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Effect of substitution degree on carboxymethylcellulose interaction with lysozyme

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ABSTRACT

Substitution degree (DS) of carboxymethylcellulose (CMC) is one of the critical internal factors for affecting CMC interacting with other polyelectrolytes. The assembly of lysozyme (Ly) and CMC with various DS were analyzed with turbidities, zeta potentials, intrinsic fluorescence, Fourier transform infrared spectra and circular dichroism. It was found that the electrostatic interactions were weakened with decreasing the ratio for Ly and CMC in the Ly-CMC complexes because of the increasing electrostatic repulsion of CMC itself. Electrostatic attractions were also reduced at neutral or alkaline pH and high salt concentration. In addition, electrostatic attractions were much stronger between Ly and CMC with higher DS. CMC with higher DS value can resist the decomposition of Ly-CMC more effectively. Our work provides a new insight for understanding the interactions between proteins and polysaccharides.

1. Introduction

Interactions between proteins and polysaccharides were the focus of research in the last several years for their applications in food and pharmaceutical industries (Souza, Goncóalves, & Gómez, 2011). Among these, one of the most frequent applications was the carrier with proteins/polysaccharides complexes to encapsulate drugs or bioactivators (Cai & Yao, 2013; Cui, Kong, Chen, Zhang, & Hua, 2014; Xu et al., 2014). The interactions mainly arise from intermolecular electrostatic interactions, hydrophobic interactions and hydrogen bonding (McClements, 2006). Among these, the electrostatic interactions were the main acting forces which were highly dependent on the degrees of ionization, the mass ratios of polyelectrolytes, the salt concentrations in the bulk solution, and so on (Zhang & Vardhanabhuti, 2014; Zhang, Hsieh, & Vardhanabhuti, 2014).

Carboxymethylcellulose (CMC), derived from cellulose by etherification of the hydroxyl groups with methylcarboxyl groups, has been widely applied in food and medical industry (Cai et al., 2011; Geng et al., 2014; Gibis, Schuh, & Weiss, 2015; Salama, Shukry, & El-Sakhawy, 2015). In addition, CMC has strong negative charges at pH 2–10 and the charge distribution was closely relevant to the degree of substitution (DS) (Jia et al., 2014). The DS, defined as carboxymethyl groups per repeating unit, is one of the most important characteristics of CMC. Generally, CMC with DS values in the range of 0.7–1.2 possesses good solubility and transparency, which is critical for food applications (Shakun, Heinze, & Radke, 2013).

Lysozyme (Ly), a scarce natural protein with positive charges, is an important natural bactericidal enzyme which was widely distributed in egg white (Koshani, Aminlari, Niakosari, Farahnaky, & Mesbahi, 2015; Li, Xu, Zhang, Chen, & Li, 2015). Furthermore, Ly is often used as food preservative and carrier material for nutraceuticals and medicines (Diarrassouba et al., 2015; Wu et al., 2015).

The complexes of Ly and CMC (Ly-CMC) can be formed at pH 2–10. Therefore, they are ideal materials used in food and biosystem at various pH values. In previous studies, we fabricated Ly-CMC which was successfully applied for drug encapsulation with electrostatic interactions (Li et al., 2015; Li et al., 2015a, 2015b; Zhu et al., 2013). However, the interaction mechanism between Ly and CMC was not intensely researched. It is generally known that the surface charge is one of the most important factors for the interactions between polyelectrolytes. Therefore, the DS of CMC is

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Abbreviation

Ly	lysozyme
CMC	carboxymethylcellulose
DS	degree of substitution
CMC 0.7	carboxymethylcellulose with substitution degree of 0.7
CMC 0.9	carboxymethylcellulose with substitution degree of 0.9
CMC 1.2	carboxymethylcellulose with substitution degree of 1.2
Ly-CMC	the complex of lysozyme and carboxymethylcellulose with ratio of 1:1, if not specially mentioned the ratio.
Ly-CMC 0.7	the complex of lysozyme and carboxymethylcellulose with substitution degree of 0.7 with ratio of 1:1, if not specially mentioned the ratio.
Ly-CMC 0.9	the complex of lysozyme and carboxymethylcellulose with substitution degree of 0.9 with ratio of 1:1, if not specially mentioned the ratio.
Ly-CMC 1.2	the complex of lysozyme and carboxymethylcellulose with substitution degree of 1.2 with ratio of 1:1, if not specially mentioned the ratio.
CD	Circular dichroism
UV	ultraviolet
FTIR	Fourier Transform Infrared Spectroscopy

one of the most important internal factors for CMC interacting with other polyelectrolytes. However, there were scarce study about the interaction mechanism between CMC with different DS and proteins.

In our work, CMC with DS values of 0.7, 0.9 and 1.2 were selected to assembly with Ly. The interactions between Ly and CMC were studied by spectroscopy means. The solution behaviors of the Ly-CMC at various pH and sodium chloride concentrations were also analyzed. Our study enriches the theoretical frame of interactions between proteins and polysaccharides, and provides guidance on the applications of complexes based on natural polyelectrolytes.

2. Materials and methods

2.1. Materials

Lysozyme (Ly) (14.3 kDa) from chicken egg white was purchased from National Medicine Group Chemical Reagent Co., Ltd. Carboxymethyl cellulose (CMC) (250 kDa) with the degree of substitution (DS) of 0.7, 0.9 and 1.2 (represented as CMC 0.7, CMC 0.9 and CMC1.2, respectively) were 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 Ly-CMC solutions

Ly, CMC 0.7, CMC 0.9 and CMC 1.2 were dissolved in phosphate buffer solution (PBS, 0.01 mmol/ml, pH 6.0) for 4 h at room temperature with the concentration of 2.0 mg/ml, respectively. Ly solution was dropwise added into CMC solution with vigorous magnetic stirring. Then the mixtures were continuously stirred for 15 min for well blending. The soluble complexes of Ly-CMC 0.7, Ly-CMC 0.9 and Ly-CMC 1.2 were prepared with fixing the final Ly concentration in the complexes as 1 mg/ml. Ratios of Ly and CMC in the complexes were restricted to 10:3, 5:2, 2:1, 5:3, 10:7, 5:4, 10:9 and 1:1, respectively.

2.3. Turbidities, intrinsic fluorescence intensities and zeta potentials of complexes with various ratios of Ly and CMC

Turbidity measurements were acquired at 600 nm by using a Shimadzu UV-1750 spectrophotometer at room temperature (25 °C). Intrinsic fluorescence intensities of Ly were carried out by using a fluorescence spectrometer (RF-5301PC) from 290 to 450 nm at an excitation wavelength of 280 nm, with the same excitation and emission slit width (5 nm) at a voltage of 400 V, 25 °C. Zeta potentials of Ly-CMC 0.7, Ly-CMC 0.9 and Ly-CMC 1.2 were determined by using a Zetasizer Nano-ZS (Malvern ZEN 3690, Malvern Instruments).

2.4. Circular dichroism spectroscopy

The CD spectra of samples were conducted at 25 °C by using a J-1500 spectropolarimeter (JASCO, Tokyo, Japan). Spectra in the far-UV region (190–250 nm) were determined with the Ly

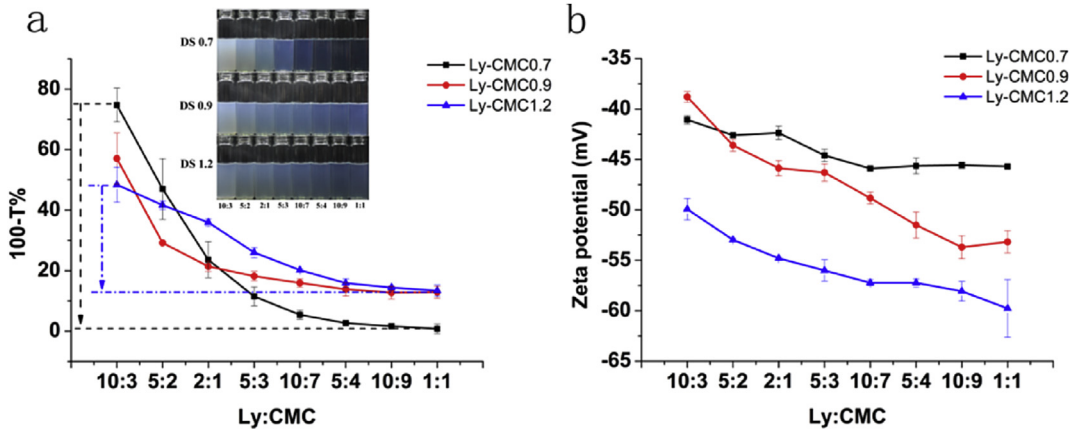

Fig. 1. a) Turbidities of Ly-CMC 0.7, Ly-CMC 0.9 and Ly-CMC 1.2 (Insert was corresponding digital picture of Ly-CMC); b) zeta potentials of Ly-CMC 0.7, Ly-CMC 0.9 and Ly-CMC 1.2, as a function of Ly and CMC ratios (Ly concentration fixed with 1×10^3 mg/L).