

Acoustic non-linearity method for estimating the ratio of bound to free water of biological media

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The acoustic non-linear properties of bound and free water in biological media are treated based on their structural differences. A model describing the different effects of bound and free water in protein-water solutions and in biological media is proposed. The ratio of bound to free water can be estimated from this model from measurements of the acoustic non-linearity parameter B/A , the sound speed, and the density of the specimens.

Keywords: biological media; acoustic properties; water

The acoustic non-linearity parameter B/A of biological media has been studied at the molecular level for aqueous macromolecular solutions through the cellular level in aqueous suspensions to *in vivo* preparations during the past decade¹⁻³. As most soft tissues contain approximately 70% water, and as many of the B/A measurements for biological materials, such as proteins, are performed in aqueous solutions, it is necessary to know and to understand the basis of the acoustic non-linear properties of water.

In general, water may exist in two states: bound water and free water. Bound water forms hydrogen bonds among water molecules or between the polar parts of some molecules (such as proteins) and water molecules. On the other hand, free water does not contain stable hydrogen bonds. NMR studies have shown that the ratio of bound water and free water is closely related to the state and nature of the soft tissues⁴. Since the structures of these two kinds of water in tissue are different, their contributions to the total B/A of tissue are different, therefore it is possible to estimate their ratio in tissue by acoustic non-linear measurements.

This paper will discuss the basis of the non-linear properties of bound and free water, propose a model for protein-water solutions and tissue, and estimate the ratio of these two kinds of water with the model from B/A measurements.

Acoustic non-linear properties of bound and free water

It is well known that water possesses unusual properties, for example, the relatively high freezing and boiling points and expansion upon transition to the solid state, which are explained by hydrogen bonding interactions among water molecules⁵. The acoustic non-linear parameter of water also shows unusual properties compared, for example, with alcohols. The B/A of water increases by about 50% when temperature increases from 0 °C to

100 °C, while the thermal variation of B/A of alcohols is negligible⁶. It has been shown that the thermal variation of B/A is proportional to the thermal expansion coefficient⁷. Since the thermal expansion coefficients of water and alcohols are very small, namely of the order of 10^{-3} and thereby negligible, the temperature variation of B/A should be negligible for both water and alcohols⁸. The inconsistency of these theoretical and experimental details is due to the existence of two kinds of water; bound and free water⁹. The two states of water have different B/A values which are nearly independent of temperature, although the ratio of these two kinds of water changes considerably with temperature. That is, increasing temperature causes an increase of thermal motion of the water molecules, which decreases the amount of bound water as the hydrogen bonds among water molecules are broken by absorption of heat energy. The B/A values of bound and free water are estimated to be $(B/A)_{\text{bound}} = 0.4$ and $(B/A)_{\text{free}} = 8.0$, respectively, by the non-linear parameter additive assumption and the thermodynamic relations for water⁶.

The considerable difference in the B/A values of these two kinds of water can be explained by the molecular structure of the two states of water; in the bound state the water molecules have a greater degree of association with each other, meaning that they are held together more strongly. The strong binding requires a larger ultrasonic pressure amplitude to stretch the intermolecular bonds into their non-linear region, which results, macroscopically, as lesser B/A values. On the other hand, in the free state, the weaker bonding allows lesser ultrasonic pressure amplitudes to stretch the intermolecular bonds into their non-linear regions, producing a larger B/A value^{9,10}.

A protein-water solution model

It has been found from experimental observation that the B/A value of protein-water solutions increases nearly

linearly with protein concentration¹. In order to understand the basis of this result, a simple model of the protein–water solution is proposed which involves application of the mixture law and considers that the ratio of bound and free water depends upon the protein concentration.

First, for pure water, or zero protein concentration, at a specific temperature, the fraction of bound and free water is described by the mixture law¹¹

$$N_w(0) = N_{wf}x_{wf}(0) + N_{wb}x_{wb}(0) \quad (1)$$

where the symbol N stands for the quantity $(B/A)/\rho c^3$ called the velocity phase shift parameter¹¹, $N_w(0)$ is the observed velocity phase shift parameter of pure water, and N_{wf} and N_{wb} are the velocity phase shift parameters of free and bound water, respectively, produced as the hydrostatic pressure of the medium is reduced sufficiently rapidly that heat is not exchanged and adiabatic conditions prevail. $N_w(0)$ is thus the weighted sum of the components. $x_{wf}(0)$ and $x_{wb}(0)$ are the volume fractions of free and bound water, respectively, in pure water, i.e. $x_{wf}(0) + x_{wb}(0) = 1$. Equation (1) can be rewritten as

$$N_w(0)v_w = N_{wf}v_{wf}(0) + N_{wb}v_{wb}(0) \quad (2)$$

where v_w is the total volume of water, and $v_{wf}(0)$ and $v_{wb}(0)$ are, respectively, the volumes of free and bound water in pure water.

Secondly, for the protein solution, prepared by dissolving dry protein in pure water at the same temperature as the above, the mixture law is expressed as

$$N = N_{wf}x_{wf} + N_{wb}x_{wb} + N_p x_p \quad (3)$$

where N is the observed velocity phase shift parameter of the solution, N_p is the phase shift parameter of protein, and the x_{wf} , x_{wb} and x_p are the volume fractions of, respectively, free water, bound water, and protein, i.e. $x_{wf} + x_{wb} + x_p = 1$. Equation (3) can be rewritten as

$$Nv = N_{wf}v_{wf} + N_{wb}v_{wb} + N_p v_p \quad (4)$$

where v and v_p are the total volume and volume of protein, respectively, and v_{wf} and v_{wb} are the volumes of free water and bound water in the solution, respectively.

It is to be noted that the volumes of free and bound water in pure water and in the protein solution are different since the protein dissolved in water forms hydrogen bonds to water molecules with its polar surface; thus, additional bound water is induced. It is assumed that the volume of additionally-formed bound water is Δv_{wb} , and that the total water volume remains unchanged [$v_{wf}(0) + v_{wb}(0) = v_{wf} + v_{wb}$] so that $v_{wf} = v_{wf}(0) - \Delta v_{wb}$, $v_{wb} = v_{wb}(0) + \Delta v_{wb}$, and Equation (4) may be expressed as

$$Nv = N_{wf}[v_{wf}(0) - \Delta v_{wb}] + N_{wb}[v_{wb}(0) + \Delta v_{wb}] + N_p v_p \quad (5)$$

or

$$Nv = [N_{wf}v_{wf}(0) + N_{wb}v_{wb}(0)] - (N_{wf} - N_{wb})\Delta v_{wb} + N_p v_p \quad (6)$$

Note that the first term on the right-hand side of Equation (6) is $N_w(0)v_w$, Equation (2), so

$$Nv = N_w(0)v_w - (N_{wf} - N_{wb})\Delta v_{wb} + N_p v_p \quad (7)$$

It has been shown that the amount of protein-induced bound water is proportional to the amount of protein in

the solution^{4,12}. Thus, substituting $\Delta v_{wb} = b_p v_p$ where b_p is a constant, yields

$$Nv = N_w(0)v_w - (N_{wf} - N_{wb})b_p v_p + N_p v_p \quad (8)$$

and dividing by v , gives

$$N = N_w(0)x_w + [N_p - b_p(N_{wf} - N_{wb})]x_p \quad (9)$$

where x_w is the volume fraction of total water. Substituting $x_w + x_p = 1$, gives

$$N = N_w(0) + [N_p - N_w(0) - b_p(N_{wf} - N_{wb})]x_p \quad (10)$$

Equation (10) shows that the observed phase shift parameter increases linearly with the volume fraction of protein.

Estimation of the ratio of bound to free water for protein–water solutions

The model discussed above is used to estimate the ratio of bound to free water, or the volume fraction of protein. The phase shift parameters of the solution, pure water, protein, bound water, and free water at a selected temperature can be measured, or estimated by using the mixture law. Then, the constant b_p can be estimated from Equation (10) if the volume fraction of protein is known. Therefore, the ratio of bound to free water can be expressed as

$$r = v_{wb}/v_{wf} = [v_{wb}(0) + b_p v_p]/[v_{wf}(0) - b_p v_p] \quad (11)$$

or dividing the numerator and denominator by v gives

$$r = [x_{wb}(0)x_w + b_p x_p]/[x_{wf}(0)x_w - b_p x_p] \quad (12)$$

The values of the non-linear parameter B/A , the sound speed c , and the density ρ were determined experimentally as a function of protein volume fraction for lysozyme solutions. The data of these measurements and the results calculated from Equation (12) for the water ratios are shown in *Figures 1* and *2* assuming the B/A of free and bound water are 8.0 and 0.40, respectively⁶. b_p can be estimated as follows, noting that all the quantities of Equation (10) are known excepting N_p and b_p . A least squares fit of the data of *Figure 1* yields the regression relation $N = 1.55 - 0.304 x_p$. Thus, for pure water, $x_p = 0$, $N(0) = 1.55$, and Equation (10) becomes

$$N_p - N_w(0) - b_p(N_{wf} - N_{wb}) = -0.304 \quad (10a)$$

Further, it is known that $N_{wf} = (B/A)_{wf}/\rho c^3 = 8.0/1 \times (1.509)^3 = 2.33$ and $N_{wb} = (B/A)_{wb}/\rho c^3 = 0.40/1 \times (1.509)^3 = 0.117$ (in a consistent set of units), considering the velocities of sound of bound and free water to be the same. N_p can be estimated by observing that 193 water molecules can be bound to a single lysozyme molecule¹². The protein volume fraction is (M_w is the molecular weight)

$$\begin{aligned} x_p &= \frac{v_p}{v_p + v_w} = \frac{M_w \rho_p / \rho_p}{M_w \rho_p / \rho_p + M_w w / \rho_w} \\ &= \frac{13900/1.37}{13900/1.37 + 193 \times 18/1.0} = 0.75 \end{aligned}$$

when all the water is bound. N_p can be obtained from the mixture law Equation (3) with the first term on the right-hand side taken as zero (no free water), giving $N = N_{wb}x_{wb} + N_p x_p = 0.117 \times 0.25 + N_p \times 0.75$. Using the regression relation above to estimate N , gives

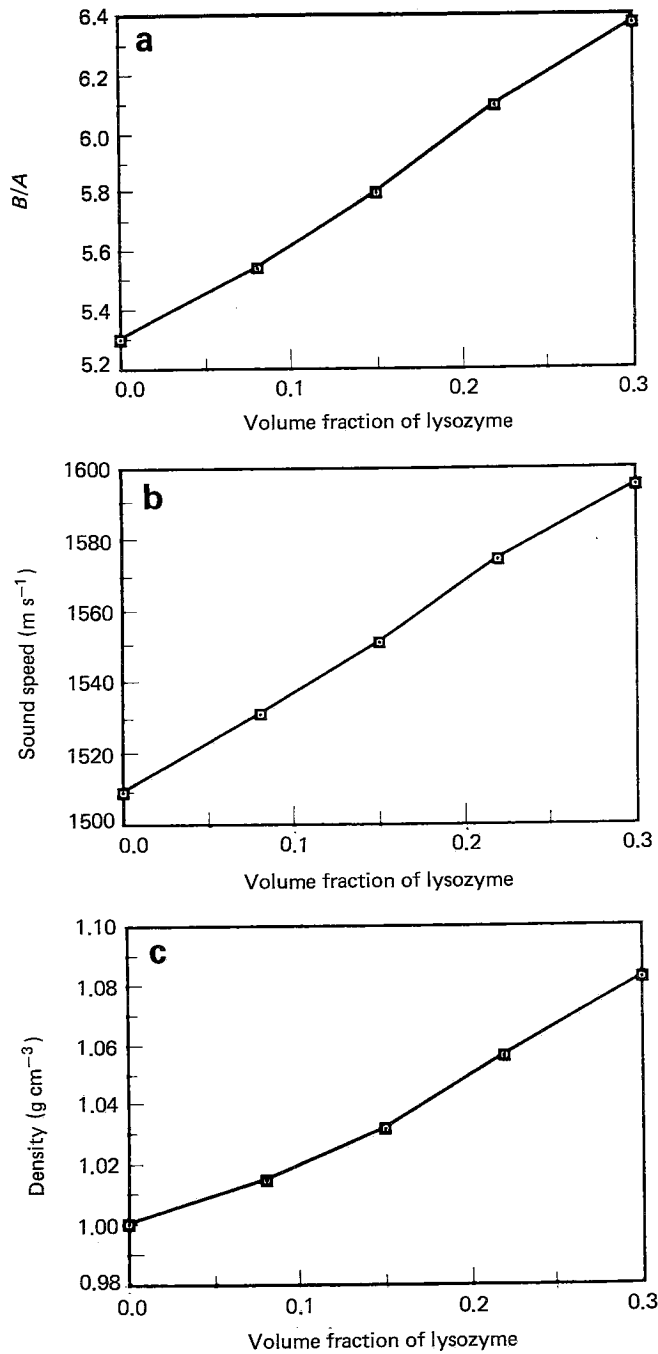


Figure 1 The measured values of B/A , sound speed, and density of lysozyme solutions as a function of volume fraction of lysozyme. (The straight lines simply connect the experimentally obtained data points)

$N_p = 1.72$. b_p is then obtained from Equation (10a)

$$b_p = \frac{N_p - N_w(0) + 0.304}{N_{wf} - N_{wb}} = \frac{1.72 - 1.55 + 0.304}{2.33 - 0.117} = 0.21$$

Alternatively, if the constant b_p has been determined by other techniques, for example from the NMR spin-lattice relaxation time (T_1), the volume fraction of protein in the solution can be estimated from measurements of B/A , sound speed, and density. It is seen that this method can be applied to determine the volume fraction of other solutes possessing polar surface regions and induce additional bound water in solution.

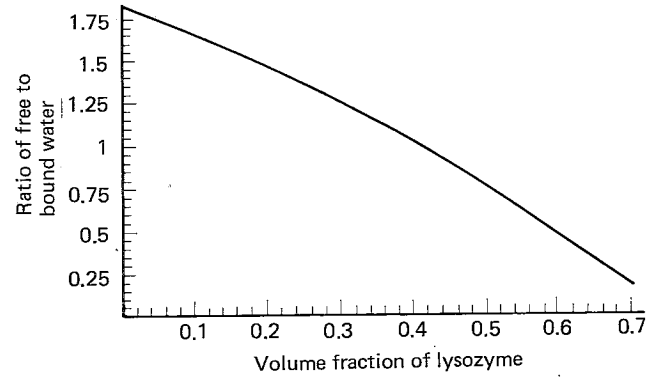


Figure 2 The calculated curve of the ratio of free to bound water of lysozyme solutions as a function of volume fraction of lysozyme

Estimation of the ratio of bound and free water for tissue

The possibility for estimating the ratio of bound and free water is discussed and shown to be promising.

The Sehgal *et al.* method¹¹ proposed for estimating the biological tissue composition is employed as it requires only knowledge of the non-linear parameter B/A and the sound speed c of the total tissue. The method starts with the mixture law

$$1/c = x_w/c_w + x_f/c_f + x_p/c_p \quad (13)$$

$$N = N_w x_w + N_f x_f + N_p x_p \quad (14)$$

where c , c_w , c_f , and c_p are sound speeds of total tissue, water, fat, and protein, respectively; and x_w , x_f , x_p are the volume fractions of water, fat, and protein, respectively.

By eliminating x_p in Equations (13) and (14) where $x_w + x_f + x_p = 1$ and applying multiple linear regression analysis to the experimentally determined values of B/A and c of tissue and of the water-fat composition, the fractions of water and fat can be expressed as¹¹

$$x_f = k_1 + k_2 N + k_3/c \quad (15)$$

$$x_w = k_4 + k_5 N + k_6/c \quad (16)$$

where k_1 , k_2 , k_3 , k_4 , k_5 , and k_6 are constants. Thus, the composition of the tissue can be estimated by measurement of B/A and c of tissue.

However, the Sehgal *et al.* method¹¹ of estimating tissue composition treats water as a single component, namely, not divided into the two categories of bound and free water. In order to take the two states of water into account, Equations (13) and (14) must be modified.

First, N_w of Equation (14) can be considered to be the 'apparent' phase shift parameter of water, with the total contribution of bound water to free water being

$$N_w v_w = N_{wf} v_{wf} + N_{wb} v_{wb} \quad (17)$$

Since protein and fat may induce additional bound water in the amount of $b_p v_p$ and $b_f v_f$, respectively, compared with that of pure water, where v_p and v_f are the volumes of protein and fat, respectively, and b_p and b_f are constants, Equation (17) is expressed as

$$N_w v_w = N_{wf} [v_{wf}(0) - b_p v_p - b_f v_f] + N_{wb} [v_{wb}(0) + b_p v_p + b_f v_f] \quad (18)$$

where $v_{wb}(0)$ and $v_{wf}(0)$ are the volumes of bound and

free water, respectively, in pure water. Equation (14) can be rewritten as

$$N = N_w(0)x_w + [N_f - b_f(N_{wf} - N_{wb})]x_f + [N_p - b_p(N_{wf} - N_{wb})]x_p \quad (19)$$

where $N_w(0) = N_{wf}v_{wf}(0) + N_{wb}v_{wb}(0)$ is the phase shift parameter for pure water. Similarly, Equation (13) can be written as

$$1/c = [1/c_w(0)]x_w + [1/c_f + b_f(1/c_{wb} - 1/c_{wf})]x_f + [1/c_p + b_p(1/c_{wb} - 1/c_{wf})]x_p \quad (20)$$

where $c_w(0)$ is the sound speed of pure water, c_{wb} and c_{wf} are the speeds of sound of bound and free water, respectively. Eliminating x_p in Equations (19) and (20) using the relation $x_w + x_f + x_p = 1$ yields

$$N = [N_p - b_p(N_{wf} - N_{wb})] + [N_w(0) - N_p + b_p(N_{wf} - N_{wb})]x_w + [N_f - N_p + (b_p - b_f)(N_{wf} - N_{wb})]x_f \quad (21)$$

$$1/c = [1/c_p + b_p(1/c_{wb} - 1/c_{wf})] + [1/c_w(0) - 1/c_p - b_p(1/c_{wb} - 1/c_{wf})]x_w + [1/c_f - 1/c_p + (b_f - b_p)(1/c_{wb} - 1/c_{wf})]x_f \quad (22)$$

Similar to the method above, by applying multiple linear regression analysis on the measured values of B/A and c , and the water-fat composition, the coefficients in Equations (21) and (22) can be determined and the results expressed as

$$N = k'_1 + k'_2x_w + k'_3x_f \quad (23)$$

$$1/C = k'_4 + k'_5x_w + k'_6x_f \quad (24)$$

where $k'_1, k'_2, k'_3, k'_4, k'_5,$ and k'_6 are constants. Solving Equations (23) and (24) gives

$$x_f = k''_1 + k''_2N + k''_3/c \quad (25)$$

$$x_w = k''_4 + k''_5N + k''_6/c \quad (26)$$

where $k''_1, k''_2, k''_3, k''_4, k''_5,$ and k''_6 are constants. Thus, the composition of tissue can be estimated from measurements of B/A and sound speed c of the tissue, and the ratio of the bound to free water can be estimated as

$$r = v_{wb}/v_{wf} = [v_{wb}(0) + b_p v_p + b_f v_f] / [v_{wf}(0) - b_p v_p - b_f v_f] \quad (27)$$

Conclusion

The study shows that estimation of the ratio of bound to free water in protein-water solutions and in tissues

by acoustic non-linearity measurements is possible. The contributions of these two states of water to acoustic non-linearity is due to their different interactions with tissue at the molecular level. The mechanism with which the non-linear parameter varies with the ratio of these two states of water provides a better understanding of the properties of acoustic non-linearity in biological systems.

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