Ultrasonic Studies of Proton-Exchange Reaction Between Hydrogen Phosphate Ions and Imidazole

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Abstract

Ultrasonic relaxation measurements for imidazole and its derivative in phosphate buffer exhibit a high peak of absorption at neutral pH. Near neutral pH, protolysis and hydrosis may be neglected and the essential reaction only consists of a direct proton-exchange. The kinetics constants and the volume changes for the proton transfer reaction with the protonated imidazole and 2-methylimidazole have been determined at 25℃. The kinetics constants are 7.2×10^8 $s^{-1}M^{-1}$ for imidazole and 1.7×10^8 $s^{-1}M^{-1}$ for 2-methylimidazole. The kinetics constants are used to estimate the spectrum of relaxation times and acoustic relaxation amplitude associated with intermolecular and intramolecular proton-exchange reactions in biological media. It is concluded that the magnitude of the acoustic absorption reasonably attributable to the perturbation of proton-transfer equilibria between imidazole and inorganic phosphate is comparable in magnitude with the acoustic absorption observed in some intact tissues.

Ⅰ. Introduction

Ultrasonic techniques[1] have been widely applied for material testing and also in the field of medical diagnostics substituting X-rays for the visualization of tissues and organs. However, in fewer cases ultrasonic wave has been used to determine the chemical properties of substances: ultrasonic measurements have contributed the results in fast chemical kinetics in liquids[2] with relaxation time $\tau \leq 1\mu s$. Therefore, in this short paper, we have applied the theory of chemical relaxation to the proton exchange, according to the Eigen scheme [3], to obtain an exact quantitative description of the variation of ultrasonic absorption α with pH and ionic concentration for imidazole and 2-methylimidazole. In addition, the volume change, convenient kinetic constsnt, and the absence of competing proton-transfer reactions at other residues combine to make the reaction represented by reaction (1) unusually amenable to study by ultrasonic techniques. We have determined the kinetics constants and volume changes ΔV for reaction (1) for imidazole and 2-methylimidazole and very briefly discuss the possible utility of the results in the estimation of the rates and relaxation amplitudes associated with intermolecular and intramolecular proton-transfer processes in biological media.

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Ⅱ. Ultrasonic Absorption Theory

The general reaction scheme of a proton-exchange process between two different acid-base pairs has been presented by Eigen[3]. Near neutral pH, protolysis and hydrolysis may be neglected and the essential reaction only consists of a direct proton-exchange of the general form:

$$AH + B \underset{k_R}{\overset{k_D}{\rightleftharpoons}} A + BH \qquad (1)$$

where AH and BH are the protonated forms of the two acid-base pairs and A and B their unprotonated forms, respectively. For the sake of simplicity the electrical charges have been omitted. kD and kR are the forward and backward rate constants.

Equilibrium (1) is characterized by a single relaxation time τ which is related to the rate constants by the following equation[3]:

$$\tau^{-1} = k_D (C_{AH} + C_B) + k_R (C_A + C_{BH}) \qquad (2)$$

where C_{AH}, C_A and C_{BH}, C_B designate the equilibrium concentrations of the two acid-base pairs.

In dilute aqueous solutions, the ultrasonic absorption excess α associated with reaction (1) as a function of angular frequency ω may be written[4]:

$$\frac{\alpha}{\omega^2} = \frac{1}{2}\frac{\rho v}{RT}(\Delta V)^2 \frac{\Gamma\tau}{1+(\omega\tau)^2} \quad (3)$$

where ρ and v are, respectively, the density of the solution and the velocity of ultrasound in the solution, R is the gas constant and T the absolute temperature, ΔV is the volume change for the process (1) and Γ[5] is a concentration factor which may be derived by means of the general relation[3].

$$\Gamma^{-1} = \frac{1}{C_{AH}} + \frac{1}{C_A} + \frac{1}{C_{BH}} + \frac{1}{C_B} \quad (4)$$

If the equilibrium concentrations of the reactants are expressed in terms of the total concentrations $C_A^\circ = C_A + C_{AH}$ and $C_B^\circ = C_B + C_{BH}$, the acid dissociation constants K_A and K_B of AH and BH respectively, the expressions for Γ and the reciprocal relaxation time τ^{-1} take the form

$$\Gamma = \frac{C_A^\circ C_B^\circ K_A K_B C_H}{K_B C_B^\circ (K_A + C_H)^2 + K_A C_A^\circ (K_B + C_H)^2} \quad (5)$$

$$\tau^{-1} = k_D C_A^\circ C_B^\circ \frac{K_B C_H}{(K_A + C_H)(K_B + C_H)} \Gamma^{-1}$$

$$= k_R C_A^\circ C_B^\circ \frac{K_A C_H}{(K_A + C_H)(K_B + C_H)} \Gamma^{-1} \quad (6)$$

$$\text{with} \quad k_D = k_R \frac{K_A}{K_B} \quad (7)$$

The activity coefficients have been assumed to be unity. Two special cases where (a) $C_A^\circ = C_B^\circ$ and (b) $K_A = K_B$ are of particular interest:

(a) $C_A^\circ = C_B^\circ = C_o$. Equations (5) and (6) indeed become

$$\Gamma = C_o \frac{K_A K_B C_H}{K_B (K_A + C_H)^2 + K_A (K_B + C_H)^2}$$

$$\tau^{-1} = k_D C_o \left(\frac{K_B + C_H}{K_A + C_H} + \frac{K_B}{K_A}\frac{K_A + C_H}{K_B + C_H}\right)$$

It can be shown that Γ and τ go simultaneously through a maximum at $pH = (pK_A + pK_B)/2$.

(b) $K_A = K_B = K$. Equations (5) and (6) reduce to

$$\Gamma = \frac{C_A^\circ C_B^\circ}{C_A^\circ + C_B^\circ}\frac{K C_H}{(K + C_H)^2}$$

$$\tau^{-1} = k_D (C_A^\circ + C_B^\circ) = k_R (C_A^\circ + C_B^\circ)$$

In this case, τ^{-1} is a pH-independent linear function of the total concentrations C_A° and C_B°, which Γ goes through a maximum at $pH = pK$.

By substituting the expressions for Γ and τ^{-1} in Eq. (3), the pH dependence of the ultrasonic absorption α/ω^2 for reaction (1) can be deduced.

pH-dependence of α/ω^2

For frequencies such that $\omega\tau \gg 1$, the dependence of the ultrasonic absorption on pH is entirely contained in the factor Γ/τ given by:

$$\frac{\Gamma}{\tau} = k_R C_A^\circ C_B^\circ \frac{K_A C_H}{(K_A + C_H)(K_B + C_H)} \quad (8)$$

It can readily be shown that α/ω^2 goes through a maximum at a pH value given by:

$$(pH_{MAX})_{\omega\tau \gg 1} = 1/2\,(pK_A + pK_B) \quad (9)$$

However, at low frequencies ($\omega\tau \ll 1$), the variation of α/ω^2 with pH is determined by the factor $\Gamma \times \tau$. Thus, when $C_A^\circ \ll C_B^\circ$, the absorption maximum is reached at the pH value[6]:

$$(pH_{MAX})_{\omega\tau \ll 1} = 1/2\,(pK_A + pK_B) - \log \delta \quad (10)$$

where δ is a function of the dissociation constants ratio $r^2 = K_A/K_B$ which may be written[6]:

$$\delta = \frac{1}{r}\left[r^2 - 1 + (r^4 - r^2 + 1)^{1/2}\right] \quad (11)$$

As a result of Eqs. (9) and (10), the change of pH_{MAX} between high and low frequencies, under the condition $C_A^\circ \ll C_B^\circ$, is given by

$$\Delta pH_{MAX} = (pH_{MAX})_{\omega\tau \gg 1} - (pH_{MAX})_{\omega\tau \ll 1} = \log \delta \quad (12)$$

Eq. (12) shows that for intermediate frequencies the location of the absorption maximum on the pH scale is necessarily frequency dependent. Under the condition $C_A^\circ \ll C_B^\circ$, the change of pH_{MAX} only depends on the pK-values difference of the reacting species. Only in the two special cases indicated above ($C_A^\circ = C_B^\circ$ or $K_A = K_B$), the values of pH_{MAX} are frequency independent.

At $pH_{MAX} = (pK_A + pK_B)/2$, the expression for α/ω^2 from Eq. (3) takes the form:

$$\frac{\alpha}{\omega^2} = \frac{1}{2}\frac{\rho v}{RT}(\Delta V)^2 \times \frac{r^2}{k_R(1+r)^2}\frac{C_A^\circ C_B^\circ}{r^2(C_A^\circ + C_B^\circ)^2 + (\omega/k_R)^2} \quad (13)$$

and equation (10) reduces to:

$$(C_B^{\circ})^2_{MAX} = (C_A^{\circ})^2 + \frac{K_B}{K_A}\left(\frac{\omega}{k_R}\right)^2$$

where $\frac{K_B}{K_A} = 10^{-\Delta pK}$

with $\Delta pK = pK_B - pK_A$.

The rate constant k_R associated with reaction(1) can readily be evaluated from a plot of $(C_B^{\circ})^2_{MAX}$ versus ω^2, which gives a straight line with intercept $(C_A^{\circ})^2$ and slope $k_D^{-2} \times 10^{\Delta pK}$.

Ⅲ. Experimental Results

Imidazole and its derivative, 2-methylimidazole, were purchased from Aldrich and used without further purification. The phosphate buffer solution (K_2HPO_4) were prepared with freshly deionised distilled water and the pH was adjusted by small amounts of HCl or KOH. The pH was measured using a calibrated Tacussel pH meter to an accuracy of 0.02 pH unit.

The ultrasonic absorption measurements were performed using an acoustic resonator method[7, 8] between 0.46 and 5.70 MHz. The temperature of the resonator was controlled to ±0.05℃ by circulating thermostated water. All the solutions studied were degassed prior to measurements.

Fig. 1 shows the variation of α/f^2 (α is the absorption coefficient of the solution and f the frequency) with pH between 6 and 9, at frequencies f ranging from 0.46 MHz to 5.70 MHz, for a 2-methylimidazole solution ($C_A^{\circ} = 5 \times 10^{-3}$M) in a potassium phosphate buffer ($C_B^{\circ} = 5 \times 10^{-2}$ M K_2HPO_4) at 25℃.

The α/f^2 value for the buffer is found to be close to that of water over the whole pH and frequency range. At all frequency values, the ultrasonic absorption α/f^2 versus pH goes through a maximum in the neutral pH range as expected, when the absorption excess is the result of a proton-exchange reaction between 2-methylimidazole and $H_2PO_4^-$.

A new fact illustrated in Fig. 1 is that the pH-value corresponding to absorption maximum is found to greatly

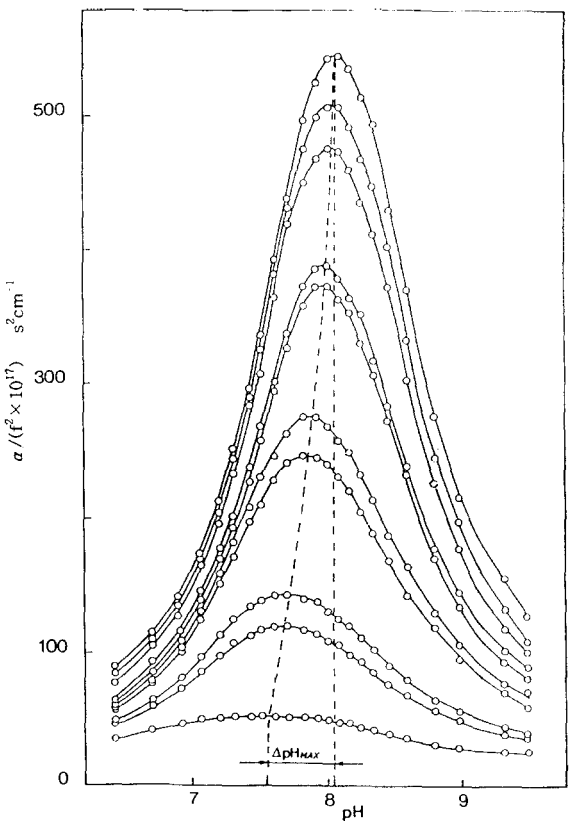

Figure 1. 2-methylimidazole, 5×10^{-3} M in 5×10^{-2} phospate buffer, T=25℃. Values of α/f^2(solution's ultrasonic absorption α divided by squared frequency) vs pH at different frequencies: (from top to bottom) 0.465, 0.611, 0.759, 0.907, 1.055, 1.499, 1.644, 2.717, 3.014, 5.708 MHz.

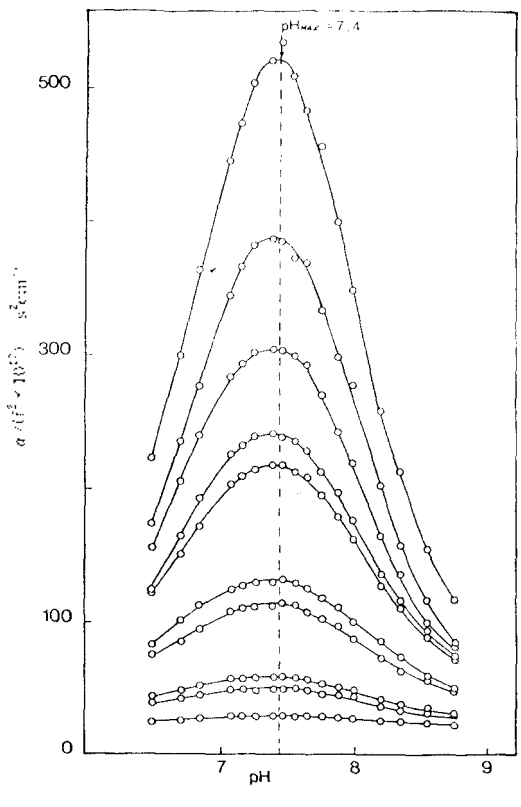

Figure 2. 2-methylimidazole, 5×10^{-3} M in 5×10^{-3} phospate buffer, T=25℃. Values of α/f^2(solution's ultrasonic absorption α divided by squared frequency) vs pH at different frequencies: (from top to bottom) 0.465, 0.611, 0.759, 0.907, 1.055, 1.499, 1.644, 2.717, 3.014, 5.708 MHz.

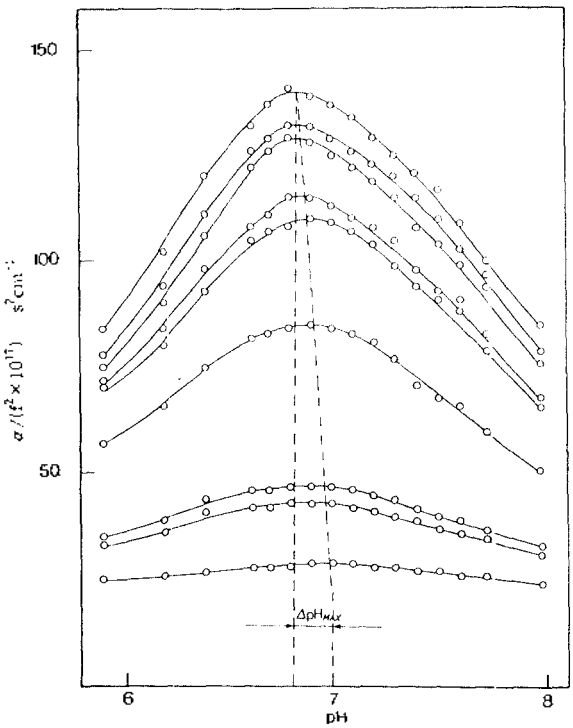

Figure 3. Imidazole, 1×10^{-3}M in 1×10^{-2} phospate buffer, T= 25℃. Values of α/f^2 (solution's ultrasonic absorption α divided by squared frequency) vs pH at different frequencies: (from top to bottom) 0.465, 0.611, 0.759, 1.055, 1.499, 1.644, 2.717, 3.014, 5.708 MHz.

depend on frequency. It is seen that the absorption peaks progressively shift to higher pH-values as the frequency decreases. The absorption maximum occurs at a pH-value $pH_{MAX}=7.60$ for 5.70 MHz and at a pH-value $pH_{MAX}=8.05$ for the lowest frequency 0.46 MHz, leading to a total pH_{MAX} difference of 0.45 pH unit. This result is in agreement with the prediction of Eq. (12) by use of $pK_A=7.75$ for 2-methylimidazole and $pK_B=7.15$ for K_2HPO_4

Fig. 2 shows the results obtained when 2-methylimidazole and K_2HPO_4 are present in equimolar amounts ($C_A^\circ=C_B^\circ=5\times10^{-3}$M). The position of the absorption maximum on the pH scale is no more frequency dependent. At all the frequency values the absorption peak is shown to occur at the same pHMAX value. The calculated value of pHMAX using relation (9) is in excellent agreement with the experimental value equal to 7.40.

Fig. 3 shows the pH-dependence of α/f^2 for a 1×10^{-3} M imidazole solution in 1×10^{-2}M K_2HPO_4. Here, the ultrasonic absorption maxima are found at pH close to 7 and, contrary to 2-methylimidazole, a pH_{MAX} decrease of about 0.20 pH unit is observed, when the frequency decreases from 5.70 to 0.46 MHz. This result is again in good agreement with the ΔpH_{MAX} value calculated by means of Eq. (12), which yields a ΔpH_{MAX} of −0.18 pH unit with $pK_A=6.95$ and $pK_B=7.15$.

The dependence on phosphate concentration C_B° of the absorption excess$(\alpha-\alpha_o)/f^2$, where α_o is the value of α in the absence of phosphate buffer, has been determined for a 5×10^{-3}M 2-methylimidazole solution, between 0.46 and 5.70 MHz, at 25℃. Results at a few representative frequencies for a pH-value of 7.40 corresponding to $pH_{MAX}=(pK_A+pK_B)/2$.

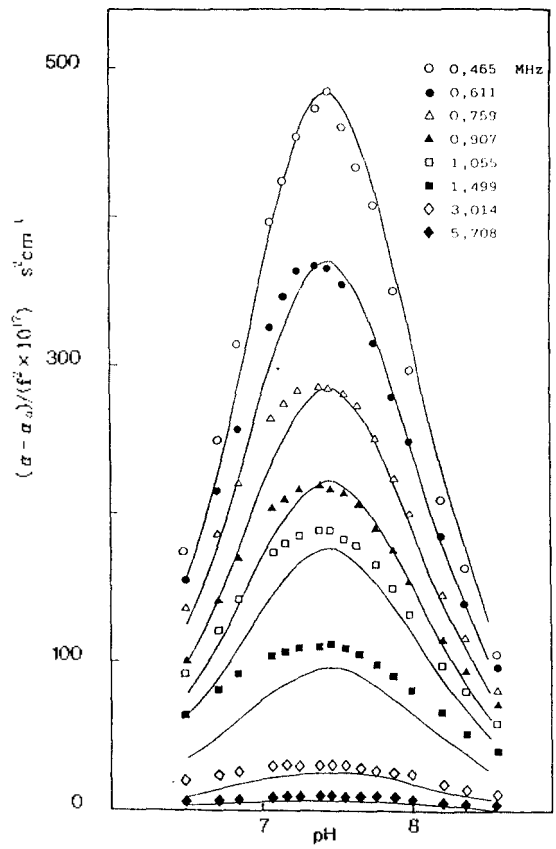

Figure 4. 2-methylimidazole, 5×10^{-3}M in 5×10^{-2} phospate buffer, T=25℃. Values of $(\alpha-\alpha_o)/f^2(\alpha_o/f^2$ for 2-methylimidazole in absence of phosphate buffer between 0.47 and 5.70 MHz) vs pH at different frequencies: (from top to bottom) 0.465, 0.611, 0.759, 0.907, 1.055, 1.499, 3.014, 5.708 MHz. (- - -) The continuous lines are each fitted to the theoretical values of ultrasonic absorption at all frequencies.

The data in Fig. 4 were fitted to theory at all frequencies, by means of Eq. (13), adjusting the two parameters ΔV and k_R, the volume change and the reverse rate constant for reaction (1), respectively. Eq. (13) gives quite a good representation of the experimental results, in the concentration range investigated. The best-fit values of the volume change ΔV and the rate constant would be

estimated to be 24.6 cm^3/mol and 7.2×10^8 $s^{-1}M^{-1}$ for imidazole, and 22.2 cm^3/mol and 6.8×10^8 $s^{-1}M^{-1}$ for 2-methylimidazole, respectively. The value of the rate constant k_D has been calculated by means of Eq. (7); 11. 4×10^8 $s^{-1}M^{-1}$ for imidazole and 1.7×10^8 $s^{-1}M^{-1}$ for 2-methylimidazole.

Ⅳ. Conclusion

From the results presented here it may be concluded that the determination of the pH dependence of the ultrasonic absorption excess of the two compounds studied provides a straightforward method for evaluating the rate constants and the volume changes associated with proton transfer equilibria in the neutral pH range. This method offers the possibility of studying the kinetics of proton transfer reactions involving the imidazole ring of more complex systems like enzymes.

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