DNA Damage Induced by Gamma-Radiation Revealed from UV Absorption Spectroscopy

SA Tankovskaia¹, OM Kotb ¹,², OA Dommes³, SV Paston¹

¹Department of Molecular Biophysics and Polymer Physics, St. Petersburg State University, Petergof, Ulyanovskaya st. 3, St. Petersburg, Russia
²Department of Physics, Faculty of Science, Zagazig University, Sharkia Gov Zagazig, 44519 Egypt
³Institute of macromolecular compounds, 199004 Saint-Petersburg, Bolshoy pr. 31, Russia

E-mail: tasva-ara1@yandex.ru

Abstract. DNA damages are the main cause of radiation-induced cell death, mutations and carcinogenesis. The study of influence of environmental conditions on the radiation damage of DNA structure is important for the wide range of medical problems. The present work is devoted to the study of alterations in DNA structure caused by γ-irradiation at the variation of counterions concentration and dose rate. The DNA solutions of the ionic strengths 0.005M and 0.15M NaCl were exposed to γ-radiation with the doses of 0-100 Gy, dose rates 2.3 Gy/s and 0.3 Gy/s. The lesions in DNA structure (base damages, partial denaturation) were studied by the methods of UV absorption spectroscopy and DNA melting. Radiation-induced base damages decrease at the rise of NaCl concentration in the solution and at the lowering of the dose rate. The stability of DNA secondary structure increase at the rise of ionic strength of irradiated solution. The influence of radiation dose rate on the DNA helicity reveals definitely only at the dose of 100 Gy. The relative fall of the DNA melting temperature after the irradiation slightly depends on the dose rate and NaCl concentration.

1. Introduction

Ionizing radiation damages all the molecules in a living cell but just injuries in the DNA are most fatal because of the unique role of this molecule. Unrepaired damages in DNA structure cause cell death, mutations, malignant degeneration [1-4]. Radiation-induced DNA lesions are extensively studied, the most frequent types of them are single- and double strand breaks (SSB and DSB), inter- and intrastrand cross-links, destruction, modification and release of nucleobases, local breakage of hydrogen bonds (partial denaturation) [1-4]. To reveal radiation-induced structural damages in DNA the model aqueous DNA solutions are widely applied in experiments [1,2]. It is possible because the secondary structure of the macromolecule in a cell and in an aqueous solution is the same and represents the B-form [5]. Besides the mechanisms of ionizing radiation action on DNA molecule in a cell and in a solution are identical: mainly it is so-called indirect action, i.e. DNA injury over its interaction with the products of water radiolysis [1,2]. The present work is devoted to the study of alterations in DNA structure caused by γ-irradiation of its aqueous solutions.
DNA conformation, its ionic surrounding, water content, temperature, presence of some low molecular compounds (radioprotectors or radiosensitizers) influence the radiation effect on the DNA, but the molecular mechanism of many of these processes are not yet clarified [1, 6-11]. Counterions concentration in the DNA solution plays an important role in the formation of the tertiary structure of the macromolecule and stability of its secondary structure, positive ions are integrated into the hydration layer of DNA [5, 12, 13]. Nevertheless there are only a few works dealing with the influence of ionic strength of solution on the DNA radiosensitivity [14-16]. We compared the effect of γ-radiation on DNA molecule in aqueous solutions of the ionic strengths \( \mu_1=0.005\text{MNaCl} \) and \( \mu_2=0.15\text{MNaCl} \) at the different radiation doses and dose rates.

The UV absorption spectroscopy can provide essential information about the DNA structure. Hyperchromic effect indicates the degree of DNA helicity [17]. The method of A.S. Spirin [18] allows to find the concentration of chromophores (nucleobases) in the DNA solution and thus estimate the amount of nucleobases destructed under the action of radiation. DNA melting process is very sensitive to any defects in the primary and secondary structure of DNA [19]. In the present work these methods were applied to reveal DNA damages induced by the γ-irradiation of DNA solutions.

2. Experimental section

2.1. Materials

DNA (Sigma) from calf thymus with a molecular mass of \((11.2\pm0.6)\cdot10^6\) Da was used. All aqueous solutions were prepared using double distilled deionized water. NaCl and HClO\textsubscript{4} were reagent-grade.

2.2. Radiation exposure

The DNA solutions were exposed to \(^{60}\text{Co}\) γ-radiation under the aerobic conditions at 20°C on “Issledovatel” apparatus at B. P. Konstantinov Institute of Nuclear Physics (St.-Petersburg). The dose rates were \(I_1=2.3\text{Gy/s}\) and \(I_2=0.3\text{ Gy/s}\). The DNA concentration in the irradiated solutions was \(C_{\text{DNA}}=(45–50)\cdot10^{-3}\) g/l.

2.3. UV absorbance spectroscopy and Spirin’ method

The hydrolysis of DNA solutions was carried out by adding 3ml of 6% HClO\textsubscript{4} to 1ml of DNA solution, subsequent exposure in a boiling water bath during 20 min and quick cooling to 0°C. The concentration of DNA in the solution was determined from the difference in UV absorption of hydrolyzed solutions at wavelengths 270 nm and 290 nm (\(D_{270}\) and \(D_{290}\)) measured at 20°C (Spirin’ method) [18]:

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

where \(V_1\) is the volume of the DNA solution and \(V_2\) is the volume of the solution after hydrolysis. The hyperchromicity of DNA was calculated by the formula:

\[
\delta = \frac{D_{260}^{\text{hyd}} - D_{260}}{D_{260}} ,
\]

where \(D_{260}\) is the optical density of the studied DNA solution in the maximum of the DNA absorption spectrum and \(D_{260}^{\text{hyd}}\) is the optical density of the studied DNA solution of the same DNA concentration after the hydrolysis. The hyperchromicity of the native DNA is about 0.4 [17]. The values of experimental errors for \(C\) and \(\delta\) were estimated from 3 experiments with unirradiated DNA solution.
UV absorbance spectra were measured on SF-56 spectrophotometer (Russia) in rectangular quartz cells of 1 cm optical path. Absorbance of corresponding solvent was subtracted from the DNA solution spectrum.

2.4. DNA melting

The melting curves of DNA samples were obtained by measuring the dependence of the optical density of DNA solution at \(\lambda=260\) 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 minute. 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. Normalized melting curves \(f(T)\) for DNA solutions were obtained using the following equation [20]:

\[
f(T) = \frac{D_{260}(T) - D_{260}^{\text{min}}}{D_{260}^{\text{max}} - D_{260}^{\text{min}}},
\]

where \(D_{260}^{\text{min}}\) and \(D_{260}^{\text{max}}\) are the minimal and maximal values of the optical density at 260 nm, corresponding to the native and denaturated states of DNA in the solutions. The melting temperature of DNA is determined as the point of maximum of first derivative of the melting curve \(df(T)/dT\). The value of experimental error for \(T_m\) was estimated from 3 experiments with unirradiated DNA solution.

3. Results and discussions

In all DNA sites where the lesions of the primary structure occur, the secondary structure of the macromolecule disturbs. One can estimate the level of DNA helicity measuring the hyperchromic effect – i.e. the relative growth of DNA UV absorbance resulted from the denaturation of the macromolecule. In this study the denaturation of DNA was carried out by heating of the solution up to 97°C and also by hydrolysis. An example of DNA absorption spectra before and after the denaturation is shown in the Figure 1. It can be seen that hydrolysis causes the more complete DNA denaturation, so for the determination of the hyperchromicity of DNA we used the value \(D_{260}^{\text{hyd}}\) corresponding to the denaturated state of DNA.

Figure 1. UV absorbance spectra of DNA solutions measured at different conditions after \(\gamma\)-irradiation with the dose of 30 Gy and dose rate \(I_1=2.3\) Gy/s; \(\mu=0.005\) MNaCl.
The obtained values for the hyperchromicity of DNA in the solutions \( \gamma \)-irradiated at the different conditions are given in the Table 1. One can see that at the larger ionic strength the degree of DNA helicity bigger both in the control solutions of native DNA and also after the radiation exposure. The stabilizing role of monovalent cations on the native DNA secondary structure is well-esteblished [5, 12]. It is interesting to note, that Na\(^+\) ions also prevent the radiation-induced breakage of DNA secondary structure. One should mention that the increase of NaCl concentration in the DNA solution leads to the decrease of relative changes of the size of the coil of double-stranded DNA caused by \( \gamma \)-irradiation [15, 16].

An influence of radiation dose rate on the DNA helicity is not so obvious (Table 1). At the doses of 30 Gy and 50 Gy the values of DNA hyperchromicity in the solutions irradiated with the dose rates differing almost 10 times are close. And only at the dose of 100 Gy the degree of DNA helicity at \( I_1 = 2.3 \text{ Gy/s} \) is sufficiently smaller then at \( I_2 = 0.3 \text{ Gy/s} \) for the solutions of both ionic strengths studied. Apparently at the large concentration of lesions the speed of their accumulation becomes influential.

Investigation of radiation-induced base damages reveals the same tendencies: at the larger ionic strength DNA nucleobases are more steady, and lowering of the dose rate leads to the decrease of base damages at all doses (Table 2). The origin of Na\(^+\) ions influence on nucleobases radioreisistance is not clear for the present. We can propose that the observed effect causes by an alteration of the DNA hydration layers at the variation of counterions concentration [5, 12]. More certain conclusions require further research.

### Table 1. Hyperchromicity of DNA after the \( \gamma \)-radiation exposure.

| (Dose±2), Gy | \( \mu = 0.005 \text{ M NaCl} \) | \( \mu = 0.15 \text{ M NaCl} \) |
|-------------|---------------------------------|---------------------------------|
|             | \( I_1 = 2.3 \text{ Gy/s} \) | \( I_1 = 2.3 \text{ Gy/s} \) | \( I_2 = 0.3 \text{ Gy/s} \) | \( I_2 = 0.3 \text{ Gy/s} \) |
| 0           | 0.46                           | 0.49                           |
| 30          | 0.40                           | 0.42                           | 0.46                           | 0.45                           |
| 50          | 0.37                           | 0.36                           | 0.45                           | 0.42                           |
| 100         | 0.24                           | 0.34                           | 0.37                           | 0.41                           |

### Table 2. Relative amount of radiation-induced nucleobases degradation. \( C_0 \) is the nucleobases concentration before irradiation and \( C \) is the nucleobases concentration after the irradiation.

| (Dose±2), Gy | \( \frac{C_0 - C}{C_0} \) ±0.005 |
|-------------|---------------------------------|
|             | \( \mu = 0.005 \text{ M NaCl} \) | \( \mu = 0.15 \text{ M NaCl} \) |
|             | \( I_1 = 2.3 \text{ Gy/s} \) | \( I_1 = 2.3 \text{ Gy/s} \) | \( I_2 = 0.3 \text{ Gy/s} \) | \( I_2 = 0.3 \text{ Gy/s} \) |
| 30          | 0.050                          | 0.053                          | 0.026                          | 0.004                          |
| 50          | 0.076                          | 0.053                          | 0.042                          | 0.014                          |
| 100         | 0.109                          | 0.094                          | 0.070                          | 0.023                          |

One of the widespread methods of study of DNA structural changes is DNA melting [19]. The helix-coil transition of DNA is very sensitive to the state of secondary structure of the macromolecule, to its interaction with biologically active agents, and also to defects of the primary structure of DNA [21-24]. The data obtained by the method of spectrophotometric melting of DNA in...
γ-irradiated solutions are shown in Table 3. An example of DNA melting curves and their first derivatives in the control solution (before irradiation) and after the expose to γ-radiation with the dose of 30 Gy at the different dose rates are presented in the Figure 2. One can see that at this dose the melting temperatures $T_m$ of irradiated DNA are equal with the accuracy of the experimental error (Table 3). As it was expected $T_m$ in the solutions of $\mu=0.15$ M are larger than $T_m$ in the solutions of $\mu=0.005$ M, which indicates the stabilization of DNA secondary structure by counterions. But the radiation effect on $T_m$ does not repeat the pattern observed in our previous results. At $\mu=0.15$ M the relative decrease of $T_m$ after the irradiation is slightly bigger than at $\mu=0.005$ M. Considering the possible DNA lesions in irradiated solutions we can divide them on two groups: 1) the lesions which decrease $T_m$ (SSB, DSB, destruction, modification and release of nucleobases) and 2) the lesions which increase $T_m$ (inter- or intramolecular crosslinks) [19, 23]. We suppose that we observe the combined influence of these two groups of lesions on the DNA melting temperature. Further investigations can help to segregate these radiation effects and estimate them separately.

Table 3. Melting temperatures $T_m$ of DNA in solutions exposed with the γ-radiation at the dose rate $I_1=2.3$ Gy/s.

| $\mu$, M NaCl | (Dose±2), Gy | ($T_m$±0.5), °C | ($\Delta T_m$±1), °C |
|---------------|--------------|-----------------|-------------------|
| 0.005         | 0            | 57.3            |                   |
|               | 30           | 50.6, 50.3*     | 7                 |
|               | 50           | 47.0            | 10                |
| 0.15          | 0            | 84.3            |                   |
|               | 30           | 77.5            | 8                 |
|               | 50           | 73.5            | 12                |

*Dose rate $I_2=0.3$ Gy/s

Figure 2. The melting curves (a) and their first derivatives (b) of the native DNA and DNA exposed to γ-radiation with the dose of 30 Gy at the different dose rates; $\mu=0.005$ M NaCl.
4. Conclusions
Radiation-induced base damages decrease at the rise of NaCl concentration in the solution (from 0.005M to 0.15M) and at the lowering of the dose rate (from 2.3 Gy/s to 0.3 Gy/s). The stability of DNA secondary structure increases at the rise of ionic strength of irradiated solution. The melting temperature of DNA $T_m$ in irradiated solutions diminishes monotonously with the radiation dose growth. The relative fall of $T_m$ after the irradiation slightly depends on the dose rate and NaCl concentration. It can be explained by the superposition of effects from different DNA lesions which decrease and increase the DNA melting temperature.

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