Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 134 (2015) 361–366

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

Protein damage and reactive oxygen species generation induced by the synergistic effects of ultrasound and methylene blue

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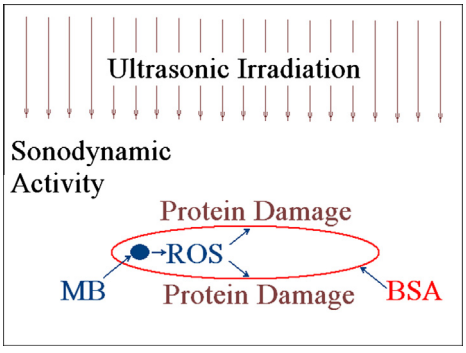
HIGHLIGHTS

- The sonodynamic damage to protein in the presence of MB were studied.
- The mechanisms of the synergistic effects of ultrasound and MB were studied.
- The protein damage induced by the synergistic effects were more serious.
- The damage of protein could be mainly due to the generation of ROS.
- Both $^1O_2$ and ·OH were the important mediators to protein damage.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 3 March 2014
Received in revised form 10 June 2014
Accepted 21 June 2014
Available online 30 June 2014

Keywords:
Methylene blue
Ultrasound
Synergistic effects
Protein damage
Reactive oxygen species
Sonodynamic treatment

ABSTRACT

The sonodynamic damage to protein in the presence of methylene blue (MB) and the various influencing factors including ultrasonic irradiation time and MB concentration on the damage of protein were studied by fluorescence and absorption spectra. In addition, the mechanisms of the synergistic effects of ultrasound and MB were studied by oxidation–extraction photometry with several reactive oxygen species (ROS) scavengers. The results indicated that the damage of protein induced by the synergistic effects of ultrasound and MB were more serious than those that ultrasound or MB alone was applied. The damage of protein could be mainly due to the generation of ROS. The damage degree of protein increased with the increase of ultrasonic irradiation time and MB concentration because of the increased quantities of ROS generation. Both $^1O_2$ and ·OH were the important mediators of the ultrasound-inducing protein damage in the presence of MB.

Introduction

Sonodynamic therapy (SDT) is a new approach for cancer treatment on the basis of photodynamic therapy (PDT). It was firstly proposed by Japanese scholars Umemura et al. in 1989, based on the synergistic effects on tumor cells damage by the combination of the hematoporphyrin and ultrasound [1]. Ultrasound can penetrate deeply into tissues while maintaining its ability to focus energy into small volumes and locally activate the cytotoxicity of the sonosensitizer that preferentially accumulates in tumor sites [2,3]. Compared with electromagnetic modalities such as laser beams or microwaves, it is a unique advantage in the application to non-invasive treatment of non-superficial tumors [4,5], which suggests that SDT has potential value in the application for targeted therapy of tumor.

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http://dx.doi.org/10.1016/j.saa.2014.06.121
1386-1425/

In the latest years, SDT has been widely investigated focusing on the antitumor effects in vivo and/or in vitro and the mechanisms for the synergism between ultrasound and drugs by using different ultrasound parameters and different sonosensitizers [6]. Most of them regarded the tumor cells as assault target, and achieved the goal of treating tumors through inducing tumor cells apoptosis [7–11]. However, the intracellular targets of SDT have seldom been studied until now. The damage to intracellular substances, especially proteins that are highly abundant in cells, might be a more effective method to kill the tumor cells [12–14]. It had been reported that the changes of cytoskeletal F-actin had some correlations with Ehrlich ascites carcinoma cells apoptosis, which suggested that protein was an important subcellular target for SDT [15]. If the proteins in the tumor cells were damaged by sonosensitizers under ultrasonic irradiation, the whole cells would undergo apoptosis abnormally.

It has been reported that many compounds have sonodynamic activity. Because of their widely different structure, it is difficult to expect a universal mechanism for the synergistic effects of ultrasound and drugs [6]. Reviewing the probably mechanisms of SDT which have been studied, most experimental evidence indicated that the cell damage induced by the synergistic effects of ultrasound and drugs may contribute to the generation of reactive oxygen species (ROS) [16–21]. ROS is a class of ubiquitous molecules including both radicals and non-radicals such as superoxide anion radical ($\cdot O_2^-$), hydrogen peroxide (H_2O_2), hydroxyl radical ($\cdot OH$), and singlet oxygen ($^1O_2$). These substances are constantly formed in the human body and have been implicated in a number of diseases due to their damage to cell structures, including lipids and membranes, proteins and nucleic acids, leading to cell death [22]. However, the ROS with strong oxidation activity can also exert beneficial physiologic effects for many diseases, especially, for various tumors [23].

Methylene blue (MB, Fig. 1) is a heterocyclic aromatic compound that has antifungal, antibacterial [24] and antimalarial activity [25]. It has been widely used to stain living organisms, treat methemoglobinemia [26], prevent ifosfamide-induced encephalopathy [27], and lately it has been investigated and used as photosensitizers of PDT against several types of tumors [28]. Moreover, it has been confirmed that MB possesses sonodynamic activities [29,30]. These results demonstrate that MB has potential to be used as a sonosensitizer in SDT. In this work, bovine serum albumin (BSA) was selected as a model of protein, the sonodynamic damage to protein in the presence of MB was studied by fluorescence and absorption spectra. The mechanisms of the synergistic effects of ultrasound and MB were studied by oxidation–extraction photometry with several ROS scavengers. It is wished that this report might offer some meaningful and valuable references to promoting the application of SDT at molecule level.

Experimental section

Materials

BSA (Fraction V) was obtained from Amresco (USA) and used without further purification. The BSA stock solution, 2.50×10^{-5} mol/L, was prepared in 0.05 mol/L Tris–HCl buffer solution (pH 7.40) containing 0.05 mol/L NaCl. MB, Diphenylcarbazide (DPCI), D-Mannitol (D-Man), L-Histidine (L-His) and Ascorbic acid (V_c) were all purchased from Sinopharm Chemical Reagent Co., Ltd (China). The MB stock solution (2.00×10^{-4} mol/L), the DPCI stock solution (2.50×10^{-2} mol/L) and the different ROS scavengers stock solution (5.00×10^{-2} mol/L) were all prepared in the same buffer solution. All the other materials were of analytical reagent grade and used without further purification. Doubly distilled water was used to prepare solutions.

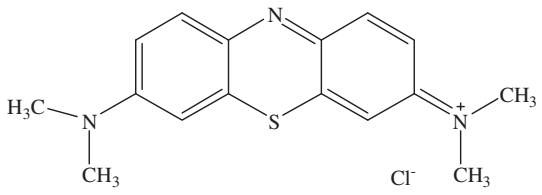

Fig. 1. Molecular structure of MB.

Apparatus

The fluorescence spectra were carried out on an F-7000 fluorescence spectrophotometer (Hitachi High-Technologies Co., Japan). Fluorescence spectra were obtained at an excitation wavelength of 280 nm, with the slit widths of both the excitation and emission set at 5.0 nm and the scanning speed of 1200 nm min^{-1}. The absorption spectra were recorded on a UV-2550 spectrophotometer (Shimadzu Co., Japan) with 1.0 cm quartz cells. The Controllable Serial-Ultrasonics apparatus (KQ5200DB, Kunshan Ultrasonic Instruments Co., Ltd. China) shown in Fig. 2 was used as irradiation source, operating at ultrasonic frequency of 40 kHz and output power of 200 W through manual adjusting. All pH measurements were made with a pHS-25 digital pH-meter (Shanghai Reaches Instrument Co., Ltd., China).

Procedures

Effect of ultrasonic irradiation time on the damage of BSA

In two conical flasks, the final concentrations of BSA were both 1.00×10^{-5} mol/L, and the final concentrations of MB were 0.00 mol/L and 1.00×10^{-5} mol/L, respectively. They were all placed in the ultrasonic irradiation apparatus and the ultrasonic irradiation time was changed from 1.0 h to 6.0 h at 1.0 h intervals. At every time interval, the solutions (10 mL) were taken out and detected by fluorescence spectrophotometer.

Effect of MB concentration on the damage of BSA

In six conical flasks, the final concentration of BSA were all 1.00×10^{-5} mol/L, and the final concentrations of MB were changed from 0.00 mol/L to 2.50×10^{-5} mol/L at 0.50×10^{-5} mol/L intervals. They were all placed in the ultrasonic irradiation apparatus for 3.0 h. Then, the solutions were taken out and detected the fluorescence spectra.

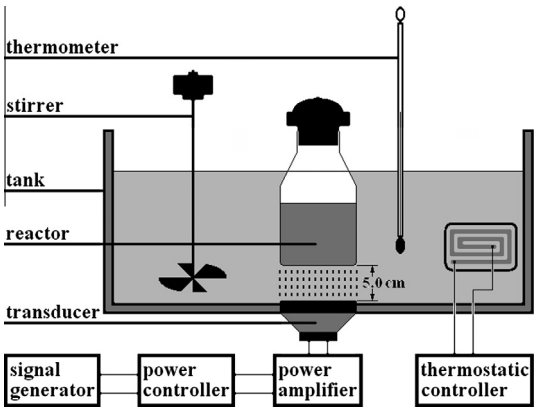

Fig. 2. The apparatus of ultrasonic irradiation.