Structure of Amylopectin (I)
Preparation and Structure of α-Amylase Macrodextrin

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From the mixture of dextrins, prepared by α-amylolytic degradation of potato starch, two homogeneous macrodextrin fractions were separated on Sephadex G-25 column. Both were characterized by molecular weight estimation, periodate oxidation, α- and β-amylolysis, thereby determining the degree of branching and structure. Macrodextrins thus obtained are branched on each fourth, or third glucose unit, whereas branching points in native amylopectin are located on each fifth to ninth glucose unit, on average. It has been concluded that the heterogeneity of branching with longer, linear chains and areas of higher branching density is typical of the structure of amylopectin.

Starch consits of two polysaccharide constituents: amylose and amylopectin in the molecular ratio of 1 : 4 to 1 : 5. Amylose was shown to be a linear α-(1 → 4)-glucan, amylopectin is regarded as α-(1 → 4)-glucan, branched by α-(1 → 6)-glucosidic linkages. The average chain length of amylopectins of different vegetable origin varies from 20 to 27 glucose units, the external chain length being 15 to 18 glucose units, the internal chain being 5 to 9 glucose units.

The former structures *I* [1] and *II* [2] proposed in 1937 were corrected in 1940 by K. H. Meyer [3], who suggested structure *III* (see Fig. 1), as the formulae did not reflect any more the experimental information obtained in this time.

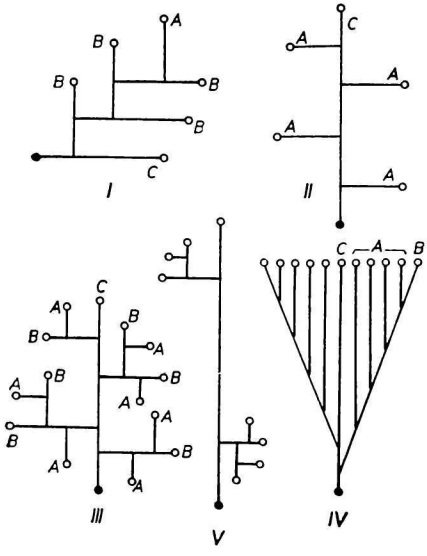

Fig. 1.

——— chain of glucose units bonded by α-(1 → 4)-glucosidic linkages;
⊥ branching of chains by α-(1 → 6)-glucosidic linkage;
● reducing terminal glucose unit;
○ non-reducing terminal glucose unit;
A, B, C chains distinguished according to branching.

All mentioned structures *I—III* differ in the ratio of chain A : B C. Based upon the latter papers, especially by Peat and Cori from 1950—1960 (cf. [4—7]) it has been evidenced by the use of various hydrolytic enzymes that structure *III* best portraits the physico-chemical properties of amylopectin and that is the reason why this structure is generally accepted nowadays. It has been assumed that the chain A : B ratio is approximately 1 1. Although these papers are in favour of Meyer's structure *III* they do not completly refute the dichotomously branched structure *IV*, as suggested for amylopectin by A. Frey-Wyssling [8] in which chain A considerably predominates over chain B.

In the structure of amylopectin *IV*, longer linear chains and sections of relative high branching density are present. This heterogeneity of branching is also possible in other structures suggested.

There are relatively few papers concerning the homogeneity and/or heterogeneity of amylopectin molecule branching. In favour of the heterogenetic branching is paper by P. J. P. Roberts and W. J. Whelan [9], who isolated from α-amylolyzed amylopectin chromatographically on charcoal with 50 % ethanol α-limit dextrins of polymerization degree 14.1. On treatment with R-enzyme the reduction power of these dextrins raised 3.44 times; the branching point was determined to be on each other glucose unit.

The branching heterogeneity of amylopectin molecule has been substantiated by K. Ohashi who prepared linear dextrins of polymerization degree 41, 34 and 28, respectively, and estimated their capability of iodine sorption; based upon this method he determined [10] amylose and linear dextrins in question in respect to each other in different kinds of starch vegetable in nature. In the next paper [11] he brought evidence that no linear dextrins but chain sections of amylopectin molecule, different in lenght are involved.

Recently, the heterogeneity of chain branching was established in the molecule of glycogen [12, 13]. These papers cover the total α-amylolysis of animal glycogen (from the rabbit liver and shellfish) and isolation of α-limit dextrins of polymerization degree 60—380 on a Sephadex G-50 column in considerable amount which were further α-amylolysis resistent. In these macrodextrins, 22—25 % of terminal glucose units (and thereby glucose units forming branching points) were determined which is indicative of two glucose units each in the internal and external chain. Starting from amylopectin, it has been succeded to isolate [12] a minute amount (0.4 %) of α-amylose macrodextrin of polymerization degree 50—80, whereas higher macrodextrins were not formed.

The branching heterogeneity in amylopectin is represented by Richter's formula *V* [14] which resulted from a combination of several structures so far presented.

Our attention was focused to the solution of the problem of branching heterogeneity in the amylopectin molecule. Consequently, the α-amylolysis of potato starch and its β-limit dextrin was done in order to obtain α-amylolytic dextrin (in top amounts). Their separation from lower oligosaccharides and salts, as well as the fractionation was performed by gel filtration on Sephadex G-25 column. We obtained two macrodextrins from potato starch and one macrodextrin from β-limit dextrin. The molecular weights were determined osmometrically, as they were on the limit of ultracentrifuge determination. Results are listed in Tab. 1.

Macrodextrins thus obtained were oxidized by periodate, and α- and β-amylolyzed. The amount of formic acid during the periodate oxidation, degree of limit enzymatic

cleaving and the average chain length calculated are listed in Tab. 2. (The amount of formic acid was approximated to the reducing terminal group.)

Dextrin *II* of lower polymerization degree (35) discloses the calculated amount of branching density as being higher and the α-amylolysis determined as being lower when compared with dextrin *I* of polymerization degree 50.

Table 1

Yield and Molecular Weight of Macrodextrins

No	Sample	Yield %	Molecular Weight (by means of ultracentrifuge)	Molecular Weight (osmometrically)	Polymerization Degree (osmometrically)
1	Macrodextrin *I*	8	8900	7560	46
2	Macrodextrin *II*	13	—	5720	35
3	Macrodextrin *III*	11	—	5160	32

α-Amylolytic macrodextrins *I* and *II* are products of potato starch, macrodextrin *III* was made from β-limit dextrin of potato starch. Yield is calculated on starch used.

Table 2

Characterization of Macrodextrins

No	Sample	α-Amylolysis %	β-Amylolysis %	HCOOH per GU	Average chain length in GU	External chain length in GU	Internal chain length in GU
1	Macrodextrin *I*	27.6	27.6	0.15	9.20	5.00	4.20
2	Macrodextrin *II*	13.3	24.6	0.19	7.50	4.35	3.15
3	Macrodextrin *III*	9.85	14.2	0.21	6.77	4.45	3.30

GU = stands for glucose unit.

When tracing the course of α-amylolysis of potato starch by paper chromatography at the beginning of the enzymatic reaction, one can prove the higher oligosaccharides only; however, α-amylolysis of macrodextrin *II* yields just from the beginning of the reaction maltose and maltotriose, whereas α-limit dextrin remained spotted on the starting point.

These results keeping in mind it could be concluded that in such a way prepared dextrins the branching point lies on each third or fourth glucose unit in contrast to the native amylopectin, where the branching point is located on each fifth to ninth glucose unit. This fact together with the high yield of dextrin prepared allowed to deduce that the heterogeneity of branching (i. e. the presence of longer linear sections and areas with higher branching density) is a characteristic feature for structure of amylopectin.

Experimental

Chemicals and Methods

The potato starch used was a product of Bohemian Starchproducing Factory, Brno, Dornych, extra fine quality.

Enzyme preparations: Suspension of crystalline α-amylase from hog pancreas, product of Reanal, Budapest; β-amylase, prepared in our Laboratory from soybean. Both preparations were properly characterized [15].

Sephadex G-25, product of Pharmacia, Uppsala.

Paper chromatography was run in the solvent system acetone—water—butanol 7 1 : 2 on Whatman paper No 1, visualization with anilinium hydrogen phthalate [16].

The diffusion coefficient was determined on a Zeiss electrophoretic apparatus 35. The sedimentation constant was estimated on a ultracentrifuge MOM 110. The value of the partial specific volume of this compound type is 0.62 [17]. The molecular weight determination was done osmometrically and tentatively estimated in single fractions by hypoiodite oxidation [18].

Preparation of Dextrins

Potato starch (6 g, i. e. 5 g dry basis) was suspended in water (300 ml) and while introducing nitrogen gas through the well stirred reaction mixture, 2 N-NaOH (50 ml) was added. The starch being dissolved, the solution was neutralized with 2 N-HCl (50 ml) and set to pH 6.8. A commercial preparation of α-amylase (66 mg suspended in 1 ml) was dissolved in a solution of 2 M urea (1 ml) and 7.0 phosphate buffer (2 ml) and added to the starch solution. Samples were withdrawn periodically to determine viscosity and reduction power. The reaction was through after 24 hours and within the additional 24 hours the increment of the reduction power was 1/2 % only. Sufficient activity of the enzyme present in the solution was checked by adding of an equal amount of α-amylase which did not result in any change within the next 24 hours.

The solution after amylolysis was evaporated to 200 ml, the enzyme was deactivated by heating at 100 °C, then cooled and centrifuged. Upon evaporation to 500 ml the supernatant contained NaCl and saccharides (about 6 g each).

Preparation of Dextrins from β-Limit Starch Dextrin by α-Amylase

To the solution of starch prepared as above a solution containing β-amylase (30 mg) was added. After 12 hours no increase of maltose content was observed and after 12 additional hours β-amylase was deactivated by heating. The α-amylolysis was proceeded as described in the preparation of dextrin from starch.

Separation of the Mixture of Dextrins

The solution of saccharides was poured on the Sephadex G-25 (column size 5 × 60 cm) prepared as described in [19]. Fractions (10 ml) were checked by anthrone and hypoiodite to estimate the contents of saccharides and their molecular weight, respectively. The first 200 ml were found to be saccharides free; further fractions contained dextrins with continuously decreasing polymerization degree (from 50 to 10, 370 ml total). The latter were combined to form 170 and 200 ml fraction and lyophilized. Elution with water was

continued: the next 100 ml of eluant was saccharide positive, further fractions began to be even chloride positive.

Lyophilized material was dissolved in water (15 ml each) and rechromatographed using the same column. The principal fractions were combined, lyophilized and rechromatographed once more. Fraction *I* of macrodextrins yielded 0.4 g (8 % starch basis), fraction *II* 0.65 g (13 %) as shown in Tab. 1. Macrodextrin from β-limit dextrin was obtained in 11 % yield (starch basis).

Periodate Oxidation of Macrodextrins

The periodate oxidation of macrodextrins was carried out in the dark using a 100 % excess of 0.02 M sodium metaperiodate for 24 hours at +2 °C. The amount of resulting formic acid was determined by iodometric titration with an amperometric indication [20].

Amylolysis of Macrodextrins

Amylolytic action of both α- and β-amylase over macrodextrins was performed as described in the case of wild apple phytoglycogen [21] and the increase of reducing groups was determined by a standard colorimetric method using dinitrosalicylic acid [22].

ŠTRUKTÚRA AMYLOPEKTÍNU (I)
PRÍPRAVA A ŠTRUKTÚRA α-AMYLÁZOVÉHO MAKRODEXTRÍNU

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α-Amylolytickým odbúraním škrobu sa pripravili dextríny a z ich zmesi sa na Sephadexe G-25 oddelili dve homogénne frakcie makrodextrínov. Tieto sa charakterizovali stanovením molekulovej váhy, jodistanovou oxidáciou, α-amylolýzou a β-amylolýzou, čím sa určil ich stupeň vetvenia a štruktúra. Získané makrodextríny sú vetvené v každej štvrtej, resp. tretej glukózovej jednotke oproti priemernému vetveniu v každej piatej až deviatej glukózovej jednotke natívneho amylopektínu. Vyvodzuje sa, že nehomogenita vetvenia s dlhšími lineárnymi reťazcami a oblasťami hustejšieho vetvenia je charakteristická pre štruktúru amylopektínu.

СТРУКТУРА АМИЛОПЕКТИНА (I)
ПОЛУЧЕНИЕ И СТРУКТУРА α-АМИЛАЗОВОГО МАКРОДЕКСТРИНА

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α-Амилолитическим расщеплением картофельного крахмала получились декстрины и из их смеси на Сефадексе G-25 отделились две гомогенные фракции макродекстринов. Эти охарактеризовались определением молекулярного веса, периодатным окислением, α- и β-амилолизом. Таким образом была определена степень разветвления и стрратуку.

Полученные макродекстрины разветвленные на каждой четвертой или третьей глюкозидной единице по сравнению с средним разветвлением на каждой пятой, даже девятой глюкозидной единице натурального амилопектина. Заключается, что неоднородность разветвления с более длинными линейными цепями и областями более плотного разветвления является характерной чертой структуры амилопектина.

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