Structure of amylopectin. II.* α-Amylase macrodextrin from wheat and maize starch

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Partial α-amylolysis of wheat starch and chromatographic separation of the obtained mixture of saccharides afforded macrodextrins. These were characterized by molecular weight, periodate oxidation, and α- and β-amylolysis. The determined structure and the branching density were compared with the known macrodextrins from potato starch.

Wheat macrodextrins contain on average 3—5 internal chains 6.0—8.5 glucose units long. The structure and yield of macrodextrins point to the irregularity and heterogeneity of branching of native amylopectin. Macrodextrins are not formed from maize starch, which provides evidence for the more regularly branched amylopectin in maize.

Although a considerable progress has been made in the study of secondary and supermolecular structure of the starch components, their biosynthesis and the structure of a starch granule, much remains to be discovered about the primary structure of amylopectin. The trend in the development of our understanding of the structure of branched α-glucans of the starch type (amylopectin and glycogen) as well as opinions of some authors about these problems have been summed up and discussed in [1].

Previously only average lengths of internal chains have been considered. It was not until the period between 1960 and 1974 that the papers dealing also with irregular branching appeared gradually [1—6]. Basing on the sorption of iodine with amylopectin, *Ohashi* [2] assumed the presence of longer linear chains in the molecule. *Roberts* and *Whelan* [3] proved by isolating α-limit dextrin with two branch points close one to another the existence of very short internal chains in the structure of amylopectin. The irregularity of branching supports also the result of enzymic degradation of starch and β-limit dextrin by isoamylase and pullulanase [4—6]; here by cleavage of branched glycosidic bonds the linear chains of different length are set free. The authors assumed that the irregularity might be caused by varying chain branching of the amylopectin molecule; they also supposed the branched starch component to be a mixture of polysaccharides of different degree of branching, *i.e.* the presence of molecules with the structure of amylopectin and glycogen.

Our finding that from potato starch and its β-limit dextrin, macrodextrins with relatively short internal chains are obtained by partial α-amylolysis [1] agrees well with the mentioned facts. On this basis and from a relatively high yield of macrodextrins we concluded that the heterogeneity of branching, *i.e.* the presence of

* For Part I see Ref. [1].

longer linear sections and areas with high branching density, is a characteristic feature of the structure of amylopectin.

The irregularity of the internal chains was confirmed unambiguously by the quoted papers. We realized that both the content and the structure of starch components of different plant origin might differ. Therefore we tried to prepare macrodextrins from wheat and maize starch.

Experimental

The wheat starch was a product of the Slovak Starchproducing Factory in Štúrovo and the maize starch in Boleráz.

β-Amylase from barley and α-amylase were the products of Koch-Light Laboratories, Colnbrook. Solutions of enzymes (1—10 mg in 10 ml) were prepared in distilled water with addition of phosphate buffer (1 ml) of pH 6.8; they were added to substrates in such amounts so that the increase of the reducing power would not exceed 10% of maximum hydrolysis within 10 min.

Other chemicals were of anal. grade.

Molecular weight determination was done on a Knauer apparatus by vapour osmometry.

α- And β-amylolysis determination, preparation of β-limit dextrin and α-amylase macrodextrin, as well as chromatography on a Sephadex G-25 (column size 5 × 55 cm) were performed as described in [1]. When chromatographing α-amylolyzed wheat starch (6.0 g), the first 150 ml was saccharides free, next 100-ml fraction containing macrodextrins was freeze-dried, and further fractions contained oligosaccharides and sodium chloride. Lyophilizate (0.38 g) was dissolved in water (5 ml) and rechromatographed. After 230 ml saccharides free eluate, 50-ml fraction of macrodextrin *IV* (0.25 g) and 30-ml fraction of macrodextrin *V* (0.13 g) were collected. Only a small amount of substance that was not worked up was obtained from β-limit dextrin of wheat starch by α-amylolysis. We failed in isolating any high-molecular highly branched fraction from maize starch.

Periodate oxidation was performed according to [7] and the methods reported in this paper were used for individual determinations.

The obtained values of determinations and the yield of macrodextrins are in Table 1. Periodate consumption in the macrodextrin oxidation was in line with the amount of formic acid released.

Table 2 contains the calculated values of the number of chains in a molecule (calculated also for potato macrodextrins *I*—*III* described in [1]) and their average lengths. The calculation was done according to the following formulas

$$\mathrm{NGU} = \left(\frac{\mathrm{HCOOH}}{\mathrm{GU}} \times \mathrm{DP}\right) - 2, \qquad \mathrm{ECL} = \frac{\mathrm{DP} \times \beta\text{-amylolysis}}{\mathrm{NGU} \times 100} + 2.5,$$

$$\mathrm{ICL} = \frac{\mathrm{DP} - \mathrm{NGU}(1 + \mathrm{ECL})}{\mathrm{NGU} - 1}.$$

Discussion

When preparing macrodextrins from wheat and maize starch, we succeeded only in the first case: we did not obtain macrodextrins with a high degree of branching from maize starch. Macrodextrin from wheat β-limit dextrin was obtained only in

a very low yield and thus the fractionation and structure determination could not be performed.

By α-amylolysis from wheat starch a mixture of saccharides was prepared. They were fractionated by repeated chromatography on a column of Sephadex to give two fractions of macrodextrins. The structure of macrodextrins was determined from the periodate consumption during oxidation and from the amount of formic acid released, from the degree of α- and β-amylolysis and by the molecular weight determination (Table 1).

In calculating the number of non-reducing end glucose units (number of external chains) we took a correction for the present reducing glucose unit (which releases two molecules of formic acid). In the calculation of the length of internal chains we did not start from the average length of the basic chain but we took into account that the number of internal chains is by a unit lower than the number of external ones. Table 2 covers also improved values for macrodextrins from potato starch described in [1]. In this paper [1] also the glucose units forming the branch point were included into internal chains.

The higher molecular wheat macrodextrin *IV* has a lower branching density (longer internal chains; Table 2) and more chains than the lower molecular *V* The average length of internal chains is in both cases longer (twofold) than with potato macrodextrins, the length of which varies within the range of one glucose unit. External chains in all macrodextrins differ virtually within this region (one glucose unit) and

Table 1

Characterization of wheat macrodextrins

Macrodextrin	Yield [%]	M_n	α-Amylolysis [%]	β-Amylolysis [%]	HCOOH per GU
IV	6.0	10 900	33.75	16.00	0.11
V	3.5	5 370	32.25	13.25	0.17

GU — glucose unit.

Table 2

Calculated values of the number and size of chains

Macrodextrin	DP [GU]	NGU	BCL [GU]	ECL [GU]	ICL [GU]
I	46	4.90	9.40	5.00	4.25
II	35	4.65	7.55	4.35	2.75
III	32	4.72	6.80	3.45*	2.95
IV	67	5.37	12.50	4.50	8.55
V	33	3.61	9.15	3.70	6.15

DP — degree of polymerization, NGU — number of non-reducing end glucose units, BCL — basic chain length, ECL — external chain length, ICL — internal chain length.
*In [1] — misprint.

also the average number of chains in the molecule, excepting macrodextrin *V*, is approximately equal.

Isolation of macrodextrins helps to prove the irregularity of branching, *i.e.* the presence of chains of different length in the structure of amylopectin. Of all proofs, this is the only one that informs about the mutual arrangement of chains in the molecule, about irregular distribution of chains of different length. This means that some short chains are concentrated in the areas with higher branching density; we speak of the heterogeneity of branching. A relatively high degree of branching of macrodextrins determined indicated that in amylopectin there are also areas of different branching density.

On this basis we proposed a new model of the structure of amylopectin [1]. Not each kind of starch has the same heterogeneity. The model is convenient for the potato amylopectin, with some corrections also for wheat, but not for the maize amylopectin. This is shown by both the yields of macrodextrins of particular starches (in the case of maize starch they were not isolated, in the case of wheat starch it was about 10%, and about 20% of potato starch) and the length of internal chains of individual macrodextrins — 6.0 to 8.5 and 3 to 4 glucose units of wheat and potato starch respectively.

The determination of the heterogeneity of branching may be thus used as a new characteristic of starches or polysaccharide fractions; this can be useful in the choice of starches for special application.

In no case was the high-molecular highly branched macrodextrin isolated, this being in agreement with paper [8]; here it was reported that amylopectin affords only a negligible amount of α-amylase macrodextrin of the maximum polymerization degree between 50 and 80. It can be therefore concluded that in the starches investigated there is no fraction of polysaccharide of glycogen structure and that the existence of phytoglycogen in sweet maize is exceptional (the study of other sorts of maize has shown that they do not contain phytoglycogen [9]). Neither the regular dichotomic branching according to *Frey—Wyssling* [10] seems to be probable.

With regard to the fact that the kinds of maize are highly amylose as well as waxy, that the maize phytoglycogen and the fraction with transition structure between amylose and amylopectin with a very low degree of branching are known, it is evident that the determined fine structure of heterogeneously branched amylopectin concerns the concrete sample of starch and cannot be generalized for the time being.

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