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Covalent Modification of DNA with Azetidinium Lipids

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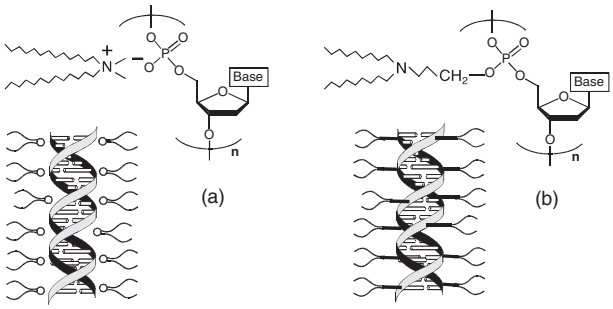
We studied covalent bond formation of an ion complex of phosphate and azetidinium and applied the reaction to preparation of DNA which was elaborated at the phosphate units with azetidinium lipids.

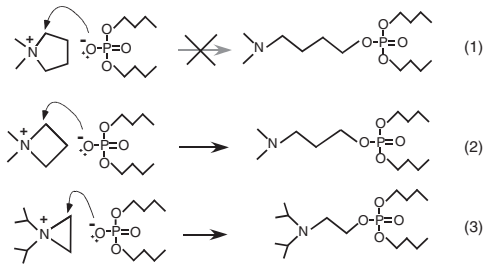
DNA has unique properties as a semiconducting polymer that shows a long rod-like duplex structure with base-pair stackings.[1] Base separation is 3.4 Å while the diameter of a duplex is about 20 Å. However, polyanion characteristics of DNA, including being soluble only in aqueous solutions, could prevent further use in material science. We have proposed DNA/cationic lipid polyion complexes as a DNA-based material (Scheme 1a).[2,3] These DNA/lipid complexes are soluble in organic media and maintain double helical structure in organic solvents.[2a,2b] Furthermore, self-supporting films can be prepared from these complexes by casting from organic solution or hot-press method.[2c,3] These films show the anisotropic conductivity along the stretched direction,[2d] however, because they are polyionic, these DNA/lipid complexes show some hydroscopicity. This characteristic is undesirable for electronic use.

In this study, we alkylated phosphate groups of DNA through a polyion complex of DNA and a reactive lipid, and the consequent hydrophobic alkylated DNA demonstrated water insensitivity in organic solvent.

We applied herein heterocyclic ammonium derivatives as phosphate alkylating agent. For instance, pyrrolidinium compounds, a five-membered ring ammonium, have been reported to react with carboxylate anions as an electrophile and to produce carboxylate esters upon ring opening.[4] Therefore, first of all, we synthesized dimethylpyrrolidinium bromide and attempted to react with dibutylphosphate in chloroform as a model reaction (eq 1 in Scheme 2). Dibutylphosphate bound to anion-exchange resins (Amberlite® IRA-400) was mixed with a methanol solution of dimethylpyrrolidinium bromide and the elution was evaporated. The resultant dimethylpyrrolidinium dibutylphosphate ion complex was dissolved in $CHCl_3$ and heated at 60 °C. However, the alkylation reaction with ring-opening was not



Scheme 1.



Scheme 2.

observed. This result would arise from the low nucleophilicity of phosphate anion compared with carboxylate.

Next, we applied a four-membered ring ammonium compound that has high strain energy to react with phosphate (eq 2 in Scheme 2). Dimethylazetidinium perchlorate was synthesized for this purpose (yield 77.8% confirmed by ^{1}H NMR).[5] Perchlorate was selected as counter anion so as not to react with azetidinium leading to a ring-opened adduct as would a nucleophilic anion such as chloride. With the same procedure as mentioned above, the ion complex of dimethylazetidinium dibutylphosphate was dissolved in $CDCl_3$ and heated at 60 °C for 0.5 h. The complete phosphate alkylation with the ring-opening reaction of azetidinium moiety was confirmed by ^{1}H and ^{31}P NMR spectroscopy (Supporting Information Figure S1).[11]

As the sufficient reactivity of the ion complex of azetidinium and phosphate was confirmed, we prepared *N*,*N*-didodecylazetidinium methanesulfonate (**1**)[6] as a phosphate reacting lipid to produce a DNA-based neutral polymer. Furthermore, the bis-*t*-butyldimethylsilyl-protected thymidine dimer **2** was also synthesized as a hydrophobic DNA model.[7] Aqueous solutions of **1** and **2** were mixed, and the resultant turbid solution was extracted with $CHCl_3$. The ion complex was purified by HPLC (Shimadzu System with TSK gel, ODS-80Ts, Toso). Confirmation of covalent-bond formation at 60 °C in chloroform was monitored by ^{1}H (Figure 1) and ^{31}P NMR spectroscopy. The ^{31}P resonance shifted from −1.6 to −1.8 and −1.9 ppm after reaction. ESI-MS of the product showed a single peak at $[M + H^+] = 1168.8$ (Supporting Information Figure S2).[11] As a result, quantitative alkylation of phosphate was observed. Furthermore, it was verified that the azetidinium amphiphile **1** does not react with thymine and ribose moieties of the DNA dimer. Therefore, we do not have to fear undesirable reaction to the nonphosphate structures of the DNA molecule. On the contrary, the more reactive three-membered aziridinium salt reacts with not only phosphates (eq 3 in Scheme 2), but also nucleobases in considerable yields.[8] Therefore, we chose the four-membered azetidinium salt instead of the three-membered aziridinium salt.

Finally, chemical modification of native DNA with the azetidinium amphiphile **1** was performed (Scheme 1b). Partially digested salmon testes DNA (ca. 300 bp, from Maruha Nichiro

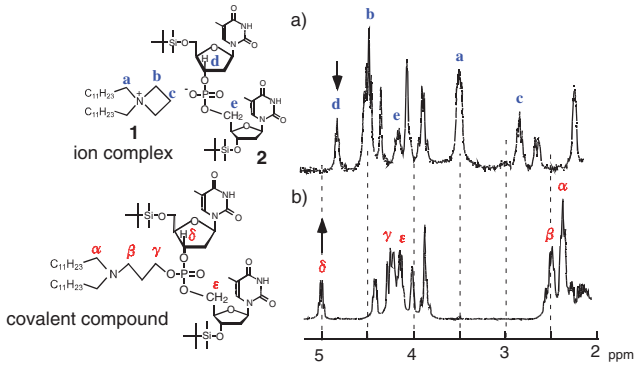


Figure 1. ^{1}H NMR spectra of (a) the ion complex of azetidinium amphiphile **1** and thymidine dimer **2** and (b) after reaction with heating at 60 °C in chloroform for 3 h. Significant signals are assigned by each character as noted in chemical structures.

Foods, Inc.)[2,3] solution was added to an aqueous solution of **1** dropwise, then the polyion complex was precipitated. After washing with water and acetonitrile, the precipitation was applied to elemental analysis, and confirmed the formation of one to one complex of **1** and DNA phosphate unit.[2] Then the polyion complex was suspended in dry chloroform, and heated at 60 °C for 10 h. The reaction solution gradually became homogeneous as the alkylation reaction progressed. We expect that all phosphate groups of DNA were alkylated with didodecylamino-propyl groups, however it was difficult to confirm it due to the broadening of both ^{1}H and ^{31}P NMR spectra and we could not get results from MALDI-TOF-MS and ESI-MS spectra. Therefore, we confirmed the effect of covalent bond formations with the azetidinium lipid **1** and DNA by CD spectroscopy.

Figure 2 shows CD spectra of the polyion complex and the alkylated neutral DNA in water-saturated and anhydrous chloroform solutions. The polyion-complex DNA showed positive Cotton effect around 270 nm with a relatively large θ value in the water-saturated chloroform, indicating that water molecules bind to the salt moiety of the minor or major groove of DNA to form B-structures.[9] In the dry chloroform, it changed to the C-form due to the dehydration from the minor or major grooves of DNA strands, showing positive Cotton effect around 290 nm with a small θ value.[10] This behavior was similar to our previous CD spectra of the polyion-complex DNA (Scheme 1a).[2,3] On the contrary, the alkylated neutral DNA showed CD spectra in between the B-form and C-form showing positive Cotton effect around 290 nm with a large θ value, and they were hardly affected by the water content of chloroform solution. This may due to the neutral triesters of phosphate containing tertiary amino groups in DNA.

In conclusion, we modified phosphate moieties of DNA covalently with a newly synthesized azetidinium amphiphile **1** in organic media. This method will provide a novel technique in the chemical modification of natural DNA. This charge-controlling reaction of DNA will be used for moisture stable engineering material that has unique conductive properties compared with native and polyion-complex DNAs. Furthermore, the tertiary amino groups of the alkylated DNA, which were introduced by the ring-opening reaction, can be cationized by protonation, and a resultant positively charged DNA derivative may demonstrate a novel conduction characteristic.

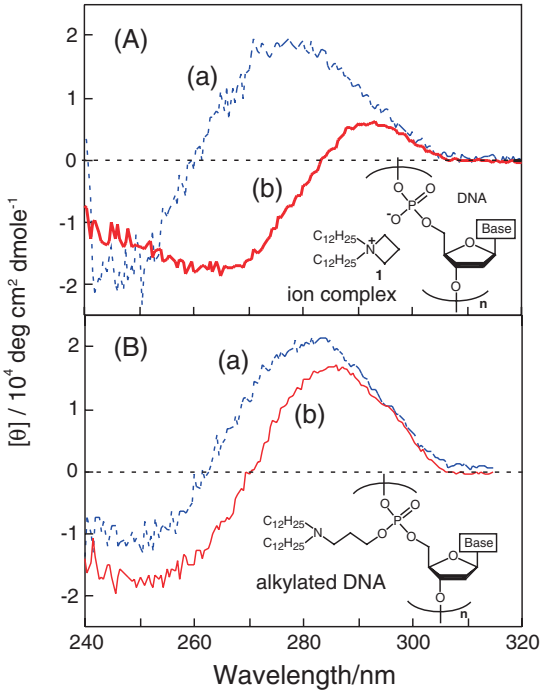


Figure 2. CD spectra of (A) the polyion complex of azetidinium amphiphile **1** and DNA (ca. 300 bp) and (B) the alkylated DNA, in (a) water-saturated chloroform and (b) in anhydrous chloroform (50 μM as DNA, at 25 °C).

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5 ^{1}H NMR (ppm, in D_2O): 2.6 (m, 2H), 3.2 (s, 6H), 4.3 (t, 4H).
6 ^{1}H NMR (ppm, in $CDCl_3$): 0.9 (*t*, 6H), 1.2–1.4 (*m*, 36H), 1.6 (*m*, 4H), 2.8 (*m*, 5H), 3.4 (*t*, 4H), 4.4 (*t*, 4H).
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11 Supporting Information is available electronically on the CSJ-Journal Website, http://www.csj.jp/journals/chem-lett/index.html.

