Ultrasonic absorption in aqueous solutions of chondroitin sulfate

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(Received 30 September 1974; revised 7 January 1975)

Ultrasonic absorption and velocity were determined in aqueous solutions of chondroitin sulfate, the major sulfated mucopolysaccharide which is distributed throughout mammalian connective tissue, as a function of ultrasonic frequency (2.39–62.4 MHz), temperature (10 °–37 °C), and *p*H (0.85–13.3). A broad distribution of relaxation frequencies is required to describe the absorption spectra. The apparent activation energy is 3.7 kcal/mole at a concentration of 0.054 gm/cc and at neutral *p*H over the frequency range 8.87–50.5 MHz. It is suggested that the same absorption mechanism(s) may be responsible in aqueous solutions of chondroitin sulfate and dextran at neutral *p*H. The absorption peaks near *p*H 2.5 and 11.5 are typical of those observed for the proton-transfer mechanism.

Subject Classification: 80.20; 35.24.

INTRODUCTION

The most extensively studied group of biological macromolecules by ultrasonic spectroscopy has been the globular proteins,[1] following early work which showed that approximately two-thirds of the ultrasonic absorption in liver tissue occurred at the macromolecular level[2] and that the ultrasonic properties of blood are determined largely by the protein content.[3] From such studies it is possible to suggest that the same mechanism(s) may be responsible for the ultrasonic absorption in aqueous solutions of globular proteins and of deoxyribose nucleic acid. Because data obtained thus far remain insufficient, the specific absorption mechanism(s) have not been elucidated for the globular proteins and the nucleic acids groups of biopolymers, nor have other similarities among biopolymer groups been identified. Chondroitin sulfate (CS) was chosen as the subject of this investigation because it is an important biopolymer, the major sulfated mucopolysaccharide (polysaccharides which contain hexasamine) of mammalian connective tissue, and because it can be compared at neutral *p*H with another polysaccharide, dextran,[4] which has received ultrasonic absorption attention. Similarities in the absorption magnitude and frequency dependence are observed with these two polysaccharides.

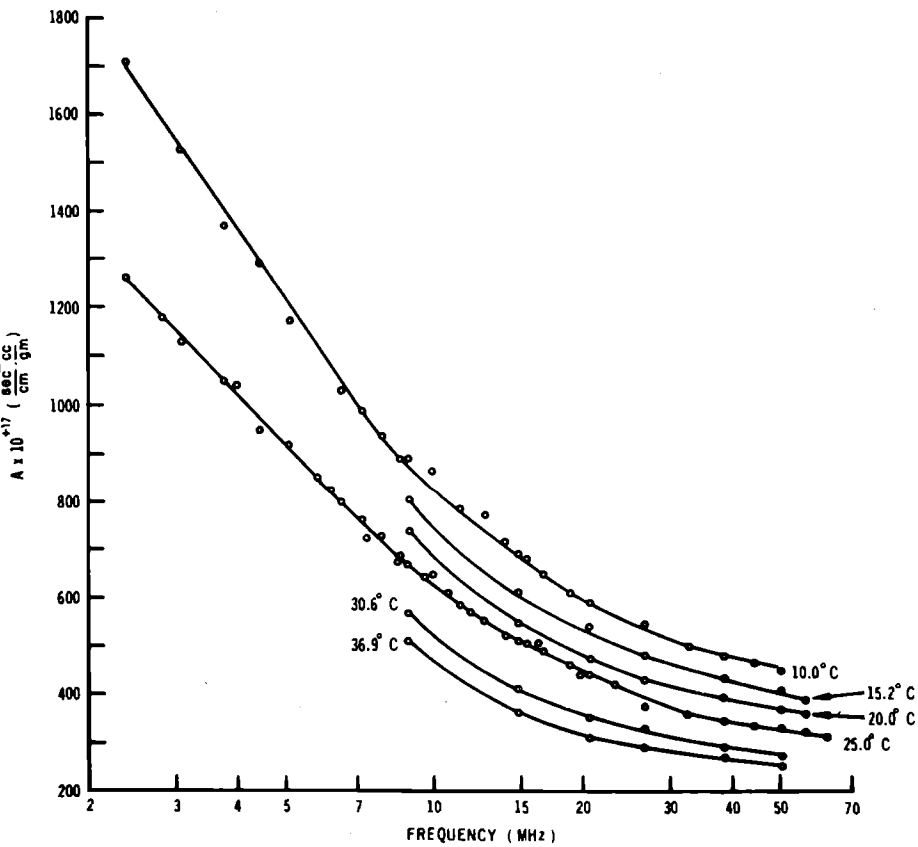

FIG. 1. Ultrasonic absorption spectrogram for aqueous solutions of chondroitin sulfate.

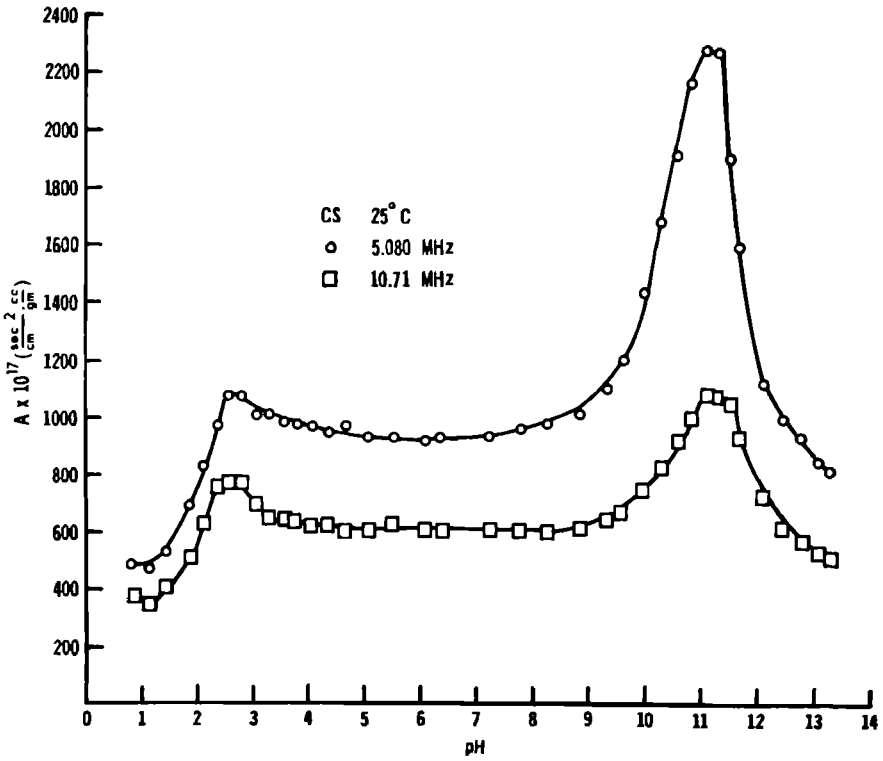

FIG. 2. Ultrasonic absorption titration curve for aqueous solutions of chondroitin sulfate.

I. MATERIALS AND METHOD

Chondroitin sulfate (source: cartilaginous tissue) is a linear unbranched polymer with a broad molecular weight distribution extending from 10 000 to 50 000.[5] The CS from cartilage is predominantly a mixture of CS-*A* and CS-*C*, both of which have the identical repeating disaccharide unit but differ in the position of the esterfied sulfate. Aqueous solutions of CS (Nutritional Biochemicals Corporation, control no. 8761) were prepared with singly deionized and distilled water and used without filtering. The two ultrasonic spectroscopic systems employed in this study have been described in detail elsewhere.[6,7] The accuracy of the ultrasonic absorption measurements, for both systems, is within ±3%. The CS concentrations were determined to an accuracy of better than ±0.3% at room temperature by evaporating 15 ml in a tared beaker over air until dry, followed by vacuum desiccation over indicating anhydrous $CaSO_4$ for 24 h. All measurements were performed at CS concentrations less than 0.1 gm/cc, a range in which it was experimentally determined in this study that the ultrasonic absorption was a linear function of concentration.

II. RESULTS AND DISCUSSION

Figure 1 shows the excess frequency-free absorption per unit concentration parameter A ($\alpha_{solution} - \alpha_{solvent}/cf^2$) as a function of ultrasonic frequency for six temperatures at neutral pH, where $\alpha_{solution}$ is the total (measured) ultrasonic absorption of the CS solution corrected for diffraction, $\alpha_{solvent}$ is the ultrasonic absorption of the solvent, water, c is the mass concentration (grams/cc) of the CS in water, and f is the ultrasonic frequency. A broad distribution of relaxation frequencies is required to describe each of the ultrasonic absorption spectra. When the 10° and 25 °C curves are plotted in log–log manner, they exhibit a straight line with a −0.5 slope and intersect 10 MHz and 840 and 620 (sec²/cm) · (cc/gm), respectively. Comparison of the ultrasonic absorption spectrum for CS at 25 °C to that of aqueous solutions of dextran, a random coil polysaccharide, shows that the absorption magnitude and frequency dependence are similar over the frequency range from 2.39 to 62.4 MHz.[4] Also, for comparison with other polymer solutions, the CS absorption spectrum is 25% to 40% less in magnitude than that of aqueous solutions of globular proteins and deoxyribose nucleic acid and 40% to 170% greater in magnitude than that of aqueous solutions of polyethylene glycol, a synthesized random coil macromolecule.[1]

Ultrasonic absorption and velocity were determined as a function of temperature, over the range 10° to 37 °C at neutral pH in aqueous solutions of CS at a concentration of 0.054 gm/cc, in order to provide information on apparent activation energies. The apparent activation energy ΔF was determined by an Arrhenius plot as the six frequencies—8.87, 14.8, 20.7, 26.7, 38.6, and 50.5 MHz—and yielded a frequency-independent value of 3.7 kcal/mole.

Thus it can be suggested that the same mechanism(s) may be responsible for the ultrasonic absorption in the polysaccharide group of biopolymers. A specific mechanism speculated for dextran is that the normal modes of the molecule merely determine the mode of coupling to some other relaxation process involving the solvent because the magnitude of the ultrasonic absorption observed at neutral pH would require a substantially greater volume concentration than would be warranted by straightforward predictions from a random-coil model.[4]

Figures 2 and 3 show A as a function of pH at 25 °C

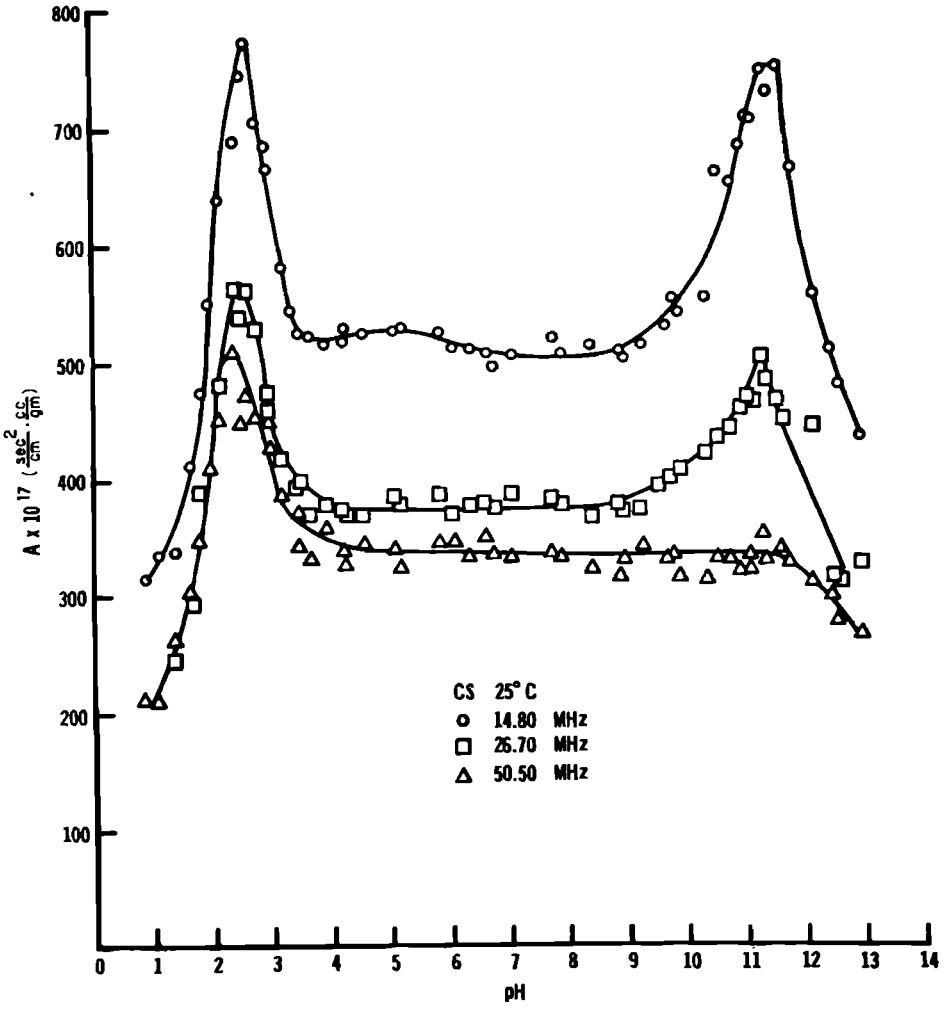

FIG. 3. Ultrasonic absorption titration curve for aqueous solutions of chondroitin sulfate.

for five frequencies. The absorption peaks at *p*H values 2.5 and 11.5 are typical of those observed for the proton-transfer mechanism.[1] The mechanism(s) responsible for the absorption peak in the acid *p*H range do not appear to begin to relax until frequencies greater than 14 MHz and have not completely relaxed by 50 MHz. Note that the ratio of A at *p*H 2.5 to A at neutral *p*H, as a function of frequency, varies only slightly from 1.1 at 5.08 MHz to 1.3 at 10.71 MHz and remains constant at 1.5 for 14.8, 26.7, and 50.5 MHz. On the other hand, the ratio of A at *p*H 11.5 to A at neutral *p*H decreases with increasing frequency from 2.5 at 5.08 MHz to 1.8 at 10.71 MHz to 1.6 at 14.8 MHz to 1.3 at 26.7 MHz to finally 1.0 at 50.5 MHz. Thus, by 50.5 MHz, the proton-transfer processes responsible in the alkaline *p*H range have completely relaxed. CS contains one carboxyl and one ester sulfate group per monomer unit. The *p*K value for CS carboxyl ranges between 3.35 and 4.41, depending upon the ionic strength.[5] The absorption peaks in the acidic *p*H range can be attributed to the carboxyl group. Additional work will be required to explain the absorption peak in the alkaline *p*H region since neither the CS molecule nor protein impurities (negative biuret reaction) can account for the peak.

In summary, it is suggested that the same ultrasonic absorption mechanism(s) may be responsible in aqueous solutions of chondroitin sulfate and dextran, both polysaccharides, at neutral *p*H, and that the absorption peaks near *p*H 2.5 and 11.5 are typical of those observed for the proton-transfer mechanism.

ACKNOWLEDGMENT

Appreciation is extended to Professor Floyd Dunn for the use of the spectroscopy equipment at the Bioacoustics Research Laboratory, University of Illinois, and Mr. Steve Goss for his assistance.

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