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Structures of building blocks in clusters of sweetpotato amylopectin

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ABSTRACT

φ,β-Limit dextrins of domains and clusters of sweetpotato amylopectin were subjected to extensive hydrolysis by *Bacillus amyloliquefaciens* α-amylase to release building blocks and reveal the internal structures of clusters. The composition of building blocks was analyzed by size-fractionation, gel permeation chromatography, and high performance anion exchange chromatography. Different domains and clusters had structurally similar building blocks with around three chains per building block and internal chain length around 2.9. Singly branched and doubly branched building blocks were the largest and second largest groups in the clusters. Type A clusters had more large building blocks and contained 5–6 blocks per cluster with an inter-block chain length (IB-CL) of 7.0, whereas type B clusters had less large building blocks and contained 3–4 blocks per cluster with IB-CL 7.9. Models on how the building blocks could be organized into type A and type B clusters are discussed.

1. Introduction

Granular starches are semi-crystalline polysaccharides, consisting of amorphous and semi-crystalline shells in the range of 100–1000 nm, which can be observed by optical microscopy.[1] The semi-crystalline shells consist of alternating layers of amorphous (3–4 nm) and crystalline (4–6 nm) lamellae with a total repeat distance unit of 9–10 nm as revealed by small angle X-ray scattering techniques.[2] Amylopectin, the major component of starch, is mainly responsible for the architecture of the repeating unit. The external chains of amylopectin interact with each other to form double helices in the crystalline lamellae, whereas the internal amylopectin builds up the amorphous lamellae.[3] The external amylopectin participating in the crystalline lamellae is essentially linear, whereas the internal portion is extensively branched. Computer modeling on the relationship between the internal molecular structure of amylopectin and the crystalline polymorph of starch suggested that certain internal chain lengths would lead to parallel double helices, and further implicated the length of internal chains as a determinant of the degree and type (A or B polymorphs) of local crystallinity.[4] Experimental data showed that the molecular structure of amylopectin is highly related to the macromolecular organization of granular starch.[5–7] Thus the molecular structure of the amorphous lamellae may be critical to the overall physical structure of granular starch.

The external part of amylopectin can be removed by exo-acting amylolytic enzymes, such as phosphorylase *a* and/or β-amylase, and the internal part can be obtained in the form of φ,β-limit dextrins (φ,β-LDs) or β-LDs, which retain all the branching points in the original amylopectin.[8] After enzymatic debranching of these LDs, the internal unit chain length distribution of B-chains of amylopectins from diverse plants showed four different types of profiles.[9] The weak correlations between the internal unit chain length distribution profiles of B-chains of amylopectins and physical properties of granular amaranth starches[10] suggested the need for further clarification of the role of fine structure of amylopectin in determining the properties relevant in starch applications.

Because of the heterogeneity of branching density in the internal part of amylopectin, the concept of 'clusters' of branching points was proposed.[11–13] Further developments of the concept have come from the isolation of clusters from the φ,β- or β-LDs of amylopectin by endo-acting amylolytic enzymes.[14–16] Diversity in the molecular structure of the isolated clusters has been observed and their relationships with the type of crystal allomorph of the granular starches has been studied.[3] The internal unit chain length distribution profile of the entire amylopectin molecule and the role of different chain categories in relation to the structure of clusters and their arrangements inside the amylopectin remain unclear. However, by subjecting the clusters to extensive hydrolysis by α-amylase from *Bacillus amyloliquefaciens*, building blocks of clusters could be isolated and the role of diverse chain categories of clusters in the molecular architecture of starch might be modeled.[17–19] With a combination of size-exclusion and ion exchange chromatography and debranching techniques, the building blocks of clusters from a very limited number of amylopectins, including cassava, amylose-free potato,

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waxy rice, amaranth, and *wxdu* and *aewx* maize mutants have been structurally characterized.[17–22] In general, the average DP of the building blocks was around 11–17. The internal chain length (ICL) was in the range of 1.5–2.8, much shorter than that of amylopectin, domains, and clusters. Consequently, the degree of branching (DB) of the building blocks was around 14–17%, higher than that of clusters, domains, and amylopectin. The inter-block chain length (IB-CL) in clusters was 7–8, and based on this, a definition of a cluster was given as a group of branches with internal chain length less than 9, according to the pattern of action of *B. amyloliquefaciens* α-amylase.[18] The clusters analyzed so far support this suggestion, but more samples need to be analyzed to draw more general conclusions.

Some of the structural parameters related to the composition of building blocks in clusters of amylopectins, from different plants so far analyzed, appear to not be directly correlated to the type of crystalline allomorph of the granular starch[19]; however the size distribution of building blocks from two maize mutants with A and B crystalline allomorphs did exhibit different patterns.[21] Thus, it seems that suitable structural parameters of building blocks may be selected that related to the different crystalline allomorphs of original granular starches between or within plant species.

Sweetpotato is the seventh most important food crop in the world, and the fifth most important in developing countries. It is cultivated in over 100 countries with a wide biogeographical range.[23] The major component of sweetpotato root is starch. In a previous study, clusters from sweetpotato amylopectin were isolated and structurally characterized.[24] In this study, building blocks in clusters from sweetpotato amylopectin were produced and structurally characterized. Thus this study may contribute to the scientific understanding of fine structure of clusters and amylopectin.

2. Experimental

2.1. Materials

The φ,β-limit dextrins (φ,β-LDs) of domains, clusters, and subclusters produced from sweetpotato amylopectin were the same samples as those used in a previous study, and some of their structural characteristics are summarized in Table 1.[24] Briefly, amylopectin was subjected to an initial stage of partial hydrolysis by *B. amyloliquefaciens* α-amylase and differential methanol precipitation to isolate the domain fractions (I). The domain fractions were further subjected to a second stage of partial α-amylolysis and methanol precipitation to isolate the cluster fractions (II). All the fractions obtained were sequentially subjected to phosphorolysis and β-amylolysis to obtain their internal part in the form of φ,β-LDs. All the samples were freeze-dried.

Liquefying type α-amylase from *Bacillus subtilis* (also known as *B. amyloliquefaciens* α-amylase, EC 3.2.1.1) was from Seikagaku Corporation, Tokyo, Japan. The enzyme activity was quantified using Amylazyme tablet assay (Megazyme, Wicklow, Ireland), based on a previous description at pH 6.5, 25 °C.[25] *Pseudomonas amyloderamosa* isoamylase (91 U/mg, EC 3.2.1.68), and *Klebsiella planticola* pullulanase (36.3 U/mg, EC 3.2.1.41), were from Megazyme International, Wicklow, Ireland. Their enzyme activities were assumed to be specified by suppliers. Linear dextrins with DP 1–7 were obtained from Sigma/Aldrich (St. Louis, MO).

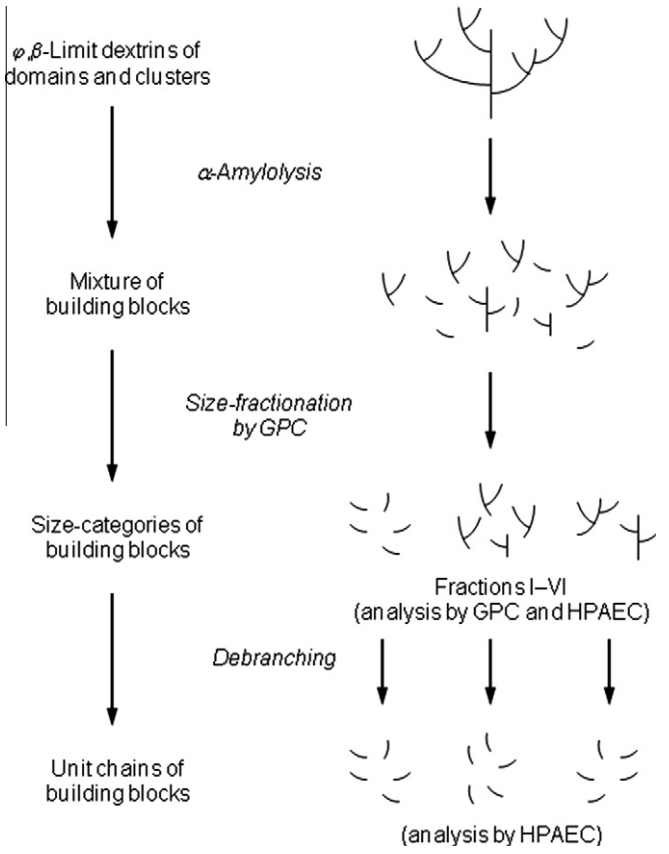
Table 1
Structural features of domains and clusters in the form of φ,β-LDs from sweetpotato amylopectin[a]

Samples[b]	DP[c]	Peak-DP[d]	CL[e]	NC[f]	Peak-NC[g]	DB[h]
I (d)	144	291	7.9	18.2	36.8	11.9
2.1.I (d)	518	718	8.3	63.2	86.5	12.0
2.2.I (d)	282	451	7.1	39.7	63.5	13.7
3.I (d)	185	222	7.4	25.0	30.0	13.0
4.I (c)	58	76	7.5	7.7	10.1	11.5
2.1.II (c)	70	109	6.8	10.3	16.0	13.3
2.2.II (c)	86	110	6.2	13.8	17.7	14.9
3.II (c)	81	109	6.5	12.4	16.8	14.1
4.II (sc)	55	68	7.5	7.3	9.1	11.5

[a] Samples and data are from a previous report.[24]
[b] I denotes first and II denotes second stage of α-amylolysis, d denotes domains, c clusters and sc sub-clusters.
[c] Average degree of polymerization of φ,β-LDs from the gel permeation chromatogram.
[d] Peak-DP in gel permeation chromatogram.
[e] Average chain length.
[f] Average number of chains per molecule.
[g] Number of chains per molecule estimated from the peak-DP.
[h] Degree of branching (%).

2.2. Time course of extensive α-amylolysis for building blocks production from φ,β-LDs of clusters

φ,β-LDs of clusters fraction 4.I (8 mg) was completely dissolved in 0.72 mL hot double distilled water with constant stirring. α-Amylase (80 μL, 30 U/mL) in sodium acetate buffer (0.01 M, pH 6.5) was added to initiate extensive α-amylolysis in a water bath (25 °C) with magnetic stirring. The concentrations for substrate and α-amylase in the reaction system were 10 mg/mL and 3 U/mL, respectively. Aliquots (80 μL) were sampled at time intervals up to 6 h, mixed with water (200 μL) and heated in a boiling water bath for 5 min to stop the reaction. If not analyzed immediately, the sample solution was stored at −18 °C. If analyzed immediately, the α-amylolysates were filtered through a Millipore filter (nylon membrane, 0.45 μm i.d.), and analyzed by gel permeation chromatography on Superdex 30 as described below.



Scheme 1. Flow diagram showing the preparation and size-fractionation of building blocks and their subsequent structural analysis. The principal molecular structures obtained are symbolized in the drawings.