average length of the chain indicated the mean length of the internal chain corresponding to 7–8 D-glucose units.

The potato amylopectin is characterized by a relatively remarkable non-uniformity of branching [1] and relatively long and short internal chains. Therefore, a very rapid α -amylolysis of long internal sections was taking place just at the beginning; this process was relatively soon stopped and the hydrolysis degree achieved did not rise any more. A more considerable amount of α -amylase macrodextrans was then found in products of partial amylolysis. On the

other hand, the α -amylase susceptibility of sorghum starch indicated a relatively equal lengths of internal chains which can be attacked by the enzyme; this facilitated a successive hydrolysis, almost uniform and in the limit deep, evidenced also by a low content of higher oligosaccharides in the hydrolyzate.

All these results show that the sorghum starch under investigation has a lower amylose content than that of currently occurring starch samples and has a usual ratio of the lengths of internal and external chains. The predominant

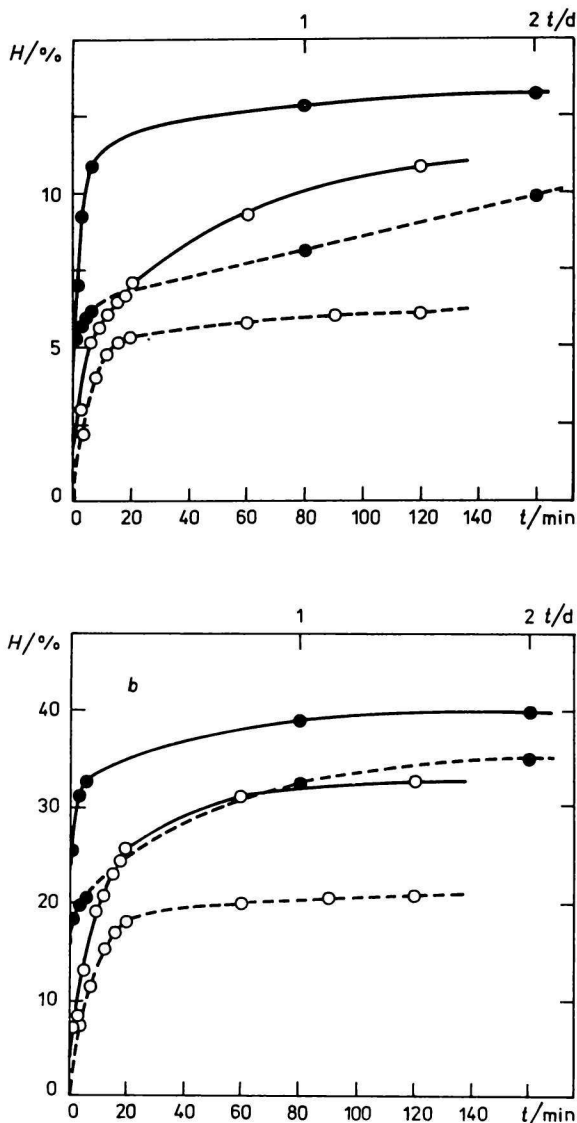


Fig. 1. Time dependence of sorghum and potato starches hydrolysis (H) by α -amylase (a) and β -amylase (b). — Potato starch, - - - sorghum starch, ○ points for the first two hours, ● points for two days.

part of internal chains has the same length close to that of the average value determined and only a small amount of short chains is statistically disseminated. These findings made the specific structure of sorghum starch different from substructural elements of other starches characterized by a considerable nonuniformity and nonhomogeneity of branching [1].

Experimental

Chemicals and material

Amylose content of the sorghum starch (laboratory preparation [6]) was $w = 16.5\%$ (iodometric determination with a biampereometric indication [13]). The soluble starch (anal. grade, Lachema, Brno) characterized in the laboratory by a blue colouration with iodine ($M_r = 5400$, *i.e.* $DP = 33.3$) was used for determination of activities and pH-optima of enzyme preparations. The potato starch (Slovenské škrobárne, Chynorany) served as reference. Standards for chromatography were the recrystallized D-glucose (anal. grade, Lachema, Brno) and sweetmeat sirup (Škrobárny, Brno) — standard for maltooligosaccharides.

α -Amylase from *Bacillus subtilis* (pH-optimum 6.1, activity 9.7 nkat mg^{-1} at 20°C and $\text{pH} = 6.5$), β -amylase of barley (pH-optimum 5.2, activity $22.7 \text{ nkat mg}^{-1}$ at 20°C and $\text{pH} = 5.1$) were commercial products (Koch-Light, Colnbrook). Their solutions were prepared in distilled water; phosphate or acetate buffers ($\text{pH} = 6.5$ for α -amylase, $\text{pH} = 5.5$ for β -amylase) were added to the substrate according to activity so as the reduction power increase would not exceed 10% of the maximal value within the first 15 min.

Other chemicals were of anal. grade.

Procedures and calculations

Oligosaccharides were chromatographed on Whatman No. 1 paper by a descending technique (over-flow 3—4 d) in acetone—water—butanol ($\varphi_r = 7 : 1 : 2$); detection with anilinium hydrogen phthalate [14].

α - and β -amylolyses were evaluated according to the increase of reduction power determined by *Somogyi* [15] and *Nelson* [16] on the basis of analytical lines determined for a chromatographically pure D-glucose at the working pH. The degree of hydrolysis catalyzed by α -amylase was evaluated by increase of the reduction power recalculated to the amount of D-glucose, with β -amylase to the amount of maltose. Procedures described in [2, 3] were applied for preparation of limit dextrans and their chromatography on Sephadex G-25 (column dimension $5 \text{ cm} \times 55 \text{ cm}$).

Amylopectin and α -amylase dextrans were oxidized with periodate according to [12]; coulometer OH-404 (Radelkis, Budapest) was used in the iodometric determination of

formic acid. The relative molecular masses (reduced to polymerization degree DP) were determined by sedimentation using the ultracentrifuge (MOM, Budapest) for the β -limit dextrin of amylopectin and by vapour osmometry (Knauer, Berlin) for α -amylase dextrans.

Values of analytical determinations of α -amylase dextrans are presented in Table 1. Following equations were used

$$N(\text{NGU}) = \frac{n(\text{HCOOH})}{n(\text{GU})} \text{DP} - 2; \quad \text{ICL} = \frac{\text{DP} - N(\text{NGU})(1 + \text{ECL})}{N(\text{NGU}) - 1}$$

where GU is the D-glucose unit, $N(\text{NGU})$ the number of nonreducing-end glucose units in molecule. ICL and ECL denote the average number of GU in internal and external chains, respectively; because the β -amylolysis of limit dextrans equals zero the ECL value is 2.5 GU.

Fractionation of the sorghum starch

Starch (2 g dry matter) was wetted by a little amount of water and stirred with 2 M-NaOH (50 cm³) at 5°C for 2 d. The solution being formed was neutralized with 2 M-HCl (indicator phenolphthalein), diluted to 1 dm³ and heated to 90–95°C. Butanol (100 cm³) was added and the mixture was left to stand at room temperature for 2 d. The solid amylose complex was centrifuged and amylopectin from the supernatant was precipitated with methanol (1.5 dm³). Both components were washed with methanol and ether and dried under diminished pressure.

Crude amylopectin was dissolved in dimethyl sulfoxide (5 cm³); water (20 cm³) and butanol (5 cm³) were then added, the mixture was centrifuged after 24 h and amylopectin precipitated by adding methanol was washed and dried as in the preceding case. Yield 1052 mg (63 % of the original content). Determination of the branching degree indicated the mean basal chain length to be 17 GU.

To crude amylose dissolved in dimethyl sulfoxide (5 cm³) water (20 cm³) was added at room temperature and butanol (5 cm³) at boiling. The mixture was cooled and left to stand for 3 d. The sediment was centrifuged off, washed gradually with methanol and ether and dried under reduced pressure. Yield 205 mg (62.1 % of the original content); purity 98.5 %.

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