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THE MECHANISMS OF ABSORPTION OF ULTRASOUND IN PROTEIN SOLUTIONS IN THE FREQUENCY RANGE 100 kHz-1MHz

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INTRODUCTION

The widespread and increasing use of ultrasound in medical practice over the last two or three decades has been associated with a growing scientific interest in the interaction mechanisms of ultrasound with soft tissue. The interest has been due to a conviction that better understanding of these mechanisms might improve the diagnostic accuracy of ultrasonic imaging techniques and also that the efficacy of therapeutic ultrasound might be increased if more were known about its mode of action. Recently, however, the need for more scientific effort in this field has been highlighted by concern about the safety of diagnostic ultrasound [1].

ATTENUATION MECHANISMS

The mode of action of ultrasound on soft tissue is complex and there are a number of identifiable energy loss mechanisms which include absorption, scattering, specular reflections, mode conversion, non-linear propagation and cavitational activity. Which of these mechanisms is dominant, will depend upon the physical parameters of the ultrasonic source and the propagation properties of the materials concerned. It cannot be assumed that the process which causes the greatest energy loss in the transmitted beam will necessarily be the dominant cause of any observed biological effect. Nevertheless, there is a growing amount of data on the attenuation coefficients of many tissues in the medical frequency range [2] which is normally taken to be predominantly between 1 and 10 MHz.

Relaxation Processes

Figures for the absorption coefficients of proteins in solution have also been published [3,4,5]. The situation has been summarised by Edmonds in a recent review paper [6] in which he concluded that up to 70% of total tissue attenuation could be accounted for by protein absorption. A number of mechanisms have been postulated to account for the observed values all of which are relaxational in nature, and therefore rely on the concept that some equilibrium (physical or chemical) is momentarily perturbed by either the local pressure or temperature fluctuations of the ultrasound wave.

A single relaxation process can be described in terms of the absorption per unit wavelength $\alpha\lambda$ [7] viz:

$$\alpha\lambda = \frac{2(\alpha\lambda)_{max}\, f/f_r}{1+(f/f_r)^2} \qquad (1)$$

where $(\alpha\lambda)_{max}$ is the peak value of $\alpha\lambda$, f if the frequency and f_r is a constant

called the relaxation frequency. This is shown in fig. 1.

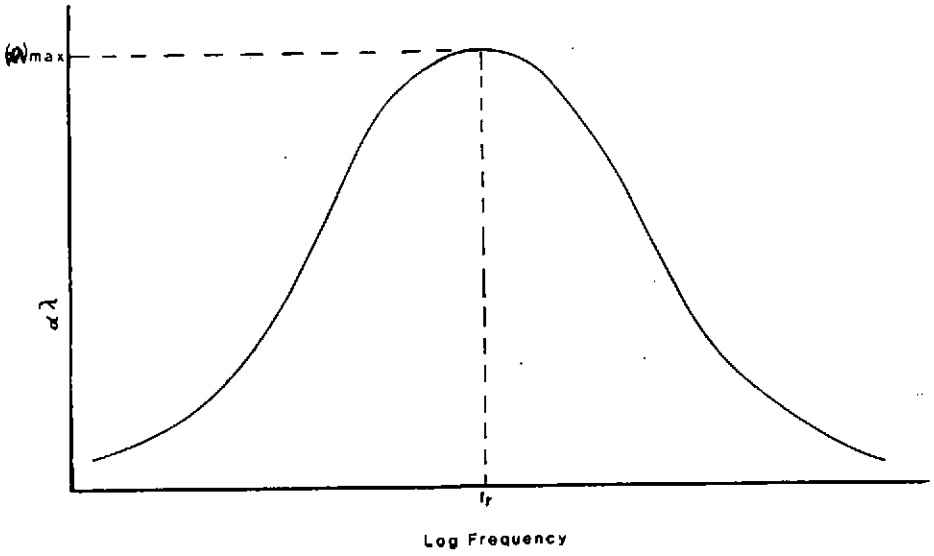

Fig. 1. Characteristic $\alpha\lambda$ curve for a single relaxation process.

It is clear from equation (1) that the $\alpha\lambda$ value falls to 20% of its peak within 1 decade of the relaxational frequency f_r. This relatively rapid fall as a function of frequency is not typical of macromolecular solutions as has been pointed out by several workers [8,9] and typically a broad spectrum of overlapping processes has been hypothesised to account for the experimental observations. It is therefore of great importance to study such systems over as wide a range of frequency as possible if the precise nature of these mechanisms is to be understood.

Measurement Techniques

The techniques most commonly applied to perform absorption measurements on macromolecular solutions have been pulse-echo techniques involving tone bursts at the frequency of interest. In the low megahertz range, the substitution method [10] has been applied and at higher frequencies the method pioneered by Pellam and Galt [11] has been widely used. More recently continuous wave cylindrical resonators have become more generally available for use in the frequency range 1-10 MHz [12]. However, below 1 MHz, there is still a significant lack of data. This is because the techniques mentioned above either require prohibitively large volumes or are diffraction limited or both.

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APPARATUS

The method used in this study has been described elsewhere [13]. Briefly, it consists of a spherical glass vessel, suspended as freely as possible with two PZT transducers bonded on to the outside (fig. 2).

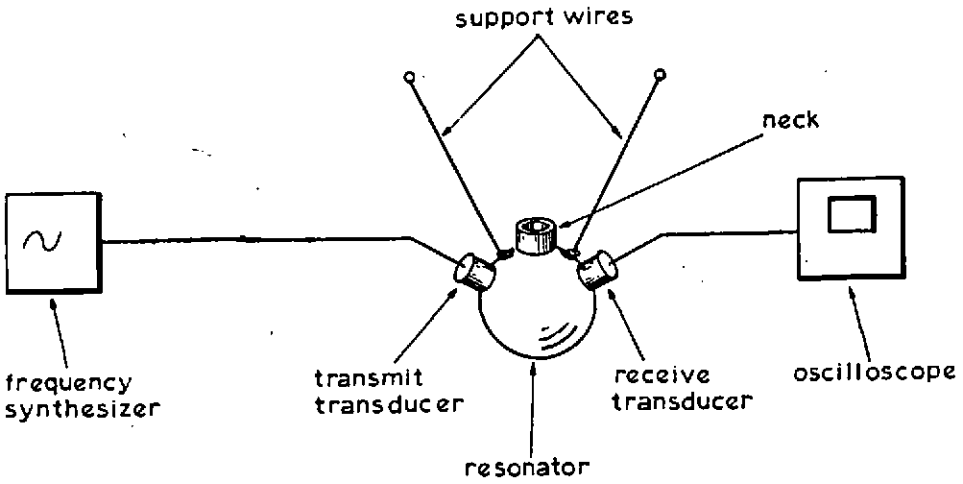

Fig. 2. Schematic Diagram of the Apparatus Used in this Study

Larger volume versions of this (up to 40 litres) have been used extensively [e.g. 14] in the frequency range 50kHz-500kHz by exciting the system with one transducer at one of its radially resonant frequencies and observing the decay on the second transducer when the excitation was stopped. In this case, we have preferred to monitor the Q value of the system at a number of resonances.

We then have:

$$Q = \frac{\pi}{\alpha\lambda} \qquad (2)$$

where $\alpha\lambda$ is the loss per wavelength of the system as a whole. This can be divided into the sample loss $(\alpha\lambda)_s$ and the unwanted container loss $(\alpha\lambda)_c$;

$$\alpha\lambda = (\alpha\lambda)_s + (\alpha\lambda)_c \qquad (3)$$

The sample loss can be found by filling the system with a liquid of known or negligible absorption but with same sound velocity as the sample and making the assumption the $(\alpha\lambda)_c$ remains constant.

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RESULTS

The study has so far concentrated on the acoustic properties of two globular proteins in solution, bovine serum albumin (B.S.A.) and haemoglobin, since these have been characterised at higher frequencies and are readily available. The B.S.A. results have already been reported [13] showing a peak in $\alpha\lambda$ at about 350 kHz under acid conditions. Haemoglobin shows similar behaviour and these results are summarised in fig. 3.

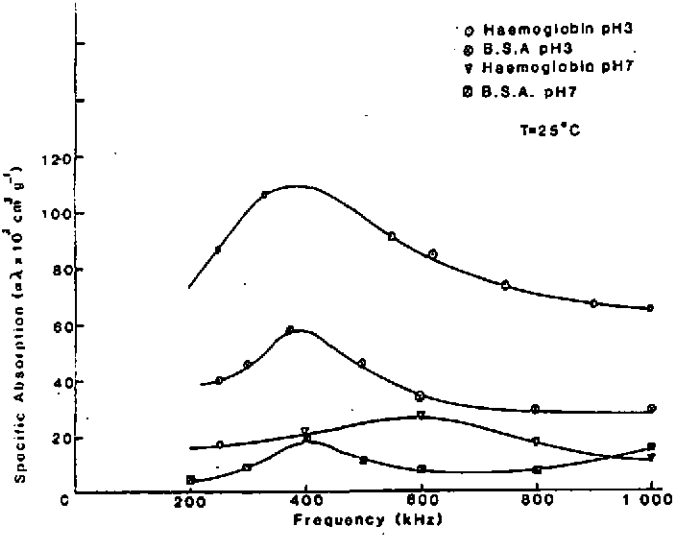

Fig. 3. Absorption ($\alpha\lambda$) per unit concentration of Haemoglobin and B.S.A. as a function of frequency

It is possible to ascribe the acid peak to a number of possible mechanisms which have been postulated but the two most probable seem to be perturbation of either a structural equilibrium (e.g. helix-random coil transition [4]) or a proton-transfer equilibrium [15] involving the carboxyl groups;

$$R\text{——}COOH \rightleftharpoons R\text{-}COO^- + H^+ \qquad (4)$$

In order to investigate this further a denaturing agent, guanidine hydrochloride (GuHCl), was added to the protein solutions, at neutral pH. The results are shown in fig. 4, where peaks in $\alpha\lambda$ can be clearly observed for both proteins. Although the maximum $\alpha\lambda$ values obtained are remarkably similar to the pH induced values (fig. 3) there is a shift in the frequency at which the maximum occurs for both proteins. It is therefore tempting to regard this as evidence of structural relaxation processes.

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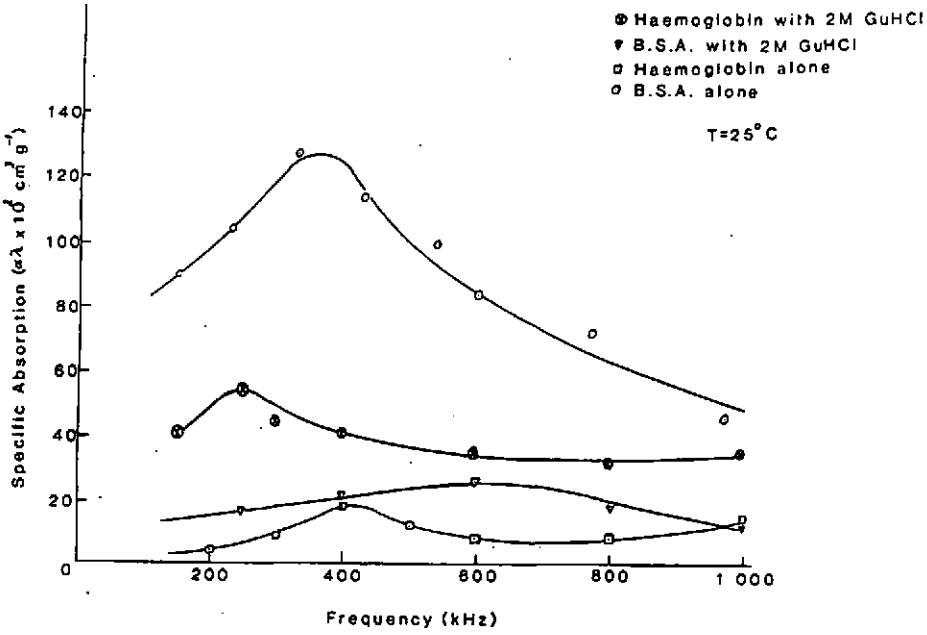

Fig. 4. Absorption ($\alpha\lambda$) per unit concentration of Haemoglobin and B.S.A. in the presence of GuHCl.

However maximum absorption occurred at roughly 2M concentration of GuHCl whereas at least 6M GuHCl is required to achieve complete denaturation [16]. Also further studies with another denaturant, ammonium sulphate, did not produce similar absorption peaks.

To test the structural relaxation hypothesis further, measurements were also performed on several amino acids under the same conditions. Since amino acids are relatively simple molecules used as protein 'building blocks' it is difficult to imagine any possible structural relaxation process involving an amino acid molecule alone.

The results of both acidification and GuHCl treatment for one amino acid, glutamic, are shown in fig. 5. It is clear that peaks in $\alpha\lambda$ occur in both cases albeit at somewhat lower frequencies than for the proteins. It should also be noted that the GuHCl induced peak is at a lower frequency than the acid peak which is also the case for the two proteins. Similar results were obtained with other amino acids.

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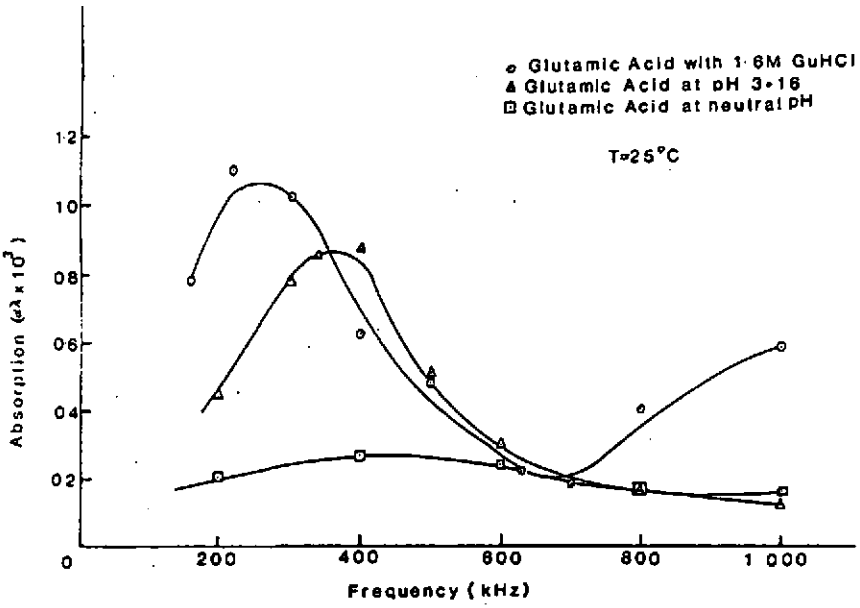

Fig. 5. Effect of GuHCl and Acidification on ($\alpha\lambda$) values of 34 mM Glutamic Acid

DISCUSSION

The results obtained from both proteins and amino acids consistently show peak values in $\alpha\lambda$ under acid conditions. The shape of the curves obtained are consistent with a single relaxation process extending well into the low MHz range. It is therefore suggested that these peaks do indeed represent maximum values of loss from processes which have already been observed at higher frequencies [17] namely those due to proton-transfer reactions of carboxyl groups.

The GuHCl results are more difficult to explain. The similarity between the magnitude and frequency of the $\alpha\lambda$ peaks when GuHCl is added with those obtained using acidification is remarkable. Since the amino acid data virtually rules out structural relaxation as being significant, it is reasonable to examine whether similar mechanisms might be involved in all cases.

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One important difference between GuHCl and the other denaturant used, ammonium sulphate, is that despite being a strong electrolyte, GuHCl can participate in proton-transfer reactions [18]. We would therefore tentatively suggest that GuHCl may be acting as a source and/or sink of free protons which can then interact with the carboxyl groups as though acid conditions had been induced. If this is the case, then it provides evidence that under some circumstances proton transfer equilibria can be extremely important at neutral pH.

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REFERENCES

[1] 'Biological Effects of Ultrasound: Mechanisms and Clinical Implications' NCRP Report No. 74. National Council on Radiation Protection and Measurements, Bethesda, Maryland, (1983).

[2] S.A. Goss, R.L. Johnston and F. Dunn, 'Comprehensive compilation of empirical ultrasonic properties of mammalian tissues'. J. Acoust. Soc. Am. 64(2) 423-457 (1978).

[3] P.D. Edmonds, T.J. Bauld, J.F. Dryo and M. Hussey, 'Ultrasonic absorption of aqueous haemoglobin solutions' Biochim. Biophys. Acta 200 174-177 (1970).

[4] L.W. Kessler and F. Dunn, 'Ultrasonic investigation of the conformal changes of bovine serum albumin in aqueous solution'. J. Phys. Chem. 73 4256-4263 (1969).

[5] H. Kanda, N. Ookubo, H. Nakjima, Y. Suzuke, M. Mineto, T. Ihara and Y. Wada, 'Ultrasonic absorption in aqueous solution of lysozyme'. Biopolymers 15 785-795 (1976).

[6] P.D. Edmonds, 'The molecular basis of ultrasonic absorption by proteins' Bioelectromagnetics 3 157-165 (1982).

[7] F. Dunn, P.D. Edmonds and W.J. Fry, 'Absorption and dispersion of ultrasound in biological media'. Chapter 3 in 'Biological Engineering' Edited by H.P. Schwan. McGraw-Hill (1969).

[8] E.L. Carstensen and H.P. Schwan, 'Acoustic properties of hemoglobin solutions'. J. Acoust. Soc. Am. 31(3) 305-311 (1959).

[9] F. Schneider, F. Muller-Landau and A. Mayer, 'Acoustical properties of aqueous solutions of oxygenated and deoxygenated hemoglobin' Biopolymers 8 537-544 (1969).

[10] E.L. Carstensen, 'Measurement dispersion of velocity of sound in liquids'. J. Acoust. Soc. Am. 26(5) 858-861 (1954).

[11] J.R. Pellam and J.K. Galt 'Ultrasonic propagation in liquids' J. Chem. Phys. 14(10) 608-614 (1946).

[12] F. Eggers and Th. Funck, 'Ultrasonic measurements with milliliter liquid samples' Rev. Sci. Inst. 44, 93-106 (1973).

[13] C. Barnes, J.A. Evans and T.J. Lewis, 'Ultrasonic absorption of bovine serum albumin solutions in the frequency range 200 kHz-1MHz'. J. Acoust. Soc. Am. 78(1) 6-11 (1985).

[14] T. Ohsawa and Y. Wada, 'Resonance reverberation method for ultrasonic measurement of liquids from 10 to 300 kilocycles' Jap. J. App. Phys. 6(12) 1351-1356 (1967).

[15] M. Hussey and P.D. Edmonds 'Proton-transfer reactions. A mechanism for the absorption of ultrasound in aqueous solutions of proteins' J. Phys. Chem. 75(26) 4012-4019 (1971).

[16] F. Tanford, 'Protein denaturation'. Adv. Protein Chem. 23 121-282 (1968).

[17] R. Zana and J. Lang, 'Effect of pH on the ultrasonic absorption of aqueous solutions of proteins' J. Phys. Chem. 74(13) 2734-2736 (1970).

[18] K.C. Tewari, N.C. Li and R.J. Kurland, 'Proton exchange between guanidinium ion and water in water-dimethyl sulfoxide mixtures'. J. Phys. Chem. 73(9) 2853-2857 (1969).