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Comparison of the Effect of High-Energy Ionizing Radiation and Hydrogen Peroxide on the Conformational Dynamics of DNA

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Abstract

Nowadays reactive oxygen species and induction of oxidative stress in cells are widely exploited for treatment of numerous types of cancer. Various endogenous and exogenous factors may generate the reactive oxygen species, such as anticancer drugs and high-energy ionizing radiation. In current project, using FTIR spectroscopy as a sensitive method for conformational DNA analysis it was proposed to use hydrogen peroxide for modeling oxidative stress and its effect on cellular components, particularly, on DNA as a main target. Moreover, the effects of different doses of high-energy ionizing radiation and different concentrations of hydrogen peroxide on DNA solutions were compared using FTIR spectroscopy. Their effects have similar characteristics, since both of them lead to generation of free radicals $OH^{\cdot}$ that are the most cytotoxic among reactive oxygen species. It was demonstrated that main changes caused by ionizing radiation include breaks of DNA chains and formation of low-weight fragments, in comparison, hydrogen peroxide treatment mainly induces oxidation of nitrogenous bases and their modifications, however, DNA remains its polymeric structure.

Keywords: high-energy ionizing radiation, hydrogen peroxide, DNA, FTIR spectroscopy, single and double stand breaks of DNA.

1. Introduction

During recent years, immense efforts were made to understand and explain the biochemical and molecular basis of sensitivity to ionizing radiation, mechanism of carcinogenesis, cancer susceptibility, and patient responses to radiotherapy [1-2]. As a result, clinical anticancer treatment has been gradually improved by introducing newly invented advancement [2]. However, despite dynamic development of anticancer drugs, ionizing radiation remains an effective and commonly employed treatment in the management of many human malignancies [3].

Ionizing radiation capacity to control tumor growth basically relies on DNA damage. Further, DNA damage triggers a number of signaling cascades that control cell cycle arrest, DNA repair, and the cell's fate. The other compound that could cause DNA damage is hydrogen peroxide [1]. It is reactive oxygen species (ROS) that arises during the aerobic respiration process on the mitochondrial membranes or as a by-product of water radiolysis after exposure to ionizing radiation [4]. Hydrogen peroxide can easily cross cellular membrane and react with different cell components [4]. In present project, it was proposed to use hydrogen peroxide for modeling oxidative stress and its effect on cellular components, particularly, on DNA as a main target. At the same time, the effect of different doses of high-energy ionizing radiation on DNA was evaluated [5] and compared with DNA damage caused by hydrogen peroxide using Fourier transform infrared (FTIR) spectroscopy.

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FTIR spectroscopy is a vibrational spectroscopic technique that is extremely sensitive to any changes in the molecular structure, and for our study this method gives us an opportunity to register and evaluate DNA damage. This method has a huge potential in medicine. It can be used for rapid and non-invasive detection of malignant transformation.

Current project was aimed at comparing the effect of high-energy ionizing radiation and hydrogen peroxide on isolated DNA using FTIR spectroscopy.

2. Experimental details

Model experiments were carried out using high molecular weight DNA of calf thymus ("Serva"). Irradiation of DNA solutions with different doses was performed using linear electron accelerator "Agrus". Two types of DNA solutions were used: aqueous (1mg DNA + 1 ml distilled water) and buffer solution (1mg DNA + 1 ml 0.15M NaCl solution). The doses of 10, 40 and 100 kGy were applied.

To assess the effect of hydrogen peroxide, 1 mg/ml DNA samples and 0.075%, 0.75%, 1.5% hydrogen peroxide solutions were prepared.

The damage of DNA molecule caused by high-energy ionizing radiation or hydrogen peroxide was evaluated by FTIR spectroscopy. The DNA solution was deposited on CaF2 substrates and then dried on air. FTIR spectra were registered on the IFS-48 Bruker instrument in the 800-4000 cm^{-1} region. The quantitative analysis of obtained spectra was performed using Opus 6.0 software program. The assignment of vibration modes was made according to the previously published data [6-9].

3. Results and Discussion

After the action of high-energy ionizing radiation and hydrogen peroxide, FTIR spectra of DNA samples showed various differences in comparison with the reference samples. The most significant ones have been observed at the complex bands at 3800-2400 cm^{-1} and 1800-1000 cm^{-1}.

The complex band at 3800–2400 cm^{-1} corresponds to stretching vibrations of OH, NH and CH molecular groups. After the action of radiation, changes in spectral characteristics were observed including an increase in the intensity and the half-width of this complex band (Fig. 1a). These changes are explained by fragmentation of DNA and formation of fragments with various molecular masses. Frequency shifts weren't registered. With increasing the dose of ionizing radiation, we observed an increase in the contribution of the shoulder to 3200 cm^{-1} corresponding to stretching vibrations of NH molecular groups (Fig. 1a), that may be linked with partial reorganization of hydrogen bonds.

The action of hydrogen peroxide on DNA molecules resulted in other type of spectroscopic particularities at 3800–2400 cm^{-1} region (Fig. 1b). The half-width of the band remained the same, while the position of its maximum was shifted by up to 40 cm^{-1} in the lower-frequency region. The observed changes indicate the rearrangement of hydrogen bonds between nitrogenous bases without fragmentation of the chains.

The 1800-900 cm^{-1} region corresponds to vibrations of double bonds C=O, C=N of thymine, guanine and cytosine. The shoulders located at about 1700 cm^{-1} correspond to hydrogen bonds, which form the double helix. Ionizing radiation effect led to broadening of

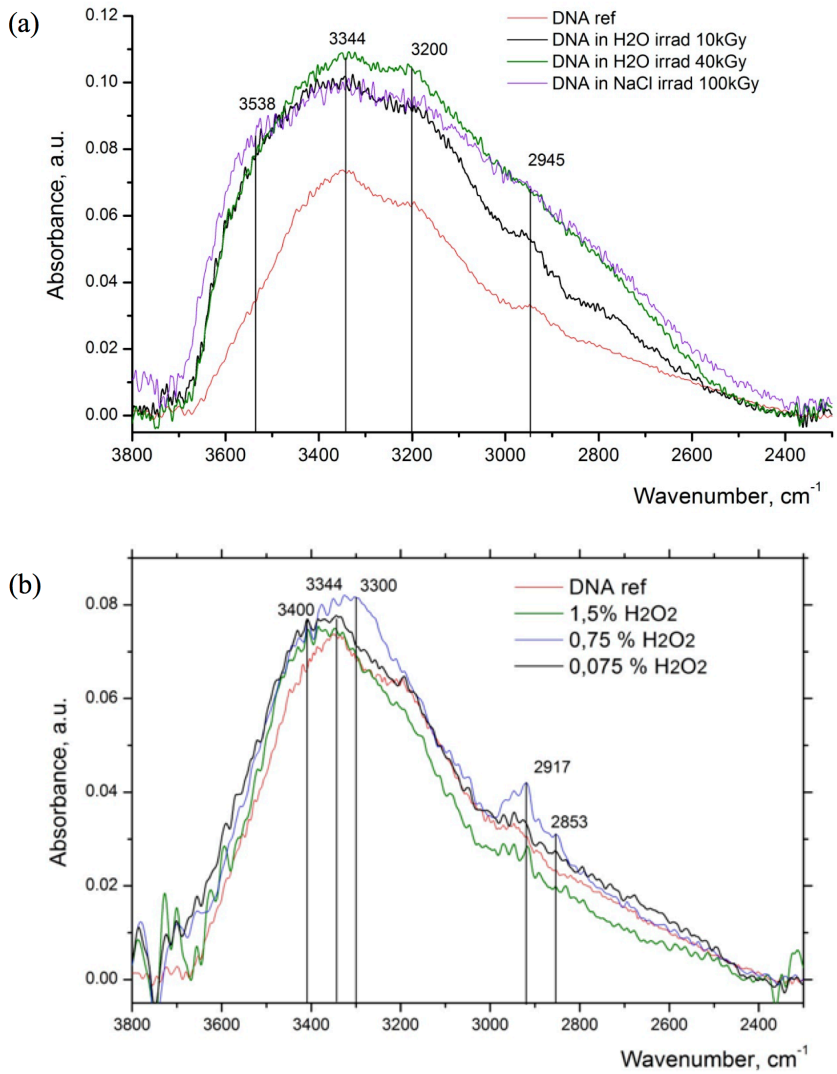

Fig. 1. FTIR spectra of DNA samples after the action of different doses of high-energy ionizing radiation (a) and different concentrations of hydrogen peroxide (b) in the 3800-2400 cm-1 wavenumber region.

the bands corresponding to C=O, C=N of nitrogenous bases (1800-1550 cm^{-1}), increasing the number of absorbance maximums with high intensity. All these changes sustain the fact that DNA fragmentation occurs. Moreover, after the action of irradiation on DNA the shoulder ~~of~~ at 1776 cm^{-1} was registered, that shows ~~the~~ possible oxidation of nitrogenous bases. After the action of hydrogen peroxide, the contribution of the shoulder at 1700 cm^{-1}, corresponding to C=O stretching band of nitrogenous bases was observed along with the increase of the higher frequency shoulder arms at 1602 cm^{-1} and 1575 cm^{-1}, which are related to the absorption of C=N in guanine (Fig. 2b).

In the region of 1300-1000 cm^{-1} (PO_2^- asymmetrical and symmetrical stretching mode) the decrease of the intensity of absorption band was observed after the action of irradiation. Low-

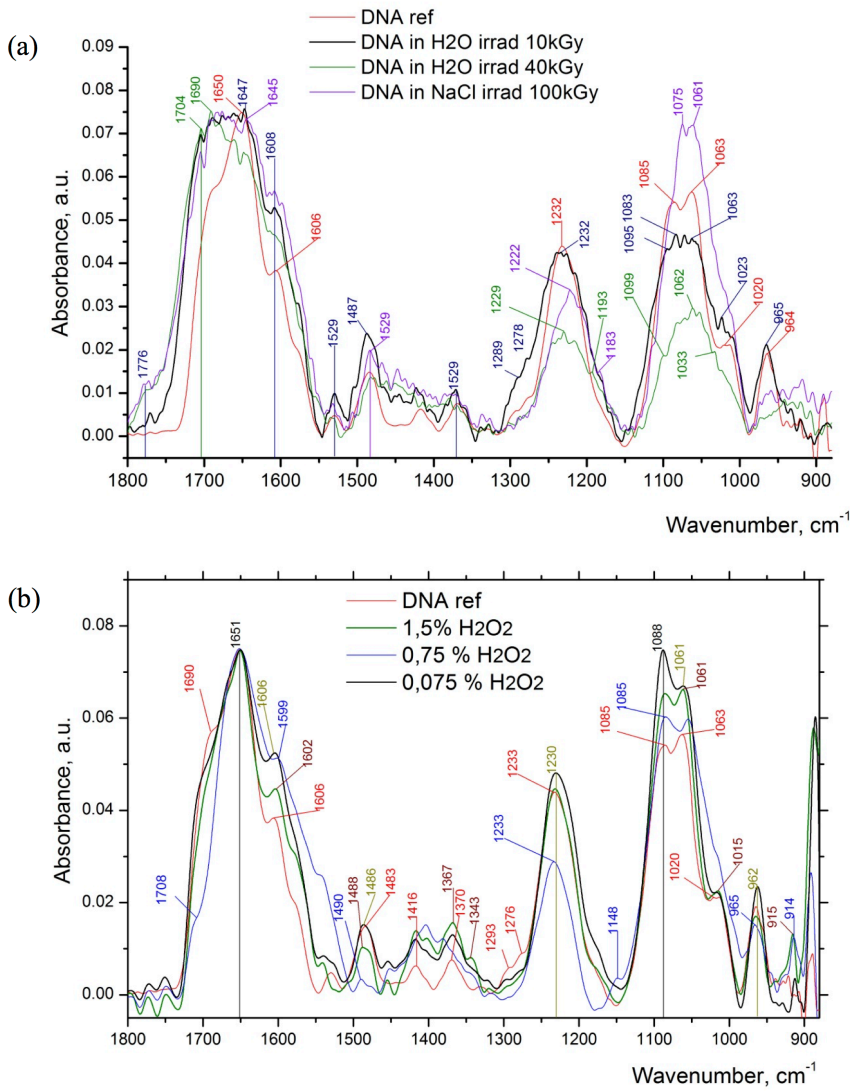

Fig. 2. FTIR spectra of DNA samples after the action of different doses of high-energy ionizing radiation (a) and different concentrations of hydrogen peroxide (b) in the 1800-1000 cm-1 wavenumber region.

frequency shift of the band corresponding to PO_2^- asymmetrical stretching mode from 1232 cm^{-1} (A-form) in reference sample compared to 1222 cm^{-1} (B-form) in the sample irradiated by 10 kGy may be a sign of partial conversion of A-form to B-form of DNA as well as increasing the contribution of the shoulder of 1289 cm^{-1} corresponding to N-H vibration of thymine (Fig. 2a). After hydrogen peroxide treatment, the decrease in the intensity was observed in the region of PO_2^- asymmetrical and symmetrical stretching mode (Fig. 2b).

The comparison of the effect of ionizing radiation on DNA aqueous and buffer solutions revealed the important results. It was clearly observed that the damage caused by the dose of 10 kGy on aqueous DNA solution is stronger than the effect of 100 kGy on the DNA buffer

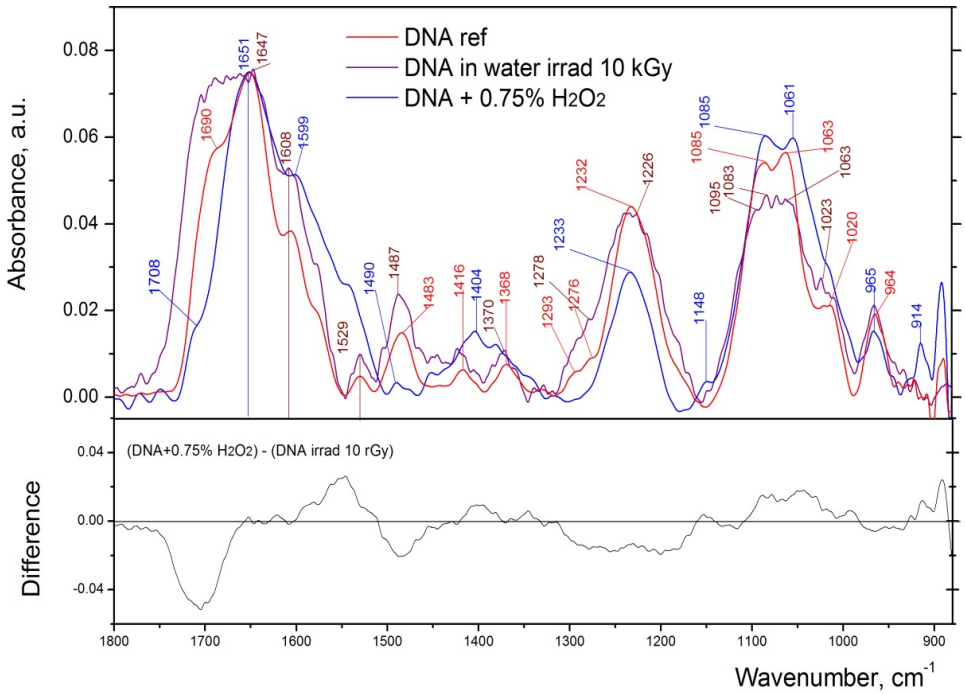

Fig. 3. FTIR spectra of DNA solutions after the action of (a) high-energy ionizing radiation or (b) hydrogen peroxide in the 3800-1000 cm^{-1} wavenumber region.

solution (Fig. 1a). Spectral features in the 3800-2400 cm^{-1} region indicated stronger restructuring of hydrogen bonds in the DNA aqueous solution. Thus, the maximum of the absorption band of OH-NH-CH molecular groups stretching vibrations for the DNA buffer solution remained the same as the control at 3340 cm^{-1}, which correspond to OH groups, but for the DNA aqueous solution the shift to 3220 cm^{-1} corresponding to NH groups was registered (Fig. 1a).

To summarize, we can maintain that ionizing radiation and hydrogen peroxide have different effect on DNA structure. Ionizing radiation leads to breaks of DNA chains and formation of low-weight fragments in the same time, while hydrogen peroxide treatment mainly causes oxidation of nitrogenous bases and their modifications, however, DNA remains its polymeric structure (Fig. 3), (Table 1).

We suppose that Na+ ions suppress a negative effect of indirect action of ionizing radiation interacting with negatively charged phosphate DNA backbone and protect it. Thus, the damage on aqueous DNA solution caused by 10 kGy was stronger that of DNA in buffer solution under 100 kGy. The charge density is so great that the effect called ion condensation occurs. Essentially the electrostatic potential interactions of the DNA are so great that it overcomes the entropic cost of localizing the ions on the DNA. This entropic cost is minimized by maintaining cations in the cloud of the highly mobile ions instead of in tightly localized salt-like. Any ions from the buffer solution should act the same way. Meanwhile H+ has no similar property and form unstable DNA structure.

Table 1. Frequencies of vibration bands [cm^{-1}] of DNA samples

Reference	10 kGy	0,75% H_2O_2	Assignment
3344	3344	3400	O-H stretching mode
3200	3189	3299	N-H stretching mode
2945	2962	2917	CH_3 stretching mode
		2853	CH_3 stretching mode
1690		1708	C=O
	1647	1651	C=N
	1608	1599	C=N
	1529		Cytosine
1483	1487	1490	Guanine, Cytosine
1416		1404	C-H bending mode
1368	1370		C-N Adenine, Guanine
1293			N-H Cytosine, Thymine
1276	1278		N-H bending mode of Thymine
1232	1226	1233	Stretching PO_2^- asymmetrical
		1148	C-O
	1095		Stretching PO_2^- symmetrical
1085	1083	1085	C-O
1063	1063	1061	C-O deoxyribose
1020	1023		C-O deoxyribose
964		965	C-O deoxyribose
		914	C-O deoxyribose

4. Conclusions

FTIR spectroscopy provides an opportunity to assess DNA damage. Ionizing radiation and hydrogen peroxide have diverse effects on DNA structure. The action of ionizing radiation on DNA resulted in the destruction of the secondary structure in the case of smaller doses, and damage to the primary structure, unpairing and formation of low-weight fragments, even separated bases, in the case of the dose of 100 kGy.

Increasing concentrations of hydrogen peroxide also leads to damaging of DNA structure, characterized by breaks, unpairing and formation of single chain DNA, oxidation of nitrogenous bases and their modifications, however, DNA keeps its polymeric form.

The effect of similar doses of ionizing radiation causes significantly less DNA damage when buffer solution was used as a solvent. Thus, NaCl buffer solution can be used as a protector from ionizing radiation for dissolved biological molecules. If DNA is presented in any solution, it becomes a main target for ionized radiation and other dissolved molecules suffer less damage.

Acknowledgements

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References

[1] A.A. Adjei and J.K. Buolamwini, Novel Anticancer Agents, Strategies for Discovery and Clinical Testing. Elsevier Academic Press, New York, 2006.

[2] Al. Baba and C. Câtoi, Comparative Oncology, The Publishing House of the Romanian Academy, Bucharest, 2007.

[3] J.L. Lopez Guerra, N. Isa, M.M. Kim, C. Bourgier and H. Marsiglia, New perspectives in radiation oncology: Young radiation oncologist point of view and challenges, *Reports of Practical Oncology and Radiotherapy* 17 (2012) 251–254.

[4] N.R. Asad, L.M.B.O. Asad, C.E.B. de Almeida, I. Felzenszwalb, J.B. Cabral-Neto, A.C. Leitão, C.E.B. Almeida, J. Cabral-Neto and A.V. Leitao, Several pathways of hydrogen peroxide action that damage the E. coli genome, *Genetics and Molecular Biology* 27 (2004) 291-303.

[5] Гродзинський Д.М., Радіобіологія, Либідь, Київ, 2000.

[6] T. Shimanouchi, M. Tsuboi and Y. Kyogoku, Infrared spectra of nucleic acids and related compounds, *Advances in chemical physics: Structure & Properties of Biomolecules*, 7 (2007) 435-498.

[7] B. Schrader, Infrared and Raman Spectroscopy. Methods and Applications, VCH, Weinheim, 1995.

[8] F.S. Parker, Applications of infrared spectroscopy in biochemistry, biology and medicines, Plenum press, New York, 1971.

[9] Z. Movasaghi, S. Rehman and I. Rehman, Fourier Transform Infrared (FTIR) Spectroscopy of Biological Tissues, *Applied Spectroscopy Reviews* 43 (2008) 134-179.