Maltase and sucrase inhibitory activities and hypoglycemic effects of carbon dots derived from charred *Fructus crataegi*

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Abstract

Although carbon dots (CDs) have been widely applied in nanobiotechnology and biomedicine, few studies have evaluated the intrinsic self–bioactivities and no reports have described the disaccharidase inhibitory activities of CDs. In this study, charred *Fructus crataegi*-derived CDs (CFC-CDs) having an average diameter of 1.3–5.6 nm are developed, without further modifications. Owing to an abundance of surface groups, CFC-CDs show distinct solubility, biocompatibility, and bioactivity. Moreover, CFC-CDs inhibit the catalytic activities of sucrase and maltase *in vitro* and reduce postprandial blood glucose levels *in vivo*, possibly acting as a disaccharidase inhibitor. This discovery provides guidance for further research on the bioactivities of CDs and supports their potential applications in the biomedical and healthcare fields.

1. Introduction

Inhibition of α-glucosidases has recently provided a new approach for the management of hyperglycemia by facilitating the maintenance of normal blood glucose levels [1] via slowing digestion and subsequent absorption of dietary complex carbohydrates [2]. Sucrase and maltase, two essential members in intestinal α-glucosidase families, are considered to be key enzymes involved in the final step of carbohydrate digestion and biosynthesis of glycoproteins [3]. Sucrase is the only intestinal enzyme able to hydrolyze sucrose [4], whereas maltase shows versatile α-hydrolytic activities [5]. Therefore, inhibitors of the two α-glucosidases described above have attracted great interest among researchers [6]. Several currently used therapeutic inhibitors against sucrase and maltase, however, have been shown to cause serious gastrointestinal side effects, and their synthesis involves tedious multistep procedures [7]. Thus, the development of more effective and safe disaccharidase inhibitors is urgently needed for the treatment of hyperglycemia.

Carbon dots (CDs), as a new kind of nanocarbon material, have attracted great interests in nanobiotechnology and biomedicine domain [8, 9] owing to their superior properties, such as water dispersity, excellent photoluminescence, and biocompatibility [10–12]. Due to their benign nature like facile synthesis, environmental friendliness and robust chemical inertness, CDs have a promising future in the application of photocatalysis, sensors and bioimaging, etc [10, 11, 13]. For any bio-applications, understanding of the interactions of CDs with biomolecules like enzymes, DNA and lipids, is of great necessity and importance. The interactions of CDs with enzymes can potentially change the structures of enzymes and alter the catalytic reactions that are essential for organisms owing to the unique structural characteristics of CDs, such as abundant nitrogen/oxygen-based surface functional groups and electron conditions [14]. CDs may have roles as sensitizers for redox enzymes [15] and peroxidase mimetic catalysts for molecule detection [16, 17]. The existed studies on the effects of CDs on enzyme catalytic activities are still rare and superficial. Only a few reports have illustrated that carbon dots can switch the catalytic activities of laccase [18, 19], rubisco enzyme [20, 21] and
porcine pancreatic lipase [22]. A recent study also discovered that the preparation of maltase/chiral CDs hybrids contributed to the partial inhibition of maltase’ activities [23]. Nevertheless, the effects of CDs on bio-enzymes and bioreactions in humans and overall animals are still unclear. Thus, in-vitro and in-vivo investigations of the potential influence of CDs on disaccharidase catalytic activities are essential.

Fructus crataegi (FC) is a traditional medicinal plant widely used in many countries which can regulate digestive function and protect the cardiovascular systems [24]. The fruit of this plant, which is abundant in carbon, oxygen and nitrogen elements, can act as an excellent biomass precursor to prepare self-passivated CDs possessing sufficient surface functional groups. Charred Fructus crataegi (CFC), processed from FC after charcoal processing, is a safe medicinal food used for the treatment of digestive diseases and obesity since 1347 A.D. in traditional Chinese medicine (TCM) [25]. Supported by clinical evidence and modern medical research, CFC has been shown to have therapeutic effects on glucose metabolism disorders and is closely associated with small-bowel disaccharidase activity [25]. However, the mechanisms underlying the pharmacological activities and of CFC are controversial, and few studies have evaluated the effects of CFC on disaccharidase activities. Additionally, from the perspective of small molecule active compound, the material basis of CFC is still less defined. Notably, CDs are generated during charcoal processing [26, 27], and we have identified CFC-CDs from CFC for the first time.

Accordingly, in this study, we established novel biocompatible CDs derived from CFC (CFC-CDs) synthesized by a simple, eco-friendly method. We then evaluated the effects of CFC-CDs on disaccharidase catalytic activities in vitro and examined the catalytic kinetics and mechanisms through which CFC-CDs exerted enzyme inhibitory activities. Finally, we evaluated the effects of CFC-CDs in mice on disaccharide digestion and postprandial blood glucose levels.

2. Methods

2.1. Chemicals

FC was purchased from Beijing Qiancao Herbal Pieces Co., Ltd (Beijing, China). Dialysis membranes (1000 Da) were purchased from Beijing Ruida Henghui Technology Development Co., Ltd (Beijing, China). Other analytical-grade chemical reagents were obtained from Sinopharm Chemical Reagents Beijing (Beijing, China). Sucrase and maltase standards were purchased from Shanghai Yuanye Biotechnology Co., Ltd (Shanghai, China). All experiments were performed using deionized water.

2.2. Preparation of CFC-CDs

First, FC (240 g) was placed into a crucible and calcined using a muffle furnace (TL0612; Beijing Zhong Ke Aobo Technology Co., Ltd, Beijing, China) at 350 °C for 1 h, yielding CFC. Then, the CFC (85 g) was boiled twice in distilled water (1 L) at 100 °C for 1.5 h each time. The obtained yellowish-brown solution was prefiltered through a 0.22-μm cellulose acetate membrane and concentrated by evaporation. To purify the CFC-CDs, the resulting solution was dialyzed using a 1000-Da dialysis membrane in deionized water for 7 days and the solution inside the dialysis membrane was collected. This CFC-CDs solution was then centrifuged to remove agglomerated particles at 11 000 rpm for 30 min to obtain clear CFC-CDs before use. The preparation process for CFC-CDs solution is shown in figure 1. Finally, the as-prepared CFC-CDs solution was dried to obtain CFC-CDs solid powder and weighed. The solid powder was dissolved in double distilled water at the concentration of 1.0 mg ml⁻¹, 0.25 mg ml⁻¹ or 0.0625 mg ml⁻¹.

2.3. Characterization of CFC-CDs

The size, morphology, and microstructure of the CFC-CDs preparation were characterized using transmission electron microscopy (TEM; Tecnai G2 20; FEI Company, Hillsboro, OR, USA). The structural details of the CFC-CDs were examined using high-resolution TEM (JEM-1230; Japan Electron Optics Laboratory, Tokyo, Japan). Ultraviolet-visible (UV–vis) adsorption spectrum of CFC-CDs were recorded by spectroscopy (CECIL, Cambridge, UK) and photoluminescence (PL) spectra were determined using a molecular fluorescence spectrometer (F-4500; Tokyo, Japan) in a standard quartz cuvette were also. The structure and crystallinity of CFC-CDs was detected by x-ray diffraction (XRD; Bruker AXS, Karlsruhe, Germany) with Cu K-alpha radiation (λ = 1.5418 Å). Raman spectra were obtained on a LabRAM HR800 Raman spectrometer (Jobin-Yvon, HORIBA Group, France) with 514 nm wavelength incident laser light. In addition, the chemical composition and structure of CFC-CDs were characterized by Fourier transform infrared (FT-IR) spectroscopy (Thermo Fisher, Fremont, CA, USA) and x-ray photoelectron spectroscopy (XPS; ESCALAB 250Xi, Thermo Fisher Scientific, Fremont, CA, USA). The CD spectra were obtained using a JASCO J-815 spectropolarimeter.
2.4. Quantum yield (QY) of CFC-CDs
The fluorescence QY of the CFC-CDs was measured according to an established procedure using quinine sulfate (Qst: 54 in 0.1 M sulfuric acid solution) as a standard sample and calculated using the following equation.

\[ Q_{QYCD} = Q_{st} \times \frac{I_{CD}}{I_R} \times \frac{A_R}{A_{CD}} \times \frac{n^2_{CD}}{\eta_R^2} \]

where Q represents the QY, I is the integrated area under the emission spectrum, A is the absorbance at 340 nm wavelength, and st is the refractive index. The subscripts CDs and R refer to CFC-CDs and standard, respectively.

To minimize the reabsorption effect, the AR and ACDs were maintained below 0.05.

2.5. Fingerprint analysis of CF and CFC-CDs by high-performance liquid chromatography
Sample preparation: To evaluate the component change of the CFC-CDs, an aqueous solution of the CFC-CDs and methanol extracts of CF were initially prepared and the solutions were filtered through a 0.22 μm. Microporous membrane before injecting of the final solution into the HPLC instrument (10 μL) for analysis.

HPLC condition: a comparative analysis of the CF and CFC-CDs was performed using a reported method with some modifications [28, 29]. An Agilent 1260 series liquid chromatographer (Agilent Technologies, Palo Alto, CA, USA) and Phenomenex Luna C18(2) 100 A column (5 μm, 250 mm × 4.60 mm, Phenomenex, USA). The components were separated by a gradient elution with water containing 0.1% phosphoric acid (solvent A) and acetonitrile (solvent B) with a constant flow rate of 1.0 ml min⁻¹. The gradient program started with 94% solvent A, followed by a linear decrease to 76% solvent A for 60 min. Each sample was injected and monitored at 210 nm. The column was held at 30 °C.

2.6. Biocompatibility evaluation of CFC-CDs
2.6.1. Cell viability assay
RAW 264.7 cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with fetal bovine serum (20%) at 37 °C in 5% CO₂. Cells were counted and seeded in 96-well plates at a density of 1 × 10⁵ cells/well for 24 h and treated with CFC-CDs at various concentrations (0.1, 10, 50, 75, 100, 250, 500, 1000, 1500 μg ml⁻¹) for an additional 24 h. Cell Counting Kit-8 (CCK-8) solution (10 μL/well) was added. After 4 h incubation, absorbance was measured at 450 nm using a microplate reader (Biotek, VT, USA). The relative cell viability was calculated using the following formula:

\[ \text{Cell viability} \% \text{ of control} = \frac{Abs_{\text{sample}}}{Abs_{\text{control}}} \times 100 \]

where Abs_sample and Abs_control represent the A450 of the experimental and control groups, respectively. The experiments were performed in triplicate, independently.

2.6.2. Animals and acute toxicity evaluations in vivo
This study was performed in accordance with the Guide for the Care and Use of Laboratory Animals and was approved by the Committee of Ethics of Animal Experimentation of the Beijing University of Chinese Medicine (IRB Code 2017BZHYLL00106). Male and female Kunming mice (weighing 30.0 ± 2.0 g) were purchased from the Laboratory Animal Center, Si Beifu with a Laboratory Animal Certificate of Conformity. The animals were
maintained under the following conditions: temperature, 24.0 ± 1.0 °C; relative humidity, 55%–65%; and a 12 h light/dark cycle, with ad libitum access to food and water.

Kunming mice (30.0 ± 3.0 g) were divided into three groups of 12 each (6 female mice and 6 male mice). Two groups of mice were exposed to CFC-CDs (20.85 mg kg⁻¹, intraperitoneal injection [i.p.]) and were sacrificed 3 and 7 days after administration. Untreated healthy mice were used as the control. The mice and major organs from the mice were harvested, fixed in 4% neutral buffered formalin, processed routinely in paraffin, sectioned into 4-μm-thick slices, and stained with hematoxylin and eosin (HE). Morphological changes were compared between the three groups.

2.7. Sucrase and maltase inhibitory activities of CFC-CDs in vitro

2.7.1. Preparation of mouse intestinal sucrase and maltase fractions

Mice were killed by exsanguinations under isoflurane anesthesia, and their small intestines were then excised and washed with physiological saline (0.9%). The small intestine tissues were homogenized (for 3 min at 5000 rpm) in 10 volumes of phosphate buffer (pH 7.0) solution using a glass Teflon homogenizer (Ultra Turrax IKA T10 Basic; Germany). The homogenate was centrifuged at 3000 × g for 30 min to remove debris. Supernatant fluid was collected for the assay.

2.7.2. Sucrase and maltase inhibitory activities of CFC-CDs in mouse intestinal fractions

The maltase and sucrase inhibitory activities of CFC-CDs were assayed as previously reported [30], with some modifications. Briefly, mouse intestine solution (300 μL) and CFC-CD solution (300 μL; 1.0 mg ml⁻¹, 0.25 mg ml⁻¹ or 0.0625 mg ml⁻¹ for high-, medium-, or low-dose, respectively) were added to a test tube. The control sample was prepared by adding phosphate buffer (pH 7.0) instead of CFC-CDs. After incubation at 37 °C for 10 min, sucrose (300 μL; 0.58 M) for sucrase inhibitory activity assays or maltose (300 μL; 0.56 M) for maltase inhibitory activity assays was added to the reaction mixture. After incubation for 60 min at 37 °C, the reaction was stopped by adding Na₂CO₃ (1000 μL, 1.0 M). Maltase and sucrase inhibitory activities were estimated by determining the difference of liberated glucose with or without CFC-CDs. Determination of glucose was performed using an Accu-Chek glucometer (Johnson & Johnson GmbH, New Brunswick, NJ, USA) based on the glucose oxidase method. The percentage of sucrase inhibition was calculated as follows:

\[
\text{Inhibitory ratio} \% = \left( \frac{[\text{glucose}]_{\text{control}} - [\text{glucose}]_{\text{Inhibit}}} {[\text{glucose}]_{\text{control}}} \right) \times 100
\]

The inhibitory activity of each sample was determined three times, and the resulting data were calculated with the same method as described above.

2.7.3. Kinetics of maltase and sucrase inhibitors

Kinetic and Lineweaver-Burk plot analyses for sucrase inhibition by CFC-CDs were performed as previously reported [31], with some modifications. First, in order to select the appropriate concentration of CFC-CDs, different concentrations of CFC-CDs were used while keeping the final concentration of the enzyme (0.38 mg ml⁻¹ sucrose dissolved in 0.1 M PBS solution) and substrate (0.27 M sucrose solution) constant. Sucrose solution was incubated with CFC-CDs at 37 °C for 10 min. The substrates of sucrose at different concentrations were then added to start the reaction at 37 °C, and after incubation for 1 h, the production of glucose was evaluated using an Accu-Chek glucometer. The concentration of inhibitor required to inhibit 50% of sucrase activity under the assay condition was defined as the IC₅₀sucrase value. The type of inhibition was determined by Lineweaver-Burk plots, as described above [31]. The concentration of CFC-CDs (0.12 mg ml⁻¹) was kept constant while changing the concentration of the substrate. The data were calculated according to Michaelis–Menten kinetics. All samples were measured repeatedly three times, and the values were expressed as the means ± standard deviations (SD; n = 3). The linear equation and correlation efficiency of the CFC-CDs as a sucrase inhibitor were estimated using Origin 8.0 software.

For determination of maltase inhibitor kinetics, the appropriate concentration of CFC-CDs was also assayed using maltose (0.14 M) and the enzyme (maltase at 3.3 mg ml⁻¹ dissolved in 0.1 M PBS). The concentration of inhibitor required to inhibit 50% of maltase activity was defined as the IC₅₀maltase Value. The Lineweaver-Burk plots were also used for evaluation of maltase inhibition by CFC-CDs using the same method as for the sucrase kinetics test above. The concentration of maltase was 3.3 mg ml⁻¹ in 0.1 M PBS, and the CFC-CD inhibitor concentration was 0.041 mg ml⁻¹.

2.8. Postprandial blood glucose reducing effects of CFC-CDs in vivo

A hyperglycemia model was established according to a previous protocol with some modifications [32, 33]. Briefly, mice (30.0 ± 3.0 g) were randomly divided into five groups and subjected to fasting for 18 h. Fasting
blood glucose was measured using blood taken from the tail vein with an Accu-Chek glucometer, according to the manufacturer’s recommendations. Mice from groups receiving intragastric gavage of maltose or sucrose (2 mg g$^{-1}$) were the model groups and treated with NS. Mice receiving both maltose or sucrose (2 mg g$^{-1}$) and acarbose (5 mg/kg) orally were the positive control groups, while mice receiving normal saline (NS) were the negative control group. CFC-CD treatment groups received both intragastric gavage of maltose (2 mg g$^{-1}$) or sucrose (2 mg g$^{-1}$) and CFC-CD administration (4.17 mg kg$^{-1}$). Blood glucose was measured at 15, 30, 60, 90, 120, 150, 180, and 210 min after induction of hyperglycemia.

2.9. Statistical analysis
Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS, version 20.0). The normally distributed data and homogeneous variances were expressed as means ± SDs. Multiple comparisons were performed using one-way analysis of variance (ANOVA) followed by the least significant difference test. Results with $p$ values of less than 0.05 were considered significant.

3. Results

3.1. Characterization of CFC-CDs
First, we characterized CFC-CDs extracted from the FC solution. As shown in figure 2(A), TEM images of CFC-CDs revealed that the CDs were nearly spherical and well separated from each other. The size distribution of the CDs was in the range of 1.3–5.6 nm (figure 2(B)). Furthermore, high-resolution TEM (figures 2(C), (D)) showed that the CDs had a lattice spacing of 0.327 nm, corresponding to the (002) spacing value of graphitic carbon [34].

UV–vis absorption was also evaluated to determine the optical properties of the CFC-CDs. The UV–vis spectrum in figure 3(A) showed a broad absorption spectrum with a weak peak at 273 nm, which could be ascribed to the π-$\pi^*$ transition of C=C. A broad emission centered at 468 nm was observed in the emission spectrum, with the strongest excitation at 323 nm. Two peaks at 275 and 323 nm appeared in the excitation spectrum, revealing that the emission might be related to two types of transitions. The fluorescence quantum yield (QY) of the CFC-CDs was measured, and the QY of CFC-CDs was 5.95%. The XRD profiles of the CFC-CDs (figure 3(B)) showed a diffraction peak located at around 21.5 degrees, which corresponded to the reported [35].
Raman spectrum of the CFC-CDs (Figure 3(C)) showed a disordered (D) band at 1358 cm\(^{-1}\), and the crystalline (G) band at 1587 cm\(^{-1}\). The value of \(I_D/I_G\) is 0.36, which indicated that the as-prepared CFC-CDs have highly crystalline nature. To gain better insights into the organic functional groups on the surface of the CFC-CDs, we further analyzed the CFC-CDs using FT-IR; the purified CFC-CD spectra (Figure 3(D)) showed characteristic peaks of 3426, 2923, 1628, 1433, and 1047 cm\(^{-1}\). A sharp peak at 1628 cm\(^{-1}\) (C=O vibrational stretch) and a peak centered at 3426 cm\(^{-1}\) (O–H vibrational stretch) revealed the existence of carboxylate and hydroxyl groups on the surfaces of the C-dots. The peaks at 1047 cm\(^{-1}\) corresponded to the symmetric stretching vibrations of C–O–C. The peak at 2923 cm\(^{-1}\) may be due to the existence of the –CH\(_2\)– stretch. The absorptions at 1433 was assigned to the C–N stretch. The FT-IR spectra of CFC-CDs demonstrated that many hydrophilic functional groups existed on the surface of CFC-CDs, leading to excellent aqueous dispersibility.

Figure 4(A) shows the full-scan XPS spectrum of CFC-CDs, from which we could observe three peaks located at 284.8, 400.1, 532.1, and 168.2 eV, corresponding to C 1 s, N 1 s, O 1 s, and S 2p in CFC-CDs, respectively. In addition, CFC-CDs contained 73.48 at% carbon, 21.42 at% oxygen, 3.75 at% nitrogen, and 0.5 at% sulfur according to the XPS results. High-resolution XPS spectra of C, N, and O were collected to illustrate the detailed bonding formation during the hydrothermal reaction process, and the results are presented in figures 4(B)–(D). The partial XPS spectrum of C 1 s could be divided into four peaks centered at 284.85 (sp\(^2\) C), 286.2 (C–O/C–N), and 288.8 eV (C=O) The high resolution O 1 s spectrum could be divided into two peaks located at 531.9 and 533.3 eV, which were assigned to C=O and C=O, respectively. Two peaks at 400.05 and 401.8 eV were observed in the high-resolution N 1 s spectrum, revealing the existence of C–N and N–H bonding. The XPS analysis suggested that N and S successfully doped with C and O of the carbon core.

3.2. Fingerprint analysis of FC and CFC-CDs by high-performance liquid chromatography

The HPLC results of this study showed that no active small molecule compounds of the FC were detected in the prepared CFC-CDs, as shown in figure 5.

3.3. Biocompatibility evaluation of CFC-CDs

3.3.1. Cell viability assay

The CFC-CDs did not affect RAW 264.7 cell growth at concentrations up to approximately 1000 µg ml\(^{-1}\) (figure 6(A)). Cell viability gradually decreased as the CDs concentration increased from 2000 to 8000 µg ml\(^{-1}\), revealing the low toxicity of CFC-CDs in vitro.
3.3.2. Animals and acute toxicity evaluations in vivo

We collected the main organs, including the livers, spleens, kidneys, and hearts, of the mice from the control and treated groups and compared histopathological changes in these organs (figure 6(B)). Overall, no apparent
histopathological abnormalities or lesions were observed in the treated groups at our injected CFC-CD doses. Our results collectively suggested that CFC-CDs were highly biocompatible at the dose used in this study.

3.4. Sucrase and maltase inhibitory activities of CFC-CDs in mouse intestinal fractions

In order to evaluate the catalytic activities of sucrase and maltase in mouse intestinal fractions under optimal proper assay conditions, a time course was performed using different concentrations of substrate (sucrose solution: 1.0, 1.2, and 1.4 mg ml$^{-1}$; maltose solution: 0.8, 1.0, and 1.2 mg ml$^{-1}$) to determine glucose production. When the substrate was sucrose (figure 7(A)), before 25 min, the glucose concentration increased in a linear pattern. From approximately 25 min to 60 min, the glucose concentration growth rate became nonlinear and reached its highest level. From these results, we selected an assay time of 20 min. As shown in figure 7(B), the substrate was maltose. Before 45 min, the glucose concentration increased in a linear pattern. From approximately 40 to 120 min, the glucose concentration growth rate slowed, reaching a plateau. Thus, an assay time of 40 min was selected to evaluate sucrase inhibitory activity. Figures 7(C) and (D) show the results of sucrase and maltase inhibitory activity assays, in which concentrations of CFC-CDs were set to 1.0, 0.25, or 0.0625 mg ml$^{-1}$ (high [H], medium [M], and low [L] dose groups, respectively). In sucrase inhibitory activity assays (figure 7(C)), glucose production levels in the H (4.12 $\pm$ 0.73 mM), M (6.27 $\pm$ 1.27 mM), and L groups (8.50 $\pm$ 0.89 mM) were all significantly lower ($P < 0.001$) than that in the PBS control group (10 $\pm$ 1.84 mM). In the maltase inhibitory activity assay (figure 7(D)), glucose production levels in the H (8.74 $\pm$ 1.7 mM) and M groups (15.80 $\pm$ 2.15 mM) were all significantly lower ($P < 0.001$ and $P < 0.01$, respectively) than in the PBS.

Figure 6. (A) Viability of RAW 264.7 cells after incubation with various concentrations of charred Fructus crataegi carbon dots (CFC-CDs) for 24 h. (B) Histological evaluation of CFC-CD toxicity in vivo. Hearts, livers, spleens, lungs, and kidneys were examined 3 and 7 days after intraperitoneal injection of CFC-CDs (20 mg kg$^{-1}$).
control group (20 ± 0.657 mM). The glucose concentration in the L group (18.88 ± 3.89 mM) did not differ significantly compared with that in the PBS control group.

From the above experiments, the enzyme inhibition rates were calculated. As shown in figure 7(E), CFC-CDs has strong sucrase and maltase inhibitory effects at H (1.0 mg ml⁻¹) and M doses (0.25 mg ml⁻¹), with sucrase inhibition rates of 58.83% ± 7.28% and 37.28% ± 8.71%, respectively, and maltase inhibition rates of 56.26% ± 9.30% and 21.02% ± 6.85%, respectively. In contrast, a low inhibition rate was observed in the presence of low-dose (0.0625 mg ml⁻¹) CFC-CDs. Thus, the extract had significant effects on sucrase and maltase enzymatic reaction when sucrase and maltase were used as the substrates.

3.5. Kinetics and mechanisms of sucrase and maltase inhibitory activities of CFC-CDs

To evaluate the kinetics of sucrase and maltase inhibitory activities, we used sucrase and maltase to facilitate stable quantification. These enzymes have been widely used in enzymatic assays to screen new α-glucosidase inhibitors owing to its availability and ease of handling [36]. The sucrase and maltase inhibitory activities were evaluated at different concentrations of CFC-CDs. In sucrase inhibition tests (figure 7(F)), the IC₅₀sucrase was approximately 0.73 mg ml⁻¹, and the sucrase inhibition rate reached up to 73.3%. In contrast, in maltase inhibition assays (figure 7(I)), the curve showed a rapid growth phase when the CFC-CD concentration was less than 0.48 mg ml⁻¹ (IC₅₀maltase 0.26 mg ml⁻¹), and the maltase inhibition rate reached almost 91%.

Next, to clarify the inhibition mode of CFC-CDs, we used Lineweaver-Burk plots (figures 7(G) and (J)). For sucrase (figure 7(G)), when CFC-CDs were added into the reaction system, hydrolysis of sucrose was obviously
shown in maltose inhibitory activities, blood glucose levels in mice were measured in sucrose and maltose loading tests. As inhibited. In both figures 7(G) and (I), the intersection of the double plot was seated at a point above the +1/[s] axis, indicating that CFC-CDs may act as a partially noncompetitive-type inhibitor of sucrase and maltase. The circular dichroism spectra of the enzymes are shown in figures 7(H) and (K). Compared with that of free sucrase (figure 7(H)), the intensities of the negative peaks at 208, 221 (α-helix), and 216 (β-sheet) for sucrase/CFC-CDs decreased, suggesting the unfolding change in sucrase on CFC-CDs and thus leading to decreased catalytic activity[37]. Compared with that of free maltase (figure 7(K)), the intensities of the positive peak at 197 nm (α-helix) and negative peaks at 208, 221 (α-helix), and 216 nm (β-sheet) for maltase/CFC-CDs increased, indicating the formation of a compact structure owing to the increased α-helix and β-sheet contents when maltase combined with CFC-CDs, allowing fewer active sites to be accessible to the substrate and thereby leading to decreased catalytic activity[18]. A schematic representation of this enzymatic hydrolysis reaction process is illustrated in figure 8.

3.6. Postprandial blood glucose reducing effects of CFC-CDs in vivo

To confirm the in vivo relevance of our in vitro findings demonstrating that CFC-CDs exhibited sucrase and maltose inhibitory activities, blood glucose levels in mice were measured in sucrose and maltose loading tests. As shown in figure 9(A), the glucose levels of the postprandial hyperglycemic model group (sucrose) increased between 0 to 30 min. Glucose levels then decreased until 150 min. In contrast, glucose levels in the CFC-CD group peaked at 15 min and then decreased steadily while acarbose group peaked at 30 min. During the test, the glucose levels in the CFC-CD groups were always lower than that in the sucrose group and during 30 to 90 min, the glucose levels in CFC-CD group were also slightly lower than acarbose group. The glucose level in the blank group remained stable. As shown in the inset in figure 9(A), the areas under the curve (AUCs) in the CFC-CD (740.499 mmol min l⁻¹), acarbose group (803.25 mmol min l⁻¹) and blank group (462.15 mmol min l⁻¹) were significantly lower than that in the sucrose group (1196.55 mmol min l⁻¹). Additionally, blood glucose levels in the sucrose and CFC-CD groups were significantly higher at 15, 30, 60, and 90 min (p < 0.05) than in the blank group (figure 9(B); unpaired Student’s t-tests). Blood glucose levels in the CFC-CDs and acarbose groups were significantly lower (p < 0.05) than those in the model group of sucrose at 15, 30, 60, and 90 min. In maltose loading tests (figure 9(C)), the glucose levels in the postprandial hyperglycemic model group (maltose), the CFC-CD group and acarbose group increased between 0 and 15 min and then decreased steadily. The AUCs (figure 9(C), inset) in the CFC-CDs (1213.575 mmol min l⁻¹), acarbose group (1128.15 mmol min l⁻¹) and blank groups (596.475 mmol min l⁻¹) were significantly different compared with those in the sucrose group (1703.425 mmol min l⁻¹). The blood glucose levels in acarbose group was a little lower than CFC-CDs group. As shown in figure 9(D), blood glucose levels in the maltose and CFC-CD groups were significantly higher

![Figure 8](image-url)

Figure 8. Schematic illustration of the enzymatic hydrolysis reaction process from the substrate [S] (sucrose or maltose) to the product [P] (glucose) catalyzed by intestinal sucrase and maltase [E] in the presence or absence of CFC-CDs [I]. [ES] represents the sucrase/sucrose or maltase/maltose complex, and [ESI] represents the sucrase/sucrose/CDs or maltase/maltose/CDs complex.
than that in the blank group. Blood glucose levels in the CFC-CD groups were significantly lower than those in the maltose groups.

4. Discussion

In this study, we developed novel eco-friendly CDs derived from CFC. These CFC-CDs had an average diameter of 1.3–5.6 nm. FC naturally contains carbon, oxygen, and nitrogen, which are necessary for the preparation of CFC-CDs and could serve both as the carbon source and as the passivation agent for CDs. Hence, no further modification or external surface passivation agents were required. Moreover, the prepared CFC-CDs were safe and biocompatible for biological use, as demonstrated by the results of in vitro CCK-8 assays and in vivo acute toxicity evaluations. Through adequate and repeated purification process including dialysis, centrifugation and filtration, we have obtained pure CFC-CDs solution. The purity of the solution has been proved by the results of high-performance liquid chromatography, in which the existence of small active molecules was not detected in the solution.

Nowadays, CDs have been studied owing to their various self-bioactivities, including anticancer activity [38], antihyperuricemic activities [39], hemostatic effects [26, 27], and etc. In our previous study, we observed the hypoglycemic effects of CDs from a charcoal TCM, Jiaosanxian [40]. Thus, we aimed to further investigate the influence of CDs on carbohydrate digestion and disaccharidase catalytic activities related to blood glucose levels.

In our experiments, we found significant inhibitory effects of CFC-CDs on sucrase and maltase in the small intestinal fractions of mice. The reaction was dose dependent, and the kinetic conditions of the assay were optimized. The IC50 value was 0.73 mg ml\(^{-1}\), and the IC50 was 0.26 mg ml\(^{-1}\), reflecting that very small doses of CFC-CDs could be effective and that inhibition of maltase was stronger. Furthermore,
Lineweaver-Burk plots suggested that the inhibitory mode of CFC-CDs against sucrase and maltase may be a partially noncompetitive type. Noncompetitive inhibitors bind to the enzyme(substrate [ES] complex and affect the breakdown of the [ES] to form a product. Partial noncompetitive inhibitors are thought to be released from the enzyme when the [ES] is broken down into the product [41, 42]. This type of inhibition often occurs where there are multiple inhibitors [43], as would be the case for CFC-CDs, a kind of nanoparticles with an uniform diameter of 1.3–5.6 nm which contain many surface functional groups, including amino, carboxyl, and hydroxyl groups, thereby contributing to their different interactions with enzymes. CD measurements showed that CFC-CDs influenced the secondary structure of the enzyme, owing to the electron accepting or donating properties of CFC-CDs and reduce degradation of the substrate [22].

To confirm the in vivo relevance of our in vitro findings, we performed disaccharide loading tests in mice. The results showed that CFC-CDs significantly decreased postprandial blood glucose levels by reducing peak blood glucose levels and AUCs of blood glucose in mice. Thus, CFC-CDs could act as sucrase and maltase inhibitors to reduce postprandial blood glucose in the complex blood glucose regulatory mechanism. This beneficial effect of CFC-CDs (i.e., postprandial blood glucose reduction) could support the application of CFC as a TCM for the treatment of diseases associated with sugar digestion, uptake, and metabolism, such as diabetes and obesity. Currently, clinical treatment of hyperglycemic disease relies on sugar moieties, such as acarbose, miglitol, and voglibose; however, these moieties are unfavorable for long-term use owing to their severe adverse side effects, including abdominal discomfort, diarrhea, and hepatotoxicity [7]. In contrast to currently available antihyperglycemic small molecule drugs, CFC-CDs as novel α-glucosidase inhibitors are biocompatible nanoparticles derived from CFC, which has long been used clinically to promote digestion and treat abdominal discomfort. It is reported that FC extracts have the antidiabetic effects [44], but excessive and improper consumption of FC can cause stomach stones and discomfort by the rich content of pectin, organic acid, tannin, and etc [45]. However, in this study, we prepared a purified sample of CFC-CDs without any small molecular components existing, which can be proved by the HPLC results and its disaccharidase inhibitory activities and hypoglycemic effects were obvious and reliable from the results of our experiment. Compared with FC, CFC-CDs retained the disaccharidase inhibitory activities with lower side-effect of gastric damage and good solubility. The CFC-CDs may have potential applications as complementary and alternative therapeutic agents for blood glucose control. Overall, our findings provide evidence and guidance for further studies of the intrinsic self-bioactivities of CDs.

5. Conclusion

In this study, novel CDs derived from CFC were developed and found to be effective for inhibiting sucrase and maltase catalytic activities. This study suggests potential applications as complementary and alternative therapeutic agents for postprandial blood glucose control. Our findings established a basis for future drug discovery and provide insights into expansion of the potential applications of CDs in nanomedical and healthcare fields.

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