An insight into thermal stability of DNA in hydrated ionic liquids from multi-wavelengths UV Resonance Raman experiments

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The utility of Ionic liquids (ILs) as alternative solvents for stabilizing and preserving for a long time the native structure of DNA may be envisaged for biotechnological and biomedical applications in the near future. The delicate balance between stabilizing and destabilizing effects of the IL-mediated interactions with the structure of DNA is complex and still not well understood. This work reports a fundamental study dealing with the effect exerted by cations and anions in imidazolium-based ILs on the thermal structural stability of large nucleic acid molecules. Multi-wavelengths UV Resonance Raman spectroscopy is used for selectively detecting heat-induced structural transitions of DNA localized on specific base-tracts. Our study reveals the establishment of preferential interactions between the imidazolium cations of ILs and the guanine bases in the DNA groove that lead to a more effective stacking between of these bases even at high temperature values. Interestingly, we observe that this trend for ILs sharing the same chloride anion is further enhanced as the alkyl chain on imidazolium cation gets shorter. The results from the present investigation lead to a more comprehensive view of the IL-mediated interactions with A-T and G-C base pairs during thermal unfolding.

A Introduction

Almost all biological molecules such as proteins and nucleic acids necessarily require a medium for their stabilization and functioning. The water environment is ubiquitously recognized to be an active partner strongly affecting the structure and application of biomolecules in most biological processes [1]. Deoxyribonucleic acid (DNA) is considered reasonably stable in aqueous solutions but at long-time storage at room temperature, DNA is vulnerable to chemical degradation and hydrolytic and oxidative damage [2]. Moreover, other conditions like non-physiological temperatures, pH and ionic strength or repeated freeze-thaw cycles can destroy the DNA helix structure. This is a severe issue in the case of DNA specimen extracted from a living organism, for example If nuclease is present in the system, DNA or RNA are readily degraded under the activity of the enzyme, making the long-term preservation of this type of samples in water impossible. This is a critical point for the DNA molecular analysis where it is essential to optimize the storage and preservation of the samples to avoid loss in DNA quality. Common strategies of conservation of nucleic acids include the storage of specimens below 0 °C or freeze-drying methods that are not particularly cheap. Other methods involve the use of reagents that deactivate nuclease but can be sometimes cytotoxic or carcinogenic. In this context, finding a medium in which DNA is soluble as well as stable for long time remains a challenging task for DNA technology [3]. Some studies proposed DNA preservation at room temperature with nanoparticles or the addition of various sugar matrices [3]. Other investigations revealed how some organic solvents, such as glycerol are able to maintain the duplex structure, while other organic compounds such as methanol, formamide, pyridine, or dimethyl sulfoxide induce unfolding and strand separation of DNA [4].

The remarkable progress in the field of ionic liquids (ILs) in the last two decades has stimulated the idea to explore their extensive use as solvents or co-solvents with water for a broad range of biotechnological applications, including DNA technology [5-9]. Several key features of ILs, such as low vapor pressure and high stability, make them suitable for use in various areas of biochemistry. ILs' solvent potential lies in their capability to form intermolecular interactions toward a broad range of polar/non-polar solute molecules, including biomolecules such as proteins and DNA [10]. Concerning common co-solutes, the large number of possible anion/cation combinations offers the chance to design tailor-made ILs for specific purposes. Both experimental and simulation studies have explored the benefits of aqueous solutions of ILs for stabilizing

and functioning DNA [9,11-13]. More recently, numerous investigations on the possible applications of ILs in nucleic acid delivery into eukaryotic cells have emerged [14,15]. Considering the promising use of ILs as new-generation solvents for DNA, the information available on DNA stabilization in ionic liquid-based systems are quite scarce [9]. Circular dichroism (CD) technique has been used to assess possible changes in the DNA structure in various ILs during long-term storage at room temperature [16]. DNA was found to be soluble and to exhibit exceptional long-term stability in hydrated ILs based on choline lactate, choline nitrate and choline dihydrogen phosphate [16]. Interestingly, it has been observed that small variations in the ionic composition of ILs can drastically change the effect on DNA properties. Several experimental and simulation studies pointed out the capability of ILs with cations based on imidazolium to be efficient co-solvents of water for improving the structural stability of DNA [17-25]. Various hypotheses on the binding mechanism between this class of ILs and the structure of DNA have been proposed for explaining the observed effects of aqueous solutions of ILs. For some authors [17,18-20,22,24] imidazolium cation can penetrate the solvation shell of DNA molecules and bind with DNA groove mainly through hydrophobic and polar forces. These strong interactions, together with the electrostatic attraction between IL cations and DNA phosphate groups are mainly responsible for the preservation of the B-form of DNA in hydrated ILs. Moreover, IL-mediated partial dehydration of DNA can hamper DNA hydrolysis [17]. A different premise [21] suggests that the increased stability of DNA is due to the intercalation binding between imidazolium cation and DNA. Since this interaction becomes stronger upon lengthening of the alkyl side chain in the IL cation, the thermal stabilization of DNA is further enhanced with length increases [21]. H-bonds and edge-to-face NH··· □□interactions between imidazolium cation and DNA bases and the formation of strong H-bonds between IL anions and the nucleobases are also supposed to stabilize B-form DNA [25].

In this work, we aim to rationalize the role played by the intermolecular interactions of cations and anions in imidazolium-based ILs on the thermal structural stability of large nucleic acid molecules. Such interactions are discussed in terms of the change of anion and variation of length of alkyl chain on imidazolium cation. The genomic DNA from salmon sperm is used as a model for explaining the network connections established between ILs and DNA molecules and the formation of peculiar nucleic acid structures in IL media.

Multi-wavelengths UV Resonance Raman (UVRR) spectroscopy offers a sensitive probing of the structure and the environment of purine and pyrimidine bases in nucleic acids [26-28]. UVRR spectra of DNA are rich in information thanks to the spectral perturbations induced from the electronic effects of base pairing and stacking interactions. Moreover, the quite different UV absorbance pattern of the various nucleobases gives promise for disentangling their Raman spectral contribution in UVRR spectra through the accurate selection of the excitation wavelength [26-28]. Recent works have shown the effectiveness of synchrotron-UV Resonance Raman spectroscopy (SR-UVRR) for finely matching the excitation wavelength with the electronic transitions of DNA nucleobases, thus offering a powerful tool for probing both the local and global DNA conformational changes during its thermal unfolding [29-31]. By exploiting the tunability of the synchrotron source, here we employ multi-wavelength UVRR experiments for systematically investigate the thermal stability of DNA in a set of hydrated imidazolium-based ionic liquids. Our experimental approach provides insights on the preferential interactions of IL cations and anions with A-T and G-C base pairs that possibly affect the structural stability of DNA in hydrated ILs.

B Experimental

The ionic liquids 1-methylimidazolium chloride [MIM][Cl], 1-ethyl-3-methylimidazolium chloride [EMIM][Cl], 1-butyl-3-methylimidazolium chloride [BMIM][Cl], 1-butyl-3-methylimidazolium bromide [BMIM][Br] and 1-butyl-3-methylimidazolium chloride [BMIM][I] were acquired from IoLiTec with a purity of 99%. Before their use, all the ILs have been dried inside a desiccator under vacuum with phosphorus pentoxide for 48h in order to remove any water contamination.

DNA sodium salt from salmon testes (sDNA) (CAS number 438545-06-3, ∽2000 base-pairs, MW= 1.3 106 Da, % G-C content: 41.2%) was purchased from Sigma-Aldrich and used without further purification. For UVRR experiments, solutions at a concentration of sDNA equal to 0.2 □M were obtained by dissolving lyophilized powder of DNA in Tris buffer 10 mM at pH 7.4 and gently stirring for 24 h to achieve a limpid sample. For the preparation of sDNA/IL samples, each IL has been added to the solution of sDNA in Tris buffer to reach the final concentration of 62 mM of IL and 0.2 □M of sDNA, corresponding to a stoichiometric ratio of sDNA/IL of 0.003 10-3. All the solutions were freshly prepared for UVRR measurements and they appeared limpid before the running of experiments and after the thermal heating.

UVRR spectra were acquired using excitation radiation in the deep UV range by exploiting the synchrotron-based UVRR set-up available at the BL10.2-IUVS beamline of Elettra Sincrotrone Trieste (Italy) [31,32]. The exciting wavelength at 250 nm was provided by the SR source. The energy of radiation was set by regulating the undulator gap and using a Czerny-Turner monochromator (Acton SP2750, Princeton Instruments, Acton, MA, USA) equipped with a holographic grating with 1800 groves/mm for monochromatizing the incoming SR. The excitation radiation at 266 nm was provided by a CryLas FQSS 266-Q2, Diode Pumped Passively Q196 Switched Solid State Laser. All UVRR spectra were collected in back-scattered geometry by using a single pass of a Czerny-Turner spectrometer (Trivista 557, Princeton Instruments, 750 mm of focal length) equipped with a holographic grating at 1800g/mm. The spectral resolution was set at 1.7 and 1.9 cm-1/pixel for the spectra collected with 266 and 250 nm of excitation wavelength, respectively. The calibration of the spectrometer was standardized using cyclohexane (spectroscopic grade, Sigma Aldrich). Any possible photo-damage effect due to prolonged exposure of the sample to UV radiation was avoided by continuously spinning the sample cell during the measurements.

UV/VIS absorption spectra were collected with a Perkin Elmer LAMBDA™ 25 UV/VIS spectrometer operating in double-beam mode and equipped with a plug-n-play single-cell Peltier with a stirrer for temperature control. For UV/VIS experiments the samples of sDNA and sDNA/IL were diluted 10 times in Tris with respect to the samples prepared for UVRR measurements.

Circular dichroism (CD) spectra were recorded using a Jasco J-810 polarimeter. All the DNA solutions were measured at room temperature in a quartz cell of 1 mm path length. Measurements were performed under a constant nitrogen flow, which was used to purge the ozone generated by the light source of the instrument. For CD measurements, the samples of sDNA and sDNA/IL were diluted about 6 times in Tris with respect to the samples prepared for UVRR measurements.

C Results and discussion

Fig. 1(a) and (b) compare the temperature behavior of UVRR spectra at excitation □□ 250 nm for sDNA in aqueous buffer and in hydrated [EMIM][Cl]. Because of the close resonance with the electronic transition at ∼ 255 nm of the imidazole ring of guanine dG, UVRR spectra of DNA in Fig. 1(a) are dominated by the signals arising from the purine moiety of this base [26-27,29-31,33]. The most prominent bands at ∼1486 cm-1 (dGI) and ∼1578 cm-1 (dGII) are indicated by the appropriate labels in Fig. 1(a). These Raman modes appear well disentangled from the vibrational contribution of ionic liquid in the UVRR spectra of sDNA dissolved in the hydrated IL (Fig. 1(b)). Several alterations in the UVRR spectra of sDNA are observed upon the increment of the temperature, both in the absence and presence of the IL, as visible in Fig. 1(a) and (b).

Figure 1: Temperature-dependent UVRR spectra of sDNA in TRIS buffer (a) and sDNA in hydrated [EMIM][Cl] (b) collected using 250 nm as excitation wavelength. The dashed line represents the spectrum

of hydrated [EMIM][Cl]. The corresponding Raman difference spectra DS are calculated as described in the text and reported for sDNA (c) and sDNA/[EMIM][Cl] (d).

These heat-induced modifications can be better visualized by looking at the difference spectra DS obtained by subtracting the spectrum measured at the lowest temperature, 300 K, from the Raman spectrum measured at the indicated temperature (Fig. 1(c) and (d)). DS spectra reflect the actual variation in the intensity of the dGI band as a function of temperature, in the presence and absence of the IL. The dGI signal at 1486 cm-1 exhibits a rapid increase in its Raman intensity that is observed mainly between 335 and 345 K for sDNA in aqueous buffer. Interestingly, the DS spectra reported in Fig. 1(d) evidence that the temperature trend for the dGI band intensity is influenced by the presence of [EMIM][Cl]. Since dGI is assigned to the coupling between the N7=C8 and C8-N9 ring stretching mode and the C8-H in-plane deformation of guanine purine group [26, 34-36] (for the numbering of atoms see Figure S1), the intensity of this band is very sensitive to base stacking interactions involving the guanine residues [28,36,37]. Figure 2 shows the temperature-dependent intensity profiles for the Raman band dGI measured for sDNA without and with different types of ILs. In each plot, the intensities have been normalized to their minimum and maximum values for a better comparison.

Figure 2: Temperature dependence of the relative intensity of dGI band for sDNA in TRIS buffer (a) and in hydrated [BMIM][Br] (b), [MIMI]Cl (c), [BMIM][Cl] (d), [EMIM][Cl] (e) [BMIM][I] (f). Continuous lines are fitting of the experimental data by eqn (1) as described in the text. The unstacking temperature T_us obtained for sDNA in TRIS is marked with dashed line in the figure.

The sudden increase of the dGI band's intensity observed for sDNA in Fig. 2(a) indicates a cooperative structural transformation of the system that takes place around ∼340 K. The intensity recovery of Raman bands associated with modes of the purine and pyrimidine rings has been largely attributed to two types of effects, i.e. i) the hydrogen bonds (H-bonds) breaking and the base unstacking that accompany the complete separation of DNA strands and ii) local structural changes such as the reversible rupture or weakening of H-bonds in the double-stranded structure of DNA as well as the bases tilting occurring without complete separation of the bases [34,35,37-42]. The different intensities of the dGI band observed in Fig. 2(a) match the marked hyperchromicity detected at ∼340 K in the UV absorbance of sDNA measured at 250 nm (see Fig. S2 in the Supporting Information). Since this striking increase of the optical density of DNA is directly connected with the disruption of stacking interactions between bases occurring in DNA upon thermal denaturation, the marked Raman hyperchromicity of the dGI band at ∼340 K reflects the unstacking of guanine bases during the melting of sDNA [20,28,30,33,37,39,40]. It has to be remarked that UV Raman cross-section compared to UV absorbance can selectively identify the structural modifications localized on specific DNA base-tracts during the melting of sDNA, i.e. on guanine-pairs in the case of dGI Raman intensity [29-31, 33]. The substantial disappearances of UV absorbance and Raman hyperchromicity above ∼345 K for the dGI band of sDNA (Fig. 2(a) and Fig. S2 in SI) indicate the complete separation of the two strands of DNA in this temperature region [39]. Conversely, the temperature-dependent variation of dGI intensity observed for T< 335 K in Fig. 2(a) does not correspond to any visible change in UV absorbance at 250 nm. This pre-melting domain marks a relatively non-cooperative structural change, probably associated with an adjustment of the backbone helical geometry and a rearrangement of the inter-guanine interactions that precede the melting of DNA [37,39,40,43,44].

Interestingly, figs 2(a)-(f) point out the effect of different types of ILs on the local structural modifications of G-tracts during the thermal denaturation of sDNA. This is revealed, at first, from the different temperature T_us at which the cooperative unstacking of guanine-pairs takes place. The value of T_us for sDNA in the absence and presence of ILs has been evaluated by fitting the plots in Fig. 2(a)-(f) with the two-state transition law already proposed in previous works [29,30,43,45]:

I_dGI (T)=(I_D + m_D· T + (I_N+ m_(N)· T)·e^(〖ΔH〗_us/R·(1/T_us -1/T)))/(1 + e^(〖ΔH〗_us/R·(1/T_us -1/T))) (1)

where I_N and I_D represent the intensities of the native and denatured state of DNA, R is the gas constant, 〖ΔH〗_us and T_us are the enthalpy variation and the temperature associated to the unstacking of guanine-pairs described by the temperature-dependent intensity of the dGI band I_dGI (T). The parameters m_D and m_(N) have been introduced in eqn(1) to account for the linear temperature-dependence of the band intensity in the pre- and post-melting regions, respectively. The values of T_us estimated for different DNA systems by fitting of the experimental data in Fig. 2 are reported in Table S1 in SI section.

The unstacking temperature obtained for sDNA in aqueous buffer solution is T_us= (341.5±0.5) K. This is consistent with the melting temperature of (338.9 ± 0.1 K) estimated for DNA by UV absorbance data (see insets of Fig. S1 in SI). The addition of [BMIM][Cl] and [BMIM][Br] to the aqueous solution of sDNA produces a small increment of T_us, (343.9±0.9) K and (345.0±0.8) K, respectively. A more marked effect is exerted on sDNA by ILs with different cations [EMIM][Cl] and [MIM][Cl], leading to T_us= (345.7±0.7) K and (347.6±1.0) K, respectively. This result can be explained by the formation of preferential interactions between the cations of imidazolium-based ILs and the guanine-tracts on DNA sequence. These strong IL cation-DNA interactions cause a more effective stacking between guanine bases even at higher temperature values, probably affecting the double helix structure stability [17, 18-20, 22]. This explanation is consistent with simulation results that unveiled the formation of a significant number of interactions between the oxygen and nitrogen of guanine and the oxygen of thymine and the imidazolium ring of ILs [17]. Interestingly, the unstacking temperature T_us of sDNA in the presence of ILs sharing the same anion (chloride in our case) shows a trend related to the alkyl chain length: T_us increases as the alkyl chain gets shorter, in the order [BMIM]<[EMIM]<[MIM]. This may be explained by experimental and simulation results indicating that the lengthening of the alkyl chain induces a charge delocalization on the imidazolium cation [47-49]. We can argue that such charge delocalization together the increasing steric effects lead to a weakening of electrostatic interaction between DNA backbones and [BMIM]+ with respect to [EMIM]+ or [MIM]+ that is reflected by its different stability effect on the structure of DNA. The increment of T_us found as the alkyl chain length of imidazolium cation decreases differs from the investigation by Liu et al. [21] that suggest the establishment of stronger intercalating interactions with DNA upon the increasing the length of the alkyl side chain in the IL cation. Our results support the conclusion of a predominance of groove binding instead of intercalation mechanism between IL imidazolium cations and DNA [17, 18-20, 22].

By looking at the plot in Fig. 2(f), the effect exerted by [BMIM][I] on sDNA appears quite peculiar concerning other ILs. A significant reduction of the unstacking temperature for sDNA is observed in the presence of [BMIM][I] (T_us= 335.8±2.8 K). This finding is probably to be ascribed to the characteristic of iodide ion when undergoes to thermal irradiation. In fact, the high dimension and the high tendency to oxidation to form molecular iodine I2 could play an important role in the unfolding process in contrast with the smaller chloride or bromide ions. At room temperature instead, similarly to the other ILs, [BMIM][I] does not affect the sDNA structure as highlighted in Fig. S3. This is confirmed by CD spectra which are very sensitive to conformational modification of double strand. It is easily shown when sDNA is subjected to increasing temperature (see spectra reported in the inset of Fig. S3), through strong variations both in negative and positive Cotton effects. On the contrary, at room temperature the CD spectra of sDNA solution with or without ILs is the same. In the case of [BMIM][I], even if its strong UV absorption under 250 nm affects the CD spectra (and only with a dilution of IL by 25 time the CD spectra does not show saturation under 250 nm) the positive Cotton effect is superimposable with the one of pure DNA confirming the preservation of sDNA structure at room temperature.

Fig. 3 displays the temperature-dependence of the wavenumber position of the Raman bands dGI and dGII for sDNA with and without ILs.

Figure 3: Temperature evolution of central wavenumber position of the Raman bands dGI and dGII for sDNA in absence and presence of ILs. Dashed lines are fitting of the experimental data by eqn(1) as described in the text.

The red-shift of the dGI mode upon the increment of the temperature has been ascribed to the formation of stronger H-bonds on the N7 site of guanine, occurring in the denatured state of DNA [29,30,35]. This effect can be explained by taking into account the trading base-base hydrogen bonds for base-water hydrogen bonds that might lower the frequency of the dGI mode [35]. It is noteworthy that the presence of [MIM][Cl] and [EMIM][Cl] induces a further red-shift in the position of the dGI band with respect to sDNA in the aqueous buffer that is observed over all the explored T range (Fig. 3). The finding is consistent with the "dehydration" effect operated by these ionic liquids on the structure of the DNA duplex [17,18], whose cations experience stronger interactions with the H-bonding sites of guanine compared to water. The data in Fig. 3 unveil a best capability of [EMIM]+ and [MIM]+ respect to [BMIM]+ cation to disrupt the well-coordinated hydration layer of water around DNA structure and take part in the solvation process. This finding seems to suggest that the balance between the hydrophilic/hydrophobic moieties in imidazolium cations is a key parameter that determines the amount of IL ions penetrating the DNA solvation layer and affecting the stability of double helix structure.

The Raman signal labeled as the dGII band is assigned to the stretching motions of N3-C4, C4-C5, and C5-N7 bonds of the purine ring of guanine [28,34,44]. Fig. 3 shows a sudden blue-shift in the frequency position of the dGII mode for sDNA that occurs around the melting temperature at ∼340 K. A similar two-state behavior is observed for the same dGII band also in presence of ILs, with the rapid upshift taking place at a characteristic temperature depending on the type of IL (Fig. 3). For the case of sDNA in hydrated [BMIM]I the analysis of the temperature evolution of dGII band does not provide reliable results due to the strong superimposition in the spectra of the Raman signals arising from sDNA and [BMIM]I. The temperature dependence of the wavenumber position found for the dGII band is closely related to the heat-induced unstacking of guanine-pairs revealed by the Raman hyperchromicity of the dGI signal (Fig. 2). Indeed, the reinforcement of the double bonds of the guanine ring reflects the major localization of the electrons within the purine structure in the single strand compared to the double strand of sDNA. The fitting of the temperature trends of the dGII frequency position (plots reported in Fig. 3) according to equation (1) provides a quantitative evaluation of the unstacking temperature T_us marked by this Raman mode. Interestingly, Fig. 4 points out that the increase or decrease of T_us for sDNA in presence of different types of ILs is fully consistent with the behaviour observed for the intensity of the dGI band.

Figure 4: Guanine unstacking temperature T_us for sDNA in absence and presence of ILs detected by the Raman hyperchromicity of dGI band intensity and by the frequency position of dGII band. The structures of the different ILs considered in this study are reported on the graph.

The agreement between the values of T_us obtained by the analysis of the spectral parameters of dGI and dGII bands confirms that the increase of the temperature induces a cooperative reduction of base stacking interactions and H-bonding strength involving G-tracts in sDNA structure. This process is strongly affected by the presence of ILs, as clearly shown in Fig. 4. In particular, the evidence for the existence of a high-temperature partially folded state of sDNA in hydrated [EMIM][Cl] and [MIM][Cl] appears like a remarkable trait of the stabilizing action operated by these imidazolium-based ionic liquids on DNA structure. The good accordance found between the values of T_us estimated by the analysis of two different Raman signals of sDNA corroborates the reliability of UVRR technique in providing sensible spectroscopic markers to localized structural changes in DNA.

Figure 5(a)-(b) displays the temperature evolution of UVRR spectra of sDNA in the absence and in presence of [EMIM][Cl] collected using a different excitation wavelength at 266 nm.

Figure 5: Temperature-dependent UVRR spectra of sDNA in TRIS buffer (a) and in hydrated [EMIM][Cl] (b) collected using 266 nm as excitation wavelength. The dashed line represents the spectrum of hydrated [EMIM][Cl]. The corresponding Raman difference spectra DS are calculated as described in the text and reported for sDNA (c) and sDNA/[EMIM][Cl] (d).

Thanks to different UV absorption patterns of nucleobases, the 266 nm UVRR spectra provide a special focus on the behavior of adenine dA and thymine dT residues during the thermal unfolding path of DNA [26,27,29]. The difference spectra DS reported in Fig. 5(c)-(d) evidence remarkable heat-induced changes in intensity and frequency of the Raman bands at ∼1307, 1339 and 1650 cm-1. The signals at ∽1307 cm-1 (dA) and ∽1339 cm-1 (dAI) are attributed to the coupled stretching vibrations of N7=C8 and C5-N7 bonds localized on the purine ring of adenine dA [26, 49,50]. In particular, the Raman band dAI is very sensitive to adenine nucleoside conformation [39] and to the rearrangement of H-bonds at the acceptor site N7 of dA [44, 51]. The Raman signal labeled as dT∼1650 cm-1 is assigned to the coupled stretching modes of C4=O and C5=C6 bonds of thymine and it is strongly reflective of any perturbations occurring at those sites of thymine [40,52, 53].

By looking at the DS spectra in Fig.5 we note a marked Raman hyperchromicity of the band intensity dAI for sDNA in the absence and presence of ILs upon the increment of the temperature. Differently from the case of the dGI signal, the dependence on the temperature exhibited by the intensity of the dAI band departs from the characteristic two-state behavior typical of the melting transition detected by UV absorbance. This is evident by comparing the temperature dependence of the dAI band intensity for sDNA in Fig. 6 and the UV absorbance of DNA at 266 nm as a function of temperature (Fig. S2 in SI).

Figure 6: Temperature dependence of the relative intensity of dAI band for sDNA in TRIS buffer (a) and in hydrated [BMIM][Br] (b), [MIMI]Cl (c), [BMIM][Cl] (d), [EMIM][Cl] (e) and [BMIM][I] (f). The dashed line marks in the graphs the pre-melting temperature at ∼304 K discussed in the text.

The complex temperature dependence reported in Fig. 6(a) for dAI band of sDNA in an aqueous buffer shows that the denaturation of DNA occurs through a multistage process including intermediate conformational changes that primarily involve A-tracts in the sequence of DNA [29,37,40,43]. As a first remark, we note the appearance of a peak with the maximum observed at ∼304 K in the temperature trends of dAI reported in Fig. 6. This feature evidences the occurring of a reversible pre-melting conformational transformation that involves adenine-pairs in the structure of DNA. Consistently with similar pre-melting phenomena observed in Poly(dA–dT) · poly(dA–dT) double-helical B DNA [39,40,53], the reversible increment in the dAI intensity at ∼304 K may be related to the stabilization of extra amount of propeller twist between the A-T base planes through the formation of a third hydrogen bond cross-strand between consecutive dA - dT pairs [40]. By looking at the trend of dAI intensity in Fig. 6(a) for temperatures above 305 K, the non-monotonic dependence on temperature of this band suggests the presence of different intermediate stacking arrangements involving adenine-pairs during thermal pathway of sDNA. These structural fluctuations in the pre-melting region could be ascribed to opening and closing of the DNA structure at the level of the adenine pairs, promoted by the increment of the temperature. To get more quantitative information on the number of these intermediate conformational states and the associated thermodynamic parameters, we model the trends of dAI intensity in Fig. 6 as the population-weighted sum of different states. We assume that each intermediate state can be described by a two-step law whose inflection point corresponds to a maximum in its temperature derivative. The first-order derivative of the temperature profiles of dAI intensity are reported in Fig. S4 of SI. Despite the multi-phasic behavior of the derivative curves, three main transitions occurring around characteristic transition temperature can be traces back. It turns out that, during the unfolding process of sDNA, the adenine residues populate different intermediate states, i.e. N ↔ I1↔ I2 ↔ D. A first minor increase of dAI intensity at about 315-320 K is followed by a much steeper rise localized at 330-340 K. The latter is likely related to the I1↔ I2 transition that probably involves the weakening of H-bonds and conformational rearrangements of adenine tracts that precede the cooperative separation of DNA strands during the melting. The last transition I2 ↔ D takes place at about 350-360 K and it is directly ascribed to the main unstacking of dA bases flanking the dissociation of the DNA strands. Interestingly, Fig. 6 points out that the presence of ILs does not produce any significant effect on the pre-melting transition of sDNA at 304 K. Consistently, we also observe that the intermediate states of sDNA unfolding described above are not significantly perturbed when DNA is in hydrated ILs. This finding, in agreement with our previous results obtained on imidazolium-based ILs [29], strongly supports the simulation studies indicating the establishment of a dominant interaction of imidazolium cations with guanine residues on the strands of DNA [17]. An exception respect to this common behavior of ILs is found by looking to the trend of dAI intensity in the case of sDNA in hydrated [BMIM]I. The rapid decrease of dAI intensity occurring after about 350 K suggest that a certain number of adenine bases are stacking presumably with the reforming of a number of inter-base hydrogen bonds. However, the reformation of double helix structure of DNA is hampered probably due to mismatching of homologous segments for the formation of loops and random-coil forms.

The picture proposed on the basis of the dAI band intensity is consistent also with the analysis of the temperature dependence wavenumber position of the dAI and dT Raman bands, displayed in Fig. 7.

Figure 7: Temperature evolution of central wavenumber position of the Raman bands dAI and dT for sDNA in absence and presence of ILs.

The red-shifts observed for the Raman signal dAI may be explained by the progressive weakening of H-bonds at the acceptor sites N7 of adenine as the temperature is raised [39,51]. This is due to the replacement of inter-adenine hydrogen bonds with weaker adenine-water hydrogen bonds as the unfolding of sDNA progresses. This process could be in turn favored by the progressive exposition to the solvent of the dA residues of DNA during the melting. Fig. 7 shows no significant modifications induced by the presence of ILs on the frequency temperature dependence of the Raman band dAI. This could be consistent with the conclusion that the predicted binding capability of ions with the minor groove of DNA [54,55] determines DNA deformations such as base tilting rather than the rupture of hydrogen bonds on adenine residues in adenine-tracts.

More interestingly, Fig. 7 displays also the temperature dependence of the dT band wavenumber position whose strength of oscillation accounts for any perturbations occurring at the C4=O site of thymine residue [26,40,51]. The marked upshift of this mode observed at ∼330 K can be correlated with a decrease in H-bonding strength on the C=O site of thymine during the transition of DNA from duplex to single-strand [29, 40]. The relatively large blue-shift (about 5-6 cm-1) observed for the dT band as a function of temperature is congruent with the occurrence in the structure of DNA of a cross-strand three-centered H-bonds between dA and dT bases [40]. Since the pre-melting transition is primarily attributed to changes in the degree of propeller twisting and in the strength of the cross-strand three-centered H-bonds, the data reported in Fig. 7 probably imply a negligible perturbation operated by ILs on the extent of these three-centered H-bonding. It has to be noted that only the presence of [BMIM]I induce a significant red-shift of the position of the dT band that persists over all the explored temperature range (Fig. 7). This finding suggests the formation of strong H-bonds between the thymine and this IL that are not observed in the case of the other considered ionic liquids. The perturbation induced by the iodide anion on the hydration shell of C=O group of thymine could be consistent with the observed strong effect exerted by [BMIM]I on the base stacking and H-bonding strength in A-T tracts of DNA.

Conclusions

Biotechnological and biomedical applications more and more often require the use of solvents alternative to water in which DNA is soluble and it can be preserved for a long time and/or under extreme conditions. The predicted capability of aqueous solutions of ionic liquids to enhance the stability and function of DNA has stimulated renewed interest in clarifying the mechanism of interaction between ILs and nucleic acids controlling the stabilization/destabilization effect on DNA structure. In this work, we address the problem to rationalize the effect exerted by cations and anions in imidazolium-based ILs on the thermal structural stability of large nucleic acid molecules. Multi-wavelengths UV Resonance Raman technique offers a selective capability for detecting both cooperative and local structural transformation specifically localized on different base tracts of DNA during the heat-induced unfolding. These structural transitions may be invisible to other analytical methods less sensitive to transitions that do not involve changes in the number of closed base pairs. By exploiting the fine tunability of the synchrotron source, we have implemented UVRR experiments that provide insights into the base stacking, hydrogen bonding and backbone conformation of DNA that possibly affect the thermal stability of nucleic acids in hydrated imidazolium-based ionic liquids. The present analysis detects the establishment of preferential interactions between the imidazolium cations of ILs and the guanine-tracts on DNA sequence. These strong cation-mediated interactions with DNA groove lead to a more effective stacking between guanine bases even at higher temperature values, probably affecting the double helix structure stability. Interestingly, we observe that this trend for ILs sharing the same chloride anion is further enhanced as the alkyl chain gets shorter, in the order [BMIM]<[EMIM]<[MIM]. The last result confirms the predominance, predicted by simulation studies, of groove binding mechanism between IL imidazolium cations and DNA with respect to the intercalation

binding. UVRR experiments at different excitation wavelengths hint that the heat-induced denaturation of DNA occurs through a multistage process including intermediate conformational changes that primarily involve adenine-pairs. These pre-melting conformational changes are detected by UVRR spectra and the data reveal that ILs do not significantly affect the intermediate states before the dissociation of the DNA strands. This is consistent with base tilting and rupture of hydrogen bonds on adenine residues in A-tracts induced by the binding of ILs ions with the minor groove of DNA.

The results from the present synchrotron-based UVRR investigation contribute to a more comprehensive view of the IL-mediated interactions with specific tracts of DNA sequence during thermal unfolding. This could facilitate designing of effective stabilizing ILs for their exploitation in biomedical and life science field.

Author Contributions

We strongly encourage authors to include author contributions and recommend using CRediT for standardised contribution descriptions. Please refer to our general author guidelines for more information about authorship.

Conflicts of interest

There are no conflicts to declare.

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