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Curcumin encapsulated in the complex of lysozyme/carboxymethylcellulose and implications for the antioxidant activity of curcumin

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ABSTRACT

A facile approach was investigated to encapsulate and protect curcumin (Cur) by self-assembly of lysozyme (Ly) and carboxymethylcellulose (CMC) of different degrees of substitution (DSs). This work studied the influence of Ly–CMC coacervates on the binding, solubility and stability of Cur. The interactions of Cur with Ly–CMC coacervates were researched by UV–vis, steady-state fluorescence, synchronous fluorescence and circular dichroism spectra. These results were explained in terms of the formation of Ly–CMC coacervates with electrostatic interaction, which led to unfold the structure of Ly for providing Cur with more hydrophobic microenvironment than free Ly. Meanwhile, the CMC of higher DS provide more negative charges, and produce more attraction to Ly than that of lower DS values. Moreover, the coacervates of Ly–CMC of higher DS are found to be superior for enhancing the stability of Cur. Our work provided a new insight for understanding the biomolecule protective system based on protein/polysaccharide complexes.

1. Introduction

Curcumin (Cur), derived from the rhizome of turmeric, is a hydrophobic polyphenol and extensively used in food and chemical industries as coloring, flavoring and preservative (Chen et al., 2014). In addition, this dietary polyphenol has recently attracted great interest due to its pharmacological effects such as antioxidant, antibacterial, anti-inflammatory and anticancer (Rosa et al., 2014). Although Cur has multiple medicinal benefits and high safety profile, the wide applications of Cur in food and medical industries have been hindered because of its poor water solubility and absorption, high hydrolytic instability at physiological pH, rapid pre-systemic metabolism and limited tissue distribution (Aditya et al., 2015; Yu & Huang, 2012).

Encapsulation is a feasible method to solve the drawbacks of Cur such as emulsion (Ahmed, Li, McClements & Xiao, 2012; Syedl, Liew, Loh & Peh, 2015; Wang et al., 2008), polymeric micelles (Zhang et al., 2015), complexes (Kunwar, Barik, Pandey & Priyadarsini, 2006), and nanoparticles (da Silva-Buzanello et al., 2015). Among these, using natural biopolymers as carriers to load Cur has received increasing interest due to their safe, biodegradable and reproducible perspective (Pan, Tikekar, Wang, Avena-Bustillos & Nitin, 2015; Sneharani, Singh & Appu Rao, 2009). Complexes based on natural proteins and polysaccharides with electrostatic interactions, hydrophobic interactions, and hydrogen bonds are more superior carriers than proteins alone because polysaccharides can restrain the aggregation of proteins especially at the isoelectric point of the proteins (Chen et al., 2014; McClements, 2006).

Lysozyme (Ly) from egg white, which has molecular weight of 14 kD and isoelectric point of 10.7, is a seldom natural protein with positive charges and maintained great attraction (Li, Xu, Zhang, Chen & Li, 2015). Carboxymethylcellulose (CMC), one of the cellulose derivatives, is a typical anionic polysaccharide, and has wide applications in the food industry (Geng et al., 2014; Song & Chen, 2015). Besides, CMC was often used as a potential carrier material due to its good biocompatibility, biodegradability and low immunogenicity (Cai et al., 2011; Li et al., 2015; Song, Zhou & Chen, 2012; Zhu et al., 2013). The characteristics of CMC are closely related to the degree of substitution (DS), defined as the carboxymethyl groups per repeating glucoside unit. However, the properties of coacervates between proteins and CMC of different values of DS have been scarcely reported (Geng et al., 2014). Besides, the DSs of

Abbreviations: Cur, curcumin; Ly, lysozyme; CMC, carboxymethylcellulose; DS, degree of substitution; DPPH, 2,2-diphenyl-1-picrylhydrazyl; CMC 0.7, carboxymethylcellulose of degree of substitution 0.7; CMC 0.9, carboxymethylcellulose of degree of substitution 0.9; CMC 1.2, carboxymethylcellulose of degree of substitution 1.2; Trp, tryptophan; Tyr, tyrosine; Phe, phenylalanine; CD, circular dichroism; *EE*, encapsulation efficiency; *LC*, loading capacity.

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CMC may influence the encapsulation and protection of the bioactive molecules for Ly–CMC coacervates as carriers.

In this work, the coacervates of Ly and CMC of different values of DS were prepared based on electrostatic interaction. Cur was introduced to the complexes to study the interaction between Cur and coacervates with spectroscopy means. The stability of Cur was evaluated by determining the residual amount of Cur in Ly–CMC coacervates and free radical scavenging rate after thermal treatment.

2. Materials and methods

2.1. Materials

Curcumin (Cur) (95% purity) and lysozyme (Ly) (14.3 kDa) from chicken egg white were purchased from National Medicine Group Chemical Reagent Co., Ltd. Carboxymethyl cellulose (CMC) (250 kDa) with the degrees of substitution (DSs) of 0.7, 0.9 and 1.2, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Aladdin Chemistry Co., Ltd. Other chemicals were all analytical grade and used without purification. Aqueous solutions were prepared using ultrapure water through a Millipore (Millipore, Milford, MA, USA) Milli-Q water purification system.

2.2. Preparation of Cur loaded samples with Ly and CMC

When the DS values are in the range of 0.7–1.2, CMC possess good solubility and transparency (Shakun, Heinze & Radke, 2013). The DS values of 0.7, 0.9 and 1.2 were selected to conduct the following experiments. Ly and CMC of various DSs were dissolved in phosphate buffer solution (PBS, 10 mM, pH 7.0) for 4 h at room temperature at the concentration of 2.0 mg/ml, respectively. A solution of Cur dissolved in ethanol at 3 mg/ml was used as stock. Firstly, the Ly was dropwise added into the same volume of CMC with vigorous stirring. Then, a small quantity of Cur stock solution was added into the mixtures to achieve Cur concentration of 26.8, 53.6 and 80.4 μM, respectively. The final obtained Cur samples included free Cur, Cur with Ly (Ly–Cur), Cur with CMC (Cur–CMC) and Cur with Ly–CMC coacervates (Ly–Cur–CMC). The concentration of ethanol in the final solutions is negligible. The particle sizes and zeta potentials were determined by using a Zetasizer Nano-ZS (Malvern ZEN 3690, Malvern Instruments).

The Cur samples were centrifuged in ultrafilters with molecular cutting off of 10,000 Da at 4000 r/min in a refrigerated centrifuge (TGL-20000cR) for 20 min. Cur in the percolated solutions was determined using Shimadzu UV-1750 spectrophotometer at 424 nm. The encapsulation efficiency (*EE*) and loading capacity (*LC*) were calculated according to Eqs. (1) and (2):

$$EE = \frac{\text{total weight of Cur} - \text{weight of unloaded Cur}}{\text{total weight of Cur}} \times 100\% \quad (1)$$

$$LC = \frac{\text{total weight of Cur} - \text{weight of unloaded Cur}}{\text{weight of Ly in coacervates}} \times 100\%. \quad (2)$$

2.3. UV–vis absorption and fluorescence measurements

The absorption spectra of Cur samples were acquired by using UV-1750 spectrophotometer at room temperature (25 °C). Steady-state fluorescence measurements were detected by using a fluorescence spectrometer (RF-5301PC) with the same excitation and emission slit width (5 nm) at a voltage of 400 V, 25 °C. The fluorescence spectra of Cur in different samples were analyzed from 450 to 700 nm with the excitation wavelength at 424 nm. The intrinsic fluorescence of Ly was measured from 290 to 450 nm at an excitation wavelength of 280 nm. Synchronous fluorescence spectra of Ly with various concentrations of Cur were measured at $\Delta\lambda = 15$ nm and 60 nm with emission slit width of 5 nm.

For determining the binding constants of Cur with Ly and Ly–CMC coacervates, different concentrations of Cur in the samples were incubated in a water bath for 30 min and detected at 25 °C. The data recorded at the fluorescence maximum of 342 nm were used to estimate the binding constants from Eq. (3) (Zhang et al., 2011).

$$\log\frac{(F_0 - F)}{F} = \log K_b + n \log[\text{Cur}] \quad (3)$$

In the equation, F_0 and F are the fluorescence intensities at 342 nm in the absence and in the presence of various concentrations of Cur, respectively. K_b is the binding constant; n is the number of binding sites and [Cur] is the concentration of Cur.

2.4. Circular dichroism spectroscopy

The circular dichroism (CD) spectra of samples were conducted by using a J-1500 spectropolarimeter (JASCO, Tokyo, Japan). The far-UV CD spectra were measured with nitrogen gas purging, 1 nm bandwidth and 1 s response time as the samples were diluted at an appropriate concentration at 25 °C. An accumulation of three scans with a scan speed of 50 nm/min was performed and the data were collected from 250 to 190 nm.

2.5. Cur protection

The samples of free Cur, Ly–Cur and Ly–Cur–CMC were prepared at two different PBS (pH 6 and pH 7), respectively. For estimating the protection of encapsulated Cur, the samples were pretreated at 60 °C for 30 min and cooled at room temperature. Then the same amount of ethyl alcohol was added to the samples and extracted for 4 h, and evaporated overnight at 40 °C under vacuum. The residual Cur was calculated through absorption at 424 nm (Xu et al., 2014).

The radical-scavenging activity of Cur was examined according to the DPPH method (Yang, Wu, Li, Zhou & Wang, 2013). Briefly, scavenging activity assay was carried out by recording the absorbance of samples containing DPPH at 517 nm in the presence and absence of the remnant Cur at room temperature with a UV–vis spectrophotometer. The antioxidant activity of Cur was assessed by the percentage of DPPH that was decreased in comparison with that of the control condition after 30 min preservation in the dark.

2.6. Statistical analysis

All data were presented as the mean ± standard deviation (SD) and were repeated in at least three independent experiments. Statistical analysis was performed by SPSS 18.0. Comparisons were performed using a two-tailed paired Student's *t* test ($^{*}p < 0.05$, $^{**}p < 0.01$).

3. Results and discussion

3.1. Fabrication of Cur loaded coacervates

The charge of Ly was 12.9 ± 1.1 mV at pH 7.0, and the charges of CMC 0.7, CMC 0.9 and CMC 1.2 were −42.9 ± 7.6 mV, −51.9 ± 2.4 mV and −60.7 ± 2.8 mV, respectively. Therefore, the electrostatic interaction is the main driving force between Ly and CMC. The particle sizes and zeta potentials of Ly–CMC coacervates were not affected after Cur encapsulation (Table 1). The polydispersity indexes (PDI) of the coacervates were all larger than 0.5, indicating that nonuniform complex produced because of the unevenlengths of CMC chains (Zhu et al., 2013). The small particle size and strong negative charges of the coacervates indicated the stability of the solutions.