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Ultrasonic Absorption in Aqueous Solutions of High-Molecular-Weight Polysaccharides*

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The ultrasonic amplitude absorption coefficient has been determined in aqueous solutions of four molecular weights (73 000, 186 000, 370 000, and 2 000 000) of dextran, a linear α $(1 \rightarrow 6)$ anhydroglucose polysaccharide of biological origin, over the frequency range 3–69 Mc/sec at 22°C. It is found that the concentrationfree absorption spectrum exhibits a remarkable similarity to that of aqueous solutions of beef hemoglobin. The latter finding leads to the conclusion that ultrasonic absorption in tissues is not dominated exclusively by constituent proteins and that the contribution to such absorption by molecular structures lacking tertiary configurations may be considerable.

THE absorption processes occurring when biological media are irradiated with ultrasound remain in an unsatisfactory state of understanding. The frequency dependence of the absorption coefficient of most investigated tissues can be described by a power function whose exponent varies between 1 and 1.3 in the frequency range 0.5–5 Mc sec (Refs. 1,2). A recent study of the temperature dependence of acoustic absorption in tissue revealed a positive temperature coefficient, contrary to that expected for a viscous mechanism.[3,4] Detailed investigations of the acoustic absorption coefficients per wavelength of blood[5] and aqueous solutions of its constituents[6] have exhibited spectra with a broad maximum in the range 5–100 Mc/sec. This property can be described by a distribution of relaxation processes extending beyond 100 Mc/sec and has been attributed to constituent proteins.[7]

The present study has been undertaken to determine the importance of the contribution of proteinaceous material to the absorption spectra of biological media and, in particular, the effect of the rigid tertiary structure on the observed absorption characteristics. Dextran, a linear $\alpha(1 \rightarrow 6)$ anhydroglucose polysaccharide byproduct of bacterial metabolism, was chosen over other biopolymers because of its chemical stability, inertness, and availablility[8] of the relatively large quantities of highly fractionated material required for use with the present apparatus, *viz.*, approximately 700 cc per sample. This molecular species is primarily a linear structure with 5% 10% glucose residues existing as branching elements. Dextran is very water-soluble and assumes random coil configurations in aqueous solution. The commercially obtained material was dissolved in distilled water, passed through membrane filters of 0.5-μ pore size to remove foreign matter, and stored at 5°C until characterization and acoustic measurements were to be made. The concentrations of the solutions were determined to better than 1% by optical rotation measurements based on a specific rotation $[\alpha]_{D_1}^{20}$ of +199°. The values of the molecular weights reported here are those specified by the manufacturer and verified in the laboratory to be accurate within 10%. The weight-average molecular weight (the sum of the products of the molecular weight by the weight fraction of each component) of each fraction was determined by measurement of the limiting viscosity

* Some of the material of this paper is taken from the M. S. thesis of L. W. Kessler, University of Illinois (1965).

[1] W. J. Fry and F. Dunn, in *Physical Techniques in Biological Research*, W. L. Nastuk, Ed. (Academic Press Inc., New York, 1962), Vol. 4, p. 261.

[2] D. E. Goldman and T. F. Hueter, J. Acoust. Soc. Am. 28, 35–37 (1956).

[3] F. Dunn, J. Acoust. Soc. Am. 34, 1545–1547 (1962).

[4] F. Dunn, in *Ultrasonic Energy: Biological Investigations and Medical Applications*, E. Kelly, Ed. (University of Illinois Press, Urbana, 1965), p. 51.

[5] E. L. Carstensen and H. P. Schwan, J. Acoust. Soc. Am. 31, 185–189 (1959).

[6] E. L. Carstensen and H. P. Schwan, J. Acoust. Soc. Am. 31, 305–311 (1959).

[7] P. D. Edmonds, Biochem. Biophys. Acta 63, 216–219 (1962).

[8] Obtained in crystalline form from Sigma Chemical Co., St. Louis, Mo., and Pharmacia Fine Chemical, Inc., New Market, N. J.

TABLE I. Absorption data and physical properties of polysaccharide solutions.

	f (Mc/sec)	Molecular Weight 73 000				186 000				370 000			2 000 000		
α(cm^{-1})	3	...	...	...	...	...	...	...	...	0.031	...	...	...	...	...
	9	0.112	0.074	0.053	...	0.143	0.096	0.058	0.052	0.095	0.069	0.044	...	...	...
	15	0.19	0.134	0.096	0.083	0.22	0.144	0.100	0.088	0.165	0.128	0.100	0.20	0.159	0.098
	21	0.34	0.27	0.185	0.143	0.37	0.25	0.18	0.155	0.33	0.22	0.179	0.34	0.26	0.170
	27	0.52	0.39	0.30	0.26	0.52	0.37	0.32	0.26	0.48	0.36	0.30	0.55	0.42	0.32
	33	0.73	0.54	0.42	0.36	0.68	0.54	0.45	0.39	0.72	0.50	0.42	0.73	0.56	0.43
	39	0.99	0.73	0.58	0.52	0.91	0.71	0.60	0.50	0.91	0.66	0.57	0.97	0.78	0.62
	45	1.14	0.99	0.68	0.67	1.07	...	0.78	0.66	1.09	0.84	0.70	1.23	0.97	0.79
	51	1.36	1.19	0.97	0.82	1.27	1.06	0.95	...	1.36	1.07	0.93	1.41	...	1.04
	69	2.73	2.01	1.56	1.29	...	...	1.78	...	...	...	1.43	2.3	2.0	1.71
C(g/100 cc)		10.8	6.9	3.7	2.2	11.4	7.2	5.8	4.2	10.5	6.5	4.1	10.9	7.8	4.9
ρ(g/cc)		1.047	1.031	1.017	1.010	1.051	1.033	1.027	1.020	1.045	1.030	1.019	1.048	1.035	1.022
η(cp)		7.94	4.36	2.24	1.60	13.21	6.15	4.54	3.23	24.63	10.45	5.28	52.31	24.75	12.68
$\lvert\eta\rvert$(dl/g)		0.241				0.324				0.503			0.696		

number.[9] Both limiting viscosity and static flow measurements were accomplished with Cannon–Ubbelohde semimicrodilution viscometers. Standard pycnometers were employed for all density measurements to an accuracy of better than 0.01%.

Standard ultrasonic pulse-echo techniques were employed to determine the absorption coefficients; i.e., the Matec[10] PR 201 pulsed oscillator was used with a variable-pathlength acoustic absorption chamber. The absorption chamber, entirely fabricated of stainless steel, employed a 0.5-in.-diam, 3-Mc/sec (fundamental thickness mode) X-cut quartz plate, ground and optically polished to an accuracy of 0.00002 in. in flatness of each major face and 0.00005 in. in parallelism of the two major faces. Coaxial gold-on-chromium electrodes were vacuum-deposited on the quartz plate. The acoustic path length was varied by a screw mechanism, which displaced the quartz plate relative to the acoustic reflector, acting through a dovetail bearing. The bearing served to eliminate lateral displacements inherent in the screw mechanism. Determination of the acoustic pathlength was made with a micrometer having a least count of 0.01 mm, the entire system consisting of the quartz plate and dovetail bearing and screw mechanism comprising a rigid structure. The construction of the acoustic reflector assembly allowed for tilting in two orthogonal planes. This feature was employed for adjusting to parallelism the quartz plate and the reflector, and was accomplished acoustically with distilled water, the absorption chamber being emptied and dried prior to the introduction of the dextran sample to be measured. The acoustic chamber was immersed in a larger chamber of high thermal mass and the entire assembly maintained at 22°±0.2°C.

Values for the ultrasonic absorption coefficient and the speed of sound in the samples were obtained from oscillographs of the exponential time-amplitude relation of the trace of the first echo, as the distance between quartz plate and reflector was continuously varied. This method allowed the speed of sound to be determined with a maximum uncertainty of ±3%. Within this limit of error, no velocity dispersion was discerned and the absorption data were converted to conventional units of nepers per centimeter, using the

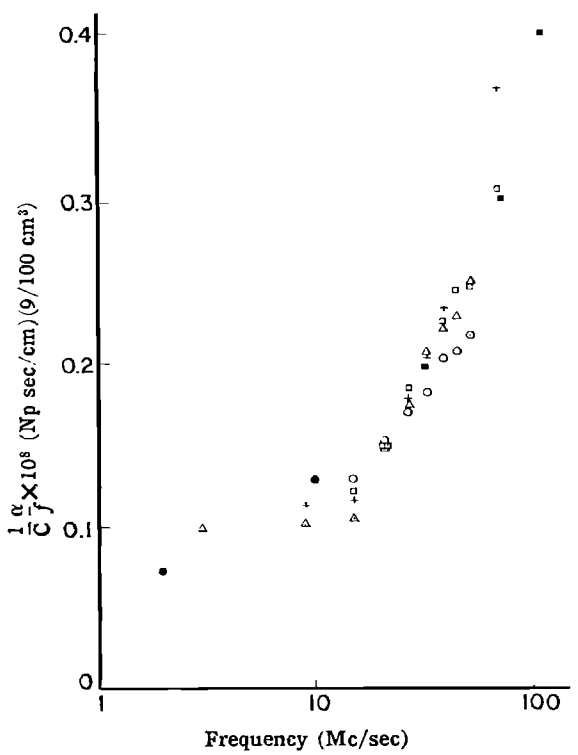

FIG. 1. Concentrationfree ultrasonic absorption per cycle vs frequency of aqueous solutions of dextran and hemoglobin. +: $\bar{M}w\sim73\,000$. ⊙: $\bar{M}w\sim186\,000$. △: $\bar{M}w\sim370\,000$. ⊡: $\bar{M}w\sim2\times10^6$. ●: Hemoglobin; Carstensen and Schwan. ■: Hemoglobin; Edmonds.

[9] F. R. Senti, N. N. Hellman, N. H. Ludwig, G. E. Babcock, R. Tobin, C. A. Glass, and B. L. Lamberts, J. Polymer Sci. 17, 527–545 (1955).

[10] Matec Instrument Co., Providence, R. I.

most probable velocity for all concentrations and molecular weights of 1.5×10^5 cm sec for the speed of sound. For frequencies below 15 Mc, sec, the absorption values were corrected for diffraction error, using the method of Carome *et al.*[11] The reported values of absorption coefficients are considered to have an accuracy of $\pm5\%$.

Table I is a complete tabulation of the absorption data for each molecular weight and all concentrations including the static flow shear viscosity and density data. Figure 1 shows the absorption per cycle per unit concentration for all the polysaccharide data, together with the results of Carstensen and Schwan[6] and Edmonds[7] on aqueous solutions of beef hemoglobin. It is evident from the Table and the Figure that the absorption coefficient per unit concentration is independent of molecular weight for the four polysaccharides considered in this frequency range; i.e., the absorption per solute molecule is about proportional to molecular weight. This result is considered important relative to the absorption observed in tissues, since the concentration of certain molecular species can vary with the physiological state of the organism; e.g., the variation of the glycogen content of liver may explain the variety of reported results[2] owing to different experimental conditions and treatments provided by the various investigators. Thus, observation of the ultrasonic absorption coefficient may provide a means for monitoring selected physiological states *in vivo*. Figure 1 illustrates the remarkable similarity of the absorption spectra of dextran and hemoglobin (molecular weight 68 000). It is concluded that molecular species possessing a rigid tertiary structure, in all likelihood, do not alone account for the observed ultrasonic absorption spectrum of tissue and that the contribution to the total absorption by nonproteinaceous material may be considerable. It must be remarked that this finding is not in conflict with the conclusions reached in the studies of the ultrasonic absorption properties of hemoglobin solutions,[5,7] since the concentration of nonproteinaceous macromolecular species is insignificant therein.

It is planned to extend these studies to other biopolymers, including a wide variety of proteins.

[11] E. F. Carome, J. M. Witting, and P. A. Fleury, J. Acoust. Soc. Am. 33, 1417–1425 (1959).

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