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Engineering Buffering and Hydrolytic or Photolabile Charge Shifting in a Polycarboxybetaine Ester Gene Delivery Platform

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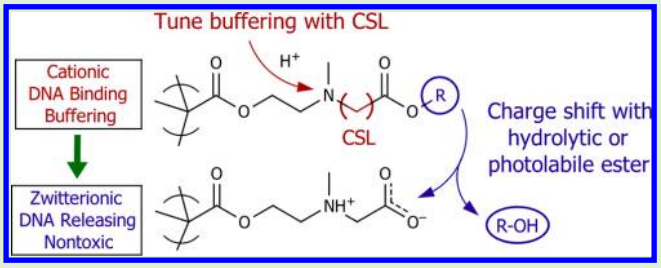
Supporting Information

ABSTRACT: Polycarboxybetaine esters (PCB-esters) can condense plasmid DNA into nanosized polyplexes for highly effective gene delivery with low toxicity. The design and characterization of tertiary CB-ester monomers and PCB-ester polymers are presented here to study the effects of molecular variation on functions important to nonviral gene transfer. Both buffering capacity and charge-shifting behavior can be tuned by modifying the distance between the charged groups and the ester size or type. A carbon spacer length (CSL) of one was found to bring the pK_a of the tertiary amine into the optimal range for proton buffering. Ester hydrolytic degradation switches this polymer from cationic (DNA binding) to zwitterionic (DNA releasing) form while conferring nontoxicity. To allow rapid and externally controlled degradation, the effect of this charge-switching behavior on DNA release from polyplexes was directly studied with a novel photolabile PCB-nitrobenzyl ester (PCB-NBE). Photoinitiated ester degradation precipitated the rapid release of 72 ± 5% of complexed DNA from PCB-NBE polyplexes. These insights reveal the key parameters important for the PCB-ester platform and the significance of charge switching to an effective and nontoxic nonviral gene delivery platform.



1. INTRODUCTION

The safe and controlled delivery of genes to targeted cells promises exceptional advancements in clinical disease treatment, next-generation vaccines, and tissue engineering.[1−3] Nonviral transfection vectors have gained increasing attention as capable platforms that mitigate the safety, immunogenicity, manufacturing, and scalability concerns of viruses.[4,5] In particular, DNA-complexing polymers are highly customizable and can be engineered with tunable parameters by which to optimize each of a vector's duties. For over a decade, polyethyleneimine (PEI) has been the benchmark in this class, mediating effective complexation and protection of anionic nucleic acids by virtue of its plentiful amines. Unfortunately, PEI remains cationic under physiological conditions and is not biodegradable, resulting in its well-known cytotoxicity through cell membrane destabilization.[6] An ideal polymeric vector would condense nucleic acids into stable polyplexes that can successfully navigate the extracellular environment and pass through the cell membrane, as well as protect genes from degradation inside and outside the target cells and assist with escape from the endosome or trafficking vesicle. However, many current polymers including PEI fall short when it comes to two final desired functions: controlled DNA release and a lack of inherent toxicity.

Many researchers have modified PEI or other well-studied cationic polymers to reduce their toxicity or boost efficiency.[7−9] Others have focused on engineering new polymers with a rational design or combinatorial chemistry approach, incorporating environmental responsiveness or biodegradability.[10−12] Most of these systems necessarily operate on a principle of compromise—one function of the vector (e.g., toxicity or DNA release) is improved at the expense of another (e.g., transfection rate or polyplex stability) until a balance is found. We have developed a gene delivery platform based on polycarboxybetaines (PCBs) modified with degradable esters, with the goal of optimizing each function without detriment to the others. PCB is a zwitterionic polymer that has recently shown many important biomedical uses.[13,14] When the anionic carboxylate groups in zwitterionic PCB side chains are esterified, the polymer is rendered cationic and thus able to bind and condense nucleic acids.[15] A tertiary amine can be introduced in place of the quaternary ammonium to make the polymer pH-responsive, buffering pH changes during polyplex trafficking and providing a mechanism via the "proton sponge" for endosomal escape.[16,17] Another key function of this platform is the hydrolytic conversion of cationic PCB esters to zwitterionic PCB. The rate of this charge shifting can be easily modified by the incorporation of different ester leaving groups and different distances between the ester and the tertiary amine groups. This appends a "smart" characteristic to this polymer; DNA is first strongly complexed by cationic PCB-esters, but is efficiently released when ester degradation reveals the anionic carboxylate groups, rendering PCB zwitterionic and nontoxic. Thus, the PCB-ester platform is of great potential

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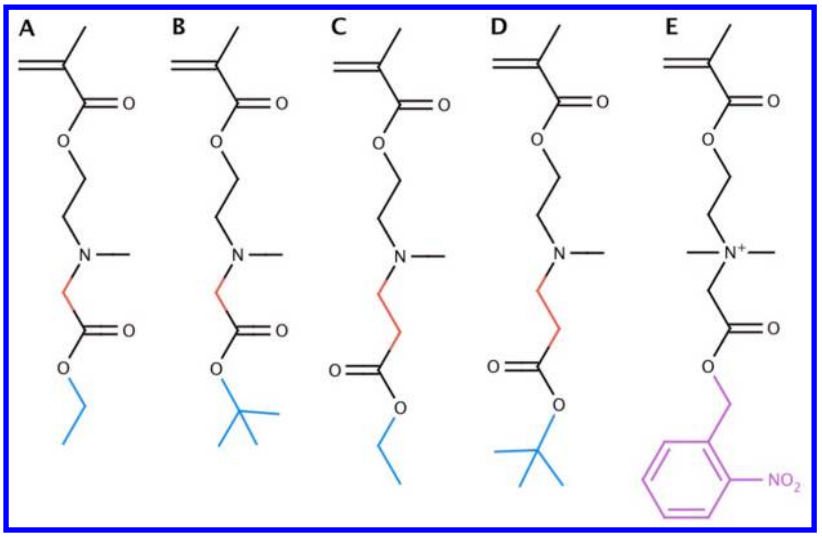
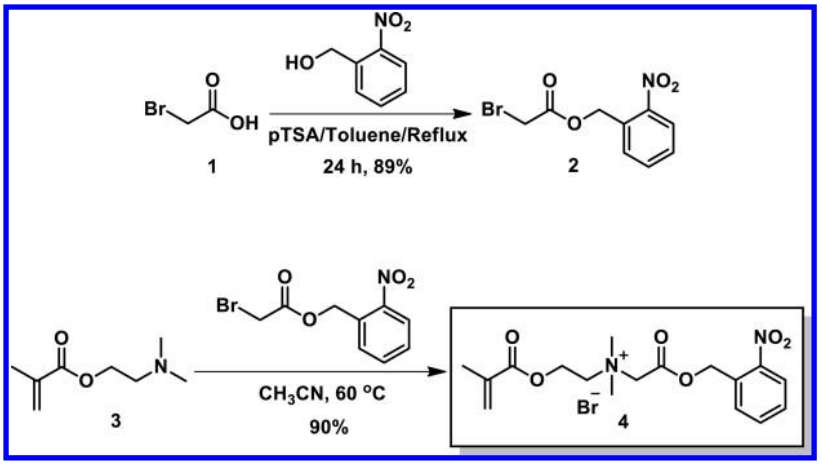


Figure 1. Structures of tertiary (A–D) and quaternary (E) carboxybetaine methacrylate esters designed in this study. Key modifications studied are color-coded: hydrolytic esters in blue, CSLs in red, and the photodegradable ester in purple. (A) CBMA1-ethyl ester (CB1-EE); (B) CBMA1-*t*-butyl ester (CB1-Tbu); (C) CBMA2-ethyl ester (CB2-EE); (D) CBMA2-*t*-butyl ester (CB2-Tbu); (E) CBMA-*o*-nitrobenzyl ester (CB-NBE).

Scheme 1. Synthesis of CBMA-*o*-nitrobenzyl-ester



because its charge-shifting ability remedies the delicate binding-release compromises of other systems.

Previously, we have demonstrated the in vitro gene transfer potential of the PCB-ester platform: an optimized tertiary/quaternary PCB-ethyl-ester copolymer mediated luciferase gene transfection approximately an order of magnitude higher than PEI, but without toxicity.[18] A similar strategy has also been applied to DNA vaccine delivery.[19] This motivated a fundamental study of how altering specific characteristics of the monomers and polymers influenced their functions. Therefore, additional tertiary monomers were developed in this study to arrive at a small library of four unique monomers. Specifically, the side chain length was varied to include either one or two carbon "spacers" denoted as a carbon spacer length (CSL) of 1 or 2 between the amine and the carboxylate/ester groups. Additionally, each chain length was synthesized with both an ethyl ester and a *tert*-butyl ester hydrolytic group. These molecular modifications result in monomers and resulting polymers with varying proton buffering capacities and hydrolytic profiles. The monomer structures are pictured in Figure 1A–D.

While the degraded, zwitterionic form of PCB does not condense DNA,[15,18] the direct contribution of the ester degradation step to DNA release deserved further study. Therefore, we developed a novel photolabile *o*-nitrobenzyl ester of polycarboxybetaine (PCB-NBE) to give this platform a UV-sensitive "switch" for active degradation control. The monomer structure of CB-NBE is shown in Figure 1E. Similar photoresponsive linkers have been utilized by others to develop photodegradable hydrogels[20,21] and release DNA from the surface of gold nanoparticles.[22] We have incorporated it into the multifunctional PCB platform to study how the charge neutralization caused by ester degradation directly catalyzes DNA release from a polyplex. The library of tertiary PCB esters and the photolabile PCB-NBE shows that the rational adjustment of buffering ability and degradation character can tune desired functions of PCB-esters and endow then with "smart" abilities.

2. EXPERIMENTAL SECTION

2.1. Materials. Chemicals used in the synthesis, purification, and characterization of CB-ester monomers and polymers, and phosphate-buffered saline (PBS, 138 mM NaCl, 2.7 mM KCl, pH = 7.4) were purchased from Sigma-Aldrich (St. Louis, MO). Dulbecco's Modified Eagle Medium, fetal bovine serum, nonessential amino acids, penicillin-streptomycin, and PicoGreen kit were purchased from

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Invitrogen Corp (Carlsbad, CA). COS-7 cells were purchased from the American Tissue Culture Collection (Manassas, VA). All water used had been purified to 18.2 mU with a Millipore Simplicity water purification system.

2.2. Synthesis of Tertiary (3°) Carboxybetaine Esters. Detailed synthesis procedures, schematics, and characterization of tertiary amine carboxybetaine ester monomers can be found in the Supporting Information, sections S1−S2.

2.3. Synthesis of CB-Nitrobenzyl Ester Monomer (CB-NBE). To design a photoresponsive ester of PCB able to fully switch from cationic to zwitterionic upon irradiation, we decided to synthesize an *o*-nitrobenzyl CBMA monomer as a starting point. Polymerization of a photolabile ester monomer ensured each PCB side chain is functionalized, for a more complete charge shift than postpolymerization esterification. The *o*-nitrobenzyl photoresponsive group was chosen based on its recent prevalence in photodegradable linker chemistries for biomedical applications.[23,24] The monomer was synthesized in a two-step process, as shown in Scheme 1. First, 2-nitrobenzyl-bromoacetate (**2**) was generated. Bromoacetic acid (11.2 g, 80.6 mmol), *p*-toluenesulfonic acid (2.78 g, 16.1 mmol), and 2-nitrobenzyl alcohol (14.8 g, 96.6 mmol) were successively dissolved in anhydrous toluene (200 mL). The solution was refluxed at 120 °C for 24 h and toluene was removed via evaporation on a rotovap. The resulting residue was dissolved in ethyl acetate (200 mL), and the organic solution was washed with H_2O (4 × 50 mL). The organic phase was dried over Na_2SO_4, after which the solvent was removed under reduced pressure and the residue was purified by silica gel chromatography using a gradient of pure hexane to hexane:ethyl acetate 3:1. The pure product was obtained as a light yellow oil (19.6 g, 71.5 mmol). Yield: 89%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.99 (d, 1H, J = 8.0 Hz), 7.59 (m, 1H), 7.43 (m, 2H), 5.49 (s, 2H), 3.89 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 166.3, 146.8, 133.7, 130.8, 128.7, 128.5, 124.7, 63.9, 25.3. Following this, CBMA-nitrobenzyl-bromoacetate (CB-NBE; **4**) was synthesized. 2-Nitrobenzyl-bromoacetate (**2**; 19.5 g, 71.1 mmol) was dissolved in anhydrous acetonitrile (150 mL). Dimethylaminoethylmethacrylate (DMAEMA; 10.8 mL, 63.3 mmol) and hydroquinone (150 mg, 1.36 mmol) were added to the mixture and the solution was stirred at 60 °C for 18 h. The solvent was removed under reduced pressure and ether (400 mL) was added to the oily residue. The resulting suspension was stirred for 4 h and the ether phase was decanted. The process was repeated three times and the residue was concentrated on the rotovap. The resulting oil was dried further under high vacuum to afford the pure product as a yellow oil (24.6 g, 57.0 mmol). Material was protected from light to avoid unintentional degradation. Yield: 90%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.05 (d, 1H, J = 8.3 Hz), 7.71 (m, 1H), 7.47 (m, 2H), 6.68 (s, 1H), 6.02 (s, 1H), 5.53 (s, 2H), 5.09 (s, 2H), 4.60 (m, 2H), 4.26 (m, 2H), 3.59 (s, 6H), 3.35 (s, 3H), 1.82 (s, 3H).

2.4. Titration of Tertiary CB-Ester Monomers. To titrate the monomers, 5 μL of each monomer was dissolved in 0.1 M NaCl to a concentration of 1 mg/mL. The pH of the solution was lowered to 2 with 1 M HCl. Initial titration curves were obtained with sequential 0.1 mL additions of 0.1 M NaOH, with sufficient time between each addition to allow for pH stabilization. After initial curves were obtained and regions of buffering were identified, more thorough titration curves were obtained with additions of 0.02 mL 0.1 M acid or base in the buffering regions. Buffering capacity was calculated with molar equivalency.

2.5. Hydrolysis Rates of Tertiary CB-Ester Monomers. Hydrolytic rates of the tertiary CBMA-ethyl-ester and CBMA-*t*-butyl-ester monomers were determined using reverse-phase high performance liquid chromatography (HPLC), with a C18 column (Econosil, 250 × 4.6 mm, 5 μm, Alltech, Deerfield, IL, U.S.A.), and a UV detector (wavelength of 227 nm). Monomers at a concentration of 1 mg/mL in 100 mM sodium phosphate (pH 7.4) or 100 mM sodium citrate buffer (pH 5.1) were held at 37 °C. A chromatography buffer solution of 0.50 vol/vol acetonitrile and aqueous sodium phosphate (100 mM) was used. This buffer caused the monomer to elute based on hydrophilicity; hydrolyzed monomers are significantly more hydrophilic due to their zwitterionic character.[25] Peak areas were normalized and compared to show hydrolytic rate over 48 h. The photodegredation rate of the UV-sensitive CBMA-nitrobenzyl-ester monomer was determined with a similar method. A hand-held UV lamp emitting 365 nm wavelength light was positioned directly above an open-top sample of a 1 mg/mL solution of CB-NBE in pH 7.4 NaAc in a shallow 3 cm^2 glass dish for a radiation rate of 10 mW cm^{-2}. A 10 μL sample was removed after each 5 min until a complete shift in peak areas confirmed photodegredation was complete.

2.6. PicoGreen DNA Binding and Release Quantification. PicoGreen quantitative binding assays were adapted from Green et al.[12] PCBMA-NBE and quaternary PCBMA-EE solutions were prepared at 1 mg/mL in 25 mM NaAc buffer, pH 5.2. Then, 50 μL/well of 30 μg/mL gWiz-luciferase DNA plasmid was added to each well of a 96-well plate. The polymer solutions were diluted to correspond to [polymer/DNA] weight ratios from 1:1 to 20:1 and added to the DNA samples. Initial DNA concentration in each well was set to 1 μg/mL and then condensed with PCB-NBE or PCB-EE in NaAc buffer. Solutions were gently mixed with pipetting and allowed to sit for 10 min for polyplex formation. Half of each sample was transferred to another open-top plate and exposed to 365 nm UV light for 1 h via a hand-held lamp. Then, 100 μL/well of PicoGreen solution was added. PicoGreen working solution was prepared by diluting 80 μL of the purchased stock into 15.2 mL NaAc buffer. After 5 min, 30 μL/well of polymer−DNA−PicoGreen solution was added to 150 μL/well of NaAC in black 96-well polystyrene plates. The plate fluorescence was then measured on a Perkin-Elmer Victor 3 plate reader using a FITC filter set (excitation 485 nm, emission 535 nm). The relative fluorescence (RF) was calculated using the following relationship:

$$\mathrm{RF} = (F_{\mathrm{sample}} - F_{\mathrm{blank}})/(F_{\mathrm{DNA}} - F_{\mathrm{blank}}) \quad (1)$$

where F_{sample} is the fluorescence of the polymer−DNA−PicoGreen sample, F_{blank} is the fluorescence of a sample with no polymer or DNA (only PicoGreen), and F_{DNA} is the fluorescence of DNA−PicoGreen (no polymer), but an equivalent amount of DNA. The binding percentage was determined by 1 − RF, because DNA bound to polymer or entrapped in a polyplex does not contribute to dye fluorescence.[12] A standard curve of free DNA concentration was used to ensure linear correlation between free DNA content and fluorescence for all measurements and quantify the free DNA concentration of the samples.

3. RESULTS AND DISCUSSION

To study the influence of molecular variations on the buffering capacity and degradation behavior of tertiary PCB esters, four monomers were synthesized for this study. These monomers are referred to as CB1-EE, CB2-EE, CB1-Tbu, and CB2-Tbu, in reference to their carbon atom spacing length (CSL) between the charged groups (1 or 2) and identity of ester leaving group (2-carbon ethyl ester or 4-carbon *tert*-butyl).

3.1. Buffering Capacity of Tertiary CB Esters. Monomers were titrated to identify the pK_a of their tertiary amines, with the goal to evaluate how the chemical structure of the monomers affects their potential buffering capacities. For clinical applications, a vector encounters changes in pH as it is trafficked to its target, which can degrade DNA if it is not sufficiently protected. Endocytosed materials are exposed to an increasingly acidic environment; the pH in the endosome is lowered via proton pumps in the endosomal membrane, from physiological pH of 7.4 to approximately 5. Therefore, materials with a pK_a and buffering capacity in this endosomally appropriate range can absorb protons as they are pumped into the endosome and help to create an osmotic pressure gradient across the endosomal membrane that may ultimately lead to membrane rupture and endosomal escape, though this "proton sponge" mechanism is still being studied.[16,17,26,27] One

reason for the high transfection efficiency of PEI may be due to its buffering capacity in this range.[28] Dimethylaminoethyl methacrylate (DMAEMA) has been used as a gene transfer vector, but mediates inferior transfection to PEI.[29,30] The pK_a of its tertiary amine is around 8.4, which is not well-suited for endosomal buffering.[31] Both of these polymers remain substantially protonated at physiological pH, leading to post-transfection toxicity.

The monomers in this study have a tertiary amine like DMAEMA, but an electron-withdrawing carboxylate ester connected to each amine. The proximity of this ester group decreases the pH required to protonate the amine, shifting the pK_a and buffering range down into the pH environment of the endosome, as compared to DMAEMA. Changing the separation distance between the amine and ester changes the extent of this shift. All of these monomers featured either a one- or two-carbon distance (CSL = 1 or 2) between the ester and amine and exhibited pK_as that were significantly lower than the pK_a of DMAEMA. The four monomers showed pK_as in two distinct subsets: CSL 1 monomers had pK_as of 5.5−6 and CSL 2 monomers had pK_as of approximately 7. Titration curves and the molar buffering capacities of the monomers are shown in Figure 2. These data show that the pK_a is sensitive to the proximity of the electron-withdrawing group down to a one-carbon difference in distance. The pK_a values imply that half of the amines on the CSL 2 monomers will be protonated (cationic) at physiological pH, which may aid in DNA packaging. Conversely, few of the amines in the CSL 1 monomers will be protonated at physiological pH, and the fraction available for endosomal buffering will greatly increase. We expect CB esters with this shorter CSL to mediate optimal DNA protection. Their inclusion in copolymers could extend the polymer buffering range to a lower pH without resulting in leftover charge that contributes to toxicity. The pK_a of a posthydrolysis CSL 1 monomer (tertiary CB1) is 8.3, very similar to that of DMAEMA. Importantly, this implies that after DNA delivery and transfection, the polymeric residue of the vector will be almost entirely protonated at the amine and unprotonated at the carboxylic acid, rendering it zwitterionic and biocompatible. The size of ester leaving group (ethyl vs *tert*-butyl) had no significant effect on the pK_a, showing that the ester size can be changed without sacrificing the desired buffering range.

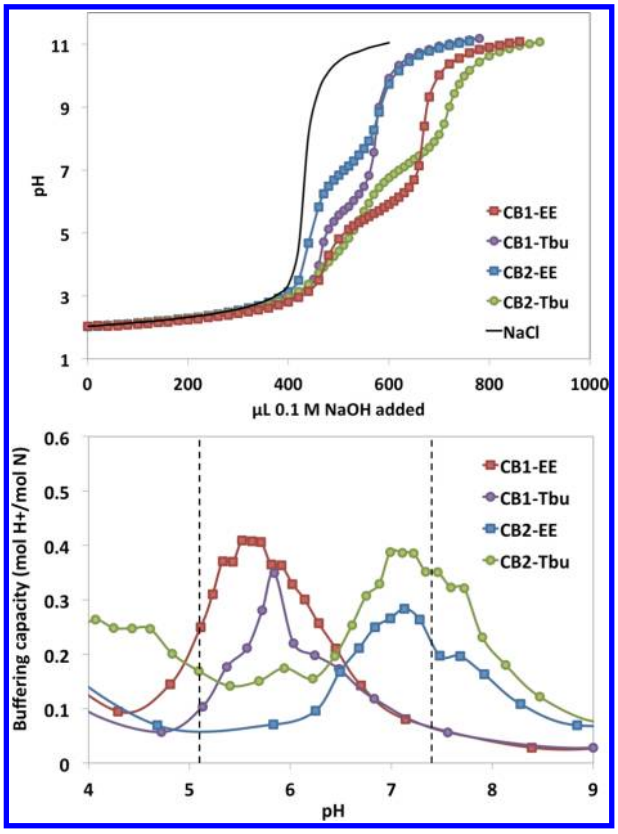

Figure 2. (Top) Titration of monomers, showing their pH buffering ability, tested in 100 mM NaCl. Region of interest is between pH 5.1 and 7.4. (Bottom) Molar buffering capacity of the tertiary amine in each monomer. Vertical dashed lines show the pH range present in the endosome. Curve peaks correspond to the pK_a of each monomer.

3.2. Hydrolytic Degradation Profiles of Tertiary CB Esters. While others[32,33] have incorporated hydrolytically degradable bonds in polymers, the wide variation in possible hydrolysis profiles necessitate a more discriminating selection of ester leaving group. For example, it could be rationalized that a bond stable at physiological pH (7.4) but labile at the lower endosomal pH (5.1) would ensure that the DNA stays protected for cellular uptake. However, such a strategy may result in DNA release and degradation within the acidic environment of the endosomal pathway.[34] An ester more stable in acidic conditions may remain intact in the endosome, but if it is overly labile at a higher pH, it may experience premature degradation. Therefore, an ester relatively stable at both pH conditions may be desirable, and it is important to study their hydrolytic profiles to help identify unsuitable leaving groups and provide design insight.

The hydrolysis of each ester was monitored over a period of 40 h with reverse-phase HPLC. As seen in Figure 3, CB2-EE and CB1-Tbu monomers exhibited pH-dependent hydrolysis behavior. At physiological conditions, the CB2-EE hydrolyzed much more rapidly, reaching more than 80% hydrolysis after 40 h, but only degrading 20% over the same time period at endosomal pH. Conversely, CB1-Tbu was more stable at physiological conditions than endosomal ones. The CB1-EE and CB2-Tbu esters were relatively stable at both physiological (pH 7.4) and endosomal (pH 5.1) conditions. These data show that the parameters of the CB-ester platform can be modified to confer ester stability while maintaining other desirable functions, such as an optimal buffering range. It is apparent that both types of molecular design variations, the ester size and amine/ester CSL, contribute to the stability of these esters. The protonation state of the amines (dependent on CSL) may thus play a role. While this could explain the ester stability difference at physiological pH between CB1-EE (deprotonated amine) and CB2-EE (protonated amine), a larger library of monomers is needed to support this hypothesis.

Overly rapid hydrolysis at any relevant pH may sacrifice polyplex stability. Of the two most stable monomers, CB1-EE features a more suitable pK_a for endosomal buffering. The hydrolytic product of ethyl esters, ethanol, also is of lowest toxicity.[35] In further platform development, we will use these insights to design additional multifunctional and nontoxic polymers and study the potential role played by enzymes in the cell.

3.3. Transfection and Toxicity of Tertiary PCB Esters. Tertiary PCB-ester copolymers were used to mediate luciferase expression in COS-7 cells, and the polymer toxicity was evaluated. As our previous work found 3:1 to be the optimal

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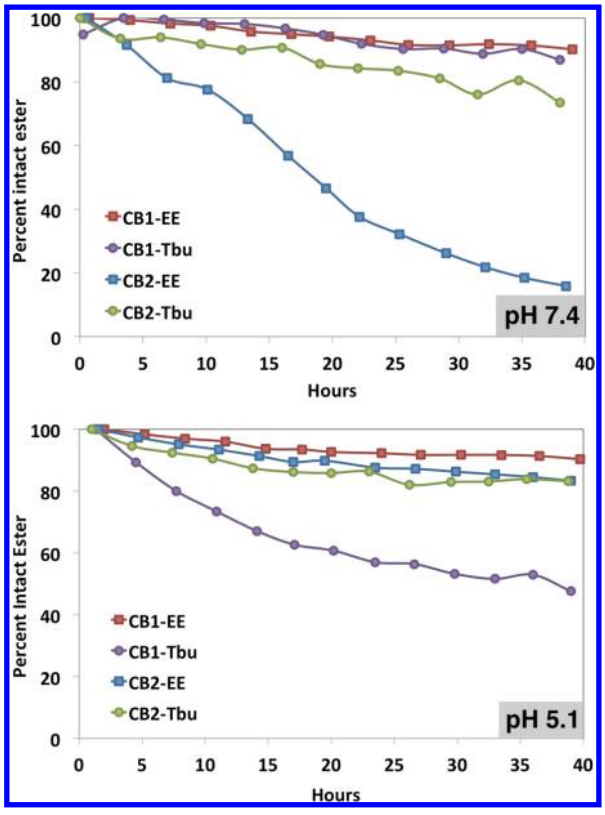

Figure 3. Hydrolysis rates of tertiary CB-ester monomers. (Top) Hydrolytic degradation of PCB-esters at pH 7.4, representative of physiological pH. (Bottom) Degradation at pH 5.1 under the same conditions, representative of lowest endosomal pH.

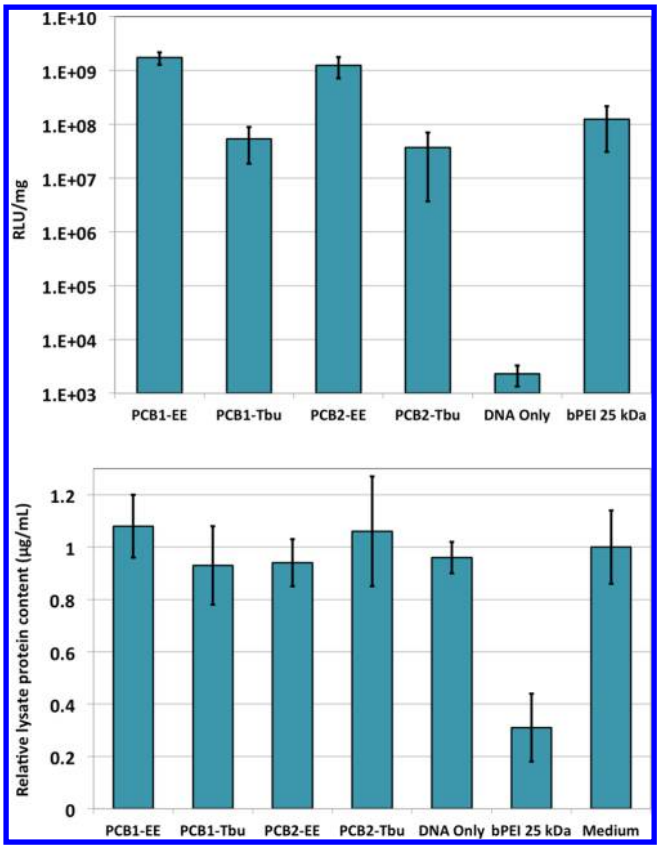

Figure 4. Transfection efficacy and toxicity of tertiary-dominated PCB-ester copolymers. (Top) Luciferase expression in COS-7 cells mediated by each tertiary copolymer variation (RLU/mg lysate protein). (Bottom) Relative toxicity, determined by BCA assay of μg protein content in mL cell lysate, normalized to medium only.

tertiary to quaternary amine ratio in PCB side chains, all polymers were generated with this monomer ratio in the reaction mixture. RAFT polymerization resulted in the generation of well-controlled molecular weights for each polymer, but increased MW beyond 10 kDa (up to 40 kDa) did not result in a statistically significant transfection increase. Therefore, 10 kDa polymers were used to control for this parameter. A nitrogen/phosphorus (N/P) ratio of 20 was found to result in optimal expression for all PCB-based polymers, while N/P ratios of 5 and 10 were used for the 25 kDa bPEI positive control. Copolymers based on the four tertiary CB-esters were all able to mediate transfection without any apparent cytotoxicity. Notably, the two copolymers featuring ethyl esters (PCB1-EE and PCB2-EE) resulted in expression about an order of magnitude higher than both those featuring *tert*-butyl esters (PCB1-Tbu and PCB2-Tbu) and bPEI. Transfection and toxicity data are shown in Figure 4. The higher stability of ethyl esters at the lower endosomal pH (PCB2-EE) or both pH conditions (PCB1-EE) might play a role in their improved transfection, potentially by delaying any DNA release until the polyplexes escape from endosomes.

While more stable esters may complex DNA more efficiently and protect it for a longer time, substantial hydrolysis delay may bottleneck gene release. As the charge-switching behavior of PCB-esters is their key feature, the direct contribution of this charge switch to DNA release from a polyplex deserved further study. This motivated the design of a PCB-ester material capable of rapid degradation upon external stimulus.

3.4. Controlled Degradation of PCB-NBE and DNA Release from Polyplexes. PCB-NBE, a photolabile *o*-nitrobenzyl ester of PCB, was developed to quantitatively study the charge-switching contribution to DNA release. Photoconversion of CB-NBE was verified with reverse-phase HPLC; after 1 h, degradation was complete.

We quantitatively measured DNA binding and release in PCB-NBE polyplexes with a PicoGreen assay, in which the fluorescence signal corresponds to the concentration of free (unbound) DNA over a wide range of magnitudes.[12,36] An initial screening (Figure S2) determined the minimum weight ratio of PCB-NBE and PCB1-EE required to condense nearly all DNA (over 95%) was 5:1. As shown in Figure 5, both PCB1-EE and PCB-NBE at this ratio complexed nearly all the DNA in each sample, quenching fluorescence. A 365 nm wavelength UV lamp was held directly above each polyplex sample for 1 h. This wavelength was chosen because it is least harmful to cells[21] but still catalyzes degradation of the nitrobenzyl ester. The released DNA in these irradiated samples bound to the fluorescent dye, restoring fluorescence. Photoinitiated ester degradation released 72 ± 5% of the DNA from the PCB-NBE polyplexes as separation of the ester converted them to zwitterionic PCB. The nonphotosensitive PCB1-EE released a statistically insignificant amount under the same conditions, as expected based on its hydrolytic profile. Previously, the postdegradation form of PCB esters have been simply shown not to *condense* DNA[15,18] due to their zwitterionic nature. Here, this photolabile polymer shows that the charge-switching step plays the key role in *releasing* DNA from an entangled polyplex, which is vital for transfection. This

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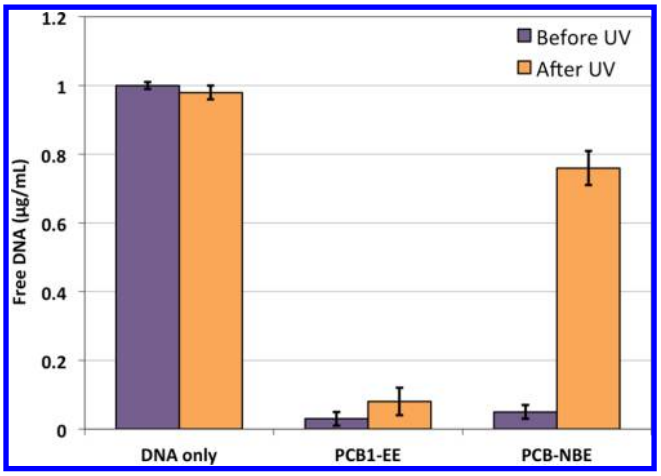

Figure 5. DNA release from polyplexes by photodegredation of esters. PCB-NBE polyplexes released 72 ± 5% of their entrapped DNA when polyplexes were exposed to 365 nm UV light to rapidly degrade side chain esters. The limited release by the nonphotolabile PCB1-EE control shows that the polyplex dissolution was due primarily to ester degradation.

result supports one of the key benefits of the PCB-ester system: since tunable hydrolysis or photoresponsive "smart" ester degradation is the primary driver of DNA release, strong initial binding need not bottleneck eventual polyplex dissociation. Thus, unlike many other platforms, PCB-esters do not need to establish a fragile balance between DNA binding and release, but these parameters can be engineered individually.

4. CONCLUSIONS

This study demonstrates how rational molecular design can tune the functionality of PCB-esters and add "smart" responsiveness to environmental stimuli. A small library of CB-esters was designed, synthesized, polymerized and characterized to study the role of small modifications on the platform's buffering and hydrolytic characteristics. A carbon spacer length (CSL) of 1 between the charged groups was found to shift the pK_a of the tertiary amine into the optimal endosomal buffering range. Ester hydrolysis converts this polymer from cationic (DNA binding) to zwitterionic (DNA releasing) form, while conferring nontoxicity. CB1-EE was most stable at both physiological and endosomal pH, while possessing the highest buffering capacity. A copolymer dominated by the CB1-EE monomer mediated transfection an order of magnitude better than bPEI, with no toxicity. To further study how the charge-switching feature affects polymer binding with DNA in polyplexes, a photolabile *o*-nitrobenzyl ester of PCB (PCB-NBE) was developed to allow rapid and externally controlled hydrolysis. Quantitative PicoGreen DNA binding assays demonstrated that photoinitiated ester degradation precipitates rapid release of approximately 72% of complexed DNA from PCB-NBE polyplexes. Further work will expand the library of CSL-1 nontoxic PCB-esters, and study the role of enzyme-catalyzed hydrolysis. These monomers provide crucial insight to the design of buffering character and charge switching in an effective and nontoxic nonviral gene delivery platform.

■ ASSOCIATED CONTENT

Supporting Information

Detailed synthesis procedures and schemes of tertiary and quaternary carboxybetaine ester monomers, polymerization, and characterization details, and the in vitro transfection protocol. 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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