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Controlled release of DNA/polyamine complex by photoirradiation of a solid phase presenting *o*-nitrobenzyl ether tethered spermine or polyethyleneimine

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Abstract—Various gene transfer and automated/monitorized analytical applications require the controlled release of nucleic acid. A solid phase with spermine or polyethyleneimines (PEI, 600 MW) tethered by *o*-nitrobenzyl linkages was synthesized with polyethylene oxide beads (Argo*Gel*-NH_2). The photolysis of test compound *O*-2-nitrophenethyl *O*,*O*-diethyl phosphate or solid phase with *o*-nitrobenzyl group as synthetic linker was completely degradable with photoirradiation at 365 nm for 10–18 min at 3.5 mW/cm^2. DNA binding with polyamine of the solid phase and releasing of DNA/polyamine were monitored by UV measurement and gel electrophoresis. The potential exists to employ a DNA-loaded solid phase for spatially, temporally, or dose-controlled release of DNA, at extracellular or intracellular sites.

Several strategies have been tested for gene delivery in vitro and in vivo. Recent studies of gene therapy employ the release of DNA from solid phase.[1] The solid phase release of DNA has been tested with suture coatings,[2] bone implants,[3] tissue engineering scaffolds,[4] vaccines,[5] polymeric stent coating,[6] and printed micro-assays.[7] The mechanism of release typically involves polymer erosion or DNA desorption. Also, various high throughput methodologies require the use of a solid phase for capture, purification or alteration of DNA.

However, the ability to control the temporal and spatial release of DNA from a solid phase presents a challenging design problem where the strong binding and specific release of DNA are competing design goals. For example, the binding of biotinylated DNA with streptavidin-coated polystyrene particles facilitates only the binding of DNA.[8]

Polyamines such as polylysine, spermidine, spermine, and polyethyleneimine (PEI) can complex and condense polyanions such as DNA via strong electrostatic interactions through multiple amine positive charges.[9–11] Linking such polycations to a solid phase presents several unique advantages with respect to nucleic acid pharmaceutic or analytical applications. The solid phase organic synthesis has emerged as a powerful methodology due to several merits such as simple reaction, easy separation of supported species and products, and application to combinatorial automation system.[12–14] Various strategies can be applied to release the DNA from the solid phase. Harding et al. reported the synthesis of the Sepharose bead containing the intercalator and a spermine linker as DNA-binding reagent and DNA releasing by base (NaOH or KOH).[15] However, the use of these bases is not amendable to work in vivo or in vitro or with enzymes typical of analytical molecular biology.

Thus, we synthesized solid phase supports presenting polyamine as efficient DNA-binding agents. Furthermore, the controlled release of DNA from the solid phase in complexation with a polyanion has also been designed. The *o*-nitrobenzyl group has been employed as a useful tool for understanding biological processes through photoirradiation.[16–19] Therefore, we selected *o*-nitrobenzyl phosphonate[20] as a photocleavable linker

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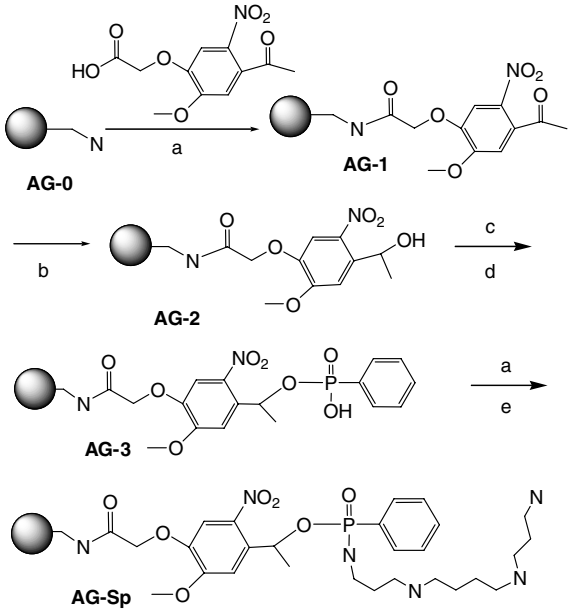
to examine controlled release of the DNA complex from their solid phase under photoirradiation. This article describes the synthesis of solid phase with polyamines, and DNA binding and controlled release of a DNA/polycation complex triggered by photoirradiation.

The synthetic procedure is shown in Scheme 1. First, a nitroacetophenone derivative with an acid group was attached to amine resin (Argo*Gel*-NH_2) to yield the derivatized resin AG-1. For this reaction, the 4-acetyl-2-methoxy-5-nitrophenoxy acetic acid (**1**)[21] was activated with *N*-hydroxysuccinimide (NHS) and *N*,*N*-dicyclohexylcarbodiimide (DCC), and reacted at a 5-fold molar excess with the amino resin to maximize yield.[22] The AG-1 resin showed negative Kaiser test, indicating complete consumption of amine. Reduction of AG-1 was carried out with $NaBH_4$ to give AG-2.[23] In the subsequent step, the AG-2 was reacted with phenyl phosphonic dichloride, followed by reaction with water to give resin AG-3 containing phosphonic acid.[24] Finally, addition of spermine or PEI was carried out with NHS, DCC, and the resin AG-3 to produce AG-Sp or AG-PEI.[25,26] All resins were characterized by ^1H NMR spectroscopy (Fig. 1). The ^1H NMR spectra of the final resins showed the signals of spermine or PEI attached to the resin as well as the other groups associated with the *o*-nitrobenzyl linkage. Additionally, the resin showed increased N content from elemental analysis, indicating the attachment of the polyamine to the resin.[27]

Prior to photocleavage studies of AG-Sp or AG-PEI, we explored the kinetics of breakdown of a soluble mimic. The ^1H NMR spectroscopic change of *O*-2-nitrophenethyl *O*,*O*-diethyl phosphate (NDP) as a control model was monitored in $CDCl_3$ to examine the photodecomposition behavior. In the spectrum after irradiation, signals of NDP completely disappeared. Signals assignable to methylene and methyl protons of *O*,*O*-diethyl phosphoric acid and nitrosoacetophenone formed by irradiation appeared at 2.72, 4.11, and 1.35 ppm, respectively. NDP showed complete decomposition at 10 min under irradiation (365 nm, 3.5 mW/cm^2). The UV spectrum of nitrosoacetophenone formed after irradiation showed absorbance around 320 nm. The decomposition by irradiation was carried out with AG-Sp.[28] After irradiation for 18 min, the reaction mixture was separated by filtration and washed with methanol. Figure 2 shows the ^1H NMR spectra of (A) AG-Sp, (B) solvent-insoluble, and (C) solvent-soluble parts after irradiation. The solvent-soluble part showed the signals of the phenyl phosphonic functionalized spermine assignable to benzene ring and methylene of spermine at 7.2–7.5, 3.1, 1.8, and 1.4 ppm, respectively (Fig. 2C). The solvent-insoluble part showed signal of only the Argo*Gel* lacking spermine (Fig. 2B). This result indicated that *o*-nitrobenzyl group on AG-Sp was completely labile with photoirradiation.



Scheme 1. Reagents: (a) NHS, DCC, THF; (b) $NaBH_4$, EtOH; (c) $Cl_2P(=O)Ph$, pyridine, CH_2Cl_2; (d) NaOH, H_2O; (e) spermine, CH_2Cl_2.

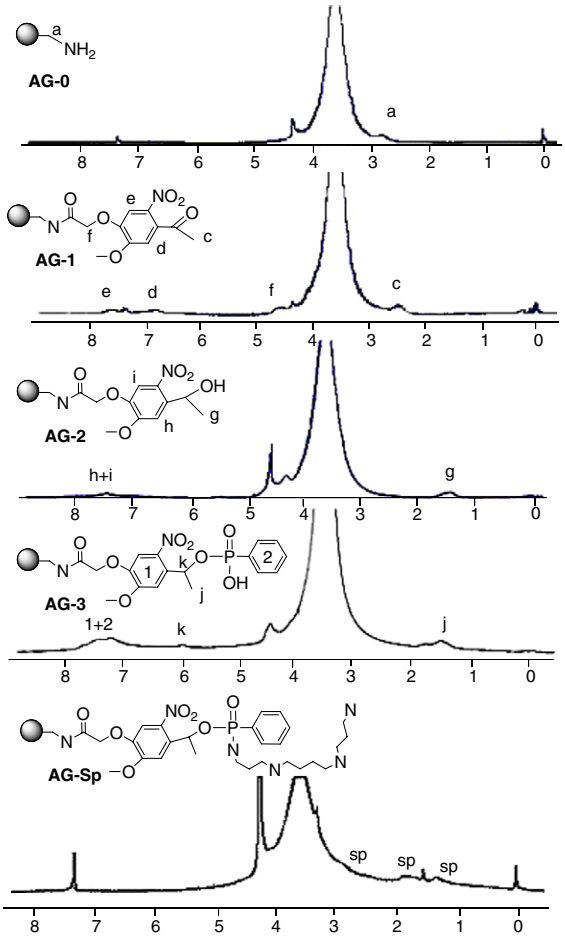


Figure 1. ^1H NMR spectra of AG-0–AG-Sp.

To test for DNA capture and photolytic release, DNA was complexed with the spermine on AG-Sp. Figure 3 shows the UV absorbance of various components. UV absorbance of only DNA solution was 0.0156 at

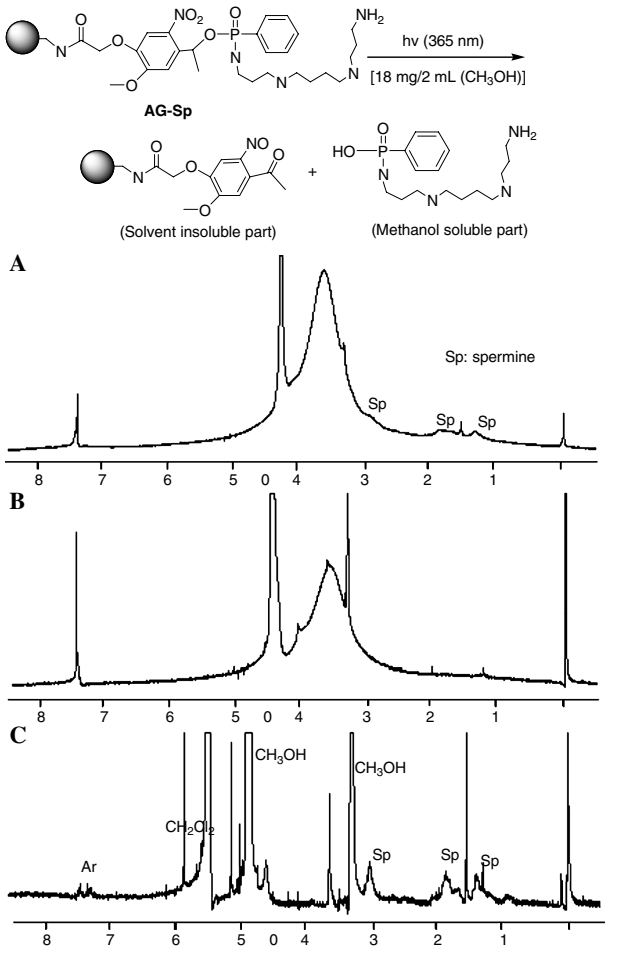


Figure 2. ^{1}H NMR spectra of (A) AG-Sp, (B) solvent-insoluble part, and (C) solvent-soluble part after irradiation for 18 min.

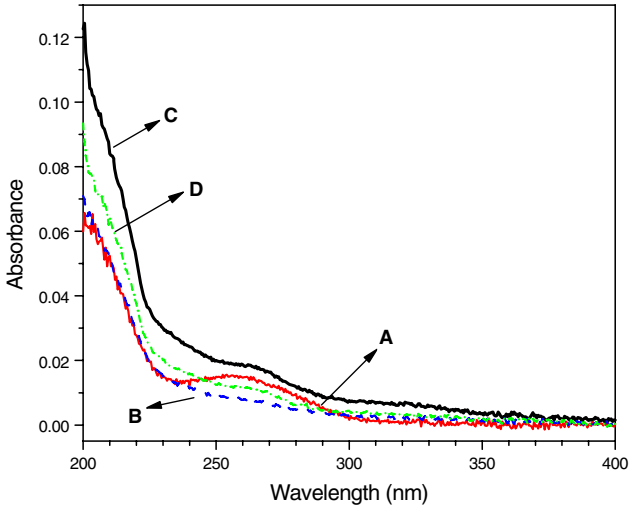


Figure 3. UV spectra of (A) only DNA, (B) before, (C) after irradiation of AG-Sp solution after addition of DNA, and (D) solution after irradiation of only AG-Sp. (DNA/nuclease-free water = 0.1099 μg/300 μL).

260 nm (A), while the supernatant solution after the addition of DNA into the AG-Sp solution showed absorbance of 0.0080 (B). This result indicates that DNA was captured by AG-Sp. Photoirradiation was carried out for the solution to examine photorelease of the DNA/spermine complex from solid phase. The absorbance of solution beads removed was 0.0199 at 260 nm after irradiation for 18 min (C). AG-Sp only as control was shown absorbance of 0.0117 after irradiation for 18 min (D). The slightly higher absorbance of the released complex was likely due to absorbance interface of AG-Sp. The UV spectrum after irradiation also showed slight absorbance as a shoulder around 320 nm due to nitrosoacetophenone formed by decomposition of AG-Sp.

To confirm the binding of DNA and release of DNA/polyamine complex by irradiation, agarose gel electrophoresis was also carried out for the solution taken from the DNA/solid phase without and with irradiation (Fig. 4).[27] Lanes b and c did not show any detectable DNA, indicating that spermine of AG-Sp completely captured the DNA. While lane e after irradiation showed some DNA in the loading well, DNA did not migrate freely through the gel because it was condensed with spermine. In order to release DNA from spermine complexes, polyanionic heparin was added to DNA/solid phase taken before irradiation (lane d) and the solution taken from the DNA/solid phase after irradiation (lane f). DNA was visualized by the addition of heparin. Lane d indicated DNA release by heparin exchange from spermine of solid phase in the condition without irradiation. Lane f demonstrated the DNA release from DNA/spermine complex formed by irradiation. However, lane f showed a partial transformation of the DNA from a supercoiled to an open circular form, maybe due to photoirradiation. Gel electrophoresis using AG-PEI showed similar result with AG-Sp.

In conclusion, we synthesized a spermine or PEI modified solid phase with *o*-nitrobenzyl group as a photolabile linker. The polyamine of the solid phase allowed capture of DNA. The DNA/polyamine complex was then released using light without the need for high temperature, high salt, or acidic/basic conditions. This approach is useful for solid phase presentation of DNA

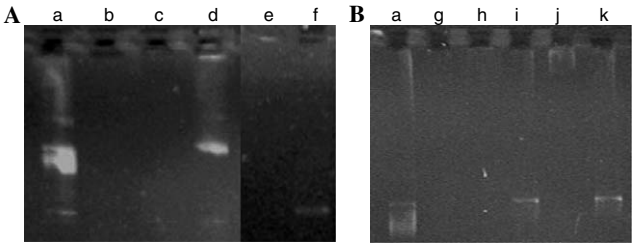


Figure 4. Gel electrophoresis of (A) AG-Sp and (B) AG-PEI. Lane a, naked DNA; lane b, solution taken from DNA/AG-Sp; lane c, heparin + solution taken from DNA/AG-Sp; lane d, heparin + DNA/AG-Sp; lane e, solution taken from DNA/AG-Sp after irradiation; lane f, heparin + solution taken from DNA/AG-Sp after irradiation. Lane g, solution taken from DNA/AG-PEI; lane h, heparin + solution taken from DNA/AG-PEI; lane i, heparin + DNA/AG-PEI; lane j, solution taken from DNA/AG-PEI after irradiation; lane k, heparin + solution taken from DNA/AG-PEI after irradiation. (DNA was visualized by ethidium bromide staining on 1% agarose gel).

with triggered release, amenable to control of time of release, position of release, and amount of DNA release. Further research on the kinetic studies of binding and releasing of DNA with polyamine of solid phase is now in progress.

Acknowledgments

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21. Compound **1**: ^{1}H NMR (CD_3OD): δ 7.62 (s, 1H, C_6H_2), 7.05 (s, 1H, C_6H_2), 4.83 (s, 2H, $-CH_2-$), 4.00 (s, 3H, OCH_3), 2.47 (s, 3H, CH_3). ^{13}C NMR (CD_3OD): δ 202.0, 171.6, 168.5, 155.9, 134.6, 110.8, 66.9, 57.3, 30.4. Anal. Calcd for $C_{11}H_{11}NO_7$: C; 49.08, H; 4.12, N; 5.20. Found: C; 48.72, H; 4.02, N; 5.24.
22. AG-1: A slurry of compound **1** (400 mg, 1.5 mmol), NHS (0.26 g, 2.3 mmol), and DCC (0.46 g, 2.3 mmol) in THF (15 mL) was stirred at room temperature for 2 h under nitrogen. Argo*Gel*-NH_2 resin (600 mg, 0.27 mmol) was poured into the activated carboxylic solution. The slurry solution was stirred at room temperature for 2 days. The solvent was filtered off and the resin beads were washed successively with DMF, THF, H_2O, and MeOH, and dried in vacuo to give the AG-1 (667 mg, 0.25 mmol) as yellow solid. The Kaiser test was performed on AG-1 beads washed three times with EtOH. Bromophenol blue was added to a bead suspension and heated at 120 °C for 5 min. AG-1 remained orange (yellow), indicating the absence of free primary amine.
23. AG-2: To a suspension of AG-1 (637 mg, 0.30 mmol) in EtOH (15 mL) was added $NaBH_4$ (0.167 g, 4.4 mmol) at 0 °C. The reaction and purification procedure was carried out similar to that used for AGg-1 to give the AG-2 (633 mg, 0.29 mmol) as light brown (orange) solid.
24. AG-3: A suspension of AG-2 (150 mg, 0.068 mmol) and phenylphosphonic dichloride (0.50 g, 2.9 mmol) in THF (10 mL) was shaken at room temperature for 2 h under nitrogen. Pyridine (1.8 mL, 23 mmol) was added into the solution via syringe under nitrogen. The reaction and purification procedure was carried out similar to that used for AGg-1 to give the AG-3 (156 mg, 0.068 mmol) as brown solid.
25. AG-Sp: A slurry of AG-3 (77 mg, 0.034 mmol), NHS (0.12 g, 1 mmol), and DCC (0.21 g, 1 mmol) in CH_2Cl_2 (10 mL) was shaken at room temperature for 2 h under nitrogen. Spermine (0.29 g, 1.4 mmol) in CH_2Cl_2 (5 mL) was added into the activated phosphonic solution. The reaction and purification procedure was carried out similar to that used for AGg-1 to give the AG-Sp (82 mg) as light brown solid.
26. AG-PEI: A slurry of AG-3 (50 mg, 0.024 mmol), NHS (0.12 g, 1 mmol), and DCC (0.21 g, 1 mmol) in CH_2Cl_2 (10 mL) was shaken at room temperature for 2 h under nitrogen. PEI (0.32 g) in CH_2Cl_2 (5 mL) was added into the activated AG-3 solution. The reaction and purification procedure was carried out similar to that used for AGg-1 to give the AG-PEI (54 mg) as light brown solid.
27. Anal. Calcd (from Aldrich) for AG-0: C; 62.05, H; 9.18, N; 0.56. Found: C; 64.66, H; 9.45, N; 0.45. Found for AG-3: C; 63.72, H; 8.85, N; 0.79. Found for AG-Sp: C; 63.11, H; 8.25, N; 1.39. Found for AG-PEI: C; 62.43, H; 8.01, N; 1.81.
28. Photolysis was performed at room temperature using Model B-100A Hi Intensity UV Lamp (UVP Inc., optical density is 3.5 mW/cm^2 at 30 cm) at a distance of 10 cm. A volume of 2 μL DNA (1.099 μg/μL) was added to 200 μL of AG-Sp (4 mg). The mixture was gently mixed and incubated at room temperature for 20 min. A volume of 50 μL of the supernatant (solution A) of the mixture or a small amount of the pellet AG-Sp (B) was taken before UV irradiation. The remaining solution C with AG-Sp and DNA was irradiated for 18 min. A 10 μL of heparin solution (2 mg/mL) was added to the solution A (25 μL), bead B, and solution C (25 μL) taken after irradiation. A 10 μL of each solution taken diluted in nuclease-free water (290 μL) for UV measurement.