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EDGE ARTICLE

Exploring the thermal stability of DNA-linked gold nanoparticles in ionic liquids and molecular solvents

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While water is the most commonly used solvent for DNA, many co-solvents have been added for various applications. Ionic liquids (ILs) are molten salts at around room temperature. ILs have been tested as a green solvent for many reactions and many biopolymers can also be dissolved in ILs. In this work, we study DNA-linked gold nanoparticles (AuNPs) in seven types of ILs. DNA-functionalized AuNPs possess a high density of negative charges and thus may generate new physical properties in ILs. We have identified the role of ILs to transit from salts to increase DNA duplex stability to solvents to decrease DNA melting temperature. The onset of this transition depends on the structure of ILs, where more hydrophobic cations destabilize DNA at lower IL concentrations. This trend is opposite to molecular solvents (e.g. ethanol, DMSO, ACN and DMF) that destabilize DNA at low solvent concentration. Specific DNA base pairing is disrupted at high DMSO concentrations, and AuNPs are held together by non-specific interactions. The other tested molecular solvents are able to maintain DNA base pairs, although strong non-specific interactions are also present. Several ILs can release proton and thus drastically change pH, which also changes the melting temperature of DNA. This study also reveals the feasibility of using ILs as solvents for DNA-functionalized nanomaterials.

Introduction

Ionic liquids (ILs) are molten salts at around room temperature (e.g. melting temperature < 100 °C). With a vapour pressure close to zero, ILs are considered non-volatile green solvents and have been tested to replace traditional organic solvents for many reactions. [1-3] The anions and cations in ILs usually have a low symmetry, disfavouring packing into crystals. With numerous possible combinations of such cations and anions, ILs are called "designer solvents" that can be customized for a diverse range of reactions. Recently, ILs have also been used to dissolve biopolymers such as proteins and DNA.[4-6] Many enzymatic reactions have been carried out in ILs with higher selectivity and better enzyme stability.[7] Choline dihydrogenphosphate (CP), one of the commonly used ILs, was reported to preserve DNA for a long time,[8] and can selectively stabilize A-T base pair while destabilizing G-C.[9-11] Practical applications have been reported for slowing down DNA translocation,[12] preparing DNA gel fibers,[13] DNA extraction[14] and capillary electrophoresis.[15] Various spectroscopic and theoretic studies have also been performed to understand the interaction between IL and DNA.[16-19]

In the past two decades, the function and application of DNA have expanded significantly. In addition to its genetic role, DNA has been used in nanostructure design,[20-22] catalysis,[23] biosensor development,[24-27] and materials science.[28-30] While salt and temperature are commonly used to modulate DNA duplex stability, the effect of solvent composition has also been tested. Water is the most commonly used solvent for DNA, but many other co-solvents have been added for various applications. For example, to mimic the macromolecularly crowded environment in cells, polymers such as polyethylene glycol (PEG) and dextran have been added.[31-36] DNA is also soluble in the presence of molecular solvents such as ethanol, dimethyl sulfoxide (DMSO), dimethylformamide (DMF) and acetonitrile (ACN), which can significantly accelerate DNA hybridization.[37-40] At the same time, these molecular solvents contain hydrophobic groups that can solvate the DNA bases and reduce DNA duplex stability. As a solvent, ILs are unique in many aspects. Unlike molecular solvents, ILs are composed of cations and anions, allowing effective screening of electrostatic interactions. ILs are not polymers and can maintain a relatively low viscosity even at high concentrations; effects such as macromolecular crowding are not expected for ILs. ILs are also different from ionic surfactants, which usually contain a long hydrophobic chain and can form self-assembled meso-structures. Equipped with the ability to harness hydrogen bonding, ionic, hydrophobic and van der Waals interactions, ILs offer a huge potential to be explored for reactions involving DNA.

Since 1996, DNA-functionalized gold nanoparticles (AuNPs) have become a major player in nanobiotechnology.[41,42] AuNPs possess an extremely high extinction coefficient and distancedependent color. Thiolated DNA can chemisorb onto AuNP surface to allow for high colloidal stability. Upon assembly by a complementary DNA, the color of AuNPs changes from red to purple (Figure 1A), popularizing their applications in colorimetric biosensor development, screening and fundamental physical chemistry studies.[24,43-45] Compared to free DNA, the DNA-AuNP conjugate displays many interesting features. For example, a high density of DNA gives polyvalent binding and cooperative DNA melting, producing very sharp melting transitions.[46,47] Compared to the broad free DNA melting transition (e.g. spanning ~20 °C), DNA melts in 2-3 °C when linked to AuNPs. In addition, the

densely functionalized AuNPs form a highly negatively charged surface that can significantly enrich counter ions. Exploring the solvent effect may generate new features that cannot be realized in aqueous buffers with free DNA.[35,40,48] In particular, this highly negatively charged conjugate in ILs may provide an interesting system to further understand the property of such hybrid materials. The classical method to study DNA melting is to measure its hyperchromicity by monitoring its temperature dependent absorbance at 260 nm, which increases by ~30% upon melting. This method, however, cannot be applied in the presence of many ionic liquids. For example, alkylammonium nitrate ILs absorb strongly in the UV region, disallowing an accurate measurement of the 260 nm absorbance. This problem can be solved by monitoring the concomitant color change of the AuNPs.

In this work, we report the property of DNA-assembled AuNPs in seven types of ILs. A comparison with four molecular solvents was also made. To the best of our knowledge, this is the first study on biopolymer-functionalized nanomaterials in ILs. By varying the concentration of ILs, we observed the role of ILs to transit from a salt to stabilize DNA duplex to a solvent to decrease DNA stability. Interestingly, this melting trend is reversed in molecular solvents.

Results and discussion

We first tested the effect of several ILs (see Figure 1E for their structures) on the colloidal stability of citrate-capped 13 nm AuNPs. Propylammonium nitrate (PN) and ethylammonium nitrate (EN) are liquid at room temperature. Reducing the alkyl chain length gives solid ILs such as methylammonium nitrate (MN) and dimethylammonium nitrate (DN). CP is also a solid at room temperature. For the initial assay, 50% (w/w) stock solutions for the solid ILs were prepared. AuNPs immediately aggregated with color changing to blue upon mixing with ILs (Figure 2A). This response is similar to the addition of NaCl to induce AuNP aggregation. Therefore, to use AuNPs in ILs, their surface needs to be protected.

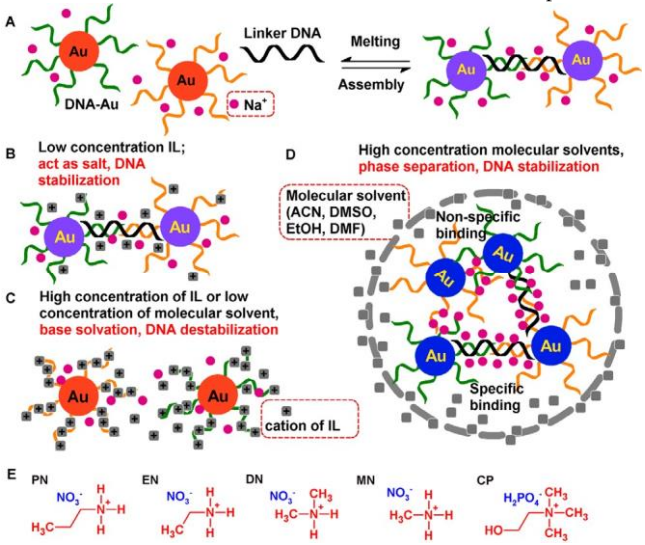

Figure 1. (A) Schematic presentation of DNA-directed assembly of DNA-functionalized AuNPs. Na^+ is added to screen DNA charge repulsion. (B) At low concentration, the cations of ILs act as a salt to stabilize DNA duplex. (C) At high concentration, cations with hydrophobic groups can solvate DNA and destabilize DNA duplex. This also occurs to low concentrations of molecular solvents. (D) With a high concentration of molecular solvent, phase separation may occur and DNA tends to precipitate out of solution together with AuNPs. The dashed ring represents the phase boundary. The duplex linkage is maintained in most molecular solvents except for DMSO. Non-specific DNA interaction is present in all the molecular solvents since AuNPs aggregate even in the absence of linker DNA. (E) Structures of some of the ILs used in this study.

The AuNPs were then functionalized with a high density of 21mer thiolated DNA and were concentrated ~50 times using centrifugation. To 49 μL of ILs, 1 μL of the concentrated AuNPs was added. As shown in Figure 2B, these AuNPs were protected in all the alkylammonium ILs since their color remained red. Note that PN and EN were at 98%, where there were more IL molecules than water (e.g. 2% water = ~ 1.1 M; 98% PN = ~10 M). CP at 49% caused a purple color, indicating AuNP aggregation. Such aggregation was reversible since after diluting the 49% CP sample with an equal volume of water and heating the sample in boiling water, red color was produced (the last tube in Figure 2B). Therefore, DNA can effectively protect AuNPs, enabling related assays in ILs.

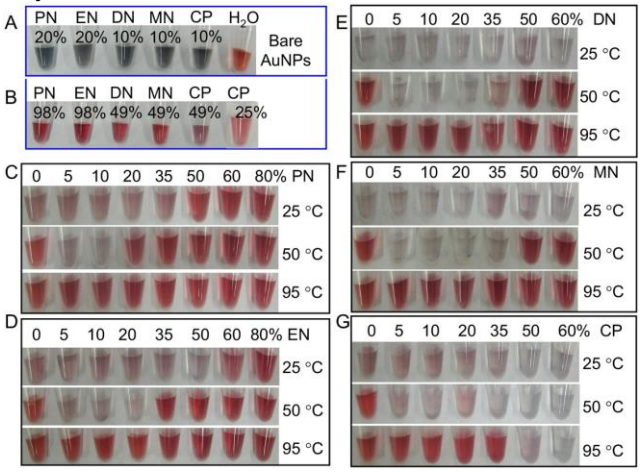

Figure 2. Photographs of citrate-capped AuNPs (A) and DNA capped AuNPs (B) mixed with ILs. The last tube in (B) was diluted from the 49% CP sample and heated in boiling water, showing AuNP aggregation in CP is reversible. (C-G) Colorimetric assay of various ILs on DNA stability. AuNP aggregates were finally dispersed in 50 mM NaCl, 20 mM HEPES, pH 7.6 with designated IL concentrations. The samples were first imaged using a digital camera followed by increasing temperature to 50 °C and then 95 °C. Red color indicates DNA melting.

After establishing the feasibility of using such AuNP probes in IL, we studied DNA melting. Two types of DNA-functionalized AuNPs were prepared and purple aggregates were obtained upon addition of linker DNA as shown in Figure 1A. Upon heating and DNA melting, AuNPs disassemble to produce a red color. To have an overall understanding, we first performed a visual screening experiment by incubating AuNP aggregates in various concentrations of ILs and monitoring color change at three temperatures. Figure 2C shows that high concentrations of PN (e.g. >50%) destabilize DNA, producing a red color even at room temperature. Warming the samples to 50 °C resulted in DNA melting in the aqueous buffer, but low concentrations of PN (e.g. 5-10%) stabilized AuNPs since the color remained purple. Heating to 95 °C melted all the samples. From this simple visual assay, we conclude that PN has a dual role on DNA stability. At low concentration, it acts as a salt to increase DNA stability; while at high concentration it destabilizes duplex stability.

PN has a hydrophobic propyl chain. To understand the destabilization effect, this chain length was reduced. For example, MN has only a methyl group and the AuNPs remain aggregated even in the presence of 60% MN at room temperature (Figure 2F). Warming the samples to 50 °C still produced red color at >50% MN

but not at lower concentrations, indicating that the destabilizing factor was still present. The behavior of EN (Figure 2D) and DN (Figure 2E) was between MN and PN, consistent with the number of hydrophobic alkyl groups. It is also interesting to compare EN and DN; the former contains an ethyl group and the latter two methyl groups. EN was more effective in destabilizing DNA since 60% EN produced a red color at room temperature but 60% DN did not.

CP has been one of the most frequently used ILs for biopolymers. While all the tested alkylammonium ILs contained nitrate as the anion, the dihydrogen phosphate anion in CP can release proton and change pH, which might affect DNA stability (Figure S3, Supporting Information). For example, 2.5% CP in water resulted in a pH of 3.2. Even with 50 mM pH 7.6 HEPES buffer, it remained difficult to keep a constant pH (Figure S4-5, Supporting Information). While CP appeared to show only a stabilization effect in Figure 2G, this is proven to be a pH related artifact. Similar to the alkylammonium ILs, CP also destabilized DNA at higher concentration (Figure S6-7, Supporting Information). To avoid potential pH artifacts, we focused our studies on alkylammonium ILs.

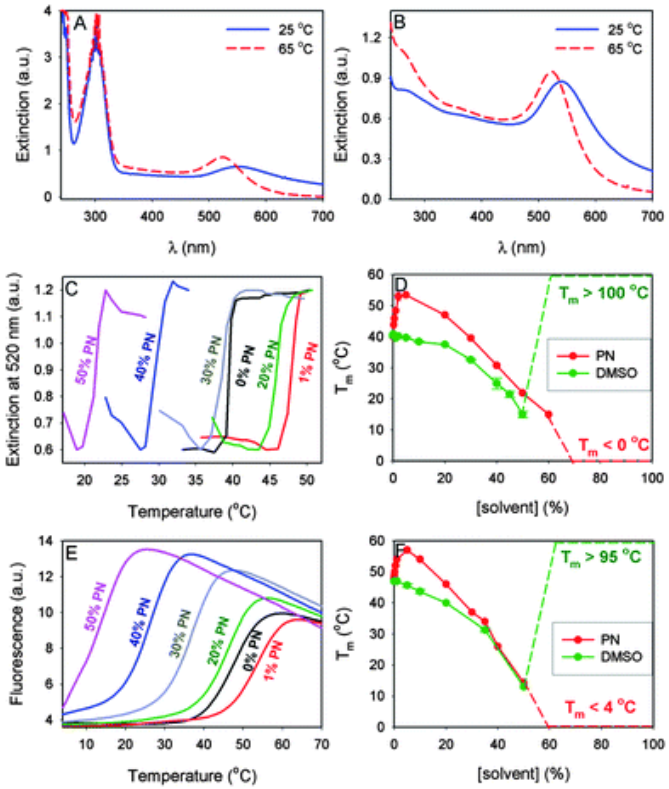

Figure 3. UV-vis spectra of aggregated and melted AuNPs in 4% PN (A) or in aqueous buffer (B). Melting curves of DNA-linked AuNPs in various concentrations of PN measured with AuNPs (C) or with free DNA (E). Plot of T_m as a function of solvent concentration measured with AuNPs (D) or with free DNA (F). Note that with greater than 60% DMSO, DNA-functionalized AuNPs aggregate even without linker DNA and such aggregates also cannot be thermally melted.

Our initial colorimetric assays have provided an overview on the interaction between ILs and DNA, which can be precisely tuned by varying the cation structure. For quantitative comparison, we next measured DNA melting temperature (T_m) using UV-vis spectroscopy. PN was chosen to highlight the hydrophobic property of ILs. With just 4% PN, a very large absorption peak at ~300 nm was observed (Figure 3A), which was due to the nitrate ions. This absorption property makes it difficult to accurately measure the 260 nm absorbance from DNA. Therefore, we chose to monitor the AuNP surface plasmon peak at 520 nm, which also increased upon DNA melting. For comparison, the spectra in the aqueous buffer are also shown, where both 260 nm and 520 nm can be used (Figure 3B). In aqueous solution, PN has a T_m of 40.5 °C (Figure 3C). Addition of just 1% PN increased the T_m by ~8 °C. Even higher PN resulted in a rapid decrease in duplex stability. For example, at 50%, melting started to occur even below room temperature.

Very sharp melting transitions were observed under all tested conditions; the color of the samples changed to red within 2-3 °C, indicating that cooperative DNA melting was still maintained in concentrated ILs. A full plot of the solvent concentrationdependent T_m change is given in Figure 3D (red dots). This quantitative measurement agrees with our colorimetric screening in Figure 2C.

To verify our data, we also performed melting analysis using a fluorescence based method, where a FAM-labeled DNA was hybridized to a complementary quencher-labeled DNA. Thus DNA melting produced fluorescence enhancement. Some representative melting traces in PN are showed in Figure 3E, where the overall trend was the same as the colorimetric melting assays in Figure 3C, although the fluorescent melting curves are broader. The change of T_m as a function of solvent concentration is plotted in Figure 3F, showing the same trend as in Figure 3D. Therefore, this fluorescence-based melting experiment supported the results from the AuNP-based assays.

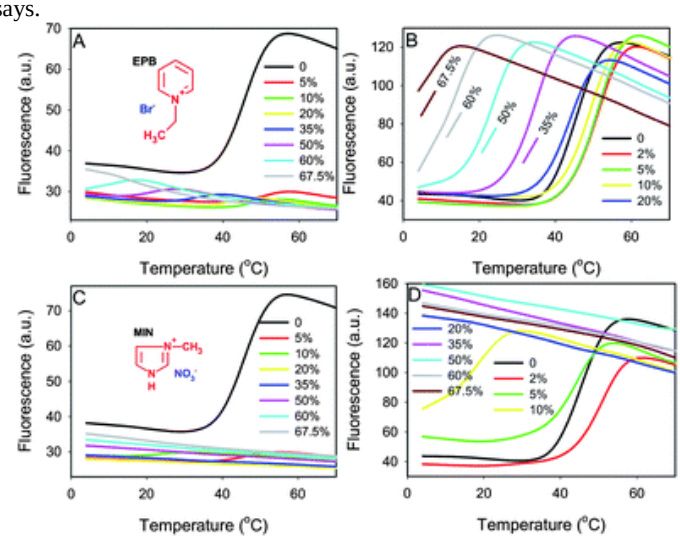

Figure 4. Fluorescence based melting curves with EPB (A and B) or MIN (C and D) ILs. The beacon contained FAM labeled (A and C) or Alexa Fluor 488 labeled DNA (B and D).

To test whether our observation is general to ILs other than alkylammonium, we further test imidazolium and pyridinium based ILs. The FAM-labeled beacon was dissolved in various concentrations of 1-ethylpyridinium bromide (EPB), which caused a significant fluorescence quenching with even only 5% EPB (Figure 4A). It is unclear about the reason behind this quenching, since pH dropping did not occur with EPB (see Figure S9, Supporting Information). By using the same DNA sequence but with an Alexa Fluor 488 label, the beacon showed normal melting curves with comparable fluorescence intensities for all the EPB concentrations (Figure 4B). The T_m increased with up to ~10% EPB and further increase of this IL caused significant DNA destabilization. IL 1-methylimidazolium nitrate (MIN) also caused a significant quenching effect on the FAM beacon (Figure 4C), which was attributable to the pH effect (Figure S9). For example, with 10% NIN, the pH dropped to 3.66 even in the presence of 20 mM HEPES (pH 7.6).

The cation part of this IL appears to be more acidic than imidazole, possibly due to its extra positive charge. By replacing FAM with Alexa Fluor 488 (a pH insensitive dye), we observed a similar stabilization-todestabilization trend (Figure 4D), although a part of the destabilization was due to pH change. These studies indicate that the DNA stabilization trend caused by ILs should be a general phenomenon. In addition, pH artifacts could be significant for ILs that can release protons.

To have a full understanding of the solvent effect on DNA melting, we next performed colorimetric tests with four common molecular solvents (Figure 5). DNA melted in 20-50% DMSO at 42 °C but remained stable in 0-10% DMSO (Figure 5A), suggesting that DNA was destabilized with increasing DMSO concentration up to 50%. For samples with greater than 60% DMSO, no melting occurred even at 95 °C. This trend is common for all four molecular solvents. Using DMSO to represent molecular solvents, we measured T_m as a function of its concentration (Figure 3D, green dots), whose trend is opposite to PN. At low DMSO concentration (e.g. <50%), T_m decreased with increasing of DMSO concentration. At even higher DMSO concentrations, melting was prohibited. The same trend has also been previously measured using several alcoholic solvents.[40] In this study, we chose to use DMSO because it can minimize sample leakage possibly due to its high viscosity. Ethanol (>25%) for example induced significant sample leakage from our UV-vis cuvettes. These molecular solvents contain hydrophobic methyl or ethyl groups, allowing them to dissolve many hydrophobic compounds. For the same reason, they can also solvate hydrophobic nucleobases and thus destabilize DNA. These molecular solvents do not contain cations, thus lacking the stabilization effect seen for ILs at low concentration.

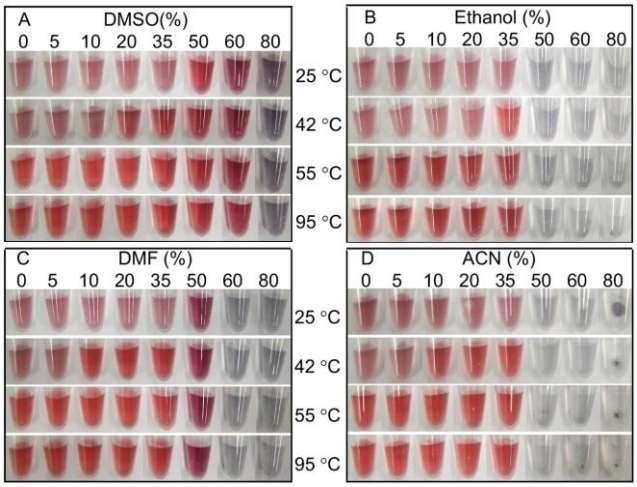

Figure 5. Colorimetric assay of the stability of DNA-linked AuNPs in various molecular solvents. AuNP aggregates were finally dispersed in 50 mM NaCl, 20 mM HEPES, pH 7.6 with designated solvent concentrations. Red color indicates DNA melting. A clear phase separation in 80% acetonitrile can be observed at 25 °C.

ILs appear to have greater solvation power; they could solvate all the molecules in the system including DNA-functionalized AuNPs, water and inorganic salts, resulting in a homogeneous phase. Concentrated molecular solvents tend to precipitate DNA and undergo phase separation, which is an indication of its poor solvation power for polar species. For example, 80% ACN clearly produced a separated phase (Figure 5D, 25 °C). Although other samples did not show such an obvious phase separation, the fact that AuNP color changed from purple to blue indicated the formation of large and closely packed AuNP aggregates (see Figure 1D for a scheme describing this state). The precipitation of DNA in concentrated ethanol with high salt is a routine operation in molecular biology, which is attributed to the formation of socalled P-DNA.[49] We reason that the same mechanism is applied when DNA is attached to AuNPs. With a high density of DNA immobilized on AuNPs, precipitation is even more favored. Compared to free DNA, the high extinction coefficient of AuNPs allows visual observation of this process.

Unlike alkylammonium ILs, which could disperse DNA-functionalized AuNPs even at 98% (Figure 2A), these molecular solvents precipitated AuNPs even in the absence of linker DNA (Figure S10, Supporting Information). Therefore, a question is whether the blue aggregates in Figure 5 were still linked by the linker DNA or just by non-specific interactions. We harvested these aggregates and re-dispersed them in buffer. The sample soaked in 80% DMSO immediately turned red, indicating that the linker DNA was removed by soaking in DMSO. The other three solvents still produced purple color, which turned red upon heating. The melting curves of these aggregates were overlapping. Therefore, it appeared that the linker DNA was still present (Figure S11, Supporting Information), although nonspecific interactions were also likely to present in these three solvents as schematically shown in Figure 1D. Therefore, concentrated molecular solvents may not selectively stabilize DNA duplex, since non-specific interaction also plays a major role.

Conclusions

In summary, we have tested the melting of free DNA and DNAfunctionalized AuNPs in seven ILs and four molecular solvents. This work has the following implications. First, we have identified the dual role of ILs. On one hand, they serve as cations to screen DNA charges and on the other hand, they solvate DNA bases. Such a dual effect was also reported in the case of the macromolecularly crowding environment, where the crowding effect increases T_m while the chemical interaction with DNA decreases it. Second, it provides another example highlighting the power of AuNP-based colorimetric assays. Compared to free DNA, AuNPs allow sharper melting transition. For alkylammonium nitrate ILs, the traditional DNA hyperchromicity 90 experiment cannot even be accurately performed; however AuNP based assays can still be successfully carried out. This work also demonstrates that functionalized nanomaterials may still maintained their functions in ILs. Third, we have shown that ILs are useful solvents for DNA-based assays. Compared to 95 molecular solvents, these ILs can effectively dissolve DNA, AuNPs, inorganic salts and water without causing phase separation. Taking together, we now have a more complete picture on the effect of solvents on DNA-based reactions.

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