Crosslinked polyion complex micelles based on biopolymers for colon-targeted drug delivery

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INTRODUCTION

Nanoparticulate drug delivery systems for oral application is a promising approach in the therapy of inflammatory bowel disease because specific uptake of nanoparticles by immune-related cells in inflamed barriers offers selective drug targeting to the inflamed tissue (Collnot 2012). Polyion complex (PIC) micelles are termed as the self-assembling nanoparticles which are formed through electrostatic interaction as the main driving force and have a core-shell structure with a core consisting of the polyion complexes and a shell consisting of the neutral segments (Lee 2009). Up to date, a lot of synthetic PIC micelles have been reported and those findings indicate that the PIC micelles can be attractive candidates for delivery of drugs, protein, DNA to specific sites. However, when using the PIC micelles to encapsulate biopharmaceuticals, there are a number of potential difficulties. Since electrostatic interaction is the main driving force of complexation, pH and ionic strength influence the stability of the micelles. Especially, stability is highly desirable for colon-targeted oral delivery because of the harsh conditions in the gastrointestinal tract.

On the other hand, the generally recognized safe (GRAS) biopolymers such as chitosan, dextran, beta-lactoglobulin are widely used as carrier matrix. The electrostatic attraction between the cationic chitosan and the anionic beta-lactoglobulin has been identified around pH 5~6 (Souza 2011). Previously, we developed chitosan/beta-lactoglobulin core-shell nanoparticles for the oral administration of nutraceuticals (Chen 2005). However, the beta-lactoglobulin shells of the nanoparticles could be degraded by digestive enzyme when transferred to simulated intestinal conditions. Dextran is non-ionic and known for its potential as colon-specific delivery systems via the oral route.

In this work, we first conjugated beta-lactoglobulin and dextran through Maillard reaction, then induced chitosan gelation using sodium tripolyphosphate (TPP) in the presence of the conjugates leading to formation of PIC micelles, and finally obtained the crosslinked PIC micelles by adding crosslinker (such as glutaraldehyde) into the PIC micelles (Scheme 1).

MATERIALS AND METHODS

Chitosan and dextran were purchased from Sigma. Beta-lactoglobulin was donated by Davisco Foods International (Le Sueur, MN, USA).

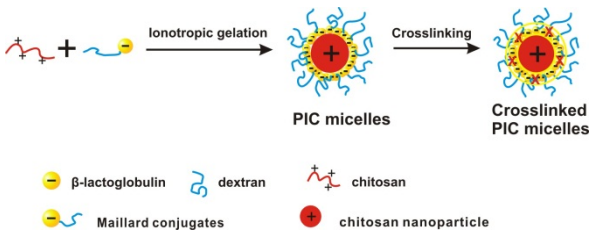
The beta-lactoglobulin-dextran Maillard conjugates were prepared by dry-heating procedure.

The PIC micelles were prepared by ionotropic gelation. Briefly, chitosan and the conjugates were dissolved and mixed together. After mixing, TPP solution was added dropwise, opalescent suspension was formed spontaneously. Finally, the obtained PIC micelles were crosslinked by adding with a small amount of glutaraldehyde and incubating overnight at room temperature.

In vitro digestibility tests were performed using the method by Lesmes and McClements (Lesmes 2012). Briefly, nanoparticles was added to equal amounts of simulated USP gastric and intestinal fluids (SGF and SIF) in the presence or absence of the digestive enzymes and incubated for up to 4 h under stirring at 37 ^{o}C.

Zetasizer NanoZS from Malvern instrument was used to characterize the Z-average diameter (D_h) and zeta-potential of the nanoparticles.

RESULTS AND DISCUSSION



Scheme 1: Preparation of the crosslinked PIC micelles.

In respect to colon-targeted oral drug delivery, factors such as proteolysis, highly acid condition and transit time influence the stability properties of the drug carriers. As shown in Scheme 1, chitosan is chosen as the reservoir of drugs, beta-lactoglobulin acts as the linker between the chitosan and dextran, while dextran plays a role as stabilizing agent against the proteolytic activity. Further crosslinking protects the PIC micelles from dilution and dissociation under high ionic strength and varied pH range.

The obtained crosslinked PIC micelles are of 120 nm in D_h. The core-shell structure of the crosslinked PIC micelles is verified by the zeta–potential measurements. The zeta–potential values of the obtained crosslinked PIC micelles are close to zero in

a wide pH range, in contrast with the positive zeta–potential values of chitosan and the absolutely higher zeta–potential values of the Maillard conjugates (Figure 1). Zeta-potential measurements further indicated the existence of electrostatic interactions between the conjugates and chitosan around pH 5~6.

We also found that the PIC micelles are quite stable over the studied pH range and no visible precipitate is observed while chitosan nanoparticles become unstable when pH is above 7.0. The result suggests that the colloidal stability of the micelles is not from the electrostatic repulsion but attributed to the steric repulsion of the conjugated dextran.

In addition, due to the crosslinking, the PIC micelles are quite stable when exposure to simulated gastrointestinal conditions. As seen in Figure 2, the size of the micelles has a little change in the presence/absence of the enzyme during the in vitro digestibility test.

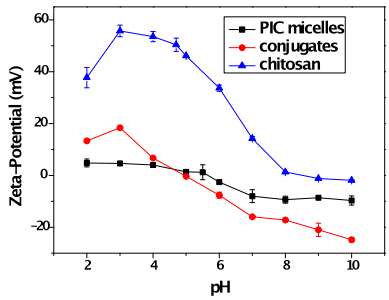


Figure 1: Zeta-potential of the PIC micelles, the conjugates, and chitosan under different pHs

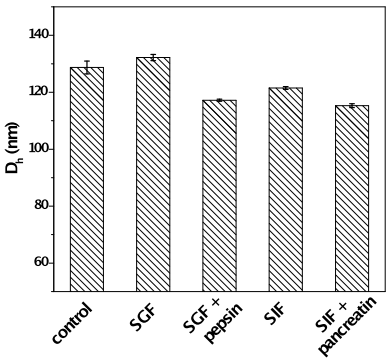


Figure 2: D_h of the nanoparticles when exposure to simulated gastrointestinal conditions.

CONCLUSIONS

Crosslinked polyion complex micelles based on biopolymers were successfully prepared. Zeta-potential measurements confirmed the micelles have a core-shell structure with a core consisting of the beta-lactoglobulin/chitosan polyion complexes and a shell consisting of the neutral dextran conjugated with beta-lactoglobulin. The crosslinked PIC micelles are stable against the harsh acidic environment and against the enzymatic digestion under gastrointestinal tract, thus showing as promising colon-targeted drug delivery systems.

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