DNA Solutions

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Long-Term Structural and Chemical Stability of DNA in Hydrated Ionic Liquids**

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There is much interest in the use of DNA as an advanced material.[1] For example, DNA has been discussed as the "ultimate" material for molecular computing[2] and there is much effort directed towards investigating this application. Recently, DNA has also been employed in templated chemical synthesis, nanomachines, and biosensors.[3–5] The use of DNA as a biomaterial from a variety of readily available sources has gained importance in recent years, especially for the surface treatment of implanted materials.[6] Since it is rich in phosphate groups it has a strong affinity for bone, and DNA has been modified for use as a bone-guiding scaffold by intercalating antibiotics between the stacked base pairs.[7] The water-insoluble form of DNA also finds a range of other applications as a biomaterial.[8,9]

Although DNA is considered to be reasonably stable in aqueous solution, there are various conditions of temperature, pH, ionic strength, depurination, deamination, solvents, etc. that can disrupt the DNA helix and cause denaturation.[10,11] The solubility and stability of DNA has been studied in a variety of nonaqueous[12] and mixed solvents[13] and it was shown that DNA loses its double-helical structure when dissolved in dimethyl sulfoxide, formamide, or methanol, whereas the structure is retained in ethylene glycol and to some extent in glycerol. Conventionally DNA is stored under refrigeration for short- and long-term applications and the influence of storage temperature has been discussed in the literature;[14] however, DNA molecules are not stable in solution at ambient temperatures for long periods ($\gtrsim$ 1 month). Therefore, finding a medium in which DNA is soluble without loss of its structure and in which it is stable for long periods of use at room temperature is an important bottleneck in this field.

In recent years, a number of biocompatible hydrated ionic liquids (ILs) have been identified, for example, choline dihydrogenphosphate (CDP) with 20% dissolved water, and shown to be good solvents for proteins.[15,16] In the ionic solution, some proteins are tremendously stabilized as compared to aqueous solutions,[17,18] thus leading to a variety of applications in drug delivery and sensor development. The presence of a solute amount of water in these systems is an important aspect of these media. The effect of ILs as solvents on the stability of other biopolymers such as DNA is unknown; hence, this work focuses on the solubility, structure, and long-term stability of DNA in a number of these novel, choline-based solvent media.

Circular dichroism spectra (Figure 1) were recorded for DNA (from salmon testes) dissolved in choline lactate (CL) IL and in aqueous solution controls, stored at different temperatures and time intervals. It can be seen from Figure 1 that the presence of the characteristic positive band at 275 nm (arising from base stacking) and a negative band at 245 nm resulting from the B conformation of DNA[19,20] (with a crossover at 258 nm) in the IL/DNA samples indicates that the native double-helical structure is indeed retained in these IL solutions. Such solubility with retention of structure is probably associated with the ability of the IL ions to engage in hydrogen bonding with the exterior of the DNA helix. Since DNA is anionic, the most likely H-bonding would be with the choline cation hydroxy group. Furthermore, these characteristic bands are still present even after 6 months of storage at room temperature. A similar storage stability was observed in CDP and choline nitrate (CN) solutions. On the other hand, the sample dissolved in water and stored at room temperature for 1 month had lost the double-helical structure.

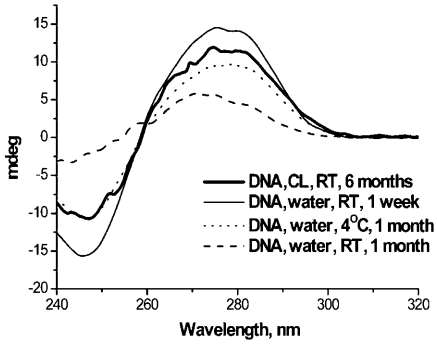

Figure 1. Circular dichroism spectra of DNA in water or IL stored for different time intervals at room temperature (RT) or 4 °C.

Fluorescence experiments[21] were also carried out to assess the effect on stability of different levels of water in the hydrated IL. The results (Figure 2) show that the samples

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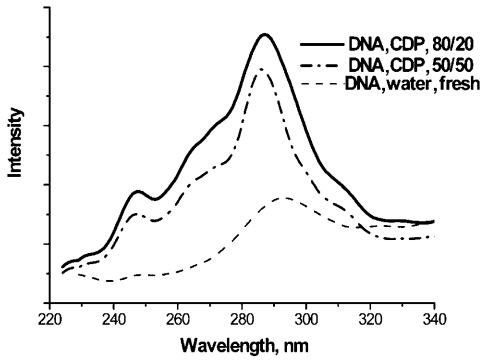

Figure 2. Fluorescence spectra of DNA samples in hydrated CDP stored at room temperature for 6 months.

produced the characteristic native DNA fluorescence emission at 287 nm in both the 50:50 and 80:20 aqueous CDP/water mixtures. It has been reported[22] that the fluorescence is a result of the presence of the hydrogen-bonded adenine base in native DNA and its intensity and band shape depend on the pH of the medium, being weak at neutral pH. CDP at 20% water content will be slightly more acidic than the 50% water and pure water samples, because of the residual acidity of the dihydrogenphosphate anion, and the intensity differences are therefore as expected.[22]

A separate set of experiments was carried out to demonstrate the effect of pH on the fluorescence emission intensity of the DNA samples; the results (Supporting Information, Figure ESI-1) clearly show that the intensity increases with acidity. This intensity increase is attributed to the increased protonation of the base involved (adenine) under these pH conditions. In a separate experiment, the fluorescence spectra of the neat ILs were obtained to confirm that there was no emission in the 240–320 nm region.

To evaluate the stability of DNA dissolved in hydrated CDP at elevated temperatures, fluorescence spectroscopy was carried out at different temperatures on DNA samples in the CDP/H_2O 50:50 medium; these samples had already been stored for 1 year at room temperature. The fluorescence was recorded over a period of 2 hours at different temperatures (40 and 90 °C and then after returning to room temperature). The results (Figure 3) show that there was only a slight change

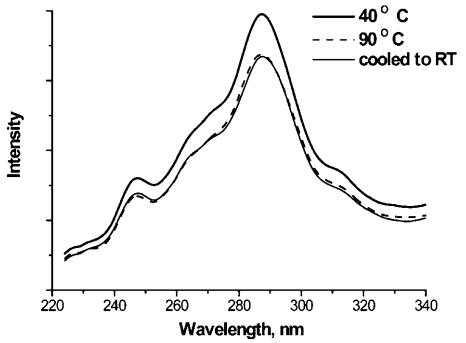

Figure 3. Fluorescence spectroscopy of DNA samples in aqueous CDP (50:50) at different temperatures.

in the intensity of fluorescence as a result of the excursion to 90 °C. Differential scanning calorimetry data (not shown) also indicate that no denaturation exotherm is observed below 100 °C. An aqueous sample would be substantially denatured by this excursion. This finding suggests that the DNA sample is stabilized at higher temperatures by the hydrated IL medium, as has been observed in the case of proteins.[15,16] This is understood to be the result of the solvent thermodynamics, which strongly favor the denatured state in an aqueous medium at elevated temperature.

CL, CN, and CDP appear to provide good stabilization, whereas it was observed that although DNA was soluble in choline formate (CF), it rapidly denatures in this IL since no fluorescence emission was observed from this sample. The detrimental effect of this IL is possibly connected with its radical activity.[23]

To determine if any degradation in molecular weight was taking place during storage, gel electrophoresis studies[24] were carried out. Figure 4 shows that the samples of DNA

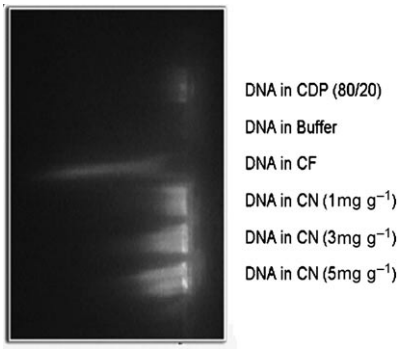

Figure 4. Gel electrophoresis of DNA samples stored at room temperature for 6 months.

in buffer and CF stored at room temperature for about 6 months underwent degradation, whereas the DNA samples in CDP (lane 1) and CN (lanes 4–6, with different DNA concentrations) remained intact.

In conclusion, DNA was found to be soluble and exhibit exceptional long-term stability in hydrated ILs based on CL, CN, or CDP. These ILs provide a mild H-bonding environment that is of relatively low water activity, hence slowing the rate of hydrolytic reactions that would otherwise slowly depolymerize or degrade the molecule.

Experimental Section

Choline salts were prepared as per literature procedures,[23] including CDP, CL, CF, and CN. Choline hydroxide (45% in methanol), nitric acid (aqueous 70%), formic acid (99%), lactic acid (99%), phosphate buffer (pH 7.4), and DNA from salmon testes were all purchased from Sigma–Aldrich, while phosphoric acid (85%) was purchased from Merck. CDP and CN are solids with melting points of 185 and 34 °C, respectively, whereas CL and CF are liquid at room temperature. CDP and CN become liquid on addition of small amounts of water; in all cases 20% (w/w) is sufficient to produce a clear, fluid liquid at room temperature. Typically, DNA (1 mg g^{-1}) was added to the hydrated ILs. The dissolution of DNA in the hydrated ILs was

slow (ca. 3–4 weeks was required for full dissolution) and depended on the water content: the higher the water content, the more rapidly soluble the DNA became. After dissolution the samples were stored in sealed vials at room temperature.

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