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Molecular encapsulation of ascorbyl palmitate in preformed V-type starch and amylose

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ABSTRACT

In the present study, we introduce a simple method to prepare inclusion complexes by "inserting" guest molecules into preformed "empty" V-type amylose helices. Ascorbyl palmitate (AscP) was used as a model guest material to investigate the effect of solvent environment, complexation temperature, annealing and guest concentration on inclusion complex formation. High complexation temperature was not necessary for encapsulating guest molecules in amylose helices, avoiding thermal degradation of guest compounds. This method would also avoid the wasting of guest materials because uncomplexed guest can be reused. It was found in the study that intermediate ethanol and acetone concentrations (generally 40–60%, v/v) at room temperature were appropriate for the complexation between V-amylose and AscP. Annealing, i.e. heat treatment in ethanol solutions at elevated temperatures (45–70 °C), was able to significantly increase the crystallinity of V-amylose and V-starch to as high as 65% and facilitate greater complexation evidenced from higher enthalpies, probably due to more regularly arranged helical cavities in larger crystalline phase. The complexation between V-amylose and AscP was also found to be enhanced with AscP concentration, while the dissociation temperature experienced a slight decrease.

1. Introduction

Starch, especially its amylose component, is well known to form inclusion complexes with a variety of small molecules. The amylose helix has a hydrophilic outer surface and a hydrophobic helical channel that accommodates the guest molecules (Immel & Lichtenthaler, 2000). The amylose helices may pack together in a crystalline structure known as V-type, with a number of subtypes. In the presence of small guest molecules, for instance iodine (Bluhm & Zugenmaier, 1981) and fatty acids (Godet, Buleon, Tran, & Colonna, 1993), amylose forms 6-fold, left-handed single helices stabilized by hydrogen bonds (Conde-Petit, Escher, & Nuessli, 2006). Crystals of such intra-helical inclusion complexes can be in the forms of V-hydrate (V_h) or V-anhydrous (V_a). The V_h form has a hexagonal unit cell with parameters $a = b = 13.65$ Å, $c = 8.05$ Å (Brisson, Chanzy, & Winter, 1991). The V_h form can transform to V_a, which has an orthorhombic unit cell structure with dimensions of approximately $a = 13.0$ Å, $b = 23.0$ Å, $c = 8.05$ Å (Rundle, 1947; Winter & Sarko, 1974; Zobel, French, & Hinkle, 1967), on losing water between the helices. When molecules bulkier than linear alcohols are included into the amylose helical cavity, crystals of larger dimensions can be obtained, e.g. the amylose-*tert*-butanol inclusion complex ($V_{tert\text{-butanol}}$, or V_7) contains 7-fold single helices with larger cavities to accommodate the guest molecules (Zaslow, 1963). A number of guest molecules, e.g. n-butyric acid (Takeo, Tokumura, & Kuge, 1973), cyclohexanol (Yamashita, Ryugo, & Monobe, 1973), menthone (Nuessli, Putaux, Bail, & Buléon, 2003; Tapanapunnitikul, Chaiseri, Peterson, & Thompson, 2007), thymol (Tapanapunnitikul et al., 2007), fenchone (Nuessli et al., 2003), geraniol (Nuessli et al., 2003), salicylic acid (Oguchi, Yamasato, Limmatvapirat, Yonemochi, & Yamamoto, 1998), and 2-naphthol (Uchino, Tozuka, Oguchi, & Yamamoto, 2001), complex with amylose to form 7-hold helices. Other than these, a few molecules, e.g. 1-naphthol (Cardoso et al., 2007; Yamashita et al., 1973) and quinolone (Yamashita et al., 1973) are able to induce amylose inclusion complexes with 8-fold helices (V_8).

Starch-guest inclusion complexes may be useful as a delivery system for guest molecules. For instance, amylose complexed with conjugated linoleic acid (Lalush, Bar, Zakaria, Eichler, & Shimoni, 2004), genistein (Cohen, Orlova, Kovalev, Ungar, & Shimoni, 2008), esters of vitamins and fatty acid (Lay Ma, Floros, & Ziegler, 2011), and long chain unsaturated fatty acids (Lesmes, Barchechath, & Shimoni, 2008; Lesmes, Cohen, Shener, & Shimoni, 2009), have been

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produced for controlled release purposes. It is expected that the active ingredients, such as essential fatty acids, lipophilic vitamins, and soy isoflavones, can be protected against the acidic environment of the stomach, and their bioavailability may be increased, since the bioactive guest compounds can be released in the lower gastrointestinal tract when amylose or starch is broken down by the action of endogenous enzymes or saccharolytic bacteria (Shimoni, Lesmes, & Ungar, 2010; Yang, Gu, & Zhang, 2009).

It is often assumed that the presence of the guest molecule is required to induce helix formation in the amylose chain, for example, Scheme 1 in (Liu, Jiang, Wang, & Zhang, 2007). Therefore, current techniques to form starch inclusion complexes involve first converting amylose to its "destructured" (i.e. random coil) state, followed by inducing coil to helix transition in the presence of guest molecules (see Fig. S1 in Supplementary data). There have been three primary methods to form amylose random coils or loose helices, and thus three methods to prepare amylose-guest inclusion complexes, namely the dimethyl sulfoxide (DMSO) method, alkali method, and high temperature method. In the DMSO method, starch or amylose is dissolved in an aqueous DMSO solution (e.g. 95%, v/v DMSO) and mixed with the desired guest. The dispersion is diluted with water at an elevated temperature (around 90 °C) and allowed to cool slowly. The inclusion complexes formed then precipitate (Lay Ma et al., 2011). In the alkali method, starch or amylose is dissolved in an aqueous alkali solution (e.g. 0.01 M potassium hydroxide) and mixed with the desired guest compound. The dispersion is then neutralized and slowly cooled prior to precipitation (Lalush et al., 2004). In the high temperature method, an aqueous solution of starch or amylose is heated to a high temperature (e.g. 155 °C) and mixed with guest compounds at a lower temperature (e.g. 90 °C). The dispersion is allowed to cool and precipitated inclusion complex collected (Tapanapunnitikul et al., 2007). For example, Kumar, Woortman, and Loos (2013) illustrate (their Fig. 1) that styrene is by necessity exposed to high temperature (160 °C) for complexation (Kumar et al., 2013), as is polytetrahydrofuran, since a low temperature (60 °C) method did not result in inclusion complex formation unless the samples were heated to high temperature in the DSC (Rachmawati, Woortman, & Loos, 2013). These methods expose the guest molecule to the same conditions necessary for destructuring the starch, namely, high concentrations of DMSO, high temperature (>90 °C), or high pH (>11.0), which may be detrimental to the guest molecule.

Starch-guest inclusion compounds have been known for over 200 years as the subject of hundreds of scientific studies, yet no technical applications have been successfully commercialized (with the possible exception of dough conditioners). A structural homologue to the V-type amylose-guest inclusion complex is the cyclodextrin-guest inclusion complex, which has been employed in numerous high-value applications, for instance, in the pharmaceutical industries to improve drug stability, solubility, bioavailability, and control drug release (Davis & Brewster, 2004; Van de Manakker, Vermonden, van Nostrum, & Hennink, 2009).

One of the greatest hindrances to successful exploitation of starch-guest complexes is the lack of a cost-efficient and reproducible method for their preparation. As aforementioned, current techniques to produce starch-guest inclusion complexes require a relatively high processing temperature (at least 70–80 °C) and a long time to allow for complexation, crystallization and precipitation (hours to days). In contrast, the preparation of cyclodextrin-guest inclusion complexes generally requires much less time and lower temperature (Duchene, 2011). Common methods include co-crystallization, co-evaporation, co-grinding, and kneading. In most cases, guest molecules enter the cyclodextrin cavities spontaneously when a particular solvent environment is satisfied. Despite easy preparation and successful commercialization, the use of cyclodextrins has certain drawbacks. Because

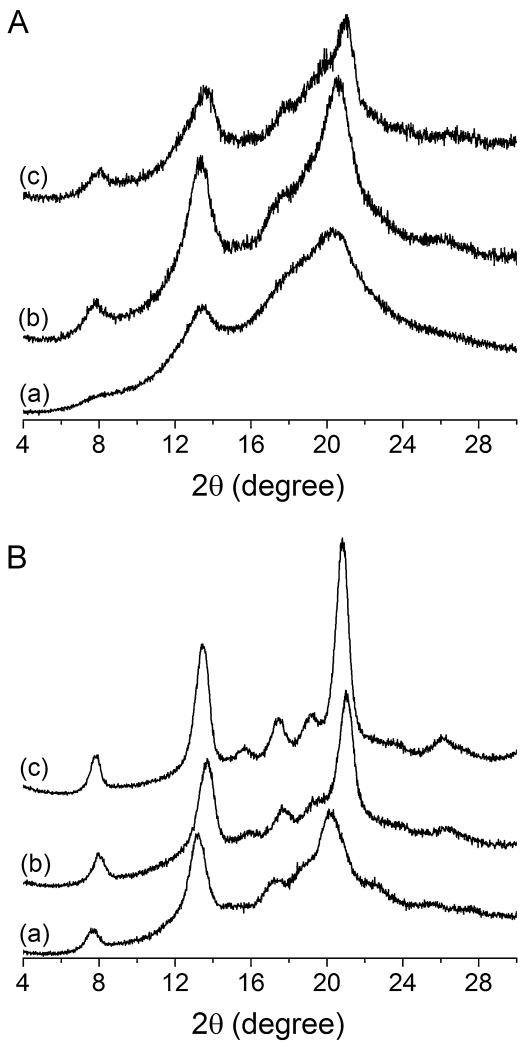

Fig. 1. (A) X-ray diffraction patterns of (a) V-starch precipitated by DMSO method, (b) V-amylose precipitated by DMSO method, and (c) V-amylose precipitated by high temperature method. (B) X-ray diffraction patterns of (a) the V-starch precipitated by DMSO method and annealed in 50% (v/v) ethanol at 70 °C, and V-amylose precipitated by DMSO method and annealed in (b) 50% and (c) 40% (v/v) ethanol at 70 °C.

they are produced from starch degradation by cycloglycosyl transferase amylases produced by various bacilli (Sicard & Saniez, 1987), cyclodextrins are costly compared with starch and amylose. When complexation efficiency is low, precipitated product is a mixture of the inclusion complex, empty cyclodextrin, and free guest. Empty cyclodextrin is wasted and production cost is increased. Finally, certain methods (e.g. co-crystallization) using aqueous solutions are restricted to β-cyclodextrin, because low solubility is required for easy precipitation. Though the product comprises inclusion complexes only, the yield is often very low.

Here we introduce a new method to create amylose-guest inclusion complexes, which involves first precipitating "empty" V-type amylose helices and then "inserting" guest molecules into the helices at temperatures well below the melting point of the V-type amylose (see Fig. S2 in Supplementary data). This method is similar to the preparation of cyclodextrin-guest inclusion complexes and has several advantages over the traditional techniques for amylose inclusion complexation. First, this method does not require a high temperature during inclusion complex formation, so degradation of heat-labile compounds can be minimized. Second, uncomplexed compounds in the solvent are not discarded.

This makes it appropriate for expensive compounds, which would otherwise be washed away in previous methods. Finally, complexation takes place rapidly when the V-type amylose and guest are mixed together. Hence, this simplified technique to produce amylose inclusion complexes has easy scalability and potential to replace the more costly cyclodextrins in a number of applications.

In this study, we demonstrate the preparation of "empty" V-type amylose/starch and the subsequent formation of inclusion complexes with a model bioactive guest compound, i.e. ascorbyl palmitate (AscP). AscP is ascorbic acid (vitamin C) esterified to palmitic acid, which has been used as a source of vitamin C and an antioxidant food additive. AscP was chosen as a model compound because its complexation with amylose has been demonstrated (Lay Ma et al., 2011) and it has higher solubility in aqueous ethanol solutions than fatty acids, e.g. palmitic acid. We use both purified amylose and high amylose starch, because the separation of pure amylose from starch is a complicated and costly process, and thus it may be more economical to use high amylose starches for most practical applications. We report the effect of complexation temperature, solvent quality, AscP concentration, and annealing of V-amylose on the ability and efficiency of inclusion complex formation.

2. Materials and methods

2.1. Materials

High amylose maize starch (Hylon VII) was kindly provided by Ingredion Incorporated (Bridgewater, NJ) and used as received. Potato amylose (essentially free from amylopectin), and ascorbyl palmitate (AscP) were purchased from Sigma–Aldrich, Inc. (St. Louis, MO). Acetone (HPLC grade) was obtained from EMD Millipore Chemicals (Billerica, MA). Ethanol (200 proof) and dimethyl sulfoxide (DMSO) were obtained from VWR International (Radnor, PA).

2.2. "Empty" V-amylose and V-starch preparation

The "empty" V-amylose and V-starch (aka V_a or $V_{ethanol}$) were prepared following two routes. In the first method, amylose or starch (5%, w/v) was dissolved in 95% (v/v) aqueous DMSO solution in a boiling water bath with constant stirring for at least 1 h. Then the hot amylose or starch dispersion was mixed into 2.5 volumes of ethanol (at ambient temperature) with vigorous stirring. The mixed suspension was then centrifuged (2000 g, 10 min). The precipitate was washed with ethanol twice and finally dried. In the second method, amylose or starch (1%, w/v) was dispersed in water at 150 °C for 30 min in a stirred pressure reactor (Parr-4592, Parr Instrument Company, Moline, IL). The amylose or starch dispersion was cooled to 90 °C and mixed into 2.5 volumes of ethanol. The V-amylose and V-starch were recovered by the same centrifugation, washing and drying procedure as described in the first method.

2.3. Annealing of the "empty" V-amylose and V-starch

The dry powders of V-amylose and V-starch were heated in aqueous ethanol solutions of varying concentration at different temperatures for 10 min. The combination of these conditions resulted in varying crystallinities of the annealed V-amylose and V-starch. Samples annealed at 70 °C in 40% (v/v) aqueous ethanol showed the highest crystallinity and were thus used in this study. Annealed V-amylose and V-starch were recovered by the same centrifugation, washing and drying procedure as described earlier.

2.4. Inclusion complex formation

Guest compounds (0.05 g) and the preformed "empty" V-amylose or V-starch (0.1 g) were mixed together into 10 mL of solvent. The solvents used were aqueous ethanol (0–100%, v/v) and aqueous acetone (0–100%, v/v) solutions. The suspension was kept at 20, 45, or 70 °C for 10 min. To determine the effect of AscP concentration, 0.05 g of V-amylose in 2 mL of 80% (v/v) ethanol that contained 0.01, 0.02, 0.1, or 0.2 g of AscP were kept at 45 °C for 10 min. Samples were collected by the same centrifugation, washing and drying procedure as described earlier.

2.5. Wide angle X-ray diffraction (XRD)

Wide angle X-ray diffraction patterns were obtained with a Rigaku MiniFlex II desktop X-ray diffractometer (Rigaku Americas Corporation, TX). Dried sample powders were exposed to Cu Kα radiation (0.154 nm) and continuously scanned between $2\theta = 4$ and 30° at a scanning rate of 2°/min with a step size of 0.02°. A current of 15 mA and voltage of 30 kV were used. Data were analyzed with Jade v.8 software (Material Data Inc., Livermore, CA). The area of the amorphous halo generated by Jade© software using the cubic spline fit option was subtracted from the total X-ray diffraction area to obtain the crystalline fraction. The degree of crystallinity was then calculated as the crystalline fraction over the total area multiplied by 100.

2.6. Differential scanning calorimeter (DSC)

Approximately 5–6 mg of sample was weighed into a 60 μL stainless steel pan (Perkin-Elmer Instruments, Norwalk, CT) and water was added to obtain a 10% (w/v) dispersion. Pans were hermetically sealed. Samples were equilibrated to 10 °C, and then heated to 170 °C at 5 °C/min in a Thermal Advantage Q100 DSC (TA Instruments, New Castle, DE). The DSC was calibrated with indium, with an empty sample pan used as the reference. Data was analyzed using the TA Universal Analysis software (Universal Analysis 2000 v.4.2E, TA Instruments-Waters LLC, New Castle, DE).

3. Results and discussion

3.1. Characterization of V-amylose and V-starch

Through both the DMSO and high temperature preparation routes, ethanol-precipitated starch and amylose demonstrated V_a-type X-ray diffraction (XRD) patterns (Fig. 1A), characterized by three major peaks at $2\theta = 7.8°$, 13.8°, and 21°. Annealed V-amylose and V-starch showed sharpened V_a patterns (Fig. 1B), with $2\theta = 7.8°$, 13.5°, and 20.8°, which correspond to d-spacing of 1.132 nm, 0.658 nm, and 0.427 nm. The annealed V-amylose also showed less intense V_a peaks at $2\theta = 15.6°$, 17.4°, and 19.2° (d-spacing of 0.561 nm, 0.508 nm, and 4.62 nm). These values were very close to those given by previous researchers (Zobel et al., 1967). Since no guest compounds were mixed before precipitation, most amylose helices formed should have "empty" helical channels, except perhaps for residue of small solvent molecules, e.g. DMSO, water and ethanol. The high temperature method resulted in V-amylose with the same crystallinity (approximately 40%) as that from the DMSO method. Using the DMSO method, crystallinity of V-starch was estimated to be lower than that of V-amylose, i.e. 33% versus 40%. This could be attributed to the presence of amylopectin in starch that constitutes about 30% (w/w) in Hylon VII, and which is structurally difficult to form V-type single helices (Lay Ma et al., 2011). The thermogram of the precipitated V-starch show a broad and low endotherm (Fig. 2a), indicating little complexation took place between starch and native lipids. Approximately 1% (w/w)

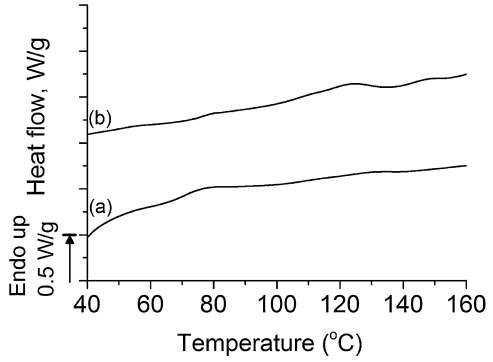

Fig. 2. DSC thermograms of (a) V-starch and (b) V-amylose prepared by DMSO method.

monoacyl lipids, e.g. palmitic, stearic, and linoleic acid, exist in native high amylose corn starch. These lipids are potentially able to form inclusion complexes with starch. The presence of various lipids may result in different structures of inclusion complexes, e.g. length of helices, and thus different thermal stabilities of the inclusion complexes (Kong & Ziegler, 2014a). This broad and low endotherm might also be attributed to the retrogradation of the amylopectin fraction of starch (Fisher & Thompson, 1997; Kohyama & Nishinari, 1991). The precipitated V-amylose did not show any noticeable endotherm below approximately 120 °C during heating (Fig. 2b), and thus no dissociation of amylose-guest inclusion complex could be detected. Two low endotherms located around 125 and 150 °C were close to those of recrystallized "spherulitic" amylose (Creek, Ziegler, & Runt, 2006) and retrograded amylose (Lay Ma et al., 2011), respectively.

V-type starch formation by alcohol precipitation can be dated back to 1933, when Katz and Derksen suggested the V-type as "an intermediate step between the amorphous conditions of the pasted starch and the B configuration" (Katz & Derksen, 1933). The V-type starch can eventually transform into B-type when sufficient water is provided (Le Bail, Bizot, Pontoire, & Buléon, 1995). However, later studies, especially X-ray diffraction and molecular modeling, regarded the V-type as a particular configuration, which differs much from the B-type (Chandrasekaran, 1997). Instead of precipitating starch from aqueous solutions, Zobel et al. described precipitation of amylose–DMSO complex using alcohol to prepare the V-type (V_a) (Zobel et al., 1967). The V_a could then be transformed to V-hydrate (V_h) by exposure to a humid environment (e.g. 85% relative humidity). The only difference between V_a and V_h is the extent of water hydration, which brings slight modifications in their crystal structure. Although the helical structure of V-type with "empty" helical cavities has been known for many decades, no other technical applications have been described, with the possible exception of the "sealed heating" treatment (>100 °C) utilized by a group of researchers (Oguchi et al., 1998; Uchino et al., 2001; Uchino, Tozuka, Oguchi, & Yamamoto, 2002). The enzymatically synthesized amylose employed showed V_6 pattern before the "sealed heating" treatment. The inclusion of 2-naphthol (Uchino et al., 2001), salicylic acid (Oguchi et al., 1998) and its analogs (Uchino et al., 2002) converted the V_6 helical structure to either V_7 or V_8 structure, depending on the size of the guest molecule. In the present study, we used V-amylose and V-starch prepared from native starch to molecularly encapsulate a model bioactive compound, ascorbyl palmitate, at much lower temperatures.

3.2. Effect of complexation temperature and solvent quality

In theory, the complexation ability and efficiency is affected by guest intrinsic solubility (S_0) and complex affinity constant (K), which can be expressed as: *complexation efficiency* = KS_0 (Duchene, 2011). An increase in the complexation efficiency can be achieved by increasing either the guest intrinsic solubility or the complex affinity constant, or both parameters simultaneously (Loftsson, Másson, & Sigurjónsdóttir, 1999). The guest solubility is mainly affected by solvent quality, whereas the complex affinity constant is governed by the driving force for complex formation. The main driving force for amylose-guest complexation may involve hydrophobic interaction, hydrogen bonding, and concentration gradient (diffusion).

Practically, there are numerous process parameters that can influence the complexation ability and efficiency in this method, for instance, solvent composition, complexation temperature, time duration, guest concentration, V-amylose concentration, V-amylose physical properties (surface area, porosity, and crystallinity), to name but a few. Initially, the effect of ethanol concentration and temperature on V-amylose complexation with AscP was studied. XRD patterns showed the presence of V-type after reaction with AscP solutions at all ethanol concentrations ≥ 20% (Fig. 3). Regardless of complexation temperature, the V-type structure was converted into B-type when reacted in water even in the presence of the guest compound. The B-type pattern obtained showed weak and diffuse peaks at approximately $2\theta = 5.5°$, 17.04°, 19.8°, and 22°. The diffraction peak at around $2\theta = 5.5°$ is characteristic for B-type starches, but its intensity is significantly dependent on the hydration level of the sample (Cleven, Van den Berg, & Van Der Plas, 1978). The $2\theta = 5.5°$ peak intensities of the B-type patterns were suppressed in Fig. 3, because the starch samples were extensively dried in a vacuum desiccator. Yet whether this conversion from V to B is due to crystal phase transition or recrystallization after dissolution is unknown. Solvent containing 20% (v/v) of ethanol resulted in a mixture of V- and B-type, while B-type was not detectable from above 40% (v/v) ethanol. A sharpening effect in the V-pattern was noticeable at intermediate ethanol concentrations (40–80%, v/v). Notably, 40% (v/v) ethanol was able to increase the crystallinity of V-amylose to about 60% at 70 °C. Amylose loses its complexation ability after conversion to B-type, but whether AscP was complexed with the V-amylose or V-amylose remained "empty" under all other conditions cannot be resolved from XRD patterns alone.

The dissociation of amylose-AscP inclusion complexes should produce an endotherm with a peak temperature around 95 °C on a DSC thermogram (Lay Ma et al., 2011). After complexation at 20 °C, V-amylose samples exposed to 40–60% (v/v) aqueous ethanol solutions containing AscP demonstrated an endotherm representing the dissociation of amylose-AscP inclusion complex (Fig. 4). At higher temperatures, i.e. 45 and 70 °C, complexation was also induced at 80% ethanol. The variation in complexation ability can be explained by two competing mechanisms, i.e. a competition between AscP solubility in the solvent and AscP-amylose inner channel hydrophobic interaction, and the effects of the solvent on mobility of amylose helices, all of which were affected by ethanol concentration and temperature. At high ethanol concentrations (80–100%, v/v) the high solubility of AscP in the solvent and the relative rigidity of amylose hinders the insertion of AscP into amylose helices. At lower ethanol concentrations (<20%, v/v), less AscP molecules were available at the liquid-solid (solvent-V-amylose) interface due to poor solubility. A further reduction in ethanol concentration (0%, v/v) would result in too much flexibility of amylose helices so that they transformed into B-type. At intermediate ethanol concentrations (40–60%, v/v), the plasticizing effect of water gave enough chain mobility for easy insertion of AscP molecules, while not so flexible as to disrupt the helical configuration. Similarly, a reduction in AscP solubility, i.e. an increase in polarity of the solvent produced conditions favoring diffusion of AscP into V-amylose. In the case of 80% ethanol, an increase in

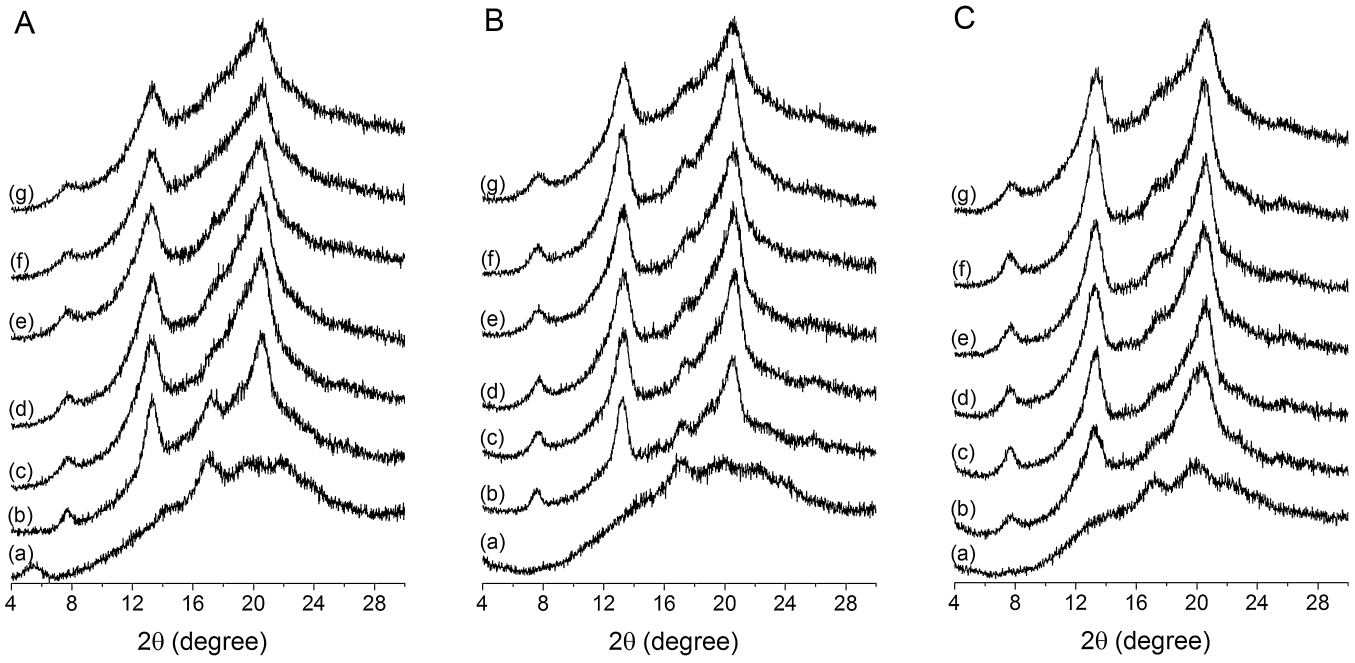

Fig. 3. X-ray diffraction patterns of V-amylose complexation with ascorbyl-palmitate (AscP) at (A) 20, (B) 45, and (C) 70 °C in different ethanol concentrations: (a) 0%, (b) 20%, (c) 40%, (d) 50%, (e) 60%, (f) 80%, and (g) 100% (v/v) aqueous ethanol solutions.

temperature changed it to a favorable condition by increasing the mobility of amylose helices. Besides the endotherm for amylose-AscP dissociation, all of the thermograms showed an endotherm with a peak temperature about 150 °C, which can be attributed to the dissociation of retrograded amylose. Uncomplexed amylose molecules tend to retrograde in the DSC pans before the heating scan. The small portion of B-type component in the V-amylose samples may also contribute to this endotherm.

Results similar to those of V-amylose were obtained with high amylose starch (V-starch) (Fig. 5). This was expected because amylose is the main component of this specialty starch. Yet V-starch did show slightly different behaviors. A very broad and flat endotherm in the range from approximately 50 to 100 °C was always observable (Fig. 6). As discussed above, this could be due to the retrogradation of the amylopectin fraction. There were no endotherms that accounted for dissociation of retrogradated amylose. The structural irregularity, especially branched amylopectin molecules, might impede perfection of retrogradated amylose phase and result in diffused thermal events that spread along the rising baselines.

The solubility of guest compounds is an important parameter for successful complexation in this method. Solvents other than aqueous ethanol solutions can also be employed, especially when guest compounds have low solubility in both ethanol and water. Here we chose to study whether aqueous acetone solutions could be used for complexation. Thermograms of V-amylose samples recovered from 40% or 60% (v/v) acetone solutions where AscP was dissolved displayed weak endotherms at the expected temperature (see Fig. S3 in Supplementary data). Aqueous acetone solution at 50% (v/v) was by far the most effective acetone concentration for inclusion of AscP.

3.3. *Effect of annealing*

The V-pattern of the precipitated V-type starch could be enhanced by a further heat treatment (or referred to as annealing) in an appropriate media. Bear (1942) stated that "starches which give V patterns after alcohol precipitation in the cold can be made to yield excellent ones if properly treated" (Bear, 1942)' i.e. 75–80% hot alcohol at 70 °C with vigorous stirring, after which precipitated and dried starch showed "three almost unbelievably sharp diffraction rings". Zobel et al. employed a similar treatment, i.e. boiling in 85% methanol, and suggested that "molecular chains conceivably would have ample opportunity to arrange into the most stable structure" by this treatment (Zobel et al., 1967). Other than these examples, no other reports have been found to systematically study the effect

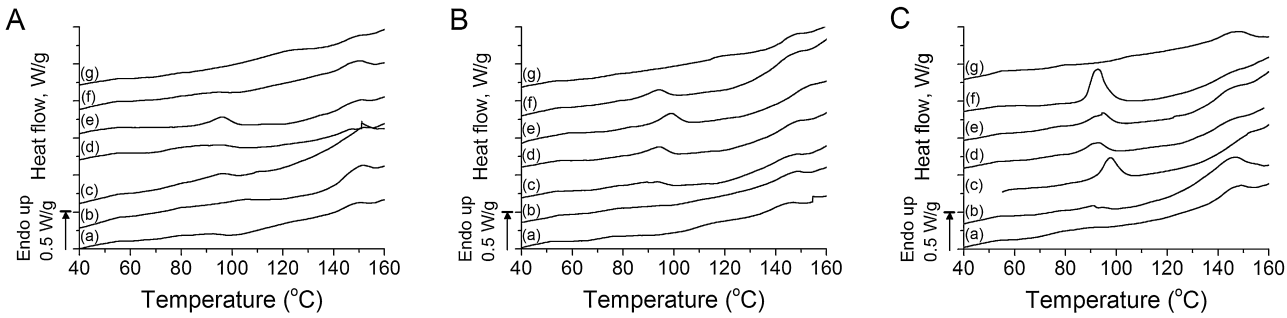

Fig. 4. DSC thermograms of V-amylose complexation with AscP at (A) 20, (B) 45, and (C) 70 °C in different ethanol concentrations: (a) 0%, (b) 20%, (c) 40%, (d) 50%, (e) 60%, (f) 80%, and (g) 100% (v/v) aqueous ethanol solutions.

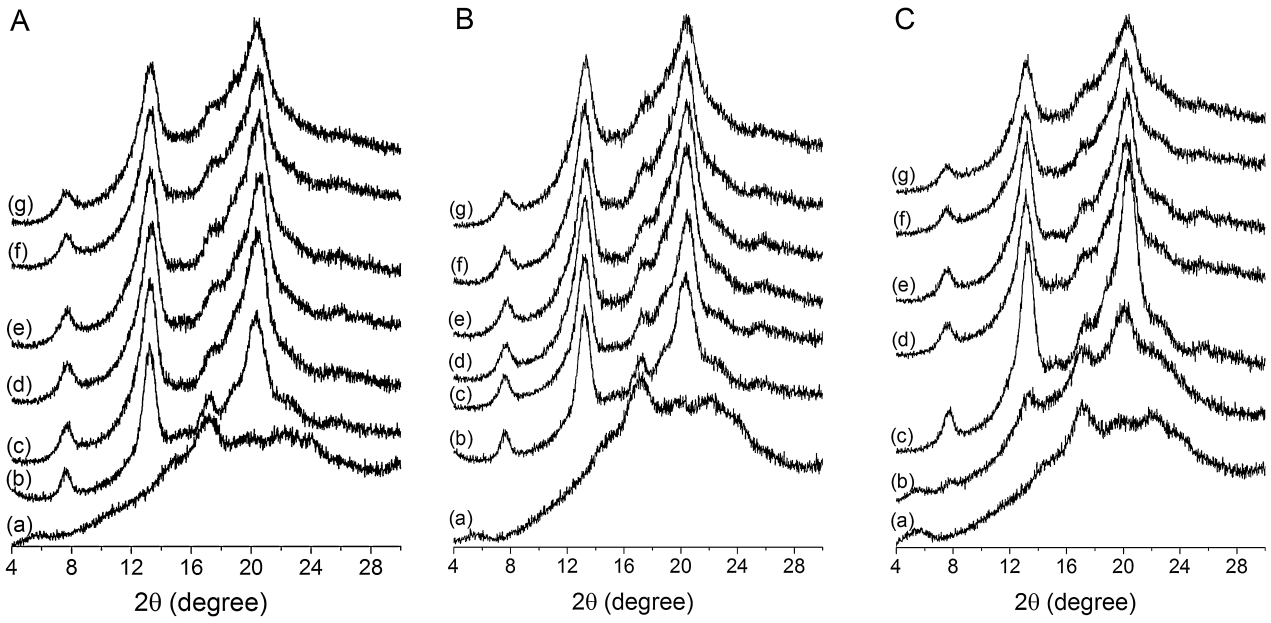

Fig. 5. X-ray diffraction patterns of V-starch complexation with AscP at (A) 20, (B) 45, and (C) 70 °C in different ethanol concentrations: (a) 0%, (b) 20%, (c) 40%, (d) 50%, (e) 60%, (f) 80%, and (g) 100% (v/v) aqueous ethanol solutions.

of heat treatment on the arrangement of amylose molecules in V-type structure. In our previous study (Kong & Ziegler, 2014b), we obtained thermograms of electrospun starch fibers (V-type from XRD evidence) in different aqueous ethanol solutions during heating. When the V-type starch fibers were scanned (by DSC) in 40–60% ethanol solutions, we observed an exotherm followed by an endotherm, the positions of which depended on the ethanol concentration. The exotherm disappeared during a reheating scan. We attributed the exotherm to the crystallization of starch helices that is evidenced as a sharpened V-pattern. The endotherm could be attributed to the dissociation of the V-type starch single helices or retrograded starch molecules.

In the current study, we found that annealing of V-amylose and V-starch powders in 40–50% (v/v) ethanol at 70 °C significantly increased the crystallinity (Fig. 1B). The crystallinities of annealed V-starch and V-amylose from 50% (v/v) ethanol at 70 °C were estimated to be 44% and 47%, respectively. Crystallinity of V-amylose could be enhanced to as high as 65% by annealing in 40% (v/v) ethanol at 70 °C. This suggested that 40% (v/v) ethanol could provide a better solvent environment, where amylose helices are flexible enough to arrange into more stable structure, but not too flexible to transition to B-type amylose as in the case of 0% and 20% (v/v) ethanol. The annealed V-amylose samples should have more well-formed helices with higher crystallinity and larger crystallite size (smaller full width at half maximum) than V-amylose without annealing. While the availability of more empty helices would provide more sites to capture guest molecules, larger crystal size might bury helices in the crystal where they may be inaccessible to guest molecules. Therefore, it would be necessary to elucidate whether highly crystalline V-amylose would facilitate inclusion complexation or not.

Compared with V-starch and V-amylose without annealing, inclusion complex formation by annealed V-amylose demonstrated a similar trend as a function of ethanol concentration when complexed at 45 °C (Fig. 7). The enthalpies ranged from 1.02 J/g (sample from 20% ethanol) to 7.79 J/g (sample from 50% ethanol). This value is greater than the largest enthalpy of V-amylose inclusion complex formed at 45 °C without annealing, i.e. 2.62 J/g. It appeared that higher crystallinity V-amylose was more efficient in capturing AscP molecules, probably due to more regular crystal structure. Microscopic observation showed that both the precipitated and annealed V-amylose sample powders are very porous with very rough surface, regardless of crystallinity (data not shown). Annealing neither altered the surface morphology nor resulted in large crystals. We suggest that surface area may be an important parameter. The variation in surface area may give difference in the amount of V-amylose helices available to accommodate guest molecules, since it would be easier for guest molecules to enter the helices on

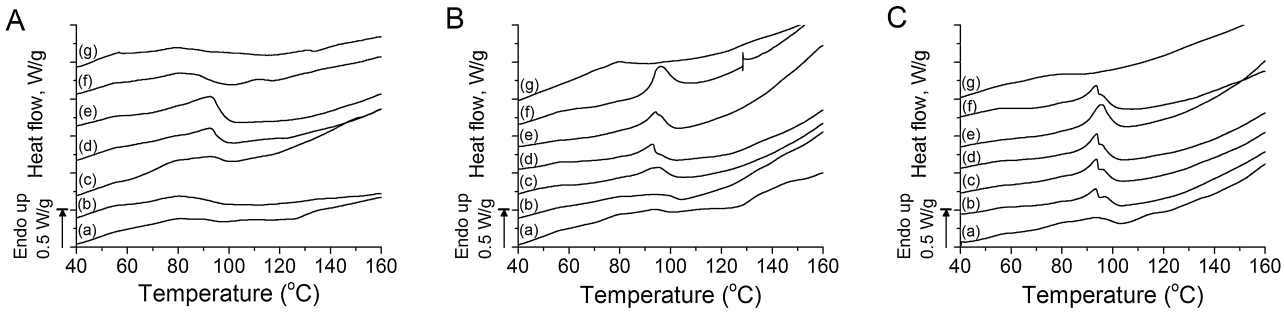

Fig. 6. DSC thermograms of V-starch complexation with AscP at (A) 20, (B) 45, and (C) 70 °C in different ethanol concentrations: (a) 0%, (b) 20%, (c) 40%, (d) 50%, (e) 60%, (f) 80%, and (g) 100% (v/v) aqueous ethanol solutions.

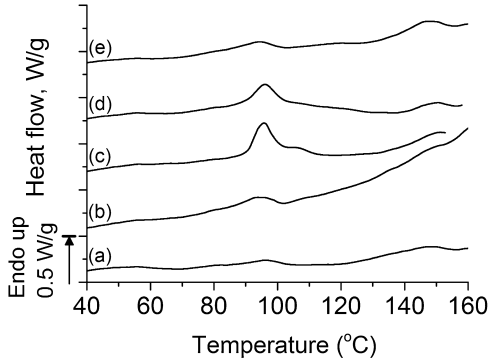

Fig. 7. DSC thermograms of annealed V-amylose complexation with AscP at 45 °C in different ethanol concentrations: (a) 20%, (b) 40%, (c) 50%, (c) 60%, and (c) 80% (v/v) ethanol aqueous solutions.

the surface of the crystals. It will be interesting in the future to evaluate the effect of particle size and distribution on the complexation efficiency.

3.4. Effect of AscP concentration

In general, the endothermic enthalpy of V-amylose-AscP complexes obtained in this study is smaller than the enthalpy obtained by the DMSO method (Kong & Ziegler, 2014a). AscP concentration may be responsible for the low complexation efficiency, even though the weight ratio of AscP to V-amylose was chosen to result in the maximum % AscP that could be complexed by starch (around 15%, w/w of starch) (Lay Ma et al., 2011). In practical terms, a greater AscP concentration is required due to the weak driving force for complexation in this method. In the AscP concentration range studied here, the proportion of V-amylose that formed inclusion complex, estimated from endothermic enthalpy, increased significantly with AscP concentration in the solution without reaching a maximum plateau (Fig. 8). The highest endothermic enthalpy, i.e. 9.54 J/g, was close to the value of the amylose-AscP inclusion complexes by DMSO method (Kong & Ziegler, 2014a). It should be noted that AscP concentration above 0.5% (w/v) was not fully soluble in the solvent, i.e. 80% (v/v) ethanol. However, as AscP was complexed from this saturated solution more was available to dissolve to maintain the concentration gradient for complexation. The dissociation temperature experienced a slight decrease with AscP concentration used, probably due to less than perfect inclusions being formed as the helices got close to saturation.

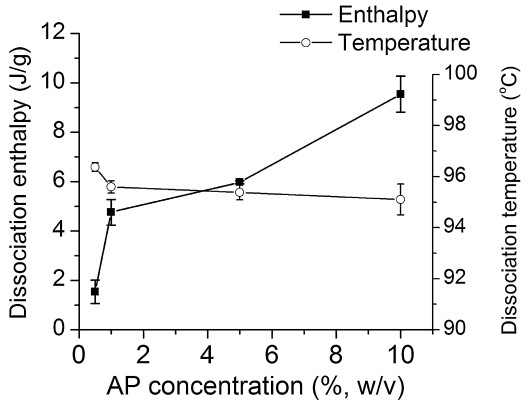

Fig. 8. Dissociation enthalpy and temperature of V-amylose complexation with different AscP concentrations at 45 °C in 80% (v/v) aqueous ethanol solution.

4. Conclusions

In conclusion, preformed V-type amylose and starch with empty helical channels could be synthesized and utilized to readily form inclusion complexes with the selected model guest compound, ascorbyl palmitate, below the melting point of the complexes. Complexation could take place in intermediate ethanol and acetone concentrations at room temperature. Annealing, i.e. heat treatment in ethanol solutions at elevated temperatures, was able to significantly increase the crystallinity of V-amylose and V-starch. High crystallinity facilitated greater complexation, probably due to more regularly arranged helical cavities in larger crystalline phase. Complexation was also affected by guest concentration.

This new method of forming starch-guest inclusion complex is easy, economical and potentially scalable for mass production. It is worthwhile to try to encapsulate more types of nutrients, drugs, and bioactive molecules, intended for food and pharmaceutical applications, especially where cyclodextrins have been heavily used. The use of solvent DMSO may be a concern for some applications, although DMSO residue in the dried powder can be eliminated. In such circumstance, the high temperature preparation method could be an alternative to prepare the V-amylose and V-starch.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2014.04.033.

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