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ARTICLE

Interaction between Amylose and β-Cyclodextrin Investigated by Complexing with Conjugated Linoleic Acid

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Interaction between amylose, a common food component, and β-cyclodextrin (βCD), an often used food additive, was investigated by incorporating a third component of bioactive conjugated linoleic acid (CLA) that could form an inclusion complex with both amylose and βCD. The existence of an amylose−βCD interaction was first evidenced by a reduced thermal stability of amylose in the amylose−βCD complex and a decrease of extractable βCD from 60 to 51.40% after their complexation. The way of their interaction was then explored in a three-component system, in which the amount of CLA is high enough to oversaturate both amylose and βCD. In comparison to the amylose−CLA and βCD−CLA complexes, a self-assemblied amylose−CLA−βCD three-component complex confirmed by differential scanning calorimetry (DSC), X-ray diffraction, and thermogravimetric analyses showed an in-between thermal stability, high acid stability, and the highest amylolytic digestibility (74.43%), which suggests that βCD is likely sandwiched between the helical amylose chains in the amylose−βCD complex. Therefore, βCD can be used to manipulate the crystallization process of amylose to modulate food product quality, and the amylose−βCD complex could also be applied to improve the delivery efficiency of CLA and other bioactive compounds.

KEYWORDS: Amylose; β-cyclodextrin (βCD); conjugated linoleic acid (CLA); complex; self-assembled interaction

INTRODUCTION

Amylose is an essentially linear molecule in starch, and its conformation and quantity have a significant influence on the gelatinization and retrogradation of starch (*1*, *2*) that are intimately associated with the quality of cereal-based foods (*3*, *4*) and their metabolic consequence in humans (*5*−*7*). β-Cyclodextrin (βCD) is a cyclic oligosaccharide composed of seven glucose units that is often used as a quality enhancer in food product development with its ability to bind ligand molecules of flavor, colorant, or functional lipids (*8*−*11*). Thus, there exists a high possibility for amylose and βCD to coexist in a food system, and their interaction should be of practical importance to modulate the physical and functional properties of food products. A recently proposed amylose−βCD complex through molecular dynamics simulation (*12*) showed that there exists an amylose−βCD interaction if they coexist in a food system. However, direct experimental evidence for an amylose−βCD interaction has rarely been reported, and the interaction mechanism is still unclear. Actually, this interaction is difficult to be directly investigated because amylose and cyclodextrins have the same primary structure and can even be produced from each other through enzyme catalysis (*13*−*15*). Nevertheless, to maintain or improve the food quality, especially for cereal-based foods in the presence of βCD, more experimental work needs to be performed to elucidate the interaction mechanism between amylose and βCD, so that βCD can be used effectively in food product development.

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It is known that amylose can form self-assembled amylose−lipid inclusion complexes through a hydrophobic interaction (*16*), and βCD with a cylindric structure, which is hydrophobic inside and hydrophilic outside, can also form inclusion complexes with lipids in a similar manner (*8*, *17*). Applicatively, both amylose and βCD have been used to deliver conjugated linoleic acid (CLA), a representive of beneficial polyunsaturated fatty acids (*18*), through self-assembled amylose−CLA and βCD−CLA complexes (*19*−*22*). Therefore, the interaction between amylose and βCD could be investigated through their complexation behaviors with CLA under the condition that the concentration of CLA is high enough to oversaturate both amylose and βCD in the system. However, no research has been carried out in this manner to investigate the interaction between amylose and βCD.

In this study, the amylose−βCD complex was first prepared to confirm the existence of an interaction between amylose and βCD, and the complexation characteristics of amylose−CLA, βCD−CLA, and amylose−CLA−βCD were compared to indirectly understand how amylose and βCD interact with each other. Additionally, owing to the fact that carbohydrates and lipids usually coexist in a food system and CLA is a bioactive and oxidatively unstable fatty acid, this study is also expected to obtain knowledge on the applications of self-assemblied structures using essential food components in protecting and delivering bioactive compounds.

MATERIALS AND METHODS

Amylose was isolated from potato starch using 1-butanol as the precipitating reagent (*23*). βCD (food grade) was obtained from China

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National Medicines Corporation Ltd. (Shanghai, China). βCD standard, heptadecanoic acid, α-amylase from porcine pancreas, pepsin from porcine gastric mucosa, and CLA, which is a mixture of *cis*- and *trans*-9,11- and -10,12-octadecadienoic acids, were purchased from Sigma-Aldrich Trading Co., Ltd. (Shanghai, China). Amyloglucosidase was from Genencor Bio-Products Pte Ltd. (Jiangsu, China).

Direct Amylose−βCD Complexation. Accurately weighed βCD dispersed in distilled water was added into amylose solution (20 mg of amylose/mL of DMSO) to make the weight ratio of amylose/βCD to be 2:3, and the mixture was stirred for 20 min at 30 °C and then vacuum-dried to make amylose−βCD complex samples. The thermal stability of the amylose−βCD complex was analyzed using a Mettler Toledo TGA/SDTA851^e thermogravimeter (Mettler Toledo Corp., Zurich, Switzerland) with STAR^e software (version 9.01) and a heating rate of 10 °C/min from 50 to 400 °C under nitrogen gas flowing at 20 mL/min. The extractable βCD content of the sample was determined using high-performance liquid chromatography (HPLC) as the following: 20 mg of sample was fully extracted with 2 mL of distilled water at 30 °C, and then the βCD content in the supernatant (free βCD or uncomplexed βCD) after 5 min of centrifugation at 5000 rpm was quantified by HPLC using the βCD standard for comparison. The HPLC instrument (Waters 600, Waters Co., Milford, MA) was equipped with a refractive index detector and a Kromasil NH_2 column (4.6 mm inner diameter × 250 mm) under a constant temperature of 30 °C. Acetonitrile/water (55:45, v/v) at a flow rate of 1 mL/min was used as the mobile phase.

Amylose−CLA−βCD Complexation. Complexation among CLA, amylose, and βCD was operated as the following: amylose solution was prepared by dissolving amylose in DMSO (20 mg of amylose/mL of DMSO) at 90 °C and then cooled to 30 °C. After dissolution of CLA (weight ratio of CLA/amylose was 1:5) in the amylose solution, distilled water (1000 times the weight of the amylose) at 30 °C was added, the mixture was stirred for 10 min, then βCD (1.5 times the weight of the amylose) dispersed in distilled water was added, and the mixture was further stirred for 20 min for complexation among amylose, CLA, and βCD followed by adding NaCl solution (5%, w/v) to facilitate precipitation of the complex. The precipitated complex was separated by centrifugation followed by washing twice with ethanol (50%, v/v) to remove uncomplexed CLA and then was vacuum-dried for further analysis. For comparison purposes, the amylose−CLA and βCD−CLA complexes were prepared using the methods described before (*22*).

X-ray Diffraction (XRD) Analysis. The crystalline structure of the complexes were analyzed using a Bruker D8-Advance diffractometer (Bruker AXS Corp., Nanjing, China) equipped with Cu Kα radiation at 40 kV and 40 mA, and the samples were scanned from 3 to 30° 2θ at a rate of 0.02°/3 s.

Differential Scanning Calorimetry (DSC) Analysis. The thermal properties of the complexes were analyzed using a Perkin-Elmer Pyris 1 DSC instrument (Perkin-Elmer Corp., Shanghai, China). The samples (5.0 mg) with 15.0 mg of distilled water were sealed in aluminum pans, equilibrated overnight, and then scanned from 30 to 150 °C at a rate of 5 °C/min.

Thermogravimetric Analysis (TGA). The thermal stability of the complexes was analyzed as described above in the Direct Amylose−βCD Complexation section.

***In Vitro* Digestion Tests.** The digestibility of amylose−CLA−βCD complex samples was measured through hydrolyzing the samples under simulated gastrointestinal conditions based on the methods described before (*22*). Briefly, 15.0 mg of samples was incubated in the simulated gastric juice (5 mg/mL pepsin at pH 2.0 and 37 °C) and small intestine solution (35 units/mL α-amylase, 35 units/mL amyloglucosidase, and 0.01 mol/L $CaCl_2$ at pH 5.2 and 37 °C) under continuous shaking. The hydrolysis degrees of the samples in the simulated small intestine solution were tested using the dinitrosalicylic acid (DNS) method, and the CLA released from the complexes under both the simulated stomach and small intestine conditions were quantified by a gas chromatography analysis after methylation. The highest amount of the CLA released from the complex during the amylase hydrolysis was regarded as the total CLA in the complex, and CLA release percentage = (weight of the released CLA/weight of the CLA in the complex) × 100%.

Statistical Analysis. Experimental data were presented as the average of duplicate or triplicate determination results. Relative crystallinity was analyzed using the Jade 5.0 software (Materials Data, Inc., Livermore, CA), and the significant difference of the free βCD content was analyzed using one sample *t* test for a mean according to SAS 8.01 software (SAS Institute, Inc., Cary, NC).

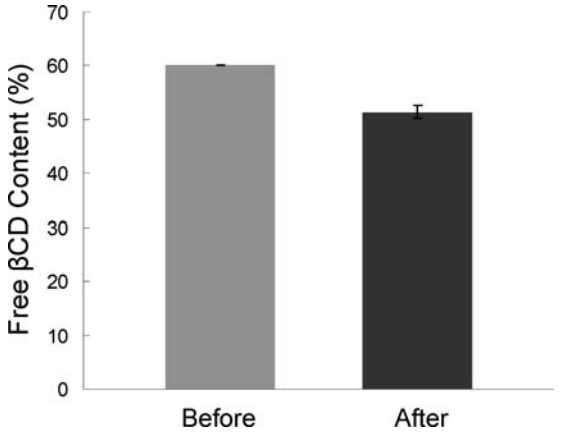

Figure 1. Free βCD content (%) before/after amylose and βCD interaction.

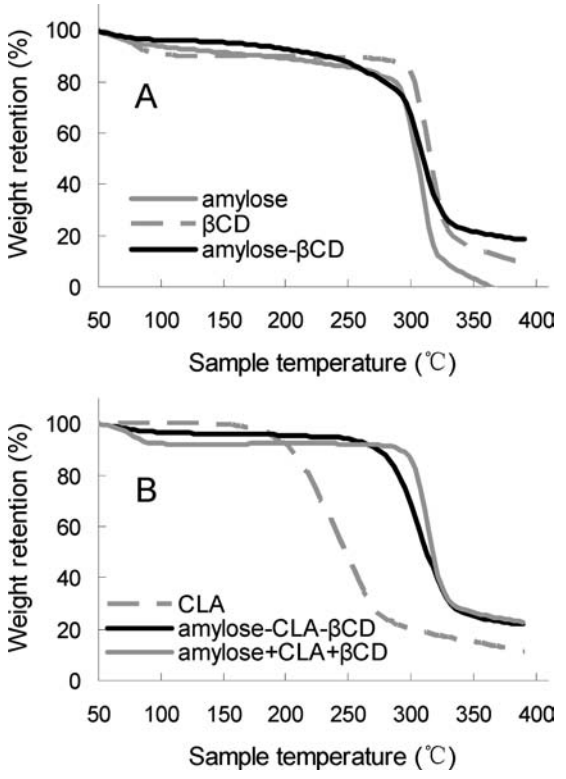

Figure 2. Thermogravimetric curves of amylose, βCD, CLA, and their complexed or physically mixed samples. (A) "amylose−βCD" refers to the amylose−βCD complex, and (B) "amylose−CLA−βCD" and "amylose + CLA + βCD" refer to complexed and physically mixed samples of CLA, amylose, and βCD, respectively.

RESULTS AND DISCUSSION

Amylose−βCD Interaction. To directly confirm the interaction between amylose and βCD, the amylose−βCD complex was prepared and the free βCD content and thermal stability of amylose were analyzed by HPLC and TGA, respectively. As shown in **Figure 1**, the content of the extractable βCD (not in the complex) after amylose and βCD complexation decreased from 60 to 51.40% ($p < 0.05$). Additionally, as shown in **Figure 2A**, the amylose−βCD complex showed a three-step thermogravimetric

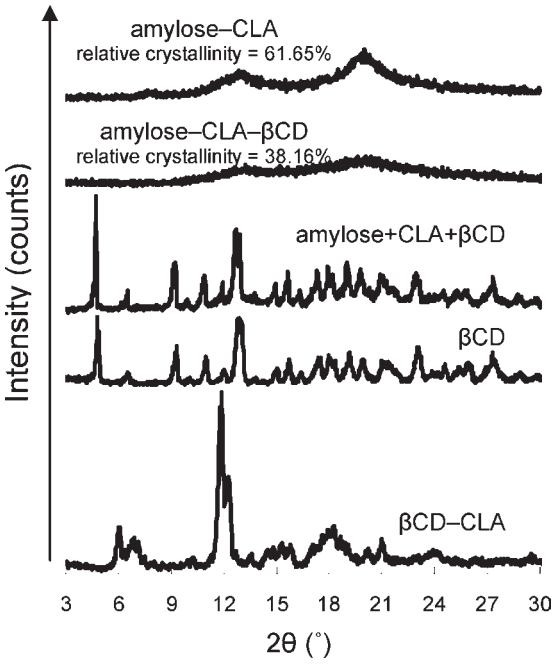

Figure 3. X-ray diffractograms of the amylose−CLA complex (amylose−CLA), amylose−CLA−βCD complex (amylose−CLA−βCD), physical mixture of CLA, amylose, and βCD (amylose + CLA + βCD), βCD, and βCD−CLA complex (βCD−CLA).

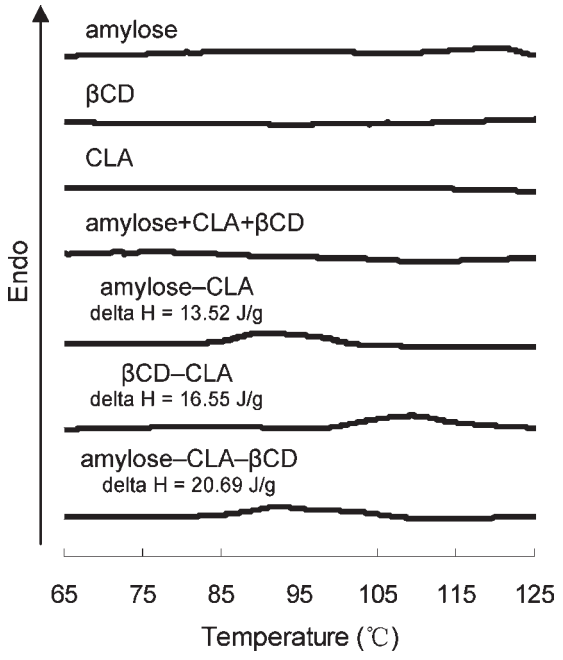

Figure 4. DSC profiles of amylose, βCD, CLA, and their complexed or physically mixed samples. "Amylose + CLA + βCD", "amylose−CLA", "βCD−CLA", and "amylose−CLA−βCD" refer to the physical mixture of CLA, amylose, and βCD, amylose−CLA complex, βCD−CLA complex, and amylose−CLA−βCD complex, respectively.

curve, which is distinctly different from the curves of amylose and βCD. Specifically, the first weight loss step of the amylose−βCD complex was caused by dehydration of βCD molecules; the second one with an initial decomposition temperature of 245.26 °C was caused by decomposition of amylose; and the third one with an initial decomposition temperature of 279.76 °C was caused by decomposition of βCD. In comparison to amylose with an initial decomposition temperature of 269.18 °C, the decomposition temperature (245.26 °C) of the amylose in the amylose−βCD complex was obviously lower, and this suggests that the thermal stability of the amylose is decreased by interacting with βCD. Apparently, the amylose−βCD interaction is supported by both the reduced free βCD content and the decreased thermal stability of the amylose in the amylose−βCD complex.

Self-Assembled Complexation among CLA, Amylose, and βCD. Amylose can form a self-assembled inclusion complex with *cis*-unsaturated fatty acids (*16*, *24*), and βCD can also form an inclusion complex with nonpolar molecules in a self-assembled manner (*25*). In our previous study, both the amylose−CLA and βCD−CLA complexes were successfully prepared (*22*). Therefore, complexation behavior among the three components of CLA, amylose, and βCD can be studied to better understand how amylose and βCD interact with each other.

XRD is a common method used to investigate structural changes of solid samples. It is known that the amylose−lipid complex shows a V-type diffraction pattern with a peak at 20.0° 2θ (*26*, *27*), and the βCD−CLA complex shows a less crystalline structure than βCD (*22*). As shown in **Figure 3**, the three-component complex containing CLA, amylose, and βCD showed a V-type diffraction pattern similar to the amylose−CLA complex, without a noticeable peak of βCD, but the pattern of the three-component complex was crystallinely not so ordered, with less sharp peaks and lower crystallinity (38.16%) than that of the amylose−CLA complex (61.65%). Comparatively, the physically mixed sample of CLA, amylose, and βCD prepared with the same material ratio as the complexed one showed a diffraction pattern similar to βCD but dramatically different from the βCD−CLA and the amylose−CLA−βCD complexes (**Figure 3**). These results suggest that the amylose−CLA complex is the main structure formed in the complexation among CLA, amylose, and βCD, and the addition of βCD influenced the packing of the amylose−CLA complex. As for βCD, its conformation in the three-component complex is changed regardless of whether it exists as a βCD−CLA complex or as uncomplexed molecules. Therefore, the amylose−CLA−βCD complex with a less ordered crystalline structure likely represents the interaction product of CLA, amylose, and βCD, despite the fact that their interaction mechanism cannot be clearly elucidated from XRD data alone.

The self-assembled complex of CLA, amylose, and βCD is also supported by the DSC profile of the amylose−CLA−βCD complex, with an endothermic peak near 93 °C (**Figure 4**) that was caused by the dissociation of the amylose−CLA complex (*22*). Comparatively, all of the thermograms of CLA, amylose, βCD, and their physically mixed sample showed no peaks around 93 °C. Thus, the amylose−CLA complex, as shown by XRD analysis, is the major secondary structure in the three-component complex. Actually, the amount of CLA used in this study (in a weight ratio of 2:10:15 for CLA/amylose/βCD) was high enough for CLA to completely complex with both amylose and βCD because $^1/_{10}$ amylose weight of CLA is adequate for its complexation with amylose (*21*, *22*) and 1 g of CLA could saturate 16 g of βCD (*20*); therefore, the βCD−CLA complex might be formed during the preparation of the three-component complex. However, the precipitation method used to obtain the amylose−CLA−βCD complex in this study was not the optimum crystallization conditions (high concentration and low temperature) for the βCD−CLA complex; thus, the βCD−CLA complex is not likely present in the amylose−CLA−βCD three-component complex according to the XRD and DSC analysis results.

Interaction Strength of the Self-Assembled Complexes. To further understand the existence of a three-component complex resulting from interactions among CLA, amylose, and βCD, the interaction strength of the self-assembled complex containing CLA, amylose, and βCD was further measured by the thermogravimetric

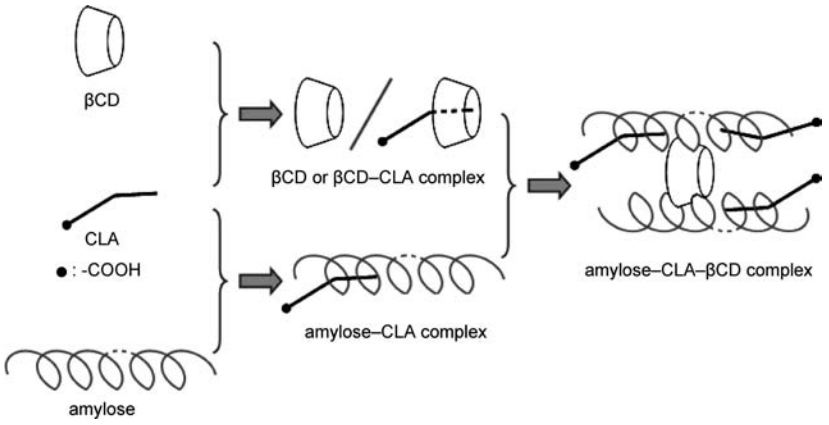

Figure 5. Schematic diagram of the self-assembly of amylose, CLA, and βCD to form the three-component complex.

analysis, which can not only differentiate structural components based on their TGA profiles but also show the thermal stability of each individual component.

In **Figure 2A**, the thermogravimetric curve of amylose showed one step of weight loss because of its thermal decomposition, while that of βCD showed two steps of weight loss caused by dehydration and the thermal decomposition of itself, and the initial decomposition temperature of amylose and βCD were 269.18 and 275.80 °C, respectively. In **Figure 2B**, both CLA and the amylose−CLA−βCD complex showed a one-step thermogravimetric curve with their initial decomposition temperature at 146.69 and 232.26 °C, respectively. Comparatively, the physically mixed sample of CLA, amylose, and βCD (amylose + CLA + βCD) showed a thermogravimetric curve with two steps of weight loss caused by the dehydration of βCD molecules and decomposition of amylose and βCD with a similar initial decomposition temperature (**Figure 2B**). Apparently, the differences of the thermogravimetric curves from the amylose−CLA−βCD complex and their physical mixture support the formation of an amylose−CLA−βCD three-component complex.

The thermogravimetric analysis results also showed the differences in the thermal stability of different samples. For the thermogravimetric curve of the amylose−CLA−βCD complex, the only one-step weight loss indicates that the sample was decomposed as a whole compound and no noticeable dehydration step suggests that the water molecules in the cavity of βCD were either displaced by CLA or not easy to evaporate because of the conformational changes in the three-component complex. Comparatively, our previous study has shown that the amylose−CLA complex was decomposed as a whole with an initial decomposition temperature at 240.60 °C and the βCD−CLA complex was decomposed in two steps caused by thermal decomposition of CLA and βCD (*22*). Therefore, the only one-step thermogravimetric curve with a lower initial decomposition temperature than that of the amylose−CLA complex indicates that the thermal stability of the amylose−CLA−βCD complex is lower than that of the amylose−CLA complex, which also demonstrates that the presence of βCD in the three-component complex might decrease the packing of the amylose−CLA complex, leading to a lower thermal stability of the amylose−CLA−βCD complex.

Digestibility of the Three-Component Complex. The digestibility of the amylose−CLA−βCD complex was studied under a simulated gastrointestinal condition to further understand the properties of the self-assembled complex among CLA, amylose, and βCD. In the simulated stomach conditions, the released CLA percentage of the amylose−CLA−βCD complex was 3.88% (±1.94%) after 2 h of incubation. After 6 h of incubation in the simulated small intestine conditions, the hydrolysis extent and the corresponding released CLA percentage from the complex were 74.43 and 93.02%, respectively. In comparison to our previous study results for amylose−CLA and βCD−CLA complexes (*22*), the released CLA percentages after 2 h of incubation in the simulated stomach conditions were 0 and 11.49%, the hydrolysis extents after 6 h of incubation in the simulated small intestine conditions were about 62 and 11%, and their corresponding released CLA percentages were about 50 and 9%, respectively (*22*). Apparently, both the amylolytic digestibility and CLA releasing property of the amylose−CLA−βCD complex are the highest in the three complexes, and the acid stability is much higher than that of the βCD−CLA complex.

It is known that amylose can be degraded by amylase but is quite stable in acidic conditions and βCD is acid-instable and resistant to enzymolysis; therefore, the amylose−CLA complex could release CLA in the simulated small intestine conditions without loss in the simulated gastric juice, while the βCD−CLA complex released more than $^1/_{10}$ of CLA in the same acidic hydrolysis conditions. In comparison to these two complexes, the superiority of the amylose−CLA−βCD complex in the digesting and releasing properties suggests that the enzymolysis sensitivity of the amylose−CLA complex is increased by the presence of βCD in the complex, which is consistent with its lower thermal stability described above. Apparently, the existence of βCD in the complex makes the three-component complex more susceptible to enzyme actions with its less rigid structure.

Self-Assembly of the Amylose−CLA−βCD Complex. To clearly understand the self-assembled complexation process among CLA, amylose, and βCD, a schematic diagram (**Figure 5**) based on the experimental results was drawn to show the way of the interaction among all of the components. As shown in **Figure 5**, the amylose−CLA complex and βCD are the basic building blocks for the three-component complex. Because the interior of both amylose and βCD are hydrophobic, their hydrophilic exterior is likely the interaction basis between amylose and βCD, leading to a sandwiched βCD between the helical chains of amylose. The special location of βCD would hinder the self-packing of both itself and the amylose−CLA complex, resulting in a less ordered amylose−CLA−βCD complex with properties of reduced crystal order, in-between thermal stability, and high susceptibility to amylase hydrolysis.

Interaction Way of the Self-Assembled Amylose−βCD Complex. The amylose−βCD complex in which βCD is likely sandwiched between amylose chains has been indirectly supported by the formation of the amylose−CLA−βCD complex. Additionally, the reduced free βCD content (from 60 to 51.40%; **Figure 1**) in the amylose−βCD interaction sample suggests that the interaction mole ratio of βCD/amylose in the amylose−βCD complex

would be about 151:1 because the degree of polymerization of potato amylose is 4920 (*28*), and this means that the average distance of two βCD molecules sandwiched between the amylose chains would be about five amylose helixes. Apparently, the existence of the amylose−βCD complex in which the βCD is sandwiched between helical amylose chains would affect the physical and functional properties of the food products containing both amylose and βCD, and this interaction would play important roles in modulating food product quality. Additionally, the amylose−βCD interaction can possibly be manipulated to effectively deliver CLA and other bioactive compounds into the small intestine because the small intestine targeted delivery efficiency of the amylose−CLA complex was improved by adding βCD to form an amylose−CLA−βCD complex.

Normally, amylose in water is thermodynamically unstable and will retrograde through self-packing if the concentration is high enough. Inferred from the self-assemblied amylose−CLA−βCD complex and the existence of the amylose−βCD complex, βCD, if coexists with amylose in a food system, would hinder the self-packing of amylose chains leading to a decrease in thermal stability, retrogradation retardation (*12*), and an increase in digestibility of amylose. However, the exact mechanism of the interaction, such as the interaction forces between amylose and βCD, still needs more investigations.

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