Pressure Dependence of Weak Acid Ionization in Deuterium Oxide Solutions

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The pressure dependence of solution pH for acetate, cacodylate, phosphate, and TRIS buffers in deuterium oxide solutions was measured with the optical pH indicators 2,5-dinitrophenol and *p*-nitrophenol up to a pressure of 300 MPa. The results give the pressure dependence of the weak acid ionization constant for these buffers in D_2O solution. Neuman et al. [*J. Phys. Chem.* **1972**, *77*, 2687] measured the pressure dependence of K_a for these buffers in H_2O solutions and used the equation $RT \ln(K_p/K_o) = -\Delta V_a^\circ P + 0.5 \Delta\kappa^\circ P^2$ to describe the observed results where ΔV_a° is the limiting volume change on ionization at atmospheric pressure and $\Delta\kappa^\circ$ reflects the pressure dependence of ΔV_a°. The present experiment shows that there is no significant deuterium isotope effect on the pressure dependence for these weak acid ionization constants.

Introduction

This work was motivated by a study of activation volumes for amide hydrogen−deuterium exchange for individual sites in T4 lysozyme.[1] Amide hydrogen−deuterium exchange rates are routinely measured by NMR methods, often in deuterium oxide solutions and are strongly dependent upon solution pH.[2] Meaningful interpretation of kinetic data requires careful control of the solution pH or pD in D_2O solutions, but maintenance of constant solution pH or pD over a range of pressures may be challenging because the ionization constants of weak acids used as buffers may change with pressure.[3] Several buffer systems have been studied as a function of pressure in H_2O, but no studies have been reported in D_2O, which is commonly used as a solvent in biomolecular NMR measurements. Therefore, we extend the measurements of Neuman et al.[3] to D_2O.

The pH of a buffered solution depends upon the pK_a of the weak acid [HA] and the concentration ratio of the acid and conjugate base, [A]:

$$\mathrm{pH} = \mathrm{p}K_a + \log\frac{[\mathrm{A}]}{[\mathrm{HA}]} \quad (1)$$

When buffer concentration is sufficiently high, the shifts in hydrogen ion concentration caused by changes in the water ionization constant are easily dominated by the second term in eq 1, and changes in pH can be attributed directly to the change in pK_a of the buffer with pH; that is,

$$\frac{\partial(\mathrm{pH})}{\partial P} \cong \frac{\partial(\mathrm{p}K_a)}{\partial P} \quad (2)$$

Solution pH may be determined with optical indicators such as *p*-nitrophenol or 2,5-dinitrophenol where the acidic form of the molecule is colorless and the conjugate base is colored,

$$\mathrm{pH} = \mathrm{p}K_I - \log\frac{[\mathrm{OD_I} - \mathrm{OD}]}{[\mathrm{OD}]} \quad (3)$$

where K_I is the dissociation constant for the acid form of the indicator, OD_I is the optical density at λ_{max} corresponding to complete conversion of the indicator to its corresponding conjugate base, and OD is the optical density at the same wavelength that is observed in the solution of interest.

If the solution pH is followed with an optical indicator, the pressure dependence of pK_a for a buffer can be determined by

$$\partial(\mathrm{p}K_a)/\partial P = \partial(\mathrm{p}K_I)/\partial P - \partial\left\{\log\frac{[\mathrm{OD_I} - \mathrm{OD}]}{[\mathrm{OD}]}\right\}/\partial \mathrm{P} \quad (4)$$

where the observed pressure dependence of the log(Δ*O*D/OD) reflects the difference in the pressure dependence of pK_I and pK_a. Therefore, if the pressure dependence of the K_I for the indicator is known, the pressure dependence of the weak acid K_a may be determined.

Neuman et al.[3] used this method to determine the pressure dependence of pK_a for several common buffers in water (H_2O) up to a pressure of 650 MPa and fit the data to eq 5.

$$K_a(p) = K_a^\circ \exp\left[\frac{-P\Delta V_a^\circ}{RT} + \frac{0.5\Delta\kappa^\circ \mathrm{P}^2}{RT}\right] \quad (5)$$

where ΔV_a° is the limiting volume change on ionization at atmospheric pressure and $\Delta\kappa^\circ$ reflects the pressure dependence of ΔV_a°.

Methods and Materials

Sample Preparation. Sodium cacodylate (dimethylarsinic acid) was obtained from Aldrich Chemical Co., Trizma base [2-amino-2-(hydroxymethyl)-1,3-propanediol] was obtained from Sigma Chemical Co, and dibasic and monobasic sodium phosphate and sodium acetate were research grade. Stock solutions of the optical indicators were prepared in D_2O (99.9% atom D, Cambridge Isotope Laboratories, Inc.) to concentrations of 1.0×10^{-2} M for *p*-nitrophenol (Aldrich Chemical Co.) and 2.5×10^{-2} M for 2,5-dinitrophenol (Aldrich). Buffer/indicator solutions were prepared just prior to use by diluting 50 μL of the indicator stock solution to 5.0 mL with D_2O containing buffer. TRIS buffer was prepared by mixing Trizma base and Trizma HCl. Phosphate buffer was prepared with dibasic and monobasic sodium phosphate, and the cacodylate and acetate

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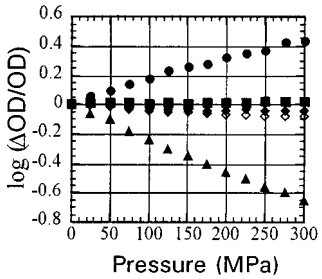

Figure 1. Plot of log[(OD_I − OD)/OD] versus pressure for *p*-nitrophenol in D_2O solutions of Tris (●), phosphate (▲), and cacodylic acid (◇), and for 2,5-dinitrophenol in D_2O solutions of cacodylic acid (◆) and acetic acid (■).

buffers were prepared by titration of the respective conjugate base with DCl (Aldrich). The final buffer concentration in all cases was 50 mM. The final concentration of the indicator was 2.5×10^{-4} M for 2,5-dinitrophenol and 1×10^{-4} M for *p*-nitrophenol. For each buffer, two solutions were prepared. A "buffered" solution was prepared by titration of the pH* (uncorrected pH meter reading) to near the pK_a with DCl (Aldrich), and a "basic" solution was prepared by titration of the pH* to greater than 10 with NaOD (Aldrich).

UV Spectroscopy at High Pressure. Samples of indicators in buffers were placed in a 1-cm-diameter quartz sample tube (S-0250, SLM-Aminico) and placed in the high-pressure optical spectroscopy vessel (HPSC-3K, SLM-Aminico). Temperature was regulated at 25 °C by circulating thermostated water through a cooling jacket surrounding the high pressure cell. High pressure was generated with a manually operated piston screw pump pressure generator (model 31-5.75-75, High-Pressure Equipment Co., Erie, PA), and ethanol was used a compression fluid. Pressure was determined with an AstraGauge pressure gauge (Astra Corporation, Ivyland, PA) rated to a 0.25% accuracy. The optical pressure vessel was fitted into a Cary 4 ultraviolet/visible spectrophotometer for optical measurements. A spectrum was recorded from 650 to 350 nm at atmospheric pressure, and the change in optical density at λ_{max} (400 nm for 2,5-dinitrophenol and 360 nm for *p*-nitrophenol) was recorded at 25-MPa increments up to 300 MPa of applied pressure. Several minutes was allowed at each pressure for temperature equilibration before the optical density was determined. The optical density measurements of the "basic" solution and "buffered" solutions at each pressure enter eq 5 as OD_I and OD, respectively. Optical densities were monitored after the pressurization step to verify stability. Some baseline shift was observed with increasing pressure, and this was subtracted when determining the optical density of λ_{max}; shifts smaller than 4 nm were observed in the position of λ_{max} over the 300-MPa pressure range. Although all the measurements of the earlier work in H_2O were not repeated, our checks of these data were in excellent agreement.

Results and Discussion

A plot of log[(OD_I − OD)/OD] versus pressure for the *p*-nitrophenol and 2,5-dinitrophenol in the buffer studies is shown in Figure 1. The value for OD_I (optical density for complete conversion of the indicator to conjugate base) is determined for each buffer/indicator solution over the entire pressure range studied and therefore, the data are corrected for an increase in optical density caused by compression of the solution, and any pressure-induced changes in the extinction coefficient for the indicator.

To determine the pressure dependence of a weak acid pK_a from the data in Figure 1, it is necessary to know pressure dependence of pK_I for one of the optical indicators, or the pressure dependence of pK_a for one of the weak acids, in D_2O. Neuman et al. were able to calibrate the pressure dependence of pK_I for 2,5-dinitrophenol based on data available for acetic acid, which was determined by another method.[4] However, we are unaware of any previous studies of the pressure dependence pK_a for these weak acids in D_2O. Neuman et al. determined that the pK_a of TRIS in H_2O is almost independent of pressure. Over a range of 650 MPa, the pK_a of TRIS decreases by less than 0.2 units, a result that may be rationalized on the basis of the conservation of net charged species in the ionization reaction.

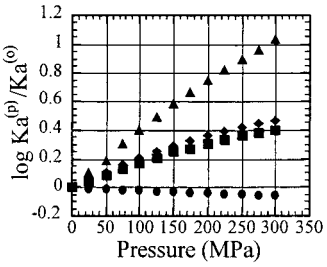

Figure 2. Plot of the pressure dependence of pK_a for phosphate (▲), cacodylic acid (◆), and acetic acid (■). The ● symbol represents a fit of $\Delta V_a^\circ = +1.0$ cm^3 mol^{-1} and $\Delta\kappa^\circ = 0$ cm^3 mol^{-1}/kg cm^{-2} for TRIS.

TABLE 1: Pressure Dependence Parameters[a]

buffer	values in H_2O[3]		values in D_2O
TRIS	ΔV_a°	∼+1	fit to +1
	$\Delta\kappa^\circ$	0	0
phosphate	ΔV_a°	−24.7	−24.95 ± 0.27
	$\Delta\kappa^\circ$	−2.0	−3.1 ± 0.22
cacodylate	ΔV_a°	−13.2	−13.39 ± 0.03
	$\Delta\kappa^\circ$	−1.94	−2.69 ± 0.03
acetate	ΔV_a°	−11.2	−11.20 ± 0.16
	$\Delta\kappa^\circ$	−1.44	−2.055 ± 0.13
p-nitrophenol	ΔV_a°	−11.3	−10.71 ± 0.33
	$\Delta\kappa^\circ$	−1.41	−1.96 ± 0.27
2,5-dinitrophenol	ΔV_a°	−11.3	−11.2 ± 0.16
	$\Delta\kappa^\circ$	−1.28	−2.06 ± 0.13

[a] The results obtained for the pressure dependence of pK_a and pK_I in D_2O by fitting the experimental data to eq 5. Values in H_2O are taken from Neuman et al.[3] [ΔV_a° (cm^3 mol^{-1}), $\Delta\kappa^\circ$ (cm^3 mol^{-1}/kg cm^{-2}) $\times 10^3$].

Assuming that the pressure dependence of pK_a for TRIS is similarly small in D_2O as in H_2O, the pressure dependence of pK_I for *p*-nitrophenol (pK_I = 7.02) in D_2O was determined by fitting the experimental data for TRIS to eq 5 using the values obtained in H_2O ($\Delta V_a^\circ = 1$ cm^3 mol^{-1}, $\Delta\kappa^\circ = 0$ cm^3 mol^{-1}/kg cm^{-2}). The optical indicator, *p*-nitrophenol, was then used to monitor the pressure dependence of pK_a for $H_2PO_4^-$ and cacodylic acid. The pressure dependence of pK_I for 2,5-dinitrophenol (pK_I = 5.10) was determined from experimental results using cacodylate, and 2,5-dinitrophenol was in turn used to determine the pressure dependence of pK_a for acetic acid.

Figure 2 shows the pressure dependence of pK_a for several of the buffers studied, and the results of fitting the data to eq 5 are summarized in Table 1. Except for *p*-nitrophenol, the values of ΔV_a° measured for D_2O solutions are the same as the values in H_2O obtained by Neuman et al., within experimental error. The values for $\Delta\kappa^\circ$ in D_2O are slightly more negative than the values reported in H_2O, in all cases. The small difference observed for *p*-nitrophenol may be a result of the initial assumption that the values for TRIS are the same for both solvents. The difference may reflect small structural changes induced in the solvent by the solute molecule caused by differences in hydrogen-bonding ability of the two solvents.

Conclusions

This work was motivated by the study of the pressure dependence of amide hydrogen−deuterium exchange kinetics of proteins. Substitution of D_2O is well-known to change pK_a values by substantial amounts; for example, the pK_a of acetic acid shifts by 0.52 units in D_2O.[5] However, these experiments demonstrate that the pressure dependence of the acetate, phosphate, cacodylate, and TRIS buffers is unchanged, for practical purposes, by the isotope substitution, which implies that the volume changes associated with the ionizations are not dominated by subtle effects involving isotope effects in the water.

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