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Understanding the structural features of high-amylose maize starch through hydrothermal treatment

Jianing Yang[a], Fengwei Xie[b,**], Wenqiang Wen[a], Ling Chen[c], Xiaoqin Shang[a], Peng Liu[a,*]

[a] School of Chemistry and Chemical Engineering, Guangzhou University, Guangzhou 510006, China
[b] School of Chemical Engineering, The University of Queensland, Brisbane, Qld 4072, Australia
[c] Ministry of Education Engineering Research Center of Starch and Vegetable Protein Processing, Guangdong Province Key Laboratory for Green Processing of Natural Products and Product Safety, College of Light Industry and Food Sciences, South China University of Technology, Guangzhou 510640, China

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ABSTRACT

In this study, high-amylose starches were hydrothermally-treated and the structural changes were monitored with time (up to 12 h) using scanning electron microscopy (SEM), confocal laser scanning microscopy (CLSM), small-angle X-ray scattering (SAXS), X-ray diffraction (XRD), and differential scanning calorimetry (DSC). When high-amylose starches were treated in boiling water, half-shell-like granules were observed by SEM, which could be due to the first hydrolysis of the granule inner region (CLSM). This initial hydrolysis could also immediately (0.5 h) disrupt the semi-crystalline lamellar regularity (SAXS) and dramatically reduce the crystallinity (XRD); but with prolonged time of hydrothermal treatment (≥2 h), might allow the perfection or formation of amylose single helices, resulting in slightly increased crystallinity (XRD and DSC). These results show that the inner region of granules is composed of mainly loosely-packed amylopectin growth rings with semi-crystalline lamellae, which are vulnerable under gelatinization or hydrolysis. In contrast, the periphery is demonstrated to be more compact, possibly composed of amylose and amylopectin helices intertwined with amylose molecules, which require greater energy input (higher temperature) for disintegration.

1. Introduction

Starch is a biopolymer that naturally exists in the form of granules in plants; and the granule is structured in a highly complex way, which has not been fully understood so far [1–3]. Starch has great potentials in a diversity of applications, with some new applications in foods (resistant starch, microporous starch, etc.) [4,5], pharmaceutical materials (drug delivery systems) [6–8], and biodegradable plastics (starch-based materials) [9–11]. A clear understanding of starch structures and how its structures change under different treatments is indispensable for the utilization of starch with desired properties.

It is worth noting that in many applications like those above-mentioned, high-amylose starch (a type of starch by genetic modification) is preferably used. High-amylose starch has some special properties, such as its heat resistance [12], which is reflected by high gelatinization temperature and the retainment of granules in boiling water [13–15], and its digestion resistibility [16,17], due to which this starch has been used as resistant starch and in drug delivery systems. In addition, high-amylose starch is especially suitable for producing thermoplastic materials [18,19], because amylose as a linear molecule can provide better mechanical properties resulting from easier formation of crystallites and entanglements. However, when preparing materials, a significant problem would be just the resistance to processing or treatment, because the complete destruction of original starch supramolecular structures is required to form a continuous integrated phase [12–15]. In other words, due to its special structural organization, native high-amylose starch is more resistant to hydrolysis or disintegration by small molecules such as water, enzymes, etc., which negatively impacts on its application.

These particular behaviors of high-amylose starch can hardly be explained by the theories obtained from waxy and regular starches, of which the structural features have been more intensively studied [1–3]. Despite the lower crystallinity, high-amylose starches have a rather compact structure without weak points or voids, which may explain those behaviors to some degree [16,17,20,21]. Nevertheless, the reason for such a compact granule organization, and how

* Corresponding author. Fax: +86 20 3936 6903.
** Corresponding author. Fax: +61 7 3346 3973.
E-mail addresses: f.xie@uq.edu.au, fwhsieh@gmail.com (F. Xie), liu_peng@gzhu.edu.cn (P. Liu).

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this compact structure is altered during hydrolysis by e.g. water, are not well understood.

There have already been many studies on the compact granule organization of native high-amylose starches [22–27]. Leach and Schoch [25] found that different types of starches showed different degrees of enzyme susceptibility, with waxy maize starch granules being the most susceptible and high-amylose maize starch the least susceptible. It has been proposed that amylose concentrated at the periphery of starch granules could interacts with amylopectin to form a hard shell [28–30], and that amylose could form crystalline structure especially in high-amylose starch granules [31,32], both of which could make the starch granules more compact. In addition, some researchers [33,34] showed that there was no correlation between crystallinity and enzyme susceptibility, and the hydrolysis residues were composed of both amorphous material and B-type crystallites; therefore, they proposed that the distribution of B-type crystallites within the granule and their influence on local granule organization is important. However, to the best of our knowledge, there has been no further exploration on the structural organization of native high-amylose starch granules which account for its compactness.

The current study aims to further explore the structural features of native high-amylose starch, which was done by monitoring the structural changes of high-amylose starch during hydrothermal treatment.

2. Experimental

2.1. Materials and Chemicals

Two varieties of commercially-available maize starches used in this work, Gelose 50 (G50), and Gelose 80 (G80), were supplied by National Starch Pty Ltd. (Lane Cove NSW 2066, Australia). Both of the two starches were chemically unmodified; and their amylose contents were 56.3% and 82.9%, respectively, and their degrees of crystallinity were 31.3% and 28.3%, respectively [35,36], both as measured previously. 8-Aminopyrene-1,3,6-trisulfonic acid trisodium salt (APTS) and sodium cyanoborohydride were purchased from Sigma–Aldrich China (Mainland) (Shanghai, China).

2.2. Hydrothermal treatment of starch

10 g (dry basis) of starch (either G50 or G80) was weighed into distilled water to form 5% suspensions. In a closed environment, these starch suspensions were heated using an oil bath (102 °C) with stirring. Once the suspension reached 50 °C, the time counting was started from zero. From 50 °C (time zero), the temperature could rapidly increase to ca. 98 °C within 5 min; afterwards with prolonged time, the temperature could be maintained at ca. 98 °C. After specific times (0.5 h, 1 h, 2 h, 4 h, 12 h), one suspension was taken out and immediately quenched in an ice bath for stopping any further changes. After that, the samples were centrifuged at 5000 rpm for 10 min. The sediments were washed with distilled water for five times, and then freeze–dried for further examinations.

2.3. Scanning electron microscopy (SEM)

The freeze-dried starch samples were adhered to specimen holders using a silver adhesive, and then coated with gold in a vacuum evaporator. The obtained specimens were viewed for their morphologies by a scanning electron microscope (JSM-7001F, Japan Electronics Corporation) at an accelerating voltage of 15 kV.

2.4. Confocal laser scanning microscopy (CLSM)

The starch samples used for CLSM observation were only washed and centrifuged without freeze–drying. APTS was used as the stain and sodium cyanoborohydride were used as the reducing agent. Specifically, 2 mg of the sample was added into a 3 μL freshly-prepared APTS solution (10 mM, with 15% acetic acid solution as the solvent), which was mixed with 3 μL of 1 M sodium cyanoborohydride solution. The mixtures with the starch samples were sealed and kept in a dark place for 15 h at room temperature. Then, the samples were washed with distilled water for five times, and dispersed in a 1:1 water/glycerol solution, before CLSM observation.

Detection of the fluorescence signals from the dye-stained starch samples was carried out using a confocal laser scanning microscope equipped with He/Ne/Ar laser (Nikon Digital Eclipse C1-Plus, Nikon Instruments Inc., Melville, NY, U. S. A.). The detail of objective lens used was Plan Apo VC 60×/1.40 oil. The excitation wavelength was 488 nm. And the emitted light was detected between 500 and 535 nm [37]. The resolution of generated images was 512 × 512 pixels. The magnification of photos was determined by the product of eye lens and objective lens (10 × 60). During image acquisition, each line was scanned for four times and averaged in order to reduce noise.

2.5. Small-angle X-ray scattering (SAXS) analysis

SAXS experiments were performed using an SAXSess system (Anton–Paar GmbH, Austria) equipped with a PW3830 X-ray generator (PANalytical B.V., Netherlands), operated at 50 mA and 40 kV, using Cu Kα radiation with a wavelength of 0.1542 nm as the X-ray source. The moisture content of freeze–dried starch samples were adjusted to 50% by adding a certain amount of water, and equilibrated at 20 °C for 24 h, before the detection. The sample was filled into a sample cell and measured for 10 min. The data, recorded using an image plate, were collected by the IP Reader software program with a PerkinElmer® Storage Phosphor System. All data were normalized, and the background intensity and smeared intensity were removed using the SAXS Quant 3.0 software program for further analysis.

2.6. X-ray diffraction (XRD) analysis

XRD analysis was performed with an Xpert PRO diffractometer (PANalytical B.V., Netherlands), operated at 40 mA and 40 kV, using Cu Kα radiation with a wavelength of 0.1542 nm as the X-ray source. The scanning was carried out with the diffraction angle (2θ) from 5° to 50° with a scanning speed of 10°/min and a scanning step of 0.033°. The samples were equilibrated at 40 °C for 24 h and the moisture content of all the samples was about 10% before analysis.

The degrees of crystallinity of the samples were quantitatively estimated according to a previous study [36]. Specifically, a smooth curve connected with the peak baseline was computer-plotted on the diffraction. The area above the smooth curve was regarded as the crystalline portion, while the lower area between the smooth curve and a linear baseline that connected the three points at 4°, 6°, and 28° was taken as the amorphous portion. The upper diffraction peak area and the total diffraction area were integrated by MDI Jade 6. The ratio of upper area to total diffraction area was taken as the degree of crystallinity.

2.7. Differential scanning calorimetry (DSC)

A PerkinElmer® Diamond DSC with an internal coolant (Intercooler IP) and nitrogen purge gas was used in the experimental work. The melting points and enthalpies of indium and zinc were