Ultrasonic Investigation of Aqueous Solutions of Deoxyribose Nucleic Acid*

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The ultrasonic absorption of aqueous solutions of salmon sperm deoxyribose nucleic acid (DNA), molecular weight of the order of 10^6, was determined as a function of *p*H in the neutral and acidic regions over the frequency range 5–50 MHz at 25°C. The viscous relaxation frequencies appear to be well below 1 MHz. At neutral *p*H values, viscosity and molecular conformation appear to contribute little in determining the ultrasonic absorption spectrum. The DNA solution absorption spectrum appears to be similar to that of globular protein aqueous solutions implying that the mechanisms may be the same. The ultrasonic absorption acid titration curves for DNA exhibit maxima around *p*H 2.6 and 12 [J. Chim. Phys. **66**, 81 (1969)] which can be attributed to either or both hydrogen bond transfer and proton transfer as the double helix DNA molecule denatures.

Subject Classification: 16.2.

INTRODUCTION

The mode of interaction of ultrasound and biological media is of growing concern as this agent receives ever increasing attention in clinical medicine. However, the interaction mechanisms are generally not well established, if known at all, thus limiting the application of ultrasound and creating doubt of its efficacy and safety. The ultrasonic properties of macromolecular conformation in aqueous solution of biomacromolecules have received considerable attention in attempts to understand fundamental processes of absorption at the molecular level.[1–7] Aqueous solutions of macromolecules which assume globular or rigid conformations, i.e., possess tertiary or quaternary structure, typically exhibit ultrasonic absorption magnitudes greater than those which assume a random coil conformation. However, destruction of higher-order structure as, for example, hemoglobin in 5*M* aqueous guanidine hydrochloride solution in which this molecule assumes a random coil conformation, appears not to produce an ultrasonic absorption spectrum significantly different from that of the native molecule in aqueous solution.[3] Thus, the importance of macromolecular spatial arrangement in aqueous solution remains unsettled. The ultrasonic examination of deoxyribose nucleic acid (DNA) aqueous solutions was considered, since this molecule possesses a conformation markedly different from those previously studied.[7–9]

Nucleic acids are among the largest macromolecules known, some ranging in molecular weight greater than 10^9. The primary structure of DNA, one particular nucleic acid, is a polyester chain of polynucleotides consisting of alternating phosphoric acid and pentose sugar with a base connected to the sugar. The nucleotide monomer consists of the phosphoric acid, sugar, and base. The four common bases found in DNA are the pyrimidines thymine (T) and cytosine (C) and the purines adenine (A) and guanine (G). The secondary structure of DNA consists of two complimentary right-handed, helical, polynucleotide chains coiled around a common axis to form a double helix with the bases, located in pairs, appearing on the inside of the helix in such a fashion that A pairs only with T and G only with C.[8] The hydrogen bonds between the complimentary base pairs are essential in determining the form and specificity of the helix, but do not appear particularly important in affecting helical stability, i.e., maintenance of the DNA double helix integrity. The main contributions to the helix stability are attributed to the electrostatic and hydrophobic interactions, viz., stacking forces, appearing between the parallel stacked bases.[9,10]

I. MATERIALS AND METHODS

The salmon sperm DNA (Calbiochem, lot Nos. 50075 and 50209) solutions were prepared by placing the appropriate quantity of crystallized DNA on the top surface of a measured volume of solvent and stirring gently at 7°C until mixing was complete, usually two weeks. The solvent, SSC, was 0.15*M* NaCl (Mallinckrodt

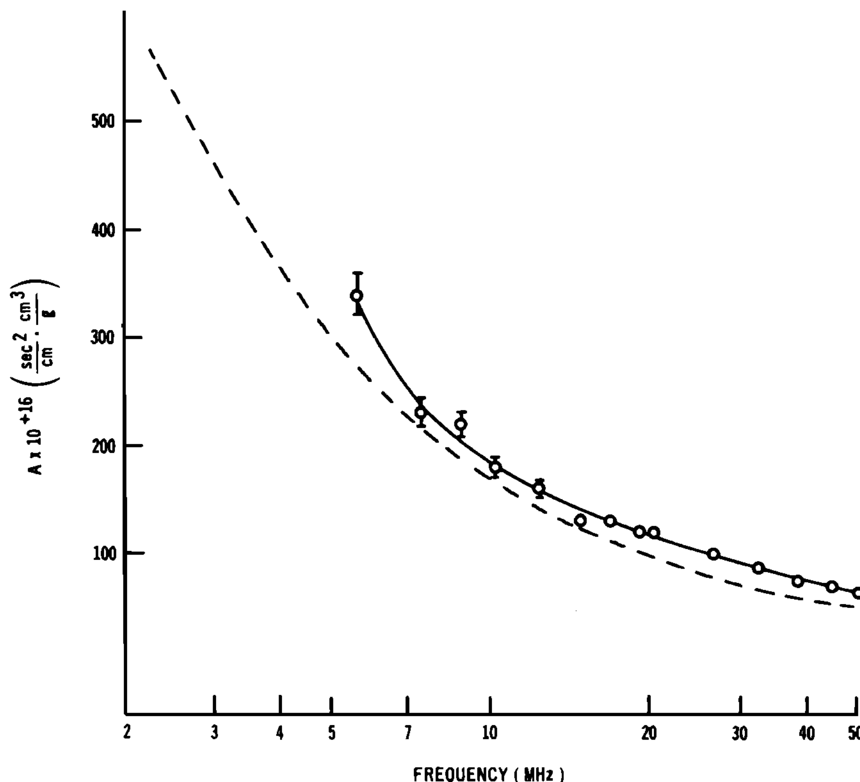

FIG. 1. Ultrasonic absorption spectrogram of aqueous solutions of salmon sperm DNA at 25°C and bovine hemoglobin at 10°C (- - -).

Analytical Reagent) plus $0.015M$ $Na_3C_6H_5O_7$ (Baker Analyzed) in singly deionized and distilled water. Bacterial growth was inhibited by the addition of chloroform (Allied Chemical Reagent Grade), which was removed prior to ultrasonic measurements. The DNA concentrations, typically about 0.004 g/cm^3 were determined with a Beckman Model DU Spectrophotometer using the extinction coefficient

$$E\,\Big|\,\frac{1\%}{260}=224$$

at pH 12. The dry wieght of protein in the dry sample of DNA was determined as 0.003% using the Folin–Ciocalteu test. Sedimentation coefficient of one DNA sample yielded a molecular weight between one and ten million.

The ultrasonic absorption measurements were performed in an automated version of the Pellam and Galt[11] apparatus described in detail elsewhere.[12,13] Recent modifications and improvements allowed for the on-line operation of the ultrasonic equipment and analysis of the data with a PDP-8 digital computer. Diffraction corrections were applied to all data,[14] although they were negligible at frequencies greater than 15 MHz. The temperature of the solutions investigated was maintained with an accuracy of ±0.1°C. The accuracy of the ultrasonic absorption data presented in Fig. 1 and 3 is within ±10% at the lower frequencies and ±3% at the higher frequencies.

II. RESULTS AND DISCUSSION

Figure 1 represents the frequency-free ultrasonic absorption per unit concentration, defined as

$$A=(\alpha_{\text{solution}}-\alpha_{\text{solvent}})/cf^2 \qquad (1)$$

as a function of frequency, where α_{solution} is the ultrasonic absorption coefficient determined for the specimen solution (corrected for diffraction), α_{solvent} is that of the solvent (SSC in the case of DNA solutions), c is the macromolecular concentration in grams per cubic centimeter and f is the ultrasonic frequency. The upper curve represents the salmon sperm DNA over the frequency range 5–50 MHz at 25°C and pH 7. The static viscosity of these DNA solutions is in excess of one poise which corresponds to a classical Stokes absorption magnitude in excess of $20\,000\times10^{-16}$ sec^2/cm $\cdot$cm^3/g, or two orders of magnitude greater than appearing in Fig. 1. When the double helix DNA is denatured by heat to single-stranded DNA, the static viscosity decreases approximately two orders of magnitude with no observed change in the ultrasonic absorption spectrum.[13,15] Thus, it is clear that the viscous relaxation frequencies of both the native and denatured DNA solutions are well below 1 MHz and, as a result, viscosity plays an unimportant role in determining the ultrasonic absorption properties in the frequency range of interest here.

When the DNA is thermally denatured, its conformation in aqueous solution is altered. The native molecule

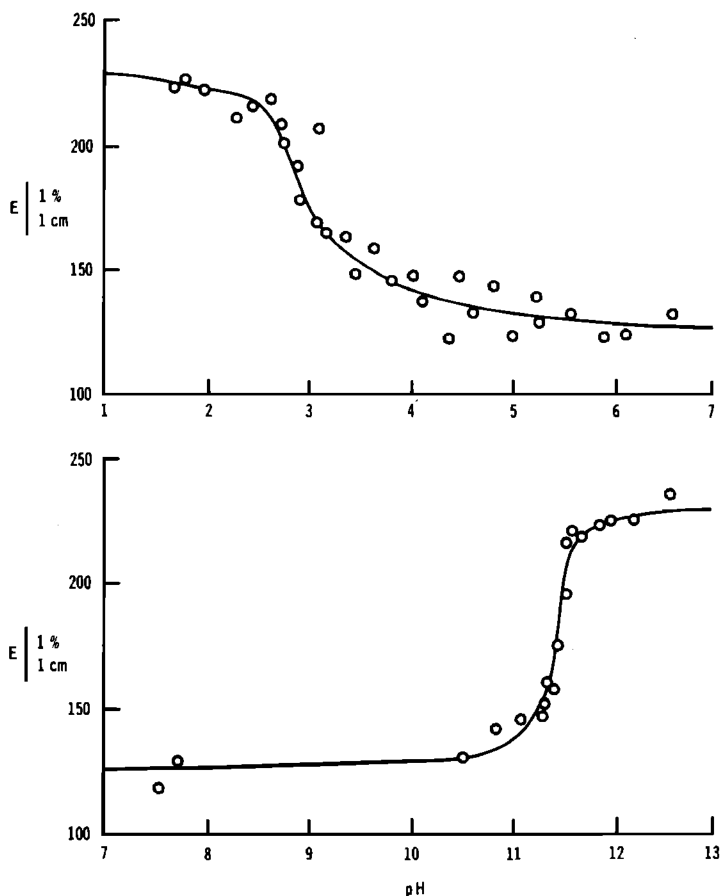

FIG. 2. Ultraviolet titration curve (259 mμ) of aqueous solutions of salmon sperm DNA at 20°C.

possesses a wormlike conformation, i.e., the structure is a slightly flexible rod 25 Å in diameter and with length linearly proportional to molecular weight. When the DNA molecule is thermally denatured, the double helix separates into two single-stranded polynucleotide chains with each chain assuming a random-coil conformation. Thus, there also appears to be little effect on the ultrasonic absorption spectrum as a result of this conformational change. A similar finding appears for the globular protein hemoglobin[3] when it is denatured chemically.

The ultrasonic absorption magnitude and frequency dependence of DNA in Fig. 1 is similar to that of hemoglobin at 10°C.[1] Other globular proteins such as bovine serum albumin at 20°C,[6] ovalbumin at 10°C,[13] and hemoglobin at 25°C,[16] posses ultrasonic absorption magnitudes within 15% of the hemoglobin curve in Fig. 1. This finding can be considered strong evidence that the ultrasonic absorption mechanisms are the same for both aqueous solutions of DNA and globular proteins. For example, one commonly occurring feature among these aqueous solutions is the existence of an increased structuring of water in the vicinity of the macromolecule. As a result, the absorption process may be due to the interaction of the ultrasonic wave and the hydration layer, with this layer being dependent upon the forces maintaining the integrity of the macromolecule. An additional speculated absorption process is related to the degree of hydrogen bonding which exists in such solutions.[17]

Ultraviolet (uv) absorption is a useful technique in characterizing aqueous solutions of nucleic acids. Figure 2 represents the uv absorption, at a wavelength of 259 mμ, as a function of *p*H for aqueous salmon sperm DNA solutions. In the upper acidic and lower alkaline titration curves, the uv absorption is shown to increase sharply (the hypochromic effect) at extreme *p*H values around 3 and 11 because the actual quantitative uv absorption is less for the double helix DNA molecule than that predicted from the summation of the absorbed uv energy by the individual nucleotides. The uv titration curve of Fig. 2, together with those for intrinsic viscosity[10] and sedimentation coefficient,[18] identify the *p*H values at which the double helix DNA molecule collapses and the two chains separate yielding two single-stranded, random-coiled polynucleotide chains. This breakdown in structure occurs around *p*H 2.5–3.5 and 11.0–11.5.

Figure 3 shows the ultrasonic absorption titration curve at acidic *p*H values over the frequency range 5–50 MHz for salmon sperm DNA at 25°C. The absorption maximum observed around *p*H 2.6 has also been shown to occur at 10°C[13] and has been observed in

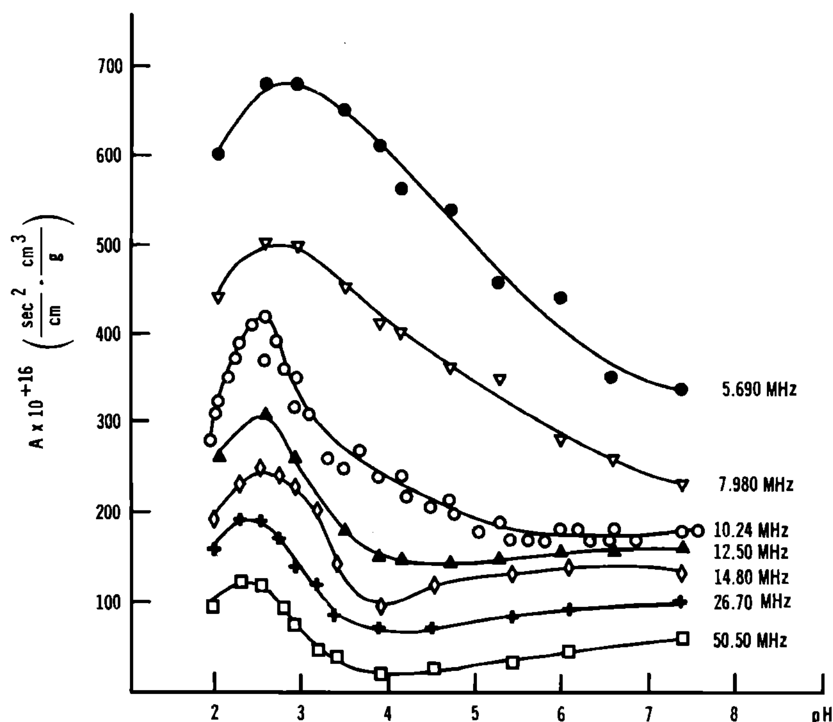

FIG. 3. Ultrasonic absorption acidic titration curve of aqueous solutions of salmon sperm DNA at 25°C.

calf thymus DNA of molecular weight 10^5 at 25°C.[19] This absorption maximum occurs at the *p*H at which the hypochromic effect reaches its maximum value. Lang and Cerf[15] have observed an ultrasonic absorption peak in calf thymus DNA solutions in the alkaline region around *p*H 12, the *p*H at which the hypochromic effect reaches its maximum value (as seen in Fig. 2). Thus, the ultrasonic absorption maxima at *p*H values 2.6 and 12 correspond to the separation of the polynucleotide chains of the double helix DNA molecule into two single-stranded, random-coiled polynucleotide chains.

The proton-transfer reaction[2] has been invoked to explain similar ultrasonic absorption maxima in aqueous solutions of globular proteins and may be considered as a possible mechanism contributing to absorption phenomena in aqueous DNA solutions. The DNA bases possess residues such as carboxyl and amino groups which can participate in the proton-transfer reaction. In the double helix structure, these bases are positioned along the helical axis and not exposed to the aqueous environment. When the polynucleoticle strands separate in response to the appropriate acid or base titration, the bases are exposed to the environment and the carboxyl or amino groups are susceptible to protonation. For the DNA concentrations utilized in this study, protonation of the carboxyl group somewhere between *p*H 3.5 and 2.5 should produce absorption maxima between *p*H 1.75 and 1.25. In the alkaline *p*H region, if the amino group is protonated between *p*H 11 and 12, the absorption maxima would occur between *p*H 12.5 and 13. Since the absorption maxima occur at 2.6 and 12, there is reasonable doubt that the proton-transfer reaction can be invoked to account for such maxima in DNA solutions.

As the helical structure of the DNA molecule denatures, the base–base hydrogen bonds between A–T and C–G are replaced by base-solvent hydrogen bonds. The energy transfer of base–base to base–solvent hydrogen bonds, stimulated by the ultrasonic energy, may be regarded as the process eliciting both absorption maxima in the extreme acidic and alkaline *p*H regions. The hydrogen bond transfer from base–base to base–solvent occurs at the same *p*H values as do the absorption maxima.

It has been suggested recently[20] that the absorption maxima appearing in the acid and alkaline *p*H regions are essentially due to proton-transfer equilibria. At alkaline *p*H values, similar ultrasonic absorption titration curves were obtained for both native and thermally denatured DNA solutions, i.e., the bases of the latter solutions were exposed to the environment. However, it has been shown that intrachain base–base hydrogen bond interaction occurs at *p*H values less than 10 for such denatured DNA[18], and while the above suggestion provides a convincing argument for the absorption maxima being due to proton transfer, the possibility of explaining these results by invoking hydrogen bond transfer from base–base to base–solvent must also be considered. However, the evidence presently available is inconclusive for favoring completely one mechanism over the other.

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