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An innovative method for preparation of hydrophobic ion-pairing colistin entrapped poly(lactic acid) nanoparticles: Loading and release mechanism study

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ABSTRACT

Hydrophobic ion-pairing (HIP) complexation has emerged as an efficient approach to enhance the entrapment of therapeutic peptides in the biodegradable polymer matrix. In the present study, we developed an innovative extraction method for preparation of HIP-colistin (CST, a polycationic peptide) using various water-insoluble anionic lipids. To determine the loading mechanism of HIP-CST entrapped poly(lactic acid) (PLA) nanoparticles (HIP-CST-PLA-NPs), the effects of anionic lipids and PLA molecular weight (Mw) on the unentrapped fraction (*uf*) of CST in PLA-NPs were investigated. And CST release mechanism from HIP-CST-PLA-NPs was also investigated by evaluating their release behavior and NP swelling. It is showed that HIP-CST retention in the PLA-NPs was imposed by their physical localization in the networks of the PLA chains, rather than the electrostatic attraction between anionic lipid and CST in serum. And HIP-CST-PLA-NPs in serum exhibited the swelling-controlled release behavior with a substantially accelerated release and NP swelling observed in comparison with that in phosphate buffer. Our results can effectively guide the preparation of biodegradable polymer based modified drug release systems with desired properties for peptides delivery.

1. Introduction

The increasing number of new therapeutic peptides and proteins has been developed as a consequence of the progress of biotechnological techniques and genetic engineering (Orive et al., 2003, Löfblom et al., 2010, Stryjewska et al., 2013). However, their clinical application is often hampered by a number of obstacles, such as poor protein stability and short in vivo half-lives (Almeida and Souto, 2007, Martins et al., 2007). One approach to overcome these problems would be the entrapment of these drugs into a modified drug release systems fabricated from biocompatible and biodegradable polymers, such as poly(lactic acid) (PLA) and its copolymers with poly(glycolic acid) (Anderson and Shive, 2012). However, one of the main problems of these techniques is the poor entrapment of water-soluble peptides or protein, mainly with those that involve a W/O/W emulsification, due to the partition of drugs from inner water phase into the continuous aqueous phase (Quintanar-Guerrero et al., 1997). In this regard, the hydrophobic ion-pairing (HIP) complexation has emerged as an efficient approach to enhance the entrapment of therapeutic peptides (Yang et al., 2009, Patel et al., 2014). The HIP complex is formed driven by electrostatic interaction between ionizable groups of peptides and the opposite charged hydrophobic counterion. The complex shows enhanced hydrophobicity without modification of their chemical structures and is able to partition readily into polymers matrix during the encapsulation process. Therefore, HIP complexation greatly increases the encapsulation efficiency of peptides in the hydrophobic polymer matrix. On the other hand, the HIP complex is reversible in nature and can easily dissociate in the presence of excess of oppositely charged ions (Agresti et al., 2008). This approach has been applied for delivery of various peptides and proteins in the biodegradable hydrophobic polymers (Yang et al., 2009, Patel et al., 2014).

HIP complexes of peptides are usually formed by a process termed precipitation ion-pairing, where the water soluble counterion (e.g. SDS) and peptides are interacted each other and precipitated in the aqueous medium (Yang et al., 2009, Patel et al., 2014). Therefore, water-insoluble counterions (like hydrophobic anionic lipids used in this study, Fig. 1) is not suitable for this method. In this regard, in this study we developed an innovative method to prepare the HIP-peptide based on the Bligh and Dyer extraction (Stuart and Allen, 2000), enabling the formation of HIP complex of water soluble peptides and various highly water-insoluble anionic lipids (Fig. 1). Colistin (CST, molecular weight (Mw) 1155.4), a polycationic antimicrobial peptide (Fig. 1), was chosen as the model peptide. HIP-CST was prepared and

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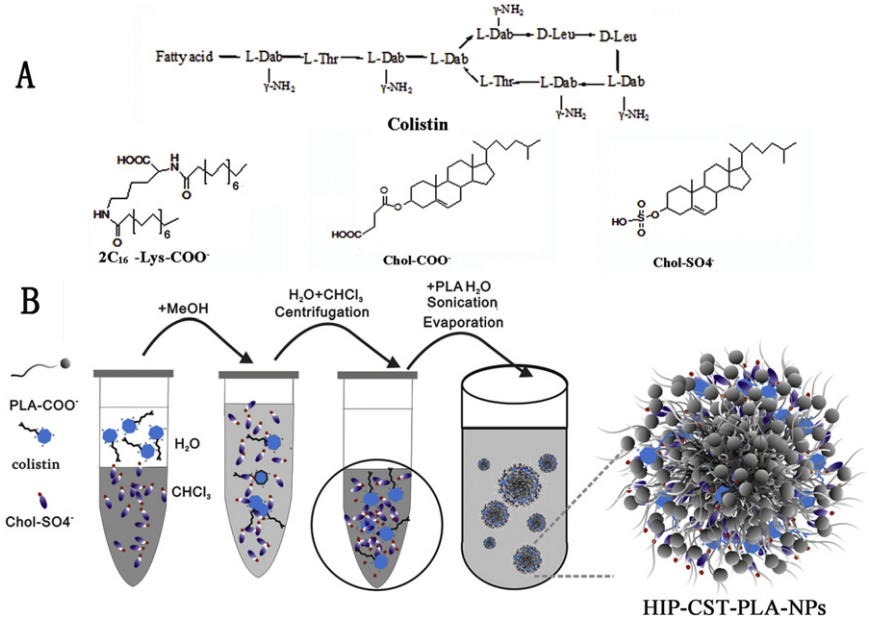

Fig. 1. (A) Chemical structures of the CST and anionic lipids including $2C_{16}$-Lys-COO^-, Chol-COO^- and Chol-$SO4^-$. (B) Schematic illustration of the preparation of HIP-CST-PLA-NPs.

subsequently entrapped into PLA nanoparticles (PLA-NPs) using an emulsification-evaporation method.

It is believed that the retention of peptides (generally having a high Mw, approximately 1100–55,000) in the PLA matrix is mainly ascribed to their physical localization in the networks of the PLA chains. The rate of the removing of physical localization depends on the infiltration of water and the degradation of polymer chains (He et al., 2015), which further determines the release rate of peptides from the PLA matrix. However, HIP-peptides are entrapped both electrostatically and physically in the polymer matrix, and the strength of electrostatic interaction is also significantly influenced by the ions or other oppositely charged components in solution (Li and Yang, 2015). Therefore, it is also very important to investigate the factors affecting the peptide retention in the HIP-peptides entrapped PLA matrix, especially in a physiologically relevant medium where various ions and proteins exist. To this end, we examined the effects of anionic lipids, PLA Mw and hydrophilic stabilizer on the drug retention of HIP-CST entrapped PLA-NPs (HIP-CST-PLA-NPs), thus to determine their loading mechanism of HIP-peptide in the PLA matrix. Another purpose of this study was to investigate CST release mechanism from HIP-CST-PLA-NPs in a physiologically relevant media (serum) by evaluating their drug release behavior and NP swelling.

2. Material and Methods

2.1. Material

The mPEG-PLA and uncapped PLA with various Mws were purchased from Daigang Biomaterial Co., Ltd. (China). CST sulfate was purchased from MEILUN Biology Technology Co., LTD. (Dalian, China). Poly (vinyl alcohol) (PVA, Mw 13,000–23,000, 87–89% hydrolyzed) was obtained from Sigma-Aldrich, and the sodium cholesteryl sulfate (Chol-$SO4^-$) was purchased from Sinopharm Chemical Reagent Co., LTD. (China). *N*,*N*-Dipalmitoyl-L-lysine ($2C_{16}$-Lys-COO^-) and cholesteryl hemisuccinate (Chol-COO^-) were synthesized in our laboratory and verified by ^{1}H NMR (Li et al., 2016). All other chemicals were analytical reagent grade and used without further purification.

2.2. Preparation of HIP-CST

HIP-CST was prepared by the Bligh and Dyer extraction using anionic lipids with various structures, including $2C_{16}$-Lys-COO^-, Chol-COO^- and Chol-$SO4^-$ (Fig. 1(B)). Briefly, 13.0 mg of CST sulfate was dissolved in the 0.4 ml of distilled water (ddH_2O). Anionic lipid (at 1.2:1 -/+ charge ratios, CST containing 5 positive charges) was dissolved in the 0.5 ml $CHCl_3$ and 1 ml MeOH. The two solutions were subsequently mixed to form the Bligh and Dyer monophase, enabling the electrostatic interaction between CST and anionic lipids (Stuart and Allen, 2000). Specially, 50 μl of 1 M NaOH was added for deprotonation of $2C_{16}$-Lys-COOH and Chol-COOH. Following 30 min at room temperature, 0.5 ml of $CHCl_3$ and 0.5 ml of ddH_2O were added, centrifuged at 2500 rpm for 15 min to obtain the HIP-CST $CHCl_3$ solution.

2.3. Preparation of HIP-CST-PLA-NPs and HIP-CST-NPs

HIP-CST-PLA-NPs were prepared by an emulsification-evaporation method. Brifely, HIP-CST $CHCl_3$ solution (100 μl, containing 1.2 mg CST) was mixed with PLA (Mw, 2500, 5000, and 50,000) $CHCl_3$ solution (100 μl, containing 25 mg PLA) and transferred into 2 ml ddH_2O containing the 10 mg stabilizer (i.e. PVA or mPEG-PLA). The mixture was sonicated under cooling to form the O/W emulsion using a probe sonicator (Jy92-2D, Scientz, China), and then the organic solvent was evaporated to form the HIP-CST-PLA-NPs. HIP-CST-NPs were prepared by the same procedure as described above without the presence of PLA.

2.4. Evaluating Strength of Electrostatic Interactions by Differential Scanning Calorimetry (DSC)

DSC was used to evaluate the strength of electrostatic interactions between the CST and anionic lipids by detecting the peak shifts and the presence/absence of specific peaks. DSC curves were performed on a calorimeter (STAR system, METTLER, Switzerland) under nitrogen atmosphere with flow rate of 50 ml/min, temperature range from 5 to 300 °C at a heating rate of 10 °C/min.

2.5. Size and Zeta Potential Analysis

The mean diameter and zeta potential of various versions of NPs were measured using a dynamic light scattering (DLS) instrument (Nano-ZS90, Malvern, England) at 25 °C. NPs were diluted by the ddH_2O before the measurement to adjust scattering light intensity to an acceptable level for measurement.