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Preparation and properties of pH-responsive, self-assembled colloidal nanoparticles from guanidine-containing polypeptide and chitosan for antibiotic delivery

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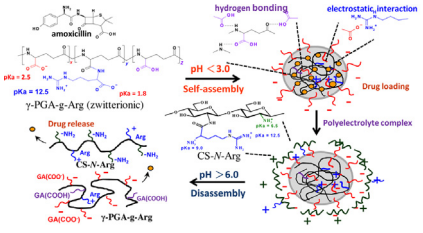
HIGHLIGHTS

- synthesis of guanidine-containing polypeptide and chitosan.
- preparation of CS-*N*-Arg/γ-PGA-*g*-Arg complex nanoparticles.
- amoxicillin release was slow at pH 2.5 and quick at pH 7.0.
- a superior antibacterial activity against the growth of *H. pylori*.

GRAPHICAL ABSTRACT



pH-responsive property of CS-*N*-Arg/γ-PGA-g-Arg complex nanoparticles

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ABSTRACT

Amoxicillin is a traditional antibiotic used to treat *Helicobacter pylori* (*H. pylori*). However, the clinical applicability was limited by low local concentrations of amoxicillin that are reached at the sites of *H. pylori* infection. In this study, a pH-sensitive, guanidine-containing polypeptide composed of poly(γ-glutamic acid) (γ-PGA) and arginine (Arg) were synthesized. The γ-PGA-*g*-Arg polypeptide can self-assemble into colloidal nanoparticles at pH lower than 3.0, and the morphological changes are reversibly switched by elevating the pH of the colloidal suspension. The chemo–physical properties of the γ-PGA-*g*-Arg polypeptide were investigated by proton nuclear magnetic resonance (^{1}H NMR), X-ray diffraction (XRD), and Fourier transform infrared (FTIR) spectroscopy. The γ-PGA-*g*-Arg colloidal nanoparticles were modified with a guanidinylated polymer, the chitosan (CS)-arginine(Ag) conjugate (CS-*N*-Arg). The effect of electrostatic complexation between γ-PGA-*g*-Arg polypeptide and CS-*N*-Arg conjugate extends the stable range of the self-assembled nanoparticles to a higher pH (pH > 6.0), and the surface charge density changes from negative to positive. The morphological changes of the CS-*N*-Arg/γ-PGA-*g*-Arg complex nanoparticles in response to environmental pH were investigated by dynamic light scattering (DLS) and transmission electron microscopy (TEM). Amoxicillin release from the CS-*N*-Arg/γ-PGA-*g*-Arg NPs was reduced at pH 2.5 (gastric fluid, fasted state) and 4.5 (the gastric mucosal surface), but the antibiotic released rapidly from the nanoparticles at pH 7.0 (the sites of *H. pylori* infection).

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The amoxicillin-loaded CS-*N*-Arg/γ-PGA-*g*-Arg complex nanoparticles showed a superior antibacterial activity against the growth of *H. pylori*.

1. Introduction

During the past few decades, a lot of research has focused on the development of stimulus–responsive drug delivery systems, particularly for those that can significantly change their drug release rates in response to pH variation [1–5]. It has been known that variations in pH occurred at several body sites and cellular compartments, including gastrointestinal (GI) tract, vagina and tumor microenvironment. The pH-responsive polymers composed of polymeric backbones with ionic pendant groups can either accept or donate a proton, depending on pH and ionic strength of the media which the polymer is exposed to. Altering the pH of the solution will generate electrostatic repulsive forces responsible for swelling or deswelling of the pH-responsive polymer-based carriers, consequently, the rate of release of encapsulated solutes are pH sensitive. Various pH-responsive carriers have been used for delivery of drugs, genes, peptides and proteins [6–11].

Polypeptides are promising materials for pharmaceutical and biomedical applications because they are biodegradable, biocompatible, and can assemble into different structures [12,13]. Polypeptides have received considerable attention as useful biomaterials for diverse applications including gene and drug delivery [14–18], and tissue engineering [19–23]. The amide bonds in the peptide backbone can act as a donor and an acceptor of hydrogen bonds. Modification of polypeptides with acidic amino acids (glutamate and aspartate) or basic amino acids (lysine, arginine, and histidine) allows for the design of zwitterionic polypeptides for a variety of biological functions [24–26]. Moreover, zwitterionic polypeptides have been used for controlling release of doxorubicin in cancer cells [27].

Arginine, the most basic amino acid, has a positively charged guanidino group. The guanidine group with a pKa of 12.48 exhibits strong basicity over a wide pH range. The positively charged terminal guanidinium group (the guanidinium cation) of the arginine side chain is involved in the antibacterial action of several antimicrobial peptides, which displayed an enhanced affinity for specifically targeting the negatively charged bacterial membranes [28]. Chemical modification of polymers with guanidino groups has attracted increasing interest due to the excellent antibacterial activity of the guanidinylated compounds, and the activities are not affected by antimicrobial resistance [29–32]. The guanidinylated polymers are a class of cationic antimicrobial polymers which attacks a wide range of gram-positive and gram-negative bacteria through electrostatic attraction between the guanidinium cation and the anionic cell surface of bacteria [33]. Moreover, guanidinylated polymers have been used for the development of effective gene delivery systems [34,35].

Helicobacter pylori (*H. pylori*) is a human-specific gram-negative pathogen which colonizes in the deep mucus layer near the stomach epithelium. Infection with *H. pylori* causes chronic inflammation, which is related to the development of gastric ulcer and cancer. Amoxicillin is a traditionally used antibiotic for *H. pylori* eradication. However, the use of the drug in clinical practice is limited by lack of enough residence time to maintain the effective concentration of the antibacterial agent in gastric mucosa. The pH of the gastric mucosa is possibly elevated to higher than 5.0 due to neutralization of gastric juices with ammonia and bicarbonate produced from urea hydrolyzed by *H. pylori* urease [36]. Thus, the pH-sensitive polymers may be used as potential materials for application in the field of gastric mucosal drug delivery because of the existence of the pH gradient between the interior and exterior of gastric mucosa.

In this work, we report the combination of the cationic arginine and an anionic polypeptide, poly(γ-glutamic acid), to obtain a pH-responsive, zwitterionic polypeptide, poly(γ-glutamic acid)-*g*-arginine (γ-PGA-*g*-Arg). The γ-PGA-*g*-Arg polypeptide was able to self-assemble into nanoparticles in simulated gastric fluid, but was capable of swelling and subsequently disassembled in the medium having a pH greater than 3.5. The pH-triggered assembly and disassembly of γ-PGA-*g*-Arg polypeptide due to the establishment and disruption of hydrogen bonding and electrostatic interactions were examined in this study. Chitosan (CS) offers promising advantages as oral drug delivery systems, such as antibacterial and mucoadhesive properties. Previous studies showed that CS-*N*-Arg conjugate was a potential cationic polymer which could be used for delivery of protein and a near-infrared fluorescence imaging dye [18,37]. Furthermore, positively charged, CS-based NPs can interact with the cell wall of *H. pylori*, and then target the site of *H. pylori* infection [36]. The effects of electrostatic complexation between γ-PGA-*g*-Arg polypeptide and CS-*N*-Arg conjugate extend the stable range of the self-assembled nanoparticles to a higher pH (pH > 6.0). The purpose of this study is to develop a pH-sensitive nanocarrier that shows a slow release of amoxicillin in gastric juice (pH 2.5, fasted state), but fast drug release in the gastric mucosa infected by *H. pylori* (pH 5.0–7.0). Moreover, antimicrobial activity of amoxicillin-loaded nanoparticles prepared from γ-PGA-*g*-Arg polypeptide and CS-*N*-Arg was evaluated. The pH-triggered disassembly of the colloidal nanoparticles, and their corresponding amoxicillin releases from the nanocarriers are shown in Scheme 1.

2. Experimental

2.1. Materials

Brain heart infusion (BHI) broth and agar for *Helicobacter pylori* culture were purchased from Difco Inc. (Detroit, MI). Arginine (Arg), amoxicillin, 1-ethyl-3-(3-(dimethylamino) propyl) carbodiimide hydrochloride (EDC), 3-(4,5-cimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and *N*-Hydroxysuccinimide (NHS) were purchased from Sigma–Aldrich (St Louis, MO). Other chemicals were of reagent grade.

2.2. Synthesis of CS-N-Arg and γ-PGA-Arg conjugates

The CS-*N*-Arg conjugate was synthesized through a reaction between CS (Mw 60 kDa and 85% DD, Koyo, Japan) and Arg according to our previous study [20]. The zwitterionic γ-PGA-*g*-Arg polypeptide was synthesized as follows. Firstly, 0.1 mg γ-PGA (Mw 100 kDa, VEDAN Enterprise Corporation, Taiwan) was dissolved in 100 ml deionized water (DI water) to obtain an aqueous γ-PGA solution (0.1% wt/v). Subsequently, 0.1 g EDC and 0.05 g NHS in 0.1 M MES buffer were mixed with the γ-PGA solution, and then the pH was adjusted to 5.0. Theγ-PGA-*g*-Arg polypeptide was prepared by reacting the EDC/NHS-activated carboxyl groups in γ-PGA (activated for 30 minutes), with the amino groups in arginine (Arg, 0.1 g) for 24 h. The synthesized γ-PGA-*g*-Arg polypeptide was