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Composition of clusters and their arrangement in potato amylopectin

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Abstract

The cluster structure of amylose-free potato starch was investigated in detail. Groups of clusters (structural domains) were produced by α-amylolysis and size-fractionated by methanol precipitation. The domains were then further hydrolysed with α-amylase until free clusters were released. In the form of ϕ,β-limit dextrins the clusters possessed mainly DP around 70 and 50–55, but a minor group of very small clusters were of DP 25. In order to release the clusters, the α-amylase hydrolysed long B-chains into shorter B1b-chains. In the initial stages of hydrolysis small, branched fragments were released, possibly from branched structures outside the clusters. In domain structures composed of two clusters a B2-chain was involved in their interconnection, but when the number of cluster increased, the number of long B2- and B3-chains was apparently higher than predicted from the generally accepted cluster model. The two-directional backbone model offered a possible solution for the interconnection of groups involving several clusters.
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Keywords: Potato amylopectin starch; Amylopectin structure; Cluster structure; Cluster interconnection; α-Amylolysis

1. Introduction

Amylopectin is the major macromolecular component of starch and is responsible for the architecture of the starch granules. It is composed of a large number of short chains of approximately 6–35 α-D-glycosyl residues connected by (1 → 4)-linkages. The chains are divided into A-chains, which do not carry other chains, and B1-chains, which carry other chains through (1 → 6)-branches (Peat, Whelan, & Thomas, 1952). In the generally accepted model by Hizukuri (1986), the short chains form clusters that are interlinked through long B2- and B3-chains with a degree of polymerisation (DP) of approximately 40–50 and 60–80, respectively. The proportion of short to long chains depends on the plant species and varies typically from roughly 5 ∼ 13 on a molar basis (Hanashiro, Tagawa, Shibahara, Iwata, & Takeda, 2002).

Within the starch granules the external segments of the clusters form double helices that crystallize into either A- or B-polymorphs (Imberty, Buléon, Tran, & Pérez, 1991). A typical representative of the latter type is potato starch (Buléon, Bizot, Delage, & Multon, 1982; McPherson & Jane, 1999; Vermeylen, Goderis, Reynaers, & Delcour, 2004) and it was shown that B-type starches generally possess somewhat longer average chain lengths (CL) and a higher proportion of long chains than A-crystalline samples (Hizukuri, 1985). The crystals form approximately 5–6 nm thick lamellae that alternate with 3–4 nm thick amorphous lamellae, in which most of the branches of the clusters are found (Jenkins, Cameron, & Donald, 1993; Sanderson, Daniels, Donald, Blennow, & Engelsen, 2006). Stacks of these structures build up semi-crystalline granular rings, often called "growth rings", that alternates with apparently completely amorphous rings. Details of these structures, like the presence of blocklets (Atkin, Abeysekera, Cheng, & Robards, 1998; Baker, Miles, & Helbert, 2001; Gallant, Bouchet, & Baldwin, 1997) or super-helices (Oostergetel & van Bruggen, 1993; Waigh, Donald, Heidelbach, Riekel, & Gidley, 1999), the latter only proposed for potato starch so far, is still under debate.

Despite a rather good knowledge and understanding of the participation of the clusters in the fine structure of the

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starch granules, the knowledge about the fine structure of the clusters themselves and their actual mode of interconnection remains comparatively poor. Clusters were so far isolated from comparatively few samples by using endo-acting enzymes. Possibly because of different action patterns, the reported sizes of the clusters differ. Thus, clusters in potato amylopectin were estimated to be of uniform size with DP (in the form of limit dextrins, in which most of the external chain segments have been removed) around 140 (corresponding to M_n 23,000) when using the maltotetraose forming amylase from *Psuedomonas stutzeri* (Finch & Sebesta, 1992), but of three different sizes from DP 40 to 140 when using cyclodextrin glycosyltransferase (Bender, Siebert, & Stadler-Szöke, 1982), or from 33 to 70 when using the α-amylase of *Bacillus amyloliquefaciens*, which corresponds to roughly 5–10 chains per cluster (Zhu & Bertoft, 1996). Other investigators used other methods and suggested that clusters in various starches are build up by 4.22 (Thurn & Burchard, 1985), 22–25 (Hizukuri, 1986), 2.1–12.9 (Hanashiro et al., 2002), or 18–34 chains (Gallant et al., 1997). This suggests an obvious lack of definition for what is meant by a cluster, which necessarily leads to arbitrary definitions based on the methods of the analyses in use. When using the α-amylase of *B. amyloliquefaciens* (also named "liquefying amylase" of *Bacillus subtilis*), we defined a cluster as a dextrin that is easily released from the amylopectin by endo-attack at internal chain segments, but comparatively resistant to further hydrolysis (Bertoft, 1986).

Our studies using the *B. amyloliquefaciens* amylase have shown that clusters from waxy types of cereals (typically low in amylose content) of A-crystalline starches (maize, barley, and rice) are of several sizes from DP 34 ~ 200 (Bertoft, 1989a; Bertoft & Åvall, 1992; Bertoft, Zhu, Andtfolk, & Jungner, 1999), and are larger than those found in normal potato starch of the B-type (Zhu & Bertoft, 1996). A similar difference was found when mutants of maize possessing A- and B-crystallinity (*wxdu* and *aewx*, respectively) were compared (Gérard, Planchot, Colonna, & Bertoft, 2000). In all cases most of the long B-chains were absent in the isolated clusters, which is in accordance with the present model for the interconnection of the clusters (Hizukuri, 1986). Clusters from waxy rice were found to be interconnected to form domains of different fine structures, either within or between the amylopectin macromolecules (Bertoft et al., 1999), and some degree of domain structure was also suggested for amylopectin from normal potato starch (Zhu & Bertoft, 1996).

The aim of the present investigation was to achieve a thorough analysis of the composition of clusters and their unit chain composition in amylose-free potato starch. The mode of the interconnection of the clusters was also of interest, especially in the light of our recently proposed two-directional backbone model, in which this aspect is fundamentally different from the classical model (Bertoft, 2004). The strategy was to isolate domain structures (groups of clusters) from the amylopectin macromolecule by an initial, short α-amylolysis, and from these samples finally isolate the clusters by a continued enzymatic hydrolysis.

2. Materials and methods

2.1. Starch sample and enzymes

Potato amylopectin starch (PAPS) was a kind gift from Lyckeby Stärkelsen, Sweden, and contained only amylopectin. α-Amylase of *B. amyloliquefaciens* [(1 → 4)-α-D-glucan glucanohydrolase; EC 3.2.1.1] was purchased from Boehringer–Mannheim (now Roche), Germany, and had an activity (Bertoft, Manelius, & Qin, 1993) of 600 U/mg. Rabbit muscle phosphorylase *a* [(1 → 4)-α-D-glucan:orthophosphate α-D-glucosyltransferase; EC 2.4.1.1], specific activity 24 U/mg, was from Sigma, Germany, and barley β-amylase [(1 → 4)-α-D-glucan maltohydrolase; EC 3.2.1.2] with a specific activity of ca 1400 U/mg was from Megazyme, Ireland. Isoamylase from *Pseudomonas amyloderamosa* (glycogen 6-glucanohydrolase; EC 3.2.1.68), activity 71,000 U/ml, and pullulanase from *Klebsiella pneumoniae* (amylopectin 6-glucanohydrolase; EC 3.2.1.41), activity 404 U/ml, were purchased from Hayashibara Shoji Inc., Japan. All enzyme activities (except for α-amylase) were given by the suppliers.

2.2. Production of fractions of α-dextrins

PAPS (10 g) was dissolved in 90% dimethylsulphoxide (DMSO, 200 mL) on a boiling water bath for 15 min and then stirred at room temperature for 4 days. The solution was then diluted with water (700 mL) and the temperature adjusted to 25 °C before a solution (100 mL) of α-amylase (0.3 U/mL) in 0.01 M sodium acetate buffer, pH 6.5, was added. The reaction was stopped after exactly 1 h by the addition of 5 M KOH (25 mL). A small aliquot (0.2 mL) was taken for analysis by gel-permeation chromatography (GPC) as described below. The pH was adjusted to 11 with 1.5 M HCl and 5 volumes of methanol was added. A precipitate was allowed to settle overnight and was recovered by centrifugation (30 min at 1800*g*) to give sample I, which represented intermediate α-dextrins obtained by a first, initial stage of α-amylolysis (Fig. 1). The methanol in a part (70 mL) of the supernatant fraction (S-I) was evaporated (Büchi Rotavapor R-3000). Because the sample contained DMSO, it could not be lyophilised and was therefore stored in a refrigerator until further analyses.

The precipitate (sample I) was dissolved in hot water. A small amount of unsolubilised material was removed by centrifugation before the carbohydrate concentration (measured by phenol–sulphuric acid (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956)) was adjusted to 10 mg/mL. Sample I was then subjected to fractional precipitation in methanol (Bertoft & Spoof, 1989) as described in the scheme in Fig. 1. An initial addition of 0.5 volumes of methanol did not give any precipitate, but successive

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