SESSION 1:6 INTERACTION OF MEGAHERTZ ULTRASOUND AND BIOLOGICAL POLYMERS

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Attempts to understand in detail the alterations induced in animal tissues by intense ultrasound have led investigators to examine such interactions at various levels of biological structure. As a result, biopolymers in solution, in particular the unique macromolecular structure Deoxyribonucleic Acid (DNA), have received considerable attention. Investigations treating proteins have also been undertaken. While it is recognized that conformation of the DNA macromolecule in vitro bears little relation to that in vivo, it is hoped that such studies can aid in assessing the mechanical stresses to which polymers in vivo may be subjected during ultrasonic irradiation.

Much interest has arisen around the point of whether or not polymer degradation can occur in the absence of cavitation. One general approach has been to effect damage under certain conditions and then to take steps to suppress cavitation. Persisting degradation at a rate less than that existing before these steps have been taken has been used as an argument for the presence of a mechanism other than cavitation. There is general agreement that the main action of ultrasound on polymers is mechanical, since experiments show that degradation proceeds to a limiting molecular weight if irradiation is continued for long periods (*1*). However when working with cavitation in aqueous solutions where free radicals are produced, Alexander & Fox (*2*) accessed that 30 percent of their breakage could be due to chemical effects.

On ultrasonic irradiation of synthetic polymers at intensities from 5 to 700 W/cm^2 over the frequency range from 210 kHz to 2 MHz and simultaneously suppressing cavitation, several investigators reported no degradation (*3-7*). Some investigators have reported observing continued degradation, though at a reduced rate, with suppression of cavitation by irradiating samples under reduced ambient pressure (*8, 9*). This latter method of suppressing cavitation has not been widely used and no quantitative measures are available on the efficacy of this technique.

The DNA molecule consists of a polymer of two strands coiled in a double helix about a common axis. The backbone of the individual strands consists of pentose residues connected together by phosphate bridges. The pentose residues, in turn, are linked to purine or pyrimidine bases. In the double helical form, the sugars and phosphates are on the exterior and the bases on the interior of the helix. The main forces holding the two strands together are the base stacking forces which are the hydrophobic interactions between the heterocyclic bases as they stack in parallel arrays at right angles to the main helix axis (*10*).

Electron micrographs of DNA fragments produced by cavitation at 7 kHz have shown that breakage of the molecule occurs by double backbone scission, i.e., the ends of the fragments were double rather than single stranded (*11, 12*). An investigation of the viscosity of sonic fragments after irradiation with cavitating ultrasound at 800 kHz and 15 W/cm^2 supports the view that double backbone scission, rather than an accumulation of singles strand breaks, is the cause of DNA degradation (*13*). Chemical studies suggest that the main bond involved in the back-bone cleavage is the C-O bond (90 percent) with 10 percent P-O rupture and no appreciable C-C damage (*14*). In an attempt to detect a mechanism for DNA breakdown which does not involve the effects of cavitation (*15*), degassed samples of DNA were irradiated at 981 kHz and 25-31 W/cm^2. The sample holder and field geometry were such that the distribution of the acoustic energy over the sample chamber did not vary by more than 10 percent. A sedimentation coefficient change from 32 to 16,

corresponding (*16*) to a molecular weight change from 2.2×10^7 to 4×10^6, occurred in about 15 seconds. Irradiation for 2 minutes produced little additional degradation showing that a limiting molecular weight had been reached. Several methods were employed simultaneously to detect the presence of cavitation and no evidence was observed suggesting its presence. It was considered then that cavitation was not present and that the degradation resulted from viscous stresses established within the molecule, due to relative motion resulting from the density difference between the DNA molecule and the solvent molecules (water). Attempts to determine the geometrical configuration of the DNA molecule in solution, in order to obtain quantitative values for the mechanical stresses developed along the length of the polymer, have not been fruitful since the sensitivity of the acoustically induced birefringence method, while substantial for some pure liquids, is insufficient for polymer solutions (*17, 18*).

The degradation of DNA of molecular weight 10^7 exposed to 1 MHz ultrasound of 10 W/cm^2 and 30 msec repeated pulses has been observed to occur when the spherical sample holder was rotated during the irradiation period (*19*). No effect was observed in degassed solutions on irradiation without rotation of the sample holder. The degradation correlated with the sonochemical release of iodine from potassium iodide and the detection of the first subharmonic of the driving frequency. It was concluded that microstreaming around stable oscillating bubbles was responsible for the effect.

In a more recent study of the mechanism responsible for degradation, calf thymus DNA in solution was irradiated in a focused sound field at 1 MHz at intensities greater than those of previous studies. Here, transient cavitation was shown to be the cause of DNA breakage at intensities of 515 W/cm^2 and higher, though such events recorded at 288 and 200 W/cm^2 were insufficient to account for the breakage observed (*20, 21*). Further, since it was not possible to link 500 kHz subharmonic activity with degradation, as previously reported (*19*), the possibility of a noncavitation—linked mechanism of degradation cannot be completely discounted and the following computation lends support to this view.

If a noncavitating mechanism depending upon a time averaged force existed, the strain, S, experienced by a molecule in the sound field would be proportional to $I^{1/2}$ and also would depend upon the relaxation time T required for the molecule to respond to the stress. The streaming velocity of the molecule along the sound beam axis is proportional to I. The values of the relaxation time required of the DNA molecule so that the strain at 200 W/cm^2 (S_{200}) may be greater than that at 400 W/cm^2 (S_{400}) may be calculated since

$$S \propto I^{1/2}\left(1 - \exp\frac{-0.693t}{T}\right)$$

where t is the time the molecule spends streaming along the beam axis. This condition is fulfilled if

$$T > 0.82t.$$

The estimated speed of a cavitation event through the focal region at 515 W/cm^2 is 1 mm/sec (*22*). This decreases to about 0.8 mm/msec at 400 W/cm^2. If it is assumed that the DNA molecule moves at the same speed as the event, then the molecule traversed the 13 mm length of the container in 16 msec. Thus T must be greater than (0.82 x 16) or 13 msec. The DNA molecule extended and oriented in hydrodynamic flow relaxes on stopping the flow suddenly with a spectrum of relaxation times (*23*), the longest of which is given as

$$T = 5.0 \times 10^{-14} M^{1.6},$$

where M is the molecular weight.

The value of T relevant to the present study is 20 msec which fulfills the condition that T be greater than 13 msec. A large fraction of the DNA relaxes with the longest relaxation time (*23, 24*). The fact that the DNA relaxation times meet the requirements of a mechanism which would be noncavitational and yet explain the strange observed intensity dependence of degradation, viz., greater degradation at 200 W/cm^2 than at 515 W/cm^2, may be fortuitous but further experimentation involving irradiation of degassed DNA will be required before deciding between it and cavitational microstreaming.

An extensive study of the effects of noncavitating ultrasound on solutions of α- chymotrypsin, trypsin, aldolase, lactate dehydrogenase and ribonuclease has been carried out in which (1) the en-

zyme solutions were irradiated and then analyzed to determine effects on the physical and chemical properties of the protein molecules and (2) enzymecatalyzed reactions were irradiated and simultaneously monitored spectrophotometrically (25). This study was conducted in the frequency range 1 MHz to 27 MHz, at intensities as high as 10^4 W/cm^2, depending upon frequency, and for time durations ranging from 0.1 sec to 10 min., depending upon intensity. It emerged from this study that cavitation is a necessary condition for ultrasonic degradation of enzymes in solution. Thus, if it is considered that the results of the studies of macromolecules in solution can be extrapolated to the tissue environment, then molecules the size of proteins must be eliminated as the sites of interaction, except perhaps for unusually intense fields (26).

A controversial report (27,28), in which the inactivation of enzymes in solution by 3 MHz ultrasound in the intensity range 1-3 W/cm^2 is reported, has been investigated. The inactivation has been shown not to be due directly to an interaction between the ultrasonic waves and the protein molecules, but rather to a reaction between the solution and the protein molecules, but rather to a reaction between the solution and the rubber material used as part of the containing vessel (29).

Because the solution environment is considerably different than that obtaining in vivo, the results of investigations concerning the fate of biological polymers must be reinterpreted and extrapolated with extreme caution. Very likely there is little that can be gleaned from these studies that lend insight to events occurring in tissue exposed to ultrasound. A different situation may well occur for the next higher level of structure, viz., specific macromolecular arrangements, and investigations of the interaction of ultrasound and biological membranes should begin to receive considerable attention (30).

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