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FINE STRUCTURE OF NÄGELI AMYLODEXTRIN OBTAINED BY ACID TREATMENT OF DEFATTED WAXY-MAIZE STARCH—STRUCTURAL EVIDENCE TO SUPPORT THE DOUBLE-HELIX HYPOTHESIS*

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ABSTRACT

Nägeli amylodextrin, acid-resistant polysaccharide, was obtained from waxy-maize starch after intensive, heterogeneous acid-hydrolysis. Amyloc extrin was fractionated by multiple-descending paper chromatography and the fine structure of each fraction analyzed by enzymic degradation with beta amylase, *Bacillus subtilis* saccharifying alpha amylase, and pullulanase. There were two main groups of polysaccharides in amylodextrin, one was d.p. 12–16 linear or glucosyl- or maltosyl-stubbed polysaccharide branched near the reducing end, the other was d.p. 28–30 in which two d.p. 14–16 unit-chains were linked by an α-D-(1→6) bond near the reducing end. The latter has a hairpin-like structure in which the parallel strands could easily intertwine. These structural features strongly support the possibility of a double-helix organization in the crystalline region of the starch molecule. Models of intertwined chains having d.p. 14–16 readily form double helices about 50-Å long, which corresponds to recent observations of amylodextrin by electron microscopy.

INTRODUCTION

In 1874, Nägeli described the preparation of acid resistant polysaccharides by protracted, heterogeneous hydrolysis of potato-starch granules in aqueous sulfuric acid at room temperature[1]. Materials obtained by this treatment are called amylo-

*Symbols and abbreviations: G_1, G_2, G_3, ... etc., denote glucose, maltose, maltotriose, and so on. B_3, B_4, ... are "branched" oligosaccharides containing a single α-(1→6) linkage. "Fraction *n*" indicates linear and branched maltosaccharides which move paper-chromatographically as fast as the linear maltosaccharide of d.p.$_n$. In symbolic formulae, O represents an α-D-glucopyranosyl unit, Ø represents a reducing-end D-glucose residue, —— is an α-(1→4) linkage, and ↓ is an α-(1→6) linkage. One International Unit (IU), as defined according to the International Commission on enzymes, is the amount of enzyme that will hydrolyze one micromole of glycosidic bonds per minute under optimal conditions.

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dextrins. Amylodextrins retain starch-granule form and give sharper X-ray patterns than those of the parent starch[2]. The average starch chains are 15–30 glucose residues in length[3] and are readily recrystallized from hot water or aqueous organic solvents[2].

Recently, several authors have described the structure of Nägeli amylodextrin and Lintnerized starch[3-6]. These materials may be regarded as low-molecular-weight models of native starch. Kainuma and French[3,4] studied the enzymic susceptibility and X-ray diffraction patterns of the Nägeli amylodextrins obtained from various kinds of starch, and proposed a left-handed, double helix as a hypothetical structure of the acid-resistant, crystalline part of B-starch. Later, the double-helix structure was supported by computer modeling[7,8] and detailed X-ray diffraction analysis[9,10].

Combining the results of branching-pattern analysis of amylopectin[11] and formation of amylodextrin by acid treatment, French proposed the "Cluster model" for the structure of amylopectin[12].

Robin *et al.*[5,6] prepared Lintnerized starch using hydrochloric acid, fractionated the dextrin by gel filtration, and examined the structure of the various fractions by enzymic degradation. Their results were in general agreement with the cluster model.

Up until now, gel filtration has been considered to be the best method to separate amylodextrin into its component fractions. However, the sharpness of the fractionation was not adequate to obtain individual fractions of different chain length for detailed structural analysis[5,6,13].

In this study, it was our goal to obtain direct evidence of the chemical structure of amylodextrin, which would relate to our previous proposal of a double-helix structure in the crystalline part of the starch molecule. This paper summarizes our structural analysis of Nägeli amylodextrin fractionated by multiple-descending paper chromatography[14], which gave sufficient resolution for separation of components differing in size by only one glucose unit up through d.p. ~25.

EXPERIMENTAL

Enzymes — Twice-recrystallized pullulanase (30 IU/mg) was purchased from Hayashibara Biochemical Research Laboratory, Japan. *Bacillus subtilis* saccharifying alpha amylase (crystallized, 395 IU/mg) and *Rhizopus niveus* glucoamylase (crystallized, 22 IU/mg) were obtained from Seikagaku Kogyo Co., Tokyo. Crude soybean beta amylase was purified to specific activity 80 IU/mg as described by Umeki and Yamamoto[15]. The reaction conditions for pullulanase and beta amylase were as follows. Crystallized pullulanase (2 IU) was incubated with 1 mg of substrate for 7 h at 40°. Substrate (1 mg) was treated with 2.3 IU of soybean beta amylase for 24 h at 40°.

Preparation of Nägeli amylodextrin. — Nägeli amylodextrin of waxy-maize starch was prepared as by Kainuma and French[3], except that the temperature was 38°. Samples were taken at 2, 4, 6, 8, 10, and 13 days, and designated as 2-day amylodextrin, 4-day amylodextrin, etc. The precipitate was washed with deionized water

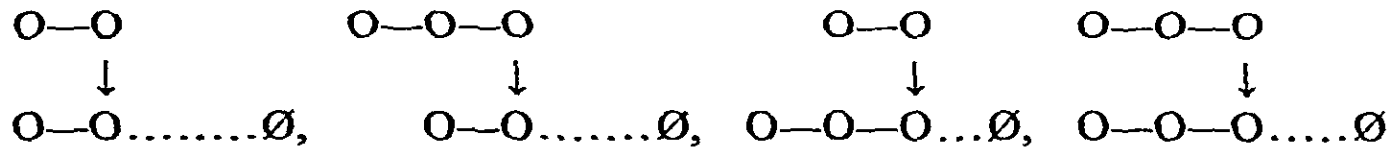
until the pH of the washings became neutral. The samples were kept under water in a refrigerator.

Carbohydrate analysis. — Total carbohydrate and the reducing value were determined by the phenol–sulfuric acid method[16] and the Park–Johnson method[17], respectively. The degree of polymerization of each amylodextrin fraction was calculated by the ratio of the total carbohydrate and the reducing value. Maltose was used as a standard for both methods.

Paper chromatography. — Descending paper chromatography was conducted on Toyo Filter paper No. 50 (60 × 60 cm) with the solvent system 1-butanol–pyridine–water in various ratios. The multiple-descending technique, using sequentially changed ratios of 1-butanol–pyridine–water (6:4:3, 1:1:1, 1:1:1.45, 1:1:1.9), allowed fractionation of linear dextrins up to[14] d.p. 25. Sugar spots were revealed by the silver nitrate dip-method after treatment[18] with glucoamylase to enhance the intensity of higher saccharides otherwise only weakly revealed. For quantitative analysis of the fraction, amylodextrin solution was streaked along a 9-cm line at the bottom of the paper. The mixture of reference oligosaccharides was spotted on the 9-cm line on both sides of the sample. Both reference spots were separated from the sample by 3 cm. After sufficient irrigation, the chromatogram was cut between the sample and the spots of reference oligosaccharides. The reference strips were developed by the silver nitrate dip-method as previously described and used as guide strips for locating the compounds on the undeveloped chromatogram. By matching the undeveloped chromatogram with guide strips, each compound on the undeveloped strip was located and the chromatogram sectioned between the area containing the compound. The quantitative amount was determined by extracting the compound with boiling water for 15 min. The carbohydrate content was determined by the phenol–sulfuric acid method.

Enzymic analysis of the structure of Nägeli amylodextrin and its fractions. — For the structural analysis of amylodextrin, various starch–hydrolyzing enzymes, of which the substrate specificities were well characterized, were used.

(*a*) *Beta amylase.* Beta amylase hydrolyzes amylodextrin exowise to form maltose from the non-reducing end and the reaction stops near the branch point. The followings are the major structures of the outer part of the branch point of beta amylase limit-dextrin[19],



The position of the branch point in amylodextrin is readily determined by analyzing the structure of the limit dextrin.

(*b*) *Saccharifying alpha amylase of* Bacillus subtilis. The specificity of *B. subtilis* saccharifying alpha amylase is characterized sufficiently for use for the structural analysis of polysaccharides[20,21]. Fig. 1 shows that the enzyme has strict product specificity to form isomaltose from structure *a*, panose from *b*, and B_4 (6^3-*O*-α-D-

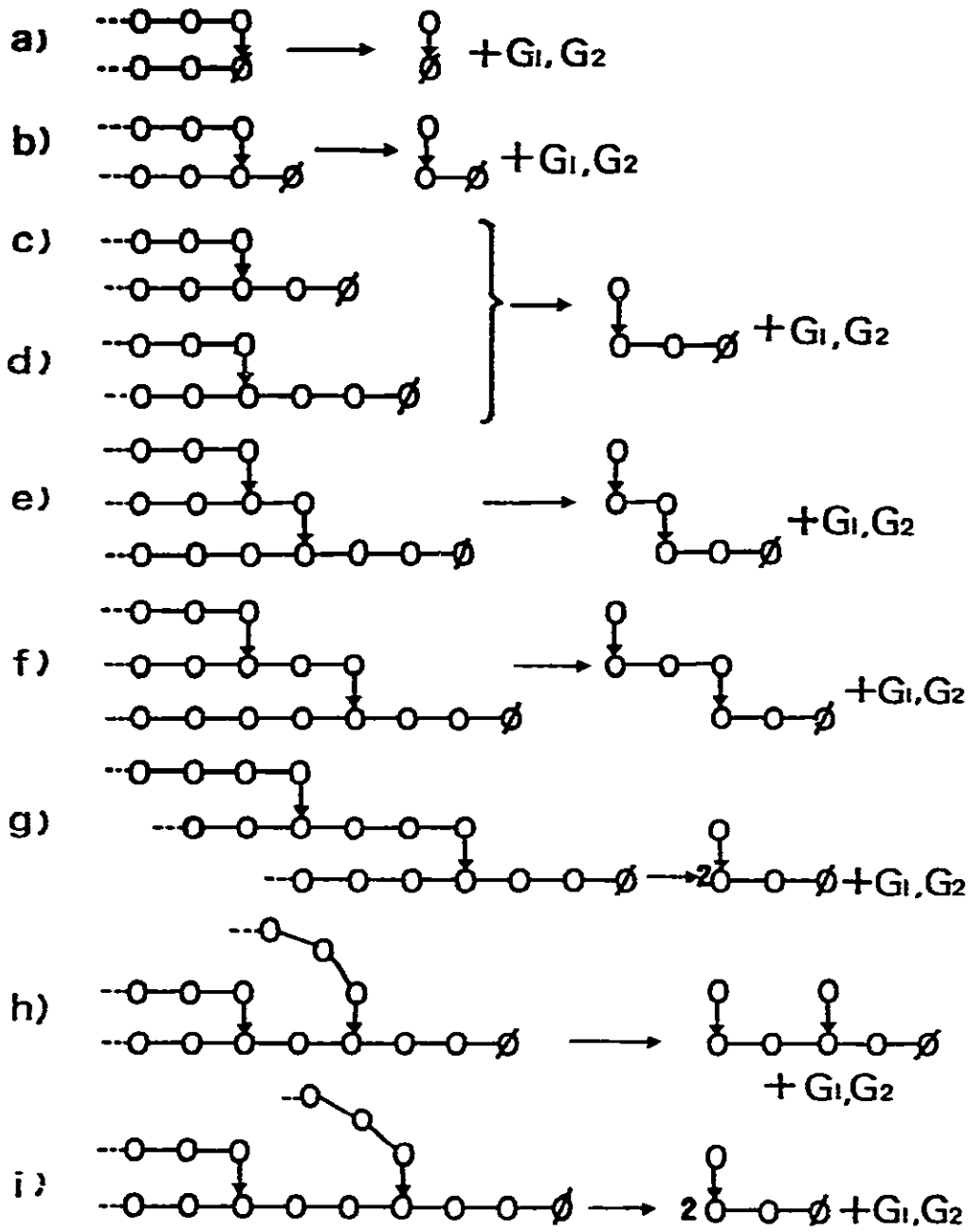


Fig. 1. Schematic presentation of the action pattern of saccharifying alpha amylase of *B. subtilis* on branched oligosaccharides.

glucosylmaltotriose) from *c* and *d*. Multiply-branched oligosaccharides are formed from *e*, *f*, *g*, *h*, and *i*. These specificities are extremely useful for locating the position of the α-(1→6) linkages in oligosaccharides, particularly in analyzing the number of glucose units at the reducing-end side of the branch point.

(*c*) *Pullulanase*. Pullulanase hydrolyzes α-(1→6) glucosidic linkages in amylodextrin, beta amylase limit-dextrin, and branched starch-oligosaccharides. The substrate specificity of pullulanase has been studied in detail for use of the enzyme for structural analysis[22,23]. In general, pullulanase cleaves maltosyl- or longer branches attached to main chain by α-(1→6) linkages.

The structures of Nägeli amylodextrin or its fractions were analyzed essentially by the methods of Kainuma and French[11], and Umeki and Yamamoto[20,21], using the enzymes already mentioned.

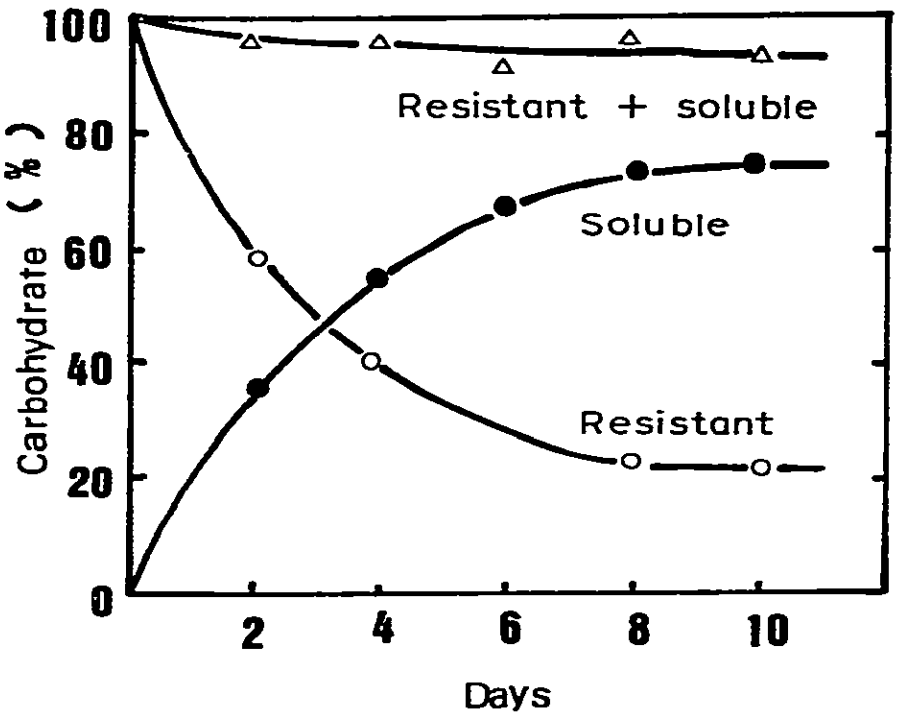


Fig. 2. Solubilization of waxy-maize starch during treatment with 16% sulfuric acid at 38°. Soluble: Solubilized carbohydrate. Resistant: acid-resistant carbohydrate.

RESULTS

1. Changes in solubility, crystallinity, average degree of polymerization, and unit chain-length during acid treatment of waxy-maize starch. — Degree of solubilization and crystallinity. It is known that the amorphous part of the starch molecule is hydrolyzed and solubilized during the course of amylodextrin preparation[3]. The degree of solubilization of the starch is shown in Fig. 2. Rapid solubilization of starch is observed during 2 days, and then proceeds slowly until the 8th day. After 8 days, 75% of the starch is solubilized and ~20% is obtained as the acid-resistant residue. Less than 10% of the carbohydrate is degraded and lost during this acid treatment. As already reported[4], the X-ray diffraction patterns of the treated starch became sharper with increased solubilization.

Average degree of polymerization and unit chain-length. Average degrees of polymerization ($\overline{\text{d.p.}}$) of the samples withdrawn at various stages of hydrolysis were determined and plotted as $\overline{\text{d.p.}}$ in Fig. 3. The same samples were debranched by pullulanase to obtain the average unit chain-length ($\overline{\text{c.l.}}$) of the amylodextrin. In the previous studies[5,6,24], it was observed that some of the amylodextrin molecules were singly or multiply branched. As may be seen in Fig. 3, both the $\overline{\text{d.p.}}$ and $\overline{\text{c.l.}}$ decreased drastically during the first 4 days, after which time they became constant. The $\overline{\text{d.p.}}$ was 17–18 and the $\overline{\text{c.l.}}$ ~14–15 after 6 days. The $\overline{\text{c.l.}}$ indicated that the acid-resistant region in the amylopectin molecule was ~14–15 glucose units long. Details of the fine structure of each fraction of different molecular size will be discussed later in this paper.

Samples (5 mg) of the amylodextrin of various stages of hydrolysis were dissolved and fractionated into different molecular-size components by descending paper chromatography. (See Fig. 4). At 2 days, it may be seen that only a small amount of carbohydrate was located between $\overline{\text{d.p.}}$ 10–15, and that most of the carbo-

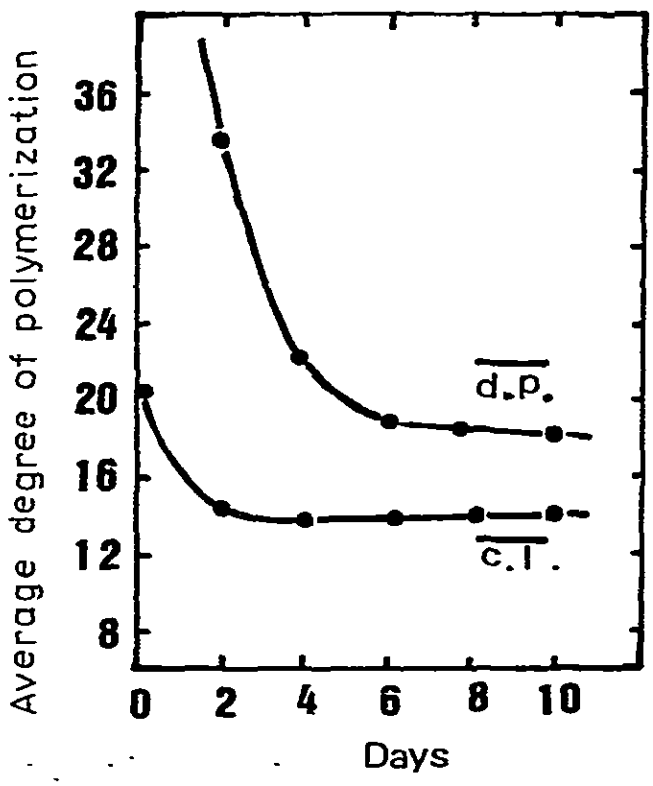


Fig. 3. Average degree of polymerization ($\overline{d.p.}$) and average unit chain-length ($\overline{c.l.}$) of Nägeli amylodextrin prepared with 16% sulfuric acid at 38°.

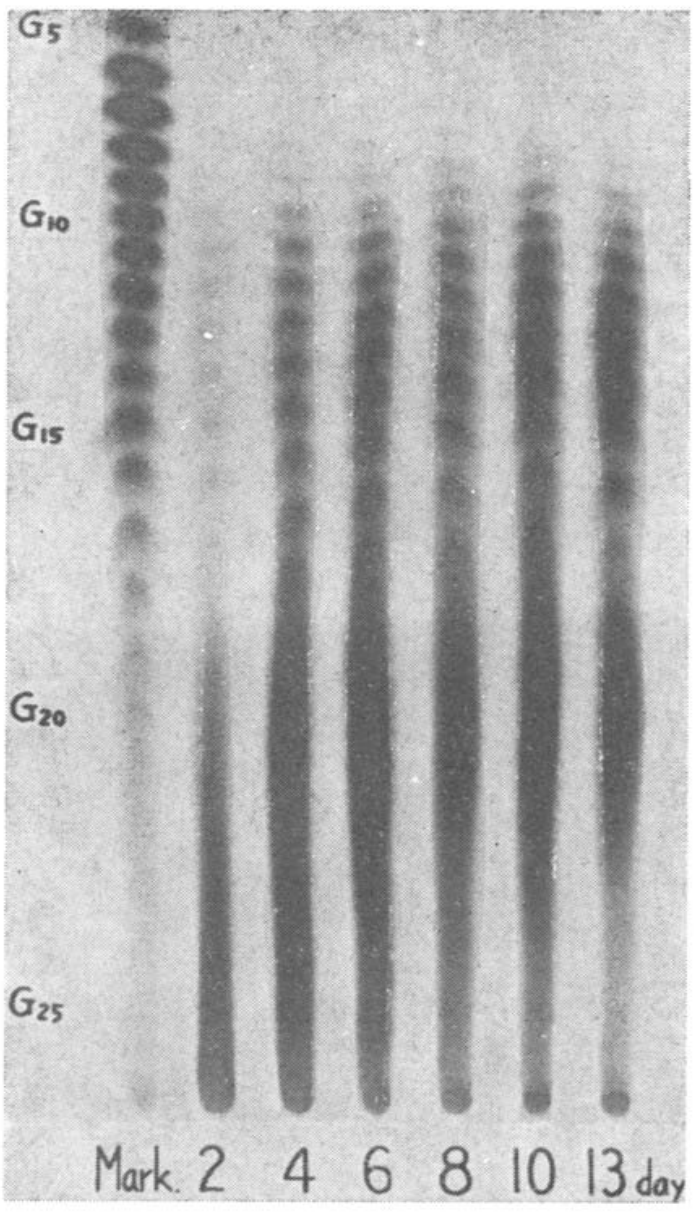


Fig. 4. Multiple-descending paper chromatogram of Nägeli amylodextrin, sampled at various stages of hydrolysis. G_n indicates reference linear maltosaccharides of d.p.n.

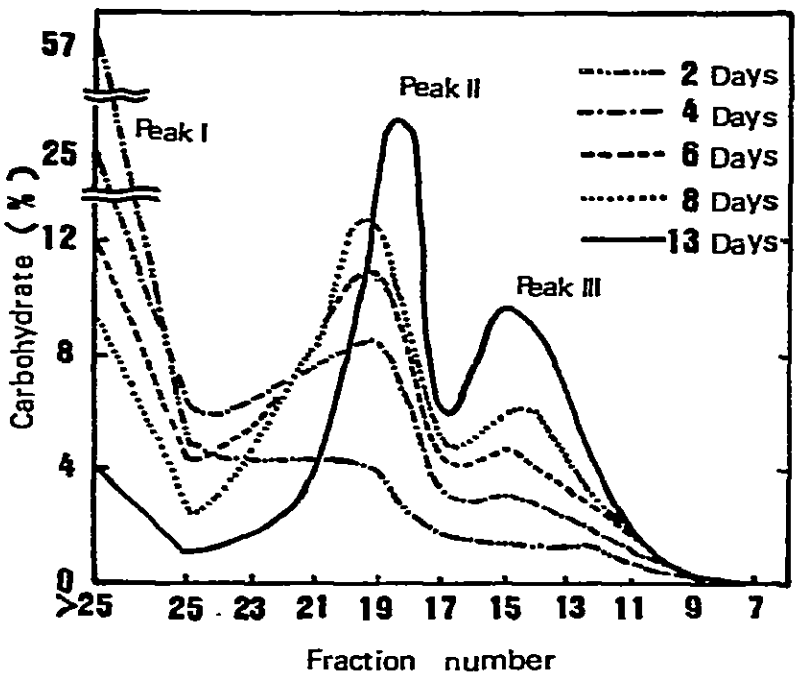


Fig. 5. Change in the distribution of molecular size of Nägeli amylodextrin during acid treatment, as determined by quantitative descending paper chromatography. "Fraction *n*" is defined as mentioned in the footnote on p. 143.

hydrate was of higher molecular weight. This distribution changed with additional reaction, as may be seen in the 4–13 day data. At 13 days, when 75% of the carbohydrate had become solubilized, there were two distinct populations of carbohydrate. One was located at the position corresponding to the reference, linear maltosaccharide of d.p. 13–15, and another was seen at d.p. ~19–22 of the linear maltosaccharides.

The same experiment was made for quantitative analysis of the carbohydrate. The paper chromatogram was cut to separate the amylodextrin fractions of various molecular size, and then each fraction was extracted by boiling water as mentioned in the Experimental section. The carbohydrate content of each fraction was determined by the phenol–sulfuric acid method and the results are shown in Fig. 5. The peak for the larger-molecular-weight fraction (>25) decreased gradually, and then two distinct peaks appeared at Fractions 18–22 and 12–16. We designate these peaks as peak I, II, and III, respectively. These peaks seem to correspond to the so-called Fractions I, II and III, which Kikumoto and French obtained by precipitation of amylodextrin[25,26] with organic solvents.

As may be seen in Fig. 5, peak I decreased with the increased solubilization and only 4% of the total carbohydrate existed in this fraction at 13 days, as compared with 57% at the 2nd day. It was found that the distinct peak II appeared in the samples after 4 days. Peak II became the preponderant population of the carbohydrate and the sharpest molecular-size distribution at Fraction 18–19 after 13 days. Peak III appeared at almost the same time as the peak II. This peak also became narrower with inereased solubilization and was located at Fraction 14.

The same amylodextrin as that in the experiment of Fig. 5 was debranched with pullulanase to determine the distribution of the unit chain-length, as shown in Fig. 6. Most of the debranched chains were located in narrower range between Fractions 12–16, after 8 days of reaction. After debranching, Fraction *n* and d.p.$_n$ were mostly

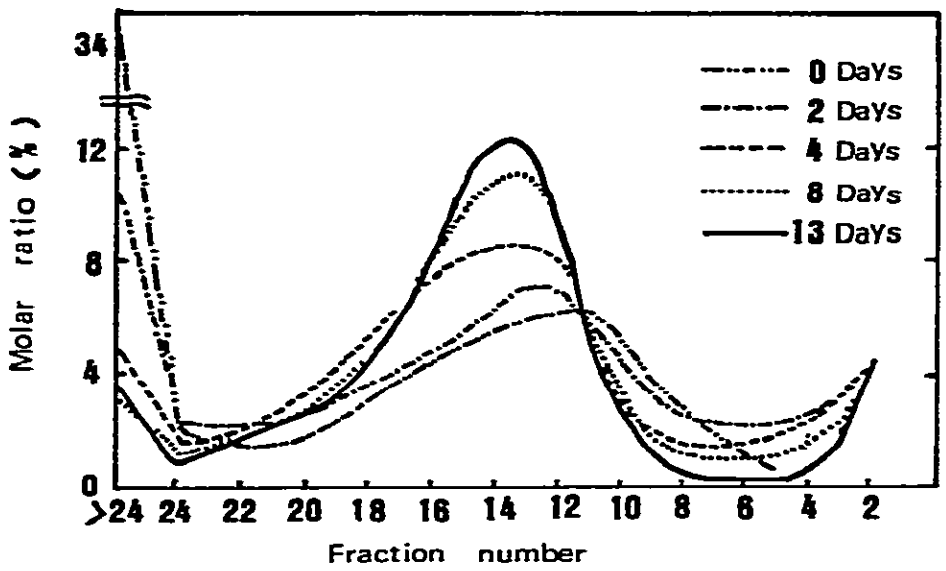


Fig. 6. Unit chain-length distribution of amylodextrin after the action of pullulanase, as determined by quantitative descending paper chromatography. Definition of "Fraction" is as in Fig. 5.

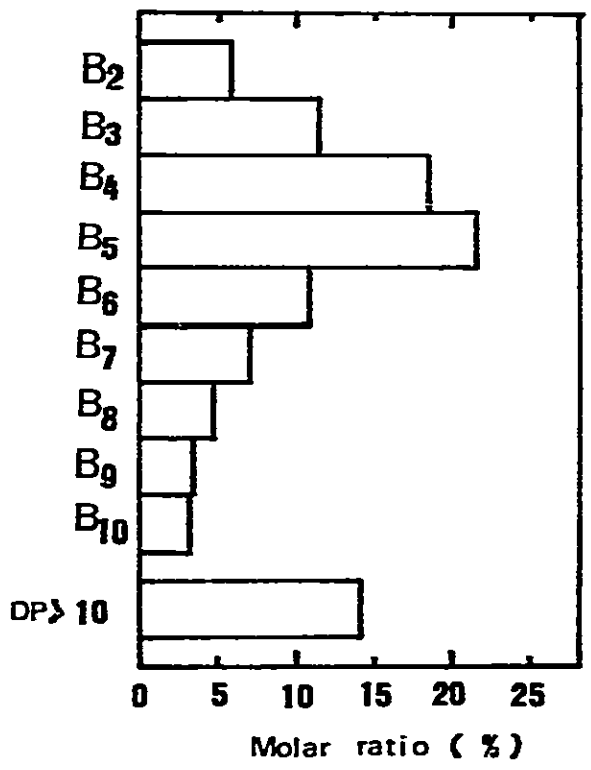


Fig. 7. Distribution of beta-limit dextrins obtained from Nägeli amylodextrin. B_n indicates singly branched oligosaccharide of d.p.n.

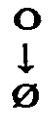
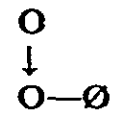
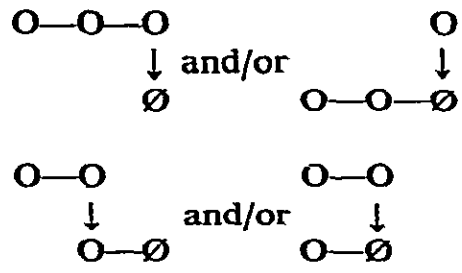
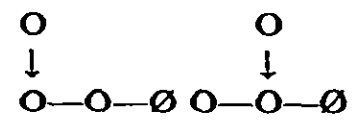
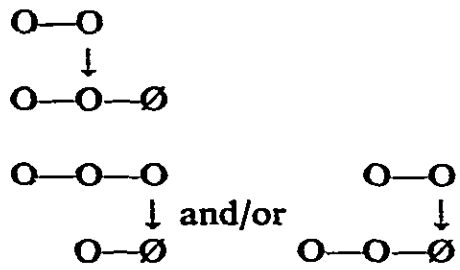
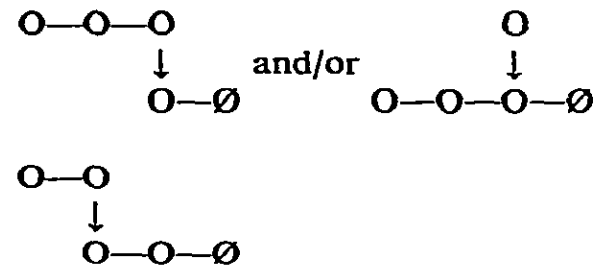
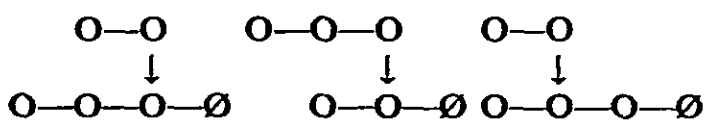
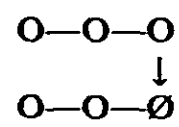
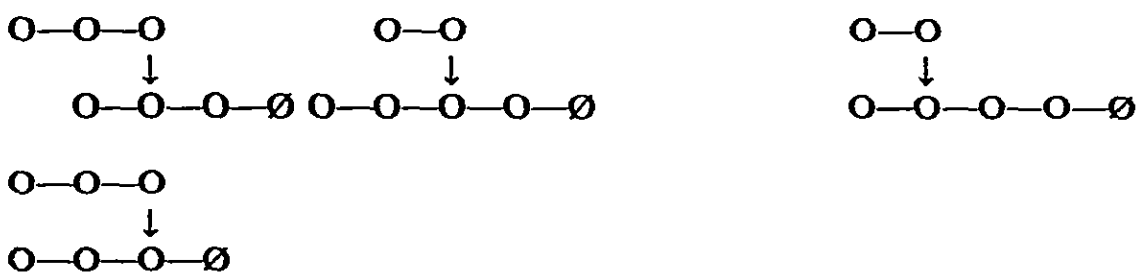
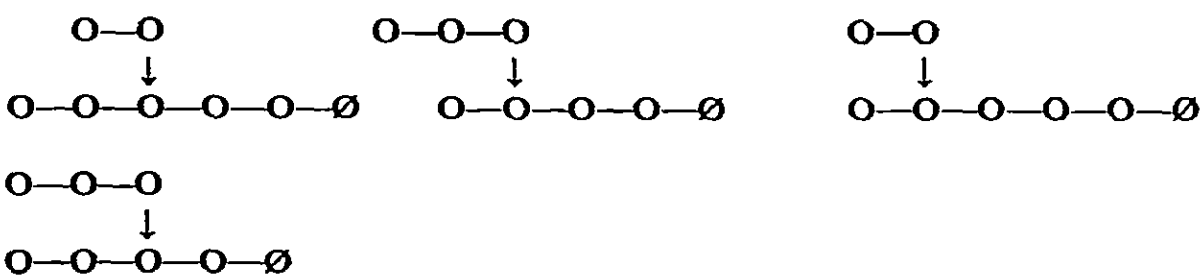
identical. From the results shown in Figs. 2–6, it is clear that the structure of amylodextrin was relatively homogeneous after 8 days of acid treatment. For further structural analysis, a large-scale preparation of Nägeli amylodextrin was conducted by using the acid treatment for 8 days.

2. Chemical structure of Nägeli amylodextrin of 8 days acid treatment. — Details of the chemical structure of the Nägeli amylodextrin, as prepared by heterogeneous acid hydrolysis of waxy-maize starch to the extent when 75% of starch was hydrolyzed and solubilized by 8 days acid treatment at 38°, were studied by using various starch hydrolyzing enzymes.

Action of soybean beta amylase on the Nägeli amylodextrin. Beta amylase converted Nägeli amylodextrin into 80% maltose and 20% of branched limit-dextrin.

TABLE I

PROPOSED STRUCTURES OF BETA-LIMIT DEXTRINS OBTAINED FROM NÄGELI AMYLODEXTRIN

Beta limit dextrin	*Main component*	*Minor component*
B_2		
B_3		
B_4		
B_5		
B_6		
B_7		
B_8		

Molecular size and molar ratios of the branched beta amylase limit-dextrin are shown in Fig. 7, where it may be clearly seen that B_4 and B_5 are the main products. The detailed structures of the beta-limit dextrins in Fig. 7 were determined by the enzymic method described later. Each fraction, such as B_2, B_3, B_4, ..., was separated by multiple descending paper-chromatography, and the structure of each fraction was determined by the action of *B. subtilis* saccharifying alpha amylase and pullulanase. The results are shown in Table I. Of the total beta-limit dextrin, 80% was oligosaccharides of d.p. 2–8, and these were singly branched dextrins. The branch point was located at the reducing end or at the adjacent glucose residue as seen in Table I, where the main structures of the limit dextrins were those terminated by isomaltose, panose, and B_4 at the reducing end. These results suggest that the branched amylodextrins were mostly singly branched near the reducing end. Judging from the specificity of beta amylase, these glucosyl stubs have not been formed by action of beta amylase, but originally existed as glucosyl-stubbed amylodextrin[19,27]. French and co-workers found panose and isopanose in the beta-limit dextrins of Nägeli amylodextrin obtained by different conditions[28].

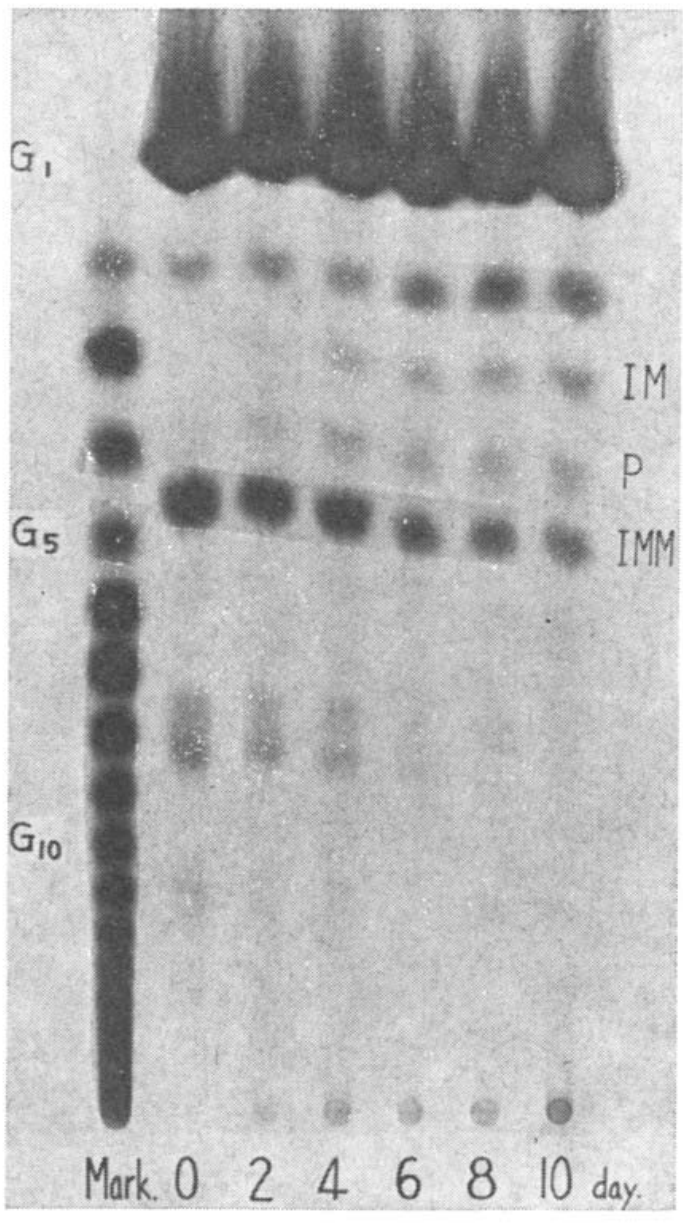


Fig. 8. Paper chromatogram showing the action of the saccharifying alpha amylase of *B. subtilis* on Nägeli amylodextrin at various stages of hydrolysis. M: Marker linear maltosaccharides; IM: isomaltose; P: panose; and IMM: Isomaltosyl-maltose (6^3-*O*-α-D-glucosylmaltotriose, B_4).

Action of bacterial saccharifying alpha amylase on the Nägeli amylodextrin. Amylodextrins obtained from various stages of the hydrolysis were treated with *B. subtilis* saccharifying alpha amylase (0.5 IU/mg carbohydrate, pH 5.4) for 48 h at 40°. The products were examined by paper chromatography (Fig. 8). From the parent starch, B_4 (Isomaltosylmaltose, IMM) was formed as the only singly branched oligosaccharide, and BB_6, BB_7 and BBB_9, and BBB_{10} were observed as multiply branched oligosaccharides. The amount of isomaltose and panose increased with prolonged acid treatment. This result strongly suggests that the structure *a* and *b* of Fig. 1 increased with the intensive acid treatment.

The products obtained by the action of the saccharifying alpha amylase of *B. subtilis* on the 8-day amylodextrin were analyzed by quantitative paper chromatography. The molar ratio of isomaltose:panose:B_4 was 45:31:24. This result indicated that 76% of the α-(1→6) linkages in the dextrin were located at the reducing end (45%) or at the glucose residue adjacent to the reducing end (31%). Multiply branched oligosaccharides of d.p. 6, 7 and 9, and 10, which were formed by the

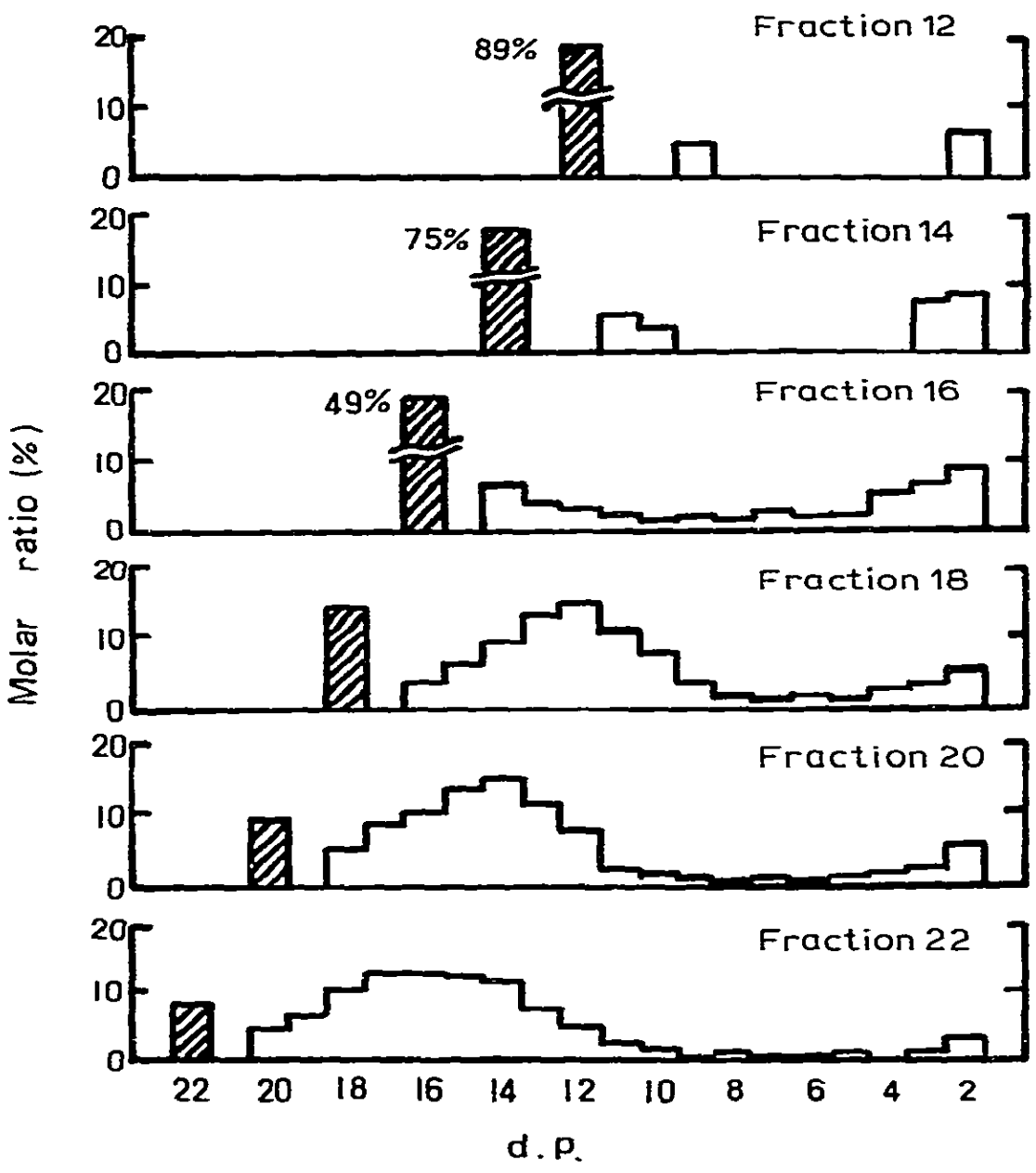


Fig. 9. Chain-length distribution of various fractions of Nägeli amylodextrin after treatment with pullulanase. Cross-hatched area: pullulanase-resistant dextrin.

action of *B. subtilis* saccharifying alpha amylase on the parent starch, gradually disappeared by the increasing solubilization. These multiply branched oligosaccharides were originally formed from the highly branched region of amylopectin[11]. These observations support the idea that highly branched regions of amylopectin are more susceptible to acid hydrolysis.

3. Structure of the paper-chromatographically fractionated amylodextrins of different molecular size. — Action of pullulanase and beta amylase on the fractionated Nägeli amylodextrin. The 8-day amylodextrin was dissolved and fractionated by multiple descending paper-chromatography as described in the Experimental section. Each fraction of different molecular size was excised and extracted by hot water, and then incubated with pullulanase to hydrolyze the branch points. Some of the results are shown in Fig. 9. Fig. 9 shows that fractions 12–16, mainly from the peak III in Fig. 5, were partly hydrolyzed by pullulanase to form maltose, maltotriose, and linear maltosaccharides that were 2 or 3 D-glucose units smaller than the original fractions. For Fraction 12, 89%, and for Fraction 14, 75%, was resistant to pullulanase action. Pullulanase-resistant dextrins were considered to be a mixture of linear maltosaccharides and glucosyl-stubbed maltosaccharides, as readily demonstrated by the action of beta amylase.

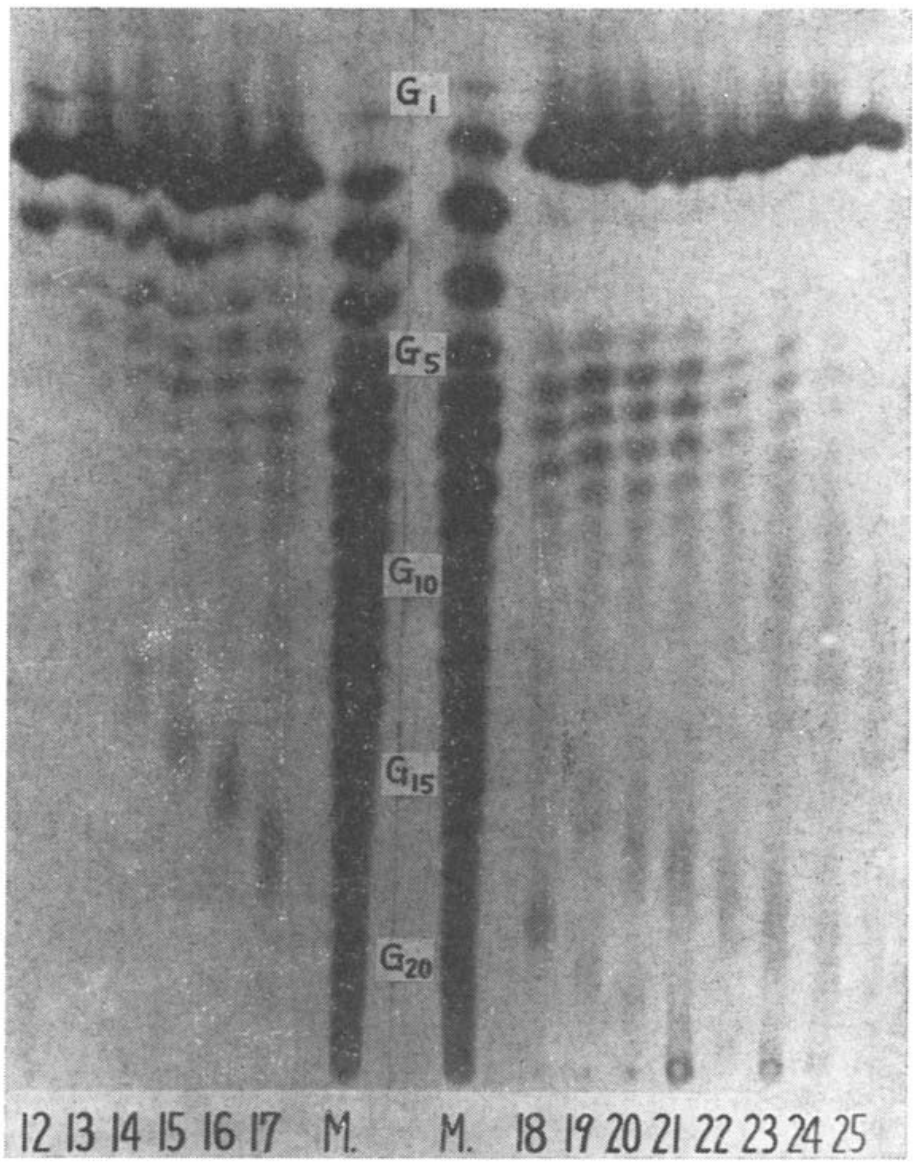


Fig. 10. Paper chromatogram showing the action of beta amylase on each fraction of Nägeli amylodextrin; M: Marker linear maltosaccharides; 12, 13, ... indicate fraction number of amylodextrin.

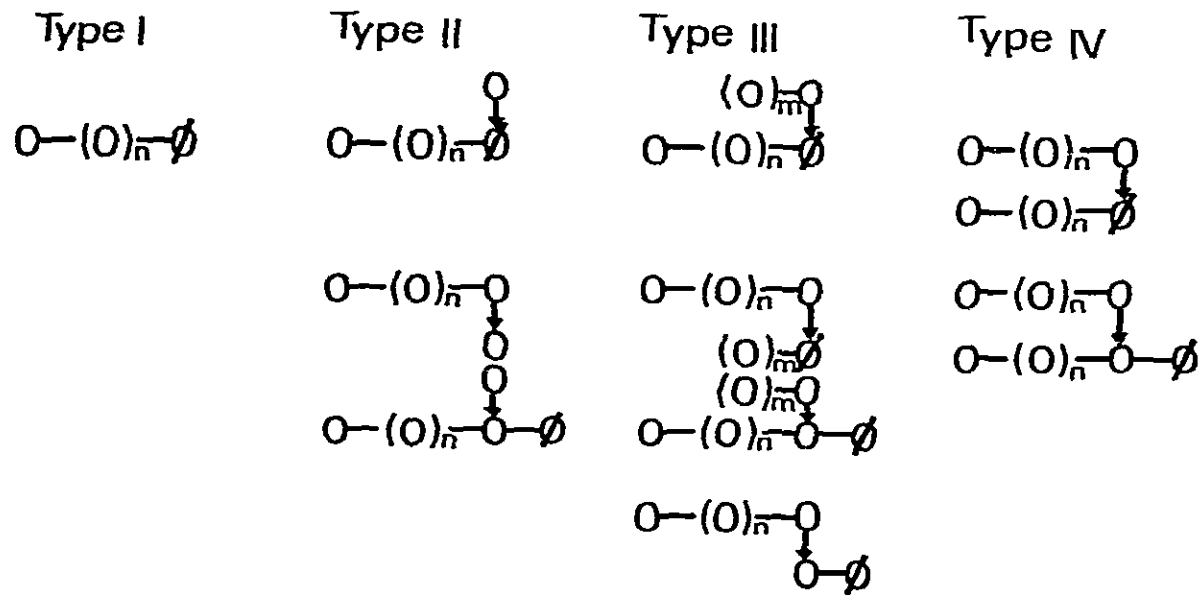


Fig. 11. Proposed structures of various types of Nägeli amylodextrin ( )$_{n,m}$: when n is 10–14, m is 1 or 2. A maltosyl or maltotriosyl branch exists most frequently in Type III.

When Fraction 17–24 (the higher-molecular-weight fractions corresponding to peak II of Fig. 5) were debranched with pullulanase, about 85–90% of them were converted into linear maltosaccharides of d.p. 12–16, and a very small amount of maltose and maltotriose as may be seen in Fig. 9. These observations indicate that most of the amylodextrin of Fraction 17–24 is composed of two chain molecules of d.p. 12–16, linked to each other by an α-(1→6) bond near the reducing end.

The action of beta amylase on separated fractions of amylodextrin was also studied. Fractions 8–11 were converted into maltose and a small amount of glucose, which indicated that these fractions were mostly linear oligosaccharides. From the fractions larger than 12, branched beta-limit dextrins were formed besides maltose and D-glucose, as seen in Fig. 10. The distribution of the limit dextrins was mostly between B_2 and B_9. We also observed, in the lower part of the paper chromatogram, 5% (molar ratio) of unreacted dextrins, which are most probably branched near the non-reducing end. Fig. 10 indicates also that most of the branched points were linked at the reducing end or the adjacent glucose unit. Fig. 11 shows that Type I, II, III, and IV are considered to be the major conformation of amylodextrin from the results of the enzyme-degradation studies. Type I is linear, and Types II and III are glucosyl- and maltosyl-stubbed amylodextrins at or near the reducing end. Type IV is composed of two molecules of Type I amylodextrin linked at or near the reducing end of the molecule.

From the results of the quantitative analysis, Type I was mainly located in Fractions 8–17 and the molar ratio of this fraction to the whole amylodextrin was 47%. Types II and III were mainly in Fractions 12–18, with a molar ratio of 16%. Type IV was in Fractions 15–24, with a molar ratio of 26%, and amylodextrin branched near the non-reducing end was ~5%. Only 6% remained on the origin of the paper chromatogram and was not amenable to analysis.

In paper chromatography, Type IV dextrins moved faster than would be expected from their d.p.; for example, Type IV dextrin of d.p. 28–30 moved as fast

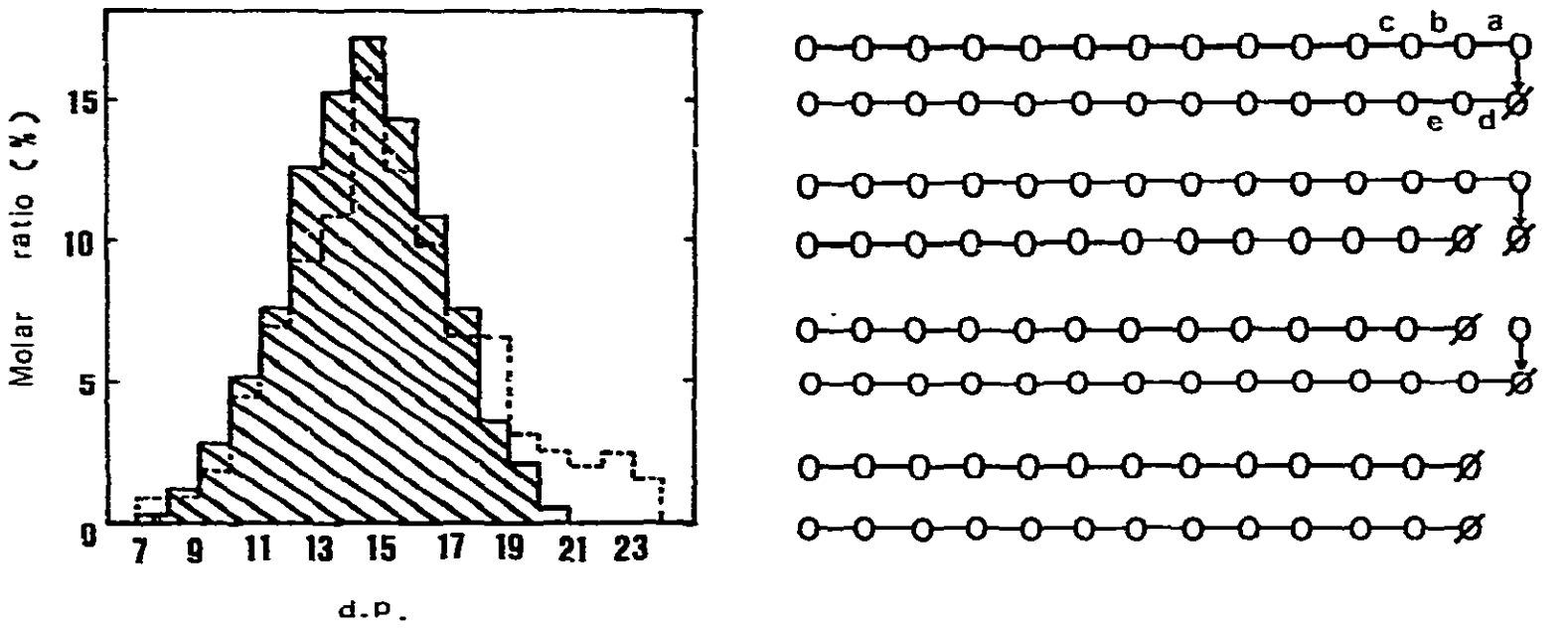


Fig. 12. Unit chain-length distribution of Type I and Type IV amylodextrin. Unit chain-length distribution of Type IV was obtained as described in the text. Area bounded by broken line: Type I dextrin. Cross-hatched area bounded by solid line: Type IV dextrin.

Fig. 13. Schematic presentation to show the formation of Type I, II, and III dextrin from Type IV. Frequently, one or two glucose residues are linked to the right-hand side of the reducing glucose residue of Type IV.

as linear dextrin of d.p. 20. This anomalous chromatographic behavior may be caused by the different conformation of Type IV dextrin, where two chains could readily intertwine.

Structure of Type IV amylodextrin and distribution of unit chain-length. Among the structures of amylodextrins shown in Fig. 11, only Types III and IV are susceptible to the action of pullulanase. Type III produces (G_2 + G_3) and equimolar amounts of linear dextrins of d.p. 12–16, which were designated as "unit chain", and Type IV forms two molecules of unit chain. Theoretically, the unit chain-length distribution obtained from Type IV may be calculated from the results of the action of pullulanase on the mixture of Type III and IV. After the reaction, the amount of unit chain formed from Type IV was calculated by deducting an equimolar amount of dextrin from that formed (G_2 + G_3) from the whole unit-chain. Differential analysis of Types I, II, III, and IV was accomplished by the extensive action of beta amylase. Type I was completely hydrolyzed to maltose and glucose, whereas Types II, III, and IV gave branched beta-limit dextrins. From the independent analyses of each separated fraction, the unit chain-length distribution of Type IV in Fraction 17–24 was calculated as already described. The results are shown in Fig. 12, where the unit chain-length distribution of Type I is superposed. In both instances, the unit chains are distributed between d.p. 7 and 20. However, a narrow distribution may be seen, having a peak at d.p. 14. These quantitative analyses suggest that Type IV dextrins are resistant to acid treatment because of the interwinding of unit chains, which are linked by an α-(1→6) bond near the reducing end.

DISCUSSION

It is demonstrated by the enzymic-degradation studies that the unit chain-length of Type I, II, III, and IV shown in Fig. 11, is identically distributed between d.p. 12–16. It may readily be seen that the Type IV is an acid-resistant structure, and often Type IV was hydrolyzed to form Type I and II, I and III, and I and I (Fig. 13). According to the results of model building of the double helix, steric hindrance of the α-(1→6)-linkage between two chains causes great distortion to the specific α-(1→4) linkages designated as *a*, *d*, *b*, and *e*, which are less organized, and the rest of the α-(1→4) linkages are packed compactly and buried inside of the double helix (Fig. 13). These structural features may contribute to the formation of Types I, II, and III amylodextrin by acid hydrolysis of the distorted linkage *a*, *d*, *b*, or *e*.

As may be seen in Figs. 6, 9, and 12, the Type IV amylodextrin consists of two chains of the same length, linked at the reducing glucose residue or at the adjacent glucose residue. This structural evidence indicates that a pair of α-(1→4)-linked glucose chains are more stable to acid treatment than a single chain, and also suggests that the crystalline regions of amylopectin are packed with a number of pairs of inter-winding dextrins of ~14 glucose residues. This structural evidence supports the idea of the double-helix model for the crystalline part of starch, as suggested the studies of X-ray diffraction under various conditions, reaction with iodine, and space-filling model building of Kainuma and French[4], who proposed the possibility of a left-handed, double helix having six interwinding glucose units in a turn of 21 Å.

This structural feature is strongly supported by recent observations by electron microscopy. Yamaguchi *et al.*[29] succeeded in obtaining electron micrographs of Nägeli amylodextrin of defatted waxy-maize starch. They observed structures of regular lamellae 50 Å thick, arranged tangentially to the growth ring of the acid-treated starch. This observation agreed well with the results of the analysis of the structure described in this paper. According to our analytical results and model building, two chains of 14 glucose units formed a double helix 50 Å in length.

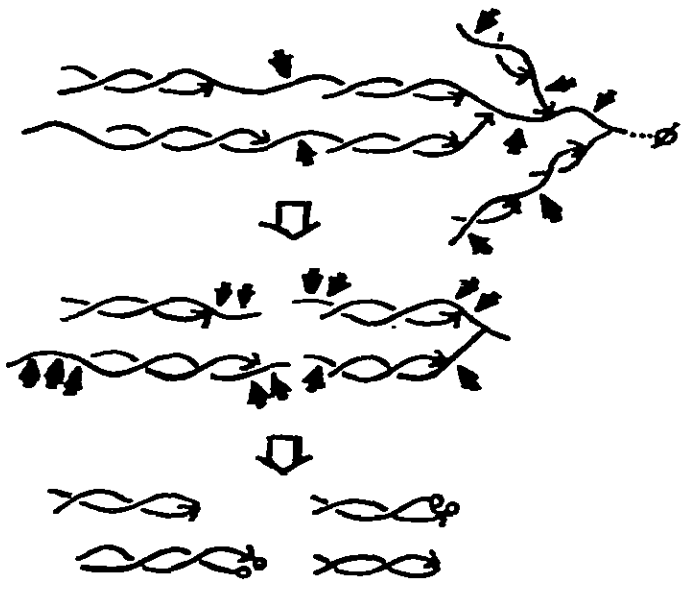


Fig. 14. Schematic explanation of heterogeneous acid hydrolysis of amylopectin. Arrows indicate the position of acid hydrolysis.

Another interesting observation on Type IV amylodextrin is its unusual chromatographic mobility. Fraction 20 migrated as fast as d.p. 20 linear dextrin, and it formed two molecules of linear dextrin (d.p. 14) by the action of pullulanase. Normally, the existence of α-(1→6) linkage decreases the chromatographic mobility of oligosaccharides. However, Type IV dextrin migrates much faster than linear fraction of d.p. 28. Although we are not certain whether or not the molecule moves as a double helix during paper chromatography, the unusual mobility may be attributed to the three-dimensional structure of the Type IV molecule.

The course of hydrolysis during preparation of amylodextrin is schematically illustrated in Fig. 14. At the initial stage of reaction, starch molecules are cleaved at random at the single chain, and densely branched regions of amylopectin. In the following steps, glucose chains not included in the double helix are removed. The distribution of $\overline{\text{d.p.}}$ and $\overline{\text{c.l.}}$ becomes narrower at this stage (as shown in Figs. 5 and 6). At the same time, some of the α-(1→4) linkages near the branch point are hydrolyzed. By these processes, the amylodextrins, which do not contain any single-chain parts, remain as an acid-resistant fraction after protracted acid treatment.

Of the branched amylodextrin, 85% was converted into low-molecular-weight beta-limit dextrin, and the structures were readily analyzed. We observed that a small fraction of the branch points were located near the nonreducing end of the amylodextrin molecule. We have not studied this fraction to determine how this branched molecule survived the extensive acid treatment.

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