Nuclear Science and Techniques **20** (2009) 223–227

The protective effect of caffeine on DNA photosensitive damage: A gel electrophoresis

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Abstract Agarose gel electrophoresis was performed to study interaction effect of caffeine on photosensitive injury of DNA caused by anthraquinone-2-sulphonic acid disodium (AQS), a model compound of strong photosensitizer, under 254 nm or 365nm UV irradiation Photosensitive injury of DNA induced by AQS under deoxidized condition was used as control. The results show that caffeine may resist effectively the injury effect of photosensitive damage and strong UV irradiation on DNA. The effects depend on the caffeine and AQS concentration, and irradiation time. Caffeine in concentration of 0.01–3.0 μg/μL, may prevent DNA from damage induced by UV light, but caffeine in concentration of >5.0 μg/μL accelerates the DNA damage. In particular, in the aqueous solution system of DNA, caffeine and AQS, at pH 6.25–7.35, the caffeine in concentration of 2.5–4.50 μg/μL may resist the photosensitive injury of DNA caused by AQS under the deoxidized condition and exposure by 254 nm UV for 10 min. And caffeine in concentration of 5 μg/μL would present a synergetic effect on the photosensitive injury of DNA. Possible molecular mechanism also is discussed.

Key words Caffeine, DNA, Photosensitive injury, Effect, Agarose gel electrophoresis

1 Introduction

DNA, the basic genetic substance in living organism, is submitted to attacks by various environmental factors. Therefore, it can be injured. This injury may occur due to DNA metabolism, chemical (reagents) induction, ionizing radiations, and activated oxygen. The photosensitive oxidizing injury of DNA may lead to generation of DNA cations and anions of photosensitizer as free radicals involved in the process[1–3]. DNA injury would affect replication and transcription of DNA and protein synthesis, hence the further effects on growth, development, heredity, metabolism, reproduction and other processes of cells. A great deal of medicine acts on living organism at the gene level, and the actions are related closely with DNA injury and repair.

It is of practical and theoretical significance to seek for natural substances that may prevent effectively DNA injury against radiations, oxidation, aging, cancer and cancer treatments. Caffeine, a psycho-stimulant of common use, exists mainly in tea leaves and coffee. There have been some reports on biological activity of caffeine. To the authors' knowledge, however, there is no report about the study on function of caffeine in preventing photosensitive injury of DNA. In this paper, we use Agarose electro phoresis to study the effect of caffeine in preventing DNA from injury by UV plus strong photosensitizer of anthraquinone-2-sulphonic acid disodium (AQS)[4–7], in an attempt to find scientific basis for exploring the biological function and application of caffeine.

2 Materials and methods

2.1 Equipments and reagents

UV-vis light spectrophotometer (CA-RY50, South East Chemical & Instrument Ltd., China); gel imaging analytic system (Vilber Lourmat, Bioprofile, Germany); DNA/protein analyzer (DV-640, Beckman

Supported by Natural Science Foundation of Fujian Province, China (2009J01032), Education Committee of Fujian Province, China (JA081336) and Specialized Foundation for Creative Team of Young and Middle-aged Teachers of Jimei University (2006A003).

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Received date: 2009-05-06

Coulter, USA); dark box UV analyzer (ZF-20C, Shanghai Gucun Electron Optic Instrument Factory, China); constant-voltage electrophoresis apparatus and horizontal electrophoretic tank (DYCP-3ID, Beijing LiuYi Instrument, China); microliter pipette (20001010, 0.5–10 μL, 5–25 μL, 100 μL, 200 μL, with the attached tips); automatic distiller of dually pure water (SZ-93, Shanghai Yarong Biochemical Instrument, China); precision pH meter (PHS-2C, Shanghai Rex Instrument, China).

Agarose (A4018, A6877), Ethidium bromide (EB), calf thymus DNA, and caffeine were purchased from Sigma Co., Ltd.; TRIS: ultra pure (BBI, Canada); AQS, ascorbic acid, green vitriol, HCl, NaOH and 30% H_2O_2 were analytical reagents (AR).

2.2 Methods

The samples were UV-irradiated (254 nm, 20 min) DNA, DNA+AQS, DNA+caffeine, DNA+AQS+caffeine, and DNA+AQS+caffeine+Fe^{2+}, in certain concentration. Photosensitive injury of DNA due to AQS was taken as control. The system of DNA+caffeine+AQS was used to study the effect of caffeine in preventing or reducing the photosensitive injury of DNA caused by AQS as photosensitizer. AQS may break DNA under UV light exposure when the AQS is transferred into its excited triplet state, and the oxygen should be removed by bubbling the solution with N_2. But the UV-induced triplet state of AQS can be quenched when oxygen and metal ions exist[1]. Therefore, our results were obtained with the oxygen being removed by Fe^{2+}.

2.3 Sample loading

Each sample was prepared with 10–20 μL aqueous solution of DNA (containing about 0.5–10.0 μg of DNA) mixed with 60–120 μL buffer, then supplemented with several μL of caffeine (the volume was determined by the size of the loading well and the sample concentration, and the total volume of the loaded sample being about 10 μL in general). The mixed liquid (consisted of loading buffer, caffeine, and UV-irradiated DNA, DNA+AQS, DNA+AQS+caffeine, DNA+AQS+caffeine+Fe^{2+} and controls) was added to the sample cell by the pipette. With a pipette over a loading well, the sample was pushed in slowly so that it might be concentrated at the bottom of well.

During the irradiations, the covers of PCR tubes were closed. Ultrapure water, boiled for 5 min and cooled to the room temperature, was used, and will be termed as water hereinafter.

3 Results and discussion

3.1 Agarose gel electrophoresis showing the protecting effect of caffeine on DNA

The electrophoretic procedures were derived after a number of early stage tests. The results show that caffeine of certain concentration does not break DNA irrespective of presence or absence of oxygen. In order to confirm whether caffeine can prevent DNA from oxidative injury by photosensitizers, a series of experimental systems have been prepared.

Considering that AQS may break DNA only under the deoxidized condition, we selected the deoxidized condition in subsequent tests for obtaining comparable results. At beginning we used ascorbic acid to remove oxygen, by using icy water to control the temperature of the whole reaction system under 10℃, as ascorbic acid shows a good effect of oxygen removal only under 10℃. However, the operation was difficult. In addition, ascorbic acid itself will injure DNA when its concentration exceeds a definite limit. Addition of Fe^{2+} was chosen to remove oxygen.

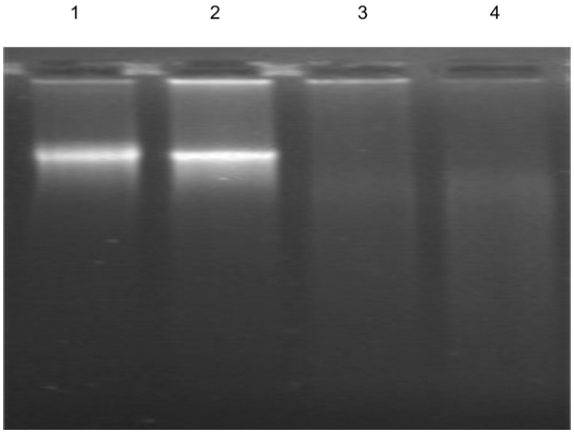

Fig.1 DNA systems irradiated by 254-nm UV for 20 min.
Agarose, 0.1441g; GE time, 90 min;
DNA concentration, 0.12 μg/μL, 5 μL;
Fe^{2+}, 0.5 μg/μL, 2 μL.
Lane 1, DNA+water, 8 μL;
Lane 2, DNA+water (1 μL)+caffeine (0.2 μg/μL, 5 μL)+Fe^{2+};
Lane 3, DNA+water (1 μL)+AQS (0.3 μg/μL, 3 μL)+Fe^{2+};
Lane 4, DNA+water (1 μL)+AQS (0.2 μg/μL, 5 μL)+Fe^{2+}.

Gel electrophoretograms (GE) of DNA, DNA+ AQS and DNA+caffeine exposed to UV (254 nm) light are shown in Fig.1. Band 2 is more distinct and brighter than Band 1 (DNA+water), indicating that the caffeine does not harm the DNA, and it may prevent DNA effectively from the injury caused by 254 nm UV irradiation when oxygen is removed by $FeSO_4$. By comparing Band 3 with Band 4, it can be seen that AQS oxidized DNA completely for both the oxidized and deoxidized.

3.2 Protective effect of caffeine on DNA

Fig.2 shows that Fe^{2+} in concentrations of (1 μg/μL, 0.5 μL), (1 μg/μL, 0.7 μL), and (1 μg/μL, 0.9 μL) is a good oxygen remover. The residual bands of DNA occur near the loading well where Fe^{2+} concentration is higher. It can be explained that Fe^{2+} is oxidized by oxygen to form $Fe(OH)_3$ colloid, which has positive charges and moves to cathode during the electrophoretic process, leading to the existence of bands near the loading well. It can be seen that the residual Bands 4–8, where Fe^{2+} concentration is (1 μg/μL, 0.9 μL), tend to leave the loading well. Considering that at low concentration (1 μg/μL, 0.5 μL) the oxygen removal effect may not be comparable with that of Fe^{2+} in concentration of 1 μg/μL, 0.9 μL, Fe^{2+} in concentration of 1 μg/μL, 0.7 μL were used to remove oxygen in subsequent tests.

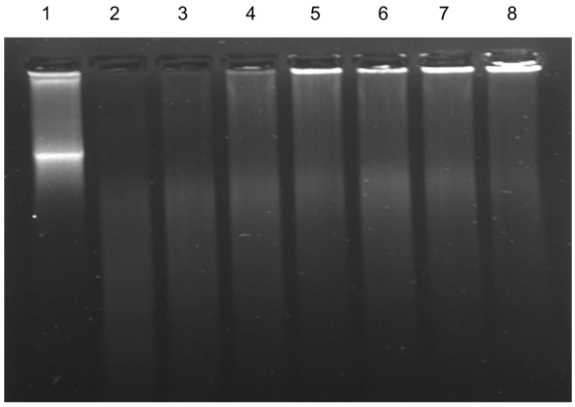

Fig.2 Tests for optimal Fe^{2+} concentration for oxygen removal.
DNA content, 0.12 μg/μL, 5 μL; AQS, 0.1 μg/μL, 5 μL.
Lane 1, DNA+water, 7 μL;
Lane 2, DNA+AQS+Fe^{2+} (1 μg/μL, 0.5 μL);
Lane 3, DNA+AQS+Fe^{2+} (1 μg/μL, 0.7 μL);
Lane 4, DNA (0.12 μg/μL, 5 μL)+AQS (0.1 μg/μL, 5 μL) +Fe^{2+} (1 μg/μL, 0.8 μL);
Lanes 5–8, DNA (0.12 μg/μL, 5 μL)+AQS (0.1 μg/μL, 5 μL) +Fe^{2+} (1 μg/μL, 0.9 μL)

3.3 Interaction between caffeine and DNA under deoxidized condition

Fig.3 shows the effect of caffeine in different concentrations on DNA. The 0.01–3.0 μg/μL caffeine intensifies the brightness of bands of DNA, especially for Bands 5, 8, and 9, indicating that caffeine exactly prevents DNA from the injury caused by strong UV light. When the caffeine concentration exceeds 5.0 μg/μL, the DNA damage becomes very serious as shown in Well 15. The last two bands, Band 16 and 17, produced in DNA+AQS, disappeared totally under the deoxidized condition. The fact reveals that AQS has photosensitive effects while caffeine in suitable concentration has protection effects involved in photopdamage of DNA exposed to UV light.

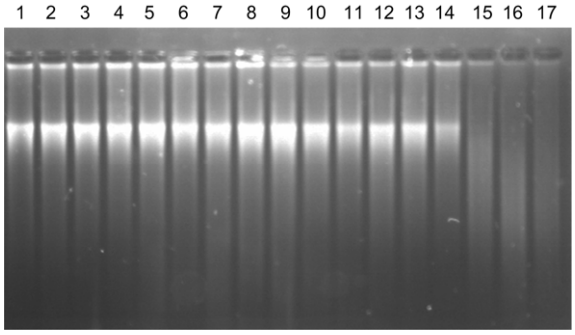

Fig.3 UV (254 nm) irradiated DNA systems containing caffeine in concentrations of 0.01, 0.05, 0.1, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 μg/μL for Lane 2–14 (5 μL each), respectively.
Irradiation, 20 min for all but Band 16 (40 min).
Agarose, 0.2880 g; GE time, 90 min;
DNA content, 0.12 μg/μL, 5 μL; Fe^{2+}, 1 μg/μL, 0.7 μL.
Lane 1, DNA (0.12μg/μL, 5μL)+Fe^{2+};
Lanes 2–14, DNA+caffeine+Fe^{2+};
Lane 15, DNA+AQS (0.1 μg/μL, 1 μL) +caffeine (>5.0 μg/μL)+water (4.3 μL)+Fe^{2+};
Lanes 16 and 17, DNA+AQS (0.1 μg/μL, 0.7 μL) +water(4.3 μL)+Fe^{2+}.

3.4 Caffeine protective effect under different minutes of 254 nm UV irradiation

Fig.4 shows the GE result of DNA (0.12 μg/μL, 5 μL) systems irradiated for different minutes by 254 nm UV light. Bands 2–6 (0–20 min irradiation) are distinct and bright, while Bands 7–15 (25–65 min irradiation) disappeared completely. This means that caffeine may be decomposed when it is in the protection action and can be exhausted completely under longer time irradiation. It also means that the photosensitive damage of DNA in the system of DNA+AQS+Fe^{2+} is more serious than that of DNA+ Fe^{2+}exposed to

254-nm UV light. Considering that AQS may have injury effect to DNA under longer time UV irradiation, the irradiation time was set at 10 min (for the most distinct Band 4) in subsequent tests.

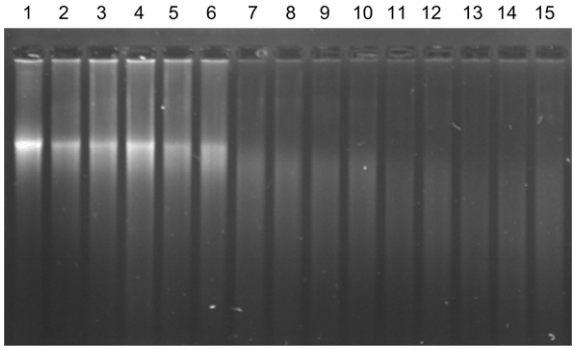

Fig.4 DNA (0.12 μg/μL, 5 μL) systems irradiated by 254-nm UV for 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60 and 65 min of Lane 1–15, respectively.
Agarose, 0.2882 g; GE time, 100 min.
Lane 1, DNA+water (6.4 μL);
Lanes 2–15, DNA+caffeine (4 μg/μL, 5 μL)
+AQS (0.05 μg/μL, 0.7 μL)+Fe^{2+} (1 μg/μL, 0.7 μL).

3.5 The system of DNA+Caf+AQS+Fe^{2+} at 37℃

In order to investigate the temperature effect on the caffeine protection of human DNA, the tests were carried out at 37℃, the close-to-body temperature (Fig.5). The caffeine shows the same antagonist effect in preventing DNA from injury by strong UV irradiation (Bands 6–9) and by photosensitizers (Bands 14–15 and Bands 17–18) at 37℃.

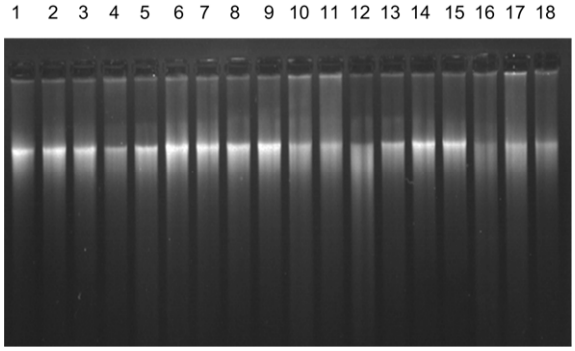

Fig.5 Suitability of caffeine system at 37℃, the close-to-the-body temperature. Bands 2–18 were incubated at 37℃ for 30 min, bands with even numbers were irradiated by 254-nm UV for 10 min.
Agarose, 0.2884 g; GE time, 100 min;
DNA content, 0.12 μg/μL, 5 μL; caffeine, 4 μg/μL, 5 μL;
AQS, 0.05 μg/μL, 0.7 μL; Fe^{2+}, 1 μg/μL, 0.7 μL.
Lanes 1–3, DNA+water (6.4 μL);
Lanes 4 and 5, DNA+AQS+water (5.7 μL);
Lanes 6–9, DNA+caffeine+water (1.4 μL);
Lanes 10–13, DNA+AQS+Fe^{2+};
Lanes 14 and 15, DNA+caffeine+AQS+water (0.7 μL);
Lane 16, DNA+AQS+Fe^{2+};
Lanes 17 and 18, DNA+caffeine+AQS+Fe^{2+}.

3.6 pH effect on the DNA+ Caf +AQS+Fe^{2+} system

As shown in Fig.6, pH value of the DNA+caffeine+AQS+Fe^{2+} system affects greatly the prevention of photosensitive injury of DNA. At pH 6.25–7.35 (Bands 5–10), the prevention of photosensitive injury of DNA is remarkable. As Fe^{2+} oxidized into Fe^{3+}, therefore the residual bands in loading Bands 11–14 may be attributed to the interaction between Fe(OH)3 colloid and DNA. According to Sigma's specification about calf thymus DNA, the DNA sample should avoid ultrasound treatment and agitation as much as possible while preparing and using, and it should not be kept in an alkaline solution at pH > 8.0, otherwise it may be degraded. Therefore the test for determining the effect of pH should be performed with caution. However, the bands shown in 2–4 wells represent the serious damage of DNA. It means caffeine has no protection action in acidic solution or DNA should be damaged in acidic solution and the reason should be explored further.

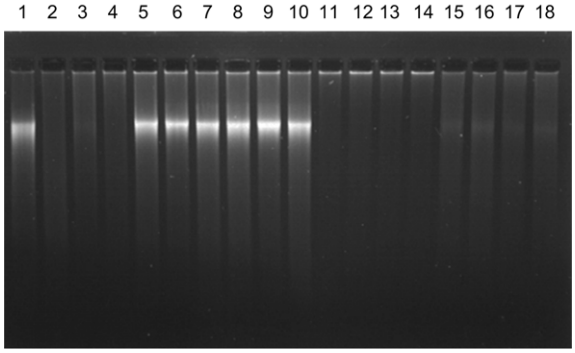

Fig.6 Effect of pH on the reaction system of DNA+ caffeine +AQS+Fe^{2+}.
Irradiation, 254 nm UV, 10 min, for Bands 2, 4, 5, 7, 8, 10, 12, 14, 16 and 18; Agarose, 0.2889 g; GE time, 100 min.
DNA content, 0.12 μg/μL, 5 μL; caffeine, 4 μg/μL, 5μL;
AQS, 0.05 μg/μL, 0.7 μL; Fe^{2+}, 1 μg/μL, 0.7 μL.
Lane 1, DNA + water, 6.4 μL;
Lanes 2–18, DNA+caffeine+AQS+Fe^{2+};
Lanes 2–4, pH=3.80; Lanes 5–7, pH=6.25;
Lanes 8–10, pH=7.35; Lanes 11–14, pH=7.55;
Lane s 15–18, pH=8.10.

3.7 Effect of 365-nm UV on caffeine containing protective system of DNA

UV light of 365 nm was used, too, to study the caffeine protection (Fig.7). All bands of the DNA systems irradiated by 365 nm UV light are bright. This may be associated with UV absorption of caffeine, DNA and AQS, because the maximal wavelength of UV absorbed by the three targets is 274.9 nm, 260.0

nm, and 254.9 nm respectively. In addition, the 365 nm absorption is weak. This means that 365 nm UV makes weak injury to DNA, and AQS does the same in a system free from oxygen (see Band 11). However, Bands 2, 3 and 5–8 are brighter, indicating that caffeine can effectively prevent DNA from UV photo-induced damage.

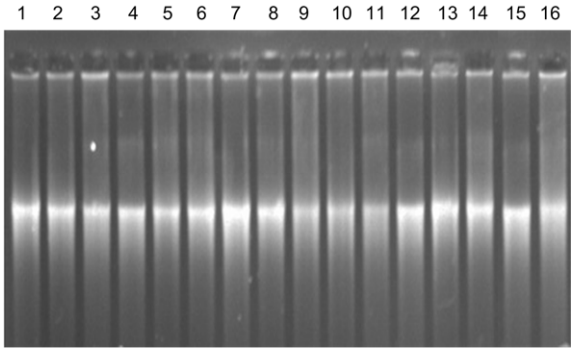

Fig.7 Caffeine protective effect in DNA system irradiated by 365 nm UV light.
Irradiation, 10 min for bands with odd numbers.
Agarose, 0.2884 g; GE time, 100 min.
DNA content, 0.12 μg/μL, 5 μL; caffeine, 4 μg/μL, 5 μL;
AQS, 0.05 μg/μL, 0.7 μL; Fe^{2+}, 1 μg/μL, 0.7 μL.
Lane 1, DNA+water, 6.4 μL;
Lane 2, DNA+water (5.7 μL)+Fe^{2+};
Lanes 3–5, DNA+AQS+water (5.7 μL);
Lanes 6–8, DNA+caffeine+water (1.4 μL);
Lane 9, DNA+AQS+water (0.7 μL)+Fe^{2+};
Lanes 10 and 11, DNA+AQS+water (5 μL)+Fe^{2+};
Lane 12, DNA+caffeine+water (0.7 μL)+Fe^{2+};
Lanes 13 and 14, DNA+caffeine+AQS+water (0.7 μL);
Lanes 15 and 16, DNA+caffeine+AQS+Fe^{2+}.

4 Discussion

AQS is a well-known model compound of strong photosensitizers. There have been many reports about the photosensitive injury of biological macromolecules caused by AQS exposed to lights. AQS can be transferred into excited triplet state in an oxygen-free system exposed to 254-nm UV. The excited triplet state of AQS can seize electrons from DNA to from AQS radical anions and DNA cations as free radicals[1]. In the reaction system of caffeine +DNA+AQS+Fe^{+2}, the excited triplet state of AQS ($^3AQS^*$) can be produced. The caffeine molecule is of planar configuration and its nitrogen atom at 9^{th} position is alkaline, which loses an electron easily. It means that caffeine can quench the $^3AQS^*$ *via* one electron transfer from caffeine to $^3AQS^*$rapidly. This prevents the DNA from photo-oxidation or electron transfer oxidation.

Caffeine in the system of DNA+caffeine shows no injury effect on DNA when oxygen is removed by $FeSO_4$. By comparing the reaction system of DNA+caffeine +AQS+Fe^{2+} with that of DNA+AQS+Fe^{2+}, we can see that caffeine can prevent the DNA from both the direct damage of DNA induced by 254 nm light and photosensitive damage of DNA induced by excited state of AQS.

The maximal wave lengths of UV absorbed by caffeine, calf thymus DNA and AQS are 274.9 nm, 260.0 nm, and 254.9 nm, respectively. Therefore, 254-nm UV as irradiation source is more effective than 365-nm UV in the photosensitive damage of DNA. In system of DNA+caffeine+AQS+Fe^{2+}, DNA is degraded almost entirely under 254 nm UV irradiation time of over 20 min, and caffeine is degraded, too.

Caffeine in system of DNA+caffeine+AQS+Fe^{2+} is capable of preventing the photosensitive damage of DNA at 37℃. This offers a convincing scientific basis at the molecular level for explaining why caffeine of suitable concentration may protect the human body.

The caffeine protection of DNA against its photosensitive injury is affected greatly by pH value of AQS in the system of DNA+caffeine +AQS+Fe^{2+}.

The study suggests that the appropriate amount caffeine, the world's most widely used refreshing substance coming mainly from coffee and tea, may be useful to health as a protector of DNA, but its excess use may be harmful to the human body in the molecular level study.

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Supported by Natural Science Foundation of Fujian Province, China (2009J01032), Education Committee of Fujian Province, China (JA081336) and Specialized Foundation for Creative Team of Young and Middle-aged Teachers of Jimei University (2006A003).

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Received date: 2009-05-06