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Transient spectra study on photo-dynamics of curcumin

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ABSTRACT

A novel mechanism of DNA damage induced by photosensitized curcumin (Cur) was explored using laser flash photolysis, pulse radiolysis and gel electrophoresis. Cur neutral radical (Cur•) was confirmed as an identical product in photo-sensitization of Cur by laser flash photolysis and pulse radiolysis. A series of reaction rate constants between Cur• and nucleic acid bases/nucleotides were determined by pulse radiolysis. Gel electrophoresis was carried out to investigate damage induced by photosensitized Cur to biologically active DNA. The results indicate that the damage to DNA may be caused by Cur• produced from the photosensitization of Cur.

1. Introduction

Curcumin has been used as a phytochemical agent in Asia traditional medicine to treat ailments like wounds [1], abdominal disorders [2], and eye or skin infections [3] for over 4000 years. It has anti-oxidizing [4], cytotoxic [5], chemo-preventive, anti-inflammatory [6] and pharmacological properties and is non-toxic to human even at high dose of 10 g/day [7]. Cur has been found to physically inhibit the activity of DNA, enzymes and growth factor receptors, etc. [8]. And Cur is sensitive in binding in minor groove of double helix of DNA [9].

Recently, literatures [10,11] indicate promising insights into the multiple mechanisms for Cur–mediated Photodynamic Therapy (PDT) photo-toxicity effects on microorganism with 8.2 ± 0.2 μM and 40 mM Cur respectively, Cur shows remarkable photo-toxicity to *Staphylococcus aureus* [12], *Escherichia coli* [13] and *Candida albicans* [14] exposed to 400–700 nm light. PDT drugs are activated through losing or accepting electron. PDT induces a sophisticated response like apoptosis or programmed cell death. It can destroy mitochondrial membrane and initiate apoptosis of cells [15,16]. The destructive reactions are contributed partly to the formation of free radicals from degradation of photo-sensitizer [17], and partly to the formation of ROS such as singlet oxygen ($^1O_2^*$) and hydroxyl radicals (•OH) from the interaction of photo-sensitizer with O_2.

Up to now, the effect of $^1O_2^*$ was usually proposed to be associated with Cur-induced photo-damage to biomolecules. But all evidences that prove Cur could cause photo-induced damage are only located on superficial layer of cells with plenty of O_2 [18–25]. Furthermore, it is still unclear whether there are other transient species derived from photo-chemistry of Cur participating in its induced photo-damage.

Radical Cur formed from photo-ionization can be observed in laser flash photolysis in our previous study [26]. Therefore, Cur radical may be also involved in photosensitized damage of biological molecules which can exactly attack the biological molecules.

Pulse radiolysis is a special method to investigate radical reactions using acetonitrile-water mixture solution system [27,28]. Transient species, neutral radical and radical cation of Cur can be formed from reaction with •OH. But till now the damage caused by Cur• radical to biomolecules, especially DNA components is not reported in literature.

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In this study, gel electrophoresis was used to investigate the DNA damage induced by photosensitized Cur on exposure to 430 nm light. Laser flash photolysis and pulse radiolysis was used to explore the reaction mechanism of Cur• with DNA components in order to understand anticancer potential and other pharmacology of Cur photosensitization.

2. Experimental materials

2.1. Chemicals

Curcumin (Cur), nucleic acid components (adenine (A), adenosine (Ad), guanine (G), thymine (T), thymidine (Th), deoxyguanylic acid (dG)), ethanol (95%), potassium rhodanate (KSCN) were purchased from Sigma-Aldrich. All chemicals were analytically pure while acetonitrile was spectroscopically pure and used as received without further purification. All solutions were prepared freshly with triple distilled water or mixed with acetonitrile and then saturated with high-purity N_2, N_2O or O_2 (99.999%) for 20 min before each experiment. And all the experiments were conducted at room temperature.

2.2. Solution systems for different experiments

Ethanol-water is usually taken as solvent of Cur in photolysis study. In our previous laser flash photolysis of ethanol-water solution, Cur and $^1O_2^*$ were formed via excited energy transfer from Cur triplet ($^3Cur^*$) to O_2 [26]. Instead of ethanol, acetonitrile-water mixture solution have been well studied by laser flash photolysis and pulse radiolysis [27–29] because biological substances(especially DNA components) and Cur can dissolve in water and acetonitrile, respectively. Considering certain toxicity of acetonitrile to biological targets, we used DMSO-water mixture solution in electrophoresis study [30,31]. According to previous report [24], photolysis and radiolysis of Cur in DMSO-water and acetonitrile-water system led to the formation of both $^3Cur^*$ and Cur•, and there was no great difference. So the reactions of Cur• with water soluble nucleic acid components were all carried out with 5:5 acetonitrile-water mixture solution in pulse radiolysis experiment meeting with the high Cur soluble concentration to 5 mM.

2.3. Determining pKa values of cur in acetonitrile-water solution

Acetonitrile-water (5:5, v/v) mixture solution containing 0.02 mM Cur was prepared. Drops of different concentration of $HClO_4$ or NaOH aqueous solution were used to adjust pH of the mixture solution. The pH was constantly monitored using a glass electrode and the absorbance of a series of Cur mixture solutions were measured immediately after pH adjustment. The titration curves were fitted by a sigmoidal function and analyzed by nonlinear least square methods. pKa values of Cur in mixture solution were obtained according to the titration curves.

2.4. Laser flash photolysis

Laser flash photolysis of 5:5 acetonitrile-water mixture solution has been conducted to explore its photochemical performance. A Nd:YAG laser (Laser NL 301 series, EKSPLA Co., Lithuania) was used to carry out laser flash photolysis experiments. The system consists of a Q-switched energy of 36 mJ at 355 nm per pulse with 5 ns duration as the excitation source. A xenon lamp was employed as detecting light source with an enhanced pulse width of 2 ms for photolytic detection to improve S/N ratio. The laser and analytical lights passed perpendicularly through a quartz cell with an effective absorption optical length of 10 mm. The transmitted light entered a monochromator equipped with an R955 photomultiplier. The output signals recorded with a HP54510B digital oscillograph were transferred to PC for processing using domestic software [32]. Detailed descriptions of the facility have been provided elsewhere [33].

2.5. Pulse radiolysis

Pulse radiolysis experiments were conducted using a linear accelerator providing a 10 MeV electron beam pulse with duration of 8 ns. A standard 100 mM N_2O-saturated KSCN aqueous solution as a thiocyanate dosimeter was used to estimate the absorbed doses of sample by taking the $\varepsilon_{480\ nm} = 7600\ dm^3\ M^{-1}\ cm^{-1}$ for $(SCN)_2^{\bullet-}$. The dose of every electron pulse was about 10 Gy. A 300 W xenon lamp was employed as the detecting light source. The electron pulse and the analyzing light beam passed perpendicularly through a quartz cell with an optical length of 10 mm. The transmitted light entered a monochromator equipped with a Hamamatsu R955 photomultiplier. The output signals recorded by a digital oscillograph were transferred to a computer for further treatment with domestic software. Detailed technical descriptions of the pulse radiolysis equipment and experimental conditions have been previously reported [34].

As shown in the following equation, the radiolytic products of water include solvated electron (e_{sol}^-), •OH, hydrogen atom (H), hydronium ion (H_3O^+) and a small amount of hydrogen peroxide (H_2O_2) (Eq. (1)). In the acetonitrile-water solution CH_3CN will be excited by electron beams to produce its excited singlet $^1CH_3CN^*$ (Eq. (2)), and solvent electron (e_{sol}^-) could be scavenged by to produce its anion radical $CH_3CN^{\bullet-}$ (Eq. (3)) [35]. However, a part of •OH will attack CH_3CN to produce its radical (Eq. (4)), and a lot of •OH still exists in acetonitrile-water solution system, which is confirmed by our experiment and other scientists [27,28]. The laser flash photolysis and pulse radiolysis of Cur have been performed with different solution system. It has been found that the polarity is water > wate-CH_3CN > water-CH_3COH > CH_3CN > CH_3COH, and may affect the electron transfer reaction. As the laser flash photolysis in both of water-CH_3COH and water-CH_3CN with the same concentration of Cur were performed, the quantum yield of $^3Cur^*$ in wate-CH_3CN was less than that in water-CH_3COH. It means that the polarity of wate-CH_3CN was more than that of water-CH_3COH.

$$H_2O \rightarrow e_{sol}^-,\quad \cdot OH\,(H), H_3O^+(H_2O_2) \tag{1}$$

$$CH_3CN \rightarrow {}^1CH_3CN^* \tag{2}$$

$$e_{sol}^- + CH_3CN \rightarrow CH_3CN^{\cdot-} \tag{3}$$

$$\cdot OH\,(H) + CH_3CN \rightarrow \cdot CH_2CN + H_2O\,(H_2). \tag{4}$$

2.6. Cleavage of DNA damaged by cur exposed to 430 nm visible light

A 500 W xenon lamp was used as irradiation light source. The light passed through a cut-off filter with a bandwidth of 415 to 465 nm, and then was focused on a 10 × 10 × 40 mm quartz cell with an irradiance of 27.5 mW/cm^2. Samples were analyzed by agarose gel electrophoresis with a BIORAD quantity one computer controlled electrophoresis power supply.

Cur was added into plasmid pUC18 DNA (form I and form II, 200 ng) in 4% DMSO-10 mM-Tris-HCl buffer at pH 6.5 solution to make its final concentration to be 0.02 mM. The samples for exposure were incubated for 30 min duration at 25 °C and avoided from light with aluminium foil.

After exposure to visible light at different times at 25 °C, loading buffer containing 0.05% bromophenol blue, 0.9% SDS and 50% glycerol was added into the samples. Electrophoresis was performed at 90 V for 30 min in Tris-acetate-EDTA (TAE) buffer (40 mM Tris-base-40 mM acetic acid-2 mM EDTA) using 0.7% agarose gel containing 0.5 μg/ml ethidiumbromide [36]. The percentage of each form of pUC 18 DNA was quantified based on intensity using Image J software.