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Structural and thermodynamic properties of rice starches with different genetic background
Part 2. Defectiveness of different supramolecular structures in starch granules

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Abstract

High-sensitivity differential scanning microcalorimetry (HSDSC), small-angle X-ray scattering (SAXS), light (LM) and scanning electronic (SEM) microscopy techniques were used to study the defectiveness of different supramolecular structures in starches extracted from 11 Thai cultivars of rice differing in level of amylose and amylopectin defects in starch crystalline lamellae. Despite differences in chain-length distribution of amylopectin macromolecules and amylose level in starches, the invariance in the sizes of crystalline lamellae, amylopectin clusters and granules was established. The combined analysis of DSC, SAXS, LM and SEM data for native starches, as well as the comparison of the thermodynamic data for native and annealed starches, allowed to determine the structure of defects and the localization of amylose chains in crystalline and amorphous lamellae, defectiveness of lamellae, clusters and granules. It was shown that amylose "tie chains", amylose–lipid complexes located in crystalline lamellae, defective ends of double helical chains dangling from crystallites inside amorphous lamellae ("dangling" chains), as well as amylopectin chains with DP 6–12 and 25–36 could be considered as defects. Their accumulation can lead to a formation of remnant granules. The changes observed in the structure of amylopectin chains and amylose content in starches are reflected in the interconnected alterations of structural organization on the lamellar, cluster and granule levels.

Keywords: Rice starches; Lamella; Cluster; Granule structure; HSDSC; SAXS; LM; SEM

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1. Introduction

For starches, four types of supramolecular structures differing in macromolecular organization and sizes can be studied in detail by DSC, SAXS, microscopic techniques and different physical approaches [1–15]. These structures include crystalline and amorphous lamellae (∼4–6 nm), amylopectin clusters (∼9 nm), semi-crystalline or amorphous rings (∼120–400 nm) and granules (∼0.5–100 μm). A recent investigation of rice starches showed that alterations in chain-length distribution of amylopectin chains and amylose content exert influence on the defectiveness of crystalline lamellae. The formation of amylopectin and amylose macromolecules with altered structures and defective crystalline lamellae are apparently interconnected processes that result from biosynthetic reactions and physical assembly processes. Indeed, analysis of the published data showed that maturation of starch-containing plants, decrease in environmental temperature during their development, and increase in granule bound starch synthase (GBSS I) activity are accompanied by the formation of defects within starch crystalline lamellae [1–9]. However, it is not possible to exclude that structural changes also influence on the

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macromolecular organization of supramolecular structures of higher levels.

Generally, both amylose content and chain-length distribution of amylopectin chains in starches exerts significant influence on the defectiveness of the crystalline lamellae through an alteration of their structural organization. Amylose chains could be localized both in amorphous and crystalline lamellae [7–10,16]. Amylose chains arranged in the amorphous lamellae and oriented transversely to lamellar stack could be considered as defects altering the structure of amorphous lamellae without exerting influence on the melting temperature of the crystalline lamellae [7–10]. Unlike such chains, amylose–lipid complexes and amylose "tie-chains" exert destabilizing effect on structure organization of crystalline lamellae and decrease their melting temperature. Amylopectin chains with DP < 13 and DP 25–36, disordered ends of double helices and double helices unpacked inside crystallites (molecular ordered structures) could be considered as defects arranged inside crystalline lamellae or at the boundaries to the amorphous lamellae [1–9,12,17–21]. Such defects promote the decrease in melting temperature of crystalline lamellae. An increase in amylose level under passing from amylopectin-rich to amylose-rich starches, or a decrease of environmental temperature during the development of starch-containing plants, is accompanied by an increase of the content of amylose defects in crystalline lamellae [4–7,9,22,23]. However, the increase in defectiveness of crystalline lamellae can be compensated by a rise of the relative amounts of amylopectin chains with DP 13–24 and >37, shown for rice starches [21].

Some correlations between the SAXS parameters and chain-length distribution of amylopectin chains were also recently shown [14]. It allowed evaluating the effects of chain distributions on structural organization of lamellae and amylopectin clusters in starches with A-, B- and C-type polymorphous structures. Particularly, the investigation showed that the degree of layer bending in B-type starches (starches with low level of short amylopectin chains) is sufficiently high, while layer bending in A-type starches (high level of short amylopectin chains) is practically absent. However, layer bending increases at increased amylose content, as shown for maize starches (A-type packing). Generally, the degree of deviation from the radial orientation of starch supramolecular layer structures proposes their defectiveness. It is implied, that chain-length distribution of amylopectin chains exerts an influence both on the defectiveness of starch lamellae and amylopectin clusters and apparently, semi-crystalline growth rings, a supramolecular structure of higher order compared with the alternating lamella structure. As assumed by Glaring et al. [24], a lack of visible growth rings in rice starch represents a deposition of crystalline amylopectin as flat layers, and therefore any strain due to amylose chains introduced by having to bend the crystalline arrays to form a circular structure would be eliminated. Summing up the above noted, it could be proposed that the chain-length distribution of amylopectin chains, amylose "tie-chains", and amylose–lipid complexes located within the lamellae could be considered as the protosource of defectiveness of different starch supramolecular structures. However, the conclusion needs additional evidences from subjects of a single origin by using different methods and approaches. As shown in our previous work, rice starches with different amylose content and chain-length distribution of amylopectin chains are promising subjects for finding the evidence to support this hypothesis [21].

The structure of amylose and amylopectin defects can be determined by the analysis of thermodynamic data for native and annealed starches [8,9,25,26]. By using this approach, the structure of amylose and amylopectin defects in crystalline lamellae of starches extracted from various cultivars of potato, sweet potato, wheat, and barley were successfully tested. Additionally, the combined DSC–SAXS approach based on analytical data produced using a one-dimensional paracrystalline model and "two-state" melting model was developed to evaluate the structure of such defects and their localization within crystalline lamellae and amylopectin clusters [8,9,25,26]. At present different approaches based on combined analysis of data produced by using different microscopic (LM, SEM, AFM, CLSEM and others), SAXS, WAXS, and chromatographic techniques are intensively developed [13–15,24,27]. Unfortunately, the analysis of literature data showed that experimental data published are in conflict with each other. For example, SAXS investigation of three rice samples showed that (i) unlike other A-polymorphous starches, the degree of layer bending for rice starches is equivalent to B-polymorphous starches, (ii) the sizes of amylopectin clusters are practically the same irrespective of amylose level, and (iii) a rise of amylose level in starches leads to increasing sizes of crystalline lamellae [13,14]. However, the latter conclusion conflicts with the results obtained from DSC-data that showed invariance in the thickness of crystalline lamellae of starches extracted from 11 cultivars of rice [21]. Additionally, the one-dimensional paracrystalline model, applied to a description of SAXS curves [14] implies parallel arrangement of alternating structures in semi-crystalline growth rings and their radial orientation. However, growth rings are not seen in microscopic images [24]. Generally, systematic investigations that allow a description of defectiveness of various supramolecular structures using different physical–chemical approaches are missing.

Recently, a combined DSC–HPAEC-PAD approach, gel-permeation chromatography and mild long-term acidic hydrolysis was employed to study the effects of amylopectin chain-length distribution and amylose defects on the assembly structures of amylopectin (crystalline lamellae, amylopectin clusters) in A-type polymorphic starches extracted from eleven Thai cultivars of rice with different amylose level [21]. Joint analysis of the data allowed to determine the contributions of different populations of amylopectin chains to the thermodynamic melting parameters of the crystalline lamellae. Additionally, the study of the total effect of amylose and amylopectin defects on the thermodynamic behavior of starches and the rates of their acidic hydrolysis allowed a subdivision of the starches into two cultivar groups. For starches from the first group, a rise of amylose level leads to an increase of the melting temperature and the melting enthalpy of crystalline lamellae and a decrease in the rate of acidic hydrolysis of the amorphous and crystalline parts of starch granule, whereas for starches from the second group these parameters remained unchanged despite the increased amylose content. Application of different physical