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Thermal and enzymatic degradation induced ultrastructure changes in canna starch: Further insights into short-range and long-range structural orders

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ABSTRACT

Slurries of canna starch treated with or without α-amylase were heated at 60 °C to investigate the mechanism of thermal and enzymatic degradation and the effects of these processes on the ultrastructure of starch residues. For this purpose, solid-state ^{13}C CP/MAS NMR, X-ray diffraction (XRD), and small angle X-ray scattering (SAXS) were used to estimate the short- and long-range orders within the structure. Crystal stabilization was observed in both samples of thermally degraded canna starch (TDCS) and enzymatically degraded canna starch (EDCS), and the critical time for the formation of double helical crystals was 3 h for thermal degradation and 5 h for enzymatic degradation. With longer times, imperfect sub-crystal started prevailed. The results showed that when degradation occurs, the crystal lamellae compact, as indicated by a higher crystal density in the structure of EDCS. Moreover, the relationship between crystalline weight and volume fraction as well as short-range and long-range orders were also investigated and found correlate well with each other.

1. Introduction

Starch is composed of two types of α-glucans joined through α-D-(1 → 4) and α-D-(1 → 6) glycosidic bonds: nearly linear amylose and highly branched amylopectin. Starch granules consist of amorphous and ordered areas, and the ordered areas are formed from clusters of short amylopectin chains. Ordered areas within starch have been identified and are composed of short- and long-range structures. Using small angle X-ray scattering (SAXS), Cameron and Donald (1992) and Oostergetel and van Bruggen (1989) found that starch was arranged in lamellae. Although SAXS can monitor crystal structure and relative amounts of crystalline and amorphous lamellae in starch, it is only sensitive to long-range order. NMR gives access to short-range, atomic level measurement. Using this technique, the amorphous, single, and double helical patterns in starch could be identified. Wilson and Pake (1953) first used NMR to determine the short-range order in two polymers and then, Tait, Ablett, and Wood (1972) applied this technique to study starch crystal.

The behavior of aqueous starch slurries is of interest because many industrial applications involve the heating of aqueous starch slurries as part of starch processing. Enzymatic degradation via α-amylase improves the crystalline perfection of starch by preferentially eroding amorphous regions over ordered crystallites (Imam et al., 2006; Sievert, Czuchajowska, & Pomeranz, 1991). This results in a porous structure in the starch residue. Unlike enzymatic degradation, thermal degradation is a physical modification, which is very similar to annealing and pre-gelatinization (Zavareze & Dias, 2011). In the process of thermal degradation, water-imparted chain rearrangement results in an increase in the flexibility or plasticity of polymers. This is especially prevalent when the plasticizer is heated, this kind of plastication may then increase (Suyatma, Tighzert, Copinet, & Coma, 2005; Zhai, Yoshii, & Kume, 2003). Besides plastication, thermal degradation facilitates the partitioning of amylose and amylopectin double helices (Tester & Debon, 2000). This results in a low level of soluble amylose. The porous structure and low amylose content in degraded starch residue has been

Abbreviations: X-ray diffraction, XRD; small angle X-ray scattering, SAXS; thermally degraded canna starch, TDCS; enzymatically degraded canna starch, EDCS; scanning electron microscopy, SEM; dextrose equivalent, DE; partially stabilized zirconium oxide, PSZ; native canna starch, NCS.

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studied using scanning electron microscopy (SEM) and nitrogen and iodine sorption (Baldwin, Adler, Davies, & Melia, 1994; Sujka & Jamroz, 2009). However, the short- and long-range structural orders have not been well characterized.

Cannas are nutrient-rich, contain over 66% starch in their rhizomes (dry weight), and are a crop that can adapt successfully to a wide range of habitats, including marginal regions. The aim of this study was to investigate the long- and short-range structural changes that occur following both thermal and enzymatic degradation in canna starch. Getting an accurate molecular description of degraded starch will help researchers unlock the mystery of starch degradation.

2. Materials and methods

2.1. Materials

Native canna starch (NCS) (30.88% amylose) was obtained from Ziyun Jiahe Chemical Co. Ltd. (Guizhou, China). The α-amylase (3700 U/g) from *Bacillus subtilis* was purchased from Beijing Aoboxing Bio-tech Co. Ltd. (China). Double distilled water was used in all experiments. Other reagents were of analytical grade and were purchased from China National Medicine Group (China).

2.2. Methods

2.2.1. Preparation of thermally degraded canna starch (TDCS) and enzymatically degraded canna starch (EDCS)

The starch slurry (10%, w/v) was prepared in phosphate buffer (pH 6.9) with α-amylase added (50 U/g starch). Then, the samples were incubated in a water bath with continuous stirring at the critical geletinization temperature (60 °C). Aliquots of each suspension were taken at different time intervals. Samples of EDCS were isolated by centrifugation and washed twice with 80% ethanol and then lyophilized. The supernatants were kept together for a subsequent dextrose equivalent (DE) determination. As a control, TDCS was prepared as above without addition of α-amylase. Starches were treated for 1, 3, 5, and 7 h, and the samples were denoted with treating method and respective treating time (eg. TDCS-1 indicates thermal degradation for 1 h, similarly, EDCS-1 indicates enzymatic degradation for 1 h).

2.2.2. X-ray diffraction analysis

XRD experiments were performed with a Bruker-AXS D8 ADVANCE powder diffractometer using Cu-Kα radiation (wavelength $\lambda = 1.54$ nm), operating at 40 kV and 40 mA. Data were collected by step-scanning at 0.02° intervals over the 2θ range of 3–40°. Degree of crystallinity was estimated using the nonlinear peak fitting method (Nara & Komiya, 1983).

2.2.3. Solid-state ^{13}C CP/MAS NMR

Solid state ^{13}C CP/MAS NMR experiments were performed at a ^{13}C frequency of 100.61 MHz on an AVANCE III 400 spectrometer (Bruker BioSpin GmbH). Approximately 100 mg of starch sample was packed in a 4-mm diameter, cylindrical, partially stabilized zirconium oxide (PSZ) rotor with a Kel-F end cap. The rotor was spun at 5 kHz at the magic angle (54.7°). The 90° pulse width was 4 ms, and a contact time of 2 ms was used for the starch sample with a recycle delay of 4 s. The spectral width was 30 kHz, acquisition time 34 ms, time domain points 2 k, transform size 4 k, and line broadening 50 Hz. A minimum of 1024 scans were accumulated for each spectrum to obtain a satisfactory signal to noise ratio. Spectra were analyzed using the high field resonance of glycine (carbonyl at 176.03 ppm) as the reference. The program PeakFitTM version 4.12 (SYSTAT Software Inc., CA) was used to peak fit all spectra including the sub-spectra according to the method described by Tan, Flanagan, Halley, Whittaker, and Gidley (2007).

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

Samples for SAXS measurements were prepared as aqueous starch slurries (~45% (w/v) starch), centrifuging at 8000*g* for 10 min after an overnight equilibration. The SAXS experiments were conducted on beamline BL16B1 at the Shanghai Synchrotron Radiation Facility. An incident wavelength of 1.24 nm was used, and the sample-to-detector distance was set to 2150 mm. Scattering was detected in the q ranges of 0.06–1.88 nm^{-1} (where $q = (4\pi sin\theta)/\lambda$, the scattering vector). The isotropic scattering patterns were radially averaged, empty scattering was subtracted, and the resulting SAXS intensity was analyzed as a function of the scattering vector q. Data processing was performed using the FIT2D software package.

2.2.5. Analysis of SAXS data

The obtained SAXS data were analyzed via the one dimensional linear correlation function $\gamma_1(x)$ (Equation (1)) to obtain the long-range order. The correlation function was calculated using a cosine transformation of the scattering intensity, I (q), where x represents the distance in real space, and denominator is the scattering invariant (Goderis, Reynaers, Scherrenberg, Mathot, & Koch, 2001).

$$\gamma(x) = \frac{\int_0^\infty I(q)q^2 \cos(qx)dq}{\int_0^\infty I(q)q^2 dq} \quad (1)$$

3. Results

3.1. Investigation of single and double helical short-range order

The NMR analysis can detect the short-range order of the double helical packing. The chemical shifts obtained in these NMR spectra are the “isotropic” values for the solid state and can be used for structural elucidation in terms of both molecular and crystal structures. Signals at 94–104, 81–84 ppm are attributed to C-1 and C-4 respectively. The signal at 59–62 ppm is assigned to the carbon atoms (C6) of the CH_2OH group. The large signal around 70–73 ppm, is for carbon atoms (C2, C3, C5) connected by −OH groups.

As mentioned previously, the natural starch can be classified into two groups, “A” and “B” starch, on the basis of X-ray diffraction data (Lan, Li, Xie, & Wang, 2015a). A similar classification of starch can be made on the basis of ^{13}C CP/MAS spectra. The “A-type polymorph” starch shows a characteristic triplet for C1, while the “B-type polymorph” starch shows a characteristic doublet. Fig. 1a and b summarize the ^{13}C CP/MAS NMR spectra of native canna starch (NCS) and degraded starches. A characteristic doublet was observed in nearly all starches at C1 except EDCS-7. This is because the threefold symmetry of adjacent helices, in the B-type polymorph, leads to two different residues per unit (Buléon, Colonna, Planchot, & Ball, 1998). Two remarkable differences are observed in Fig. 1b between NCS and EDCS. Firstly, the intensity of C1, C-2,3,5 and C6 resonances in samples of EDCS is found to increase with increasing time. And the characteristic resonances for B-type allomorph (C1) in Fig. 1b change to the characteristic triplet for A-type allomorph, though this is not very obvious. This proves that there is a transformation of B- to A-type allomorph during the enzymatic degradation of canna starches. Samples of TDCS, on the other hand, exert trivial difference in their NMR resonances.