Thermodynamics of Macroscopic and Microscopic Proton Ionization from Protonated 4-Aminobenzoic Acid in Aqueous Solution from 298.15 to 393.15 K

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Macroscopic and microscopic thermodynamic quantities (equilibrium constants and enthalpy, entropy, and heat capacity changes) for proton ionization from protonated 4-aminobenzoic acid in aqueous (H_2O and D_2O) solutions at temperatures from 298.15 to 393.15 K have been determined by a combination of NMR, potentiometric, and calorimetric methods. There are mathematical relationships between the thermodynamic quantities associated with the macroscopic and microscopic reactions. The reactions occurring during the proton ionization are much better understood by examining the thermodynamic quantities for the microscopic rather than for the macroscopic reactions. The changes of the $\Delta H°$ and $\Delta S°$ values for the microscopic reactions with temperature give insight into the interactions that occur during proton ionization since the trends of these quantities for a given ionization with temperature vary depending on whether the other functional group is protonated. There is a direct relationship between the temperature variations of the $\Delta H°$ and $\Delta S°$ values and the $\Delta C_p°$ values. Therefore, a knowledge of the $\Delta C_p°$ values, which can only be obtained by making measurements at more than one temperature, is helpful in gaining an understanding of the interactions of the reactants and products with the solvent and with each other. Large negative or positive $\Delta C_p°$ values indicate that extent of interaction between the solvent molecules with the reactants and products is very different. Equations typically used to describe the temperature dependence of pK, $\Delta H°$, $\Delta S°$, and $\Delta C_p°$ for simple ionic reactions in aqueous solutions are used to correlate these values for the microscopic reactions. The trends of the macroscopic constants with temperature are not those typically found in simple ionic reactions.

Introduction

Proteins are ubiquitous and essential components of living organisms. They are assembled from various combinations of 20 amino acids.[1] A remarkable and distinguishing feature of proteins is the unique manner in which they adopt well-defined structural conformations in solution. Anfinsen has pointed out that in the native state, proteins are folded in unique conformations that are determined by the sequence of their constituent amino acids.[2] There has been great interest in the nature and energetics of the bonds resulting when proteins assume their particular configurations.[2−7] In addition to the internal hydrogen bonds and van der Waals forces associated with a given protein conformation, interactions of constituent protein groups with surrounding water molecules play an important role in determining protein structure.[5]

An important aspect of protein structure is the effect that neighboring groups have on each other. Results from flow calorimetric and NMR studies[8−11] as a function of temperature are valuable in establishing the effect of neighboring functional groups on each other in amino acids. Values of pK, and of $\Delta H°$, $\Delta S°$, and $\Delta C_p°$ ($\Delta X°$) for protonation of a number of amino acids from 298.15 to 393.15 K show interesting trends. First, pK values for ionization of $-NH_3^+$ groups decrease while those for carboxylate groups increase over this temperature range.[8−10] Second, the change in $\Delta H°$ with temperature for $-NH_3^+$ ionization is quite different depending on whether the neighboring group is $-COO^-$ or $-COOH$, suggesting that the charge

and type of the neighboring group can have a sizable effect on the $\Delta H°$ and $\Delta C_p°$ values.[9] Third, the change in $\Delta H°$ with temperature for $-COO^-$ protonation is quite different depending on whether a methyl or $-NH_3^+$ is the neighboring group.[10] Finally, the changes in the $\Delta H°$ and $\Delta S°$ values with temperature show a strong correlation with amino acid-solvent and amino acid intramolecular interactions.[8−11]

In this paper, proton ionization from fully protonated 4-aminobenzoic acid (**I**) from 298.15 to 393.15 K is investigated. This amino acid was chosen for study because the pK values for the carboxylate and amino groups in this temperature range are close enough together for microspecies to occur, thus affording the opportunity to investigate in some detail the effect of neighboring protonated and nonprotonated carboxylate and amino groups on proton ionization. The magnitude of the pK value for either of these groups depends on whether the other group is protonated.[12] Microconstants for this system as a function of temperature are determined by an NMR technique described by Rabenstein and Sayer[13] and subsequently used by them[13−15] and by Noszál[16] and Noszál and Kassai-Tánczos.[17]

Proton ionization from **I** is illustrated in Figure 1. There are two macroscopic reactions, 1 and 2, which can be studied directly using calorimetric, potentiometric, spectroscopic, or other techniques, and five microscopic reactions, a, b, c, d, and e which have been studied at 298.15 K but not at other temperatures. Equilibrium constants for these reactions are labeled in Figure 1 and are related to each other in eqs 1−4.

$$K_1 = K_a + K_b \tag{1}$$

The effect of neighboring groups on proton ionization from a

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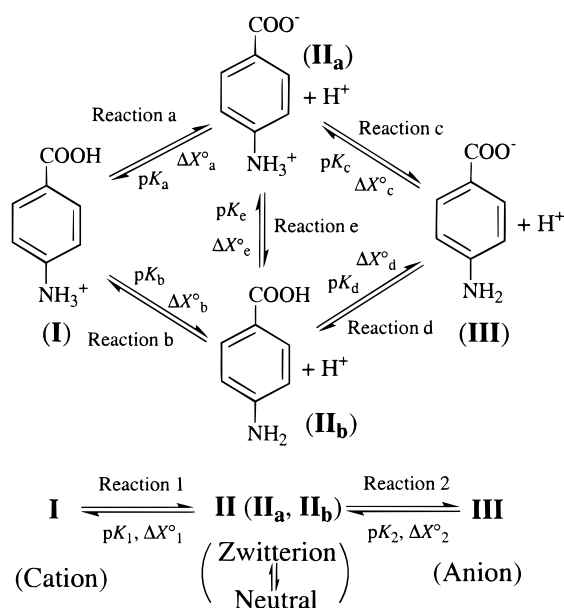[‡] Department of Chemical Engineering.

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Figure 1. Schematic of proton dissociation from fully protonated 4-aminobenzoic acid. $X = H, S, C_p$.

$$K_2 = K_c K_d / (K_c + K_d) \tag{2}$$

$$K_1 K_2 = K_a K_c = K_b K_d \tag{3}$$

$$K_e = K_a / K_b = K_d / K_c \tag{4}$$

given functional group is illustrated by comparing reaction a with reaction d and reaction b with reaction c. In each case, proton ionization is from the same group, but the neighboring groups differ in whether they are protonated. The result is that K_a is markedly different from K_d and K_b is markedly different from K_c at 298.15 K.[12,18,19]

Several studies have been performed to estimate the microscopic constants involving I, II, and III at 298.15 K.[12,18,19] Robinson and Biggs estimated K_b using ionization constants of the conjugate acids of the related esters 4-$COO(CH_2)_n$H−aniline.[18] Estimates of the microscopic constants K_a and K_c were obtained by van de Graaf, Hoefnagel, and Wepster from the dissociation data of 4-(trimethylammonium)benzoic acid.[19] Christensen and associates calculated K_e using the ethyl ester analogue and related microconstants from macroenthalpy and macroentropy changes of the dissociation reactions involving **I**.[12] No microscopic constants have been reported for proton ionization from **I** using a direct measurement. The macroconstants and microconstants in this paper were determined directly using an NMR titration method. The measurements were performed at temperatures from 298.15 to 348.15 K in D_2O solutions. These constants were converted to values valid in H_2O solutions using a method reported by Delgado et al.[20] Combination of these latter values with appropriate heat data obtained calorimetrically in H_2O solutions at 298.15 to 393.15 K allowed the calculation of $\Delta X°$ values valid in H_2O. Values of $\Delta X°$ valid in D_2O were calculated by using the van't Hoff equation and the equation which relates the pK values valid in H_2O and D_2O.

Experimental Section

Materials. The purity of the 4-aminobenzoic acid (Aldrich) was found to be >99.9% by titration with standardized NaOH. The NaOH solutions used were made by diluting a 50% NaOH aqueous solution (Alfa) and were standardized using potassium

hydrogen phthalate (acidimetric standard, Fisher Scientific) that had been dried for 24 h at 383.15 K. All solutions were prepared using deionized CO_2-free water and were degassed for 5 min in an ultrasonic bath. Deuterium chloride (DCl, 20 wt % in D_2O, Aldrich) and sodium deuterioxide (NaOD, 40 wt % in D_2O, CIL) solutions were standardized using potassium hydrogen phthalate and NaOH solutions, respectively.

^{1}H and ^{13}C NMR Measurements. NMR spectra were recorded on a VXR 500S (500 MHz) high-resolution spectrometer in D_2O at probe temperatures of 298.15, 323.15, and 348.15 ± 0.1 K. Sodium 2,2-dimethyl-2-silapentane-5-sulfonate was used as the internal standard. To calculate the deprotonation constants, a series of 4-aminobenzoic acid solutions (0.02 M) with pD values from 0.4 to 11 were prepared and their NMR spectra were taken. The pD values were adjusted by adding different amounts of DCl and NaOD solutions. Each solution was prepared in a 2 mL volumetric flask. A 0.6−0.8 mL portion of each solution was transferred to a high-resolution NMR tube for spectral recording and the pD value of the remaining solution was measured. Because of limited solubility of the 4-aminobenzoic acid in water, the pre-acquisition delay time was set to be long enough (d1 = 2 or 3) to allow the carbon atoms to relax to their ground state. Values of pD below 2 and above 8 were used to determine the NMR response to the fully protonated and deprotonated amino acid, respectively. Values of pD between 2 and 8 were used in the data reduction.

pD Measurements. All pD measurements were made with an Orion model EA 940 m. The electrode used was an Orion glass micro-combination pH electrode (model 9826). Precision buffer solutions, pH 4.000 ± 0.002 and 7.000 ± 0.002 at 298.15 K (Cole-Parmer), were used to calibrate the pH meter. This procedure resulted in pH (pD*) values. The pD* values are the pH values obtained in a solution of D_2O using a pH meter that is calibrated using buffered solutions in H_2O. The pD* values are related to pD values by[20−24]

$$pD = pD* + 0.40 \tag{5}$$

Temperatures of the solutions were controlled to 298.15, 323.15, and 348.15 ± 0.1 K using a circulating water bath (VWR Scientific model 1130A).

Heat Measurements. The heats of dilution and heats of reaction for the systems studied were measured in H_2O at 298.15, 323.15, 348.15, and 393.15 K using a high-pressure flow calorimeter. The procedure and equipment used have been described.[25] These data were used to calculate the pK_1, pK_2, $\Delta X_1°$, and $\Delta X_2°$ values as a function of temperature.

Calculations

Calculation of Macroscopic and Microscopic Proton Ionization Constants. All measurements were made in the absence of an inert electrolyte. Activity coefficients for the species in solution were used to account for the varying ionic strength during an experiment. Macroscopic and microscopic proton ionization constants were determined in D_2O by a method described by Rabenstein and Sayer.[13] Both ^{1}H and ^{13}C NMR measurements were made. Because the chemical shifts of benzo ring protons of 4-aminobenzoic acid depend on the state of protonation of both the carboxylate and ammonium groups, these chemical shifts belong to a common resonance. To determine whether the ^{13}C chemical shift of the carboxylate carbon is independent of the protonation state of the amino group, the ^{13}C NMR spectra of methyl 4-aminobenzoate were measured from pH 1.90 to 11.08 in a solution of 30 vol % methanol in water. The methanol was added to the water so that sufficient

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TABLE 1: Chemical Shifts of the ^{13}C NMR Spectra for the Carboxylate Carbon in Methyl 4-Aminobenzoate in 30/70 CH_3OH/H_2O (v:v) at 25 °C as a Function of pH

pH	δ (ppm)	pH	δ (ppm)
1.9	164.94	4.56	166.10
2.12	165.15	6.20	166.11
2.81	165.78	7.72	166.13
3.29	166.00	11.08	166.12
3.94	166.10		

ester for the measurements could be dissolved into the solution. As shown in Table 1, the chemical shift of the carboxylate carbon is dependent on the pH of the solution; and therefore, on the protonation state of the amino group. Comparing the total change in shift of the ^{13}C NMR spectra for the carboxylate carbon in D_2O with the change in shift of the ^{13}C NMR spectra for the carboxylate carbon in the ester shows that the protonation of the amino group accounts for about 20% of the change in the shift. The macroconstants (K_1^D and K_2^D) in D_2O were calculated using the ^1H NMR and the ^{13}C NMR versus pD data. The chemical shifts are given by

$$\delta_{obs} = [a_{D+}{}^2\delta_{D_2A} + \gamma K_1^D a_{D+}\delta_{DA} + K_1^D K_2^D \delta_A]/[a_{D+}{}^2 + \gamma K_1^D a_{D+} + K_1^D K_2^D] \quad (6)$$

where a_{D^+} is the activity of the D^+ ion in the solution; δ_{obs} is the experimentally observed chemical shift of a proton signal; δ_{D_2A}, δ_{DA}, and δ_A are proton chemical shifts for the fully deuterated I (δ_{D_2A}), partially deuterated IIa + IIb (δ_{DA}), and nondeuterated III (δ_A); and γ is the activity coefficient of a singly charged species. Values of δ_{D_2A} and δ_A were obtained from the NMR spectra of solutions sufficiently acidic to ensure complete deuteration and sufficiently basic to ensure complete ionization, respectively. Values of K_1^D, K_2^D, δ_{DA} for the proton, and δ_{DA} for the ^{13}C δ_{DA} in eq 6 were calculated by use of nonlinear least-squares minimization of both the ^1H and ^{13}C NMR data. Values of γ were calculated using the Pitzer form of the Debye−Hückel equation.[26] The limiting slope A_ϕ was found using the physical properties of D_2O instead of H_2O. Several ^1H NMR and ^{13}C NMR spectra of 4-aminobenzoic acid in D_2O at different pD values are shown in Figures 2 and 3, respectively and the chemical shifts as a function of pD are plotted in Figures 4 and 5.

The microconstants were then obtained by combining the ^{13}C NMR data for the 4-aminobenzoic acid and the methyl 4-aminobenzoate. It was assumed that the change of the ^{13}C chemical shift of the carboxylate carbon due to the protonation of the amino group was the same for the 4-aminobenzoic acid and the methyl 4-aminobenzoate and that the total ^{13}C chemical shift is a linear sum of the shifts due to the protonation states of the carboxyl group and the amino groups. These assumptions lead to the following equations:
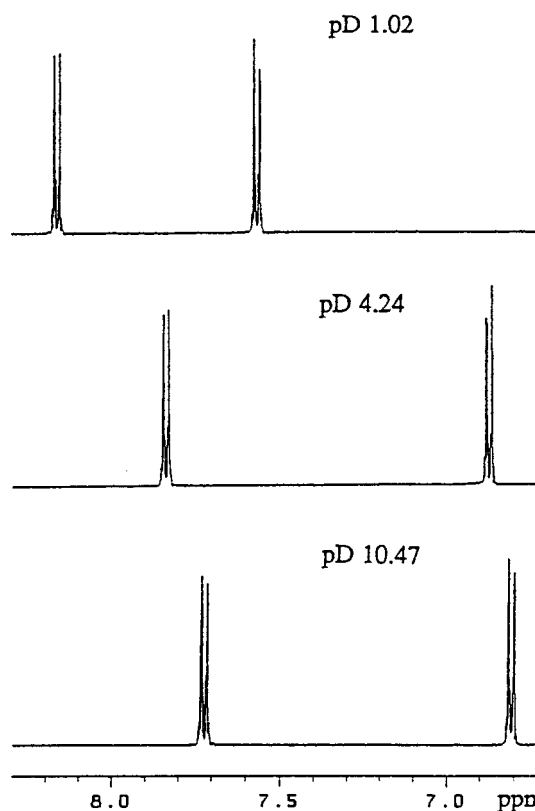
$$\delta_{DA}(IIb) = \delta_{D_2A} + \Delta\delta \quad (7)$$

where $\delta_{DA}(IIb)$ is the chemical shift for species IIb, $\delta_{DA}(IIa)$ is

$$\delta_{DA}(IIa) = \delta_{D_2A} + \delta_A - \delta_{DA}(IIb) \quad (8)$$

the chemical shift for species IIa, and $\Delta\delta$ is the difference in the chemical shifts between the protonated and the nonprotonated methyl 4-aminobenzoate. The value for K_e^D can then be found using the following equation.

$$K_e^D = [\delta_{DA}(IIb) - \delta_{DA}]/[\delta_{DA} - \delta_{DA}(IIa)] \quad (9)$$

Figure 2. ^1H NMR spectra at 323.15 K of 4-aminobenzoic acid in D_2O at different pD values.

The remaining microconstants K_a^D, K_b^D, K_c^D, and K_d^D can be calculated from eqs 1−4.

Values of pK^D were converted to pK^H values. From an examination of pK^D and pK^H data for over 100 different Brønsted acids of various types, Delgado et al. have concluded that a linear correlation exists between pK^D and pK^H values and proposed several linear equations.[20] We used the following equation to calculate pK^H from pK^D values

$$pK^D = Q + 1.044(pK^H) \quad (10)$$

where Q is 0.33 in the case of the carboxylate and 0.62 in the case of the amino group. Equation 10 is similar to the equation recommended by Delgado et al. which is

$$pK^D = 0.32 + 1.044(pK^H) \quad (11)$$

The correlation recommended by Delgado et al. is general and is the best fit for many acids although it does not give an exact relationship for all acids. Equation 10 gives the correct relationship between the pK_1^D and pK_2^D values measured in this study and those found earlier by a calorimetric technique.[12]

Calculation of $\Delta H^{\circ H}$, $\Delta S^{\circ H}$, and $\Delta C_p{}^{\circ H}$ Values. The calorimetric heat of reaction data were analyzed by a computer program to derive pK^H and $\Delta H^{\circ H}$ values at each temperature.[27] To obtain these values, which are by definition those valid at zero ionic strength, the Pitzer form of the Debye−Hückel equation[26] was used to calculate γ and $(\partial \ln \gamma/\partial T)_p$ in the interpretation of the heat data.

The dependence of the microconstants on temperature is represented by

$$\ln K_i = q^{(1)}{}_i + q^{(2)}{}_i/T + q^{(3)}{}_i \ln \rho \quad (12)$$

where ρ is the density of water; $q^{(1)}$, $q^{(2)}$, and $q^{(3)}$ are constants

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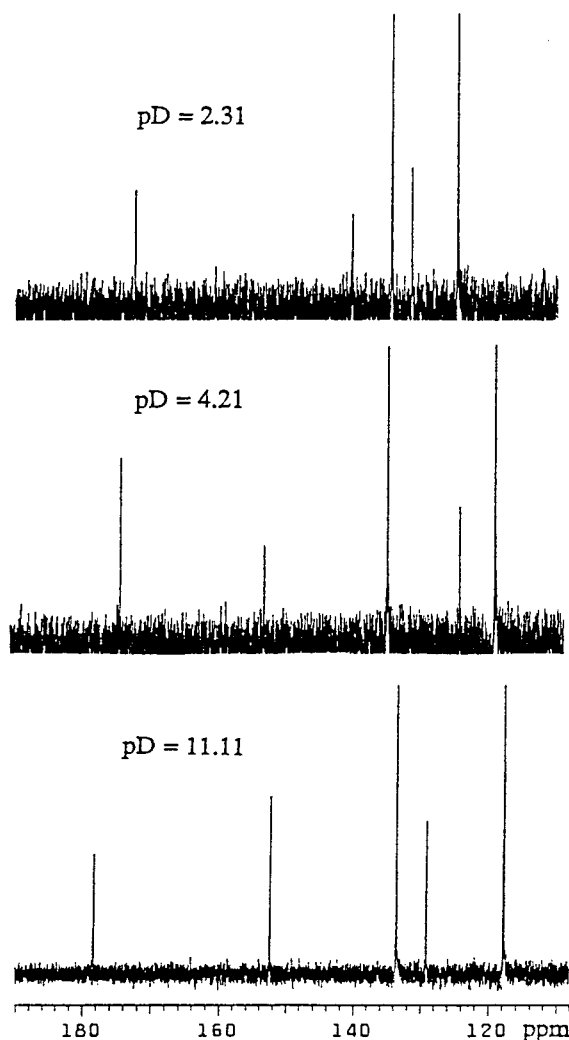*J. Phys. Chem. B, Vol. 104, No. 35, 2000* **8601**

Figure 3. ^{13}C NMR spectra at 298.15 K of 4-aminobenzoic acid in D_2O at different pD values.

(see Table 2); and i = a, b, c, or d representing the different microreactions as shown in Figure 1. Equation 12 has been effective in fitting ion dissociation reactions as a function of temperature when the difference between the maximum and minimum temperatures is less than 150 K. Thermodynamic consistency requires that $q^{(j)}_d = q^{(j)}_a + q^{(j)}_c - q^{(j)}_b$, where j equals 1, 2, and 3. Use of eq 3, the van't Hoff equation, and definitions of $\Delta S°$ and $\Delta C_p°$ gives

$$\Delta H_i° = -q^{(2)}_i R + q^{(3)}_i RT^2(\partial \ln \rho/\partial T) \qquad (13)$$

where R is the universal gas constant.

$$\Delta S_i° = q^{(1)}_i R + q^{(3)}_i R \ln \rho + q^{(3)}_i RT(\partial \ln \rho/\partial T) \qquad (14)$$

$$\Delta C_{pi}° = 2q^{(3)}_i RT(\partial \ln \rho/\partial T) + q^{(3)}_i RT^2(\partial^2 \ln \rho/\partial T^2) \qquad (15)$$

The thermodynamic quantities for macrospecies ionization can be found using the appropriate combination of the quantities for microspecies ionization. Since only three of the reactions involving the microspecies are independent (see eq 3), there are nine parameters which were regressed using an optimization routine.

$$pK_1 = -\log(e^{\ln K_a} + e^{\ln K_b}) \qquad (16)$$

The constants in eqs 13−21 were changed by use of eq 10 and

$$pK_2 = -\log\{[e^{(\ln K_c + \ln K_d)}]/[e^{\ln K_c} + e^{\ln K_d}]\} \qquad (17)$$

$$\Delta H_1° = (\Delta H_a° K_a + \Delta H_b° K_b)/(K_a + K_b) \qquad (18)$$

$$\Delta H_2° = (\Delta H_c° K_d + \Delta H_d° K_c)/(K_c + K_d) \qquad (19)$$

$$\Delta C_{p1}° = (\Delta C_{pa}° K_a + \Delta C_{pb}° K_b)/(K_a + K_b) + [(\Delta H_a° - \Delta H_b°)^2 K_a K_b]/[RT^2(K_a + K_b)^2] \quad (20)$$

$$\Delta C_{p2}° = (\Delta C_{pc}° K_d + \Delta C_{pd}° K_c)/(K_c + K_d) - [(\Delta H_c° - \Delta H_d°)^2 K_c K_d]/[RT^2(K_c + K_d)^2] \quad (21)$$

the van't Hoff equation in order to obtain the values valid in D_2O.

The uncertainties for each set of thermodynamic quantities reported in Tables 3-6 were estimated to be less than 3% according to two or three replicated NMR and calorimetric measurements.

Results and Discussion

Thermodynamic quantities for proton ionization from protonated 4-aminobenzoic acid in aqueous solutions (both in H_2O and D_2O) as a function of temperature are listed in Tables 3−6. Values for the macroscopic reactions are given in Tables 3 (H_2O) and 4 (D_2O) while those for the microscopic reactions are given in Tables 5 (H_2O) and 6 (D_2O).

NMR Spectra. ^1H resonances of 4-aminobenzoic acid in D_2O are observed only for benzo protons. At pD 4.24 (4-aminobenzoic acid itself, without DCl or NaOD added), as shown in Figure 2, two sets of double peaks are present at 6.88 and 7.85 ppm. As pD values decrease (DCl added), these peaks show a downfield shift due to an addition of deuterons to the amino and carboxylate groups. As pD values increase (NaOD added), on the other hand, the benzo proton signals show an upfield shift. Five ^{13}C peaks are observed for 4-aminobenzoic acid (Figure 3). At pD 4.21, two strong peaks at 118.4 and 134.3 ppm are peak resonances of unsubstituted benzene carbons. Resonances of the C1, C4, and the carbon in the carboxylate group are observed (pD 4.21) at 123.6, 152.6, and 173.9 ppm, respectively.[28]

Reaction Types. Proton ionization reactions in aqueous solutions can be classified in three groups. The generic forms of these reactions together with examples are shown in Table 7. The first type occurs when a protonated species dissociates to form a proton and a species with a net charge greater than that of the protonated species. The second type is an iso-Coulombic reaction where the protonated species (with a positive charge) dissociates, to a neutral species and a proton. Hence, there is a single positive charge on each side of the equation representing the reaction. In the third type of reaction, proton ionization creates a zwitterion or a zwitterion dissociates to form a proton and an anion.

The first type of reaction has a profound effect on the structure of water since proton ionization results in a net increase of charge resulting in additional water molecules bound to the charged product species. These additional water molecules come from the bulk water, as indicated by the second term in the example of the type 1 reaction in Table 7. Reactions of this type are usually exothermic and they have negative $\Delta S°$ values because the disorder of the system decreases. The additional water molecules lose the vibrational, rotational, and translational
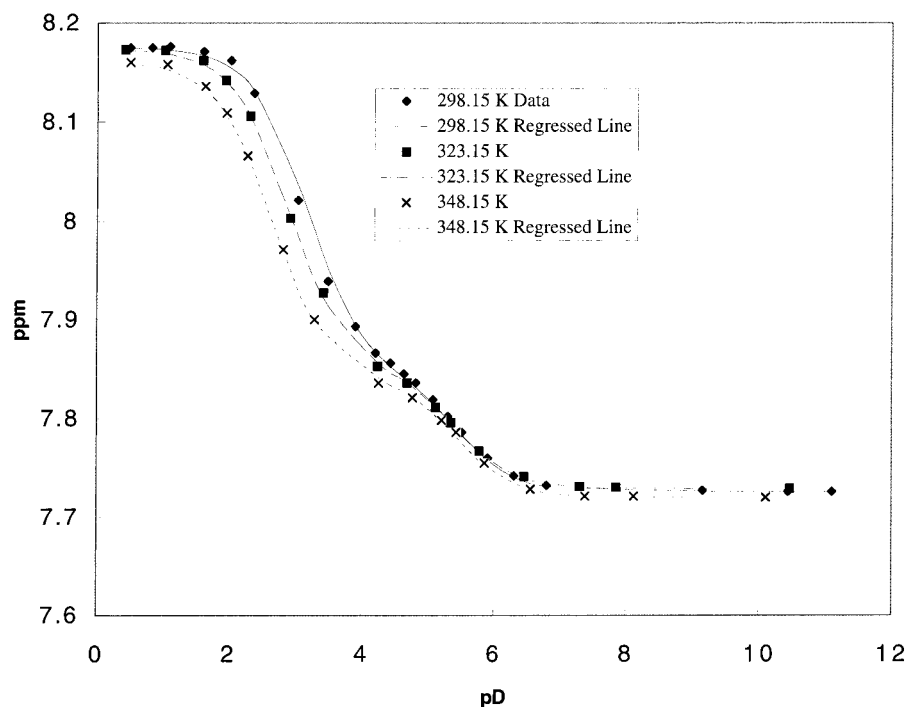
Figure 4. Plots of chemical shifts for ^{1}H NMR spectra of 4-aminobenzoic acid in D_2O as a function of pD values. Symbols are data and lines are values calculated using regressed parameters.

Figure 5. Plots of chemical shifts for ^{13}C NMR spectra of 4-aminobenzoic acid in D_2O as a function of pD values. Symbols are data and lines are values calculated using regressed parameters.
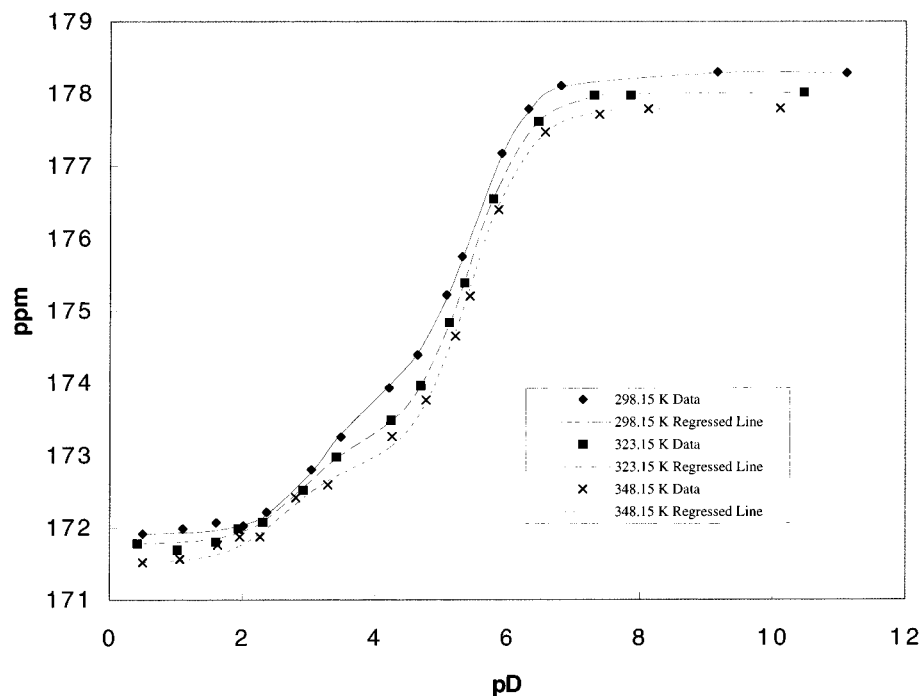
TABLE 2: Constants for Eq 12

parameter	K_a	K_b	K_c	K_d
$q^{(1)}$	−2.054	2.796	−4.541	−9.391
$q^{(2)}$	−1944.2 K	−2537.2 K	−1133.4 K	−540.4 K
$q^{(3)}$	6.9	0.25	3.3	10.0

motion that they had in the bulk water. These reactions characteristically have large, negative $\Delta C_p°$ values that increase in the negative direction as temperature increases.[10,11] As the temperature increases, the extent of hydrogen bonding in the bulk water decreases,[29,30] resulting in an increasing number of

non-hydrogen bonded water molecules in the bulk water. Since free water molecules have higher energy and disorder than do the hydrogen bonded water molecules, the H and S values for the bulk water system increase with temperature. The normal increases in H and S with temperature are enhanced by this effect. Since the H and S values for the solvated species are not expected to change significantly with temperature, the observed decreases in $\Delta H°$ and $\Delta S°$ with temperature for reactions of this type are primarily due to the increased H and S values for the bulk water. The reduction of the dielectric constant as temperature increases causes further decreases in

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TABLE 3: Thermodynamic Quantities for Macroreactions 1 and 2 in H_2O

T, K	pK	$\Delta H°$, kJ·mol^{-1}	$\Delta S°$, J·mol^{-1}· K^{-1}	$\Delta C_p°$, J·mol^{-1}· K^{-1}
		Reaction 1		
298.15	2.46[a,b]	20.7	22	−2
323.15	2.18[a]	20.7	22	−2
348.15	1.94[a]	20.6	22	−2
373.15	1.73	20.6	22	−2
393.15	1.58	20.5	22	−2
		Reaction 2		
298.15	4.90[a,b]	2.9	−84	−83
323.15	4.88[a]	0.9	−91	−84
348.15	4.88[a]	−1.4	−97	−94
373.15	4.90	−3.9	−104	−109
393.15	4.94	−6.2	−110	−126

[a] Measured in D_2O with NMR and converted to values valid in H_2O and measured in H_2O using calorimetry. All other pK values and all $\Delta H°$ values were measured using calorimetry. Values of $\Delta C_p°$ were found by differentiating $\Delta H°$ values with respect to temperature. $\Delta S°$ values were calculated by combining pK and $\Delta H°$ values. [b] Calorimetric data from ref 12 were used (see text).

TABLE 4: Thermodynamic Quantities for Macroreactions 1 and 2 in D_2O

T, K	pK	$\Delta H°$, kJ·mol^{-1}	$\Delta S°$, J·mol^{-1}· K^{-1}	$\Delta C_p°$, J·mol^{-1}· K^{-1}
		Reaction 1		
298.15	3.17[a,b]	21.4	11	−3
323.15	2.88[a]	21.4	11	−2
348.15	2.63[a]	21.3	10	−1
373.15	2.42	21.3	11	−2
393.15	2.27	21.3	11	−2
		Reaction 2		
298.15	5.46[a,b]	3.3	−94	−87
323.15	5.43[a]	1.1	−101	−88
348.15	5.43[a]	−1.2	−108	−99
373.15	5.46	−3.9	−115	−115
393.15	5.49	−6.3	−121	−132

[a] Measured in D_2O with NMR and measured in H_2O using calorimetry and converted to values in D_2O. All other pK values and all $\Delta H°$ values were measured using calorimetry. Values of $\Delta C_p°$ were found by differentiating $\Delta H°$ values with respect to temperature. $\Delta S°$ values were found by combining pK and $\Delta H°$ values. [b] Calorimetric data from ref 12 were used (see text).

$\Delta H°$ and $\Delta S°$ with temperature. This amplifies the temperature effect since there is an increase in the number of water molecules affected by the charged species as a result of the decrease in the dielectric constant of water.

In the second type of interaction, the net charge on reactants and products is the same and the net change of the water structure due to the reaction is the smallest of the three reaction types. The quantity $(\Sigma_i a_i - \Sigma_j b_j)$ is small and can be either positive or negative. Thus, $\Delta H°$, $\Delta S°$, and $\Delta C_p°$ are relatively small and do not change appreciably with temperature.

In the third type of reaction, a zwitterion either ionizes or is formed. In this case, the magnitude of $(\Sigma_i a_i - \Sigma_j b_j)$ depends on the degree of the interaction between the opposite charges on the zwitterion. The degree of interaction shows a strong dependence on the distance between the charges. When the distance between the charges is small, the behavior of the zwitterion toward the solvent molecules is similar to that of a neutral molecule, i.e., the effectiveness of the charges in neutralizing each other increases. As the distance increases, the behavior of the zwitterion approaches that of two separate ions.[10]

The magnitude of $\Delta C_p°$ has been found[11] to correlate better with the type of reaction than do the magnitudes of pK, $\Delta H°$, and $\Delta S°$. A possible explanation for this better correlation follows. Values for pK are a result of the appropriate combination of $\Delta H°$ and $\Delta S°$ values and can best be explained in terms of these latter quantities. The main contributions to the $\Delta H°$ and $\Delta S°$ values for a reaction are (1) enthalpy and entropy

TABLE 5: Thermodynamic Quantities for Microreactions a through e in H_2O

T, K	pK	$\Delta H°$, kJ·mol^{-1}	$\Delta S°$, J·mol^{-1}· K^{-1}	$\Delta C_p°$, J·mol^{-1}· K^{-1}
		Reaction a		
298.15	3.73[a]	14.8	−22	−58
323.15	3.54[a]	13.4	−26	−58
348.15	3.39[a]	11.9	−31	−65
373.15	3.28	10.2	−36	−75
393.15	3.22	8.6	−40	−87
		Reaction b		
298.15	2.48[a]	21.0	23	−2
323.15	2.20[a]	21.0	23	−2
348.15	1.95[a]	20.9	23	−2
373.15	1.74	20.9	23	−3
393.15	1.59	20.8	22	−3
		Reaction c		
298.15	3.63[a]	8.8	−40	−28
323.15	3.51[a]	8.1	−42	−28
348.15	3.42[a]	7.4	−44	−31
373.15	3.35	6.5	−47	−36
393.15	3.31	5.7	−49	−42
		Reaction d		
298.15	4.88[a]	2.6	−85	−84
323.15	4.86[a]	0.5	−91	−83
348.15	4.86[a]	−1.7	−98	−93
373.15	4.89	−4.2	−105	−109
393.15	4.93	−6.5	−110	−126
		Reaction e		
298.15	1.25[a]	−6.2	−45	−56
323.15	1.34[a]	−7.6	−49	−55
348.15	1.44[a]	−9.0	−54	−62
373.15	1.54	−10.7	−58	−73
393.15	1.62	−12.3	−62	−84

[a] Measured in D_2O with NMR and converted to values in H_2O. All other values were calculated using eqs 13 through 21. The constants in eqs 13 through 21 were found by optimizing eqs 13 through 15 using both calorimetric and NMR data.

TABLE 6: Thermodynamic Quantities for Mircoreactions a through e in D_2O

T, K	pK	$\Delta H°$, kJ·mol^{-1}	$\Delta S°$, J·mol^{-1}· K^{-1}	$\Delta C_p°$, J·mol^{-1}· K^{-1}
		Reaction a		
298.15	4.23[a]	15.5	−30	−60
323.15	4.03[a]	14.0	−34	−60
348.15	3.87[a]	12.4	−39	−67
373.15	3.76	10.6	−44	−79
393.15	3.69	8.9	−48	−91
		Reaction b		
298.15	3.21[a]	22.0	12	−2
323.15	2.91[a]	21.9	12	−2
348.15	2.66[a]	21.9	12	−2
373.15	2.44	21.8	12	−3
393.15	2.28	21.7	12	−3
		Reaction c		
298.15	4.41[a]	9.2	−54	−29
323.15	4.29[a]	8.5	−56	−29
348.15	4.19[a]	7.7	−58	−32
373.15	4.12	6.8	−61	−38
393.15	4.07	6.0	−63	−44
		Reaction d		
298.15	5.42[a]	2.7	−95	−87
323.15	5.40[a]	0.5	−102	−87
348.15	5.41[a]	−1.7	−109	−97
373.15	5.44	−4.4	−116	−114
393.15	5.47	−6.8	−122	−131
		Reaction e		
298.15	1.02[a]	−6.5	−42	−58
323.15	1.12[a]	−7.9	−46	−58
348.15	1.21[a]	−9.5	−50	−65
373.15	1.32	−11.2	−55	−76
393.15	1.41	−12.8	−60	−88

[a] Measured in D_2O with NMR. All other values were calculated using eqs 13 through 21. The constants in eqs 13 through 21 were found by optimizing eqs 13 through 15 using both calorimetric and NMR data.

changes due to the dissociation of the solute species in the absence of solvent and (2) the changes in the number of water molecules bound to the solute species, $(\Sigma_i a_i - \Sigma_j b_j)$. Changes in $\Delta H°$ and $\Delta S°$ with temperature in the absence of solvent

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TABLE 7: Types of Proton Ionization Reactions in Aqueous Solution

Type 1

generic $HA = H^+ + A^-$

example $b_1H_2O \cdot C_2H_3COOH + (\Sigma_{i=1}^2 a_i -$
$\Sigma_{j=1}^1 b_i)H_2O = a_1H_2O \cdot C_2H_3COO^- + a_2H_2O \cdot H^+$

Type 2

generic $AH^+ = A + H^+$

example $b_1H_2O \cdot NH_4^+ + (\Sigma_{i=1}^2 a_i - \Sigma_{j=1}^1 b_i)H_2O =$
$a_1H_2O \cdot NH_3 + a_2H_2O \cdot H^+$

Type 3

generic $HAH^+ = H^+ + {}^+HA^-, {}^+HA^- = H^+ + A^-$

example $b_1H_2O \cdot {}^+H_3NCH_2COOH + (\Sigma_{i=1}^2 a_i -$
$\Sigma_{j=1}^1 b_i)H_2O = a_1H_2O \cdot {}^+H_3NCH_2COO^- + a_2H_2O \cdot H^+$
$b_1H_2O \cdot {}^+H_3NCH_2COO^- + (\Sigma_{i=1}^2 a_i -$
$\Sigma_{j=1}^1 b_i)H_2O = a_1H_2O = a_1H_2O \cdot H_2NCH_2COO^- + a_2H_2O \cdot H^+$

molecules are expected to be small. However, the *H* and *S* values for the H_2O molecules in the bulk water system are larger and increase more rapidly with temperature than do *H* and *S* for the solute-bound molecules. Therefore, the principal contribution to the change of $\Delta H°$ and $\Delta S°$ with temperature is the change of *H* and *S* for the bulk water with temperature. The $\Delta C_p°$ values reflect this change with temperature. Thus, the $\Delta C_p°$ values are sensitive indicators of the extent of interactions between the water molecules and the reactants and products.

Macroscopic Quantities. Each macroscopic reaction in Figure 1 is a combination of two microscopic reactions (eqs 1 and 2). Macroscopic reaction 1 is a combination of reactions of type 2 (reaction b) and type 3 (reaction a) while macroscopic reaction 2 is a combination of reaction type 1 (reaction d) and type 3 (reaction c). Reaction type 3, involving a zwitterion, is common to both macroscopic reactions 1 and 2. It is impossible to predict with confidence the changes of thermodynamic quantities for macroscopic reactions with temperature without considering their constituent microscopic reactions. It is sufficient to know the values for the macroscopic reactions in many practical applications such as in calculating the pH of the solution. However, an understanding of the chemistry involved requires a knowledge of the microscopic reactions. The overall trends for the macroscopic reactions with temperature as seen in Tables 3 and 4 are listed below.

1. pK_1 values decrease but pK_2 values are relatively constant with increasing temperature.

2. $\Delta H_1°$ values are relatively constant but $\Delta H_2°$ values decrease with increasing temperature.

3. $\Delta S_1°$ values are relatively constant and $\Delta S_2°$ values decrease with increasing temperature,.

4. $\Delta C_{p1}°$ values go from small negative to small positive values while $\Delta C_{p2}°$ values are large and negative and become more negative with temperature.

Microscopic Quantities. The p*K*, $\Delta H°$, $\Delta S°$, and $\Delta C_p°$ values as a function of temperature are given in Tables 5 and 6. The traditional approach in explaining the changes of thermodynamic quantities associated with a reaction is to emphasize the p*K* and $\Delta H°$ values, if measured, and then report the $\Delta S°$ and $\Delta C_p°$ values as calculated from the first two. Since the $\Delta C_p°$ values most closely reflect the interaction of solute species with water, we have taken the approach of examining the $\Delta C_p°$ values as a guide to understand the reactions and the solute−solvent interactions. The $\Delta C_p°$ and $\Delta C_p°/T$ values are the derivatives of the $\Delta H°$ and $\Delta S°$ values with respect to temperature, respectively. The p*K* values are a combination of the $\Delta H°$ and $T\Delta S°$ values. The p*K* values by themselves provide little information concerning solute−solvent interactions. The $\Delta C_p°$ values for the several microscopic reactions follow the trends seen in our earlier studies of the three types of reactions.[11] Reaction d is of type 1 and, as expected has the largest negative $\Delta C_p°$ values. Reaction b is of type two and has the smallest $\Delta C_p°$ values consistent with its iso-Coulombic nature. The $\Delta C_p°$ value for reaction b is very close to that for proton ionization from the protonated amino group of aniline (See Table 8). Reactions a and c are of type 3 and the $\Delta C_p°$ values for these reactions are intermediate between those found for reactions b and d. In the case of straight chain aliphatic amino acids, the magnitude of the $\Delta C_p°$ values for proton ionization from the protonated amino groups depends on the number of methylene groups between the amino and carboxylate groups. This effect is shown in the first four examples in Table 8. For the α-amino acids (glycine and α-aminobutyric acid), the $\Delta C_p°$ values are large and negative, −77 and −80 J·mol^{-1}·K^{-1}, respectively. The introduction of methylene groups between the functional groups in the case of 4-amino butyric acid and 6-amino hexanoic acid results in low $-\Delta C_p°$ values. The $\Delta C_p°$ values for proton ionization from protonated amino groups of these latter amino acids are intermediate between those for α-amino acids and that of diglycolamine (DGA) which is a primary straight chain amine (Table 8). This result is consistent with the observation that as the distance between the functional groups increases (the number of methylene groups increases) the $\Delta C_p°$ values for type 3 reactions become less dependent on the second functional group.

The magnitudes of the $\Delta C_p°$ values for proton ionization from the carboxylate groups (Table 8) are relatively independent of the chain length or the functional group attached to the acid. This may be due to the fact that the oxygen atoms of the carboxylate group are separated from the other groups by one or more carbon atoms. The effect of the benzene ring, which is not very large, can be seen by comparing the $\Delta C_p°$ values for aniline and DGA. The results of the present study indicate that the presence of a charge on the carboxylate group has a moderate effect on proton ionization from the amino group. This effect can be observed by comparing the $\Delta C_p°$ values for reaction b

TABLE 8: Thermodynamic Quantities at 50 °C for Proton Ionization from Selected Amino Acids, Benzoic Acid, Aniline, Acetic Acid, and Diglycolamine (DGA)

	carboxylate group			amino group		
compound	$\Delta H°$, kJ·mol^{-1}	$\Delta S°$, J·mol^{-1}·K^{-1}	$\Delta C_p°$, J·mol^{-1}·K^{-1}	$\Delta H°$, kJ·mol^{-1}	$\Delta S°$, J·mol^{-1}·K^{-1}	$\Delta C_p°$, J·mol^{-1}·K^{-1}
glycine[a,b]	0.9	−41	−119	40.6	−50	−77
α-aminobutyric acid[a,b]	−1.1	−47	−105	43.3	−43	−80
4-aminobutyric acid[a,b]	−2.1	−84	−126	51.2	−30	−26
6-aminohexanoic acid[a,b]	−5.0	−100	−127	56.6	−17	−31
benzoic acid[c]	−3.3	−91	−124			
aniline[c]				31.1	16	4
acetic acid[c]	−4.1	−104	−96			
diglycolamine[d]				51	−11	12

[a] Reference 9. [b] Reference 10. [c] Reference 31. [d] Reference 32.

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with those for reaction c. The difference between the ΔC_p° values of reactions b and c shows that interaction between the carboxylate group and amino group in 4-aminobenzoic acid is about the same as it is in 4-aminobutyric and 6-aminohexanoic acids. In reaction a, which most closely resembles a reaction of type 1 the neutral carboxylic acid group loses a proton and forms a negatively charged carboxylate group and a positive proton with resultant large negative ΔC_p° values. In reaction c, the positively charged ammonium ion releases a proton and forms a neutral amino group. This reaction is similar to that of an iso-Coulombic reaction in which ΔC_p° is small. If the two groups were closer together as in glycine, the magnitude of the ΔC_p° values would be expected to be larger since the zwitterion would more closely resemble a neutral molecule.

The derivatives of the ΔH° and ΔS° values with respect to temperature can be explained in terms of ΔC_p°. The magnitudes of the ΔH° and ΔS° values at a given temperature can be explained in terms of the addition of the ΔH° and ΔS° values in the absence of solvent to the contribution to these values by the solvent–solute interactions with the reactants and products. All dissociation reactions of this type in the absence of water would be expected to have positive ΔH° and ΔS° values. The larger the value of $(\Sigma_i a_i - \Sigma_j b_j)$ the more negative will be the contribution of the solvent interaction to the ΔH° and ΔS° values of the reaction. Reaction b is an iso-Coulombic reaction and the value of $(\Sigma_i a_i - \Sigma_j b_j)$ should be very small, so the observed large positive values of ΔH° and ΔS° and their invariance with temperature are consistent with this model. Reaction d is a type 1 reaction so the value of $(\Sigma_i a_i - \Sigma_j b_j)$ should be large and as expected, this reaction has the lowest ΔH° and ΔS° values. The values of ΔH° and ΔS° for reactions a and c are between those for reactions b and d, as expected. The pK values for all reactions are the result of the combination of the ΔH° and ΔS° values.

Conclusions

An understanding of the thermodynamic quantities associated with macroscopic reactions that consist of two microscopic reactions can be gained by investigating their constituent microscopic reactions. The microscopic reactions follow the trends with temperature typical of their type and can be modeled using the fitting equations typically used for ionic reactions in aqueous solutions. The appropriate mathematical combination of such reactions can then be used to describe the thermodynamic quantities involved with the macroscopic reactions. The ΔC_p° value is a strong function of the difference between the effect of the reactants and products, respectively, on the solvent. The calculation of the ΔC_p° values requires that calorimetric measurements be made at several temperatures. Therefore, measurement of the thermodynamic quantities as a function of temperature gives considerably more information than would be obtained if the measurements were made at only one temperature. NMR measurements were required in order to determine the pK values for the microreactions. The combination of spectroscopic with calorimetric data gives a good description of the interactions occurring in microreactions.

In biochemical systems, consisting of proteins, nucleic acids, etc., there are many proton ionization sites. The present study demonstrates that the influence of these sites on each other is strongly reflected in the thermodynamic quantities for proton ionization. From our preliminary investigations, calorimetric and NMR studies of such molecules should provide much valuable information on such systems.

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