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Melting of the solvent structure around a RNA duplex: a molecular dynamics simulation study

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Abstract

From three 2.4-ns molecular dynamics simulations of the r(CpG)$_{12}$ duplex conducted at 5, 25 and 37 °C, a strong temperature dependence of the dynamics of the water molecules and ions located in the first nucleic acid coordination shell is observed. At 5 °C, the highest residence times of bound water molecules exceed 1 ns while, at 37 °C, they decrease to 0.5 ns in agreement with available NMR data. Similar temperature dependencies are observed for the potassium ions bound to the duplex. In this temperature range, the structure of the RNA helix remains essentially unchanged. Thus, the observed alterations correspond to a 'premelting' of the solvent structure around the duplex. It is proposed that, before the nucleic acid structure melts, the entropy of the solvent increases to a point where it is no longer compensated by the enthalpic contribution of solute–solute and solute–solvent interactions. At this stage, the weakest structural elements start to melt. In other terms, the experimentally observed melting processes are preceded by a melting of the more labile solvent structure. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

Experimental and theoretical studies have led to a better understanding of the structural properties of the hydration shell of RNA molecules [1–4]. Yet, while several NMR studies investigated the dynamical characteristics of the DNA hydration shells [5–11], only two studies reported partial data related to the kinetics of the hydration shell of RNA duplexes [12,13]. Molecular dynamics (MD) simulations, which are able to provide detailed views of the hydration of DNA structures in agreement with experimental data [14–17], have been used in order to complement this lack of information for RNA molecules [15,16]. At room temperature (25 °C), several long-lived hydration patterns ($\approx$800 ps) were characterized in the deep groove while shorter residence times ($<$300 ps) were calculated for water molecules located in the

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shallow groove. Given the symbiotic nucleic acid/solvent relationship, it is clear that these hydration patterns are involved in the stabilization of RNA structures through enthalpic/entropic compensation processes [18]. However, the temperature-dependent features of these molecules at the microscopic level are poorly understood. Here, in order to shed light on the temperature-dependent properties of the hydration shell of a RNA helix, three 2.4-ns molecular dynamics simulations of the $r(CpG)_{12}$ duplex at 5 °C (278 K), 25 °C (298 K; room temperature) and 37 °C (310 K; physiological temperature) are analyzed.

2. Computational methods

Three 2.4-ns MD trajectories of the $r(CpG)_{12}$ duplex at constant temperature (5, 25 and 37 °C) and constant pressure (1 atm) have been generated with the AMBER 5.0 molecular dynamics simulation package [19,20] by using protocols which have been described elsewhere (the 25 °C simulation is identical to that analyzed by Auffinger and Westhof [15,16]). The simulation box contains 72 K^+ and 26 Cl^-, as well as 5697 SPC/E water molecules [21], corresponding to an excess ionic concentration of KCl close to 0.25 M. It has to be noted that for all three simulations, the starting configuration, i.e. position of the solute, the ions and the water molecules, is exactly the same. The particle mesh Ewald (PME) summation method has been used for the treatment of the long-range electrostatic interactions [22,23] to circumvent severe artifacts generated by the use of truncation methods [24–26]. In order to avoid the inclusion of potential end effects in the statistics and exclude the equilibration phase, only data gathered from the last 1.5 ns of each 2.4-ns trajectory and the underlined central 12 base pair segment of the $r[(CpG)_3(\underline{CpG})_6(CpG)_3]_2$ duplex were analyzed.

The methods used to calculate the densities of water molecules around the r(G=C) pairs and the residence time profiles for water molecules have been described by Auffinger and Westhof [15,16,27]. In short, the water molecules in direct contact with the base pairs were used in order to construct ‘hydrated building blocks’. Subsequently, these ‘hydrated building blocks’ were Fourier transformed in order to obtain water molecule ‘pseudoelectron’ densities. The residence time profiles are based on hydrogen bonding contact times that correspond to the maximum time during which a water molecule establishes a hydrogen bond interaction with a solute atom. Hence, the residence time profiles represent the hydrogen bonding time of water molecules entering into contact with specific nucleic acid atoms plotted vs. the number of water molecules attached to these atoms.

3. Results

For a given contour level, although qualitatively similar, the water molecule densities around the r(G=C) pairs are less well defined at 37 °C than at 5 °C (Fig. 1). At 5 °C, density peaks corresponding to all the 22 hydration sites, which are part of the first hydration shell of the r(G=C) pair [15], are clearly visible, while at 37 °C, only 17 density peaks can be identified.

As expected, the residence times of water molecules connected to the r(G=C) base pair (Fig. 2) decrease with an increase in temperature, inline with a calculated increase of the diffusion coefficient of water [28]. This effect is observable for all the solute atoms but is the most pronounced for the OR and OS anionic oxygen atoms of the phosphate groups followed by the deep groove atoms of the bases. At 5 °C, water molecules with calculated residence times longer than 1 ns are found in the vicinity of the OR, N7 and O6 atoms while, at 37 °C, the longest residence times barely exceed 500 ps. As noted elsewhere [15,16], the average residence times of water molecules bound to the shallow groove is shorter than that of water molecules bound to the deep groove, although the calculated temperature dependence is similar. Consequently, the activation energy for the displacement of a deep groove water molecule is higher than the one of a shallow groove water molecule. In a kinetic sense, chemical groups belonging to drugs, amino acids and other nucleotides may replace the latter more easily.

For the K^+ ions, despite a limited sampling due to the ‘relatively’ short simulation times, the same trend is observed. At 5 °C, three ions with residence times exceeding 1 ns form direct contacts

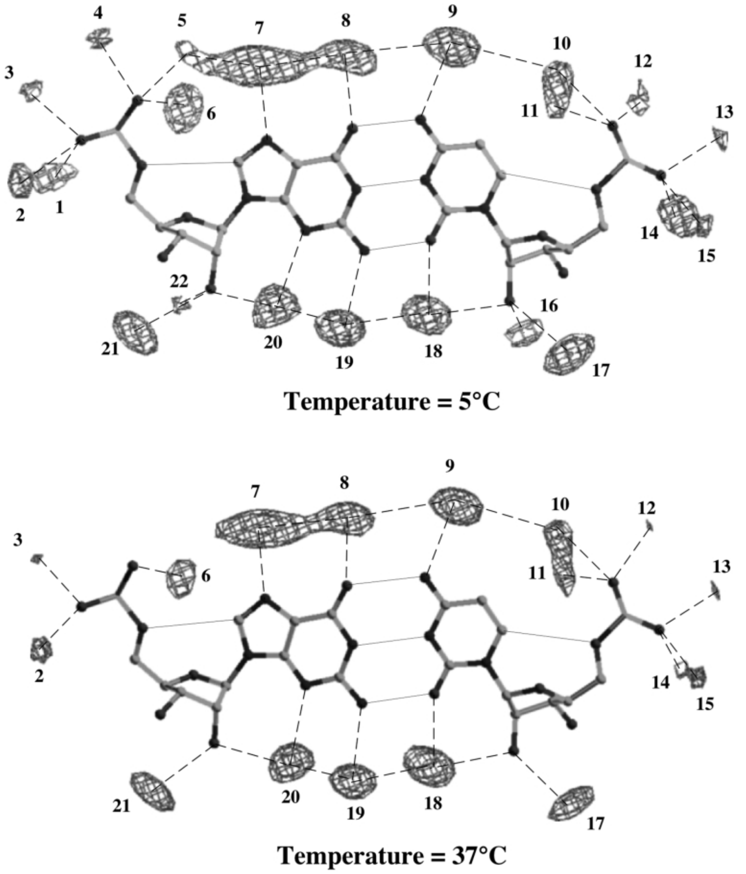

Fig. 1. Water and ion densities surrounding an r(G=C) base pair represented at a similar contour level at 5 °C (top) and 37 °C (bottom). As shown by Auffinger and Westhof [15,16], the K^+ ions are considered as water molecules.

with atoms of the deep groove of the duplex, while at 37 °C, the longest calculated residence time does not exceed 700 ps. As described elsewhere, the K^+ ions with the longest residence times are essentially bridging the O6 atoms of GpC steps but not CpG steps due to steric and electrostatic hindrance [15,16] (Fig. 3).

Since such drastic changes in the residence times of water molecules and ions were calculated, it is interesting to note that the conformation of the RNA helix was not altered in a significant manner when raising the temperature from 5 to 37 °C (Fig. 4), although a slight increase in the mobility of the backbone atoms can be noted: the average B-factors calculated for all the heavy atoms of the 12 central base pairs increase from 33 ± 10 Å^2 (5 °C) to 40 ± 11 Å^2 (25 °C) and to 42 ± 12 Å^2 (37 °C).

4. Discussion

The implications of the temperature-dependent stability of the first solvent shell described above are multiple. They concern the interpretation of crystallographic and NMR data, the thermal denaturing process and methodological aspects.

4.1. Methodological issues

In this study, three MD simulations were conducted in a relatively narrow range of temperature (5–37 °C). Since the force-fields and water models

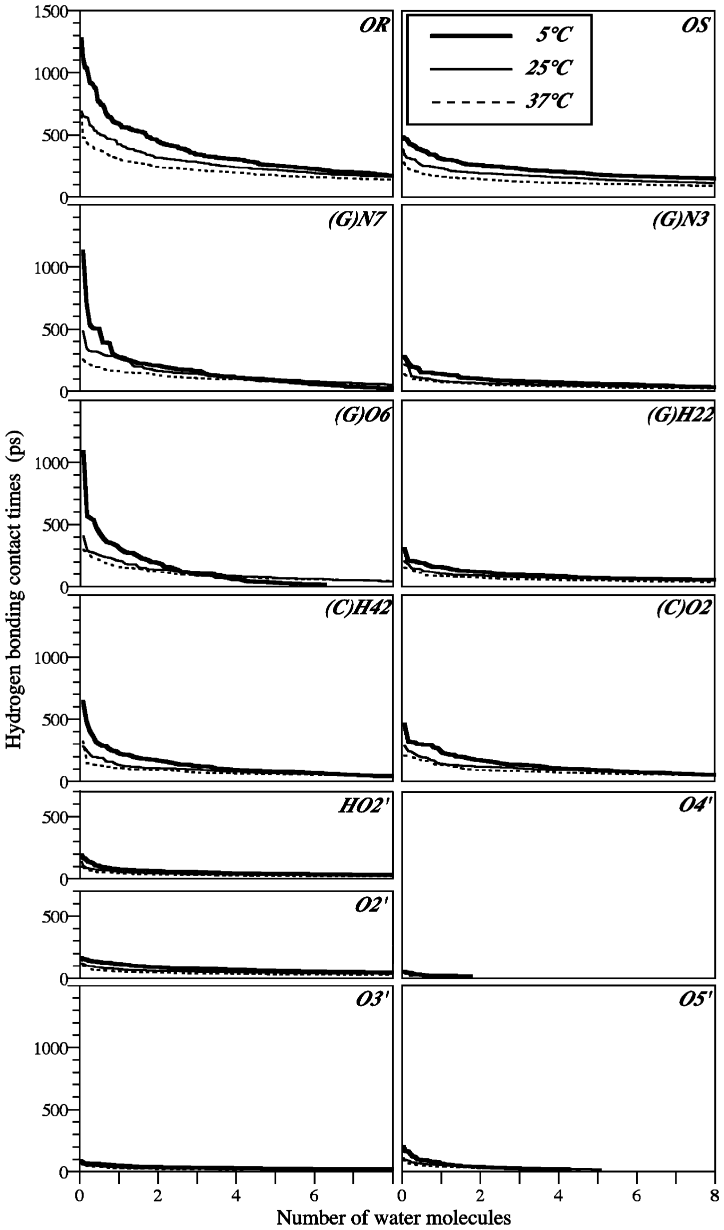

Fig. 2. Residence time profiles for water molecules. The number of water molecules bound to specific hydrophilic atoms of the central G = C base pairs of the RNA duplex is plotted as a function of their hydrogen bonding contact times. The data have been normalized according to the number of identical atoms present in the duplex and, therefore, differ from the non-normalized data from previous work [15,16].

are calibrated for the 25 °C room temperature, small differences in the results may occur due to deviations from this 'ideal' temperature. For these reasons, and due to other approximations inherent to the MD methodology, such as the neglect of polarization and charge transfer effects, the calcu-

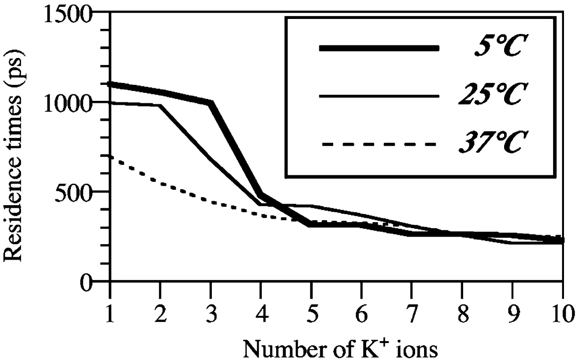

Fig. 3. Residence time profiles for K^+ ions. The number of potassium ions present in the first coordination shell of the twelve central base pairs of the $r(CpG)_{12}$ duplex (see Fig. 4) is plotted as a function of their residence times.

lated residence times may not be quantitatively exact. However, their order of magnitude and the temperature dependence of the water molecule residence times are in agreement with NMR data as detailed below and give credit to the results extracted from these MD simulations.

4.2. NMR

NMR is the only experimental method that gives access to the kinetics of the solvent molecules. Model independent residence times of 0.9 ns (±0.1 ns) at 4 °C were reported for five water molecules located in the minor groove of the d(CGCGAATTCGCG) duplex [7]. At 10 and 27 °C, the residence time for these water molecules was estimated to be close to 0.6 and 0.2 ns, respectively. Consistently, at 10 °C a 0.6-ns residence time was determined for water molecules located in the minor groove of the same duplex [11] and residence times close to 11 ns have been characterized at −20 °C [9]. Temperature-dependent ion binding times were also reported [8,29]. These data on DNA duplexes are in agreement with those extracted from present MD simulations on the $r(CpG)_{12}$ duplex. The latter provide detailed information about the location and the binding strength of the solvent molecules located in the first solvent shell that may be of use in the interpretation of NMR data.

4.3. Crystallography

Not many temperature-dependent crystallographic studies have been reported. While for most high-resolution crystal structures, data are collected at 100–160 K after flash cooling, the crystals are usually grown at temperatures above 0 °C. For a left-handed DNA hexamer, it has been shown that crystals grown at 37 °C diffract much better than those grown at 18 °C, while no crystals could be obtained at 5 °C [30]. The same oligomer structure was found in both crystals, but a more complete hydration shell was observed from the crystals obtained at high temperature. Yet, many DNA crystals are grown at ≈4 °C while RNA crystals are usually grown at ≈37 °C, illustrating that temperature is an important crystallization parameter. As shown above, although the structure of the RNA duplex (Fig. 4) and of its hydration shell (Fig. 1) is qualitatively similar for the three investigated temperatures, the dynamics of the solvent molecules differ considerably. Thus, a high solvent entropy may favor the crystallization of rigid RNA molecules. For the more flexible and adaptable DNA structures [15,16], a high solvent entropy may not compensate for the loss of solute structure, therefore, crystallization at low temperatures is preferable.

4.4. Melting

In contrast to NMR, which tend to favor the enthalpic over the entropic component of the free energy of the system by working at low temperatures, melting experiments use the temperature dependence of the free energy in order to determine the stability of specific structures and the associated thermodynamic parameters [31,32]. Yet, implications at the atomic scale of these measurements are not as well understood as are the enthalpy/entropy compensation processes at play in these systems. Present data suggest that the melting may occur in at least two stages. First, before the nucleic acid melts, the water and ionic shells surrounding the solute increase their entropy by reducing the number and strength of the solute/solvent interactions. When the temperature further increases, the solvent (ion and water) loses its

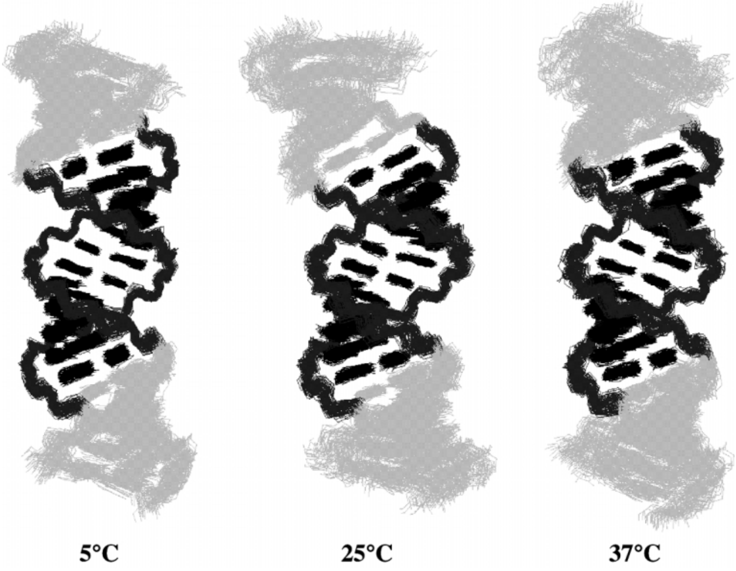

Fig. 4. Superposition of 50 snapshots of the $r(CpG)_{12}$ duplex extracted from the last 500 ps of three 2.4-ns MD simulations conducted at 5, 25 and 37 °C. The highlighted 12 central base pairs of the duplex have been used for superposition.

cohesion to a point where the solute/solvent interactions are no longer sufficiently strong to help in the stabilization of the solute/solute interactions. Then, the weakest structural elements of the nucleic acid system start to melt [33,34]. Thus, the observed melting processes are preceded by a melting of the more labile solvent structure, probably similar to the one extrapolated from present MD simulations. It is proposed that the melting of the solvent structure corresponds to a 'premelting' phase of the nucleic acid structure.

Does the melting of the solvent structure coexist with a conformational transition of the nucleic acid structure? Premelting of DNA structures containing phased A-tracts has been reported near 30 °C by numerous groups [35–38]. This premelting is associated with a transition from a bent structure at low temperature toward an unbent structure at higher temperature (over 40 °C). Yet, such transitions are certainly related to the unusual structure adopted by the A-tracts of DNA duplexes that are associated with small activation energies, since no such conformational changes were reported for other DNA sequences [35] or more rigid RNA duplexes. Thus, A-tract sequences exhibit a larger susceptibility to the 'shaping' effect of the solvent (water and ions) than other DNA sequences. Clearly, this 'shaping' effect is more pronounced at low than at high temperature and affects more DNA than RNA helices, which are known to be more rigid than DNA duplexes of similar sequence [15,16].

5. Conclusion

Nucleic acid structures are known to be stable over a given range of temperature where enthalpic/entropic compensation processes involving the solute as well as the solvent are at play. Here, we show that MD simulations conducted at different temperatures can give some insight into the microscopic phenomena that underline these macroscopic thermodynamic effects by being able to distinguish and categorize the ions and water molecules into those with long residence times and those with short residence times. This partitioning may lead to a better understanding of nucleic acid melting, RNA/protein recognition, RNA/drug association and enzymatic mechanisms that are all related to the dynamics of the solvent bound to the RNA structure, and more specifically to solvent molecules located at interfaces or residing in active sites.

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