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Block ionomer complexes of PEG-*block*-poly(4-vinylbenzylphosphonate) and cationic surfactants as highly stable, pH responsive drug delivery system

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ABSTRACT

A new family of block ionomer complexes (BIC) formed by poly(ethylene glycol)-*block*-poly(4-vinylbenzylphosphonate) (PEG-*b*-PVBP) and various cationic surfactants was prepared and characterized. These complexes spontaneously self-assembled in aqueous solutions into particles with average size of 40–60 nm and remained soluble over the entire range of the compositions of the mixtures including stoichiometric electroneutral complexes. Solution behavior and physicochemical properties of such BIC were very sensitive to the structure of cationic surfactants. Furthermore, such complexation was used for incorporation of cationic anti-cancer drug, doxorubicin (DOX), into the core of BIC with high loading capacity and efficiency. The DOX/PEG-*b*-PVBP BIC also displayed high stability against dilution, changes in ionic strength. Furthermore, DOX release at the extracellular pH of DOX/PEG-*b*-PVBP BIC was slow. It was greatly increased at the acidic pH mimicking the endosomal/lysosomal environment. Confocal fluorescence microscopy using live MCF-7 breast cancer cells suggested that DOX/PEG-*b*-PVBP BICs are transported to lysosomes. Subsequently, the drugs are released and exert cytotoxic effect killing these cancer cells. These findings indicate that the obtained complexes can be attractive candidates for delivery of cationic drugs to tumors.

1. Introduction

A very special class of drug delivery systems based on block and graft copolymers containing ionic and non-ionic water-soluble segments ("block ionomers") was proposed in mid-90-ies [1–4]. Such drug delivery systems are spontaneously formed in aqueous solutions upon electrostatic binding of block ionomer with oppositely charged molecules. The resulting complexes represent micelle-like particles termed "polyion complex micelles"[5–7], or "block ionomer complexes" (BICs) [8,9]. They have attracted great attention in various applications for delivery of low molecular mass drugs [10–13], proteins [14,15], polynucleotides (antisense oligonucleotides [16,17], plasmid DNA [18,19], siRNA [20,21]) and imaging agents [22,23]. For example, due to extended blood circulation and ability to circumvent renal excretion, known as the "enhanced permeability and retention (EPR) effect" [24,25], BICs loaded with anticancer drugs exhibit preferential accumulation and delivery of their cargos to solid tumors [26].

A special class of BICs was previously prepared by reacting block ionomers with surfactants of opposite charge, resulting in the spontaneous formation of the nanomaterials, which differ in sizes and morphologies, such as micelles and vesicles [27–34]. These materials contain a hydrophobic core formed by the surfactant tail groups and a hydrophilic shell formed by nonionic chains of the block copolymer (for example by PEG). These BICs can incorporate charged surfactant drugs, such as retinoic acid that binds to the polyelectrolyte chains, as well as hydrophobic non-charged drugs that solubilize in the hydrophobic domains formed by surfactant tail groups [35]. They display an ability to respond to the changes in the environmental parameters such as pH and ionic strength and can be designed to release drugs

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triggered by the environment in the target cell [36]. The simplicity of design and availability of diverse surfactant components for preparation of such BICs makes them quite attractive for development of carriers for drug delivery.

Here we explore BICs formed by anionic poly(ethylene glycol)-*block*-poly(4-vinylbenzylphosphonate) block ionomer (PEG-*b*-PVBP) and cationic surfactants (Scheme 1). The structure of PEG-*b*-PVBP is quite different compared to regular "double hydrophilic" block ionomers, such as PEG-*block*-poly(methacrylic acid) (PEG-*b*-PMA), that were previously studied by us and others [37,38]. Specifically, the polyion segment of PEG-*b*-PVBP contains hydrophobic styrene moiety in every repeating unit, which can strongly affect the self-assembly and colloidal stability of the resulting BICs. Furthermore, in addition to a regular cationic surfactant we explored the use of a cationic surfactant drug, doxorubicin (DOX) for preparation of BICs. Since such BICs are potential drug delivery vehicles their loading efficacy with respect to DOX, the release rate of DOX at different environmental pH and *in vitro* cytotoxicity in cancer cells were also examined. Overall the results of this study further advance the potential use of BICs in drug delivery.

2. Materials and methods

2.1. Materials

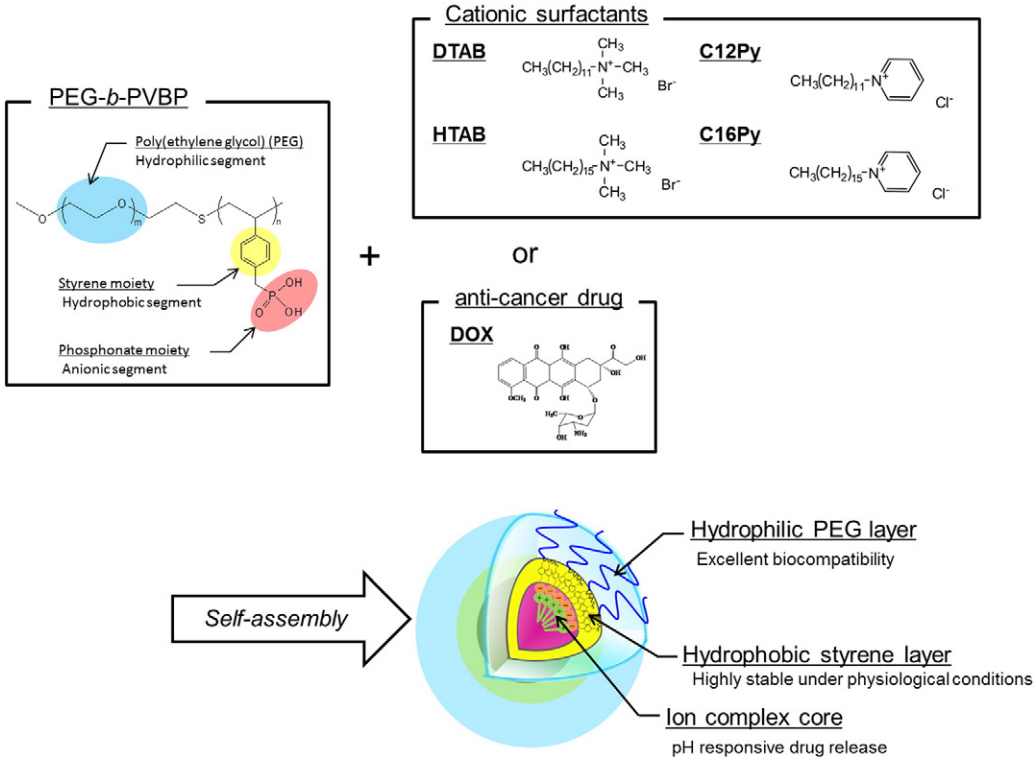
PEG-*b*-PVBP block copolymer was synthesized as described in our previous report [39]. The repeating units for PEG and PVBP blocks were 113 and 20, respectively. Methanol, dodecyltrimethylammonium bromide (DTAB), hexadecyltrimethylammonium bromide (HTAB), and cetylpyridinium chloride (C16Py) were purchased from Sigma-Aldrich (St Louis, MO). Dodecylpyridinium chloride (C12Py) was purchased from Tokyo Chemical Industry, Co., Ltd, (Tokyo, Japan). Doxorubicin hydrochloride (DOX) was kindly provided by Dong-A Pharmaceutical Company (Korea). Lysotracker Green, fetal bovine serum (FBS), and Dulbecco's Modified Eagle's Medium (DMEM) were purchased from Invitrogen Inc (Carlsbad, CA). MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was purchased from Research Products International (Mount Prospect, IL). All other chemicals were used without further purification.

2.2. Preparation of BICs formed by PEG-b-PVBP and cationic surfactants

Cationic surfactants (DTAB, HTAB, C12Py, and C16Py) and PEG-*b*-PVBP were separately dissolved in water and methanol, respectively. Their concentrations were 50 mM and 22 mM, respectively. An aqueous solution surfactant and water were added to a vial followed by addition of 0.2 mL of PEG-*b*-PVBP solution in methanol to achieve the desired composition of the mixture. Final volume of the solution was adjusted to 2.4 mL with water. Methanol was removed by evaporation. The composition of the mixture (Z value) was expressed as the molar ratio of the concentration of the surfactant to the concentration of 4-vinylbenzylphosphonate (VBP) units, Z = [surfactant]/[VBP]. For example, when the 0.088 mL of surfactant solution was mixed with 0.2 mL of PEG-*b*-PVBP solution, the Z value of the mixture was 1.0. Surfactant composition of the BIC is labeled in accordance with the surfactant nomenclature, i.e. DTAB/PEG-*b*-PVBP refers to the BIC formed from DTAB surfactant and copolymer.

2.3. Fluorescence measurements

Fluorescence spectra and intensity measurements were carried out using Fluorolog3 (Horiba Jobin Yvon, France). Pyrene solubilization method was used to detect the onset of surfactant aggregation [40,41]. The known amounts of pyrene in acetone were added to empty vials, followed by acetone evaporation. Solutions of surfactants or surfactant/PEG-*b*-PVBP mixture in phosphate buffer (10 mM, pH7.0) containing 5 vol% of methanol were added to the vials and kept overnight under constant stirring at room temperature. Pyrene



Scheme 1. Schematic illustration of self-assembly of the block ionomer complexes.