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Full Length Research Paper

Effect of high power ultrasound on aqueous solution of DNA

Monira M. Rageh[1]*, Ali El-Lakkani[1], Mohamed H.M.Ali[2], Ahlam M. M. Abd El-Fattah[2] and A. El-Ghreeb Raafat[3]

[1]Biophysics Department, Faculty of Science, Cairo University, Cairo, Egypt.
[2]National Institute for Standards (NIS), Ultrasound Department. El-Haram-Giza, Egypt.
[3]Pharmacology Department, National Cancer Institute, Cairo University, Cairo, Egypt.

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The effect of (20 kHz continuous wave) ultrasound (power from 17 to 77 W) on the damage of nitrogen bases, the loss of double-helical structure, strand breaks and fragmentation of Calf spleen DNA were studied *in vitro*. The relative ultrasound sensitivity of the hydrogen bonded base pairs A-T and G-C were also studied. The techniques used in this investigation were ultraviolet absorption spectroscopy and constant-field gel electrophoresis (CFGE). The results showed that ultrasound decreased the hydrogen-bonded nucleotides. The ultrasound sensitivity was greater for the A-T base pairs than for the G-C base pairs. It was also shown that high power ultrasound damaged the nitrogen bases. The loss of the double helical structure by the ultrasound was mainly due to the separation of certain sections of the DNA by single strand breaks (SSB) and also to a small extent due to base damage. The size of the DNA fragments decreased as the ultrasound power increased and finally a plateau effect was seen at the highest powers.

Key words: DNA damage, ultrasound, spectroscopy.

INTRODUCTION

Ultrasound has played an important role in the practice of medicine over the last decade. Ultrasound is commonly used today in clinical imaging modalities and has become essential in the studies of cardiology, obstetrics and gynecology. Ultrasound is also commonly used in therapeutic applications such as bone healing, selective killing of cancer cells and fragmentation of kidney and gall bladder stones (Dyson 1985; Ziskin et al., 1990).

Deoxyribonucleic acid (DNA) has a fundamental role in the maintenance of vital processes. It is a key molecule in the storage and transmission of genetic information and in the readout of this information during protein synthesis. Because of this central importance, the DNA has been subjected to extraordinarily intensive research. Several studies have been focused on the effect of ultrasound on the conformation structure of DNA. Pinamonti et al. 86) Reported that diagnostic ultrasound causes single strand breaks (SSB) in the DNA of leukocytes exposed *in vitro*. Miller et al. (1989) showed that continuous ultrasound of high intensity (161 W/cm^2) and frequency (1.45 MHz) could produce SSB effect in the DNA of fresh human leukocytes. This effect is comparable in magnitude to that resulting from of one Gy of ^{60}Co gamma-ray exposures and may have resulted from the chemical action of transient cavitations. Ultrasound cavitations can produce free radicals and longer lived sonochemicals (Riesz and Kondo, 1992), which are capable of producing bioeffects. One of the long-lived sonochemicals is hydrogen peroxide (Suslick, 1988) which causes SSB via free radicals from the Haber-Weiss reaction in the cell nucleus (Mello Filho and Meneghini, 1984). Miller et al. (1991) and Miller and Thomas (1996) found that under high-power conditions, ultrasound cavitations could produce SSB in the DNA of fresh Chinese hamster ovary (CHO) cells by the action of hydrogen peroxide generated in phosphate buffered saline. The DNA damage may also

*Corresponding author: E-mail: moniragheh@yahoo.com.

Figure 1. Main components of sonifier model 450.

occur directly by the mechanical forces produced by the collapsing bubbles (Miller et al., 1995).

Alter et al. (1998) demonstrated that high power and low frequency ultrasound induces structural and functional changes in sonicated cells. Ashush et al. (2000) showed also that ultrasound of high power and low frequency caused DNA fragmentation and induced apoptosis in cultured myeloid leukemic cells.

Therefore, the search for conditions and mechanisms by which ultrasound may damage cellular DNA is important for risk and benefit considerations. The specific aims of the present work were to determine the effects of low frequency (20 kHz) continuous ultrasound, high power (ranging from 17 – 77 W) on hydrogen bonding of nucleotides, damage to nitrogen bases and DNA fragmentation for DNA in aqueous solution.

MATERIALS AND METHODS

Samples preparation

High polymerized and purified samples of DNA isolated from Calf spleen (DNA content and DNAase absence are satisfactory) by the method described in Alexander and Griffiths (1993) were dissolved in standard saline citrate (SSC) buffer using distilled water (0.15 M NaCl, 0.015 M Na_3 citrate, pH 7.0). The concentration of the stock solution was 20 mg/ml.

Ultrasound exposure

The source of ultrasound used in this work was the 450 Sonifier (made by Branson Ultrasonics Corporation in Danbury, U.S.A.). The main components of the 450 Sonifier (Figure 1) were the power supply, the converter (lead zirconate titanate electrostrictive element) and horn. The converter vibrates in a longitudinal direction and transmits this motion to the horn tip immersed in the solution. The energy is most concentrated within ½ an inch (12.7 mm) diameter of the face of the tip. A volume approximately equal to 30 ml concentrated DNA solution (20 mg/ml) was placed in an inner cylinder glass tube 2.5 cm in diameter and 8 cm in length, closed at its end and surrounded coaxially with another tube in which a current of water (at a temperature of 22°C) flowing during the ultrasound exposure to prevent thermal effect of ultrasound. The sonifier was adjusted on continuous operation mode for 10 min and the tip of its horn was immersed about ½ an inch (12.7 mm) in the DNA solution. The actual powers delivered to the samples used in this work were 17,34,47,65 and 77 W at a fixed frequency of 20 kHz (19.850 - 20.05 kHz). The values of the power are obtained from the readings of the loading meter and the chart supplied with the sonifier. It was written in the instruction manual of the sonifier that it can be used for DNA fragmentation and cells disruption.

Thermal denaturation of the DNA helix

Fresco et al. (1963) described a method which they successfully applied to estimate the proportions of the A-T and G-C base pairs in a DNA molecule. Rafi et al. (1968) extended the use of this method to obtain the average base pair composition of the helical segment of DNA surviving thermal denaturation after different doses of γ-irradiation. This method uses the equation:

$$\frac{\Delta A_\lambda}{C} = f_{A-T} \times \Delta\varepsilon_{A-T\lambda} - f_{G-C} \times \Delta\varepsilon_{G-C\lambda} \quad \text{---------- (1)}$$

where ΔA_λ is the difference between the absorbencies of the DNA solution at high (90°C) and low (30°C) temperatures at wavelength λ, C is the molar nucleotide concentration, $\Delta\varepsilon_{A\text{-}T\lambda}$ is the change in extincttion coefficient at wavelength λ corresponding to the disruption of 1 mole of A-T pairs, $f_{A\text{-}T}$ is the mole fraction of A-T pairs and $\Delta\varepsilon_{G\text{-}C\lambda}$ and $f_{G\text{-}C}$ are the corresponding quantities for G-C pairs. On rearrangement, equation (1) takes the forms.

$$\frac{\Delta A_\lambda}{\Delta\varepsilon_{A-T\lambda}} = C\left[f_{A-T} + f_{G-C} \times \frac{\Delta\varepsilon_{G-C\lambda}}{\Delta\varepsilon_{A-T\lambda}}\right] \quad \text{------(2)}$$

and

$$\frac{\Delta A_\lambda}{\Delta\varepsilon_{G-C\lambda}} = C\left[f_{G-C} + f_{A-T} \times \frac{\Delta\varepsilon_{A-T\lambda}}{\Delta\varepsilon_{G-C\lambda}}\right] \quad \text{--------(3)}$$

The plots of $\frac{\Delta A_\lambda}{\Delta\varepsilon_{A-T\lambda}}$ against $\frac{\Delta\varepsilon_{G-C\lambda}}{\Delta\varepsilon_{A-T\lambda}}$ and $\frac{\Delta A_\lambda}{\Delta\varepsilon_{G-C\lambda}}$ against $\frac{\Delta\varepsilon_{A-T\lambda}}{\Delta\varepsilon_{G-C\lambda}}$ should both be linear and the values of slope / (slope intercept) for these lines give $f_{G\text{-}C}$ and $f_{A\text{-}T}$ respectively. The main assumption implicit in this method is that the contribution of each base pair, A-T and G-C, to the total ultraviolet absorption is additive and independent of its neighbors (Rafi et al., 1968).

Ultraviolet spectroscopy

The spectrophotometer used in this work is LKB Ultraspec II manufactured by LKB in England. Initial absorbencies of the DNA samples (60 µg/ml) at wavelengths 232, 234, 238, 240, 243, 250, 256, 261, 264, 266, 267, 268, 270, 271, 273, 274, 276 and 277 nm

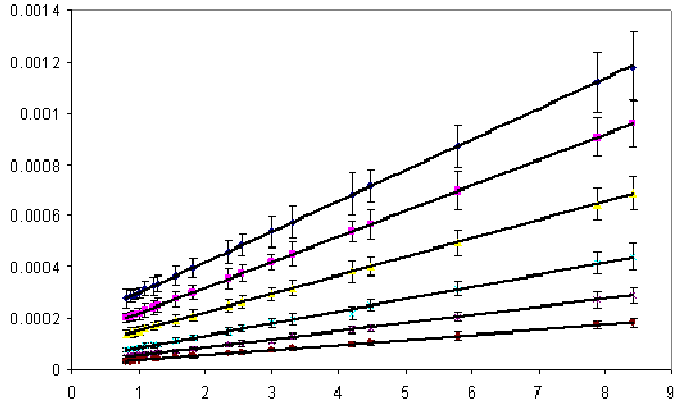

Figure 2. Graphs for DNA samples constructed by using equa-tion (2) to calculate f_{G-C}. (a) without exposure and (b, c, d, e and f) 10 min-post ultrasound exposure with powers 17, 34, 47, 65 and 77 W respectively. Each data point is the average of measurements on three identical samples ± SD.

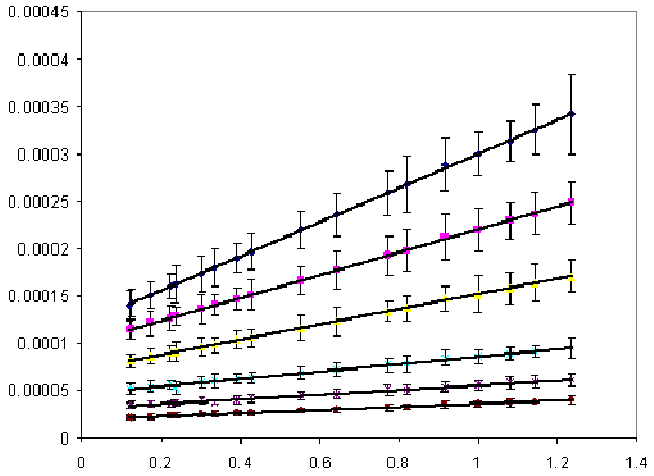

Figure 3. Graphs for DNA samples constructed by using equation (3) to calculate f_{A-T}.
(a) without exposure and (b, c, d, e and f) 10 min-post ultrasound exposure with powers 17, 34, 47, 65 and 77 W respectively. Each data point is the average of measurements on three identical samples ± SD.

are recorded at 30°C. Final absorbencies of the DNA samples at the same wavelengths are recorded at a high temperature of 90°C (after denaturation of the DNA).

Base destruction

Destruction of the constituent bases of DNA is associated with some loss of ultraviolet absorption. The measurement of this loss at 266 nm and at a high temperature of (90°C) in air-saturated solutions of the DNA would give a reasonable estimate for the extent of base destruction (Rafi et al., 1968).

Separation of DNA fragments by constant-field gel electrophoresis (CFGE)

DNA fragments resulting from treatment with different powers of ultrasound were separated using CFGE according to the method of Dahm-Daphi and Dikomey (1996).

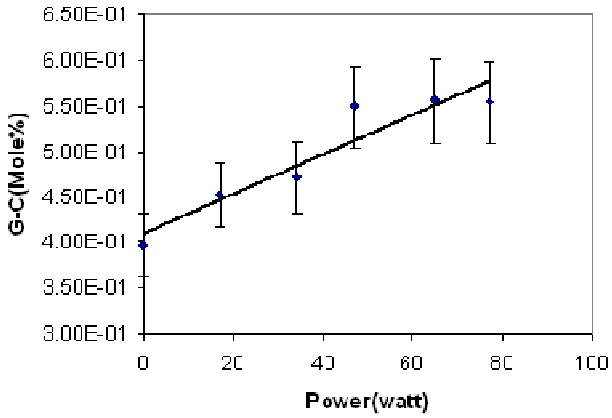

Figure 4. Effect of different powers of ultrasound on the mole % of hydrogen bonded G-C base pairs remaining in the DNA samples after exposure.The error bars represent the propagated uncertainties.

RESULTS

Absorption spectroscopy

Using a computer program, the experimental values of ΔA_λ at the eighteen wavelengths for the DNA samples (un-exposed and 10 min after exposure to different powers of ultrasound) with the corresponding values of the parameters $\Delta\varepsilon_{A-T\lambda}$ and $\Delta\varepsilon_{G-C\lambda}$ (Bloomfield et al., 1974) were inputted on the computer to obtain the least squares best fit to equations 2 and 3. The computer generated the values of slopes and intercepts and the propagated uncertainties in each.

Figures 2 and 3 show the results for the unexposed DNA sample (a) and 10 min after exposure of the samples to different powers of ultrasound (b, c, d, e and f). The least squares fit values and the linearity of these plots seem to confirm that this method is sensitive and satisfactory (within the experimental error) for evaluating the base composition of the DNA helical region, which survived thermal denaturation after exposure to ultrasound.

Figures 4 and 5 show the mole fraction of G-C and A-T base pairs respectively of the DNA's helical regions which survived denaturation after exposure to ultrasound for the different DNA samples (unexposed and 10 min after exposure to different powers of ultrasound). The decrease in the value of f_{A-T} and increase of the value of f_{G-C} with increasing ultrasound power can be attributed to greater ultrasound sensitivity of the A-T base pairs with respect to the G-C base pairs.

The values of f_{A-T} obtained 10 min after exposure to ultrasound of power 77 W is found from the results to be 27% less than the initial value in the native DNA.

The intercepts of the straight lines represented by equa-

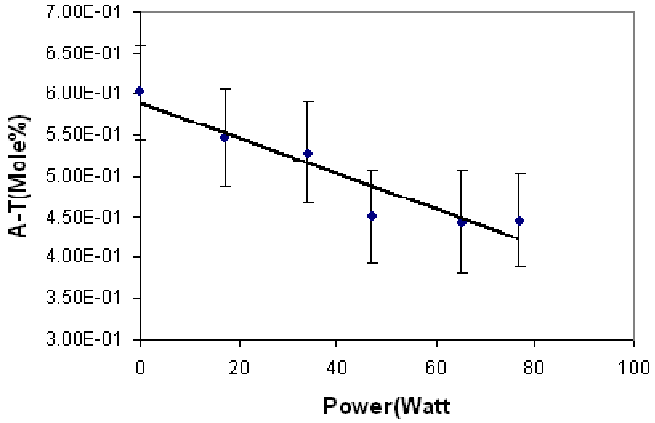

Figure 5. Effect of different powers of ultrasound on the mole % of hydrogen bonded A-T base pairs remaining in the DNA samples after exposure. The error bars represent the propagated uncertainties.

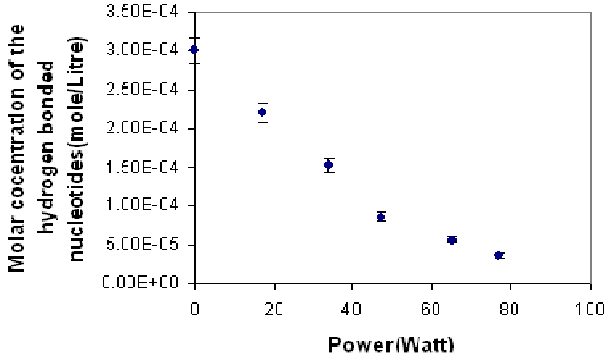

Figure 6. Molar concentrations of hydrogen bonded nucleotides of the DNA samples (unexposed and 10 min after exposure to different powers of ultrasound) calculated from the intercepts of the graphs in Figures1 and 2. The error bars represent the propagated uncertainties.

tions 2 and 3 are C f_{A-T} and C $f_{G-C,}$ respectively. The sum of these intercepts represents the hydrogen bonded nucleotides concentration (C). Figure 6 shows the molar concentration of the hydrogen bonded nucleotides (C) of the DNA samples (unexposed and10 min after exposure to different powers of ultrasound). The value of (C) obtained for the sample exposed to a power of ultrasound 77 W is 88% less than the initial value in the native DNA.

The linear graph (Figure 7) shows a high temperature absorption at λ = 266 nm (which is proportional to the number of intact nitrogen bases) of the DNA samples (unexposed and 10 min after exposure to different powers of ultrasound). From this graph, the number of the nitrogen bases for the sample exposed to a power of ultrasound 77 W is 14% less than the initial value in the native DNA.

Gel electrophoresis

From the gel electrophoresis photograph, the corresponding mean molecular size (in base pair) of the DNA fragments in the samples unexposed and 10 min after exposure to different powers of ultrasound have been calculated and shown in Figure 8.

The distributions of the fluorescent intensities for the same migration distance of the bands in the gel electrophoresis photograph for the DNA samples were obtained by using a Dual-Wavelength Flying-Spot Scanning Densitometry P/N 205 -13300. Figure 9 shows the numerical value of the area under the curve for the same migration distance versus the corresponding powers of ultrasound. The area under the curve for the same migration distance increases with increasing ultrasound power, which indicates that as the ultrasound power increases the fragmentation of DNA increases.

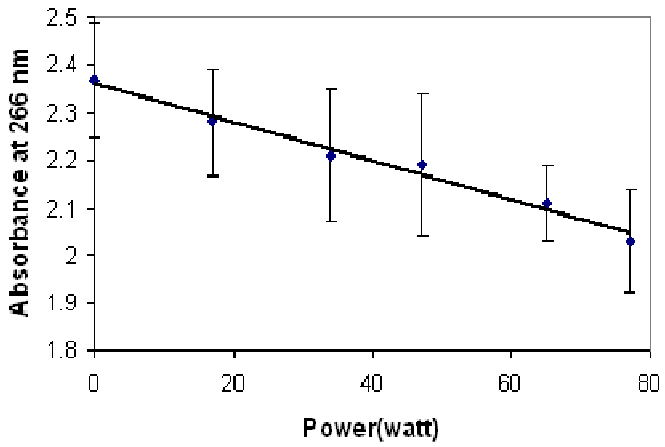

Figure 7. Effect of ultrasound power on the high temperature absorbance of DNA samples at 266 nm and 90°C in 1 cm cell. Each data point is the average of measurements on three identical samples ± SD.

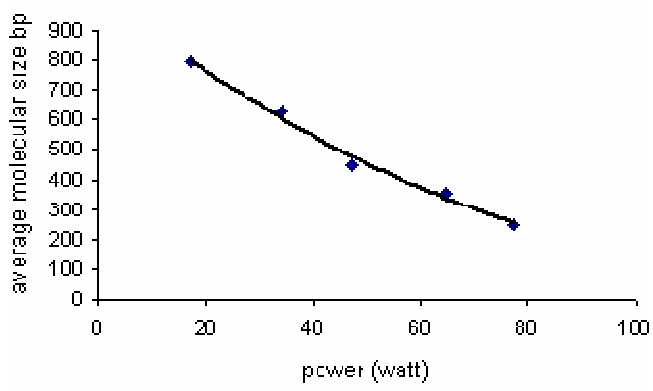

Figure 8. Effect of ultrasound on fragmentation of DNA.

DISCUSSION

Absorption spectrophotometer measurements showed

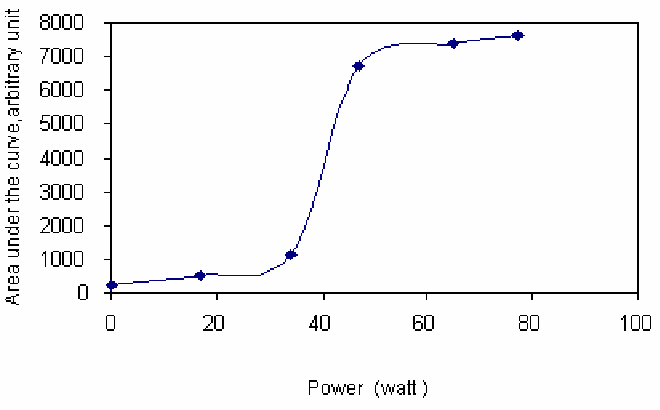

Figure 9. Numerical values of the area under the curve for the same migration distance versus ultrasound power.

that the number of hydrogen-bonded nucleotides decreased to 12% of the initial value in the native DNA for the DNA sample 10 min post-exposure with a power of 77 W, (Figure 6).

Some authors (Casarett, 1968) postulate that any of these bonds that are broken will tend to reform immediately and the irreversible damage will only occur when very large numbers of hydrogen bonds are broken at the same time. The increased value of the mole fraction f_{G-C} and the decreased value of the mole fraction f_{A-T} of the DNA's helical regions which survived denaturation by increasing the power of ultrasound for the DNA samples 10 min post-exposure indicated greater ultrasound sensitivity of the A-T base pairs with respect to the G-C base pairs. These results agree with that obtained by Rafi et al. (1968) for irradiation with γ-rays.

Nitrogen base damage can lead to local breakage of hydrogen bonds between the bases of opposite strands and to disturbance of base stacking (Georgakilas et al., 1998). The decrease of 14% in the number of the nitrogen bases of the native DNA sample due to exposure to ultrasound of power 77 W (Figure 7) compared to the percentage decrease in the hydrogen bonded nucleotides of 88% at the same power of 77 W (Figure 6), indicates that nitrogen base damage is not the only reason for the decrease of the hydrogen bonded nucleotides. The other reason for the decrease of hydrogen bonded nucleotides may be the damage of the deoxyribose-phosphate backbone which causes scission of the backbone and ends in the breakage of a single strand and separation of certain sections of the DNA. This can be shown from the small value of the average molecular weight corresponding to an ultrasound power of 77 W obtained from constant-filed gel electrophoresis, (CFGE) (Figure 8).

At a low power of ultrasound, there is a slight increase in fragmentation with increasing power of ultrasound, (Figure 9). For an ultrasound of power of about 30 to 48 W, there is rapid increase in fragmentation by increasing the power of ultrasound; this may be due to the formation of violent cavitation bubbles. The ultrasound power of 48 W is considered as a saturation power that is a very slight increase in fragmentation occurs (Figure 9). From Figures 8 and 9 it is quite clear that molecular weights below about 200 bp are hard to be obtained. The same result was obtained by Jernberg et al. (2001) for the DNA of (V79) cells when exposed to ultrasound. This effect may be due to a particular property of ultrasound not being able to make very short fragments significantly shorter than the distance between microcavitation sites. This effect may indicate that the damage obtained for the DNA is from the direct mechanical effect of cavitation.

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