

Ab Initio Molecular Dynamics of Na⁺ and Mg²⁺ Counteranions at the Backbone of RNA in Water Solution

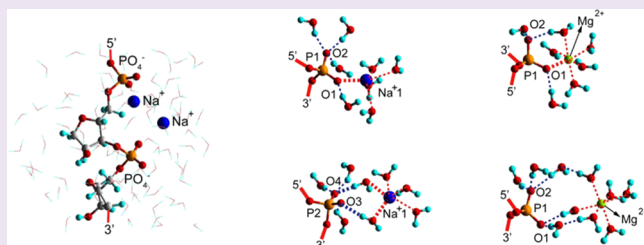
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S Supporting Information

ABSTRACT: The interactions between sodium or magnesium ions and phosphate groups of the RNA backbone represented as dinucleotide fragments in water solution have been studied using ab initio Born–Oppenheimer molecular dynamics. All systems have been simulated at 300 and 320 K. Sodium ions have mobility higher than that of the magnesium ions and readily change their position with respect to the phosphate groups, from directly bonded to completely solvated state, with a rough estimate of the lifetime of bonded Na⁺ of about 20–30 ps. The coordination number of the sodium ions frequently changes in irregular intervals ranging from several femtoseconds to about 10 ps with the most frequently encountered coordination number five, followed by six. The magnesium ion is stable both as directly bonded to an oxygen atom from the phosphate group and completely solvated by water. In both states the Mg²⁺ ion has exactly six oxygen atoms in the first coordination shell; moreover, during the whole simulation of more than 100 ps no exchange of ligand in the first coordination shells has been observed. Solvation of the terminal phosphate oxygen atoms by water molecules forming hydrogen bonds in different locations of the ions is also discussed. The stability of the system containing sodium ions essentially does not depend on the position of the ions with respect to the phosphate groups.



Metal ions (Na⁺, K⁺, Mg²⁺, Ca²⁺) play important roles in the stability, folding, and functioning of nucleic acids.^{1,2} Their presence is essential for the structural integrity of the chromosomes of different living cells, including mammalian.^{3,4} These ions help maintain the spatial configuration of DNA^{5,6} and RNA^{7,8} not only by neutralizing the negative charges of the phosphate groups but also mediation by some of them of the interactions between parts of the macromolecules. Metal ions stabilize the tertiary structure of RNA in the ribosome^{9,10} and the ribozymes^{11–14} or directly participate in the interactions between different components of the ribozymes. Metal ions could also be used as doping agents to alter the electronic properties of nucleic acids in order to create new materials for the photonics and electronics¹⁵

The interactions between metal ions and various DNA and RNA sequences have been experimentally studied employing various techniques. The preferred binding sites of monovalent and divalent metal ions in solution and crystal phase have been explored by NMR^{5,6,8,16–18} and vibrational spectroscopy methods including IR^{19,20} and Raman^{21–26} spectroscopy. The interactions between the ions and phosphate groups have been analyzed by measuring symmetric and asymmetric PO₄[−] vibrations. The assignment of the vibrational frequencies for this analysis was based on the vibrational spectra of low molecular weight phosphoric acid esters.^{27–29} Low frequency vibrations, arising from the presence of alkali ions near the phosphate groups of DNA, are also studied.³⁰ The locations of

the ions in crystal phase may be determined by X-ray crystallography,^{5,6,9,10,31–35} but often their positions are not clear due to irregular location of the cations and difficulties in distinguishing them from the water molecules.⁵ An additional complication comes from the fact that the structure of the biomolecules in the crystalline phase differs from that in solution. The experimental results obtained in solution more closely match the biological systems, but the methods, applied in these studies (NMR, IPR, vibrational spectroscopy) give only indirect information for the interactions and the structural information derived by them is to a large extent qualitative.

To complement this deficiency of the available experimental methods, theoretical approaches, including classical molecular dynamics^{36–38} and Monte Carlo methods,³⁹ are also used to study interactions of metal ions with nucleic acids. While these methods are very powerful for modeling dynamics of biomolecules as proteins, DNA, or RNA, the description of the local interactions in these methods is predetermined by the parametrization of the applied force field. Such local interactions are properly described with quantum chemical methods, although they are restricted by the size of the modeled system. The first-principles approaches based on quantum chemical methods intrinsically account for all types of

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Table 1. Data from Dynamical Simulations^a

cation ^b	temp (K)	period (ps)	M–O1 (pm)	M–O (pm)	% N ^c	comment
Na ⁺ 1	300	1–18	235	235	19/61/20/0	Na ⁺ 1 bonded to O1
Na ⁺ 2			665	235	9/79/12/0 ^d	Na ⁺ 2 solvated
Na ⁺ 1	300	18–66	455	235	2/88/8/1	Na ⁺ 1 solvated
Na ⁺ 2			645	235		Na ⁺ 2 solvated
Na ⁺ 1	300	66–95	255	245	1/32/52/15	Na ⁺ 1 bonded to O1
Na ⁺ 2			645	245		Na ⁺ 2 solvated
Na ⁺ 1	300	95–104	355	235	1/81/18/0	Na ⁺ 1 solvated
Na ⁺ 2			665	235		Na ⁺ 2 solvated
Na ⁺ 1	320	104–125	455	245	3/82/14/1	Na ⁺ 1 solvated
Na ⁺ 2			665	235	3/78/19/0 ^d	Na ⁺ 2 solvated
Mg ²⁺ bonded	300	1–61	205	215	0/0/100/0	Mg ²⁺ bonded to O1
Mg ²⁺ bonded	320	61–140	205	215	0/0/100/0	Mg ²⁺ bonded to O1
Mg ²⁺ solvated	300	1–61	465–565	215	0/0/100/0	Mg ²⁺ solvated
Mg ²⁺ solvated	320	61–140	425	215	0/0/100/0	Mg ²⁺ solvated

^aIncluding the temperature used; period; distances (peaks of the RDF) between the cations, O1 oxygen atom, and the oxygen atoms from the first coordination shell; and percents of time during which the ion coordinates different numbers of oxygen atoms, % N. ^bFor notation of the atoms see Figure 1 and the description in the text. ^cThe numbers a/b/c/d in the column denote the percentage of time in the corresponding simulation period during which the coordination number of the ion is 4, 5, 6, and 7, respectively. The highest value is in bold. ^dFor Na⁺2 the values are calculated for the whole periods of simulation at 300 and 320 K, 1–104 and 104–125 ps.

interactions between the atoms, ions, molecules, or functional groups in the system as electrostatic interaction, electron transfer (including variation of the charge of the species during simulation), mutual polarization, Pauli repulsion, electron exchange and correlation. Some of these interactions are included in the classical potentials for MD simulations. Since the parameters of these potentials are derived to reproduce certain properties of the systems to be modeled, they cannot necessarily provide correct results for other systems or for other properties (for a recent example see ref 40). Since the quantum chemical methods are derived from first principles, they as such are not system-specific and can be used to simulate different properties or characteristics of the system without special parametrization. For this reason quantum chemical calculations are often used as targets in derivation of the parameters of classical potentials or for evaluation of their accuracy with respect to certain properties.⁴¹ A detailed discussion of the advantages, disadvantages and typical applications of the classical, quantum mechanical and hybrid quantum mechanical-molecular mechanical (QM/MM) methods for simulations of RNA is provided in the recent paper of Ditzler et al.⁴²

The application of quantum chemical methods has so far been limited to optimization of the structure of RNA fragments with Na⁺ and Mg²⁺ ions in gas phase or water modeled as continuum⁴³ as well as using QM/MM schemes.^{42,44} On the basis of cluster calculations using a continuum solvent model, it was suggested that both sodium and magnesium ions have affinity for binding to the phosphate groups of RNA, although the affinity of Mg²⁺ is higher by about 116 kJ/mol, assuming that Mg²⁺ is bonded to two phosphate groups while the Na⁺ binds only to the one.⁴³ In order to understand the complexity of the interactions, in addition to looking at a local minimum, one could simulate the evolution of the system by ab initio molecular dynamics. Such type of modeling has been used to describe the behavior of the metal ions in different systems such as in water solution of carbonates,⁴⁵ but ab initio MD simulations of the interaction of metal cations with RNA or DNA fragments has not been reported so far.

In the present paper we report ab initio molecular dynamic simulation of sodium or magnesium ions in the vicinity of the

backbone fragment of a nucleic acid in water solution. The model system includes two phosphate groups and two ribose moieties compensated by two sodium or one magnesium cation. The whole system is solvated in 106 or 110 water molecules for the model systems with two Na⁺ ions or one Mg²⁺, respectively. With these calculations we were able to clarify the actual location and mobility of these cations with respect to the phosphate groups of the backbone in water and to shed light on the picoseconds dynamic of the system up to 125 ps. We have shown that sodium cation is very mobile and moves between the first and second coordination sphere of the phosphate in periods of ca. 30 ps, while magnesium ion keeps its coordination sphere rather strong (no exchange of the ligands in its first coordination shell is observed) and does not move notably with respect to the phosphate.

RESULTS AND DISCUSSION

Dynamics of the Sodium-Containing Systems. *Mobility of the Sodium Ions.* First we report the results from the ab initio molecular dynamics simulation related to the interactions of sodium ions with the phosphate groups from RNA backbone. The length of the simulation is in total 125 ps. In Table 1, the summary of the conditions and duration of all performed simulations is presented. During the first 104 ps, the temperature (thermostat) is adjusted to 300 K, while in the last 21 ps the temperature is set at 320 K in order to speed up the dynamics of the system.

After 1 ps (necessary for thermal equilibration to 300 ± 20 K), both sodium ions stay close to their initial positions: one of the Na⁺ ions (denoted as Na⁺1, Figure 1b,c) is directly bonded to an oxygen atom (O1) from the phosphate group (P1), while the other Na⁺ ion (Na⁺2) interacts with one of the phosphate groups via one water molecule and is denoted as solvated in water (Figure 1b,c). The Na⁺1 ion remains coordinated directly to the O1 atom until the 18th ps of the simulation. The radial distribution function (RDF) for the Na⁺1–O1 distance during the 1–18 ps period, presented in Figure 2a, has a maximum at 235 pm, which indicates a direct contact between the sodium ion and the oxygen atom from the phosphate group. The solvation of the Na⁺1 ion begins after the 18th ps. The Na⁺1–

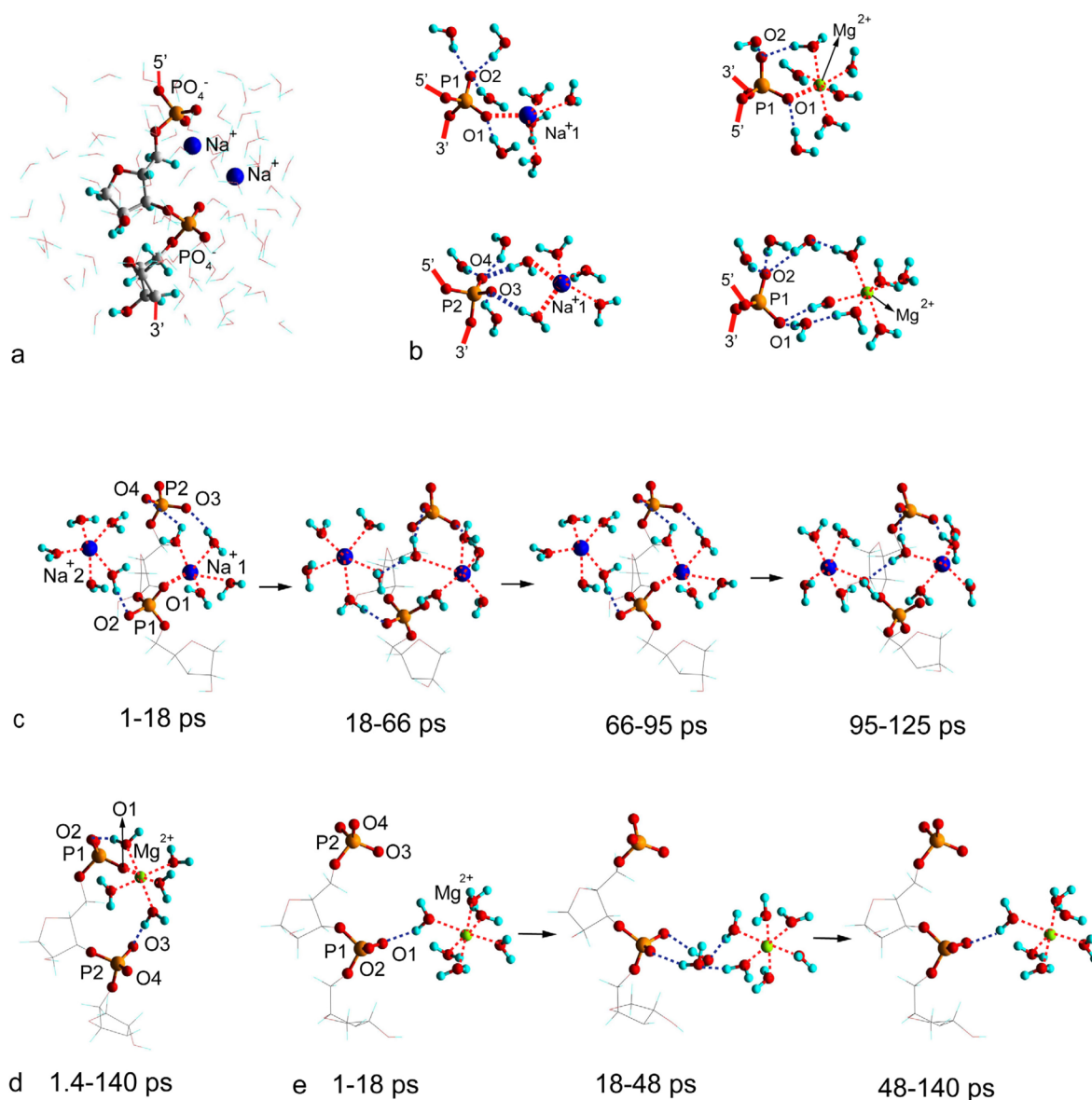


Figure 1. Selected snapshots from the simulations: (a) the simulation box with the RNA backbone model and two sodium ions (water molecules are shown only as lines); (b) water molecules solvating P1 or P2 phosphate groups and the counterions in bonded and solvated state; (c) sodium ions in different time periods; (d) bonded magnesium ion; and (e) solvated magnesium ion in different time periods.

O1 radial distribution function for the 18th to the 66th ps simulation time (Figure 2a) has several small maxima and corresponds to the process of the movement of sodium ion into the solution without direct contact with the phosphate oxygen atom. The final maximum is at 455 pm, indicating indirect interaction of the Na^+ ion with the oxygen atoms from the phosphate group through two water molecules. After this period the Na^+ ion is bonded again to the O1 oxygen atom for about 30 ps (from the 66th to 95th ps) with Na^+ –O1 RDF maximum of 255 pm. After the 95th ps the ion again starts transition to the solution and in the period 104–125 ps (simulated at 320 K) is already completely solvated. The maxima of the RDF (Na^+ –O1) for the periods 95–104 ps and 104–125 ps are 335 and 455 pm, respectively. The continuous time evolution of the Na^+ –O1 distance is presented in Figure 2a, and snapshots typical for the consecutive periods are shown in Figure 1c.

It should be noted that the Na^+ ion coordinates to the phosphate group in a monodentate manner, as observed for Na^+ ion in the periods 1–18 ps and 66–95 ps, remaining in a close contact with only one oxygen atom from the phosphate group, Figure 1b. The second terminal oxygen atom of the phosphate group, O2, remains in these periods at more than 100 pm farther from the cation than O1. This can be seen from the maxima of the RDF for Na^+ –O2 distances in the time periods 1–18 ps and 66–95 ps, 405 and 385 pm, respectively (Figure 2c,d).

When Na^+ ion is not directly bonded to O1, it interacts with each one of the O1 and O2 phosphate oxygen atoms via two water molecules. During the major part of the simulation (5–125 ps), Na^+ interacts with the terminal oxygen atoms of the distant phosphate group, O3 and O4, via a single water molecule from the first coordination shell of Na^+ ion, forming an eight-membered cycle, Figure 1b.

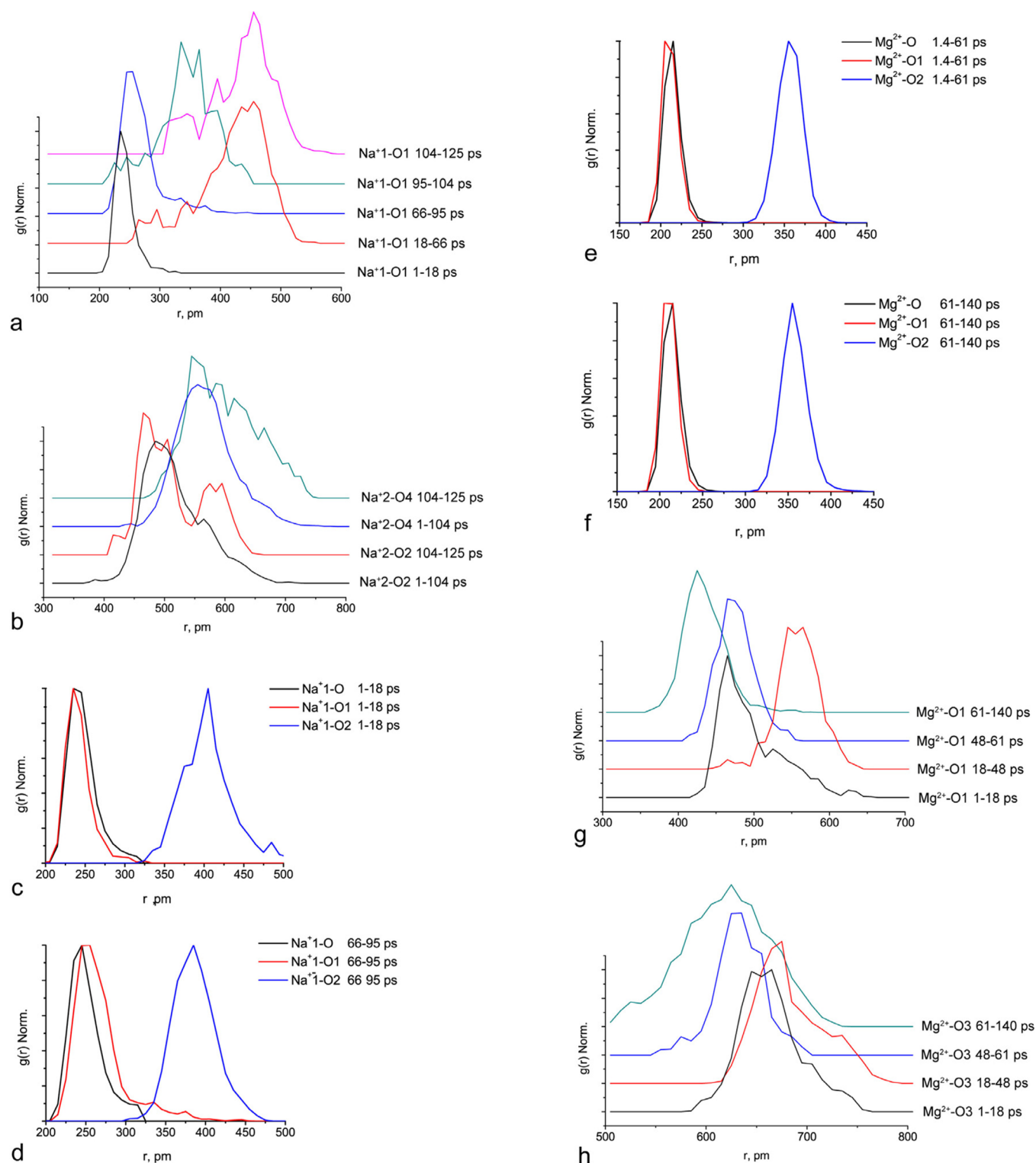


Figure 2. Radial distribution functions, RDF, of various species: (a) Na⁺1 and (b) Na⁺2 ions to the nearest O center from a phosphate group in different time intervals of the simulations. (c, d) Na⁺1 to all O centers and to O1 and O2 centers of P1 phosphate group in the periods when this cation is bonded, 1–18 ps (c) and 66–95 ps (d). (e, f) Bonded Mg²⁺ ion to all O centers and to O1 and O2 centers of P1 phosphate group in the periods simulated at 300 K (e) and at 320 K (f). (g, h) Solvated Mg²⁺ ion to O1 (g) and O3 (h) centers of P1 and P2 phosphate groups in different simulation periods at 300 K (1–61 ps) and at 320 K (61–140 ps). For notation of the atoms see Figure 1 and the description in the text.

The second sodium ion Na⁺2 remains completely solvated during the whole simulation time, 1–125 ps. The time evolution of the Na⁺2–O2 distance is presented in Figure 2b. The closest phosphate oxygen atom to Na⁺2 is O2, followed by O4, which is farther by about 100 pm during the simulation

(also shown in Figure 1c). Na⁺2 interacts with O2 through a single water molecule during the initial period of the simulations, 1–24 ps. During the 24–37 ps period, Na⁺2 interacts with O2 through two water molecules and, during the 37–120 ps period, again through a single water molecule.

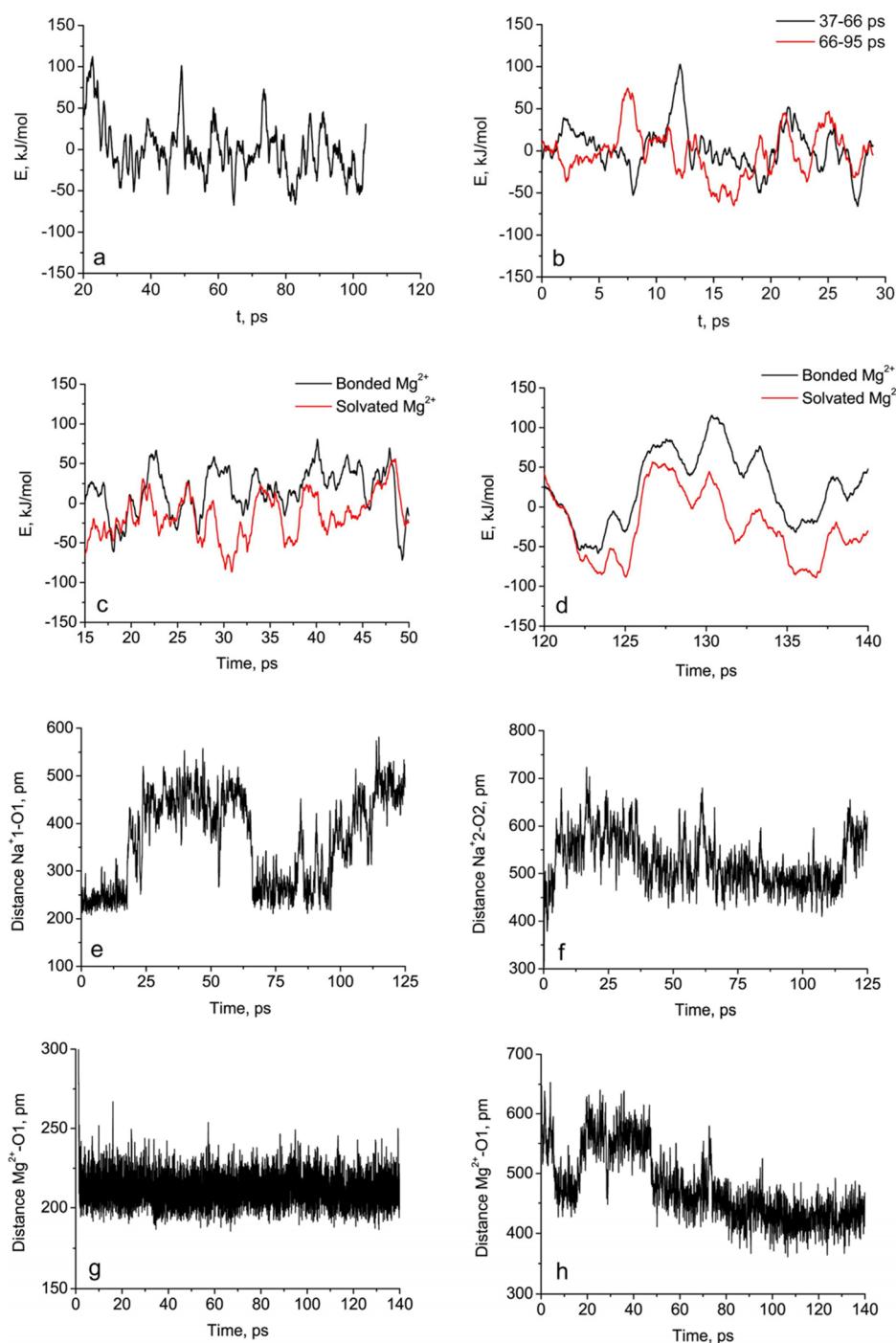


Figure 3. (a) Variation of the potential energy of the simulated system with two sodium cations for the interval 20–104 ps, simulated at 300 K, and (b) comparison of the variations in the potential energy for the periods 37–66 ps (solvated Na^+1) and 66–95 ps (bonded Na^+1). The values are averaged over 1000 points. Zero energy corresponds to the average value for the whole period 20–104 ps, -2193.71634 hartree. (c, d) Variation of the potential energy of the simulated systems with bonded and solvated magnesium cation for the intervals 15–50 ps at 300 K (c) and 120–140 ps at 320 K (d). The values are averaged over 1000 points. Zero energy for panel c is -2169.04305 hartree, which is the mean value between the average values for the bonded and for the solvated Mg^{2+} in the 15–50 ps period. Zero energy for panel d is -2169.04721 hartree, which is the mean value between the average values for the bonded and for the solvated Mg^{2+} in the 120–140 ps periods. (e–h) Variation of the distance between the metal cation and the oxygen center from phosphate group closest to it for Na^+1 (e) and Na^+2 (f) ions in the simulation with two sodium ions and for Mg^{2+} in the simulations with bonded (g) and solvated (h) magnesium ion.

During the last 5 ps of the simulation Na^+2 interacts with O2 through two water molecules. The maximum at around 475 pm (Figure 2b) indicates interaction through a single water molecule, and the peak around 575 pm indicates interaction via two water molecules.

Energy of the System. Potential energy of the system with two sodium cations for the period 20–104 ps is presented in Figure 3a. To improve visibility the plot is smoothed by averaging at every 1000 points of the simulation. It should be noted that local minima and maxima of the potential energy do

Table 2. Characteristics of the First and Second Peaks in the RDF for the Interatomic Distances between Na⁺1, Na⁺2, and Mg²⁺ Ions and All Oxygen or Hydrogen Atoms in the System for Different Time Periods

distance/period ^a	first peak				second peak			
	start	maximum	end	integral	start	maximum	end	integral
2 Na⁺ ions								
Na ⁺ 1–O/1–18	205	235	315	5.0	315	445	535	23.7
Na ⁺ 1–O/18–66	205	235	325	5.1	325	435	515	20.8
Na ⁺ 1–O/66–95	205	245	315	5.8	315			
Na ⁺ 1–O/104–125	205	245	315	5.1	315	445	515	21.2
Na ⁺ 1–H/1–18	215	295						
Na ⁺ 1–H/18–66	225	295	355	12.0	355			
Na ⁺ 1–H/66–95	215	295	395	16.1	395	495	535	36.4
Na ⁺ 1–H/104–125	215	295	375	14.6	375			
Na ⁺ 2–O/1–104	205	235	325	5.1	325	445		
Na ⁺ 2–O/104–125	205	235	325	5.2	325	435		
Na ⁺ 2–H/1–104	215	295	385	14.5	385	495	565	48.1
Na ⁺ 2–H /104–125	215	295	385	14.5	385	485		
Mg²⁺ bonded								
Mg ²⁺ –O/1.4–61	185	215	295	6.0	295	415	495	20.2
Mg ²⁺ –O/61–140	185	215	305	6.0	305	425	505	20.8
Mg ²⁺ –H/1.4–61	215	275	365	12.5	365	485		
Mg ²⁺ –H/61–140	225	275	375	12.6	375	495		
Mg²⁺ solvated								
Mg ²⁺ –O/1–18	185	215	285	5.9	285	425	505	21.3
Mg ²⁺ –O/18–48	185	215	305	6.0	305	425	515	23.0
Mg ²⁺ –O/48–61	185	215	275	6.0	275	435	505	21.5
Mg ²⁺ –O/61–140	185	215	285	6.0	285	425	485	19.1
Mg ²⁺ –H/1–18	225	275	345	12.4	345	475		
Mg ²⁺ –H/18–48	225	275	325	12.5	325	465		
Mg ²⁺ –H/48–61	235	275	325	12.6	325	475		
Mg ²⁺ –H/61–140	225	275	345	12.6	345	485		

^aFor notation of the atoms see Figure 1 and the description in the text.

not correlate with some specific position of the metal ions with respect to the phosphate groups but to a large extent reflect the variation of the arrangement of the water molecules in the system since potential energy variations of the same order of magnitude are observed in simulation of bulk water.

To compare the energy of the two distinct positions of the sodium ion Na⁺1, bonded to the phosphate oxygen atom O1 and solvated, the potential energies of the corresponding periods 37–66 ps and 66–95 ps (both periods include 29 ps simulation) are plotted in Figure 3b. The average value of the potential energy during the 66–95 ps period, when the Na⁺1 ion is directly bonded to the O1 phosphate oxygen atom, is by 6 kJ/mol lower than that for the period with solvated Na⁺1 ion. Having in mind the large variations in the potential energy of the system, the energy difference of 6 kJ/mol can be considered as negligible for the studied systems and could be attributed to minimal variations in the arrangement of the water molecules. So, both locations of the sodium cation can be considered energetically equivalent, and the energy of the system does not depend strongly on the position of the sodium ions toward the phosphate groups, being directly bonded or interacting through a water molecule.

Solvation Shell of the Sodium Ions. The radial distribution functions of the distances between Na⁺1 and Na⁺2 ions and all oxygen and hydrogen atoms in the system with two sodium cations are presented in Figures S1 and S2 of the Supporting Information and in Table 2, along with the coordination numbers $n(r)$ obtained from the integral of the RDF.

We will start with the Na⁺2 ion, which is solvated during the whole simulation, 1–125 ps. Its first solvation shell has Na⁺2–O distances between 205 and 325 pm, with a maximum at 235 pm, indicating the most probable distance between the ion and the oxygen atoms from the surrounding water molecules. The RDF value at the first minimum, 325 pm, does not reach zero, which indicates a poorly defined first coordination shell.⁴⁶ The coordination number for the first coordination shell is 5.10 and 5.22 for the periods 1–104 ps and 104–125 ps, respectively, suggesting the solvation of sodium ion by 5 water molecules, but states at which the sodium ion is solvated by 4 or 6 water molecules can also be observed during the simulation.

For the Na⁺1 ion, which is bonded to the phosphate oxygen atom O1 during the periods 1–18 ps and 66–95 ps of the simulation, the first coordination shell begins at 205 pm and ends at 315–325 pm, with a maximum within the interval 235–245 pm and the integrated value corresponding to about 5 oxygen atoms participating in the first coordination shell of the sodium ion, oxygen atoms from water molecules and one oxygen atom from the phosphate group (Table 2). An exception is the period 66–95 ps in which sodium ion has 6 oxygen atoms in the first coordination shell, 5 water molecules and one oxygen atom from the phosphate group. The second maximum of the RDF for Na⁺1–O distance appears at 435–445 pm, and the integrated value suggests that the second coordination shell of the sodium ion contains 13 water molecules. It should be noted that during the whole simulation (1–125 ps) the oxygen atoms from the ribose do not contribute to either the first or the second coordination shell

of the sodium ion Na^+ , but the ribose moiety occupies some space in vicinity of the Na^+ ion, which for the solvated ion is occupied by water molecules.

The characteristic minima and maxima in the RDF of the Na^+ –H distances, see Figure S1 of the Supporting Information, are similar to those for the Na^+ –O distance (Supplementary Figure S2), but the integrated values $n(r)$ include some hydrogen atoms from the ribose and depend on the proximity of Na^+ ion to the dinucleotide. The integral of the second maximum, about 36.4, includes, beside water molecules, 4 hydrogen atoms from the ribose. Thus, one can estimate that in addition to the 13 water molecules in the second coordination shell (estimated from Na^+ –O RDF, see previous paragraph) in this area fall 6 hydrogen atoms from neighboring H_2O molecules forming hydrogen bonds.

In order to compare the interaction of the sodium ion Na^+ with the oxygen atoms O1 and O2 from the phosphate group and from the water molecules, we present the radial distribution functions between Na^+ and O1 and O2 and between Na^+ and all oxygen atoms in the system except O1 and O2 denoted as Na^+ –O (Figure 2c,d) for the periods when Na^+ is bonded to the phosphate group. For the period 1–18 ps, both maxima are located at 235 pm, indicating equal average distances between the sodium ion and the oxygen atoms from water molecules or O1 from the phosphate group. For the period 66–95 ps, the average Na^+ –O1 distance is somewhat longer, 245–255 pm, due to coordination of two proton-donating water molecules at O1 atom for this period (see below). Na^+ –O2 distance is spread in a wider interval with maxima at 410 and 390 pm.

Solvation Shell of the Phosphate Oxygen Atoms. The radial distribution functions $\text{O}_n\text{--H}$, where O_n represents terminal oxygen atoms of the phosphate moieties, O1, O2, O3, and O4, and H represents all hydrogen atoms in the system with the exception of the hydrogen atoms from the dinucleotide, are used to assess the hydration of the phosphate oxygen atoms that are not bonded to the alkyl residue (Figure S3 of the Supporting Information and Table 3). The first hydration shell around O1 starts at 145 pm (1–104 ps) or 135 pm for the 104–125 ps (at 320 K) periods with the most probable distance O1–H of 175 pm, except for the 66–95 ps period, where it is longer by 10 pm at 185 pm. During the 1–18 ps period one water molecule ($n(r) = 0.96$) is hydrogen bonded to the O1 atom in addition to the Na^+ cation coordinated to it, while for the second period with bonded Na^+ cation, 66–95 ps, two water molecules are coordinated to O1 ($n(r) = 1.94$), although at somewhat longer distance. During the periods with solvated Na^+ cation, 18–66 ps (at 300 K) and 104–125 ps (320 K), the integrated values of the first peak are 1.78 and 1.58, corresponding to two water molecules hydrogen bonded most of the time to the phosphate oxygen atom as the $n(r)$ value at 320 K is by 0.2 lower than the value obtained at 300 K, i.e., the hydration of this O atom is reduced with the increasing of the temperature.

The O2–H, O3–H, and O4–H radial distribution functions start at about 125 pm, and their maxima are at 175 pm, which suggests formation of strong hydrogen bonds as should be expected since these oxygen centers are formally negatively charged. The first minima, about 245 pm, are clearly distinguished, suggesting a well ordered first hydration shell, similarly to the O1–H RDF. For O2 and O4 the $n(r)$ values clearly suggest three water molecules hydrogen bonded to the phosphate oxygen atoms, whereas for O3, the value is close to

Table 3. Characteristics of the First Peak in the RDF for the Interatomic Distances between Different Oxygen Centers of the Two Phosphate Groups and Hydrogen Atoms in the System for Different Time Periods

distance/period ^a	first peak			
	start	maximum	end	integral
2 Na⁺ ions				
O1–H/1–18	145	175	235	1.0
O1–H/18–66	145	175	265	1.8
O1–H/66–95	145	185	245	1.9
O1–H/104–125	135	175	255	1.6
O2–H/1–104	125	175	245	2.8
O2–H/104–125	145	175	245	3.0
O3–H/1–104	135	175	245	2.2
O3–H/104–125	125	165	245	2.0
O4–H/1–104	125	175	255	2.9
O4–H/104–125	125	175	245	2.9
Mg²⁺ bonded				
O1–H/1.4–61	145	185	235	1.0
O1–H/61–140	135	185	235	0.9
O2–H/1.4–61	135	175	255	2.1
O2–H/61–140	135	175	255	2.1
O3–H/1.4–61	135	175	265	2.3
O3–H/61–140	135	175	255	2.0
O4–H/1.4–61	125	175	245	2.9
O4–H/61–140	135	175	265	3.2
Mg²⁺ solvated				
O1–H/1–61	135	175	245	2.1
O1–H/61–140	135	175	245	2.1
O2–H/1–61	135	175	265	2.9
O2–H/61–140	125	175	255	3.0
O3–H/1–61	135	175	255	2.9
O3–H/61–140	135	175	245	2.6
O4–H/1–61	135	175	255	2.4
O4–H/61–140	145	175	255	2.6

^aFor notation of the atoms see Figure 1 and the description in the text.

two hydrogen bonded water molecules, similarly to the O1 oxygen atom described above. The reason for this difference is the 5′-CH₂ groups of the two ribose moieties that remain close to O1 and O3 centers, at about 260 pm, and prevent the approach of the third water molecule to these negatively charged oxygen centers. The other two oxygen atoms, O2 and O4, are farther from the ribose and are able to coordinate three water molecules. The influence of the temperature on the hydration for the two groups of oxygen atoms, as estimated by $n(r)$, is different: the $n(r)$ for O1 and O3 decreases by 0.2 when the temperature increases by 20 K, while for O2 and O4 $n(r)$ increases or remains unchanged, respectively.

During the simulation all water molecules that form hydrogen bonds with the phosphate oxygen atoms participate with only one of their hydrogen atoms as a donor of the hydrogen bond, as presented in Figure 1b. The dynamic behavior of the water molecules coordinated to the terminal phosphate oxygen atoms is not uniform: the fastest exchange of the water molecules forming hydrogen bonds to the phosphate oxygen atoms occurs for 20–30 ps, while some of the water molecules remain bonded to the oxygen atoms during the whole simulation of 125 ps.

Dynamics of the System with Directly Bonded Magnesium Ion. *Solvation Shell of the Directly Bonded Magnesium Ion.* Initially, in this simulation the magnesium ion

was placed in the space between the two phosphate groups, at a distance of about 450 pm from both phosphorus atoms. After 1.4 ps, the Mg^{2+} ion is directly bonded to the O1 oxygen atom from the P1 phosphate group (Figure 1b). The magnesium ion remains bonded to O1 during the whole simulation: 1.4–61 ps at 300 K and 61–140 ps at 320 K (Figure 1d). According to the RDFs for the Mg^{2+} –O1 distance (Figure 2e,f), the most probable distance between the magnesium ion and the oxygen atom O1 is 205 pm both at 300 K (1.4–61 ps) and at 320 K (61–140 ps). The magnesium ion binds to the phosphate group in a monodentate manner, remaining in direct contact with only the O1 oxygen atom, while the Mg^{2+} –O2 distance (Figure 2e,f) remains around 355 pm. The magnesium ion interacts with O2 and O3 through single water molecules (see Figure 1d).

The radial distribution functions Mg^{2+} –O (Figure 2e,f) clearly indicate that the first coordination shell of the Mg^{2+} ion is well-defined, at variance from the coordination shell of the sodium ion. The integrated values at the first peak for both periods are exactly equal to 6.00 (Table 2 and Figure S4 of the Supporting Information), indicating 6 oxygen centers bonded to the magnesium ion, five of which from water molecules and one from the O1 oxygen atom from the phosphate group. The peak maximum is located at 215 pm, longer by 10 pm than the Mg^{2+} –O1 distance, likely due to stronger electrostatic attraction between the Mg^{2+} ion and the negatively charged phosphate O1 center. The second maximum of the Mg^{2+} –O RDF is located at 415 pm (1.4–61 ps at 300 K) and 425 pm (61–140 ps at 320 K), and $n(r)$ values correspond to 9 or 10 water molecules (taking into account the oxygen atoms from the backbone) in the second coordination shell of the Mg^{2+} ion at 300 and 320 K, respectively. The Mg^{2+} –H radial distribution functions (Figure S4 of the Supporting Information and Table 2) suggest the same number of water molecules in the first coordination sphere of the cation. At variance from Na^+ , for Mg^{2+} hydrogen atoms from the ribose moiety do not participate in the second coordination sphere.

Solvation Shell of the Phosphate Oxygen Atoms. The analysis of the solvation of the O1, O2, O3, and O4 oxygen atoms from the P1 and P2 phosphate groups is based on the corresponding On–H RDFs including all hydrogen atoms in the system with the exception of the hydrogen atoms from the dinucleotide (Figure S5 of the Supporting Information and Table 3). The first hydration shell around O1, which is directly bonded to the Mg^{2+} ion, contains about one water molecule during the whole simulation, and the most probable length of the hydrogen bond O1–H is 185 pm.

For the other three phosphate oxygen atoms, O2, O3 and O4, the most probable length of the hydrogen bonds with the water molecules is 175 pm, shorter by 10 pm than O1–H. The integral of the first peak corresponds to two water molecules hydrogen bonded to O2 and O3 oxygen atoms during the whole simulation, while for the O4–H radial distribution functions, the $n(r)$ values are 2.86 (1.4–61 ps) and 3.21 (61–140 ps), corresponding to about three hydrogen bonded water molecules. Thus, as expected, the O1 oxygen atom, which is in direct contact with the Mg^{2+} ion, coordinates fewer water molecules (one) than the other phosphate oxygen atoms, which are hydrogen bonded to 2 or 3 water molecules. The main difference from the solvation of the phosphate oxygen atoms in the presence of bonded Na^+ is the reduction of the number of water molecules in the O2 coordination shell likely due to

blocking of the space around O2 center by the rigid $\text{Mg}^{2+}(\text{H}_2\text{O})_5$ moiety.

Dynamics of the System with Solvated Magnesium Ion. Solvation Shell of the Solvated Magnesium Ion. In this simulation the magnesium ion was initially solvated by water molecules at about 500 pm from the O1 oxygen atom from the phosphate group P1 interacting with it through a single water molecule (Figure 1e) and remains solvated during the whole simulation (1–140 ps). In the initial period, 1–18 ps, the magnesium ion interacts with O1 via a single water molecule (Figure 1e) with a Mg^{2+} –O1 distance of 465 pm and interacts with O2 and O3 oxygen atoms through two water molecules. During the period 18–48 ps, the Mg^{2+} ion moves away and interacts with O1 and O2 atoms through two water molecules with Mg^{2+} –O1 distance of 565 pm, 100 pm longer than in the previous position (Figure 2g). After the 48th ps of the simulation, the Mg^{2+} ion moves back, closer to the O1 oxygen atom, and interacts with it through a single water molecule with Mg^{2+} –O1 distance of 465 pm, as in the first period. During this period the magnesium ion interacts with O2 and O3 through 2 water molecules. In the last period of the simulation, 61–140 ps, run at 320 K, the Mg^{2+} ion interacts with the O1 oxygen atom through a single water molecule with Mg^{2+} –O1 distance of 425 pm, shorter by 40 pm than the distance for the analogous position at 300 K (periods 1–18 and 48–61 ps).

Similarly to the magnesium ion bonded to the phosphate group, the first coordination shell of the solvated magnesium ion has an RDF maximum of 215 pm and contains exactly 6 oxygen atoms (Table 2, Figure S6 of the Supporting Information) all belonging to water molecules. The second maxima of the Mg^{2+} –O RDF are located at about 425 pm for all simulation periods, but the amount of water molecules in the second coordination shell differs in different periods: it is 17 when magnesium ion interacts with the phosphate oxygen atoms through 2 water molecules, and decreases to 14 for Mg^{2+} interacting through single water molecule since part of the solvation shell is replaced by the phosphate oxygen atoms. The simulation at 320 K (61–140 ps) resulted in a further decrease of the number of water molecules in the second coordination shell of Mg^{2+} ion to 12. The Mg^{2+} –H RDFs with first and second maxima at 275 pm and 465–485 pm suggest similar number of water molecules in the first and second coordination shell of the cation as the Mg^{2+} –O distances.

Solvation Shell of the Phosphate Oxygen Atoms in the System with Solvated Magnesium Ion. For all oxygen atoms O1, O2, O3, O4 the first coordination shell, consisting of water molecules, hydrogen bonded to the phosphate oxygen atoms, begins at about 135 pm (Table 3, Figure S7 of the Supporting Information). Similarly to the phosphate oxygen atoms (nonbonded to Mg^{2+} ion) from the previous simulation, the most probable length of the O...H hydrogen bond is 175 pm. In average five water molecules are coordinated to the two terminal oxygen atoms of each phosphate group. For P1 group O1 and O2 centers have most of the time two and three coordinated water molecules, due to variation of the position of the ribose five-atomic ring with respect to these centers. The ribose is oriented on the side of O1 at the distance of 350 pm, which is responsible for the lower coordination number of O1. The number of water molecules hydrogen bonded to O3 and O4 oxygen centers varies between 2.9 and 2.4 during the first 61 ps of the simulation. The O4 oxygen atom is closer to the 3' hydrogen atom from the ribose by 10 pm than the O3 to the 5' hydrogen atom, the nearest hydrogen atoms (245 and 255 pm

mean distances), which may explain the slightly lower coordination number of O4. During the last period of the simulation, 61–140 ps, when both O3 and O4 oxygen atoms coordinate the mean number of 2.6 water molecules, no structural differences between the O3 and O4 neighboring structures are observed.

Energy of the Systems with Magnesium Ions. The variations of the potential energy of the systems with directly bonded or solvated magnesium ions are presented in Figure 3c,d. The potential energies are plotted from the 15th to the 50th ps (300 K), corresponding to the period when the solvated Mg^{2+} interacts with O1 through 2 water molecules, and the 120–140 ps period (320 K), the last 20 ps of the simulation, when Mg^{2+} interacts with O1 through 1 water molecule. During both periods no energy drift is observed. Like in the simulation with sodium ions, local minima and maxima of the potential energy do not correlate with the position of the metal ions with respect to the phosphate groups but likely reflect the arrangement of the water molecules in the system.

Comparison between Sodium and Magnesium Ions.

Solvation of the Sodium and Magnesium Ions. During the whole simulation (both at 300 and 320 K) both directly bonded and completely solvated magnesium ions have exactly 6 oxygen atoms in their first coordination shell. Moreover, the ligands are strongly coordinated to magnesium cation, and no exchange of water molecule or phosphate oxygen atom occurs in the first coordination shells during the whole simulations.

On the other hand sodium ions readily exchange water molecules between the first and second coordination shells and change coordination numbers. During the simulation sodium ions coordinate 4–7 oxygen atoms from water molecules or phosphate groups. The rapid changing of the coordination number for the Na^+ ions is evident in Figure 4, where coordination number for Na^+1 and Na^+2 ions is plotted against time for the last 21 ps of the simulations and for the Na^+1 the 66–95 ps period, when the ion is directly bonded to the phosphate group ($\text{Na}-\text{O}$ distance cutoff is 320 pm).

Water molecules from “bulk” solution participate in the exchange between the coordination shells, while water molecules hydrogen bonded to phosphate groups are much less mobile. For this reason, water molecules from the first coordination shell of the sodium ions that form hydrogen bonds with phosphate oxygen centers are not frequently exchanged.

The variation of the coordination numbers of the sodium cation can be also followed as the fractions of time (in percents) during which the ion coordinates different numbers of oxygen atoms (Table 1). During the whole simulations the most frequently encountered coordination number for the sodium ions is five (with 61–88% for the Na^+1 ion in different time intervals and 78–79% for the Na^+2 ion), followed by coordination number six. Exception is the 66–95 ps period, when the Na^+1 ion is directly bonded to the O1 phosphate oxygen atom, when the most frequently encountered coordination number is six (with 52%), followed by five and seven. No clear influence of the temperature on the coordination number is observed.

Comparison with Experimental and Previous Computational Results. In some cases the positions of the metal ions, Na^+ or Mg^{2+} , with respect to the phosphate groups of RNA backbone have been determined by X-ray crystallography.^{9,32} Since Na^+ is particularly difficult to be identified in the nucleic acid X-ray crystal structures,^{5,34} we present only an example of

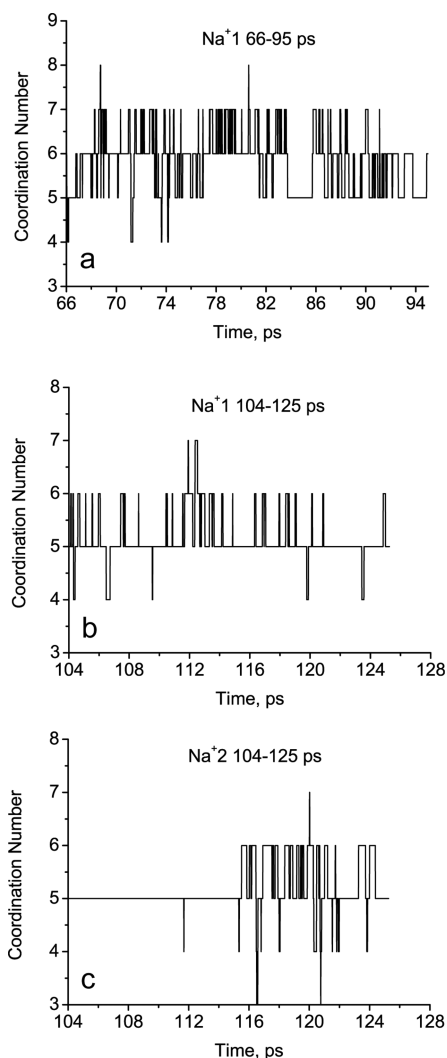


Figure 4. Variation of coordination number of Na^+1 and Na^+2 cations in selected simulation periods.

sodium ion, directly bonded to a phosphate group, see Supplementary Figure S8a (Na^+ ion number 315 from PDB 2OJ0 from ref 31 with resolution 260 pm). The distance between the ion and the oxygen atom from the phosphate group is 245 pm, which is in agreement with the typical $\text{Na}^+1-\text{O}1$ distance found in our simulations, 235–255 pm.

In the PDB files from the crystallographic data, magnesium ions are observed either directly bonded to the phosphate groups or interacting through water molecules.^{9,32,33} The $\text{Mg}^{2+}-\text{O}(\text{P})$ distance for the magnesium ions interacting directly with phosphate oxygen atoms in the large ribosomal subunit is estimated by Klein et al.⁹ to be shorter than 240 pm. According to the data reported by Subirana et al.³⁴ the average $\text{Mg}^{2+}-\text{O}(\text{P})$ distance to phosphate oxygen atoms (averaged over Mg^{2+} ions in a sample studied at very high resolution, 89–120 pm) is 203 pm, which is very close to the corresponding average distance derived from our ab initio MD simulations. An example of such position of magnesium ion is presented in Supplementary Figure S8b (Mg^{2+} ion number 200 from PDB 354D from ref 33 with 150 pm resolution) with $\text{Mg}^{2+}-\text{O}(\text{P})$ distance of 205 pm. The $\text{Mg}^{2+}-\text{O}(\text{P})$ distances when the phosphate oxygen atoms and the Mg^{2+} ion are separated by one water molecule may vary by a large interval (Figure 2h). An

example for such location observed experimentally is shown in Supplementary Figure S8c (Mg^{2+} ion number 201 from PDB 354D from ref 33) with Mg^{2+} –O(P) distance of 466 pm, close to the average value from our simulation for the analogous location of the magnesium ion (Figure 1e).

Using geometry optimization approach with a DFT method Petrov et al.⁴³ reported interatomic distances very similar to the optimal values obtained in our calculations. The calculated distances Mg^{2+} –O(P) were about 200 pm and Mg^{2+} –O(Water) about 215 pm; Na^+ –O(P) about 235 pm and Na^+ –O(Water) about 245 pm. Note that in their calculations the RNA fragment forms a chelate complex with the magnesium ion with two neighboring phosphate groups coordinated to it.

We also compared the results from the ab initio MD simulation with classical MD simulation of dinucleotide with sodium or magnesium ions (at 300 K) using CHARMM and AMBER force fields. For Na^+ the optimal Na^+ –O distance to the oxygen atoms from the phosphate and water molecules in the classical MD simulation with AMBER is the same as the distance in the ab initio MD, 235 pm. However, the location of the cation differs (Figure S9 of the Supporting Information). In the classical MD most of the time Na^+ ion is located close to both terminal oxygen atoms of one of the phosphate groups (O1 and O2), while in the ab initio MD only one of these oxygen atoms enters in the first coordination shell of the cation and a bidentate complex is not observed. The simulation with CHARMM resulted in monodentate location of Na^+ with respect to the phosphate group, but the Na^+ –O(P) distances are around 215 pm (Supplementary Figure S10a,b), shorter by 20 pm than in the ab initio MD. Similarly to the ab initio MD, in the classical MD simulations we also observed mobility of one of the Na^+ ions (Supplementary Figures S9a, S10a) although for different duration; it moves away from the phosphate oxygen in the periods 40–49 ps (AMBER) and 44–49 ps (CHARMM).

The classical MD simulation for magnesium ion resulted in very similar position of the cation with respect to the phosphate group, monodentate coordination (Supplementary Figures S9b, S10c). However, the maximal values of the RDF for Mg^{2+} –O distances were found to be 10–30 pm shorter than those in ab initio simulation of bonded Mg^{2+} ion: Mg^{2+} –O1 to the oxygen atom from the phosphate is 175 pm (CHARMM) and 185 pm (AMBER), and Mg^{2+} –O(w) to the oxygen atoms from the water molecules is 195 pm both with CHARMM and AMBER (Figure S10c).

Effect of Temperature. Since our simulations are performed at two temperatures, 300 and 320 K, we may compare some characteristics of the systems obtained at these temperatures. The first maxima of the Na^+ –O and Mg^{2+} –O RDF are located essentially at the same distances for the two temperatures (see Table 1). The coordination numbers for magnesium are also the same, exactly six. A small increase of the average coordination number of the solvated sodium ion with the temperature is observed. At 300 K it is 5.03 and at 320 K it is 5.16, due to the increase of the time in which the cation is six coordinated (see Figure S11e of the Supporting Information). Although this difference is rather small, it follows the general trend for increase of the average number of ligands in the first solvation shell of Na^+ in water with the temperature, observed in separate simulation: when the temperature increases from 303 to 353 K, the sodium coordination number increases by 0.39.

The mobility of the cations at different temperature can be estimated by the difference between the minimal and maximal values of the root-mean-square deviation (RMSD) of their position for certain simulation period. In Supplementary Figure S11a–d we present the variations of RMSD for Na^+ and Mg^{2+} ions during the simulations. ΔRMSD values for magnesium and solvated sodium ions (Table S1 of the Supporting Information) are higher for the simulations at 320 K, which indicates higher mobility of the Na^+ and Mg^{2+} ions at the higher temperature.

Vibrational Analysis of the Phosphate Groups in the Modeled Systems. Complete vibrational analysis of the studied systems explicitly including also the water molecules has been performed for the cations (Na^+ or Mg^{2+}) in solution or directly bonded to the phosphate groups. We summarize here only the symmetric ν_s and asymmetric ν_{as} vibrations of the phosphate groups (Figure 5 and Supplementary Table S4), since they are

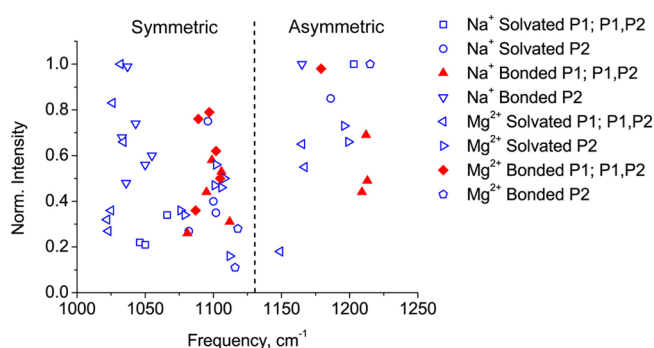


Figure 5. Calculated frequencies of the symmetric and asymmetric phosphate groups' vibrations and their normalized intensities. The filled red symbols correspond to vibrations of a phosphate group with countercation bonded to it, and the empty blue symbols correspond to vibrations of a phosphate group surrounded only by water molecules without a countercation bonded to its oxygen centers.

considered depending on the positions of the metal ions. As the frequencies of these vibrations depend also on the phosphate groups conformations,^{47,48} we present the conformations of the structures used for vibrational analysis (Table S2 of the Supporting Information). During the simulations (Supplementary Table S3) one of the phosphate groups (P1 in the systems with 2 Na^+ and with solvated Mg^{2+} , and P2 in the system with bonded Mg^{2+}) remains always in gauche–gauche (gg) conformation, which was reported to be slightly more stable than the trans–gauche (tg) conformation for phosphate esters.^{47,48} The conformation of the other phosphate group changes irregularly between tg and tt as the 3' end remains always in trans configuration.

The frequencies of the symmetric vibration ν_s of the phosphate groups that are not directly bonded to metal ions appear in two regions, a lower frequency region around 1035 cm^{-1} and a higher frequency region around 1100 cm^{-1} , which can be compared to the experimental vibrational frequencies measured in different samples around 1023–1040 cm^{-1} and 1072–1115 cm^{-1} .⁴⁹ All frequencies for ν_s bands of the phosphate groups bonded to a Mg^{2+} or Na^+ ion are located only in the higher frequency region around 1100 cm^{-1} . The asymmetric frequencies are observed in the 1150–1215 cm^{-1} region, but no clear correlation between their values and the positions of metal ions was observed. These results suggest that the bands for symmetric vibrations of the phosphate groups can be used experimentally for determination of the position of the

sodium or magnesium ion with respect to the phosphate moieties, in the absence of a directly bonded counterion both low and high symmetric frequency bands will appear, while in presence of a counterion directly bonded to it the lower frequency band will disappear and the intensity of the high frequency band is expected to increase.

Similar trends have been experimentally observed by Raman spectroscopy of RNA crystals in water medium, complemented by XRD measurements.²⁴ The ν_s frequency of the RNA phosphate groups in NaCl solution was measured 1100 cm^{-1} , while after addition of Mg^{2+} ions in the solution a band at 1117 cm^{-1} (by 17 cm^{-1} higher than the initial value) is evident. Although the exact values of the vibrational frequencies cannot be reproduced precisely with this computational method and model, the observed experimental trend suggests that the sodium ions are staying mainly in hydrated form in the solution and phosphate groups did not interact directly with the counterion. On the other hand, magnesium ions are directly bonded to the phosphate groups in solvated RNA for long times that correspond to the high frequency band of the symmetric vibrations. The direct bonding of Mg^{2+} ions to the phosphate groups was confirmed by XRD measurement.²⁴ It should be noted these experimental data are obtained with double stranded RNA, while our calculated value correspond to the backbone of single stranded RNA.

In the previous quantum chemical simulations of the vibrational frequencies of the phosphate group in some model compounds the solvation of the phosphate and the metal ion was taken into account by a continuum solvation models and partially by explicit water molecules.^{48,49} Although our model system is more realistic since it includes explicitly the complete first and second solvation shells of both metal ions and the phosphate moieties, the calculated characteristic vibrational frequencies cannot be used for direct comparison with the experiment by several reasons: (i) typical error of the computational method; (ii) anharmonicity of the vibrations; (iii) variation of the local arrangement of the water molecules solvating the phosphate oxygen atoms in the real solution resulting in broadening of the experimental bands; (iv) influence of the conformations of the phosphate groups,^{47,48} described above, etc.

Conclusions. The interactions between sodium or magnesium ions and phosphate groups of the RNA backbone in water solution have been studied using ab initio Born–Oppenheimer molecular dynamics. Sodium ions have higher mobility (Figure 3e,f) than the magnesium ions (Figure 3g,h) and readily change their position with respect to the phosphate groups, from directly bonded to completely solvated state. Within the 104 ps simulation at 300 K the lifetime of bonded Na^+ was about 20 to 30 ps, during which the sodium ion binds to only one of the oxygen atoms from the phosphate group at about 235–255 pm (Figure 3e). One of the studied sodium ions remain completely solvated during the whole simulation and interacts with the phosphate groups through one or two water molecules.

Sodium ions coordinate 4–7 oxygen atoms from water molecules or phosphate group in their first coordination shell with the most frequently encountered coordination number being five (about 80% of the simulation time), followed by coordination number six (about 15% of the time). The coordination number of the sodium ions frequently changes in irregular intervals ranging from several femtoseconds to about 10 ps. The distance between the sodium ion and the oxygen

atoms from the first coordination shell is about 235 pm and does not depend on the origin of the oxygen atom, from water molecule or phosphate group.

Two states of the systems containing magnesium ions, with Mg^{2+} directly bonded to the phosphate group and with completely solvated ion, were found stable for 140 ps simulations. The Mg^{2+} binds to one of the oxygen atoms of one of the phosphate group of the selected RNA fragment with optimal $\text{Mg}^{2+}\cdots\text{O}=\text{P}$ distance of about 205 pm. The completely solvated Mg^{2+} interacts with the phosphate groups through one or two water molecules. Both the directly bonded and completely solvated magnesium ions have exactly six oxygen atoms in their first coordination shell. Moreover, during the whole simulation at 300 and 320 K no exchange of ligand in the first coordination shells has been observed. The distance between the magnesium ion and the oxygen atoms from the first coordination shell is about 215 pm.

The terminal phosphate oxygen atoms are solvated by water molecules forming hydrogen bonds with typical distance $\text{H}\cdots\text{O}=\text{P}$ of about 175 pm. In the simulation involving solvated sodium or magnesium ions both phosphate groups are solvated by about 5 water molecules. The presence of a directly bonded ion reduces the number of the water molecules at the corresponding phosphate group by one for part of the simulation with sodium and by two for directly bonded Mg^{2+} ion.

The stability of the system containing sodium ions does not depend strongly on the position of the ions with respect to the phosphate groups, being directly bonded or interacting through a water molecule. The energy difference between the bonded and solvated states of one of the cations is only 6 kJ/mol (simulated at 300 K), negligible for the system, and could be attributed to minimal variations in the arrangement of the water molecules.

Classical MD simulations with AMBER or CHARMM force fields gave similar behavior as the ab initio MD simulations but the concrete distances, cationic positions and time scales differ and depend on the selected force field.

■ COMPUTATIONAL METHODS

Ab initio Born–Oppenheimer molecular dynamic simulations were performed using the CP2K/Quickstep package.^{50,51} Density functional theory was applied within the generalized gradient approximation (GGA), using Perdew–Burke–Ernzerhof (PBE) functional.⁵² Basis set DZVP-MOLOPT-SR-GTH,⁵³ which is optimized for calculating molecular properties in gas and condensed phase, was applied for all atoms in the studied systems. For reducing computational cost Gaussian and Plane-Wave (GPW) method was used.^{51,54,55} This method uses an atom-centered Gaussian-type basis to describe the wave functions and an auxiliary plane wave basis to describe the electron density.⁵¹ Only the valence electrons are explicitly treated. Their interaction with the remaining ions is described using the pseudopotentials of Goedecker–Teter–Hutter (GTH).^{56,57} The charge density cutoff of the finest grid level is equal to 400 Ry. The number of used multigrids is 5.

All Born–Oppenheimer molecular dynamic simulations were carried out in NVT ensemble with a time step of 1 fs. The temperature was set to constant (at 300 or 320 K, as described below) using canonical sampling through velocity rescaling (CSVR) thermostat. Statistics were gained after thermal equilibration. The studied systems consist of nucleic acid dinucleotide fragment from RNA (with ribose) without the nucleobases, and the metal ions necessary to neutralize the negative charges of the phosphate groups, two Na^+ or one Mg^{2+} ion. The whole system is hydrated in a periodic box with dimensions: 1264.5 pm \times 1700.0 pm \times 1700.0 pm (see Figure 1a).

For simulations with sodium and magnesium ions the box contains 106 and 110 water molecules, respectively, that assures density of the water phase equal to 0.99 g/cm³. The dinucleotide skeleton is oriented along the shortest axis *x* (1264.5 pm) and forms a monotonic infinite chain of a single-stranded nucleic acid backbone (e.g., RNA) with average distance between the phosphorus atoms equal to 635 pm. The initial state (atom coordinates) of the dinucleotide are taken from a section with single-stranded RNA from an atomistic classical MD simulation of the ribosome in water solution based on crystallographic structure by Harms et al.⁵⁸ In the present simulation the cations, necessary to neutralize the negative charges of the phosphate groups, are initially located as follows:

- Simulation with 2 sodium ions: one Na⁺ ion directly coordinated at the phosphate group P1 and the second Na⁺ ion separated from P1 and P2 by water molecules
- Simulation with one bonded Mg²⁺: one Mg²⁺ ion directly located at the phosphate group P1 and interacting with the P2 phosphate group through a single water molecule
- Simulation with one solvated Mg²⁺: one Mg²⁺ ion separated from the phosphate group P1 by a single water molecule and from the phosphate group P2 by two water molecules

Initial simulations were performed at 300 K and after 61 ps for the simulations with magnesium ions and 104 ps for the simulation with sodium ions the temperature was set to 320 K in order to speed up of the dynamics still preserving temperature of the system reasonably close to the biological one.

Harmonic vibrational frequencies were calculated in order to compare the results with trends in the experimental data from the literature. The procedure we used is as follows: selection of several local potential energy minima of the dynamics steps, corresponding to the most abundant coordination number of the phosphate oxygen atoms and the metal ions. Then the geometry of the selected structures was optimized without constraints and vibrational analysis was performed explicitly including all atoms in the systems. The same basis set and exchange-correlation functional, used for the dynamical simulations were used for the geometry optimization and vibrational analysis. None of the studied structures has imaginary frequencies following this procedure. Typically the calculated harmonic vibrational frequencies of isolated modes are corrected for the error of the computational method (which for the DFT calculations with PBE functional corresponds to scaling by a factor of 1.026) and for the anharmonicity. In case of interacting modes, as the symmetric and asymmetric modes of the phosphate groups, these errors affect the calculated frequencies in different directions and with different magnitude. Due to this problem the calculated frequencies cannot be corrected simultaneously in an unbiased way. By this reason we report uncorrected harmonic frequencies.

The classical MD simulation of dinucleotide with sodium or magnesium ions were performed with GROMACS package version 4.4.5⁵⁹ with two different force fields, CHARMM27^{60,61} and AMBER (ver. 2.0).⁶² With both force fields the water molecules were described by TIP3P potential.⁶³ The time step was set to 1 fs, and the frame was recorded for every 10th fs of the simulation. NVT ensemble at *T* = 300 K was used. VMD package was used for visualization of the results.⁶⁴

■ ASSOCIATED CONTENT

■ Supporting Information

Figures with radial distribution functions and integrals for the modeled systems and results from the classical MD simulations; tables with conformations of the phosphate groups and calculated frequencies of the phosphate groups vibrations and their intensities; and a movie for the ab initio MD simulation for the system with sodium ions. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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