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Spectroscopic assessment of argon gas discharge induced radiolysis of aqueous adenine and thymine

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ABSTRACT

Ionizing radiation influences life profoundly for it can modify genetic materials. It is a long-standing task to investigate the interaction between energetic particles and DNA together with its components such as nucleotides, nucleosides and bases so as to predict and assess the potential biological effects. In this study, argon gas discharge was employed to produce energetic ions and electrons. The gas discharge caused the radiolysis of aqueous bases and the involved reactions were analyzed by means of spectroscopic tools including UV–vis absorption, fluorescence and Fourier transformation infrared (FTIR) spectroscopy, also assisted by liquid chromatography/mass spectrometry (LC/MS). It was found that the discharge resulted in the adenine-derived lesions such as 4,6-diamino-5-formamidopyrimidine, 8-OH-Ade and 2-OH-Ade in the radiolysis of aqueous adenine, as well as the thymine-derived lesions such as thymine glycol, 5-hydroxy-6-hydrothymine and/or 6-hydroxy-5-hydrothymine, 5-hydroxymethyluracil and 5-formyluracil in the radiolysis of aqueous thymine. The study of radiosensitivity showed that adenine was more resistant to the discharge. The mechanisms of the involved reactions were studied in detail, confirming that the hydroxyl radical played a dominant role.

1. Introduction

Ionizing radiation exist ubiquitously in the environment and may affect life on earth profoundly. On one hand, ionizing radiation shows toxic, mutagenic and carcinogenic effects because it can cause DNA damage, chromosome aberration and cell inactivation (von Sonntag, 1987). Generally, ionizing radiation causes DNA damage mainly through hydroxyl radicals, leading to DNA base modifications (Dizdaroglu, 1992; Gajewski et al., 1990; Hartmanna et al., 2007), protein–DNA cross-linking (Oleinick et al., 1987; Dizdaroglu, 1998) and sugar lesions (von Sonntag, 1981, 1984). Six decades ago, Scholes et al. (1949) initiated the research on ionizing radiation induced damage of nucleic acids. Since then, most studies have focused on the effect of ionizing radiation on DNA damage, which causes cancer and non-cancerous diseases. Yet it is still a big challenge to elucidate the involved mechanisms in detail (Cooke et al., 2003; Evans et al., 2004). On the other hand, ionizing radiation may have important implications on the origin of life because it might have caused some specific chemical events on the ancient earth. For example, a large amount of energetic particles were generated by thunder, lightning and emission of radioactive elements from the eruption crust, and these particles impacted molecules especially in water leading to the formation of organic molecules such as amino acids(Yu et al., 1998). While the abiotic synthesis of amino acids by electrical discharge was first demonstrated by Miller–Urey experiment where the electrical discharge was applied to a gas mixture containing CH_4, CO, N_2, H_2 and H_2O to form amino acids (Miller, 1953), it is still a puzzle how the genetic materials such as bases and nucleotides are produced. A most recent study reported that activated pyrimidine ribonucleotides may be formed in a short sequence that proceeds through arabinose amino-oxazoline and anhydronucleoside intermediates instead of free ribose and the nucleobases (Powner et al., 2009).

In this work, we focused on the study of low-energy-particle induced damage of bases, the most important components in nucleosides and nucleotides. Bases are also the major targets of ionizing radiations (Scholes et al., 1960; Ward and Kuo, 1973; Deeble and von Sonntag, 1986). On the other hand, oxidized bases are of mutagenic potential to cause alterations of the genetic information in vivo (Kamiya, 2003). For example, the base-derived lesions such as 8-OH-Ade, 2-OH-Ade and thymine glycol are the derivation products due to the ionizing radiation that can exert significant distortions on the duplex DNA molecules (Wallace, 2002; Evans et al., 2004).

To scrutinize the involved interactions, which may cause the base derivatives and lesions, we applied gas electrical discharge to produce low-energy particles (including ions and electrons), and employed spectroscopic tools including UV–vis absorption, fluorescence and Fourier transformation infrared (FTIR) spectroscopy combined with other analytic methods such as high performance

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liquid chromatography/mass spectrometry (HPLC/MS) to analyze the reaction products. Previously, our laboratory had already been engaged in the study of nitrogen gas discharge-induced damages of adenine (Shi et al., 2002a) and cytosine (Shi et al., 2001a) and had some interesting findings such as mass deposition effect for the formation of new products containing -NO_2, -NH_2 and C═O (Shi et al., 2001a, 2002a). In the past, the ionizing radiations were usually obtained from γ-rays or X-rays radiations, and for the analysis, the following methods were often employed, including paper chromatography, ion-exchange chromatography and ultra-violet absorption spectra (Conlay, 1963; Ponnamperuma et al., 1963; Vanhemme and Bleichro, 1971; Teoule et al., 1974), gas chromatography mass spectrometry (GC/MS) and liquid chromatography/mass spectrometry (LC/MS) (Alexander et al., 1987; Dizdaroglu, 1985; Gajewski et al., 1990; Berger et al., 1992; Dizdaroglu, 1992, 1993; Dizdaroglu et al., 2002; Frelon et al., 2000; Theruvathu et al., 2007). In the present study, we aimed to study the radiolysis of aqueous bases using a home-made gas discharge apparatus, and put emphasis on the employment of spectroscopic tools to take the advantage that they can dissect the involved reactions and provide quantitative analysis effectively (Huang et al., 2010; Ke et al., 2010a, 2010b). Adenine and thymine were chosen as representatives for they represent the respective purine and pyrimidine bases, the two main constituents of nucleic acids. By means of combined spectroscopic measurements, the gas discharge induced radiolysis of aqueous bases was thus investigated both qualitatively and quantitatively.

2. Materials and methods

2.1. Materials

Adenine, thymine (Bio Basic Inc., Canada) and 4, 6-diamino –5-formamidopyrimidine (Sigma, USA) of either chromatographic or reagent grade were commercially available. They were used without further purification. Deionized water was obtained through a Milli-Q system (Millipore Corporation).

2.2. Experimental set-up

Previously our lab had built the gas discharge apparatus for radiolysis of small biomolecules in aqueous solutions (Shi et al., 2001b, 2001c, 2002b). Later, we made some technical improvement to facilitate discharge (Ke et al., 2010b). The gas discharge apparatus is shown in Fig. 1. Briefly, a stainless steel needle used as anode (A) was placed 5 mm over the solution surface, whereas a stainless steel plate used as the cathode (C) was placed at the bottom of the container (B). Argon gas was introduced into the chamber through the gas inlet orifice (I) and expelled through the gas exit orifice (O). The gas was introduced into the sample solution for at least 10 min before discharge in order to remove the air inside. When the discharge was steady, the current was about 20 mA, and the total voltage was about 1350 V (± 10%). The samples were prepared as 25 mL of 2 mM adenine and 25 mL of 2 mM/10 mM thymine in aqueous solutions.

During the discharge, the gas discharge was triggered at atmospheric pressure to generate plasma ions and the cathode was immersed in the solution. The ions were injected into the sample solution after crossing the plasma sheath at energy of several hundreds eV (Yu, 2006).

2.3. Sample analysis

2.3.1. Liquid chromatography/mass spectrometry (LC/MS)

LC/MS spectra were obtained with a liquid chromatography/mass spectrometry (LCQ Advantage, Thermo-Finnigan, USA) equipped with a PDA detector and an automatic injector. A ZORBAX Eclipse XDB C18-reversed-phase column (150 mm × 4.6 mm *i.d.*, 5 μm particle size) (Agilent Technologies) was used with an eluent of 10% methanol in deionized water and the pH was adjusted to 3.6 with acetic acid. The flow rate was 0.2 ml/min. The tested samples were 2 mM adenine solution (at pH 6.3) and 10 mM thymine solution (at pH 6.3), prepared with deionized water. The injection volumes were 20 μl.

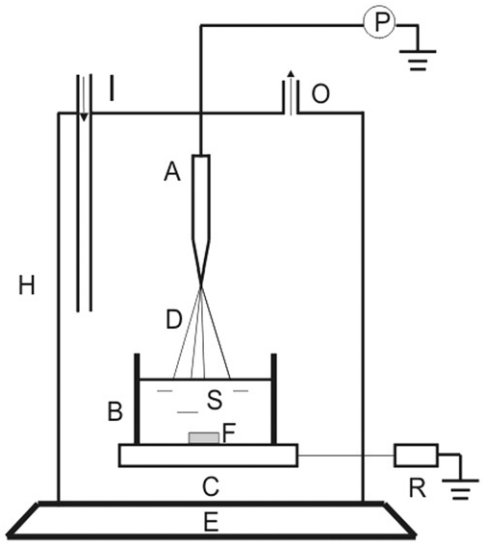


Fig. 1. Argon gas discharge set-up. A: stainless steel needle; B: sample chamber; C: stainless steel plate; D: gas discharge zone; E: magnetic stirrer; F: magnet bar; S: sample solution; H: cooling container; I: gas inlet orifice; O: gas exit orifice; P: power supply; R: electrical resistance.

2.3.2. UV–vis, fluorescence and FTIR spectroscopy

Samples at different discharge time (0, 5, 10, 20, 30 min) were examined using UV–vis spectrometer (SHIMADZH UV-2550), fluorescence spectrometer (VARIAN Cary Eclipse) and Fourier transform infrared spectroscopy (FTIR) (BRUKER ALPHA-T) instruments.

3. Results

3.1. Radiolysis of aqueous adenine

3.1.1. Formation of adenine-derived lesions

After gas discharge on the aqueous adenine, new products were generated, which were analyzed by LC/MS, FTIR, UV–vis and fluorescence spectroscopy. The majority of reaction products are identified as 4,6-diamino-5-formamidopyrimide (FAPyAde), 8-hydroxyadenine (8-OH-Ade) and 2-hydroxyadenine (2-OH-Ade). As shown in Fig. 2, the component 1 with retention time 5.6 min (corresponding to 7.04 min in Fig. 3) is identified as FAPyAde because it shows the same retention time as the reference sample FAPyAde (peak 4 in the HPLC spectrum of Fig. 2) and it has a MS peak MH^+ at *m/z* 154 (MS spectra in Fig. 3). Moreover, it shows the characteristic IR bands of FAPyAde at 1646, 1387, 778 cm^{-1}, as compared with the reference sample FAPyAde in Fig. 4. This product has the absorption band at 264 nm. The component 2 in the HPLC chromatogram in Fig. 2 is identified as 8-OH-Ade verified by the subsequent MS measurement with MH^+ peak at *m/z* 152 (retention time: 8.95 min) as shown in Fig. 3. It has the absorption band at 272 nm (Ponnamperuma et al., 1963; Conlay, 1963; Cavalieri and Bendich, 1950). Fig. 4 also shows the FTIR spectra of the sample

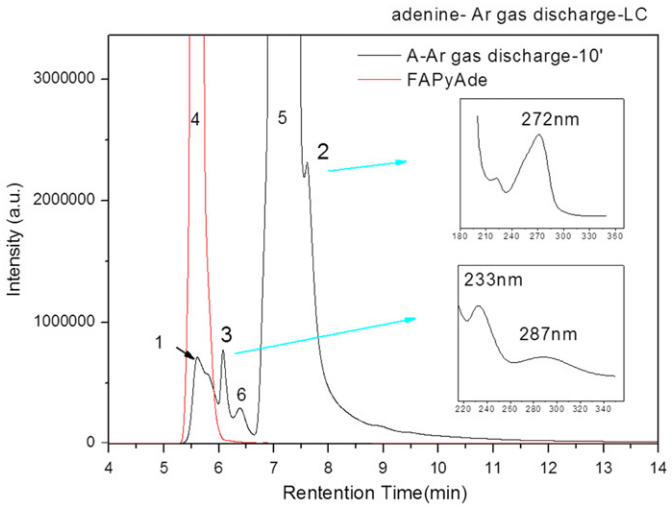


Fig. 2. HPLC chromatogram of the samples: aqueous adenine irradiated by argon gas discharge (black curve) and the reference sample FAPyAde (red curve). Inset: the UV–vis absorption spectra of the components represented by peak 2 (upper) and peak 3 (lower). Assignment: peak 1 (retention time=5.6 min): FAPyAde; peak 2 (retention time=7.6 min): 8-OH-Ade; peak 3(retention time=6.1 min): 2-OH-Ade; peak 4: reference sample FAPyAde; peak 5: adenine; peak 6: unidentified components. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

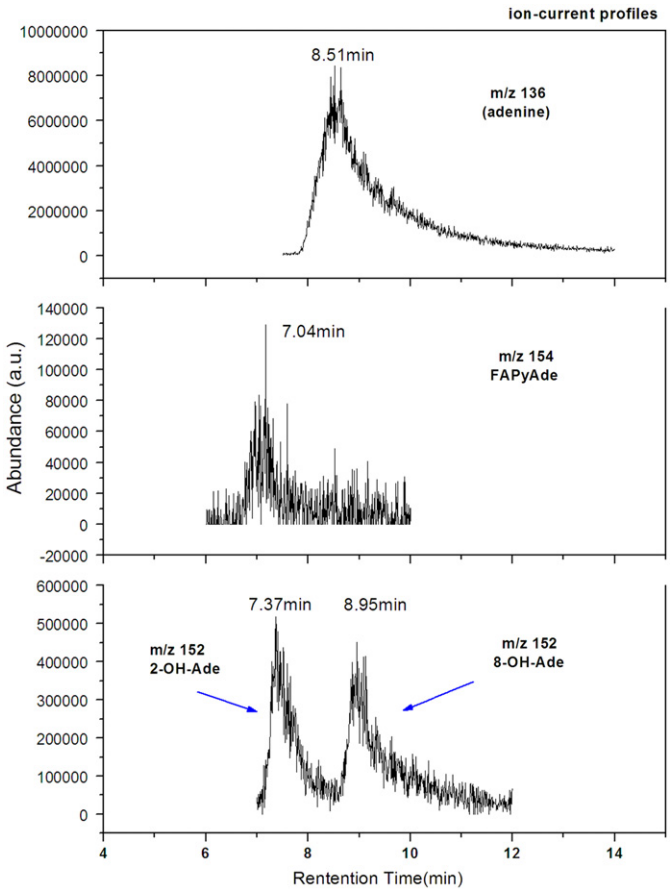


Fig. 3. Ion–current profiles at *m*/*z* 136 (adenine), *m*/*z* 154 (FAPyAde), *m*/*z* 152 (8-OH-Ade and 2-OH-Ade) recorded during LC/MS-SIM analysis of the irradiated adenine solution samples.

after the discharge treatment, where the bands (1728–1646 cm^{-1}) are attributed to the C=O of 8-OH-Ade and its tautomers (Gu et al., 2000). The component 3 in the HPLC chromatogram in Fig. 2 is

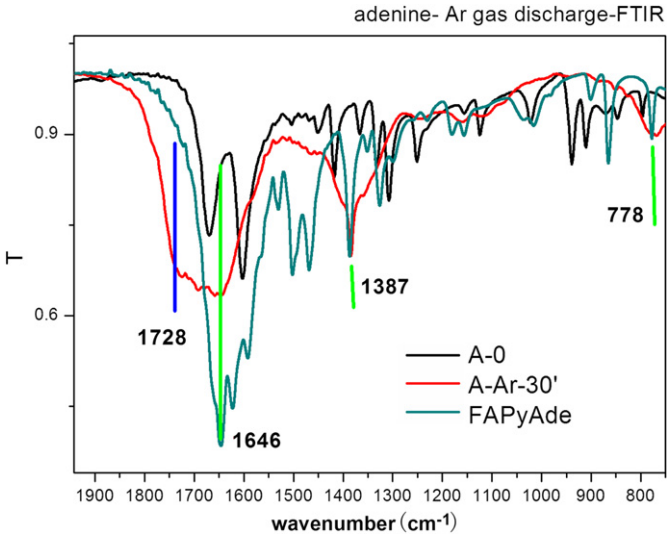


Fig. 4. FTIR spectra of the pure adenine (the black curve), adenine irradiated by argon gas discharge for 30 min (the red curve) and the reference sample FAPyAde (the blue curve). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

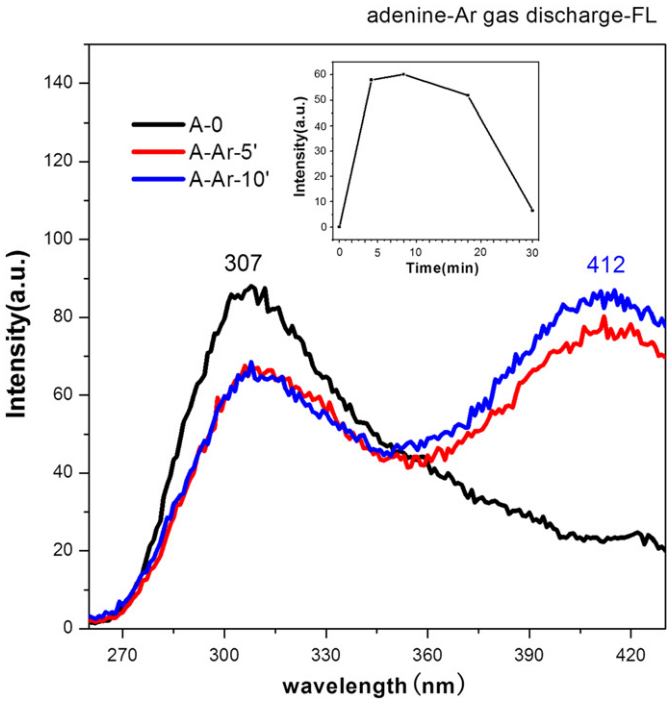


Fig. 5. Fluorescence spectra of the following samples: adenine (the black curve), aqueous adenine irradiated by argon gas discharge for 5 min (the red curve) and 10 min (the blue curve). The excitation was at 227 nm. Inset: the content of 8-OH-adenine and 2-OH-adenine, measured by the intensity of fluorescence band at 412 nm. Sample concentration: 50 μM; pH=6.3. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

identified as 2-OH-Ade, which is verified by the MS measurement with MH^+ peak at *m*/*z* 152 (retention time: 7.37 min) as shown in Fig. 3. It has the absorption band at 287 nm (Ponnamperuma et al., 1963).

Fig. 5 shows the fluorescence spectra of the reaction products, where the overall fluorescence intensity is obviously enhanced and the adenine's fluorescence peak at 307 nm is red-shifted to 412 nm, indicating the formation of hydroxyadenine, including

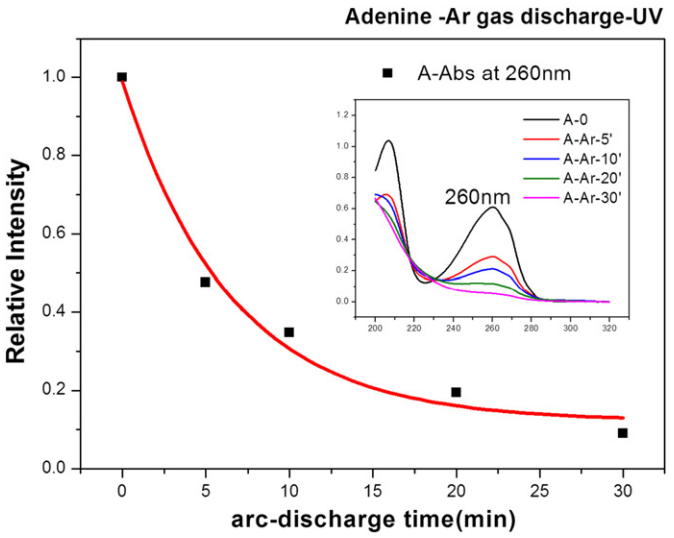
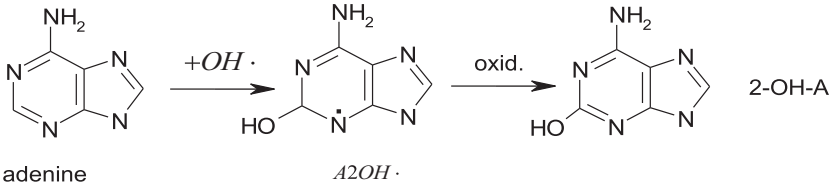


Fig. 6. Argon gas discharge induced degradation of adenine solution changes with the discharge time. Initial concentration is 2 mM with pH=6.3. Inset: the UV–vis spectra of the irradiated sample at concentration=50 μM.

8-OH-Ade and 2-OH-Ade. This is because the addition of hydroxyl group to the aromatic ring and the pyrimidine ring of adenine gives rise to electron-adding effect for the π–π^* transition. The inset in Fig. 5 estimates the yields of the new products 8-OH-Ade and 2-OH-Ade, showing that they increase rapidly in the beginning of discharge ($t<10$ min), then decrease slowly during the time 10 min-$<t<$20 min, and then decrease rapidly again afterwards. So there is an optimal condition for yield of new products.

3.1.2. Loss of adenine

The contents of the new products are extremely low, and they have different absorption bands from that of adenine (Fig. 2). So their contribution to the absorption spectra in the region around 260 nm (the adenine absorption band) can be neglected, and it is convenient to achieve the quantitative analysis for the damage of adenine by the measurement of absorbance at 260 nm. Fig. 6 shows that the content of adenine decreases exponentially with discharge time.



3.2. Radiolysis of aqueous thymine

3.2.1. Formation of thymine-derived lesions

Fig. 7 shows the HPLC chromatogram of the thymine solution after argon gas discharge irradiation. The new peaks in Fig. 7 indicate the formation of new products. Combined with the MS results in Fig. 8, these new products are identified as follows: thymine glycol (MH^+ at m/z 161), 5-hydroxy-6-hydrothymine and/or 6-hydroxy-5-hydrothymine (MH^+ at m/z 145), 5-hydroxymethyluracil (MH^+ at m/z 143) and 5-formyluracil (MH^+ at m/z 141).

3.2.2. Loss of thymine

Similarly, the quantitative analysis of the loss of thymine can be achieved through absorption measurements. Fig. 9 shows the decrease of thymine with argon gas discharge time, measured from the absorption band at 265 nm.

4. Discussion

4.1. Reactions through hydroxyl radical

As we know, argon gas discharge can produce excited argon atoms Ar* and energetic electrons, which then interact with water molecules in water vapor causing dissociative excitation of the water molecule at the gas–liquid interface (Lukes and Locke, 2005; Hoeben et al., 2000; Yu, 2006). The following reactions occur:

$$Ar^* + H_2O \rightarrow Ar + OH^\bullet + H^\bullet \quad \text{dissociaion}$$

$$e^- + H_2O \rightarrow H^\bullet + OH^\bullet + e^- \quad \text{dissociaion}$$

$$e^- + H_2O \rightarrow H_2O^+ + 2e^- \quad \text{dissociaion}$$

$$H_2O^+ \rightarrow H_2O \rightarrow H_3O^+ + OH^\bullet$$

The reactions mainly through hydroxyl radical were also tested by adding scavengers. For this purpose, 0.5 M mannitol as a hydroxyl radical scavenger was prepared and added to the 2 mM adenine solution, followed by irradiation under the same discharge condition as described above for comparison. Fig. 10 shows that adenine was much less damaged with the addition of mannitol. This verifies that the hydroxyl radical played a dominant role in the radiolysis process, and so the damage of the bases in solution was mainly via an indirect interaction.

4.1.1. Radiolysis of aqueous adenine

Hydroxyl radical interacts with adenine giving rise to C4-OH-, C8-OH-, C2-OH-adduct radicals. C4-OH-adduct radicals undergo dehydration and yield oxidizing purine(-H)* radical, which reconstitutes adenine upon reduction. One-electron oxidation and one-electron reduction of C8-OH adduct radicals may give rise to 8-OH-Ade and FAPyAde, respectively (Scheme 1) (Steenken, 1989; Vieira and Steenken, 1990; Breen and Murphy, 1995; Dizdaroglu et al., 2002). Correspondingly, one-electron oxidation of C2-OH-adduct radicals may give rise to 2-hydroxyadenine (2-OH-Ade) (Evans et al., 2004).

For the formation of 2-OH-Ade, the reaction may occur as follows:

4.1.2. Radiolysis of aqueous thymine

The hydroxyl radical also reacts with thymine by the addition and abstraction reactions. Addition to the C5=C6 double bonds results in the formation of C5–OH– and C6–OH-adduct radicals; the H-abstraction leads to the allyl radical of thymine. The OH-adduct radicals and the allyl radical are oxidized or reduced depending on their redox properties, environment and reaction partners (Evans et al., 2004). Scheme 2 illustrates the formation of thymine glycol, a result of the oxidation of the major C5–OH-adduct radical of thymine, followed by addition of OH-(or addition of water followed by deprotonation) in the absence of oxygen; the oxidation of the minor allyl radical of thymine yields 5-hydroxymethyluracil and 5-formyluracil, and the reduction of the major C5–OH-adduct radical and minor C6–OH-adduct radical

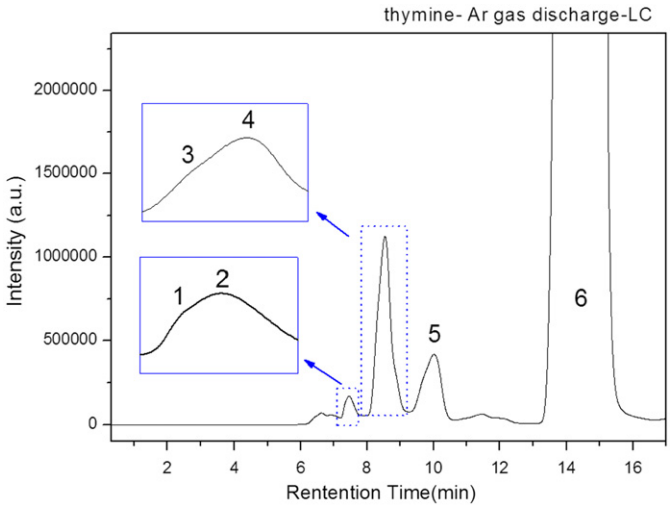


Fig. 7. HPLC chromatogram of the aqueous thymine irradiated by argon gas discharge. Inset: zooming of the corresponding peaks to show the peak overlaps. Assignment: peak 1 (retention time=7.3 min): 5-hydroxymethyluraci; peak 2, 4 (retention time=7.5, 8.5 min): thymine glycols; peak 3 (retention time=8.2 min): 5-hydroxy-6-hydrothymine and/or 6-hydroxy-5-hydrothymine; peak 5: unidentified components; peak 6: thymine.

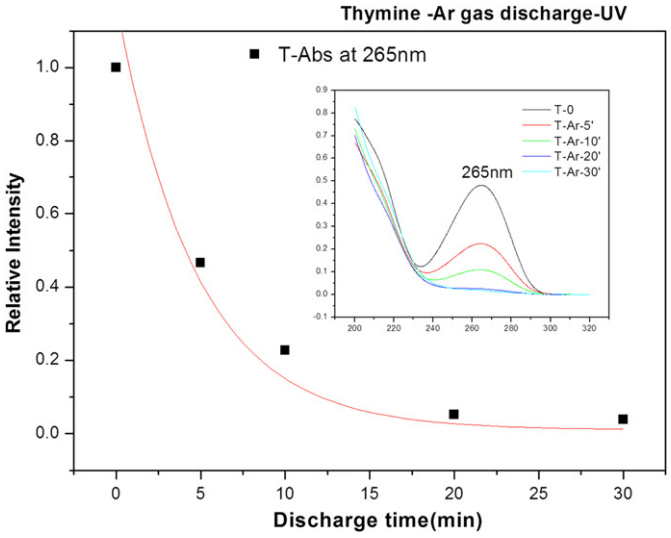


Fig. 9. Argon gas discharge induced degradation of aqueous thymine changes with discharge time. Initial concentration is 2 mM with pH=6.3. Inset: the UV–vis spectra of the irradiated aqueous thymine at 50 μM.

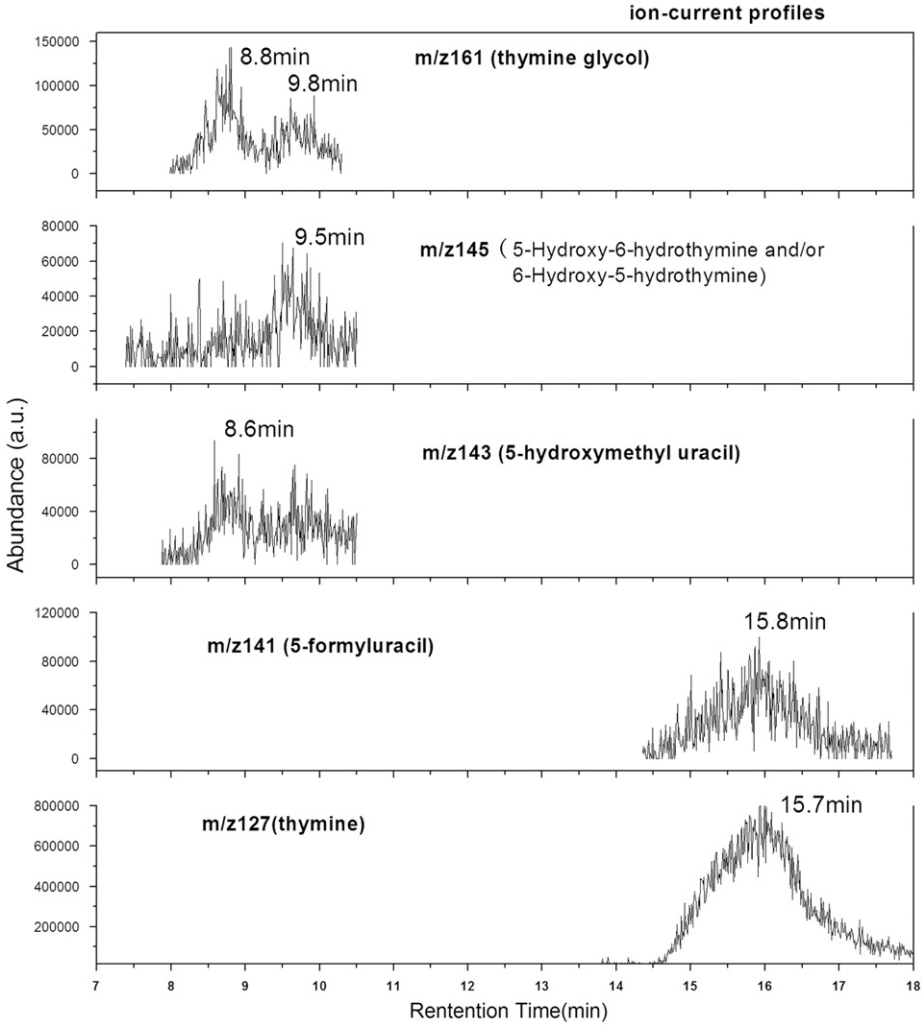


Fig. 8. Ion–current profiles at *m*/*z* 127 (thymine), *m*/*z* 161(thymine glycol), *m*/*z* 145 (5-hydroxy-6-hydrothymine and/or 6-hydroxy-5-hydrothymine), *m*/*z* 143 (5-hydroxymethyluracil) and *m*/*z* 141 (5-formyluracil) recorded during LC/MS-SIM analysis of the irradiated aqueous thymine sample (10 mM).

occurs in the absence of oxygen, followed by protonation to give 5-hydroxy-6-hydrothymine and 6-hydroxy-5-hydrothymine, respectively (Evans et al., 2004; Fujita and Steenken, 1981).

4.2. Comparison of radio-sensitivity of adenine and thymine to argon gas discharge

As shown in Figs. 7 and 10, the amounts of adenine and thymine decrease exponentially with discharge time, respectively. The fitting curves are obtained by the following equation:

$$\ln(C_0/C_t) = kt$$

where C_0 represents the concentration of primary adenine/thymine solution. C_t represents the concentration of adenine/thymine treated by gas discharge for t min, and k is a constant. To illustrate the difference regarding discharge radio-sensitivity, Fig. 11 plots the comparison result where $k_{adenine}=0.08366$ for adenine solution and $k_{thymine}=0.12344$ for thymine solution. Therefore, thymine is easier to be damaged by argon gas discharge than adenine.

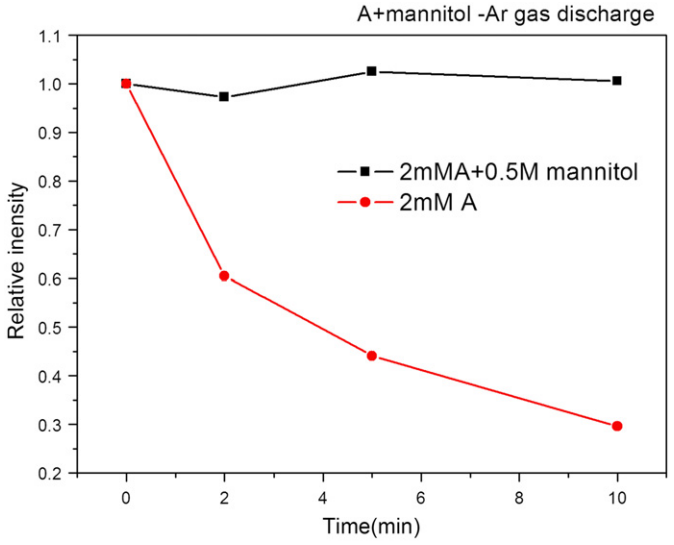


Fig. 10. Argon gas discharge induced degradation of aqueous adenine, with or without addition of mannitol. The content of adenine was estimated by measuring the absorbance at 260 nm.

Interestingly, this result is consistent with the result that thymine is more radio-sensitive than adenine when exposed to X-ray irradiation as reported by Scholes et al. (1960) and Hems (1960). In X-ray irradiation, thymine solution shows a higher G value (the number of molecules changed per 100 eV of absorbed radiation energy) (0.40) than that of adenine solution (0.22) under X-ray irradiation. Although adenine has a lower reduction potential (-2.52 V) than that of thymine (-2.18 V) (Seidel et al., 1996), it may be easily oxidized by $OH^{\bullet}$ radical than thymine; adenine has a back reaction mechanism, which leads to the re-formation of itself, i.e., as seen in Scheme 1, C4-OH-adduct radicals undergo dehydration and yield oxidizing purine(–H)* radical, which can reconstitute adenine upon reduction (Steenken, 1989; Vanhemme and Bleichro, 1971; Dizdaroglu et al., 2002; Scholes et al., 1960). This might explain why adenine shows less damage than thymine.

4.3. Influence of discharge accompanied UV radiation on radiolysis of aqueous bases

The plasma discharge is also a radiation source with a significant portion of the radiation in the UV region of the spectrum (Sun et al., 1998). Therefore, in our discharge experiment, there is inevitable ultraviolet irradiation accompanied with

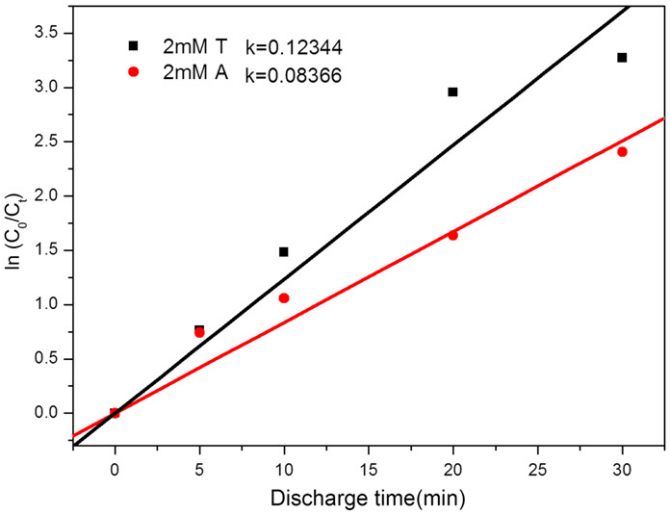
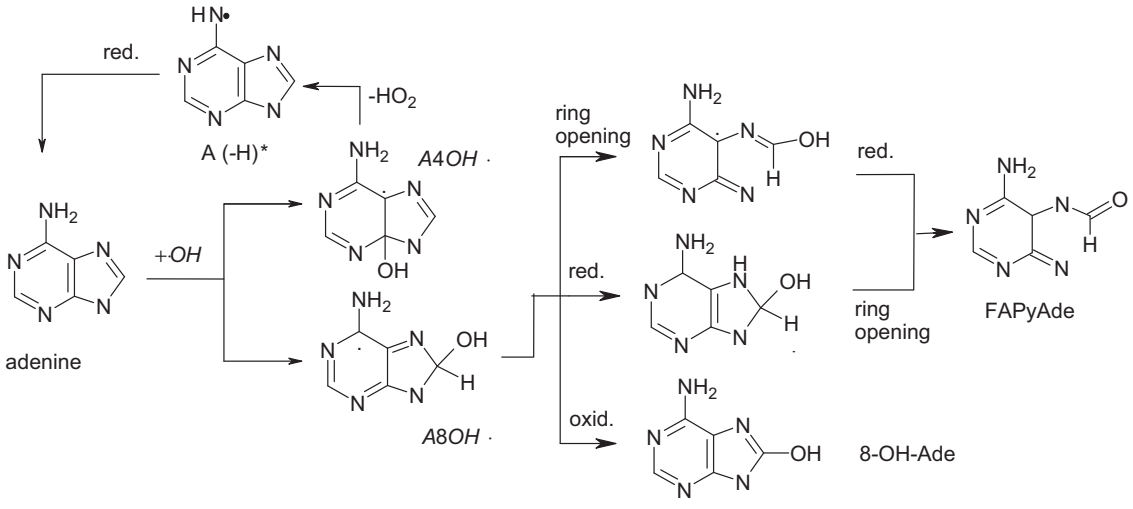


Fig. 11. Curves of $\ln(C_0/C_t)$ vs discharge time (t).



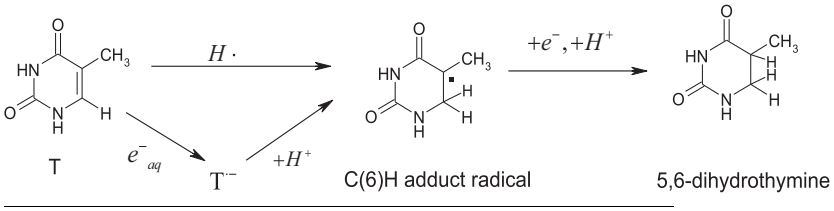
Scheme 1. Reactions and products for the radiolysis of aqueous adenine.

plasma discharge. This UV-irradiation can induce formation of dimeric pyrimidine photoproducts (Fisher and Johns, 1970; Cadet et al., 2005; St-Jacques et al., 2010; Douki et al., 2000; Sinha and Hader, 2002). However, in our experiments, the amounts of these products are too low to be detected by our current method by means of LC/MS. So compared to the hydroxyl radical attack, the damage from UV-induced radiolysis is insignificant during the short discharge time and thus is neglected in our quantitative analysis.

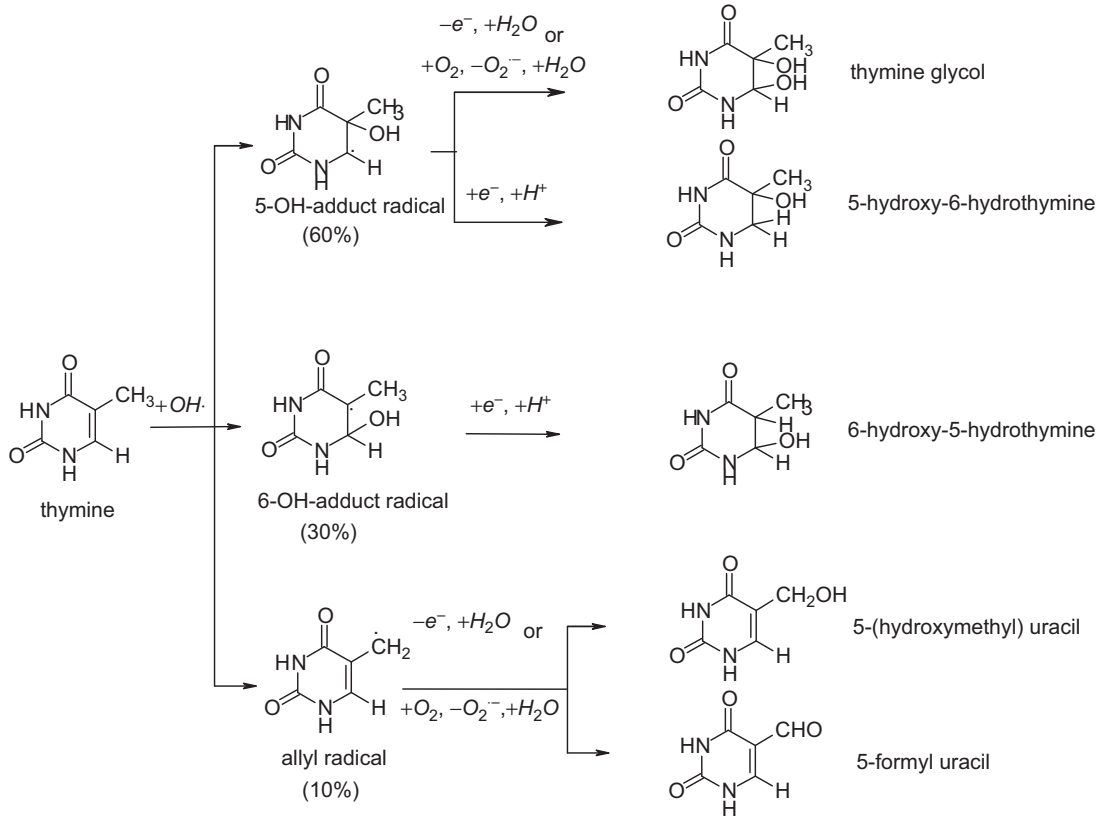
4.4. *Different radiolysis effect compared to other forms of ionizing radiations*

In gas discharge, the radiolysis of the aqueous bases leads to many new products. However, we did not detect dihydrothymine, which is abundant in radiolysis in other forms of ionizing radiations such as γ-ray radiation. For example, Ekert (1962) and Ali and Scholes (1979) found that γ-ray caused radiolysis of thymine in deaerated aqueous solution yielding hydroxymethyluracil and dihydrothymine. Wade et al. (1982) even gave the quantitative result in terms of *G*-values, and estimated about 34% of 5,6-dihydrothymine in all the radiolysis products, more than double of thymine glycol. Gajewski et al. (1990) investigated the modification of DNA bases in mammalian chromatin in aqueous suspension by γ-ray radiation, also found that the yield of 5,6-dihydrothymine is 0.48 ± 0.04 (nmol J^{-1}), exceeding ten times that of thymine glycol (0.045 ± 0.004).

This difference indicates different radiolysis pathways and mechanisms. In general, the radiation chemistry of aqueous pyrimidines indicates that the attack stems mainly from the primary species such as H atom, OH. and e^-_{aq} produced from water (Ali and Scholes, 1979). von Sonntag (1987) even quantified the contributions from these three species, i.e., hydroxyl radical (OH.) (yield: 0.28 μmol J^{-1}), hydrated electron (e^-_{aq}) (yield: 0.27 μmol J^{-1}) and hydrogen (H atom) (yield: 0.057 μmol J^{-1}) upon water exposed to γ-ray radiation in the presence of argon. However, the comparison of our discharge experiment to other ionizing radiation experiments show that the hydroxyl radical plays a dominant role under argon gas discharge, whereas e^-_{aq} and H radical may play an important role in other ionizing irradiations. Normally, the product of 5,6-dihydrothymine can be produced through the attack of thymine by the hydrated electron (e^-_{aq}) or hydrogen (H atom) (Breen and Murphy, 1995) due to the following reactions:



But this reaction via e^-_{aq} takes place very fast as revealed by the experiment of nanosecond pulse radiolysis of aqueous thymine solution (Theard et al., 1971). On X-ray and γ-ray irradiations,



Scheme 2. Reactions and products for the radiolysis of aqueous thymine.

the reaction rate of e_{aq}^- with thymine is $k = 1.5-1.8 \times 10^{10} M^{-1} s^{-1}$, much quicker than $8 \times 10^8 M^{-1} s^{-1}$ for the reaction of H atom with thymine (Loman and Blok, 1968). Yamamoto and Fuji (1985) demonstrated that the hydrated electrons are also involved in the decomposition of thymine in oxygen-free aqueous solutions (in γ-ray irradiation). However, in argon gas discharge experiment, more OH. radicals and H atoms can be produced (Hoeben et al., 2000; Lukes and Locke, 2005; Sun et al., 1998). Due to this the lifetime of e_{aq}^- is too short to take part in the reaction, and that H atoms produced in the discharge can be recombined as $H^{\cdot} + H^{\cdot} \rightarrow H_2$ (Breen and Murphy, 1995), therefore, in our discharge experiment, the contributions from these two species are negligible compared to that from hydroxyl radical.

5. Conclusions

It is important to understand how the DNA is damaged under indirect interaction of ionizing radiation, which is mediated through different radicals produced during the irradiation on the aqueous solution. In this work, we have investigated the argon gas discharge induced radiolysis of aqueous adenine and thymine and analyzed the involved reactions using spectroscopic tools combined with LC/MS analysis. It is verified that hydroxyl radical plays a dominant role in the argon discharge induced radiolysis of aqueous bases. The radiolysis of aqueous adenine leads to formation of FAPyAde, 8-OH-Ade and 2-OH-Ade and the radiolysis of aqueous thymine leads to formation of thymine glycol, 5-hydroxy-6-hydrothymine and/or 6-hydroxy-5-hydrothymine, 5-hydroxymethyluracil and 5-formyluracil. The analysis for the loss of adenine and thymine shows that adenine has lower radio-sensitivity than thymine. This work also demonstrates that combined use of spectroscopic tools can effectively dissect the involved radiolysis reactions and give quantitative assessment of the damage of the nucleotide bases under gas discharge induced indirect interaction through radicals.

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References

Alexander, A.J., Kebarle, P., Fuciarelli, A.F., Raleigh, J.A., 1987. Characterization of radiation-induced damage to polyadenylic-acid using high-performance liquid-chromatography tandem mass-spectrometry. Anal. Chem. 59 (20), 2484–2491.

Ali, K.M.I., Scholes, G., 1979. Radiolysis products analysis of aqueous thymine and N_2O solutions. J. Radiat. Res. 20, 146–156.

Berger, M., Cadet, J., Berube, R., Langlois, R., Vanlier, J.E., 1992. Reversed-phase high-performance liquid chromatography thermospray mass spectrometry of radiation-induced decomposition products of thymine and thymidine. J. Chromatogr. 593 (1–2), 133–138.

Breen, A.P., Murphy, J.A., 1995. Reactions of oxyl radicals with DNA. Free Radical Biol. Med. 18 (6), 1033–1077.

Cadet, J., Sage, E., Douki, T., 2005. Ultraviolet radiation-mediated damage to cellular DNA. Mutat. Res.—Fundam. Mol. Mech Mutagenesis 571, 3–17.

Cavalieri, L.F., Bendich, A., 1950. The ultraviolet absorption spectra of pyrimidines and purines. J. Am. Chem. Soc. 72 (6), 2587–2594.

Conlay, J.J., 1963. Effect of ionizing radiation on adenine in aerated and de-aerated aqueous solutions. Nature 197 (486), 555–557.

Cooke, M.S., Evans, M.D., Dizdaroglu, M., Lunec, J., 2003. Oxidative DNA damage: mechanisms, mutation, and disease. FASEB J. 17 (10), 1195–1214.

Deeble, D.J., von Sonntag, C., 1986. Radiolysis of poly(U) in oxygenated solution. Int. J. Radiat. Biol. 49 (6), 927–936.

Dizdaroglu, M., 1985. Application of capillary gas-chromatography mass-spectrometry to chemical characterization of radiation-induced base damage of DNA-implications for assessing DNA-repair processes. Anal. Biochem. 144 (2), 593–603.

Dizdaroglu, M., 1992. Measurement of radiation-induced damage to DNA at the molecular-level. Int. J. Radiat. Biol. 61 (2), 175–183.

Dizdaroglu, M., 1993. Quantitative-determination of oxidative base damage in DNA by stable isotope-dilution mass-spectrometry. FEBS Lett. 315 (1), 1–6.

Dizdaroglu, M., 1998. Mechanisms of free radical damage to DNA. In: Aruoma, O.I. (Ed.), DNA and Free Radicals: Techniques, Mechanisms and Applications, OICA International, Saint Lucia, pp. 3–26.

Dizdaroglu, M., Jaruga, P., Birincioglu, M., Rodriguez, H., 2002. Free radical-induced damage to DNA: mechanisms and measurement. Free Radical Biol. Med. 32 (11), 1102–1115.

Douki, T., Court, M., Cadet, J., 2000. Electrospray-mass spectrometry characterization and measurement of far-UV-induced thymine photoproducts. J. Photochem. Photobiol. B 54 (2–3), 145–154.

Ekert, B., 1962. Effect of γ-rays on thymine in de-aerated aqueous solution. Nature 194 (4825), 278–279.

Evans, M.D., Dizdaroglu, M., Cooke, M.S., 2004. Oxidative DNA damage and disease: induction, repair and significance. Mutat. Res. 567 (1), 1–61.

Fisher, G.J., Johns, H.E., 1970. Ultraviolet photochemistry of thymine in aqueous solution. Photochem. Photobiol. 11 (6), 429–444.

Frelon, S., Douki, T., Ravanat, J.L., Pouget, J.P., Tornabene, C., Cadet, J., 2000. High-performance liquid chromatography-tandem mass spectrometry measurement of radiation-induced base damage to isolated and cellular DNA. Chem. Res. Toxicol. 13 (10), 1002–1010.

Fujita, S., Steenken, S., 1981. Pattern of OH radical addition to uracil and methyl- and carboxyl-substituted uracils. Electron transfer of OH adducts with N,N,N′, N′-tetramethyl-p-phenylenediamine and tetranitromethane. J. Am. Chem. Soc. 103 (10), 2540–2545.

Gajewski, E., Rao, G., Nackerdien, Z., Dizdaroglu, M., 1990. Modification of DNA bases in mammalian chromatin by radiation-generated free radicals. Biochemistry 29 (34), 7876–7882.

Gu, J., Tian, A., Li, W.K., Wong, N.B., 2000. Intramolecular proton transfer in the tautomers of C8 oxidative adenine: a DFT study. J. Phys. Chem. B 104 (45), 10692–10698.

Hartmanna, J., Quint, R.M., Getoff, N., 2007. Radiolysis of aqueous adenine (vitamin B4) and 8-hydroxyadenine. Radiat. Phys. Chem. 76, 834–840.

Hems, G., 1960. Chemical effects of ionzing radiation on deoxyribonucleic acid in dilute aqueous solution. Nature 186 (4726), 710–712.

Hoeben, W.F.L.M., van Veldhuizen, E.M., Rutgers, W.R., Cramers, C.A.M.G., Kroesen, G.M.W., 2000. The degradation of aqueous phenol solutions by pulsed positive corona discharges. Plasma Sources Sci. Technol. 9, 361–369.

Huang, Q., Ke, Z.G., Su, X., Yuan, H., Zhang, S.Q., Yu, Z.L., 2010. Quantitative assessment of amino acid damage upon keV ion beam irradiation through FTIR spectroscopy. Plasma Sci. Technol. 12 (3), 378–384.

Kamiya, H., 2003. Mutagenic potentials of damaged nucleic acids produced by reactive oxygen/nitrogen species: approaches using synthetic oligonucleotides and nucleotides. Nucleic Acids Res. 31 (2), 517–531.

Ke, Z.G., Huang, Q., Dang, B.R., Lu, Y.L., Yuan, H., Zhang, S.Q., Yu, Z.L., 2010a. A study of low-energy ion induced radiolysis of thiol-containing amino acid cysteine in the solid and aqueous solution states. Nucl. Instrum. Methods Phys. Res. Sect. B 268 (17–18), 2729–2734.

Ke, Z.G., Huang, Q., Su, X., Jiang, J., Wang, X.Q., Yu, Z.L., 2010b. A paradigm study for assessment of phenylalanine damage under arc-discharge irradiation. Nucl. Instrum. Methods Phys. Res. Sect. B 268 (10), 1618–1625.

Loman, H., Blok, J., 1968. On the radiation chemistry of thymine in aqueous solution. Radiat. Res. 36, 1–13.

Lukes, P., Locke, B.R., 2005. Plasmachemical oxidation processes in a hybrid gas–liquid electrical discharge reactor. J. Phys. D: Appl. Phys. 38 (22), 4074–4081.

Miller, S.L., 1953. A production of amino acids under possible primitive earth conditions. Science 117 (3046), 528–529.

Oleinick, N.L., Chiu, S.M., Ramakrishnan, N., Xue, L.Y., 1987. The formation, identification, and significance of DNA–protein cross-links in mammalian cells. Br. J. Cancer Suppl. 8, 135–140.

Ponnamperuma, C., Calvin, M., Lemmon, R.M., 1963. Radiation decomposition of adenine. Radiat. Res. 18 (4), 540–551.

Powner, M.W., Gerland, B., Sutherland, J.D., 2009. Synthesis of activated pyrimidine ribonucleotides in prebiotically plausible conditions. Nature 459 (7244), 239–242.

Scholes, G., Stein, G., Weiss, J., 1949. Action of X-rays on nucleic acids. Nature 164 (4173), 709–710.

Scholes, G., Ward, J.F., Weiss, J., 1960. Mechanism of the radiation-induced dagradation of nucleic acids. J. Mol. Biol. 2 (6), 379–391.

Seidel, C.A.M., Schulz, A., Sauer, M.H.M., 1996. Nucleobase-specific quenching of fluorescent dyes. 1. Nucleobase one-electron redox potentials and their correlation with static and dynamic quenching efficiencies. J. Phys. Chem. 100 (13), 5541–5553.

Shi, H.B., Shao, C.L., Wang, X.Q., Yu, Z.L., 2001a. Preliminary study on the damage effects of low energy N^+ for cytisine solution. J. Radiat. Res. Radiat. Process. 19 (3), 231–235.

Shi, H.B., Shao, C.L., Wang, X.Q., Yu, Z.L., 2002a. Analysis of the spectrum of damage effects of low energy N^+ ions on adenine aqueous solution. Spectrosc. Spectral Anal. 22 (2), 278–280.

Shi, H.B., Shao, C.L., Yu, Z.L., 2001b. Preliminary study on the way of formation of amino acids on primitive earth under non-reducing conditions. Radiat. Phys. Chem. 62, 393–397.

Shi, H.B., Shao, C.L., Yu, Z.L., 2001c. Synthesis of amino acids by arc-discharge experiments. Nucl. Instrum. Methods Phys. Res. Sect. B 183 (5–6), 369–373.
Shi, H.B., Shao, C.L., Yu, Z.L., 2002b. The preliminary study on the chemical synthesis between graphite and water by glow-discharge experiments. Radiat. Phys. Chem. 64 (4), 289–292.
Sinha, R.P., Hader, D.P., 2002. UV-induced DNA damage and repair: a review. Photochem. Photobiol. Sci. 1 (4), 225–236.
St-Jacques, A., Anichina, J., Schneider, B.B., Covey, T.R., Bohme, D.K., 2010. UV-induced bond modifications in thymine and thymine dideoxynucleotide: structural elucidation of isomers by differential mobility mass spectrometry. Anal. Chem. 82 (14), 6163–6167.
Steenken, S., 1989. Purine bases, nucleosides, and nucleotides: aqueous solution redox chemistry and transformation reactions of their radical cations and e^- and OH adducts. Chem. Rev 89 (3), 503–520.
Sun, B., Sato, M., Harano, A., Clements, J.S., 1998. Non-uniform pulse discharge-induced radical production in distilled water. J. Electrostat. 43 (2), 115–126.
Teoule, R., Bonicel, A., Bert, C., Cadet, J., Polverel., M., 1974. Identification of radioproducts resulting from breakage of thymine moiety by gamma-irradiation of *Escherichia coli* DNA in an aerated aqueous-solution. Radiat. Res. 57 (1), 46–58.
Theard, L., Peterson, F., Myers, J., L., 1971. Nanosecond pulse radiolysis studies of aqueous thymine solutions. J. Phys. Chem. 76 (26), 3815–3821.
Theruvathu, J.A., Jaruga, P., Dizdaroglu, M., Brooks, P.J., 2007. The oxidatively induced DNA lesions 8,5′-cyclo-2′-deoxyadenosine and 8-hydroxy-2′-deoxyadenosine are strongly resistant to acid-induced hydrolysis of the glycosidic bond. Mech. Ageing Dev. 128, 494–502.
Vanhemme, J.J., Bleichro, J.F., 1971. Decomposition of adenine by ionizing radiation. Radiat. Res. 46 (3), 444–456.
Vieira, A., Steenken, S., 1990. Pattern of OH radical reaction with adenine and its nucleosides and nucleotides-characterizaion of two types of isomeric OH adduct and their unimolecular transformation reactions. J. Am. Chem. Soc. 112 (19), 6986–6994.
von Sonntag, C., 1981. γ-radiolysis of DNA in oxygenated aqueous solution: alterations at the sugar moiety. Int. J. Radiat. Biol. 40 (4), 347–354.
von Sonntag, C., 1984. Carbohydrate radicals: from ethylene glycol to DNA strand breakage. Int. J. Radiat. Biol. 46 (5), 507–519.
von Sonntag, C., 1987. The Chemical Basis of Radiation Biology. Taylor and Francis, New York.
Wade, T., Ide, H., Nishimoto, S.-i., Kagiya, T., 1982. Radiation-induced hydroxylation of thymine sensitized by nitro compounds in N_2O-saturated aqueous solution. Chem. Lett. 11 (7), 1041–1044.
Wallace, S.S., 2002. Biological consequences of free radical-damaged DNA bases. Free Radical Biol. Med. 33 (1), 1–14.
Ward, J.F., Kuo, I., 1973. Deoxynucleotides-models for studying mechanisms of strand breakage in DNA. II. Thymidine 3′5′-diphosphate. Int. J. Radiat. Biol. 23 (6), 543–557.
Yamamoto, O., Fuji, I., 1985. Degradation of thymine in aqueous solution containing 3HHO. Comparison with 60Co gamma-radiolysis. J. Radiat. Res. 26, 257–268.
Yu, Z.L., 2006. Introduction to Ion Beam Biotechnology. Springer, New York.
Yu, Z.L., Han, J.W., Wang, X.Q., Huang, W.D., Shao, C.L., 1998. The role of radiation in the origin and evolution of life. Viva Origino 26, 175.