Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 117 (2014) 754–762

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

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

# Spectroscopic investigation on interaction and sonodynamic damage of Riboflavin to DNA under ultrasonic irradiation by using Methylene Blue as fluorescent probe

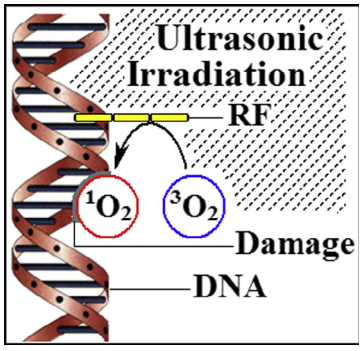
Qi Wang, Qiong Wu, Jun Wang *, Dandan Chen, Ping Fan, Baoxin Wang

*College of Chemistry, Liaoning University, Shenyang 110036, PR China*

## HIGHLIGHTS

- Riboflavin was used to study the interaction and sonodynamic damage to DNA by spectrometry.
- Methylene Blue was used as fluorescence probe to study interaction of Riboflavin and DNA.
- Methylene Blue was used as fluorescence probe to evaluate sonodynamic damage of Riboflavin to DNA.

## GRAPHICAL ABSTRACT




## ARTICLE INFO

*Article history:*
Received 16 July 2013
Accepted 7 September 2013
Available online 19 September 2013

*Keywords:*
Deoxyribonucleic acid (DNA)
Riboflavin (RF)
Methylene Blue (MB)
Sonodynamic damage
Ultrasonic irradiation

## ABSTRACT

In this paper, the Riboflavin (RF) as a sonosensitizer and Methylene Blue (MB) as a fluorescent probe were used to study the interaction and sonodynamic damage to Deoxyribonucleic Acid (DNA) by fluorescence and UV–vis spectroscopy. The results showed that the RF could efficiently bind to DNA in aqueous solution and exchange with the MB through competing reaction. And then, under ultrasonic irradiation, the RF could obviously damage the DNA. In addition, the influencing factors such as ultrasonic irradiation time and RF concentration on the sonodynamic damage to DNA were also considered. The experimental results showed that the sonodynamic damage degree increase with the increase of ultrasonic irradiation time and RF concentration. Perhaps, this paper may offer some important subjects for broadening the application of RF in sonodynamic therapy (SDT) technologies for tumor treatment.



## 1. Introduction

In recent years, the photosensitive drugs for tumor treatment through photochemical reactions have attracted attention of scholars. Because the light was used as driving force, this method was called as photodynamic treatment (PDT) [1–3]. However, the PDT method can only be used to cure shallow tumors such as skin cancer and lung cancer, because of the short penetration distance of irradiation light. Obviously, this lower penetrability restricts its application range. On contrary, the ultrasound can overcome those disadvantages of light. The ultrasound can not only reach deep-seated tumor but also maintain the focus energy in a small volume [4]. Therefore, some researchers have paid great attention to use the ultrasonic irradiation instead of light to activate the photosensitive compounds. Accordingly, this new method of tumor treatment was defined as the sonodynamic treatment (SDT) [5–7]. The primary mechanism involved in SDT is known to be based on the cavitation effect resulted from the ultrasonic irradiation. It has been well known that, under ultrasonic irradiation, some micro-bubbles in the fluid form, grow and col-lapse. On the collapsing

* Corresponding author. Tel.: +86 024 62207859; fax: +86 024 62202053.
*E-mail address:* wangjun888tg@126.com (J. Wang).

1386-1425/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.saa.2013.09.039

process of the empty micro-bubble, the gas inside may receive adiabatic compression, which results in short and local hot spots and sonoluminescence [8]. Just using the hot spots or sonoluminescence, the sonosensitizers (most of photosensitive compounds) could generate a large number of reactive oxygen species (ROS) to kill the tumor cells [9,10].

Previously, during the study on SDT, most researchers adopted the tumor cell as the target, that is, through the oxidation of cell membrane leading to tumor cell necrosis, thus to achieve therapeutic effect. In fact, for a tumor cell, the cell membrane accounts for the majority of cell quality. Therefore, it is difficult to kill tumor cell only through the destruction of cell membrane. Besides, the fluidity and rapid repair of cell membrane also reduce the damage degree of sonodynamic reaction. In order to achieve a better therapeutic effect of tumors, we have been advocating that killing tumor cells should be performed from cell inside. That is, the better way is to make sonosensitizers enter the tumor cell, and then, under ultrasonic irradiation, directly damage the biological molecules with various functions, such as DNA, protein and enzyme and so on, to promote the necrosis of the tumor cells. Apparently, the utilization of small doses of targeting sonosensitizers to treat tumor will not result in severe side effect for the patient [11]. If so, it is important to confirm the target biological molecules and select the appropriate sonosensitizer with high efficiency and low toxicity.

Deoxyribonucleic acid (DNA) is an important biological macromolecule that can store and transmit genetic information, and plays an important role in life processes [12]. The studies of the binding of small molecule compounds with DNA have become a very extensive research topic due to its relation to both genetic effects and carcinogenicity [13]. As a main target biological molecule, DNA was selectively attacked by many anticancer drugs in clinical. Through interaction, anticancer drugs can destroy the structure of DNA, thereby affecting gene regulation and replication function. As an important genetic and replication material for proliferation and differentiation of cells, DNA is the most suitable to be selected as the target biological molecule. For a cell the breakage of DNA is fatal, in other words, the damage of DNA can lead to cell death more easily.

Riboflavin (RF, also called as Vitamin B2), a mammal necessary nutriment, is widely distributed in human tissues in free and conjugated forms. In addition, RF is also an important metabolizable photosensitizer [14,15]. Under light irradiation, it even participates in some biological photochemical reactions, for example, causing the damage of biological molecules and other cell matrix components [16–18]. In fact, the photochemical treatment using RF has been applied for the development of an effective and safe inactivation technology of pathogens present in blood components [19,20]. Under ultravoilet light irradiation, RF was found to be able to selectively damage the guanine bases in DNA molecules of pathogenic microorganisms [21]. Therefore, like most of the photosensitizer, RF should also display sonodynamic activity under ultrasonic irradiation [22]. Meanwhile, due to the aromatic tricyclic planiform structural property the RF should also be endowed with natural antitumour activity [23–26]. Here, based on the plane structure and photochemical activity, RF is adopted as a special sonosensitizer to study the interaction to DNA and the sonodynamic activity in the damage of DNA.

In order to detect the interaction of small molecular compounds with DNA molecules, many technologies have been applied such as fluorescence and UV–vis spectra. However, it is limited that some biological properties of DNA were investigated by directly using the fluorescence emission spectrum due to the very weak fluorescence intensity [27]. In the fluorescent methods, some organic dyes with intercalating performance are generally employed as fluorescent probes for the DNA-drug interaction study [28]. Particularly,

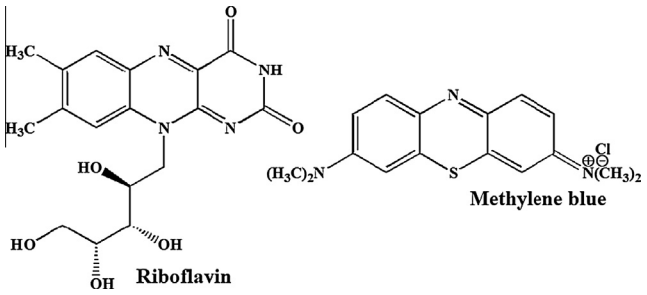



**Fig. 1.** Molecular structures of Riboflavin (RF) and Methylene Blue (MB).

in recent years, many obvious effects have been got on the observation of DNA damage by using the organic dye probes. Generally, the usable fluorescent probes include those organic dyes such as ethidium bromide (EB), acridine orange (AO), oxazide yellow homodynes, nile blue, neutral red and so on [29]. However, most of these used DNA fluorescent probes are highly toxicity, especially the combination mode of some probes with DNA is irreversible. Methylene Blue (MB) is one of the most studied organic dyes in biochemistry due to its high extinction coefficient that can be used in low concentration [30]. And the MB also has many advantages such as low toxicity, high selectivity and high sensitivity [31]. So, in this work the MB was selected as fluorescent probe to examine the change of structure and composition of DNA.

In this study, the RF and MB were selected as sonosensitizer and fluorescence probe, respectively, to investigate the interaction with DNA and sonodynamic damage to DNA under ultrasonic irradiation by UV–vis and fluorescence spectroscopy. Moreover, several influences factors, such as ultrasonic irradiation time and RF concentration, on the damage of DNA were also considered. It is wished that these workers could expand the application of sonodynamic therapy (SDT) in future cancer treatment for humans. The molecular structures of RF and MB used in the experiment are presented in Fig. 1.

## 2. Experimental section

### 2.1. Materials

Calf thymus-deoxyribonucleic acid (ct-DNA, purity >99.0%) was procured from Beijing Abxing Biological Technology Company (China) and stored in refrigerator at 4.0 °C. Riboflavin (RF) and Methylene Blue (MB) were analytical reagent grade and obtained from Sinopharm Chemical Reagent Co., Ltd. (China). Tris (hydroxyl-methyl) aminomethane (Tris), HCl and NaCl were all of analytical reagent grade and purchased from Sinopharm Chemical Reagent Co. Ltd. (China). All other chemical reagents were commercial products of analytical grade and used as received. Double distilled water puried by Aquelix 5 Millipore water purification system (US) was used throughout experiment.

### 2.2. Apparatus

UV–vis spectrum apparatus (Cary-50, Varian Company, USA) and fluorescence spectrometer (Cary-300, Varian Company, USA) were adopted to track the change of the mixed solutions of DNA, Riboflavin (RF) and Methylene Blue (MB). The solution pH value was measured with a pH meter (PHS-3C, Shanghai Leici Instrument Company, LTD, China). The Controllable Serial-Ultrasonics apparatus (DB-100, 40 kHz and 100 W, Kunshan Apparatus Company, China) as ultrasonic irradiation source was used to irradiate the mixed solutions of DNA with Riboflavin (RF) and Methylene Blue (MB).