Ultrasonics Sonochemistry 27 (2015) 178–186

Contents lists available at ScienceDirect

Ultrasonics Sonochemistry

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

Sonochemical transformation of thymidine: A mass spectrometric study

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ARTICLE INFO

Article history:
Received 9 February 2015
Received in revised form 14 May 2015
Accepted 15 May 2015
Available online 20 May 2015

Keywords:
Sonochemical transformation
Thymidine
Mass spectrometry
Product analysis
DNA

ABSTRACT

Ultrasound is extensively used in medical field for a number of applications including targeted killing of cancer cells. DNA is one of the most susceptible entities in any kind of free radical induced reactions in living systems. In the present work, the transformation of thymidine (dT) induced by ultrasound (US) was investigated using high resolution mass spectrometry (LC–Q-ToF–MS). dT was subjected to sonolysis under four different frequencies (200, 350, 620 and 1000 kHz) and at three power densities (10.5, 24.5 and 42 W/mL) in aerated as well as argon saturated conditions. A total of twenty modified nucleosides including non-fully characterized dT dimeric compounds were detected by LC–Q-ToF–MS. Out of these products, seven were obtained only in the argon atmosphere and two only in the aerated conditions. Among the identified products, there were base modified products and sugar modified products. The products were formed by the reaction of hydroxyl radical and hydrogen atom. Under aerated conditions, the reactions proceed via the formation of hydroperoxides, while in argon atmosphere disproportionation and radical recombinations predominate. The study provides a complete picture of sonochemical transformation pathways of dT which has relevance in DNA damage under ultrasound exposure.

1. Introduction

The reactive oxygen species (ROS) induced modification of DNA is a much investigated topic of research because of its important physiological as well as pathological consequences. Hydroxyl radical ($^{\cdot}$OH) is one of the major ROS formed in the body by transition metal mediated Fenton reactions and on exposure to ionizing radiation, causing oxidative degradation of cellular DNA [1,2]. It is capable of producing double and single strand breaks, abasic sites and modified bases which if left unrepaired accumulate in cells and leads to mutations and cancer [3,4]. The analysis of the initially formed transient species and end products in the DNA constituent level helps to elucidate the otherwise complex mechanism of interaction of DNA with the ROS [5–7]. A wealth of knowledge is available in literature on the transformation of the various nitrogen bases, nucleosides and nucleotides under gamma radiation, UV radiation and photosensitized electron transfer and Fenton reaction [2,7–11].

It is known that exposure to low frequency high power ultra sound (US) causes oxidative stress on the cells due to the production of $^{\cdot}$OH [12]. It is substantiated by the decrease in the glutathione content after US exposure. Ultrasound is extensively used in medical field as an imaging technique, for bone healing, disruption of kidney stone, and targeted killing of cancer cells [13]. Even the diagnostic US, which is pulsed and high frequency, is found to produce single strand breaks in human leukocytes *in vitro* [13]. Identification of these lesions is important as most of them have great biological implications due to their lethal, mutagenic and genotoxic effects.

The physical or chemical activity of US is due to a phenomenon called acoustic cavitation which is the formation, growth and collapse of gas filled bubbles when it is passing through liquids. High temperature and pressure are created by the final collapse of the bubble. Thermolysis of water produces the hydrogen atom (H$^{\cdot}$) and highly oxidizing $^{\cdot}$OH [14]. There are studies showing evidence for the production of $^{\cdot}$OH in medical US using terephthalate dosimeter and degradation of polymers [15]. All these evoke the interest on the effect of US on biomolecules especially DNA as it is an important target for these reactive species [16].

The effect of US on DNA was reported *in vitro* and by taking nitrogen bases as model compounds [17,18]. Among the bases, thymine was found to be the most reactive [17]. The end products of the three pyrimidine bases on sonolysis were documented by TLC and GC–MS analyses and it was found that the products obtained were identical to that obtained by radiolysis of their

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http://dx.doi.org/10.1016/j.ultsonch.2015.05.016
1350-4177/

aqueous solutions [19–21]. Fuciarelli et al. reported several base lesions in DNA solution by the cavitational activity of ultrasound at a frequency of 2.17 MHz [18]. Studies have also been done on the sonochemical activity of thymine over a range of US intensities which also help to understand the kinetics of the reaction [22]. Even though there are many studies on the sonochemically modified nitrogen bases, sonochemical transformation studies on nucleosides would give a better understanding of the mechanistic aspects of effect of US on cellular DNA. In the present study, we investigated the effect of US on thymidine (dT), a relevant nucleoside used as a target of various ROS generating systems [1,10]. The variation of the sonochemical activity of dT with US frequency at three different power densities is explained along with the influence of argon on sonolysis. Moreover, the study is a reinvestigation of the free radical induced transformation products of dT using high resolution mass spectrometry. Argon atmosphere simulates the hypoxic conditions in cells and hence details of the products in its presence would be highly beneficial. The accurate mass measurement using high resolution mass spectrometer as well as the MS/MS facility helps to get an insight into the structure [23].

2. Materials and methods

2.1. Sonolysis of dT

dT was purchased from Pharma Waldhof and used as such. All solutions were prepared in water purified by a Cascada™ Lab Water Systems and of resistivity 18.2 MΩ cm. Solutions of dT (10^{-4} mol/L, 250 mL) was subjected to sonolysis at four different frequencies (200, 350, 620 and 1000 kHz) at three different power densities, 10.5, 24.5 and 42 W/mL for 2 h. Two frequencies (620 kHz and 1 MHz), among the four studied, fall under the range used for the surgical non disruptive applications. The sonolysis was carried out in a glass reactor with an L3 ELAC Nautik ultrasound generator powered by an Allied Signal R/F generator (T & C power conversion, Model AG 1006). For the experiments under argon saturated atmosphere, the solution was bubbled with argon gas for half an hour at a pressure of 5 psi prior to sonolysis and Ar was bubbled throughout the experiment. Sonolysis was carried out only at a power density of 42 W/mL under argon atmosphere, where there was maximum degradation in the case of aerated conditions. Two transducers were used with resonances 212 and 620 kHz and 350 and 1000 kHz respectively. The temperature was maintained at 25 ± 1 °C.

2.2. HPLC analysis

The percentage degradation of dT on sonolysis was monitored by HPLC (Shimadzu prominence UFLC, LC 20 AD) connected with a diode array detector (SPD-M20 A). An Enable C18 (25 cm × 4.5 mm × 5 μm) column was used at a flow rate of 1 mL/min. The sonolysed mixture was eluted isocratically with water and methanol (95:5) as mobile phases.

2.3. Analysis of end products by LC–Q-ToF–MS

The end products were analyzed by Waters Xevo G2 QToF with an electrospray ionization (ESI) source. It was coupled to Acquity H class UPLC with BEH C18 (50 mm × 2.1 mm × 1.7 μm) column and Acquity TUV detector. The instrument was tuned with the parent compound, dT. The ionization conditions are as follows: capillary voltage: 2.5 V, sampling cone voltage: 30 V and extraction cone voltage: 4 V. The source and desolvation temperatures were kept at 135 °C and 350 °C respectively. Nitrogen was used as the desolvation and nebulizing gas at a flow rate of 50 and 900 L/h, respectively. For the mass spectrometric analyses, the dT was sonolysed for 1 h under normal conditions and for 30 min. in the case of argon saturated conditions at a power density of 42 W/mL. The modified nucleosides were eluted isocratically with 0.1% formic acid in water and methanol (95:5) and detected in negative mode of ionization. For mass spectrometric analyses dT samples were sonolysed for 60 min. at frequency 620 kHz and power density 42 W/mL.

3. Results and discussion

3.1. Effect of frequency and power on the transformation

The dT solutions (10^{-4} mol/L) were subjected to four US frequencies (200, 350, 620 and 1000 kHz) at three different power densities (10.5, 24.5 and 42 W/mL) under aerated conditions. The degradation profile is given in Fig. 1. At a power density of 10.4 W/mL the degradation of dT was higher at a frequency of 350 kHz, but as power increases to 24.5 W/mL the degradation at 350 and 620 kHz became comparable. At 42 W/mL, the degradation was found to be almost equal for 350, 620 and 1000 kHz. As power increases, the rate of degradation increases at all frequencies. The reaction was found to follow pseudo first order kinetics as inferred from Fig. 1. The experiment was repeated in the presence of argon atmosphere at 620 kHz and power density of 42 W/mL. The solution was saturated with argon and an argon atmosphere was maintained throughout the sonolysis. A comparatively higher rate of decomposition of dT was observed in argon atmosphere as shown in Fig. 1(c).

The results clearly indicate that the rate of the sonochemical reaction depends on the power and frequency of ultrasound. The increased sonochemical activity observed at frequencies 350 and 620 kHz is expected as it was reported that the overall free radical production was greater in this range of frequency [24]. When power density increases, there is an enhancement in the energy of cavitation facilitating the production of higher number of free radicals [25] which explains the general increase in the rate of degradation with power.

The chemical transformations of molecules on sonolysis are caused by pyrolysis and reaction with the $^{\cdot}$OH and H$^{\cdot}$ produced by water pyrolysis [14,26,27]. Since dT is non volatile, the contribution from the pyrolytic reactions can be considered negligible [28] and the major transformation pathway could be the reaction with free radicals. The intermediate radicals formed from dT on sonolysis by the addition of $^{\cdot}$OH and H$^{\cdot}$ were detected earlier by spin trapping studies [28] which further support the free radical mediated mechanism. From the pulse radiolysis studies, it is reported that $^{\cdot}$OH adds to the base with a reaction rate of the order of $3–10 \times 10^9$ L/mol s and abstracts hydrogen from the methyl group of thymine as well as the sugar ring with a rate constant of 2×10^9 L/mol s, while the rate constant of H$^{\cdot}$ reaction is of the order ten times lesser than the former ($1–5 \times 10^8$ L/mol s) [29]. The pseudo first order nature of the reaction observed in the present case proposes that the major reactive species is the highly reactive $^{\cdot}$OH radical.

A slightly higher rate of decomposition of dT is observed under argon atmosphere. Compared to air, argon possesses higher polytropic index and lower thermal conductivity. Also, it has higher solubility which facilitates more cavitation events to take place compared to the aerated conditions [30]. This results in an efficient production of free radicals contributing to the increased rate of decomposition of dT in the presence of argon.

3.2. Mass spectrometric analysis of the transformed products

Sonolysis of dT yielded a mixture of products which were analyzed using LC–Q-ToF–MS. The total ion chromatogram obtained