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Application of spectral methods for studying DNA damage induced by gamma-radiation

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ABSTRACT

Spectral methods can provide a variety of possibilities to determine several types of radiation-induced DNA damage, such as nucleobase destruction and local denaturation. DNA UV absorption and CD spectra measured at room temperature undergo noticeable alteration under the action of γ-radiation. We have applied the Spirin method of total nucleobases determination, and have measured the molar extinction coefficient of DNA and DNA CD spectra for solutions with different NaCl concentrations (3 mM–3.2 M) and containing $MgCl_2$, exposed to γ-radiation with the doses of 0–10^3 Gy. The melting temperatures of DNA in irradiated solutions at the doses of 0–50 Gy were obtained with the help of spectrophotometric melting. It was found that the amount of destructed nucleobases and radiation-induced loss of DNA helicity significantly decreases with the rise of the ionic strength of the irradiated solution. Substitution of a portion of Na^+ ions on Mg^{2+} while keeping the total ionic strength constant ($\mu = 5$ mM) does not affect the considered radiation effects. The role of the structure and composition of the DNA secondary hydration layer in the radiation-induced damages is discussed.

1. Introduction

DNA damage is the main origin of cell death, mutation and cancer transformation. Therefore DNA is considered to be the main target of biological action of ionizing radiation [1–4]. The most frequent types of radiation-induced DNA damages are single- and double strand breaks, modification and destruction of nucleobases and also local breakages of hydrogen bonds (partial denaturation) in the lesion sites of the macromolecule [1–4]. The common methods to investigate the modified and destroyed nucleobases in DNA are immunochemical techniques, ^{32}P-postlabeling assay, comet assay, high-performance liquid chromatography (HPLC) with electrochemical detection (ECD) [5–7]. The use of tandem mass spectrometry (MS/MS) connected to HPLC is considered to be most versatile and sensitive of the modern techniques [6–9]. HPLC-MS/MS methods are highly developed, demand a little amount of DNA sample (about 30 μg) and allow to detect modified nucleosides whose frequency is around a few lesions per 10^7 nucleosides [7]. Application of these techniques supports to reveal plenty of nucleobase modifications appearing in a cell not only due to irradiation, but to other normal or pathologic oxidative processes as well as a result of DNA methylation and hydroxylmethylation [7–10]. At the same time these methods are labour intensive and require perfect biotechnology for the accurate DNA digestion [7–10]. Traditional methods of spectral analysis of DNA structure are simpler and require no more material then HPLC-MS/MS, but now they are not widely used for study DNA damage received in vivo evidently because of complexity for data interpretation. However different new applications of UV absorption spectroscopy, CD, spectrophotometric melting, Raman and fluorescent spectroscopy are appeared to be useful in detection various DNA structural changes induced by radiation, reactive oxygen species or biologically active agents [11–15]. Among the advantages of such methods are abilities to visualizing the radiation-induced damage in a single DNA molecule [16], to observe radiation-induced biochemical changes in single cells [17], to obtain synchronous information about DNA secondary structure and its binding with proteins and other molecules [18]. Spectroscopy techniques are proper and prompt for in vitro model experiments, for example in revealing a pro- or antioxidant properties of low-molecular additives [19–21].

In particular a total amount of undestroyed nucleobases in irradiated DNA can be obtained spectrophotometrically in a relatively simple manner. This approach does not require DNA digestion to monomers by enzymes. UV absorption spectroscopy is commonly used for determination of nucleic acids concentration from different sources, i.e. the concentration of nucleic acids chromophores – nucleobases [22–32]. Several of these methods include heat, mild acid or DMSO treatment of nucleic acids solution [25–31] to denaturate double stranded sites for accurate removal the hypochromicity. It should be

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noted that these methods were validated by other precise techniques of nucleic acids concentration determination such as HPLC [25,29] and quantitative NMR [31,32].

Nucleic acids denaturation before spectrophotometric concentration measurement is of special importance when the tested sample contains unknown ratio of single- and double-stranded nucleic acids, as it takes place in the case of irradiated DNA. Earlier in irradiated DNA solutions the so-called "loss of hyperchromicity" has been reported [33]: the authors observed that the absorption intensity maximum of DNA (at λ = 260 nm) measured at 80 °C decreases under the action of γ-radiation. Thus to detect the loss of the chromophores one should first destroy the secondary structure of DNA. The point is that radiation-induced changes in DNA structure influence its UV absorption spectrum in different ways: a partial denaturation causes hyperchromic effect, while the destruction of the nucleobases results in hypochromism. To disjoint these two effects we propose to apply the Spirin method of total nucleobases determination [27]. This method suggests the treatment of DNA sample with 6% perchloric acid and heating at 100 °C for 20 min in order to complete denaturation to avoid the secondary structure influence on the DNA absorption spectrum [30].

Circular dichroism (CD) spectrometry is widely used for studying of DNA secondary structure under variety of conditions. It provides essential information in the investigation of nucleic acids of different nucleobase compositions and different conformations [34–38], of DNA interaction with ions, proteins and other biologically active compounds [38–44], and also in the recognition of radiation-induced DNA lesions [11,45,46]. This method can give an additional information about the DNA conformation (without DNA denaturation) because the destruction of nucleobases and a partial denaturation result in the same changes in DNA CD spectra: the decrease in intensity [42]. Also we engage the spectrophotometric melting to evaluate a partial denaturation of the irradiated DNA. The DNA melting (heat induced helix-coil transition) is very sensitive to the state of the macromolecule secondary structure [47] and is frequently applied in the investigation of DNA complexation with ions and other biologically active agents [48,49].

The ionic surrounding of DNA plays a key role in its structure and radiosensitivity. Most of alkaline metal ions (Na^+, Li^+, K^+) and alkaline earth metal ions (Mg^{2+}, Ca^{2+}, Ba^{2+}) interact mainly with negatively charged oxygen atoms of phosphate groups in DNA and stabilize its secondary structure [50,51]. In a cell and in a solution DNA exists as a polyanion in a complex with ions immersed into a hydration shell of the macromolecule and forming a diffuse cloud around it [50–53]. The DNA-cations interaction determines its rigidity and volume in a solution, i.e. its tertiary structure [51,53]. Nevertheless there are only a few works dealing with the influence of ionic strength of solution on the DNA radiosensitivity [45,54,55]. Data obtained from hydrodynamical and optical experiments show that the radiation effect on DNA in a solution depends on the ionic surrounding of the macromolecule: the structural damages revealed in a decrease of DNA coil volume in a solution diminish with the increase of electrolyte concentration [54,]. It can be explained by various causes. First, the increase in the ionic strength leads to the decrease in the volume of macromolecular coil [51], i.e. the size of the target decreases. Second, in this case the changes in the DNA hydration shell take place: the amount of bounded ions rises and the degree of hydration reduces [51,53]. Study of the yields of double- and single strand breaks [56-58], and the released undamaged nucleobases [36] in DNA samples with different degree of hydration shows that the DNA radiosensitivity decreases with the reduction of the degree of hydration. The authors [57–59] point out that the structure of the DNA hydration shell influences on the radiation effect at least for two reasons: the change of the DNA secondary structure and the alteration in quantity of water molecules bonded to DNA, which are a part of the radiation target. The alteration of the target dimension changes the relation between direct and indirect radiation effects [1,56]. In addition the products of radiolysis of bonded water molecules and of bulk water differ [60,61]. Finally, the larger is the counterions concentration, the smaller is the DNA charge, hence, the smaller is its interaction with charged and polar water radiolysis products.

In the present work we have studied the aqueous DNA solutions with different NaCl concentrations (3 mM–3.2 M) and Mg^{2+} ions, exposed to γ-radiation with the doses up to 10^3 Gy. The above-mentioned spectral methods were applied to determine the amount of destructed nucleobases and the degree of helicity of the irradiated DNA. The aims of this research were to compare the abilities of the methods used to reveal these sorts of radiation-induced DNA damages and to study the influence of Na^+ and Mg^{2+} ions on DNA radiosensitivity in a solution.

2. Materials and Methods

DNA (Sigma) from calf thymus with a molecular mass of $(11.2 \pm 0.6)\cdot 10^6$ Da was used. All aqueous solutions were prepared using a double distilled water. NaCl, $MgCl_2$ and $HClO_4$ were reagent-grade. The melting temperature of the used DNA sample in 0.15 M NaCl solution is in good correspondence with the known dependency of melting temperature on GC-content [50]. The hypocromism of the used DNA sample in 5 mM NaCl solution is 35%, in 0.15 M NaCl solution is 38%. These data allow to conclude that double stranded DNA is the predominant form in this sample, as it is stated by the manufacturer.

The denaturation of DNA was carried out by adding 3 mL of 6% $HClO_4$ to 1 mL of DNA solution, and a subsequent exposure in a boiling water bath during 20 min and quick cooling to 0 °C. Acid medium prevents DNA renaturation. The concentration of DNA in the solution was determined using Spirin method from the difference in UV absorption of treated solutions at wavelengths 270 nm and 290 nm (D_{270} and D_{290}) measured at room temperature (20 ± 2) °C [27]:

$$C = \frac{10.1(D_{270} - D_{290})}{0.19}\ \frac{V_2}{V_1}$$

where V_1 is the volume of the DNA solution and V_2 is the volume of the solution after acid and heat treatment. Using the obtained value of C and the UV absorption intensity in the original non-treated solution at the maximum (D_{260}) we calculated the molar extinction coefficient, which gives an information about the degree of DNA helicity [22]:

$$\varepsilon_{260}(P) = \frac{31 \cdot D_{260}}{0.099 \cdot Cl}$$

were l – is the optical path length (in this work $l = 1$ cm), 0,099 = 9,9% is the phosphorus percentage in DNA, 31 is the phosphorus atomic mass.

The absorbance of DNA solutions was measured on SF-56 spectrophotometer (LOMO, St. Petersburg, Russia). The CD spectra were measured on a Mark IV dichrograph (Jobin-Yvon, France). These experiments were carried out at room temperature. The absorbance and CD of the corresponding solvent were subtracted from the spectra of DNA solution.

The melting curves of DNA samples were obtained by measuring the dependence of the optical density of DNA solution at λ = 260 nm on the temperature ($D_{260}(T)$). The experiment was carried out on Specord 210 Plus (Analytik Jena, Germany) with the Peltier equipment in a 0.5° step mode, the velocity of heating was 1° per min, the DNA concentration $C_{DNA} = 46\cdot 10^{-3}$ g/L. The data were collected with the help of the software WinASPECT (Analytik Jena, Germany) supplied with the instrument, further data processing were carried out via OriginPro. The melting curves $D_{260}(T)$ were smoothed by the Savitzky-Golay method. The melting temperature of DNA is determined as the point of maximum of the first derivative of the melting curve $dD_{260}(T)/dT$.

The DNA solutions were exposed to ^{60}Co γ-irradiation under the aerobic conditions at room temperature on an "Issledovatel" apparatus at the B. P. Konstantinov Institute of Nuclear Physics (St. Petersburg). The dose rates were 2.3 Gy/s (for the results in the Section 3.1) and