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A poly(L-lysine)-based hydrophilic star block co-polymer as a protein nanocarrier with facile encapsulation and pH-responsive release

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ABSTRACT

A hydrophilic star block co-polymer was synthesized, characterized, and evaluated as a protein nanocarrier. The star block co-polymer was composed of a hyperbranched polyethylenimine (PEI) core, a poly(L-lysine) (PLL) inner shell, and a poly(ethylene glycol) (PEG) outer shell. The model protein insulin can be rapidly and efficiently encapsulated by the synthesized polymer in aqueous phosphate buffer at physiological pH. Complexation between PEI–PLL–*b*-PEG and insulin was investigated using native polyacrylamide gel electrophoresis. The uptake of enhanced green fluorescent protein into Ad293 cells mediated by PEI–PLL–*b*-PEG was also investigated. The encapsulated insulin demonstrated sustained release at physiological pH and showed accelerated release when the pH was decreased. The insulin released from the star block co-polymer retained its chemical integrity and immunogenicity.

1. Introduction

Protein drugs have emerged as potent medicines for various types of human diseases and have drawn significant interest due to their high specificity and activity at relatively low concentrations in comparison with small chemical drugs [1]. However, the use of protein-based biotherapeutics faces several challenges, such as their rapid elimination from the circulatory system, poor bioavailability, low cell permeability, and inefficient endosomal escape. The future success of proteins as therapeutic agents is critically dependent on the development of appropriate delivery systems. An ideal protein carrier should have a reasonably high protein encapsulation efficiency and loading capacity, and sustained release of the loaded protein while retaining bioactivity. In recent decades hydrogels [2–5], micro- and nano-particles [6–10], liposomes [11], polymersomes [12,13], polyelectrolyte microcapsules [14–17], and polyion complex micelles [18,19] have been investigated as carriers for controlled protein delivery. While many of these delivery approaches have shown the advantages of high loading capacities and stimuli-responsive release properties, some of them result in disassembly of the carriers under dilute conditions and/or require the use of organic solvents that may lead to protein denaturation. For example, the liposomal system is mechanically unstable in the infinitely dilute environment encountered after introduction into the bloodstream; disruption of the micellar structure leads to burst release of the entrapped protein molecules. The use of hydrophobic polymers and organic solvents involved in the microencapsulation procedure (e.g. the double emulsion–solvent extraction/evaporation method) in microparticle carrier systems frequently leads to the denaturation of proteins. A facile encapsulation of proteins under hydrophilic conditions with retained physical and chemical stability remains largely unexplored.

Here we report a hydrophilic star block co-polymer (PEI–PLL–*b*-PEG) with a hyperbranched polyethylenimine (PEI) core, a poly(L-lysine) (PLL) inner shell, and a poly(ethylene glycol) (PEG) outer shell (structure shown in Fig. 1) as a potential unimolecular protein nanocarrier. PEG is employed as the outer shell of the star block co-polymer owing to its good water solubility, high degree of biocompatibility, and prolonged circulation time in the blood. The star block co-polymer contains a biodegradable PLL inner shell. Therefore, at physiological pH, at which the PLL inner shell is positively charged while most proteins carry negative charges, these macromolecules could encapsulate protein molecules in aqueous conditions via electrostatic interactions. Furthermore, when the pH is decreased below the pI of the protein charge switching of the protein molecules may lead to their accelerated release from the star block co-polymer.

2. Materials and methods

2.1. Materials

Hyperbranched PEI (M_w 10 kDa) and poly(ethylene glycol) monomethylether (PEG) (M_w 2 kDa) were obtained from

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