Structure of amylopectin. III. α-Amylase macrodextrin of some barley starches

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By partial α-amylolysis of starches from three sorts of barley followed by separation of products on Sephadex columns macrodextrins were obtained and characterized by determination of molecular weight, periodate oxidation, and β-amylolysis. The determined degree of branching was compared with the same characteristic of the other plant starches.

The structure of isolated barley macrodextrins indicates the irregular and heterogeneous branching in the starting amylopectins. The obtained results are discussed with respect to the mode of biosynthesis of amylopectin.

Были приготовлены макродекстрины ячменя в результате частичного действия α-амилазы на крахмал трех сортов ячменя и гель-хроматографического разделения на Сефадексе. Они были охарактеризованы на основании определений молекулярного веса, окисления периодатом и действия β-амилазы. Найденная степень разветвления сравнивалась со значениями α-макродекстринов других растительных крахмалов.

Структура α-макродекстринов ячменя обнаруживает неупорядоченное и гетерогенное разветвление исходных амилопектинов. Полученные результаты обсуждаются с точки зрения биосинтеза амилопектина.

In our previous studies [1, 2] we determined and compared the structures of potato, wheat, and corn α-macrodextrins. The results indicated irregularity of branching, *i.e.* the presence of linear chains of different length in the molecule of amylopectin. The amylopectins exhibited also the characteristic heterogeneity of branching, namely the concentration of shorter chains to the regions with higher degree of branching in their molecule.

Based on the method of preparation and the determined structure of α-macrodextrins we proposed a structural model of amylopectin [1]. The suggested structure is in full accordance with the structure of amylopectin deduced on the basis of the results of enzymatic degradation of Φ- and β-macrodextrins prepared by degradation of amylopectin and glycogen with isoamylase and pullulanase [3].

In the present study we subjected to partial α-amylolysis the starches obtained from three sorts of barley. From the products of α-amylolysis we isolated by means of gel chromatography α-macrodextrins and determined their structures. The latter served us as a basis for the determination of structural features of barley amylopectin.

Experimental

The samples of barley starches were prepared in the Research Institute for Grain Cultivation in Kroměříž from different barley sorts and designed as Merkur 65, Peroga 65, and Valtický 66. *Bacillus subtilis* α-amylase and barley β-amylase were purchased from the Koch-Light Laboratories, Colnbrook, England. All other chemicals used were of anal. grade.

The amylose contents in starch samples were determined iodometrically with biamperometric indication [4]. Molecular weights were determined by a vapour osmometry in the osmometer Knauer, West Germany.

The preparation of α-macrodextrins from barley starches (always 5 g of dry starch used), their purification by gel chromatography and rechromatography on Sephadex G-25 (column size 55 × 5 cm) were performed as described previously [2].

The 150 ml carbohydrate fractions appearing after the void volume in the first chromatography were twice rechromatographed in order to remove the low-molecular weight oligosaccharides. From the diffuse carbohydrate peaks after the second rechromatography the first 50 ml of the eluate were pooled, while the remaining 80 ml contained higher oligosaccharides in the case of Merkur starch. In cases of Peroga and Valtický starches from the 100 ml carbohydrate containing diffuse peaks always the first 50 ml were taken as macrodextrin portions.

Determination of the extent of β-amylolysis (expressed in %) was performed as in [2], the reducing power was measured according to *Nelson* [5] and *Somogyi* [6]. Periodate oxidation and determination of formic acid was done as previously described [7].

The results of analytical determinations and calculations of the characteristic values for macrodextrins are summarized in Tables 1 and 2. The average degree of polymerization (DP) of isolated oligosaccharides was 8.8 and the average number of nonreducing end

Table 1

Starting starches and the prepared macrodextrins

Barley starch	Amylose content %	Yield of macrodextrins		M	DP
		mg	%		
Merkur	22.7	300	6.0	1945	11.9
Peroga	19.6	360	7.2	1645	16.2
Valtický	21.8	357	7.1	2660	16.3

Table 2

Characterization of prepared macrodextrins

Macrodextrin	$NaIO_4$/GU moles	HCOOH/GU moles	β %	NGU	ECL GU	ICL GU
Merkur	1.32	0.37	10.26	2.40	3.05	1.55
Peroga	1.23	0.28	9.99	2.55	3.25	3.50
Valtický	1.31	0.34	8.99	3.55	2.95	0.90

glucose units (NGU) was 1.54. For the calculation of NGU, average external chain length (ECL), and average internal chain length (ICL) the following equations were used

$$\mathrm{NGU} = \left(\frac{\mathrm{HCOOH}}{\mathrm{GU}} \times \mathrm{DP}\right) - 2$$

$$\mathrm{ECL} = \frac{\mathrm{DP} \times \beta}{\mathrm{NGU} \times 100} + 2.5$$

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

where HCOOH/GU are moles of formic acid released per mole of glucose and β is the percentual yield of β-amylolysis.

Discussion

The α-amylolysis of starches obtained from three different sorts of barley followed by gel chromatography afforded α-amylase macrodextrins that were characterized by their molecular weight, periodate oxidation — determination of the oxidant consumption and the amount of released formic acid — and by β-amylolysis. The periodate consumption corresponded to the amount of released formic acid. The results of the individual determinations as well as the yields of macrodextrins and the amounts of amylose in the starting starches are summarized in Tables 1 and 2. From the analytical data of the individual macrodextrins we calculated the average chain length of the inner and the outer chains, and the number of branches in the molecules of macrodextrins (Table 2). The calculations took into account the amount of formic acid released by periodate oxidation per mole of reducing end glucose units (RGU) as well as the fact that the number of branching points is by one unit lower than the number of nonreducing end glucose units.

Owing to that the isolated fractions had low-molecular weight due to the low number of inner chains and to their relative shortness, it was not possible to isolate absolutely homogeneous products. It is for these reasons that the isolated macrodextrins were apparently contaminated with low-molecular weight dextrins. The inner chains (the sections between two branching points) contained 1 to 4 glucose units; one molecule of isolated polysaccharides contained 1 to 3 such inner chains.

The degree of branching in the isolated barley macrodextrins was higher (the lower average ICL) than in the amylopectins of some other plant species studied earlier [1, 2]. The yields of macrodextrins (7.8—9.1% when related to amylopectin) as well as the isolation of oligosaccharides containing a single branch point are indicating that the structure of amylopectin contains in addition to individual branches with longer linear chains also the regions with high degree of branching. This fact indicates that the barley amylopectin is markedly inhomogeneously branched.

The macrodextrins of the individual barley sorts exhibited only minor differences so that the described structural feature of amylopectin is characteristic of all investigated barley starches. This characteristic supports the structural model of amylopectin [1] and also is compatible with the results of acid hydrolysis of amylopectin, its degradation with pullulanase and β-amylase as well as with the structure of the products of these degradations isolated by gel filtration on Sephadex [8]. The obtained macrodextrins represent the isolated regions with high degree of branching in the amylopectin molecule (Fig. 1).

The obtained results indicate that the inhomogeneity of branching is characteristic of the molecule of amylopectin. This property of amylopectin gives the possibility to use the variability in the inhomogeneity of branching for the characterization of starches of different origin or of starches obtained from different sorts of the same plant species. The knowledge of the primary structure of amylopectin gives the possibility to learn its secondary and tertiary structures and the structure of the starch grain, mode of deposition of amylopectin in the starch grains as well as its aggregation with the linear constituent; the detailed structure in turn determines the physicochemical properties of the starch and its behaviour in the solution.

The inhomogeneous branching of the amylopectin structure can also help to understand the mode of biosynthesis of the starch. The study of the mechanism of action of the branching enzyme (Q-enzyme) with amylose [9] has shown that the actual substrate of the branching enzyme is an associate consisting of two linear amylose molecules. The association of amylose (retrogradation in solution) has been investigated in our previous studies [10, 11]. We have found that the association of the amylose molecules proceeds continually as a two-step process; in the first step the association takes place in the solution until the amylose associate

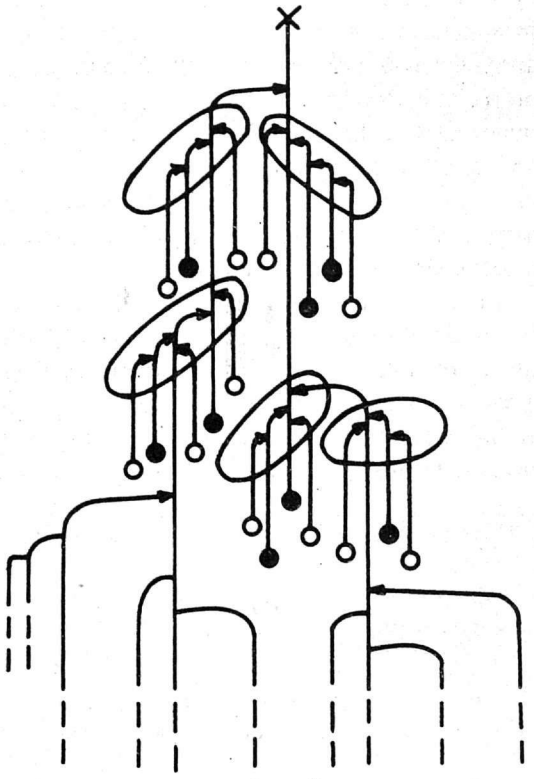

Fig. 1. Model of the detailed structure of amylopectin.
× RGU; ○ NGU (*A*); ● NGU (*B*).
——— Linear chain with α-1,4-linkages;
branching point (α-1,6-linkage);
— - - continuing chains;
region with high density of branching (origin of α-macrodextrins).

reaches certain critical size; from then on the associate precipitates and further association proceeds on the surface of the solid phase.

It can be assumed that the formation of starch (the biosynthesis of the polysaccharide and the formation of starch granules) proceeds in such a way that the formed amylose molecules after reaching certain size begin, depending on the physicochemical conditions and other factors, to associate. Under the catalytic action of branching enzyme several consecutive branches are formed between the two associated amylose molecules giving rise to a densely branched structure. The branching increases the water-solubility of the associate, which can lead to its dissociation. In the formed polysaccharide structure containing multiply branches

are the outer chains further lengthened until the linear regions reach certain size and associate again and the whole process is repeated. In case that an associate containing several amylose molecules is formed the branching enzyme is ineffective and the amylose precipitates in the form of solid aggregate.

The presented hypothesis on the mode of biosynthesis of starch granules is in agreement with the results described in [12] but it is oposed to *Erlander*'s hypothesis [13] that considers the formation of amylopectin as a result of debranching of glycogen. The results described in [12] were deduced on the basis of the ratio of *A* to *B* chains in the molecules of amylopectin and glycogen. *Gunja-Smith et al.* [3] assume that amylopectin and glycogen possess entirely unlike structures differing in the degree of branching as well as in the shape of the molecule — the glycogen molecule is a three-dimensional spheroid while the amylopectin molecule is a two-dimensional structure. A similar structure of amylopectin was, on the basis of viscosimetric and other physicochemical measurements and comparison of different amylopectins and glycogens, proposed also by *Greenwood* [14].

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