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Research paper

Structural foundation for DNA behavior in hydrated ionic liquid: An NMR study

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ABSTRACT

A well known rule of high thermal stability of GC-rich DNA helices can be reversed with the use of certain ions, rendering AT-rich duplexes more stable. We have sought to elucidate the structural basis of this phenomenon for choline dihydrogen phosphate, an ionic liquid known for extension of long-term chemical stability of biomolecules. NMR experiments complemented with CD spectroscopy revealed subtle changes of GC and AT-rich double helix structures in choline dihydrogen phosphate compared to NaCl solution. Chemical shift changes observed for different environments were used as a guide to determine choline ions' localization hotspots. For d(5′-AAATATATTT-3′) choline ions are localized in the central part, especially in the minor groove near sugar protons of thymidine and H2 protons of adenine residues. In agreement with NMR data, thermodynamic analysis points to the involvement of choline ions in the hydration network as a crucial part of thermal stabilization of AT-rich helices. Analysis for GC-rich d(5′-GGGCGCGCCC-3′) oligonucleotide showed preference of choline ions for major groove with less clearly defined localizations spots than in the case of its AT-rich counterpart.

1. Introduction

The structure and stability of DNA oligonucleotides stabilized via Watson–Crick base-pairing as well as their modulation are of interest in diverse research fields, including medical, pharmaceutical and materials sciences [1–3]. Hydrogen bonding and base stacking play major roles in the specific recognition of complementary donor and acceptor functionalities, structural features, water accessibility and thermal stability of Watson–Crick base-pairs [4]. Moreover, cationic molecules that are abundant in living cells also influence the stability and structure of poly-anionic DNAs. Several research lines have taken advantage of structure and stability changes induced by cationic molecules to develop DNA-based materials such as logic devices, circuits and other bio-inspired nanodevices [2,3,5,6]. Quantitative information regarding the molecular level interactions between cationic molecules and DNA is important not only to further our understanding of the stabilities and structures of DNA in cells, but also for use of DNA for nanotechnology. In principle, cations primarily bind to the negatively charged sugar-phosphate backbone of DNA, thus stabilizing folded, well-defined DNA structures by reducing the repulsive Coulombic forces amongst the phosphate groups [7–9]. More importantly, certain cations prefer binding to specific sites in DNA grooves. For example, some alkylammonium ions, osmolytes found in cells, bind to A–T base-pairs in the minor groove of a DNA duplex, thermally stabilizing regions of A–T base-pairs [10–14]. Glycine betaine, which is a zwitterionic osmolyte with alkylammonium-derivative ions, thermally destabilizes G–C base-pair rich DNA duplexes by binding to single-stranded DNA, especially unpaired guanines at high salt concentrations [15,16].

Ionic liquids (ILs) have been a focus of tremendous interest as solvents in the field of nanotechnology, because they provide favorable environments for a wide range of chemical reactions and

Abbreviations: CD, circular dichroism; ChdhP, choline dihydrogen phosphate; DNA, deoxyribonucleic acid; NMR, nuclear magnetic resonance.

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are environmentally friendly solvents [17,18]. Choline dihydrogen phosphate (ChdhP), a representative ionic liquid, ensures long-term chemical stability of biomolecules like DNA [19] and proteins [20–23]. We have recently shown that A–T base-pairs are thermally more stable than G–C base-pairs in the hydrated IL of ChdhP [24], although G–C base-pairs are thermally more stable than A–T base-pairs in aqueous buffered solutions with physiologically relevant ion concentrations and pH values. These results suggested that the thermal stability difference between ChdhP and aqueous buffer is due to specific stabilizing interactions between choline ions and DNA. Recent MD simulations offered microscopic insights into interactions [25,26]. The narrow groove of A–T base-pairs in a duplex allows multiple hydrogen bonds between choline ions and DNA atoms. At the same time, choline ions can be sterically accommodated into the minor groove, which leads to the thermal stabilization of duplex consisting with A–T base-pairs. The choline ions were bound also to the grooves of triplexes and induced their thermal stabilization [27]. Moreover, Portella et al. also investigated interactions in great detail and found by MD simulations and NMR that molecular ions including choline preferentially localize in the minor groove of DNA [25]. Furthermore, free energy calculations showed that single-stranded GC-rich sequences exhibit more favorable solvation by choline ions than single-stranded AT-rich sequences. The unique binding of choline ions to DNA leads to increased thermal stability in a sequence-specific manner.

In the current study, we have undertaken a ^{1}H and ^{31}P NMR spectroscopy-based structural study on ODN7, d(5′-AAATATATTT-3′), and ODN8, d(5′-GGGCGCGCCC-3′), in combination with CD spectroscopy and thermodynamic analysis for elucidation of the AT/GC-dependent thermal stability at increased concentrations of NaCl and ChdhP in aqueous solutions. Earlier studies have shown that high concentration of ionic liquid solvents and deep eutectic solvents, while having profound effect on the stability of DNA, do not induce extensive structural changes of the double helix [24,28,29]. Therefore, both AT and GC-rich oligonucleotides ODN7 and ODN8 were anticipated to retain main structural characteristics with increased NaCl and ChdhP concentrations. Moreover, our previous study by thermodynamic analyses and molecular dynamic simulations suggested the preferential binding of the choline ions to a single stranded GC-rich DNA [24,26], which was expected to be reflected in diminished thermal stability of hydrogen bonded GC-rich double helix. Indicators of the relative stability of complementary base-pairs in the local duplex environment are changes in solvent exposure and consequently increased exchange rates with bulk water molecules, which are demonstrated experimentally through linewidth changes of the ^{1}H NMR signals of the imino proton resonances. At the outset, line broadening and decreased NMR signal intensity were anticipated for hydrogen-bonded imino protons of ODN8, but not ODN7 upon increase of ChdhP concentration. Strong and long-lived interactions of ChdhP with either of oligonucleotides were expected to lead to intermolecular NOE contacts. On the other hand, weak and transient binding was not anticipated to result in appearance of additional NOE cross-peaks. Nevertheless, it should give rise to chemical shift changes of protons in the proximity of the interaction site(s). Additionally, chemical shift changes are also indicators of variations in stacking interactions; up- and downfield shifts of resonances suggest increase and decrease in the ring current contributions [30–32]. Finally, ^{31}P NMR can provide important structural and dynamic information on nucleic acids [33]. In shorter oligonucleotides such as ODN7 and ODN8 separate ^{31}P signals were expected to be observed for each of the phosphodiester connectivities of two neighboring residues, and thus, ^{31}P NMR is potentially able to probe the conformational dynamics along the entire sugar-phosphate backbone in response to its (Coulombic) interactions with ionic liquids and monovalent cations.

2. Materials and methods

2.1. Sample synthesis and preparation

All oligodeoxyribonucleotides used in this study were high-performance liquid chromatography grade (Japan Bio Service). Single-strand concentrations of DNA oligonucleotides were determined by measuring the absorbance at 260 nm and 80 °C and using single-strand extinction coefficients calculated from the mononucleotide and dinucleotide data according to the nearest-neighbor approximation model. The absorbance was measured using a Shimadzu 1700 spectrophotometer connected to a thermo programmer. The hydrated ionic liquid, choline dihydrogen phosphate, was purchased from Ionic Liquids Technologies Co. Ltd. and used without further purification.

Oligonucleotides ODN7 and ODN8 were dissolved in 0.1, 0.5 and 1 M NaCl or ChdhP solutions containing 50 mM MES and 1 mM EDTA, pH 6. Samples were annealed with heating to 80 °C for 3 min and cooled down to 0 °C. 8% of $^{2}H_2O$ was added to the samples prior NMR measurements.

2.2. CD spectroscopy

CD spectra were recorded on an Applied Photophysics Chirascan CD spectrometer at 25 °C using a 0.1 cm path length quartz cell. The wavelength was varied from 200 to 320 nm. Four scans were averaged for each CD spectrum. Samples for CD measurements were prepared at 0.1 mM oligonucleotide and 0.1 M NaCl or ChdhP concentration in 50 mM MES buffer, pH 6, and 1 mM Na_2EDTA. A blank containing only buffer was used for baseline correction.

2.3. NMR experiments

1D ^{1}H, 2D NOESY (τ_m of 150 ms) and 2D TOCSY (τ_m of 40 ms) spectra were recorded at 0.1 mM strand concentration on Agilent VNMRS 800 MHz NMR spectrometer at 0 °C. Watergate pulse sequence was used to suppress signals of water, ChdhP and buffer components. ^{31}P spectra were recorded on Unity INOVA 300 MHz NMR spectrometer at 25 °C. Spectra were processed and analyzed using programs VNMRJ (Agilent Technologies) and NMRPipe [34]. Cross-peak assignment and integration with Gaussian fit procedure was performed using SPARKY (UCSF) [35]. In calculation of distances we have restrained our analysis to the non-overlapping peaks only. Average volumes of H6–H7 cross-peaks of residues T4, T6, T8 and H5–H6 cross-peaks of residues C8, C9, C10 were used as the distance reference for ODN7 and ODN8 distance calculations, respectively.

3. Results

3.1. Proton NMR spectra

1D ^{1}H NMR spectra of ODN7 and ODN8 were recorded at 0.1, 0.5 and 1 M concentrations of both aqueous NaCl and ChdhP with the initial hypothesis that chemical shift differences will become more apparent at higher salt concentrations (Fig. 1). While the most profound effects on base-pair dependent thermal stability of the AT and GC-rich oligonucleotides were observed in 4 M ChdhP solution in an earlier study [24], such high concentration was inaccessible to NMR characterization due to the high viscosity of the ChdhP solution and prohibitive problems of high dynamic range. In general, downfield and upfield shifts of proton resonances were observed