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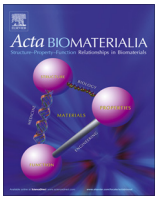

Full length article

Photoinduced inhibition of DNA unwinding *in vitro* with water-soluble polymers containing both phosphorylcholine and photoreactive groups ☆

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ABSTRACT

Nile blue (NB)-tagged DNA helix-targeting amphiphilic photoreactive 2-methacryloyloxyethyl phosphorylcholine (MPC) polymer, poly(MPC-*co*-3-methacryloyloxy-2-hydroxypropyl-4-oxybenzophenone-*co*-2-trimethylammonium ethyl methacrylate chloride) (PMHT–NB), containing a cationic group to facilitate cell membrane penetration and a benzophenone (BP) group to promote photoinduced conjugation with DNA helix was synthesized using radical polymerization method. Ultraviolet light (UV)-visible light absorption spectra of PMHT–NB showed absorption peaks at wavelengths 254, 289, and 600 nm, suggesting successful incorporation of BP and NB groups. PMHT–NB was highly sensitive to photoirradiation with UV irradiation at the second level, as confirmed based on the degradation spectra of UV absorption peaks for the BP group in PBS (pH = 7.4). PMHT–NB showed good solubility in both aqueous solution and in ethanol. In a cell culture medium containing 10 mg/mL PMHT–NB, the NB group showed fluorescence peaks at an emission wavelength of 650 nm and excitation wavelength of 633 nm. PMHT–NB also showed low cytotoxicity and good cell membrane permeability toward cancerous HeLa cells. Further, photoinduced PMHT–NB effectively inhibited the unwinding of a molecular beacon with a hairpin structure, indicating that synthetic photoreactive MPC polymers photoregulated the unwinding of DNA.

Statement of Significance

Natural and synthetic genetic hybrid biomaterials consisting of well-designed polymers loaded with oligonucleotide fragments are considered as an attractive alternative to conventional transgene systems and chemical methods for precisely and rapidly modulation of intracellular gene expression. Containing versatile functional moieties, the effectiveness of well-designed cytocompatible polymers themselves without oligonucleotide fragments on gene regulation is rarely investigated. In the present study, a 2-methacryloyloxyethyl phosphorylcholine (MPC) polymer composed of a tumor/DNA-targeting moiety and photo-controllable unit demonstrated low cytotoxicity, rapid cell membrane permeability and effective inhibitive ability on DNA unwinding under a light irradiation. The synthetic polymer was considered as promising material to effectively inhibit intracellular partial DNA unwinding for cancer/gene therapy.

1. Introduction

Compared to conventional extracellular chemical reactions, intracellular bioreactions involving proteins, enzyme, and nucleic acids are relatively complicated and extremely important for the effective regulation of cell behavior, especially in cancer therapy. Intracellular gene regulation by using transgene systems and chemical methods has been widely investigated to precisely modulate the expression of desired genes [1–3]. Transgene systems requiring strict transfection may need time to achieve an observable expression of genetically encoded biomolecules and may affect the efficiency of real-time temporal regulation of gene expression. When conventional chemical methods are used, small molecules freely and non-specifically diffuse inside cells, thus making it difficult to regulate gene expression at desired intracellular locations.

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Natural and synthetic genetic biomaterials composed of well-designed polymers loaded with oligonucleotide fragments are an attractive alternative because cell membrane permeability and target specificity of these polymer–gene hybrids can be optimized by altering the intrinsic properties of both the polymers and the loaded oligonucleotide fragments [4–7]. To our knowledge, limited studies have been performed on the intracellular regulation of gene molecules by using only synthetic polymeric biomaterials. Polymeric biomaterials with well-defined architectures, molecular weights, and functions can be precisely designed and synthesized using various chemical polymerization methods and show promising potential for use in biomedical applications. As a clean trigger, light is an ideal and effective tool to remotely, accurately, and rapidly control interactions between polymeric materials and genes even in a diminutive space of the intracellular environment [8–10]. Therefore, preparation of a well-designed polymeric biomaterial containing a DNA-targeting moiety and a photocontrollable unit is promising for intracellular regulation of DNA behavior.

Zwitterionic polymers with phosphorylcholine [11–13], carboxybetaine [14–16], and sulfobetaine [17–19] groups show excellent cytocompatibility and biocompatibility as polymeric biomaterials. The phosphorylcholine group contains a phosphate anion and a trimethylammonium cation, with an inner salt structure. Because of its simple molecular design and synthesis, phosphorylcholine group containing 2-methacryloyloxyethyl phosphorylcholine (MPC), a methacrylate monomer, is widely polymerized with other functional monomers by using versatile radical polymerization methods [20–23]. A photoreactive MPC polymer poly(MPC-*co*-3-methacryloyloxy-2-hydroxypropyl-4-oxy benzophenone [MHPBP]) (PMH) containing the phosphorylcholine group and a photosensitive benzophenone (BP) group was synthesized and used as a surface functionalization reagent for biomaterials [24]. Photoirradiation with ultraviolet light (UV) induces a chemical reaction between the BP group and aliphatic hydrogen-donating groups. Nile blue (NB), a fluorescent dye with comparably long excitation wavelength, is a well-known DNA-binding probe with low cytotoxicity and good sensitivity because the planar hydrophobic phenoxazine moiety of NB facilitates its intercalation into the relatively non-polar interior of a DNA helix [25,26]. Moreover, NB selectively localizes to tumor tissues and possibly inhibits tumor growth [27,28]. The amphiphilic MPC polymer with an optimized monomer unit composition can cross the lipid bilayer and rapidly penetrate into the cytoplasm of living cells [29–31]. Therefore, an NB-tagged photoreactive MPC polymer can penetrate living cells, embed into DNA helix, and regulate DNA unwinding after photoirradiation.

In this study, we synthesized an NB-tagged amphiphilic photoreactive MPC polymer containing a cationic group to facilitate cell membrane penetration and a BP group to promote photoinduced conjugation with DNA molecules. Photoreactivity and cell membrane penetration capacity of this synthetic polymer and its interaction with DNA under UV irradiation were explored. Further, a molecular beacon (MB) with a hairpin structure was used to evaluate the effect of different polymer types and their concentrations on the unwinding of DNA.

2. Materials and methods

2.1. Materials

MPC was purchased from NOF Co., Ltd. (Tokyo, Japan) where it was synthesized according to a previously reported method [32]. Glycidyl methacrylate (GMA), tetramethylammonium chloride (TMAC), 2-trimethylammoniumethyl methacrylate chloride (TMAEMA), and 2, 2′-azobisisobutyronitrile (AIBN) were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). 4-Hydroxybenzophenone (4-HBP) and Tween 20 were purchased from Sigma-Aldrich (St. Louis, MO, USA), and NB acrylamide was purchased from Polysciences, Inc. (Warrington, PA, USA). Cyclic polyolefin (CPO) was obtained from Sumitomo Bakelite Co., Ltd. (Tokyo, Japan), and lactate dehydrogenase (LDH) cytotoxicity test kit was purchased from Wako Pure Chemicals Co., Ltd. (Osaka, Japan). MBs with a hairpin structure were synthesized by Medical & Biological Laboratories (Nagoya, Japan), and their 5′ and 3′ ends were modified by adding fluorophore Cy3 and black hole quencher-2 (BHQ-2), respectively. Cell culture medium and its supplements (Dulbecco's modified Eagle's medium (DMEM) with or without phenol red, fetal bovine serum (FBS), and trypsin/EDTA) and ultrapure distilled water were purchased from Invitrogen (Grand Island, NY, USA). Other reagents and solvents were commercially available and were used without further purification.

2.2. Synthesis of MPC polymers

MHPBP, a methacrylate monomer with a photoreactive group, was synthesized by performing non-solvent epoxide ring opening and addition reactions between GMA and 4-HBP by using TMAC as a catalyzer, as described previously [24]. Phospholipid polymers poly(MPC) (PMPC), poly(MPC-*co*-TMAEMA) (PMT), and poly(MPC-*co*-MHPBP-*co*-TMAEMA) (PMHT) that were covalently labeled with NB were synthesized by performing conventional radical polymerization method, with AIBN as an initiator. Briefly, desired concentrations of MPC, MHPBP, and TMAEMA (total monomer concentration, 0.50 mol/L); NB acrylamide (0.5 mmol/L); and AIBN (10.0 mmol/L) were dissolved in 30 mL ethanol in a reaction flask at room temperature. Polymerization was performed in a sealed flask with an atmosphere of argon gas and in an oil bath (65 °C). Next, the reaction mixture was diluted with ethanol and was added to a solvent made of ether:chloroform (90:10, v/v) to precipitate the polymers. The precipitated polymers were purified by performing dialysis against methanol (3 days) and then against distilled water (3 days) by using a standard regenerative cellulose membrane (molecular weight cutoff, 3.5 kDa; Spectrum Laboratories, Inc., Rancho Dominguez, CA, USA). Finally, the polymers were lyophilized with EYELA FDU-1100 freeze-dryer (Tokyo Rikakikai Co., Ltd., Tokyo, Japan) at −47 °C for 48 h to convert them into a light blue powder. Chemical structures of the synthetic polymers were confirmed by performing ^{1}H NMR (Fig. S-1a, b, and c) and by using a UV-visible light (UV-vis) spectrophotometer (V-560; JASCO, Tokyo, Japan; Fig. S-2). Monomer unit composition of each polymer was assessed by performing ^{1}H NMR and by quantitatively determining phosphorus element in each polymer. Average molecular weights of the polymers were measured using a gel permeation chromatography (GPC) system (JASCO) with a water: methanol mixture (30:70, v/v) containing 10 mmol/L lithium bromide (LiBr). Poly(ethylene oxide) (Tosoh Co., Tokyo, Japan) was used as a standard to obtain a calibration curve. Chemical structure of PMHT-NB is shown in Fig. 1. Molecular properties of the synthetic polymers are summarized in Table 1.

2.3. Photoreactivity of the MPC polymers

PMHT polymers were dissolved in phosphate-buffered saline (PBS; 1×, pH = 7.4) at a concentration of 0.20 mg/mL. The polymer solutions (3.0 mL) were then added to a rectangular cuvette (capacity, 3.0 mL) with a 1.0-cm optical path length. The cuvette was irradiated with UV (wavelength: 250–400 nm; intensity: 10 mW/cm^2) for 3600 s, and the samples were measured at intervals of 10 s (0–300 s), 60 s (300–900 s), and 180 s (900–3600 s). UV absorption curves corresponding to the degradation of the photoreactive BP

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