doi: 10.15407/ujpe61.03.0219

D.V. PIATNYTSKYI, O.O. ZDOREVSKY, S.M. PEREPELYTSYA, S.N. VOLKOV
Bogolyubov Institute for Theoretical Physics, Nat. Acad. of Sci. of Ukraine
(14-b, Metrolohichna Str., Kyiv, 03680, Ukraine; e-mail: snvolkov@bitp.kiev.ua)

PACS 87.14.Gg, 87.15.-v, 87.15.He

FORMATION OF COMPLEXES OF HYDROGEN PEROXIDE MOLECULES WITH DNA

A possibility for hydrogen peroxide molecules to form stable complexes with atomic groups in the DNA backbone under the irradiation of the cell medium with high-energy ions has been studied. The energy of complexes is estimated, by taking the electrostatic and van der Waals interactions into account in the framework of the atom-atom potential function method. The interaction with metal counterions, which neutralize the surface charge of a macromolecule under natural conditions, is also taken into consideration. Stable configurations are determined for various complexes consisting of the atoms belonging to a DNA phosphate group, H_2O_2 and H_2O molecules, and a Na^+ metal ion. The complexes of hydrogen peroxide molecules with DNA phosphate groups and a counterion are shown to be not less stable than their complexes with water molecules. The attachment of an H_2O_2 molecule to a phosphate group of the double helix backbone can block the processes of DNA biological functioning and can deactivate the genetic mechanism of a cell.

K e y w o r d s: DNA, hydrogen peroxide, Bragg peak, ionic therapy.

1. Introduction

The invention of effective means to fight against cancer is one of the most challenging topics in modern science. The financial resources allotted for the development of more effective oncologic drugs increase for every year, but the number of oncological diseases is not reduced [1]. To a great extent, this situation stimulates the development of new methods of anticancer therapy, which are based on the pharmacological mechanisms of drug action.

In particular, the therapy of malignant tumors with the use of the beams of high-energy ions got a wide application lately. This method is based on the Bragg effect [2–5]. Namely, ions (as a rule, these are $^{12}C^{6+}$ or H^+), after having been accelerated to high energies of an order of 100 MeV and even more, are used to irradiate a malignant tumor. In the organism tissue, ions are slowed down and release almost all of their initial energy at the end of their trajectory to form an energy peak (the Bragg peak). The initial energy of ions is so selected to provide the coincidence of the Bragg peak with the tumor position in patient's body (tumor can be located at a depth of 10–20 cm from the surface). A significant piece of energy that is released in the tissue gives rise to the death of cancer cells and, as a consequence, to the tumor destruction.

Owing to the local action of the Bragg peak, the ionic therapy is much more efficient than the X-ray one. Moreover, in the case of hard-to-reach regions in the organism, e.g., in the brain, this method becomes irreplaceable [3–5]. Despite the significant practical interest, as well as the fact that a large number of specialized clinics for the ionic therapy of cancer diseases have already been built around the world, the molecular mechanisms of action by high-energy ions still remain far from a complete understanding.

Since the early studies [6], the action of ionizing radiation on living organisms is known to be associated, first of all, with the damage of DNA molecules. This

damage results in a violation of the mechanisms for storing and transferring the genetic information and in the cell death. The destruction of a DNA macromolecule is assumed to occur owing to the creation of a considerable number of secondary electrons and radicals [7–9]. At the same time, the corresponding calculations [9] showed that the number of damaged sections in the DNA molecule induced by secondary electrons and radicals is not sufficient for the cell death.

In recent years, the intense search for additional blocking mechanisms of the genetic activity of DNA by its irradiation with high-energy ions has been carried out. In particular, in works [10, 11], a mechanism of shock wave formation was proposed, which can explain the propagation of the ion beam action in the cellular medium. Simulations using the molecular dynamics method demonstrated that the corresponding shock wave can result in the formation of a certain number of double strand breaks in the double helix [12]. However, in view of the presence of the efficient mechanisms of DNA structure restoration in a cell, the proposed mechanism turned out not enough for understanding the processes that run in a vicinity of the Bragg peak.

At the same time, the DNA molecule can also be deactivated owing to a change in properties of the surrounding medium under the action of high-energy ions. Really, the irradiation of the cell bulk, 80% of which consists of water, gives rise to the formation of new molecular products. This fact has to be taken into account, when considering the possible mechanisms of ionic beam action.

In this work, by analyzing available experimental data and carrying out theoretical calculations, a necessity to study the processes of formation of a complex between molecular products of the water decay and the atomic groups in a DNA double helix is substantiated. The stability of complexes between hydrogen peroxide molecules and DNA phosphate groups, which are nonspecific centers of the macromolecule recognition by enzymes, is demonstrated. A new mechanism is proposed to describe the action of high-energy ionic beams on a cell.

2. Changes in the Medium Under Irradiation

The passage of ions through an aqueous medium invokes the processes of water molecule radiolysis, as well as various chemical and nuclear reactions [13–20]. The results of researches [3–5, 9] showed that the fragmentation of water molecules gives rise to considerable variations in the composition and properties of the medium. At the radiolysis of water molecules, there arise secondary electrons (e^-), radicals ($OH^\bullet$ and $HO_2^\bullet$), molecules (H_2O_2 and H_2O), and other products ($H^\bullet$, OH^-, H_3O^+) [16–18].

In a vicinity of the Bragg peak, one should expect the formation of a considerable number of molecular products, because the most amount of energy is released just there. The simulation results obtained with the use of the Monte Carlo method [19] for the radiolysis in water under its irradiation with 250-MeV carbon $^{12}C^{6+}$ ions showed that the contributions of secondary electrons and radicals are crucial at the initial stage of radiolysis (within a time interval of an order of 10^{-12} s). However, after a time period of about 10^{-6} s, owing to the reactions between reaction-active products (these are radicals and secondary electrons), the contribution of molecular products becomes dominating over all the others [19]. Hence, the molecular products of radiolysis, namely, H_2O_2 and H_2 molecules, become the most important for biological processes, which are much slower than 10^{-6} s.

In this work, using the data of Monte Carlo calculations [19, 20], the distributions for the products of water radiolysis near the Bragg peak are determined. The results of this analysis showed that, in a time period of 1 μs after the passage of a high-energy ion, the number of hydrogen peroxide molecules in the endocellular medium is the largest among all the water decay products. The content of H_2 molecules is also high, but they are not highly reactive and, as a results, are not potentially dangerous for biological macromolecules. Radicals, which are also present in a significant amount, are gradually transformed into neutral water or hydrogen peroxide molecules. Therefore, the composition of the cell medium after the passage of a high-energy ion changes. A considerable amount of new molecular components appears in the cell; in particular, hydrogen peroxide.

A hydrogen peroxide molecule can interact with the atomic groups in the double helix of a DNA molecule that are located at those chains of the DNA backbone, which are the most accessible for external contacts. It is known that the phosphate groups in the double helix backbone have a negative charge under natural conditions. As a rule, this charge is neutral-

ized by metal ions, e.g., Na^+ or K^+ [21]. Therefore, the most probable event is the binding of a hydrogen peroxide molecule with a phosphate group in the DNA backbone with the participation of metal ions. By their structure and atomic charges, hydrogen peroxide molecules are comparable with the phosphate groups in the DNA backbone and should interact with them first of all.

In this work, the interaction of a hydrogen peroxide molecule with a phosphate group in the backbone of a DNA double helix and sodium cations is studied, and the structures of the most probable complexes are determined. In the next section, the method of calculation of the interaction energy between a hydrogen peroxide molecule and atoms in the DNA double helix is described. The results of calculations of the energy for various complexes are presented in Section 4. In Section 5, the structures of the most probable complexes between the hydrogen peroxide molecule and the DNA, as well as possible mechanisms of hydrogen peroxide biological action, are discussed.

3. Models and Methods of Calculation

In this work, in order to determine the character of the interaction between the hydrogen peroxide molecule and the DNA, the energies of various complexes consisting of a DNA phosphate group, a hydrogen peroxide molecule, and a Na^+ ion are calculated. The molecules that form the aggregates are considered to be rigid structures. While calculating the energy of interaction between the hydrogen peroxide molecule and the phosphate group PO_4, only two oxygen atoms in the latter are taken into consideration. Making allowance for the internal degrees of freedom in the molecules belonging to the complex and taking the other atoms of a phosphate group into consideration would be important for obtaining the exact energy values, but are not essential for the determination of the relative stability of examined molecular complexes. The schematic structures and the structural parameters of molecules that compose the studied complexes are depicted in Fig. 1.

The energies of complexes are calculated using the method of atom-atom potential functions [22, 23]. It is based on the application of the well-known potentials, whose parameters are optimized for the description of the physical properties of molecular systems, including the DNA. If calculations are performed in

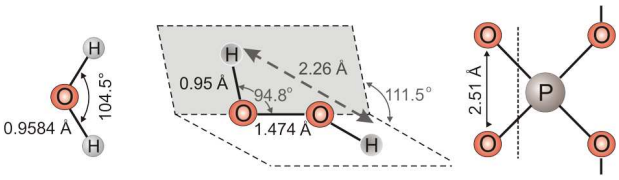

Fig. 1. Schematic structure diagrams for water and hydrogen peroxide molecules and the phosphate group of a DNA backbone

the framework of this method, they provide a sufficient level of accuracy for the comparative stability analysis of analyzed molecular structures.

In the framework of the method of atom-atom potential functions, the energy of any complex is the sum of pair interaction energies between the atoms belonging to different structural elements. In the general form, it can be presented as a sum of two contributions,

$$U = \sum_{ij} [U_{\rm el}(r_{ij}) + U_{\rm vdw}(r_{ij})], \tag{1}$$

where $U_{\rm el}(r_{ij})$ and $U_{\rm vdw}(r_{ij})$ are the potential energies of the electrostatic and van der Waals interactions, respectively, between atoms i and j that are located at the distance r_{ij} from each other.

The electrostatic interaction between the atoms was determined, by using the Coulomb law,

$$U_{\rm el}(r_{ij}) = \frac{q_i q_j}{4\pi\varepsilon\varepsilon_0 r_{ij}}, \tag{2}$$

where q_i and q_j are the charges of atoms i and j, respectively; ε_0 is the electric constant, and ε the dielectric permittivity of the medium. The atomic charges in water and hydrogen peroxide molecules are determined on the basis of the known values of dipole moments of corresponding molecules: 2.1 D for H_2O_2 and 1.86 D for H_2O [24]. The total charge of oxygen atoms in the phosphate group was taken to equal $-e$, which corresponds to the natural state of the PO_4^- group in the DNA (Table 1). In order to compare the relative energies of various complexes, the dielectric permittivity $\varepsilon = 1$ was used in calculations.

The van der Waals interaction energy was determined, by using the Lennard-Jones potential,

$$U_{\rm vdW}(r_{ij}) = -A_{ij} r_{ij}^{-6} + B_{ij} r_{ij}^{-12}, \tag{3}$$

where A_{ij} and B_{ij} are constants describing the attraction and repulsion, respectively, between the atoms

Calculation parameters

Atomic charges			Parameters of potential (4) [25, 26]		Parameters of potential (3) [22, 23]		
	H	O		Na^+			O–H
H_2O	+0.33 (e)	–0.66 (e)	q (e)	+1.00	A_{ij} (Å^6 kcal/mol)	200	86
H_2O_2	+0.41 (e)	–0.41 (e)	r_0 (Å)	2.35	B_{ij} (Å^{12} kcal/mol)	12 900	31 300
PO_4^-	–	–0.50 (e)	b (Å)	0.3			

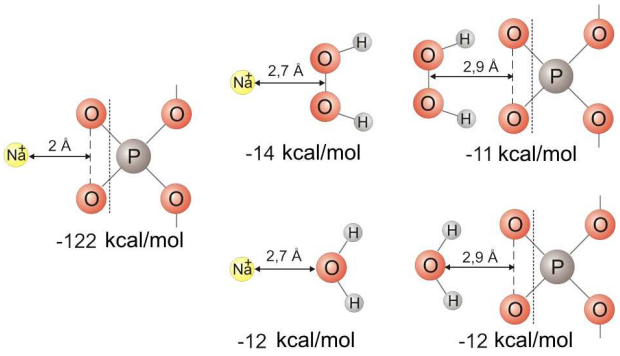

Fig. 2. Most stable two-component complexes H_2O_2–PO_4, H_2O–PO_4, Na^+–PO_4, H_2O_2–Na^+, and H_2O–Na^+

(Table). The first term in Eq. (3) describes the van der Waals attraction between the atoms, and the second one the repulsion between them at short distances.

For the atoms that form hydrogen bonds, the attraction energy was calculated using a modified potential [23], in which the exponent in the first term of formula (3) was substituted by -10. This substitution describes a short-range character of hydrogen bonds.

The interaction of an ion with charged oxygen atoms is described, by using the Born–Mayer potential, rather than potentials (2) and (3). It involves contributions of the electrostatic attraction and repulsion at short distances,

$$U_{\rm bm}(r_{ij}) = \frac{q_i q_j}{4\pi\varepsilon\varepsilon_0 r_{ij}} \left[1 - \frac{b r_{ij}}{r_0^2} \exp\left(\frac{r_0 - r_{ij}}{b}\right)\right], \qquad (4)$$

where the parameter b characterizes the repulsion between ions at short distances, and r_0 is the equilibrium distance (Table). This potential is used to describe the interaction of counterions with phosphate groups in the double helix backbone [25, 26].

Using formulas (1)–(4) with the parameter values quoted in the table, the energies of complexes consisting of a DNA phosphate group, a hydrogen peroxide molecule, a water molecule, and a sodium ion were calculated.

4. Calculation Results

In the framework of the proposed approach, the energies of two- (H_2O_2–PO_4, H_2O–PO_4, Na^+–PO_4, H_2O_2–Na^+, and H_2O–Na^+) and three-component (H_2O_2–Na^+–PO_4 and H_2O–Na^+–PO_4) complexes were calculated. For each complex, the most stable configuration was determined.

4.1. Two-component complexes

The most stable two-component complex is Na^+–PO_4^- in the configuration, where the distance between the ion and the O–O segment midpoint amounts to 2 Å (Fig. 2). This complex corresponds to the real neutralization of DNA phosphate groups by counterions. The complexes of a sodium ion with a hydrogen peroxide or a water molecule have an energy that is approximately an order of magnitude lower than that with the phosphate group. In the case of Na^+–H_2O_2 complex, the sodium ion is located in the plane arranged symmetrically with respect to the H atoms and on the straight line that perpendicularly intersects the center of the O–O segment. The energy of the complex has a minimum, when the distance from the sodium ion to the O–O segment midpoint equals 2.6 Å. In the case of Na^+–H_2O complex, the ion and the water molecule lie in the same plane, and the ion is located on the bisectrix of the H–O–H angle (2.74 Å). Under natural conditions, the water molecules in the complex with the Na^+ ion compose a part of the hydration shell of the latter.

In H_2O_2–PO_4 complex, the hydrogen peroxide molecule is so arranged that the O–O segments in this molecule and in the phosphate group are parallel to each other, and the hydrogen atoms are located

symmetrically with respect to the plane of complex symmetry (analogously to their arrangement in Na^+–H_2O_2 complex) (Fig. 2). This structure has an energy minimum at a distance of 2.85 Å between the middle points of O–O segments in the molecules. In the case of H_2O–PO_4 complex, the most stable configuration is obtained, when the water molecule and the phosphate group lie in the same plane, and the oxygen atom in the water molecule is located on a straight line that intersects the O–O segment midpoint (at a distance of 2.9 Å), is perpendicular to it, and is a bisectrix of the H–O–H angle (Fig. 2).

In the cases of H_2O_2–PO_4 and H_2O–PO_4 complexes, the equilibrium configurations depend not only on the distance between the molecules, but also on their relative orientation. If the H_2O_2 molecule is rotated around the axis that passes through the centers of O–O segments, the strongest bond is reached at a rotation angle of about 60° (an energy of −12.8 kcal/mol), and the weakest one at an angle of about 144° (an energy of −9.6 kcal/mol) (Fig. 3, *a*). If the molecule is rotated around the axis that passes through the O–O bond, the energy is maximum at an angle of 0°, whereas, at an angle of 180°, the interaction in the complex is absent (Fig. 3, *b*). In vicinities of angles of 70° and 290°, there appear shallow minima owing to the van der Waals repulsion and the Coulomb attraction (dashed curves in Fig. 3, *b*). In the case where the H_2O molecule is rotated, the obtained results are qualitatively similar.

The account for hydrogen bonds, which can arise between the H atoms in water or hydrogen peroxide molecules and the O atoms in the phosphate group, shows that they weakly affect the total energy values. In the case of the strongest hydrogen bond, i.e. when the O–H–O atoms are aligned, the calculated binding energies turned out lower in comparison with the energies of the most stable complexes. In the configuration where the H_2O_2 or H_2O molecule has simultaneously two hydrogen bonds with two corresponding oxygen atoms in the PO_4 group, the O–H–O angle exceeds 30°, which makes such hydrogen bonds very weak.

4.2. Three-component complexes

For H_2O_2–Na^+–PO_4 and H_2O–Na^+–PO_4 complexes, the dependences of the energy on the position of a hydrogen peroxide (or water) molecule were calcu-

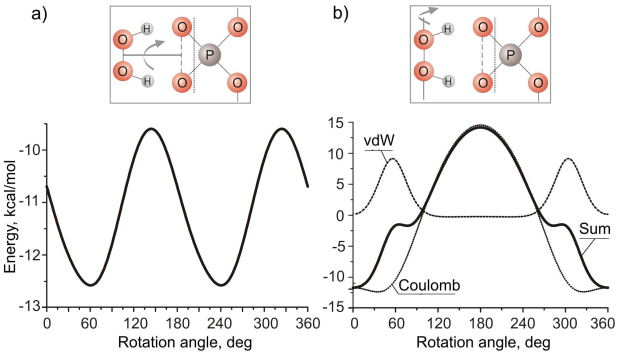

Fig. 3. Dependences of the energy of H_2O_2–P O_4 complex on the rotation angle for various rotation axes: around the horizontal axis (*a*) and around the O–O bond (*b*). The axes and directions of molecule rotation are shown in the insets

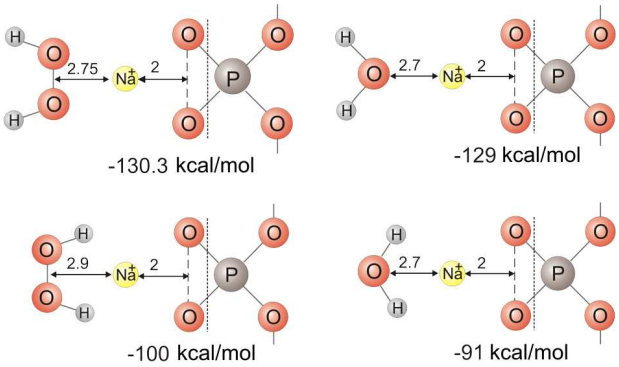

Fig. 4. Structures of the most stable three-component complexes H_2O_2–Na^+–PO_4 and H_2O–Na^+–PO_4

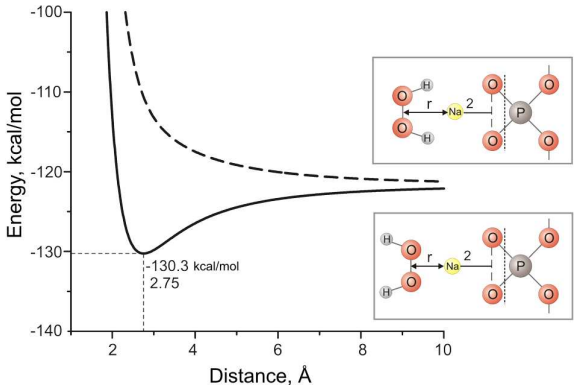

Fig. 5. Dependences of the binding energy in the H_2O_2–Na^+–PO_4 complex on the distance between the H_2O_2 molecule and the ion-phosphate complex Na^+–PO_4. The insets illustrate the initial complexes H_2O_2–Na^+–PO_4 with different orientations of hydrogen atoms in the H_2O_2 molecule

lated. The distance between the ion and the phosphate group was taken to be fixed and equal to 2 Å in accordance with the calculation results obtained for the structure of two-component Na^+–PO_4 complex (Fig. 2). This position of a sodium ion is the most beneficial energetically. Therefore, other variants of ion arrangement were not considered in this work. The structure and the energies of equilibrium configurations for three-component complexes are exhibited in Fig. 4.

Let us consider the cases where the hydrogen atoms in the H_2O_2 or H_2O molecule are turned toward or away from the phosphate group. The calculation results showed that, in the former case, the system has no energy minimum, which is associated with the electrostatic repulsion between the hydrogen atoms and the sodium ion. As the molecule moves away, the energy monotonically decreases to a value corresponding to the two-component Na^+–PO_4 complex (Fig. 5). On the contrary, when the hydrogen atoms are turned to the opposite side, there emerges a minimum in the energy of the system owing to the electrostatic attraction between the ion and the oxygen atoms in the molecule. The rotation of the hydrogen peroxide or water molecule around the axis that passes through the center of the O–O segment gives a very insignificant contribution to the binding energy variation, because this energy is mainly affected by the interaction of the ion with the oxygen atoms, so that it does not change at this rotation.

5. Discussion and Conclusions

The results of calculations of the energy of two- and three-component complexes demonstrate that the most stable structures emerge if a sodium ion is present (Fig. 4). The counterion attaches itself to the phosphate group in the double helix backbone and neutralizes the negative charge of oxygen atoms, which increases the probability for the hydrogen peroxide and water molecules to interact with the DNA. In this case, the stabilities of three-component complexes containing a hydrogen peroxide or a water molecule are almost identical.

Our calculations have shown that the energy of three-component complexes is the sum of the energies of corresponding two-component complexes to an accuracy of 5%. Using the additive approach, we have estimated the energy of a hydrated ion. The estimations testify that the stability of systems with the hydrogen peroxide molecule is not lower than that of systems with the water one. At the same time, since the mass of H_2O_2 molecule is larger, the lifetime of the complex with it will be longer than that with the water molecule. Therefore, we may assume that the hydrogen peroxide molecule will stay on the DNA molecule for a considerable time period. By interacting with active groups in the double helix, H_2O_2 molecules can block the processes of DNA double helix recognition by enzymes and, accordingly, terminate the biological functioning of DNA.

Moreover, during the long action of high-energy ions in the course of ionic therapy, hydrogen peroxide molecules that are located near the phosphate groups in the DNA backbone can decay into the $OH^\bullet$ and $HO_2^\bullet$ radicals. In this case, those radicals are not formed in the cell bulk, but immediately at the chains of the sugar-phosphate backbone. Therefore, the probability of damages in the DNA strands and the formation of double strand breaks considerably increases. The both mechanisms, which were proposed to describe the action of a hydrogen peroxide molecule on the DNA, are rather plausible under natural conditions.

Hence, we have demonstrated that the stable complexes of hydrogen peroxide molecules with the DNA can be formed, which can deactivate the functioning of the genetic mechanism of a cell.

The work was sponsored by the Program of Fundamental Research of the Division of Physics and Astronomy of the National Academy of Sciences of Ukraine (Project No. 0112U000056).

1. L. Gravitz, Nature **491**, S49 (2012).
2. A. Brown and S. Herman, Radiol. Oncol. **73**, 265 (2004).
3. G. Kraft, Prog. Part. Nucl. Phys. **45**, S473 (2000).
4. H. Suit *et al.*, Radiother. Oncol. **95**, 3 (2010).
5. C.D. Schlaff, A. Krauze, A. Belard, J.J. O'Connell, and K.A. Camphausen, Radiat. Oncol. **9**, 88 (2014).
6. N.V. Timofeev-Resovskii, A.V. Savich, and M.I Shalnov, *Introduction to Molecular Radiobiology* (Meditsina, Moscow, 1981) (in Russian).
7. B. Boudaoiffa, P. Cloutier, D. Hunting, M.A. Huels, and L. Sanche, Science **287**, 1658 (2000).
8. N. Hamada, J. Radiat. Res., **50**, 1 (2009).
9. A.V. Solov'yov, E. Surdutovich, E. Scifoni, I. Mishustin, and W. Greiner, Phys. Rev. E **79**, 011909 (2009).
10. A.V. Yakubovich, E. Surdutovich, and A.V. Solov'yov, Nucl. Instrum. Methods B **279**, 135 (2012).

11. E. Surdutovich, A.V. Yakubovich, and A.V. Solov'yov, Sci. Rep. **3**, 1289 (2013).
12. E. Surdutovich and A.V. Solov'yov, J. Phys.: Conf. Ser. **438**, 012014 (2013).
13. I. Pshenichnov, A. Botvina, I. Mishustin, and W. Greiner, Nucl. Instrum. Methods B **268**, 604 (2010).
14. E. Haettner, H. Iwase, and D. Schardt, Radiat. Prot. Dosim. **122**, 485 (2006).
15. J. Soltani-Nabipour, M A. Popovici, and Gh. Cata-Danil, Romanian Rep. Phys. **62**, 37 (2010).
16. B. Pastina and J.A. LaVerne, J. Phys. Chem. A **103**, 1592 (1999).
17. S. Le Caer, Water **3**, 235 (2011).
18. V. Wasselin-Trupin, G. Baldacchino, S. Bouffard, and B. Hickel, Radiat. Phys. Chem. **65**, 53 (2002).
19. M.S. Kreipl, W. Friedland, and H.G. Paretzke, Radiat Environ Biophys, **48**, 11 (2009).
20. S. Uehara and H. Nikjoo, J. Radiat. Res. **47**, 69 (2006).
21. W. Saenger, *Principles of Nucleic Acid Structure* (Springer, New York, 1984).
22. V.B. Zhurkin, V.I. Poltev, and V.L. Florentiev, Molek. Biol. **14**, 1116 (1980).
23. V.I. Poltev, and N.V. Shulyupina, Molek. Biol. **18**, 1549 (1984).
24. *Brief Chemical Encyclopaedia, Vol. 1* (Sovetskaya Entsiklopediya, Moscow, 1961) (in Russian).
25. S.M. Perepelytsya and S.N. Volkov, Ukr. J. Phys. **49**, 1074 (2004).
26. S.M. Perepelytsya and S.N. Volkov, Eur. Phys. J. E **24**, 261 (2007).

Received 11.03.15.
Translated from Ukrainian by O.I. Voitenko

Д.В. П'ятницький, О.О. Здоревський,
С.М. Перепелиця, С.Н. Волков

УТВОРЕННЯ КОМПЛЕКСІВ
МОЛЕКУЛ ПЕРОКСИДУ ВОДНЮ З ДНК

Р е з ю м е

Досліджено можливість утворення стабільних комплексів фосфатних груп ДНК з молекулами пероксиду водню під час опромінення високоенергетичними іонами середовища біологічних клітин. Енергія комплексів визначалася з урахуванням електростатичних і ван-дер-ваальсівських взаємодій за методом атом-атомних потенціальних функцій. Враховувалася взаємодія з протиіонами металу, які в природних умовах нейтралізують заряд фосфатних груп ДНК. Визначено стабільні конфігурації різних комплексів, які складаються з атомів фосфатної групи остова ДНК, молекул H_2O_2 і H_2O, та іона металу Na^+. Показано, що комплекси молекул пероксиду водню з фосфатними групами ДНК і протиіоном є не менш стабільними, ніж відповідні комплекси з молекулами води. Приєднання молекули H_2O_2 до фосфатної групи остова подвійної спіралі може блокувати процеси біологічного функціонування ДНК і призводити до дезактивації генетичного апарату клітини.