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Nanoparticles from Cationic Copolymer and DNA That Are Soluble and Stable in Common Organic Solvents

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The unique double helical structure of DNA along with its reversible recognition properties, ability to completely control the length and content of oligonucleotides, and the wealth of enzymes available for its modification have made DNA extremely suitable for several novel applications that are even nonbiological. Miniaturization of biosensors and biochips into the nanometer scale regime, fabrication of nanometric objects using DNA as a template, and replacement of silicon devices with single DNA molecule-based computational systems are some of the emerging fascinating applications of DNA.[1−15] Although DNA is extremely easy to fine-tune for such novel, tailor-made applications, it also has a major limitation. DNA is either insoluble or partly soluble in any common organic solvent whence it loses its base pairing recognition properties. Making DNA soluble in organic solvents such that it retains its biologically important properties is thus a challenge. In this letter, for the first time, we demonstrate an easy method for making DNA soluble in different organic solvents. We believe that this could lead to the design of new materials based on DNA.

In the past few years, cationic amphiphiles such as surfactants and lipid molecules have been used to make DNA soluble in organic solvents, mainly chloroform.[16,17] Yet these amphiphile-mediated DNA complexes are not soluble either in water or in other common organic solvents. In recent years, it has been demonstrated that polycation grafts with water-soluble polymers form nanoparticles that are stable in water when they interact with DNA molecules. DNA present in these nanoparticles is inaccessible to DNase I and can be transfected into the cell.[18−21] Such polymer-based nanoparticles are potential candidates for a wide multitude of applications.[19] Recently, we have shown that random cationic copolymers from methoxy poly(ethylene glycol) monomethacrylate (MePEGMA) and (3-(methacryloylamino)propyl)trimethylammonium chloride (MAPTAC) are also able to form stable water-soluble nanoparticles when they interact with DNA.[22,23] Here, we report that these nanoparticles from DNA and cationic random copolymer (RCP) with 94 mol % PEG content are not only stable in water media but are also stable in different organic solvents such as acetonitrile (AN), benzene, dimethyl sufoxide (DMSO), and tetrahydrofuran (THF).

Figure 1 shows an atomic force microscopic image of the DNA−RCP system when DNA (calf thymus) and RCP are mixed at a charge ratio ($Z_{+/-}$) of 2 where $Z_{+/-}$ is the ratio of the concentration of cationic units of the polymers to the concentration of negative charges present in DNA. It is quite obvious from the image that when these are mixed together at a charge ratio of 2, particles are formed. An image analysis showed that they are spherical in nature and 75−100 nm in diameter. These nanometric cores of cationic units are neutralized by phosphate anions, surrounded by a shell of hydrophilic segments. Figure 2a depicts the CD spectra of the free and RCP bound DNA at $Z_{+/-} = 2$. The CD spectrum of the free DNA is typical of a duplex in the "B" conformation;[24] addition of copolymer results in CD spectra with similar shapes revealing that the DNA in the nanoparticle retains its helical structure. Figure 2b shows the melting profiles of free DNA and DNA present in the nanoparticles in 10 mM sodium phosphate buffer. A sigmoidal type of melting profile was observed in both of the cases, confirming that DNA in nanoparticles is in the double-stranded helical form, and they are more stable in nanoparticles than when free as they have a higher melting temperature (T_M) for the helix-coil transition.

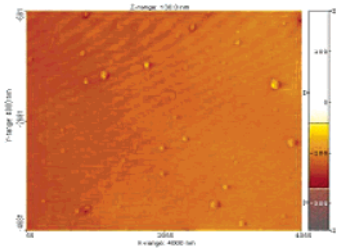

Figure 1. Atomic force microscopy image of nanoparticles obtained from random cationic polymer (RCP) and CT-DNA. DNA and RCP solutions in 10 mM sodium phosphate buffer (pH 7.0) were mixed together to obtain RCP/DNA nanoparticles of charge ratio = 2.

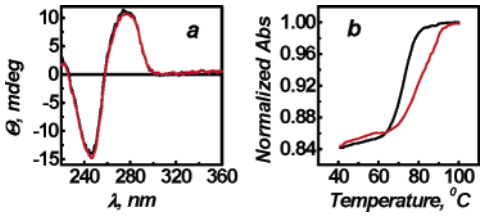

Figure 2. (a) Circular dichroism spectrum and (b) UV-melting curves of CT-DNA (black) and CT-DNA in the nanoparticle (red) in 10 mM sodium phosphate buffer (pH 7.0).

To study the solubility behavior of these DNA containing nanoparticles in different organic solvents, we have initially lyophilized the particles and redissolved them in different organic solvents, viz. AN, benzene, DMSO, and THF. Figure 3 represents AFM images of the nanoparticles dissolved in these solvents. As seen in the figure, nanoparticles neither break down in these solvents, nor aggregate to form bigger particles. They retain almost their original size and shape as is observed in aqueous medium. As PEG is soluble in all of these solvents tested, the solvophilic effect arising from PEG segments of the nanoparticles probably keeps these particles soluble in these nonaqueous solvents. This solvophilic shell at the exterior of the nanoparticles not only makes them soluble but also prevents aggregation due to steric repulsion and reduces nonspecific interactions among the particles themselves or with surroundings, thereby making them stable too.

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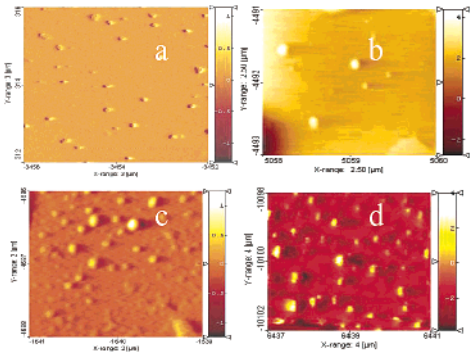

Figure 3. Atomic force microscopy image of nanoparticles obtained from cationic polymer and CT-DNA dissolved in (a) acetonitrile, *x* scale = 3 μM and *y* scale = 3 μM, (b) benzene, *x* scale = 2.5 μM and *y* scale = 2.5 μM, (c) DMSO, *x* scale = 2 μM and *y* scale = 2 μM, and (d) THF, *x* scale = 4 μM and *y* scale = 4 μM.

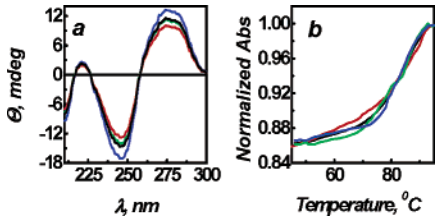

Figure 4. (a) Circular dichroism spectrum and (b) UV-melting curves of CT-DNA present in the nanoparticles recovered from organic solvents, acetonitrile (blue), benzene (red), DMSO (green), and THF (black). These nanoparticles were redissolved in 10 mM sodium phosphate buffer (pH 7.0) after removal of organic solvents under reduced pressure.

CD spectra of DNA−RCP nanoparticles dissolved in different organic solvents (Figure S4a in Supporting Information) showed a positive band above 240 nm and a negative band below 240 nm, indicating that the secondary structures of DNA are retained. As the boiling temperatures of all of the organic solvents studied here other than DMSO are low, hence we perform UV-melting experiments of the nanoparticles dissolved in DMSO (Figure S4b in Supporting Information). The hyperchromic effect in the melting profiles was observed, confirming that DNA in nanoparticles is in the double-stranded helical form. All of the solutions containing nanoparticles were practically transparent, and no aggregation was observed over a course of several weeks. Consequent measurements of turbidity at 550 nm and size measurements showed that the turbidity of the solution and the size of the particles remain unchanged. These observations revealed that the systems are quite stable and remained soluble over a period of time. To confirm this, the solvents were evaporated under reduced pressure and particles were redissolved in the same buffer in which they were present earlier. CD spectra and UV-melting profiles were recorded in each case. No characteristic change in the CD spectrum confirmed that the helical structure had not been affected at all in the course of this treatment (Figure 4). The melting temperatures in each case also match those recorded in the earlier experiments (Figure 4b). This is, to our knowledge, the first report describing the process of making DNA soluble in a variety of nonbiological solvents using cationic polymer.

Thus, DNA can be made soluble in different organic solvents with the help of PEG-based random cationic copolymer and thereby preserve its helix conformation. The methodology used in this study in making DNA soluble in organic solvents would definitely be of supreme relevance in designing DNA-based advanced materials and make DNA more compatible with organic building blocks and solvents for applications such as reaction catalysis.[10] Another possible application is the long-term stability of the particles in a Dnase free environments which can be important for gene delivery. It will also allow us to apply traditional physicochemical methods to study the molecular characteristics of such DNA-based complexes in organic solvents.

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Supporting Information Available: Synthetic procedure and characterization of the polymer, experimental protocols, UV and CD studies of the nanoparticles in organic media, and AFM of DNA and polymer alone (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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