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Spectroscopic investigation on protein damage by ciprofloxacin under ultrasonic irradiation

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ABSTRACT

In recent years, sonodynamic activities of many drugs have attracted more and more attention of researchers. The correlative study will promote the development of sonodynamic therapy (SDT) in anti-tumor treatment. In this work, bovine serum albumin (BSA) was used as a protein model to investigate the intensifying effects of ciprofloxacin (CPFX) ultrasonically induced protein damage by UV–vis and fluorescence spectra. Meanwhile, the conformation of BSA is changed upon the addition of CPFX and metal ions under ultrasound (US) so that the damaging site of BSA is considered. Various influencing factors, such as US irradiation time, metal ions, solution temperature and ionic strength, on the ultrasonically induced BSA damage are discussed. It was showed that CPFX could enhance ultrasonically induced BSA damage. The damage degree of BSA was aggravated with the increasing of US irradiation time, solution temperature, ionic strength as well as the addition of metal ions. Furthermore, the reactive oxygen species (ROS) in reaction system were detected by oxidation-extraction photometry (OEP). Experimental results also showed that US could activate CPFX to produce ROS, which were mainly determined as superoxide radical anion ($^{\bullet}O_2^-$) and hydroxyl radical ($^{\bullet}OH$).

1. Introduction

Ultrasound (US) is a sound whose frequency is too high for humans to hear. It is used for a growing variety of purposes in diverse areas, for example cell disruption, extracorporeal lithotripsy, physiotherapy, medical imaging, chemical analyses and sonochemistry, etc. [1,2]. US is able to produce these effects through the physical, mechanical and chemical results of acoustic cavitation process, which involves the formation, growth and violent collapse of small bubbles in liquid, as a result of acoustic pressure fluctuation [3]. Sonodynamic therapy (SDT) of cancer is based on preferential uptake and/or the retention of a sonosensitizer in tumor tissues and subsequent activation of sonosensitizer by US irradiation, which can penetrate deeply into tissues and can be focused into a small region of a tumor [4]. The most widely used sonosensitizers were hematoporphyrine (Hp) and its derivatives (HpD). However, they were liable to cause severe photo-dermatitis and difficult to be used in clinical practice extensively [5]. In this regard, it becomes increasingly important to explore novel sonosensitizers with fewer side effects. It was reported that some non-steroidal anti-inflammatory drugs, such as piroxicam and tenoxicam, were also found to have a synergistic anti-tumor effect with US. But they were still far from ideal for clinical use because of high systemic doses having potential toxicity [6–8]. Recently, we are investigating the sonodynamic activity of fluoroquinolones (FQs). Prophase work showed that LVFX combined with US can make the damage of BSA more serious, and the damage degree of BSA was aggravated with the increase of US irradiation time and solution temperature [9].

Ciprofloxacin (CPFX) was a kind of FQs, which has a broad spectrum of activity against Gram-positive and Gram-negative bacteria. It also showed anti-tumor activity in several cancer cell lines, such as colon cancer, bladder cancer and leukemia [10–13], and in an *in vivo* animal model [14]. The anti-tumor activity was linked with the anti-topoisomerase II activity of the FQs, which were demonstrated in eukaryotic and tumor cells [15,16].

Proteins are major targets for many drugs due to their important roles both in normal cells or tumor cells. The most abundant protein in the circulatory system is serum albumin. In general, bovine serum albumin (BSA) was selected as studied protein because of its medically important, low cost, ready availability, unusual ligand-binding properties [17]. BSA and human serum albumin's (HSA)

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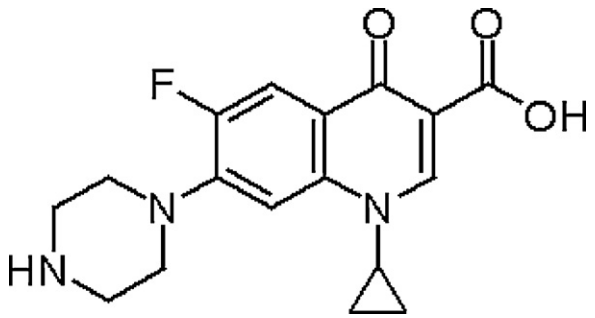

Fig. 1. Molecular structure of ciprofloxacin (CPFX).

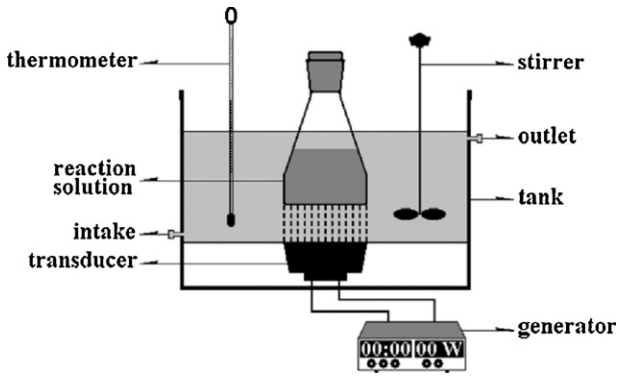

Fig. 2. The apparatus of US irradiation.

tertiary structures are similar in 76% and all the study results are consistent with the fact that HSA and BSA are homologous protein [18,19].

In this study, ultrasonically induced damage effects of ciprofloxacin (CPFX, the structure shown in Fig. 1) on BSA are investigated by UV–vis and fluorescence spectra. In addition, by synchronous fluorescence spectra, the damaging site of BSA is also considered. Herein, the experiments were carried out in two steps. Firstly, sonodynamic damage of BSA in the presence of CPFX was investigated. Meanwhile, the influencing factors, such as US irradiation time, metal ions, solution temperature and ionic strength, on BSA damage were also studied systemically. Secondly, the generated reactive oxygen species (ROS) in reaction system were detected by means of oxidation-extraction photometry (OEP) [20]. This report may offer a valuable reference to promote the application of SDT in tumor treatment at molecule level and simplify the detection of ROS.

2. Materials and methods

2.1. Chemicals

Ciprofloxacin (CPFX, ≥99.0%, Jinan Dachpharm Development Co., Ltd., China) as sonosensitizer and bovine serum albumin (BSA, ≥98.0%, Beijing Aoboxing Biotechnological Company, China) as model protein were purchased. Tris–HCl buffer solutions (0.05 M, pH 7.4, containing NaCl of 0.05 M) were used to prepare the BSA storage solutions (2.00×10^{-5} M, stored at 0–4 °C) and CPFX storage solutions (5.00×10^{-5} M). $CuSO_4$, $CaCl_2$ and $CoCl_2$ were obtained from Sinopharm Chemical Reagent Co., Ltd., China. All solutions were prepared using doubly distilled water. Diphenylcarbazide (DPCI) as trapping agent and sodium azide (NaN_3), 2,6-di-tert-butyl-4- methylphenol (BHT) and vitamin C (VC) as quenchers of reactive oxygen species (ROS) were obtained from Sinopharm Chemical Reagent Co., Ltd., China. All measurements were carried out at 25.00 ± 0.02 °C.

2.2. Sonication

The experimentations of BSA damage under ultrasound (US) irradiation were carried out in a Controllable Serial-Ultrasonics apparatus (KQ5200DB, Kunshan Ultrasonic Instrument Co., Ltd. China) shown in Fig. 2. Its frequency and power were 40 kHz and 1 W/cm^2, respectively.

2.3. Absorption spectra

To test the influence of US on CPFX, absorption spectra of these compounds were studied. This information could be useful to explain the mechanism of their synergistic effect. A UV-2100 (Beijing Purkinje General Instrument Co., Ltd., China) UV–visible spectrophotometer was used to measure the absorption spectra of the CPFX and CPFX–BSA solutions before and after sonication.

2.4. Fluorescence spectra

To study the effect of US on BSA located in BSA–CPFX solutions, the fluorescence spectra of the BSA–CPFX solutions before and after US exposure were recorded in a luminescence spectrometer (LS-55, PerkinElmer Inc., USA) with the excitation wavelength of 280 nm, and the excitation and emission slit width (each 10 nm), scan rate (1200 nm/min) were constantly maintained for all the experiments.

2.5. Sonodynamic damage of BSA under US irradiation combined with CPFX

Firstly, six clean conical flasks were marked with a, b, c, d, e and f, respectively. Four 12.50 mL BSA storage solutions with concentration of 2.00×10^{-5} M were taken exactly and put into conical flasks a, b, c and d, respectively, and then four 5.00 mL of CPFX storage solutions with concentration of 5.00×10^{-5} M were added to conical flasks c, d, e and f, respectively. Finally, six 5.00 mL of Tris–HCl–NaCl buffer solutions with concentration of 250 mM was added to above six conical flasks, respectively, to maintain the solution acidity (pH 7.4) and ionic strength (50 mM). At last, all conical flasks were diluted to 25.00 mL with twice distilled water. The final concentration of BSA and CPFX were both of 1.00×10^{-5} M. Afterwards, among them the conical flasks a, c and e were placed in a lucifuge irradiation apparatus. Others were only placed in the dark. After 3.0 h, the UV–vis and fluorescence spectra of each sample solution were determined to evaluate the damage of BSA molecules. Furthermore, in order to investigate the BSA damage process systematically, the effects of US irradiation time, the effects of metal ions Cu^{2+}, Ca^{2+} and Co^{2+}, solution temperature and ionic strength were also examined.

2.6. Identification of the reactive oxygen species (ROS)

Firstly, two aliquots of 2.00 mL DPCI and 2.00 mL CPFX were taken exactly and put into volumetric flask (a) and (b), respectively. Then, the solutions were diluted to 10.00 mL with Tris–HCl–NaCl solutions, making the final concentration of DPCI and CPFX were 5.00×10^{-3} M and 1.00×10^{-4} M, respectively. Afterwards, one of them was transferred into 50 mL conical flask and placed in an US irradiation apparatus, the other was kept in the dark. After 1.0 h, the solutions were extracted repeatedly with Benzene–CCl_4 (1:1) mixed extractant. The extracted liquids were diluted to 10.00 mL with the extractant and detected by UV–vis spectrophotometer. In order to investigate systematically the changes of ROS level under US irradiation, the effects of CPFX concentration and US irradia-