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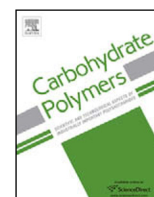

Controlled uptake and release of lysozyme from glycerol diglycidyl ether cross-linked oxidized starch microgel

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ABSTRACT

A biodegradable microgel system based on glycerol-1,3-diglycidyl ether (GDGE) cross-linked TEMPO-oxidized potato starch polymers was developed for controlled uptake and release of proteins. A series of microgels were prepared with a wide range of charge density and cross-link density. We found both swelling capacity (SW_w) and lysozyme uptake at saturation (Γ_{sat}) increased with increasing degree of oxidation (DO) and decreasing cross-link density. Microgel of DO100% with a low cross-link density ($R_{GDGE/polymer\ (w/w)}$ of 0.025) was selected to be the optimum gel type for lysozyme absorption; Γ_{sat} increased with increasing pH and decreasing ionic strength. It suggests that the binding strength was the strongest at high pH and low ionic strength, which was recognized as the optimum absorption conditions. The lysozyme release was promoted at low pH and high ionic strength, which were considered to be the most suitable conditions for triggering protein release. These results may provide useful information for the controlled uptake and release of proteins by oxidized starch microgels.

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1. Introduction

Microgels have been of great interest to scientists in the past few decades because of their favorable biocompatible properties and sensitivity to external stimuli, such as temperature (Drapala et al., 2014), pH (Koetting & Peppas, 2014), light (Schesny et al., 2014), ionic strength (Li, Norde, & Kleijn, 2012), and applied electric or magnetic fields (Bardajee & Hooshyar, 2013; Gonzalez et al., 2014; Guilherme et al., 2012). Microgels can be applied in tissue repairing (Lau & Wang, 2013), drug delivery systems (Singh, Bala, & Chauhan, 2008), biosensors (Liu et al., 2015), chromatography (Qu et al., 2014) and medical devices (Li et al., 2014). The three-dimensional network of microgels allows a controlled uptake and release of functional ingredients, bioactive compounds and drugs for various purposes (Lynam et al., 2014).

Both synthetic polymers, such as poly(acrylic acid) (PAA) (Bysell & Malmsten, 2006), poly(ether-urethane) (PEU) (Li et al., 2014), poly(ethylene glycol) (PEG) (Tomic, Veeman, Boerakker, Litvinov, & Dias, 2008) and natural polymers, such as DNA (Costa, Valente, Pais, Miguel, & Lindman, 2010), chitosan (Zamora-Mora, Velasco, Hernandez, Mijangos, & Kumacheva, 2014), starch (Mehyar, Liu, & Han, 2008), cellulose (Grznarova, Yu, Stefuca, & Polakovic, 2005; Spagnol et al., 2012), hyaluronic acid (Widjaja et al., 2014), dextran (Imren, Gümüsderelioglu, & Güner, 2010), and Konjac glucomannan (Chen et al., 2014; Shahbuddin, MacNeil, & Rimmer, 2012), can be employed to prepare functional microgels. Microgels based on natural polymers have attracted considerable attention in biomedical applications because of their biocompatibility and biodegradability (Li et al., 2009). In order to protect the active components and release them at the targeted place where required, the development of effective encapsulation systems consisting of natural polymers has become an important issue.

Previously, we have developed a microgel carrier system based on TEMPO-oxidized potato starch. It is a microgel system consisting of a sodium trimetaphosphate (STMP) cross-linked oxidized starch polymer (Li et al., 2010, 2009). These microgels have unique advantages such as well-controlled charge density and cross-linking density. Since these microgels are sensitive to external conditions, e.g., ionic strength and pH, the absorption and release of active components inside the gels can be controlled by environmental conditions. The negatively charged starch microgel can absorb positively charged functional ingredients such as proteins (Li et al., 2011a, 2011b) and anthocyanins (Wang, Li, Chen, Xin, & Yuan, 2013) through electrostatic interactions. Additionally, release of the active ingredients can be triggered under certain conditions, e.g., high pH, high salt concentrations and enzymatic attack (Li

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et al., 2011a, 2011b). In this study, we developed a new microgel system based on glycerol diglycidyl ether (GDGE) cross-linked oxidized starch polymers. A series of microgels with a wide range of charge density and cross-link density were prepared.

Many drugs are proteins or polypeptides, such as insulin, and they may be degraded by proteases in the body before they arrive at the target site (Hosseininasab et al., 2014). Therefore, such proteins need a carrier system to protect them and deliver them to the targeted areas. We chose lysozyme as a model protein to study its uptake and release behavior in GDGE cross-linked starch microgels. Positively charged lysozyme can be absorbed by negatively charged starch microgels via electrostatic interactions. The pH and ionic strength may greatly affect protein absorption on a microgel by tuning the electrostatic interaction between them. Many studies have focused on the protein absorption capacity as a function of pH and ionic strength in order to determine the optimum absorption conditions. Cai, Bakowsky, Rytting, Schaper, and Kissel (2008) found that increased pH facilitated the adsorption of lysozyme by poly(lactide-co-glycolide) (PLGA) microspheres. Loading efficiency decreased with increasing ionic strength above the optimum salt concentration of 50 mM.

In this study, we first characterized the microgels with respect to their surface morphology, size distribution and swelling capacity. Next, the protein uptake capacity was measured as a function of the charge density and cross-link density, with the aim of selecting the optimal gels for encapsulation applications. Then, the protein uptake capacity was examined as a function of pH and ionic strength, in order to determine the optimum absorption conditions. Finally, the percentage of protein release was investigated at various pH levels and ionic strengths to assess the release behavior upon different conditions. The influence of these factors on protein uptake and release provided us with useful information for applying these microgel systems in biomedical applications.

2. Materials and methods

2.1. Materials

Potato starch polymer was kindly offered by AVEBE, The Netherlands. The oxidation catalyst 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) and the globular protein lysozyme (from chicken egg white, Mw = 14,400 g/mol, activity>20,000 μm/mg) were obtained from Sigma–Aldrich, USA. The cross-linking agent glycerol-1,3-diglycidyl ether (GDGE) was provided by Amresco, USA. Purified Milli-Q water was used throughout. All other chemicals used were of analytical grade.

2.2. Methods

2.2.1. The preparation of oxidized starch microgel

The primary alcohol groups at the C-6 position on potato starch polymer were selectively oxidized into carboxyl groups by TEMPO-mediated oxidation. In this way, the oxidized starch of various degree of oxidation (DO) 30%, 50%, 70%, 100% were prepared, following the procedure developed by de Nooy (1997). The DO was controlled by the amount of sodium hypochlorite added during oxidation. The accurate DO was determined in our previous study by proton titration method (Li et al., 2009).

Microgel were prepared by cross-linking the oxidized starch polymer with GDGE at pH 10. According to literature, diepoxy GDGE are more likely to react with carboxyl groups under basic condition than the hydroxyl groups on polymer (Fleisher & Vigh, 2005). So that in our case, most likely that major carboxyl groups and minor hydroxyl groups participated in the cross-link reaction. GDGE forms intermolecular linkage between two different starch polymers. First, 12 g potato starch polymers were dissolved in 80 mL distilled water at room temperature. Then 1 M NaOH was added into the polymer solution to adjust the pH into pH 10. Thereafter, the cross-linker GDGE was added into the polymer solution and the mixture was heated at 40 °C for 10 min. Then the preformed hydrogels were put in an oven at 40 °C for 72 h for further cross-linking. The weight ratio of cross-linker to starch polymer ($R_{cross\text{-}linker/polymer}$) were 0.025, 0.04, 0.05, and 0.065. After the hydrogels were formed, the whole piece of gel was grinded, and the grinded gel pieces were passed through a sieve (mesh size: 1 mm) covered with a nylon cloth of 200 mesh (mesh size: 0.074 mm), in order to obtain reasonably uniform microgel particles. The gel particles were washed three times with distilled water using the nylon covered above a sieve again to remove the salts. Thereafter, the hydrogel particles were washed three times again with 100% ethanol to remove water and three times with 100% acetone to wash out ethanol and last traces of water. Finally, the hydrogel particles were dried in oven at 40 °C overnight. The dried microgel powder was again grinded by a glass mortar to achieve small and homogenous particles.

2.2.2. The size distribution of microgel particles

The size distribution of microgel particle in aqueous suspension was measured using a Malvern Mastersizer 2000 (Malvern, UK). In the laser diffraction measurement, particles pass through the focused laser beam and scattered light at an angle that is inversely proportional to their diameter. A dual wavelength detection system (blue light combined with red light) was used to promote detective performance and sensitivity. Before the measurements, the microgel powder was dissolved in distilled water for at least 4 h to allow equilibrium. Then the gel suspensions were sonicated for 10 min to get finely dispersed gel particles. The gel particles were diluted 1:100 to avoid multiple scattering effects. Gel particles diameter was estimated by the average of three measurements.

2.2.3. Scanning electron microscopy

The surface morphology of microgel particles was examined by scanning electron microscopy (SEM) using SEM (S-4700, Hitachi Company, Japan) with an accelerating voltage of 15 kV. Microgel particles were freeze dried by lyophilizing under vacuum (Dura-Dry TMMP, USA). Then microgel particles were sputter-coated with gold prior to observation.

2.2.4. Weight swelling capacity (FSC) measurements

A known weight of dried microgel powder (around 0.01 g) was immersed in an excess of distilled water to equilibrium swelling at room temperature. The swollen microgels of various DO and cross-link densities were immersed in the distilled water for 2 h to reach equilibrium. Then the excess water was wiped off with papers until there were no visible water droplets, and the swollen gel cannot flow freely. Swelling ratio (SW_w) is defined as the weight ratio of the swollen gel to the dried gel:

$$SW_w = \frac{W_{\text{swollen-gel}}}{W_{\text{dry-gel}}} \quad (1)$$

2.2.5. Zeta potential measurements

Zeta potential of microgel particles in buffer of various pH and ionic strength was determined using a Malvern Nanosizer ZS2000 (Malvern, UK). The instrument measures the direction and velocity of a macromolecule or particle in an applied electrical field via phase analysis light scattering and laser Doppler velocimetry. The calculated electrophoretic mobility is converted into zeta potential values using the Smoluchowski model. The temperature of all samples was controlled at 25 °C. Before the measurements, the microgel suspensions were sonicated for 15 min, in order to attain

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