Research Article

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Alteration in the molecular structure of the adenine base exposed to gamma irradiation: An ESR study

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Abstract: Adenine polycrystals were obtained from their powder form under effective crystallization conditions by adjustment of the concentration of chemical solutions. The adenine samples were irradiated with a ^{60}Co gamma-ray source at room temperature for 12, 24, 48, and 72 h, and then these samples were investigated between 240 and 400 K using an electron spin resonance (ESR) spectrometer. No signals were observed in the non-irradiated samples irradiated for 12, 24, and 48 h; however, when irradiated for 72 h, the samples exhibited complex ESR spectra. The analysis indicated the presence of a radical structure, $\dot{N}H$. The hyperfine splitting and *g*-values were calculated from the spectra by simulation programs. Also, during the measurements, it was observed that the shapes of the ESR lines changed slightly with the temperature.

Keywords: Adenine, ESR, gamma-irradiation, radical

1 Introduction

Nowadays, exposure to ionizing radiation has become an inseparable part of our lives, which causes considerable damage to organ-forming cells such as deoxyribonucleic acid (DNA) breaks, DNA base damage, etc. [1,2]. The occurring genetic changes, mutations, are critical features in the development of healthy cells into cancer cells. In recent years, for medical treatment, radiation treatment known as radiotherapy has been largely applied [3–6] in which tumor cells are killed directly or their mass is reduced. Nonetheless, there are several supportive cells in the solid tissue's environment and these cells can be damaged by the radiation. This method can result in several aforementioned side effects [7–10].

DNA plays an active and important role in the living body and has been identified as essential to all living beings. The nucleic acid contains adenine (A), guanine (G), cytosine (C), and thymine (T) bases. Both adenine and guanine are fused-ring skeletal structures derived from purines, and therefore are called purine bases; cytosine and thymine are simple-ring structures derived from pyrimidine and so these bases are called the pyrimidine bases. Up to now, many methods such as surface-enhanced Raman spectroscopy, high-performance liquid chromatography, NMR, and electron spin resonance (ESR) have been developed for the analysis of these bases [11–16]. The purine bases are susceptible to radical formation, so ESR spectroscopy is a versatile tool to study the structure of characteristic free radicals; in addition, this method is cost-effective. In the past, some studies were performed to examine adenine bases; for example, Conlay studied the radiolysis of adenine in an aqueous solution in the absence of air and also exposed to X-rays of 40 kV and gamma rays of 0.680 kGy/h. His experiments resulted in the formation of 8-hydroxyadenine [17]. Similarly, Hartmann et al. studied radiolysis of aqueous adenine saturated with argon [18]. They, as well, found similar results to those of Conlay. However, no studies are found on polycrystal adenine bases in the literature.

This study aimed to find how adenine in the polycrystal form responds to ionizing radiation (gamma rays). Effective crystallization conditions were applied to obtain it in the polycrystal form. Then, polycrystal adenine samples were irradiated with a ^{60}Co gamma source for 12, 24, 48, and 72 h.

2 Materials and methods

Adenine in powder form was purchased from Sigma (London). Adenine was annealed at 4°C and its crystallization occurred at room temperature. The obtained polycrystal samples were

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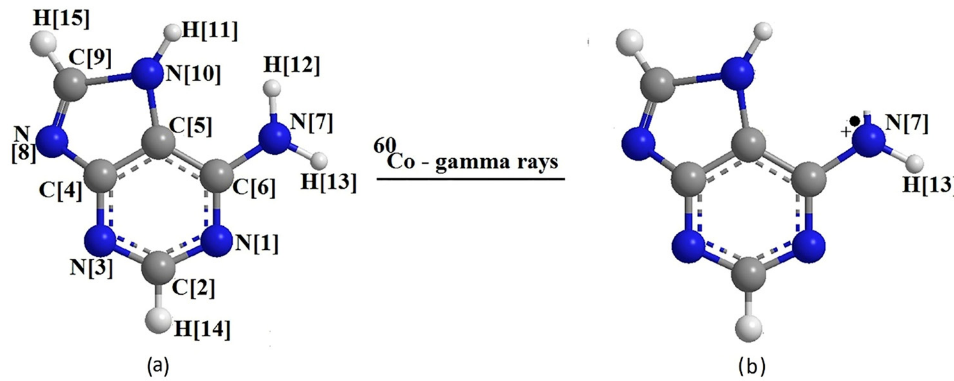


Figure 1: Molecular structure (a) and radical structure (b) of the polycrystal adenine.

kept at 25°C overnight. The buffers used in the crystallization of adenine in powder form are glutamic acid and glacial acetic acid. Oligonucleotides are usually not sensitive to the pH of the crystallization solution. However, acceptable adjustments of the pH were useful in obtaining crystals with high quality. Moreover, during the crystal growth, several methods were developed to improve the quality of adenine polycrystals. The characteristics of crystals are the most important factor for acquiring a high-resolution structure. Adenine in the polycrystal form was irradiated for 12, 24, 48, and 72 h with Cobalt-60 (^{60}Co) gamma-ray source (0.980 kGy/h) in the irradiation facility, called TENMAK (Turkish Energy, Nuclear and Mineral Research Agency). Then, the measurements of each sample were carried out using a Bruker EMX 081 X-Band Spectrometer (Germany). The spectrometer was set as follows: 1 mW of microwave power, 100 kHz of modulation frequency, 0.4 mT of modulation amplitude, and 9.409 GHz of microwave frequency. The temperature ranged between 240 and 400 K and was controlled with a temperature

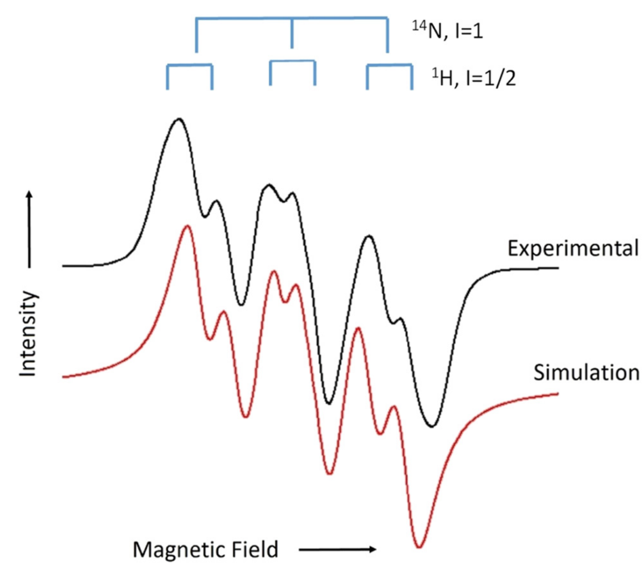
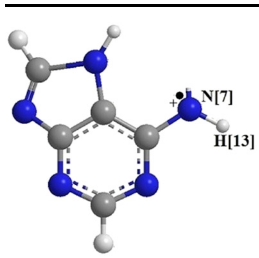


Figure 2: The ESR spectrum (black line) of the adenine sample; simulation (red line) of the spectrum of the adenine radical.

Table 1: ESR parameters obtained from the experimental spectra recorded for the adenine sample irradiated for 72 h by simulation programs

Radical	Hyperfine splitting values	Simulation values	*g* values
	aN(7) = 2.8 mT aH(13) = 1.050 mT	2.7 mT 1.1 mT	2.0095

ESR parameters of free radicals produced by gamma irradiation in polycrystals of adenine. The error for all calculated *g*-values is estimated as ±0.0005 and for hyperfine splitting values, it is ±0.05 mT. Where, a is hyperfine splitting constant.

control unit. SimFonia and WIN-EPR (Bruker) programs were used in the simulations.

3 Results and discussion

ESR signal was observed for the samples irradiated for 12, 24, and 48 h. The radicals may have formed but were short-lived. Only, the spectra were recorded from the adenine sample irradiated for 72 h at room temperature. On careful examination of the spectra, it was evident that one type of radical structure was present in the polycrystal adenine after irradiation for 72 h. After analysis of both the recorded spectra and the molecular structure (Figure 1a), it was found that the bond between the hydrogen atom labeled 12 and the nitrogen atom labeled 7 was broken after irradiation, and the unpaired electron residue was localized on the nitrogen atom which interacted with the hydrogen atom labeled 13. As a result, the line was split into a 1:1:1 intensity ratio due to the nuclear spin of ^{14}N (I = 1). Again, each line was split to a 1:1 intensity ratio due to the nuclear spin of ^{1}H (I = 1/2). The interaction of the polycrystal sample with gamma rays is shown in Figure 1b.

The radical structure formed was attributed to $\dot{N}H$, and is the same as one of the radicals found by Close et al. [19], Nelson et al. [20], and the radical structure found by Adhikary et al. [21]. However, while Close et al. and Nelson et al. studied deoxyadenosine monohydrate single crystals irradiated by X-ray at 10 K, Adhikary et al. as well investigated the deoxyadenosine monohydrate in solution

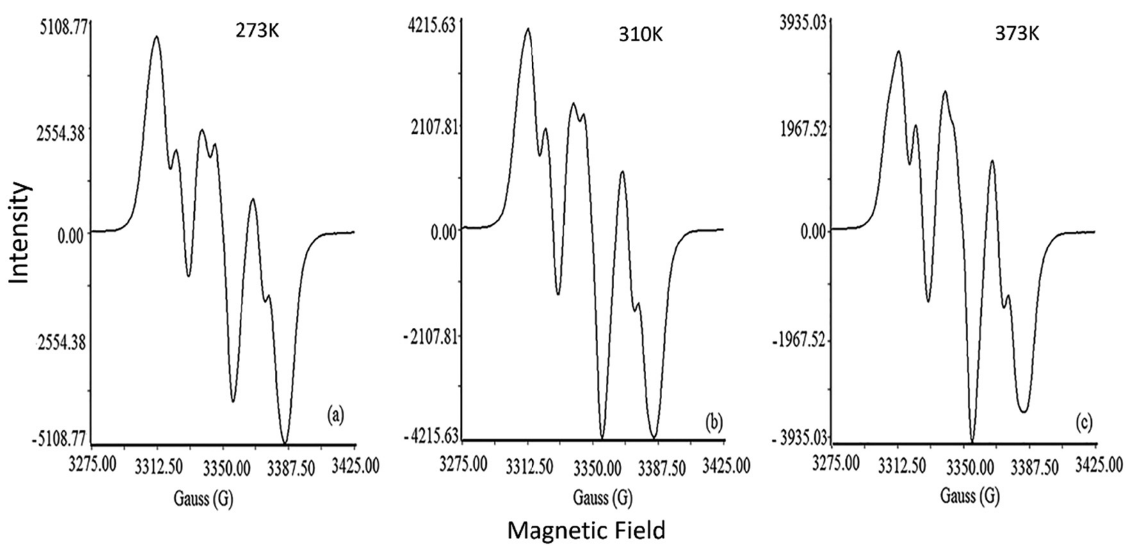


Figure 3: The temperature dependence of the ESR absorption signal at 273 K (a), 310 K (b), and 373 K (c).

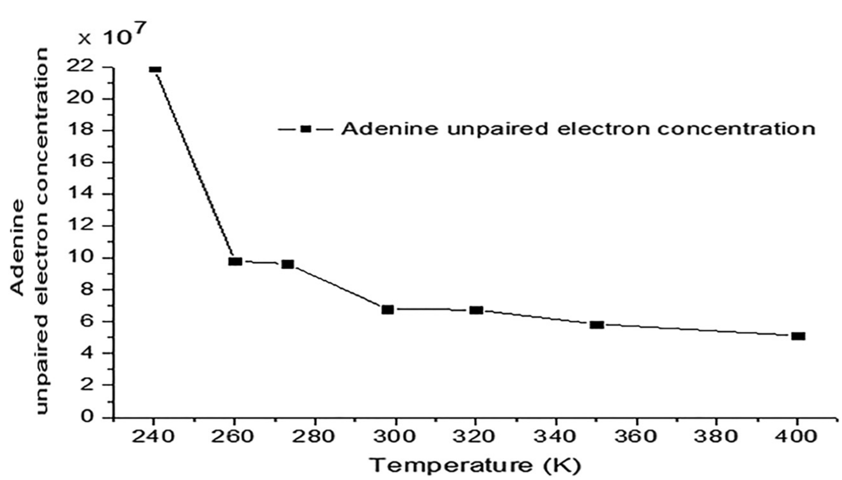


Figure 4: Unpaired electron concentration in the adenine polycrystal irradiated as a function of temperature.

at 77 K. However, the ESR parameters in this study were different from those of Adhikary et al. These differences were due to the working conditions of the samples investigated because they performed the measurements on the sample in solution. The calculated ESR parameters in Table 1 are in accordance with the values in earlier studies [12,22,23]. In the present study, the hyperfine splitting and *g*-values were determined using the spectra simulations (Figure 2) using SimFonia and WIN-EPR (Bruker) programs. In addition, the electron spin density (q) in connection with the proton was determined using the McConnell equality ($a = Qq$), where Q is a resonance multiplier. For the nitrogen atom, Q_N = 2.8 mT [23], and the q value was found to be 0.96. This value implies that unpaired electrons spend much time on the nitrogen proton.

The temperature dependence of the spectra, which gives information about the moving unpaired electron, was studied between 240 and 400 K. It was found that in the above temperature range, the intensities and the shapes of the lines in the spectra changed slightly (Figure 3), implying that the radical is resistant to the temperature.

The approximate measurements of unpaired spin concentration were performed despite many difficulties such as various factors, temperature, etc., which could affect the unpaired spin concentration in the substance. After the first integral form of the recorded spectra was obtained, the change in the unpaired spin concentration with temperature was calculated from the areas under the ESR signals (Figure 4). The results indicate that the unpaired electron concentration in the adenine sample decreased steadily with increasing temperature.

Moreover, both observations and measurements were carried out on the adenine samples for 2 days, at regular intervals. It was found that the radical structure on the adenine was stable, and the white color of the sample changed to dirty white with time in the absence of light.

4 Conclusions

When the present study was compared to other studies on adenine, it was found that the adenine base gave similar responses to both X-rays and gamma rays because of similar radical structures. The analysis of the spectra revealed that one type of radical was formed by the adenine polycrystalline on gamma irradiation, had a long life, and was stable against increasing temperature. However, the unpaired electron concentration in the adenine decreased steadily with the temperature. In brief, it may be concluded that although irradiations with high energies damage the adenine base, this base protects its general molecular structure. It is worth noting that the damaged adenine can induce mutations and lead to cell transformation [24,25].

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Ethical approval: The conducted research is not related to either human or animal use.

Data availability statement: Derived data supporting the findings of this study are available from the corresponding author on request.

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