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New Conjugated Polymers for Photoinduced Unwinding of DNA Supercoiling and Gene Regulation

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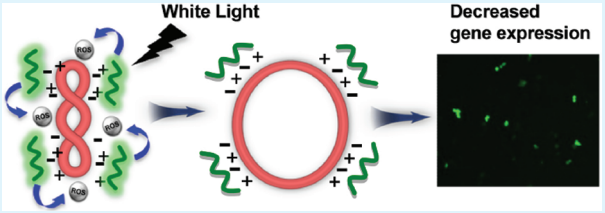
Supporting Information

ABSTRACT: Three cationic polythiophene derivatives (**P1**, **P2**, **P3**) were synthesized and characterized. Under white light irradiation (400−800 nm), they sensitize oxygen molecule in the surrounding to generate reactive oxygen species (ROS) that can efficiently unwind the supercoiled DNA in vitro. Further study shows that this relaxation of the DNA supercoiling results in the decrease of gene (pCX-EGFP plasmid) expression level. The ability of these conjugated polymers for regulating gene expression will add a new dimension to the function of conjugated polymers.



KEYWORDS: *conjugated polymers, reactive oxygen species, photoinduced unwinding, DNA supercoiling, gene regulation*

1. INTRODUCTION

The understanding of the gene function offers important information on the biological processes and gene misregulation in human diseases. DNA supercoiling refers to the over- or under-winding of a DNA strand, which plays a key role in a number of biological processes, such as transcription, DNA replication and chromosomal segregation.[1] Through changing the shape of DNA, DNA supercoiling can affect gene expression at the level of promoter activity by acting as a second messenger.[2] Duplex DNA can be converted to nonduplex DNA structures which control both turning on and off of transcription and the rate of transcription firing by supercoiling, even at considerable distances from the transcriptional start site. These nonduplex DNA structures are amenable to small-molecule targeting in some tumors, in which MYC is an important oncogene. This dynamic system provides a unique opportunity for the treatment of cancer.[3] Reactive oxygen species (ROS) can attack DNA and produce base oxidation besides DNA break, which may greatly influence the gene expression. It was reported that singlet oxygen that generated by chemical compounds under light irradiation could break frank DNA strand, which induces the supercoiled plasmid DNA to the relaxed circular species.[4] Fluorophore-assisted laser inactivation (FALI), a technique with great potential to inactivate proteins with spatial and temporal precision by using an antibody to direct a suitable fluorophore specifically to the protein of interest, has been used in protein function study.[5−9] Light irradiation stimulates local generation of oxygen radicals by the fluorophore to react with the nearby antigen and inactivates it. However, the same strategy has been less pursued in the gene study. In view of the important role of DNA supercoiling in the biological process, the photounwinding of supercoiled DNA may offer considerable potential for the study of gene expression.

Conjugated polymers (CPs) have evolved as one of the most advanced materials over the past three decades due to their delocalized electronic structures that exhibit appealing physical/chemical properties, in particular electrical, magnetic, optical, and electronic flexibility.[10−19] Research has been currently focused on the highly sensitive detection[14−17] and imaging applications of the CPs.[18,19] Recently, Whitten and co-workers initiate new application of CPs as antibiotic agents upon exposure to light for killing bacteria because of the generation of ROS.[20,21] We have shown that CPs can specifically inactivate proteins under light irradiation through their assemblies with proteins.[22] CPs have great potential for gene expression regulation. First, the light-harvesting ability of CPs results from considerable light absorbing units in the backbone is more beneficial for the generation of ROS. Second, the CPs can form complex with genes by electrostatic interactions, which meets the distance requirement for ROS damage of the target. Third, the feasibility of further modification of CPs, such as incorporation of targeting group and NIR light emitters to the side chains, should also permit the development of specific photodamaging probes in vivo. In this paper, three cationic polythiophene derivatives (**P1**, **P2**, **P3**) were synthesized for photounwinding the supercoiled plasimid DNA by the generation of ROS under white light exposure. It is found that the relaxation of the DNA supercoiling results in the decrease of gene expression level. The present work will add a new dimension to the function of CPs.

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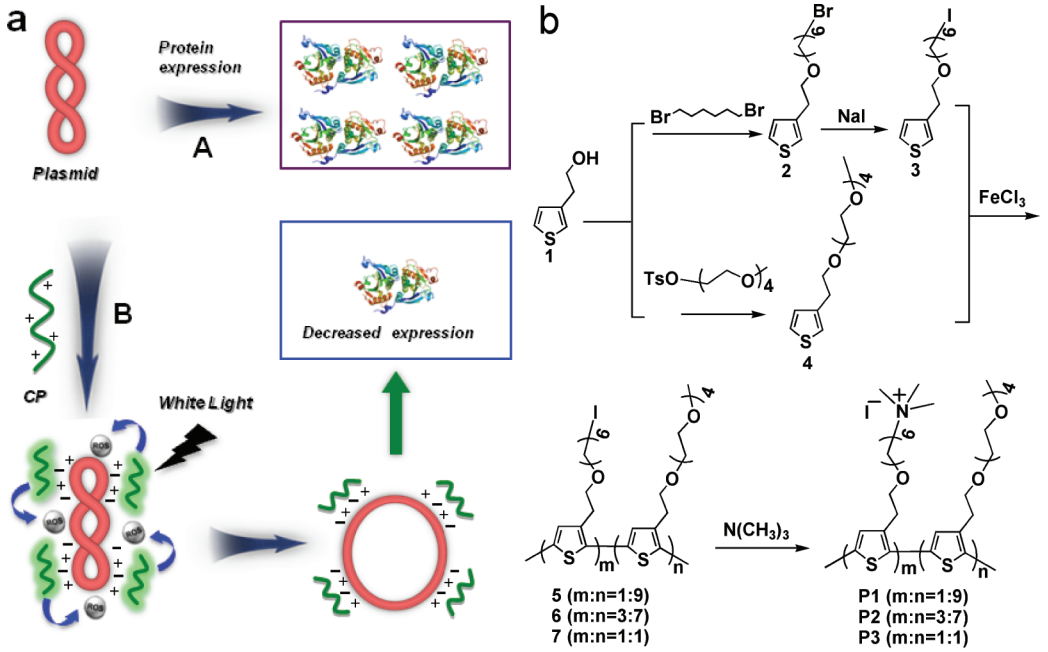
Scheme 1. (a) Schematic Mechanism of CPs for Photoinduced Unwinding of DNA Supercoiling and Gene Regulation under White Light Irradiation: Situation A, Normal Protein Expression; Situation B, Decreased Protein Expression Affected by ROS; (b) Synthetic Route of the Polymers P1, P2, and P3



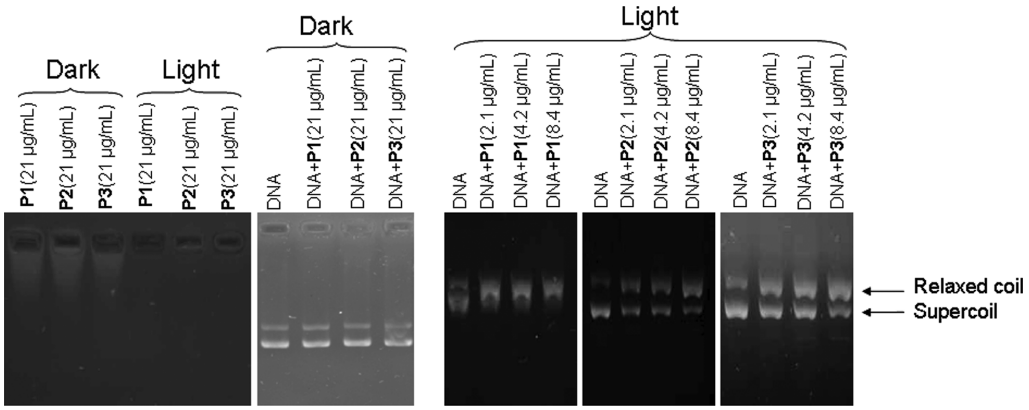


Figure 1. ROS damage activity of the polymers **P1**, **P2**, and **P3** to pBR322 plasmid DNA by agarose gel assay. Plasmid DNA (0.1 μg) was mixed with different amounts of **P1**, **P2**, and **P3** in 10 μL of phosphate buffer (50 mM, pH 7.4). White light (400−800 nm, 78 mW/cm^2) was used to irradiate each sample for 30 min and loaded onto a 1% agarose gel. Gels were stained with Goldview DNA dye. "Light" stands for samples irradiated by light; "Dark" indicates DNA samples without light irradiation; "Supercoil" stands for plasmid DNA in supercoiled state; and "Relaxed coil" stands for the plasmid DNA in relaxed state, which can be formed by the unwinding of the supercoiled plasmid DNA.

2. RESULTS AND DISCUSSION

The proposed principle of CPs for photoinduced plasmid inactivation is illustrated in Scheme 1**a**. Negatively charged plasmid DNA and cationic CPs form the complex through electrostatic interactions. Upon white light irradiation, the excited CPs sensitize oxygen molecule in the surrounding to generate ROS. The supercoiling of plasmid DNA is damaged by ROS and unwinds to form the relaxed state DNA. This unwinding greatly down regulates the protein expression (Situation B) compared with the plasmid DNA with intact supercoiling structure (Situation A).

Scheme 1b depicts the synthetic routes of three cationic polythiophene derivatives **P1**, **P2**, and **P3**. In these polymers, hydrophilic tetraethylene glycol side chain can increase the water solubility and ammonium salt side chain is used for electrostatic binding with DNA. Reaction of 2-(3-thienyl) ethanol **1** with 1,6-dibromohexane in the presence of sodium hydride affords 3-(2-(6-bromohexyloxy)-ethyl) thiophene **2** in 31% yield. Reaction of compound **2** with sodium iodide in acetone yields compound **3** in 95% yield. The 2-(3-thienyl) ethanol **1** reacts with monotosyl tetraethylene glycol monomethyl ether[23] to give compound **4** in 28% yield. Polymers **5**, **6**, and **7** were synthesized through oxidative polymerization of monomer **3** and **4** with different molar ratios in chloroform with $FeCl_3$ as the oxidizing agent. Quaternization of polymers **5**, **6**, and **7** by trimethylamine 33 WT% alcoholic solution in tetrahydrofuran (THF) gives the cationic polymers **P1**, **P2**, and **P3**. The optical properties of the polymers were investigated in water (see Figure S1 in the Supporting Information). The polymers **P1**, **P2**, and **P3** exhibit similar maximum absorption at 420 nm, which exhibits that they can be excited under white light (400−800 nm).

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To test the photoinduced damage ability of the polymers to genes, a negatively supercoiled plasmid DNA, pBR322, was used as a model in the ROS damage experiment.[24,25] The DNA samples with varying amounts of **P1**, **P2**, and **P3** in 10 μL of phosphate buffered solution at pH 7.4 were irradiated under white light (400−800 nm, 78 mW/cm^2). The samples were treated in the dark as controls. The results of agarose gel assay are displayed in Figure 1. In the dark, even in higher concentration of polymers (21 μg/mL), the plasmid DNA can migrate through the gel like the free, which shows that polymer **P1**, **P2** and **P3** can not form tight complex with the plasmid DNA. However, after irradiation with white light, CPs show comparable efficiency of DNA supercoiling unwinding with the generation of ROS. The supercoiled DNA almost disappeared and turned to the relaxed coil at the concentration of 8.4 μg/mL. It is noted that the **P1**, **P2**, and **P3** with different molar ratios of cationic moiety to oligo (ethylene glycol) show similar binding and photoinduced damage ability to plasmid DNA, which means that they possess similar abilities to produce ROS.

To make sure that the ROS generates under light irradiation and plays a vital role in DNA supercoiling unwinding, ROS-sensitive 2,7-dichlorofluorescin (DCFH-DA) was used to probe the generation of ROS.[26] The DCFH-DA can detect all the oxidizing species, such as superoxide anion radical, hydrogen peroxide, hydroxyl radical and singlet oxygen, thus providing a global approach to evaluating drug phototoxicity. As shown in Figure 2, the generation ability of ROS by polymer

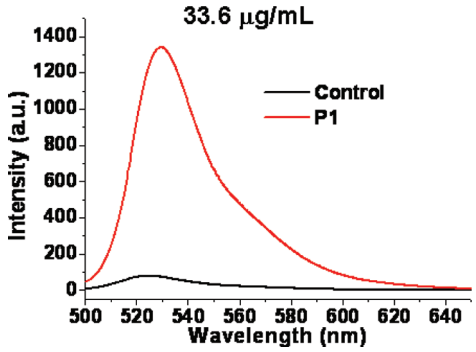

Figure 2. Fluorescence intensity of activated DCF (40 μM) at 525 nm was measured after 1 min irradiation in the presence of **P1**. [**P1**] = 33.6 μg/mL. Control experiment was performed in the absence of **P1** under the same irradiation condition. White light (400−800 nm, 78 mW/cm^2) was used for the irradiation experiment.

P1 was evaluated upon exposure to white light. Compared with the control experiment in the absence of polymer, there is a significant increase of fluorescence intensity of activated 2,7-dichlorofluorescein (DCF, 40 μM) at 525 nm upon light irradiation, which exhibits noticeable ROS generation by polymer **P1**. The photostability of **P1** in aqueous solution and the effect of irradiation time on the generation of ROS were investigated (see Figure S2 in the Supporting Information). As shown in Figure S2a in the Supporting Information, with the increase of irradiation time, the photobleaching of **P1** occurs in a fast speed. Only 31% of fluorescence intensity remains after 4 min white light exposure (78 mW/cm^2). Figure S2b in the Supporting Information shows the effect of irradiation time on efficiency of ROS generation for **P1**. As shown in Figure S2b in the Supporting Information, with the increase in irradiation time, the normalized fluorescence intensity of activated DCF decreases, which infers the decreased efficiency of ROS generation for **P1**.

The cytotoxicity of **P1**, **P2**, and **P3** toward Hela cell line was determined by MTT assay. As shown in Figure 3a, **P1**, **P2**, and

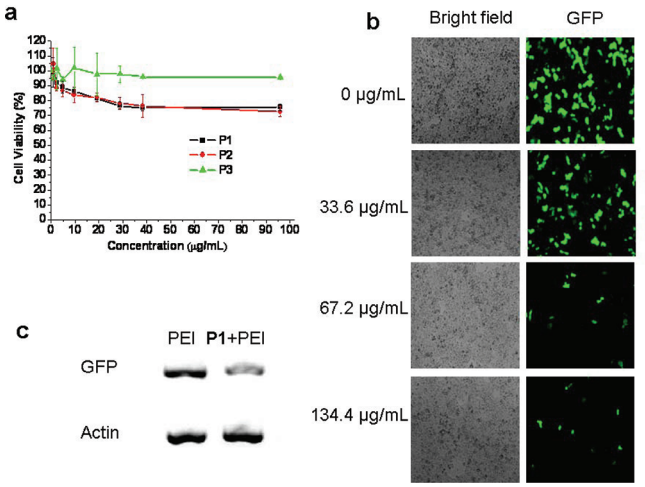

Figure 3. (a) Cell viability of Hela cells treated with **P1**, **P2**, and **P3** using MTT assay. (b) The regulation of GFP expression by polymer **P1**. (c) Down-regulation of GFP mRNA expression by polymer **P1** under light irradiation. β-actin was used as the internal control. Fluorescence images of GFP were recorded using a 455/70 nm excitation filter.

P3 show minimal cytotoxicity, even at a high concentration (96 μg/mL). To further test whether the relaxation of DNA supercoiling is directly related to the gene expression or not, we used the expression of green fluorescence protein (GFP) in Hela cell line as a gene expression model for the gene regulation test of the polymers. The plasmid DNA expressing GFP (pCX-EGFP) was first treated with different amounts of **P1** under white light irradiation for 30 min, and then polyethyleneimine (PEI) was used as the delivery vector of plasmid DNA for the evaluation of GFP expression in cells. As revealed in Figure 3b, compared with the plasmid DNA itself treated under light irradiation, the GFP expression level shows concentration-dependent decrease with the increase in **P1** amount. When the amount of **P1** reaches 134.4 μg/mL, the fluorescence microscopy image shows very low level of GFP expression. The plasmid DNA treated with **P1** without light irradiation does not show the decreased GFP expression (see Figure S3 in the Supporting Information). The similar results were also obtained for polymer **P2** and **P3** (see Figure S4 in the Supporting Information). To further confirm the gene regulation of the polymers, we next measured the mRNA level of the GFP in cells. As shown in Figure 3c, after 48 h transfection, compared with the plasmid DNA without treatment with **P1** but light irradiation, the mRNA level of GFP dramatically reduced. Thus, the unwinding of the plasmid DNA by **P1**, **P2**, and **P3** can greatly decrease the expression of the DNA.

3. CONCLUSION

Three cationic polythiophene derivatives (**P1**, **P2**, **P3**) were synthesized and characterized. Under white light irradiation (400−800 nm) they sensitize oxygen molecule in the surrounding to generate reactive oxygen species (ROS) that can efficiently unwind the supercoiled DNA in vitro. Further

study shows that this relaxation of the DNA supercoiling results in the decrease of gene (pCX-EGFP plasmid) expression level. The ability of these conjugated polymers for regulating gene expression will add a new dimension to the function of conjugated polymers.

4. EXPERIMENTAL SECTION

Synthesis of Polymers P1, P2, and P3. To a solution of **5**, **6**, or **7** in THF was added excessive trimethylamine 33 wt % alcoholic solution. After stirring for 24 h at room temperature, the solvent and excessive trimethylamine were removed under vacuum. Dialysis of the product through a membrane with a molecular weight cutoff of 3500 for 2 days yields **P1**, **P2**, or **P3**. ^{1}H NMR (400 MHz, CD_3OD-d_4, ppm) for **P1**: 7.18−7.25(br), 3.48−3.78(br), 3.12(br), 3.06(br), 2.84(br), 1.29(br); **P2**: 7.26(br), 3.48−3.78(br), 3.13(br), 3.06(br), 2.84(br), 1.29(br); **P3**: 7.18−7.25(br), 3.48−3.78(br), 3.13(br), 3.06(br), 2.84(br), 1.30(br).

Gel Electrophoresis. Plasmid pBR322 (0.1 μg/sample) was mixed with polymer **P1**, **P2** or **P3** in 10 μL of phosphate buffer (50 mM, pH 7.4). The resulting complexes were irradiated under white light (78 mW/cm^2) for 30 min at room temperature. To rrradiated solutions was added agarose gel loading buffer and loaded onto a 1% agarose gel containing Goldview DNA dye. Gels were run at 100 mA for 26 min, visualized in Chemi-Doc (Bio-Rad, USA). DNA bands were quantified by Quantity One software (Bio-Rad).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Synthesis of monomers, experimental details for transfection, mRNA analysis, RT-PCR, and ROS measurements. This material is available free of charge via the Internet at http://pubs.acs.org/.

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Notes

The authors declare no competing financial interest.

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