Molecular dynamics study of the competitive binding of hydrogen peroxide and water molecules with DNA phosphate groups

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Abstract
The interaction of hydrogen peroxide molecules with the DNA double helix is of great interest for understanding the mechanisms of anticancer therapy utilising heavy ion beams. In the present work, a molecular dynamics study of competitive binding of hydrogen peroxide and water molecules with phosphate groups of the DNA double helix backbone was carried out. The system of DNA double helix in a water solution with hydrogen peroxide molecules and Na+ counterions was simulated. The results show that the hydrogen peroxide molecules bind to oxygen atoms of the phosphate groups of the double helix backbone replacing water molecules of its hydration shell. The complexes of hydrogen peroxide molecules with the phosphate groups are stabilized by one or two hydrogen bonds and by Na+ counterions, forming ion-mediated contacts between phosphate groups and hydrogen peroxide molecules. The complex characterized by one H-bond between the hydrogen peroxide molecule and phosphate group is dominant, the other complexes are rare. The hydrogen peroxide molecule bound to the phosphate group of the double helix backbone can inhibit the formation of hydrogen bonds indispensable for the DNA biological functioning.

Keywords DNA · Hydrogen peroxide · Water · Counterion · Molecular dynamics

Introduction
Hydrogen peroxide molecule (H2O2) is always present in the living cell in micromolar concentration, which is controlled by the specific enzymes (Halliwell et al. 2000; Malinouski et al. 2011). The concentration of H2O2 molecules is significantly elevated in the tissues after the action of accelerated heavy ions (usually C6+n nuclei) used in therapy of cancer (Kraft 2001; Kreipl et al. 2009; Boscolo et al. 2018). The hydrogen peroxide molecules are formed as a product of reactions between radicals due to water radiolysis. The radicals damage the structure of DNA macromolecule directly, inducing the formation of single- and double-strand breaks of the double helix. However, the detailed analysis of the action of heavy ion beams on DNA has shown that the activity of radicals and direct irradiation effects are not sufficient to stop the biological function of DNA. Therefore, other mechanisms induced by high-energy radiation have been proposed (Solov’yov et al. 2009; Yakubovich et al. 2012; Piatnytskyi et al. 2015, 2016). In particular, the blocking of the biologically active centers of the DNA double helix by hydrogen peroxide molecules has been considered as an alternative mechanism in anticancer therapy (Piatnytskyi et al. 2015, 2016). In this regard, the study of the interaction of hydrogen peroxide with structural elements of the DNA double helix might increase our understanding of the molecular mechanisms of ion beam therapy. The use of molecular dynamics simulations can give important insights to the mechanisms of interaction of hydrogen peroxide molecule with the DNA double helix.

The biologically most prevalent B-DNA has the double helix structure with the minor and major grooves appearing as a result of twisting of opposite polynucleotide chains around each other (Saenger 1984). The hydrogen peroxide molecule can bind to DNA in a specific way through atoms of the nucleotide bases in the grooves and non-specifically through oxygen atoms of the phosphate groups of the
macromolecule backbone. The calculations with the use of atom-atom potential functions (Piatnytskyi et al. 2015, 2016) and the methods of quantum mechanics of molecules (Piatnytskyi and Volkov 2018; Zdorevskyi et al. 2019) have predicted that the complexes of phosphate groups with hydrogen peroxide are more stable than those with water molecules. The possibility of blocking the DNA specific recognition sites and the process of base pairs opening by hydrogen peroxide molecules (Zdorevskyi and Volkov 2019) has also been raised out. The realistic modeling of interaction processes of hydrogen peroxide with the structure elements of DNA should include the interactions with the surrounding water molecules and ions of the solution, which can be done within the framework of atomistic molecular dynamics methods. However, no detailed molecular dynamics studies of the interaction of hydrogen peroxide with the DNA double helix have been done yet.

To perform the molecular dynamics simulations at the atomistic level, the potential functions describing the intramolecular and intermolecular interactions for all atoms of the system should be determined. In the standard parameter sets for the potential functions, known as the force fields (Phillips et al. 2005; MacKerell et al. 1998; Foloppe and MacKerell 2000; MacKerell and Banavali 2000), the parameters for the hydrogen peroxide molecule were not implemented, and H₂O₂ molecule was parameterized in different ways (Praprotnik and Janežič 2005; Campo and Grigera 2005; Martins-Costa and Ruiz-López 2007; de Vera et al. 2018). In Ref. (Praprotnik and Janežič 2005), the force constants for intramolecular potentials of H₂O₂ molecule were obtained comparing the results of normal mode analysis with the experimental spectra of hydrogen peroxide (Giguere 1950). In Ref. (de Vera et al. 2018), the intermolecular parameters for H₂O₂ molecule have been transferred from TIP3P water model (Jorgensen et al. 1983), while the intramolecular parameters were taken from (Praprotnik and Janežič 2005). The study (de Vera et al. 2018) has been focused on a problem of irradiation-induced chemical bond breaks of DNA macromolecule in the atomistic molecular dynamics simulations by the method developed in Ref. (Sushko et al. 2016), and the interaction of hydrogen peroxide with the DNA double helix was out the scope of interest. The consistent development of the model of hydrogen peroxide molecule for the atomistic simulations was performed only recently (Orabi and English 2018), where the parameters for the both intermolecular and intramolecular potentials were carefully elaborated. Using the data obtained, the study of interaction of hydrogen peroxide molecules with the DNA double helix may be carried out now with the use of standard method of atomistic molecular dynamics simulations.

The goal of the present research is to perform the molecular dynamics study of the competitive binding of hydrogen peroxide and water molecules with the phosphate groups of DNA backbone. To analyze the problem, the molecular dynamics simulations of DNA in a water solution with H₂O₂ molecules and sodium ions have been carried out. The simulation details and the methods of the analysis are described in the following section. In the subsequent section, the structure of the solvation shell of the phosphate group in a complex with the hydrogen peroxide and water molecules is described using the radial distribution functions. Finally, the probable molecular mechanism of influence of hydrogen peroxide molecule on the DNA biological functioning is discussed.

**Materials and methods**

The molecular dynamics simulations have been carried out for five different systems: three systems contained DNA macromolecule in the solution and two systems—the solutions without DNA. The DNA was modelled as the infinite chain of canonical B-DNA consisted of repeating 20 base pairs fragment with the following nucleotide sequence: d(CGCCAATTCCGGAATTCCG). The nucleotide sequence is comprised of two overlapping motifs known as the Drew–Dickerson dodecamer (Drew et al. 1981), the model polynucleotide widely studied in the molecular dynamics simulations [see for example (Dans et al. 2016; Perepelytsya 2018; Perepelytsya et al. 2019)]. The DNA double helix in the simulation box was solvated by the solution consisting of about 4 thousands of water molecules, 40 Na⁺ counterions, and hydrogen peroxide molecules of different concentration. The number of positively charged Na⁺ counterions was taken equal to the number of the negatively charged phosphate groups. The initial positions of the counterions were randomly generated to be more apart than 5 Å from each other and at least 7 Å from the DNA double helix. The initial positions of hydrogen peroxide molecules were generated to create nonoverlapping molecules placed at distances larger than 2.5 Å from DNA atoms. The simulation box of each system contained about 15 thousands of atoms and was of rectangular shape with the initial XYZ dimensions 54Åx54Åx70Å. In the simulations, the periodic boundary conditions were used. To mimic an infinite double helix, the ends of the DNA fragment were linked with their images in the adjacent boxes. An example of the initial state of the simulated system with DNA is shown in Fig. 1.

The number of hydrogen peroxide molecules in the systems with DNA was 0, 36, and 432, which corresponds to the concentrations 0 M, 0.3 M and 3.5 M of H₂O₂, respectively. Accordingly, these simulation systems were denoted as DNA0, DNA03 and DNA35. In the present study the systems DNA35 and DNA03 are referred to the systems of DNA with high and low concentrations of hydrogen peroxide, respectively. It should be noted that the amount of H₂O₂...
in the both DNA03 and DNA35 systems is much higher than the amount of hydrogen peroxide in a living cell. As known, under the physiological conditions, the concentration of hydrogen peroxide stays within micromolar concentrations (Halliwell et al. 2000; Malinouski et al. 2011), while in our system even one $\text{H}_2\text{O}_2$ molecule in the simulation box corresponds to about 8 mM. However, in the anticancer therapy by the heavy ion beams the concentration of hydrogen peroxide increases (Kreipl et al. 2009; Boscolo et al. 2018). In particular, the calculations (Boscolo et al. 2018) showed that one $\text{C}^{6+}$ ion can induce the increase of $\text{H}_2\text{O}_2$ concentration to about tens of $\mu$M during the time $\sim$ 1 μs. Considering that hydrogen peroxide can accumulate during a therapy session, its amount can reach significant concentrations. Therefore, in the present study, the concentration of hydrogen peroxide molecules in the DNA03 and DNA35 systems was taken considerably higher than under the natural conditions.

To characterize the influence of hydrogen peroxide molecules on the solution structure, two systems with hydrogen peroxide but without DNA have been also simulated. These systems were denoted as WP03 and WP35. The content of WP03 and WP35 was as follows: $\text{H}_2\text{O}, \text{H}_2\text{O}_2, \text{Na}^+$ and $\text{Cl}^-$. The concentrations of hydrogen peroxide molecules and sodium ions in the systems WP03 and WP35 were the same as in the systems DNA03 and DNA35, respectively. Chlorine ions were added to the systems WP03 and WP35 to make them electrically neutral. The detailed description of the content of the simulated systems DNA0, DNA35, WP03, and WP35 is in the Supplemental Materials (Table S1).

The potential energy is described as a sum of pair interactions that may be written as follows (MacKerell et al. 1998):

$$
U = \sum_{\text{bonds}} K_b(b - b_0)^2 + \sum_{\text{angles}} K_\theta(\theta - \theta_0)^2 + \sum_{\text{dihedrals}} K_\chi(1 + \cos(n\chi - \delta)) + \sum_{\text{improvers}} K_\psi(\psi - \psi_0)^2 + \sum_{\text{LJ}} \left( \frac{q_i q_j}{r_{ij}} \right)^{12} - 2 \left( \frac{q_i q_j}{r_{ij}} \right)^6 + \sum_{\text{Coul}} K_C q_i q_j e r_{ij},
$$

Here, the first term describes the energy of deformation of chemical bonds with the force constant $K_b$ and the change of bond length $(b - b_0)$. The second term describes the energy of deformation of the valence angle $\theta$ with the force constant $K_\theta$, and the change of valence angle $(\theta - \theta_0)$. The third term is the energy of dihedral angles $\chi$ formed by four sequentially bonded atoms in the molecule; here, $K_\chi$ is the force constant, $n$ is the integer number that denotes the periodicity of the rotational barrier (multiplicity), $\delta$ is the phase angle of the dihedral. The fourth term describes the energy of the atoms separated by two covalent bonds (Urey–Bradley term); here, $K_{UB}$ is the force constant, $(S - S_0)$ is the change of the distance between atoms. The fifth term describes bending of some molecular structural elements (improper torsion); here, $\psi$ is the angle, $K_\psi$ is the force constant, and $(\psi - \psi_0)$ is the change of the angle. The last two terms in Eq. (1) describe the van der Waals and Coulomb nonbonded interactions. The van der Waals interactions are described by the Lennard-Jones potential with the parameters $\epsilon_{ij}$ and $\sigma_{ij}$; $r_{ij}$ is the distance between atoms $i$ and $j$. The Coulomb term is determined by the partial charges $q_i$ and $q_j$ on the atoms, $\epsilon$ is the dielectric constant and $K_C$ is the Coulomb’s constant.

The parameters in the potential energy (1) are developed for each bond type in the molecular system. The sets of parameters are unified to the force fields. In the present study, the CHARMM force field (MacKerell et al. 1998; Foloppe and MacKerell 2000; MacKerell and Banavali 2000; Denning et al. 2011; Hart et al. 2012) has been taken for the simulation of the system of DNA in water solution with $\text{Na}^+$ ions and hydrogen peroxide molecules. In particular, for the nucleic acids the CHARMM36 parameter set has been used (Denning et al. 2011; Hart et al. 2012). To link the ends of the DNA fragment with their images in the adjacent boxes, the special patch of CHARMM36 force field (LKNA)
has been applied to the end residues of the simulated poly-nucleotide. The TIP3P model of water molecule (Jorgensen et al. 1983) has been taken. For the Na⁺ ions the parameters developed by (Beglov and Roux 1994) have been used.

The parameters necessary for the simulation of hydrogen peroxide molecules, are not implemented in the standard force fields. At the same time, in Ref. (Orabi and English 2018), the detailed study has been carried out to develop an additive potential model for the simulation involving hydrogen peroxide molecule. Thus, in Ref. (Orabi and English 2018), using the methods of quantum mechanics of molecules, the structure parameters for H₂O₂ molecule and the frequencies of vibrations have been calculated at different levels of theory, and the parameters describing the energy of chemical bonds stretching (O–H and O–O), valence angles (H–O–O), and dihedral angle (H–O–O–H) have been determined by comparing the calculated frequencies of vibrations with the experimental vibrational spectra of H₂O₂. The potentials for van der Waals and Coulomb nonbonded interactions have been determined analyzing the calculated structures of the systems of hydrogen peroxide with water molecules. The developed four-site additive model for H₂O₂ molecule reproduces the experimental properties of hydrogen peroxide–water systems calculated together with TIP3P water model. The parameters for the hydrogen peroxide molecule from (Orabi and English 2018) have been used in present study. The parameter values for H₂O₂ model are shown in Table 1.

The computer simulations have been performed using the NAMD software package (Phillips et al. 2005). The lengths of all bonds with hydrogen atoms have been constrained using the SHAKE algorithm (Ryckaert et al. 1977). The long-range electrostatic interactions have been treated using particle mesh (Ewald method Darden et al. 1993). The switching and cut-off distances for the long-range interactions have been set to 8 Å and 10 Å, respectively. The integration time step is 2 fs. The temperature is kept at 300 K using a Langevin thermostat for all heavy atoms (damping constant 5 ps⁻¹). The oscillation time and damping time constants for the Langevin piston are taken equal to 100 fs and 50 fs, respectively. The target pressure was 101,325 Pa. The simulations with the constant pressure and temperature (NPT ensemble) have been carried out for the case of the systems with flexible cell (NAMD setting) allowing the fluctuations of the cell volume. After several nanoseconds of simulation in the NPT ensemble, the simulation was switched to constant volume and temperature mode (NVT ensemble). At the production stage, the systems have been simulated in NVT ensemble with a time step of 2 fs. The simulation trajectory with a length of 200 ns (trajectory 1), and for the system DNA35 two additional simulation trajectories have been obtained: trajectory 2 (200 ns) and trajectory 3 (500 ns). The simulation protocol is described in the Supplemental Materials (Table S2).

To check the convergence of the obtained simulation trajectories the detailed analyzes has been performed (Supplemental Materials, Section S2). The calculations of root mean square deviations (RMSD) for the atoms of DNA with the selection of all atoms of the macromolecule have shown that the RMSD dependencies reach the plateau at the very beginning of the trajectory (Supplemental Materials, Fig. S1). The analysis of the volume density maps (VDM) has shown that in the case of the systems DNA35, DNA03, and DNA0 the densities of the solution subsystems are governed by the DNA macromolecule present in the simulation box (Supplemental Materials, Figs. S2, S3, S4). The density distributions of water, hydrogen peroxide, and counterions are sufficiently reproduced after the first 100 ns of the simulation trajectory. In the case of the systems without DNA (WP35 and WP03), the density of the solution subsystems are characterized by the uniform distribution starting from the first time interval 1–100 ns (Supplemental Materials, Figs. S5, S6). Thus, the comparison of obtained volume density maps has shown that the time interval of 100 ns is enough to reach the sufficient homogeneity of the systems. Therefore, in the present work the time interval from 0 to 100 ns of the simulation trajectory has been discarded as a time period necessary for equilibration of the system, and the following part of the simulation trajectory has been used for the analysis.

The analysis of obtained simulation trajectories has been carried out using VMD software package (Humphrey et al. 1996).

### Table 1 The parameters for H₂O₂ molecule (Orabi and English 2018)

| Bonds          | $K_b$ (kcal/mole·Å²) | $b_0$ (Å) | $K_\theta$ (kcal/mole·rad²) | $\theta_0$ (°) | $K_x$ (kcal/mole) | $n$ | $\delta$ (°) | $\epsilon_j$ (kcal/mole) | $R_{j}^{min}/2$ (Å) | $q$ (e) |
|---------------|---------------------|-----------|-----------------------------|---------------|------------------|-----|-------------|--------------------------|---------------------|-------|
| O–O          | 285.500             | 1.442     |                             |               |                  |     |             | -0.20384                 | 1.67423             | -0.41 |
| O–H          | 521.000             | 0.963     |                             |               |                  |     |             | -0.046000                | 0.224500            | 0.41  |
| H–O–O        | 60.400              | 99.92     |                             |               |                  |     |             |                          |                     |       |
| H–O–O–H      | 2.02                | 2         | 0.00                        |               |                  |     |             |                          |                     |       |

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Results

Radial distribution functions

The structures of complexes of counterions, water molecules, and hydrogen peroxide molecules with the phosphate groups of DNA backbone have been studied using the radial distribution functions (RDFs). These functions have been calculated using the plug-in (Levine et al. 2011) implemented in the VMD software package (Humphrey et al. 1996). The radial distribution functions, \( g(r) \), have been calculated in accordance to the following definition (Levine et al. 2011):

\[
g(r) = \lim_{\Delta r \to 0} \frac{p(r)}{4\pi r^2 \Delta r N_p/V},
\]

where \( p(r) \) is the average number of atomic pairs, found at the distance within \( r, r + \Delta r \), \( N_p \) is the number of pairs of selected atoms for which the radial distribution functions is constructed, \( V \) is the total volume of the system, \( \Delta r \) is the thickness of the sphere slice, which in our calculations has been take equal to 0.1 Å. As follows from Eq. (2), the RDF describes the ratio of the local concentration of the particles in a sphere slice at the distance \( r \) to their average concentration in the system. The number of particles within the sphere slice between \( r_1 \) and \( r_2 \) distances is determined by the direct integration of the function \( g(r) \):

\[
n_{12} = \int_{r_1}^{r_2} r^2 g(r) \, dr,
\]

\( \rho = N_p/V \) is the density of atomic pairs in the system. The number of particles that are localized within RDF maximum is known as the coordination number. The coordination number has been calculated by integrating the peak of the radial distribution function from one minimum to another.

The RDFs relative to oxygen atoms \( O_1 \) and \( O_2 \) of the phosphate groups for oxygen atoms of hydrogen peroxide molecules (RDF\(_{\text{PER}O_1}^{O_1} \) and RDF\(_{\text{PER}O_2}^{O_2} \)), oxygen atoms of water molecules (RDF\(_{\text{W}O_1}^{O_1} \) and RDF\(_{\text{W}O_2}^{O_2} \)), and Na\(^+\) counterions (RDF\(_{\text{ION}O_1}^{O_1} \) and RDF\(_{\text{ION}O_2}^{O_2} \)) have been calculated. The radial distribution functions have been calculated for different time intervals of the simulation trajectories. In the case of the DNA35 system, which has 3 simulation trajectories—trajectory 1 (200 ns), trajectory 2 (200 ns), and trajectory 3 (500 ns), the mean RDFs have been obtained by averaging each time interval of 100 ns, starting from 101 ns (in total 6 intervals). In the case of the other systems the RDFs have been calculated for time interval 101–200 ns. All radial distribution functions have been calculated for the systems containing the DNA macromolecule (DNA03, and DNA35) are shown in the Supplemental Materials (Section S3).

Each of the oxygen atoms of the PO\(_4^–\) group (\( O_1 \) and \( O_2 \)) is bonded by one chemical bond with the phosphorus atom. The atom \( O_2 \) is directed toward the major groove of the double helix, while the atom \( O_1 \) is directed outward the macromolecule (Fig. 2). As the result, these atoms of the phosphate group of the DNA backbone are accessible for the molecules and ions of the solution in different extent that is observed by the shapes of obtained RDFs.

The mean radial distribution functions for oxygen atoms of hydrogen peroxide molecules with respect to the phosphate groups of DNA backbone (RDF\(_{\text{PER}O_1}^{O_1} \) and RDF\(_{\text{PER}O_2}^{O_2} \)) in the case of the system with high concentration of \( \text{H}_2\text{O}_2 \) (DNA35) are shown in Fig. 3, while the RDFs for different time intervals of the simulation trajectory of the same system are shown in the Supplemental Materials (Fig. S7). The RDF\(_{\text{PER}O_1}^{O_1} \) and RDF\(_{\text{PER}O_2}^{O_2} \) for the case of the system with lower concentration of hydrogen peroxide (the system DNA03) have qualitatively the same structure as in the case of the system DNA35 (Supplemental Materials Fig. S10). The radial distribution functions are characterized by the peaks arising due to the higher local concentration of the particles in the peak region comparing to their average concentration in the system. The first peak (peak A) and the second peak (peak B) of the RDF\(_{\text{PER}O_1}^{O_1} \) and RDF\(_{\text{PER}O_2}^{O_2} \) are localized at the distance near 2.6 Å and near 3.3 Å, respectively (Fig. 3a). The peak A is about two times higher than the peak B. The intensity of the RDF peaks is determined by the relative increase of the local concentration of oxygen atoms of \( \text{H}_2\text{O}_2 \) molecules.

![Fig. 2 The schematic representation of the DNA macromolecule.](image)
compared to the average concentration of oxygen atoms of H$_2$O$_2$ molecules at this distance in the system. The broad band C within from 4 to 6 Å is centered at about 5 Å and has very weak intensity.

The integrals of the radial distribution functions RDF$_{O1}$ and RDF$_{O2}$ for the peak A (the coordination number $n_A$), characterizing the number of particles near the atoms O$_1$ and O$_2$ of the phosphate group, have the values $n_A \approx 0.65$ and $n_A \approx 0.75$, respectively. In the case of the peak B, the values of the integrals (the coordination numbers $n_B$) are $n_B \approx 1.10$ and $n_B \approx 1.20$ for the atoms O$_1$ and O$_2$ of the phosphate group, respectively (Fig. 3b). The average number of oxygen atoms of hydrogen peroxide molecules at the distance closer than 3 Å to the oxygen atom of the phosphate group is about twice lower than the number of the oxygen atoms localized at the distance range from 3 to 4 Å. Taking this into consideration, it may be concluded that the peak A arises due to the oxygen atom of H$_2$O$_2$ molecule bonded by the H-bond to the oxygen atom of the phosphate group, while the peak B appears due the contribution of the second oxygen atom in the same hydrogen peroxide molecule and due to the contribution from the other hydrogen peroxide molecules localized in the region of this peak. The origin of the broad band C of the RDFs is related to the oxygen atoms of hydrogen peroxide molecules that are localized mostly in the region of neighbor phosphate groups.

To characterize the distribution of water molecules around DNA phosphate groups the radial distribution functions of oxygen atoms of water molecules regarding to O$_1$ and O$_2$ atoms of the phosphate group have been built. The mean radial distribution functions for oxygen atoms of water molecules with respect to the phosphate groups of DNA backbone (RDF$_{W}$ and RDF$_{O}$) in the case of system with high concentration of H$_2$O$_2$ (DNA35 system) are shown in Fig. 4, while the RDFs for different time intervals of the simulation trajectory of the same system are shown in Supplemental Materials (Fig. S8). The RDF$_{O1}$ and RDF$_{O2}$ for the case of systems with lower concentration of hydrogen peroxide (DNA03 system) and without hydrogen peroxide (DNA0 system) have qualitatively the same structure as in the case of DNA35 system and are shown in Supplemental Materials (Figs. S11 and S13a). The RDF$_{O1}$ and RDF$_{O2}$ have an intense peak at the distance 2.7 Å that corresponds to the H-bond length between water molecule and oxygen atom of the phosphate group. The broad band from 3.5 to 5.5 Å is related to the second hydration shell of PO$_4$ group (Fig. 4a).

The integrals of RDF$_{O1}$ and RDF$_{O2}$ for counterions are qualitatively the same for all simulated systems with DNA are shown in Supplemental materials (Figs. S9, S12, and S13b). The RDF$_{O1}$ and RDF$_{O2}$ for the case of systems with lower concentration of hydrogen peroxide (DNA03 system) and without hydrogen peroxide (DNA0 system) have qualitatively the same structure as in the case of DNA35 system and are shown in Supplemental Materials (Figs. S11 and S13a). The RDF$_{O1}$ and RDF$_{O2}$ have an intense peak at the distance 2.7 Å that corresponds to the H-bond length between water molecule and oxygen atom of the phosphate group. The broad band from 3.5 to 5.5 Å is related to the second hydration shell of PO$_4$ group (Fig. 4a).

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RDFs are shown in Fig. 5. The obtained RDFs are characterized by two peaks (Fig. 5a). The first peak at the distance 2.25 Å corresponds to the direct contact of ions with oxygen atom of PO$_4^-$ group. This distance is lower for about 0.1 Å than a sum of Pauling radii of Na$^+$ ion and oxygen atom (Kittel 1954) that is caused by the electrostatic attraction. Na$^+$ usually binds to one of the two oxygen atoms of the phosphate group (O$_1$ or O$_2$), which is associated with the structure-making character of hydration of the counterion (Perepelytsya 2018). The second peak at the distance about 4.6 Å is related to counterions that form water-mediated contacts with oxygen atoms of the PO$_4^-$ groups. The complexes of Na$^+$ counterions with the phosphate group are shown in the inset of Fig. 5a.

The integrals of the RDF$^{O1}_{\text{Ion}}$ and RDF$^{O2}_{\text{Ion}}$ show that the average number of counterions at the distance of the first peak (the coordination number $n_1$) are about 0.03 for the both O$_1$ and O$_2$ atoms of PO$_4^-$ group (Fig. 5b). Such values of coordination numbers indicate that the both O$_1$ and O$_2$ atoms of phosphate group are equally accessible for direct contact with Na$^+$ ions. The integrals for the second peak (the coordination number $n_2$) equal to 0.17 and 0.22 for O$_1$ and O$_2$ atoms of the phosphate group, respectively. The obtained ratio of coordination numbers, $n_2 > n_1$, reveal the water-mediated mode of interaction of Na$^+$ ion.
Na\(^+\) counterions with the phosphate groups, which is due to the structure-making character of hydration of Na\(^+\) ion (Perepelytsya 2018). At the distance 7.4 Å the integral of RDFs results the average coordination number about 0.76 (Fig. 5b). This is in a good agreement with counterion condensation theory (Manning 1978, Frank-Kamenetskii et al. 1987), predicting such number of condensed counterions per one phosphate group in the case of B-form of DNA double helix.

**Complexes of H\(_2\)O\(_2\) with DNA phosphate group**

The obtained radial distribution functions for hydrogen peroxide, water molecules and Na\(^+\) counterions with respect to the phosphate groups of the double helix backbone are the result of physical features of the solvation shell of PO\(_4\) group. The positions of the RDFs peaks indicate the most probable localization of the atoms, while the values of integrals indicate the mean number of particles at the peak regions. Analyzing the structure form of the RDFs, the structure of the complexes of H\(_2\)O\(_2\) molecule with the DNA phosphate group may be determined. Thus, taking into consideration the obtained radial distribution functions (RDF\(_{O1}^{\text{PER}}\) and RDF\(_{O2}^{\text{PER}}\) Fig. 3), the structure of H\(_2\)O\(_2\) molecule and the complexes of hydrogen peroxide molecule with the DNA phosphate group have been characterized. The structures of the most probable complexes are shown in Fig. 6.

In Complex I, the hydrogen peroxide molecule is bonded to one of the atoms of phosphate group (O\(_1\) or O\(_2\)) of DNA backbone through one H-bond. In this case, one oxygen atom of H\(_2\)O\(_2\) molecule is tethered to the phosphate group contributing to the peak A of RDF and another one is directed outward the phosphate group, contributing to the peak B of RDF. Complex II is similar to Complex I with the difference that the second oxygen atom of H\(_2\)O\(_2\) molecule does the contribution to the peak A and peak B of different RDFs (RDF\(_{O1}^{\text{PER}}\) and RDF\(_{O2}^{\text{PER}}\)). In Complex III the hydrogen peroxide molecule is bonded to the PO\(_4\) group through two H-bonds that are formed with O\(_1\) and O\(_2\) atoms. In Complex IV the hydrogen peroxide molecule is bonded to O\(_1\) or O\(_2\) atoms of PO\(_4\) group through one H-bond and the binding with participation of Na\(^+\) counterion. In this case, Na\(^+\) counterion has a direct contact with one of oxygen atoms of phosphate group and with one oxygen atom of hydrogen peroxide molecule. The comparison of the RDFs for H\(_2\)O\(_2\) molecules (Fig. 3 and in Supplemental Materials Figs. S7 and S10) with the RDFs for H\(_2\)O molecules (Fig. 4 and in Supplemental Materials Figs. S11 and S13a) has shown that in Complex I and II the hydrogen peroxide molecule substitutes one water molecule from the solvation shell of the phosphate group, while in the case of Complexes III and IV the number of substituted water molecule is two or more.

To understand which of the complexes are the most frequently encountered (dominant), the analysis of the distribution of hydrogen peroxide molecules around the phosphate groups has been carried out using the radial distribution functions, where special conditions for the position of the atoms have been considered. To test Complexes II and III, the following requirement has been imposed in the calculations of the RDFs: only those oxygen atoms have been taken into consideration which are in the structure of H\(_2\)O\(_2\) molecule having one of the oxygen atoms within 3 Å of non-reference atom of the PO\(_4\) group. Thus, two radial distribution functions of such kind have been calculated RDF\(_{O1}^{\text{PER}}\) and RDF\(_{O2}^{\text{PER}}\). The radial distribution function RDF\(_{O1}^{\text{PER}}\) has been built with respect to the atom O\(_1\) of the PO\(_4\) group (reference atom) for only those oxygen atoms which belong to the H\(_2\)O\(_2\) molecules having one of two oxygen atoms localized within 3 Å of O\(_1\) atom of phosphate group (non-reference atom). The radial distribution function RDF\(_{O2}^{\text{PER}}\) has been built by the similar scheme as RDF\(_{O1}^{\text{PER}}\), but in this case, O\(_2\) atom has been taken as the reference atom, while O\(_1\) as non-reference one.

The occurrence of Complex IV has been tested through the radial distribution functions that have been calculated fulfilling the conditions, imposed on the positions of oxygen atoms of H\(_2\)O\(_2\) molecule and Na\(^+\) ion with respect to PO\(_4\) group. According to the conditions, only those oxygen atoms in H\(_2\)O\(_2\) molecules have been taken into

![Fig. 6](Image)

**Fig. 6** The structure schemes of the complexes of H\(_2\)O\(_2\) with the phosphate groups of the DNA double helix backbone. The short- and log-dashed lines mark the distances of the peak A and the peak B of the radial distribution functions RDF\(_{O1}^{\text{PER}}\) and RDF\(_{O2}^{\text{PER}}\) in Fig. 3a. The water molecules are not shown for clarity of presentation.
account that are within 3 Å of the Na\(^+\) counterion bonded to the non-reference atom of the PO\(_4\) group. The counterion has been taken into consideration if found within 3 Å of oxygen atom of the phosphate group. Thus, two radial distribution functions of such kind have been calculated \(\text{RDF}_{O1-NaO2}^{\text{PER}}\) and \(\text{RDF}_{O2-NaO1}^{\text{PER}}\). The radial distribution function \(\text{RDF}_{O1-NaO2}^{\text{PER}}\) has been built with respect to O\(_1\) atom of the phosphate group for only those oxygen atoms of H\(_2\)O\(_2\) molecules which have one of two oxygen atoms localized within 3 Å of Na\(^+\) ion that is attached to the non-reference atom O\(_2\) of the PO\(_4\) group. The scheme of construction of the \(\text{RDF}_{O2-NaO1}^{\text{PER}}\) is the same as in the case of the \(\text{RDF}_{O1-NaO2}^{\text{PER}}\), but O\(_2\) atom has been taken as reference atom, and O\(_1\) as non-reference one.

The obtained RDFs for the system with high concentration of hydrogen peroxide (DNA35 system) averaged over different time intervals are shown in Fig. 7, and the RDFs for each time interval of the simulation trajectory of the same system are shown in Supplemental Materials (Figs. S14, S15). The RDFs for the system with lower concentration of hydrogen peroxide (DNA03 system) have qualitatively the same character as the RDFs for the system DNA35 and are shown in Supplemental Materials (Figs. S16, S17). Due to the imposed constraints the volume of localization of the selected particles is much smaller than the volume of the system \((V)\). As the result, the normalization of considered RDFs, which is the denominator in formula (2), must be different from the normalization of RDFs without imposed conditions for the positions of the atoms selected. Therefore, the obtained RDFs with special conditions may be compared only qualitatively. In this regard, the radial distribution functions without normalization also present the interest for the analysis of the complexes (Supplemental Materials Figs. S14–S17).

The results of calculations have shown that the RDFs obtained for the analysis of Complexes III and IV have one peak and one broad band (Fig. 7). The peak is at the distance about 2.6 Å that corresponds to direct contact of oxygen atom of hydrogen peroxide molecule with the phosphate group. The broad band with maximum at the distance about 5 Å appears due to oxygen atoms of hydrogen peroxide molecules that satisfy the imposed conditions, but do not form direct contact with the oxygen atoms of the phosphate groups. The observation of the peak in this region proves the formation of Complex III and Complex IV. At the same time, the mean number of oxygen atoms at the distance about 2.6 Å is rather low: about 0.03 and 0.003 in the case of Complex III and Complex IV, respectively (peak values at the distance 2.6 Å of the unnormalized RDFs in Figs. S14, S15 of Supplemental Materials). The comparison of these values with the coordination number \(n_A\), obtained for the peak A of the mean RDFs for hydrogen peroxide molecules (Fig. 3a), has shown that in the case of Complex III mean value of oxygen atoms is an order of magnitude lower than \(n_A\) (Fig. 7a), while in the case of Complex IV it is even less (Fig. 7b). Taking this into consideration it may be concluded that the most common complex is Complex I. Complex II has rather similar structure as Complex I and may be considered as its variate. Complex III and Complex IV are rare, but not negligible.
Discussion

The present molecular dynamics study of the DNA double helix in aqueous solution with hydrogen peroxide and Na\(^+\) counterions has shown that H\(_2\)O\(_2\) molecules take part in the formation of solvation shell of the phosphate group of macromolecule backbone. The analysis of the structures of complexes formed by the solution species with the phosphate group has shown that four complexes of H\(_2\)O\(_2\) molecule and PO\(_4\)\(^{3-}\) group are most probable (Fig. 6). The complex, where the hydrogen peroxide molecule is linked with one of the oxygen atoms of the phosphate group (O\(_1\) or O\(_2\)) by one H-bond (Complex I), has been found as dominant. The hydrogen peroxide molecule in the complex of such type may do the rotations around the H-bond and does not have any constraints for the conformational change of the molecule. At the same time, the complexes, where H\(_2\)O\(_2\) molecule is tethered to the phosphate group by two H-bonds (Complex III), or by H-bond and counterion-mediated contact (Complex IV), are characterized by fixed positions of oxygen atoms of hydrogen peroxide molecule with respect to the oxygen atoms of phosphate group, and they have been observed rarely in the simulation. Taking into consideration the relative mobility of hydrogen peroxide molecule in Complex I and II they may be considered as “opened” state, while due to the constrained position of H\(_2\)O\(_2\) molecule in Complex III and IV such complexes may be related to the case of “closed” state (Fig. 6).

Interacting with the phosphate group of the DNA backbone in the case of “opened” state complexes a hydrogen peroxide molecule replaces one water molecule bonded to one of oxygen atoms of the phosphate group. In the case of Complex III two H\(_2\)O molecules are removed from the hydration shell of PO\(_4\)\(^{3-}\) group and substituted by one H\(_2\)O\(_2\) molecule. The formation of Complex IV is facilitated due to the presence of Na\(^+\) counterions with the hydration shell where two H\(_2\)O molecules are replaced by one H\(_2\)O\(_2\) molecule. The counterions with attached hydrogen peroxide condensing on DNA macromolecule deliver H\(_2\)O\(_2\) molecules to the double helix. The hydrogen peroxide molecule of the counterion near DNA can leave the hydration shell of the ion or remain in it, which makes it possible to form all the complexes considered.

The influence of H\(_2\)O\(_2\) molecule on biological mechanisms of DNA may be considered as a multistage process with consequent formation of the “opened” state and “closed” state complexes (Fig. 6). The hydrogen peroxide molecule from the solution binds to one of oxygen atoms of the phosphate group (O\(_1\) or O\(_2\)) forming the “opened” state complex. Staying some time in this state H\(_2\)O\(_2\) molecule can dissociate from the phosphate group back to the solution or pass to the “closed” state complex. In the “closed” state complex the atoms of phosphate group of DNA double helix are not accessible for the formation of the H-bond with the other molecules. At the same time, the formation of two hydrogen bonds with the atoms of DNA is indispensible for the process of nucleic-protein recognition (Bruskov 1975; Seeman et al. 1976), and makes it extremely accurate. Therefore, the binding of hydrogen peroxide molecules to the phosphate groups of the double helix backbone can induce the inhibition of this biological process.

To elucidate the probable effects of hydrogen peroxide on DNA, the studies of the stability of the double helix and the complexes of H\(_2\)O\(_2\) with phosphate groups and other structural elements of the macromolecule should be carried out further. The hydrogen peroxide is expected to have an influence on DNA structure, since, as we observed in our simulations, the values of root mean square deviations of the atoms in DNA structure increase in the case of the systems with H\(_2\)O\(_2\) (Supplemental materials, Fig. S1c). So, the mobility of different structural elements of the double helix may have different sensitivity to the interaction with the hydrogen peroxide and be of particular interest for further research.

Conclusions

The molecular dynamics simulations of the DNA double helix in water solution with hydrogen peroxide molecules and Na\(^+\) counterions have been carried out to study the competitive binding of hydrogen peroxide and water molecules with the phosphate groups of macromolecule backbone. The structure of solvation shell of the phosphate group has been studied by constructing the radial distribution functions with respect to the oxygen atoms O\(_1\) and O\(_2\) of phosphate groups. The results show that hydrogen peroxide molecules bind to oxygen atoms O\(_1\) and O\(_2\) of phosphate groups of the double helix backbone substituting water molecules from the hydration shell. The analysis of the possible structures that may be formed by H\(_2\)O\(_2\) molecule in complex with the DNA phosphate group has shown that four complexes are the most probable. The complexes, where hydrogen peroxide molecule is linked with one of oxygen atoms of the phosphate group by one H-bond, are found as dominant, while the complexes, where H\(_2\)O\(_2\) molecule is bonded to the phosphate group by two H-bonds, or by H-bond and counterion-mediated contact, are observed rarely in the simulation. Thus, it should be expected that the hydrogen peroxide molecules could inhibit the formation of H-bonds with phosphate groups of the backbone chain of the double helix, interfering with the biological functioning of DNA.

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