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Paclitaxel loaded nano-aggregates based on pH sensitive polyaspartamide amphiphilic graft copolymers

Kwangwon Seo [a], Seung Woo Chung [b], Youngro Byun [b], Dukjoon Kim [a,*]

[a] School of Chemical Engineering, Theranostic Macromolecules Research Center, Sungkyunkwan University, Suwon, Kyunggi 440-746, Republic of Korea
[b] School of Pharmacy, Seoul National University, Seoul, Republic of Korea

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ABSTRACT

Polyaspartamide (PASPAM) derivatives grafted with 1-(3-aminopropyl)imidazole (API), *O*-(2-aminoethyl)-*O*′-methylpolyethylene glycol (MPEG), and octadecylamine (C18) groups were synthesized and their pH-sensitive structure and Paclitaxel (PTX) load/release properties were investigated. C18/MPEG/API-g-PASPAMs systems synthesized showed a strong pH-dependent phase transition behavior near pH 6.7. Large amount of PTX up to 60–75%, depending on polymer composition, was possibly loaded into the C18/MPEG/API-g-PASPAMs nano-aggregates using a solvent-free protocol. Its pH dependent release pattern was affected correspondingly by the phase transition behavior associated with the composition of graft substituents. The pure C18/MPEG/API-g-PASPAMs systems did not show cell toxicity but the PTX-loaded copolymer systems showed a similar cell toxicity to a Taxol-type PTX. From the in vivo animal study, PTX-loaded nano-aggregates showed the much improved inhibition effect on tumor growth compared to the conventional PTX formulation.

1. Introduction

Polymeric micelles or aggregates have been attracting great interest in their application as nano- to microscaled carriers for bioactive materials, including drugs, genes, cells, peptides, and enzymes since nanotechnology was embodied into biomedical (diagnotherapic) technology. While a variety of amphiphilic block copolymer systems have been employed towards formation of micelles or aggregates by self-assembly in aqueous media (Bae et al., 2005; Cho et al., 2010; Nishiyama and Kataoka, 2006; Savic et al., 2006; Zhang and Zhang, 2005), the graft copolymer systems have become more emphasized in their synthesis, functionalization, and phase transition behavior. In the application of an anticancer drug delivery, the pH sensitive sol–gel transition or swelling/de-swelling behaviors of polymeric micelles or self-assembled aggregates are of importance, as the drug delivery carriers are specifically located at cancer cells by the enhanced permeability and retention (EPR) effect (Campbell, 2006), along with a triggered release allowed at the relatively low pH environment of cancer (Borisov and Zhulina, 2005; Rodriguez-Hermandez and Lecommandoux, 2005; Shim et al., 2006, 2007). The pH sensitivity of the polymer micelles or aggregates also plays an important role in endosomal lysis for effective intracellular delivery (Park et al., 2006c). Recently, several cationic or anionic polymeric systems such as poly(ethyleneimine) (PEI), cationic peptide, polyhistidine, modified chitosan, polyamidoamine, polyamidoamine dendrimer, and poly(propylacrylic acid) have focused on this objective (Berna et al., 2006; Lavignac et al., 2005; Park et al., 2006a,b; Swami et al., 2007; Wu et al., 2005). Although each system shows promising results in part, it still needs development of a new polymeric system that satisfies a simpler synthetic process, lower material toxicity, and more utilizable pH range and strength.

Polypeptides and their related synthetic poly(amino acid)s have attracted a great deal of attention as they are biocompatible and biodegradable with less toxicity (Kricheldorf, 2006; Kulkarni et al., 2005). Polyamino acids with refined molecular weights can be synthesized via a well-known *N*-carboxylanhydride (NCA) method, but its synthetic route is quite complex. Poly(L-aspartic acid) has garnered much attention since its simple synthetic route via thermal condensation was first reported. Poly(succinimide) (PSI), an intermediate in the synthesis of poly(amino acid), has an advantageous molecular structure, enabling easy chemical modification through alkylation, hydrolysis, and aminolysis. This produces biodegradable derivatives such as poly(aspartic acid), poly(asparagine), and poly(hydroxyethylaspartamide). Moreover, it can be easily functionalized by linkage of hydrophilic/hydrophobic ligands, peptides, and even drug (Caliceti et al., 2001; Cavallaro et al., 2003; Chen Xu et al., 2005; Licciardi et al., 2006; Jeong et al., 2005; Moon et al., 2006; Takeuchi et al., 2006).

Paclitaxel (PTX) is one of the most effective and commonly used drugs for ovarian, esophageal, breast, and lung cancer treatment. However, its poor water-solubility (<0.1 $\mu L\,mL^{-1}$) is a serious

* Corresponding author. Tel.: +82 31 290 7250; fax: +82 31 290 7270.
E-mail address: djkim@skku.edu (D. Kim).

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problem in drug formulation. In clinics, it is usually injected into the human body as a Cremophor EL (polyethoxylated castor oil) and ethanol (1:1) solution. It is sometimes reported to bring about hypersensitivity reactions such as breathing difficulty, rash, flushing, and nephrotoxicity. Solutions to those problems require new types of drug release methods or structures, and one of them is the nano- to microscaled micelle or aggregate structures prepared from amphiphilic graft copolymers (Elkharraz et al., 2006; Hu et al., 2006; He et al., 2007; Liu et al., 2005; Seow et al., 2007).

In this lab's previous report (Seo et al., 2009, 2010; Seo and Kim, 2010), the synthesis and pH-sensitive properties of a new class of polyaspartamide (PASPAM) derivatives, C18/MPEG/API-g-PASPAMs, possessing 1-(3-aminopropyl)imidazole (API), *O*-(2-aminoethyl)-*O*′-methylpolyethylene glycol (MPEG), and octadecylamine (C18) groups, were discussed. As they showed a strong pH-dependent phase transition and aggregation behavior near pH 6.7, it was noted that they possess high potential in the application as pH-sensitive anti-cancer drug carriers. In this study, the PTX was loaded into the polyaspartamide derivatives using a solvent-free protocol, and their reversible aggregation behavior and inhibition effect on tumor growth was investigated both in vitro and in vivo.

2. Materials and methods

2.1. Materials

L-Aspartic acid (>98%) was purchased from Aldrich (Milwaukee, WI, USA) and used as a monomer; octadecylamine (Aldrich), 1-(3-aminopropyl)imidazole and *O*-(2-aminoethyl)-*O*′-methylpolyethylene glycol (M_w: 5000, Fluka, Buchs, Switzerland) were used as graft reagents. *O*-phosphoric acid (98%) and phosphoric acid (85%) were used as catalysts. Phosphate buffered saline (PBS 7.4), *N*,*N*-dimethylformamide (DMF), mesitylene, sulforane, dimethyl sulfoxide-d_6 (DMSO-d_6), octadecylamine, 1-(3-aminopropyl)imidazole, *N*,*N*-diethylnicotinamide, ethanol, acetonitrile, and Cremophor EL were used as solvents. All chemicals were purchased from Sigma–Aldrich except for *O*-(2-aminoethyl)-*O*′-methylpolyethylene glycol. Paclitaxel (99%, Genexol®; Samyang Genex, Korea) was used as received. All other chemicals purchased were of sufficient quality for use without purification.

2.2. Methods

2.2.1. Synthesis of C18/MPEG/API-g-PASPAMs

The PSI was first synthesized from L-aspartic acid. Octadecylamine (C18) (0.27–1.08 g, 0.001–0.004 mol) was added to a solution of PSI (1.0 g, 0.01 mol) in dry DMF (50 mL) under a N_2 atmosphere. After stirring for 24 h at 60 °C, the reaction mixture was poured into methanol for purification. The MPEG and API were grafted onto the prepared C18-g-PSI, sequentially. The MPEG and C18-g-PSI were each dissolved in DMF. The MPEG solution was then added dropwise into the C18-g-PSI solution for its graft reaction at 70 °C for 48 h. After the MPEG graft reaction, the API was added for its graft reaction for 24 h. Finally, the prepared C18-conjugated MPEG/API-g-PASPAM was dialyzed using a dialysis membrane (molecular weight cut-off = 10,000–12,000 g/mol) for 3 days and then freeze-dried for storage. The schematic synthetic procedure is illustrated in Fig. 1.

Chemical structure was confirmed using Fourier transform nuclear magnetic resonance spectroscopy (Varian Unity Inova 500 MHz HMR, Agilent Technologies, Santa Clara, CA, USA). Samples were dissolved in DMSO-d_6. The degree of substitution of the MPEG, C18, and API was determined from comparison of the NMR characteristic peak intensities associated with each component. The average molecular weights of the synthesized copolymers were measured using a gel permeation chromatography (GPC, model 410, Waters, USA) system equipped with KF-803L and KF-802.5 columns (Shodex, Showa Denko KK, Japan) in series. For GPC measurements, samples were dissolved in *N*,*N*-dimethylformamide (DMF) containing lithium bromide (50 mM), and the solution was injected into the column at the rate of 1.0 mL min^{-1} at 50 °C. The data was analyzed by means of an RI detector (RI-101, Shodex). Polystyrene standards (Waters) were used to determine the molecular weight (M_w).

2.2.2. Phase transition and buffering behavior

The pH-dependence of the light transmission intensity was measured for the polymer solution using the UV/vis spectroscopy (Hitachi, U-3210, Japan) at a 500 nm wavelength. The transmission % is given by the ratio of light transmission intensity for the sample to that of pure PBS solution at pH 7.4. Samples were prepared by dissolving each polymer in pH 7.4 PBS solution at 1.0 mg mL^{-1}; the pH was adjusted using 0.1 N HCl and 0.1 N NaOH. The buffering

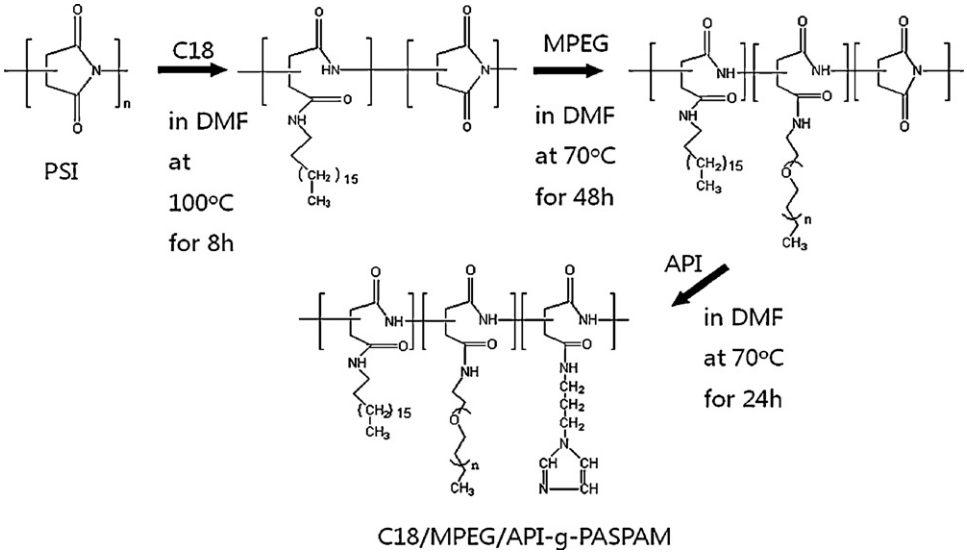

Fig. 1. Synthetic pathway of C18/MPEG/API-g-PASPAM.