Effect of temperature and pressure on the protonation of glycine

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ABSTRACT Flow calorimetry has been used to study the interaction of glycine with protons in water at temperatures of 298.15, 323.15, and 348.15 K and pressures up to 12.50 MPa. By combining the measured heat for glycine solutions titrated with NaOH with the heat of ionization for water, the enthalpy of protonation of glycine is obtained. The reaction is exothermic at all temperatures and pressures studied. The effect of pressure on the enthalpy of reaction is very small. The experimental heat data are analyzed to yield equilibrium constant (K), enthalpy change (ΔH), and entropy change (ΔS) values for the protonation reaction as a function of temperature. These values are compared with those reported previously at 298.15 K. The ΔH and ΔS values increase (become more positive), whereas log K values decrease, as temperature increases. The trends for ΔH and ΔS with temperature are opposite to those reported previously for the protonation of several alkanolamines. However, log K values for proton interaction with both glycine and the alkanolamines decrease with increasing temperature. The effect of the nitrogen atom substituent on log K for protonation of glycine and alkanolamines is discussed in terms of changes in long-range and short-range solvent effects. These effects are used to explain the difference in ΔH and ΔS trends between glycine protonation and those found earlier for alkanolamine protonation.

INTRODUCTION

The existence of thriving colonies of life forms at high temperatures and high pressures has raised questions about how organisms can survive under conditions where most proteins become denatured (1, 2). A recent differential scanning calorimetry study (3) of *Escherichia coli* whole cells and cell components showed that denaturation of different components occurred at different temperatures over the temperature range 20–130°C. Denaturation was irreversible in some, but not all, cases. The effect of temperature on the mechanism of folding and stabilization of proteins has been the subject of numerous studies, and the thermodynamic features of these processes and their relation to those of dissolution of organic compounds in water have been discussed (4, 5). Statistical thermodynamic theories have been formulated to account for the stabilities of globular proteins (6). On the other hand, answers to the questions raised concerning organism survival are hampered by the lack of relevant data about the effect of temperature and pressure on reactions involving either proteins or their constituent amino acids. We have initiated a program to quantitate interactions of amino acids and dipeptides with protons and water at high temperatures and pressures to evaluate how these variables affect the proton ionization thermodynamics of simple constituents and how this effect is transmitted to larger biomolecules.

In the present work, the protonation of glycine at temperatures of 298.15, 323.15, and 348.15 K and pressures from 0.43 to 12.5 MPa was studied by means of a high-pressure flow calorimeter. The ionization equilibria of glycine during titration with NaOH can be represented by the equations

$$^+NH_3CH_2COOH + OH^- = {}^+NH_3CH_2COO^- + H_2O \quad (1)$$

$$^+NH_3CH_2COO^- + OH^- = NH_2CH_2COO^- + H_2O. \quad (2)$$

Titration of the zwitterion with NaOH reduces its concentration in the solution and increases the formation of glycinate ions and water molecules. By substracting the ionization equilibrium of water from reaction 2, the protonation reaction of glycine, Eq. 3, is obtained:

$$NH_2CH_2COO^- + H^+ = {}^+NH_3CH_2COO^-. \quad (3)$$

Thus, by combining the calorimetric data for reaction 2 with the enthalpy of ionization of water, the equilibrium constant (K), enthalpy change (ΔH), and entropy change (ΔS) values for the protonation of glycine may be obtained and the changes in these thermodynamic quantities with temperature and pressure may be studied. These changes can then be compared with those reported previously for the protonation of alkanolamines (7) as a function of temperature in order to

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observe the effect on the thermodynamic quantities of substitution at the nitrogen atom (Fig. 1), and of the presence in glycine of a negatively charged carboxyl moiety. The trends in the thermodynamic quantities are explained in terms of long-range and short-range solute-solvent interactions.

MATERIALS AND METHODS

Materials

Glycine (Aldrich Chemical Co., Milwaukee, WI; gold label, 99% pure) and NaOH solution 50.7% wt/wt (Mallinckrodt Inc., St. Louis, MO; analytical reagent) were used. The solutions were prepared using distilled, deionized water and degassed by submersion in an ultrasonic bath for 10 min. The NaOH solutions were standardized by titration using a known hydrochloric acid solution.

Method

The calorimetric determinations were made using a high-pressure flow calorimeter described elsewhere (8, 9). The measurements were made in a steady-state, fixed composition mode. The flow rate of the glycine solution was kept constant at 8.3×10^{-3} $cm^3 \cdot s^{-1}$. In each run, the flow rate of the NaOH solution was varied from 2.2×10^{-3} $cm^3 \cdot s^{-1}$ to 1.3×10^{-2} $cm^3 \cdot s^{-1}$, in 1×10^{-3} $cm^3 \cdot s^{-1}$ increments. Because the concentration of both solutions was ~0.5 m, these flow rates correspond to base:glycine molar ratios ranging from 0.25:1 to 1.6:1. Data were obtained at 298.15 K and 0.43 MPa; 323.15 K and 0.43, 10.49, and 12.50 MPa; and 348.15 K and 0.43, 7.49, 10.49, and 12.50 MPa. Details of the data reduction method are available (10). The densities of the glycine solutions as a function of pressure and concentration were calculated at the pump temperature and pressure using the Tait equation coefficients given by Yokoyama and Takahashi (11). NaOH solution densities were calculated from the correlation given by Hershey et al. (12).

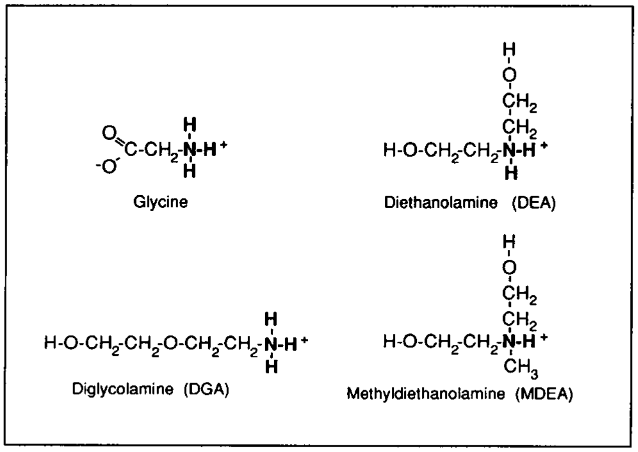

FIGURE 1 Structures of several protonated amines.

RESULTS AND DISCUSSION

Table 1 gives the calorimetric data at each temperature and pressure for the reaction of glycine with NaOH, reaction 2. The experimental heats of reaction are shown in Fig. 2. Reaction 2 is exothermic at all of the temperatures and pressures studied. The enthalpy of reaction becomes less negative as either the temperature or the pressure increases. The temperature effect is much larger than the pressure effect. The enthalpy of reaction versus the total mass flow of solvent was used to derive K and ΔH values at null ionic strength ($I = 0$). The volumetric flow rates were converted to molar flow rates using the densities and molalities of the solutions. Activity coefficients, based on Lindsay's modified Meissner model (13–17), were calculated in the program and were used to extrapolate log K values at the I values of the experiments to the log K values at $I = 0$ and to account for the enthalpies of dilution due to changes in ionic strength. A multivariate Newton method (OPTDES.BYU optimization routine, reference 18) was used to find the log K and ΔH values that gave the best agreement between the predicted and measured heats. By use of this program, water formation is taken into account (19) and the operator may input other reactions that are believed to be significant in the calculations. The correct selection of these reactions is crucial to the success of the calculation procedure. In this study, the protonation of glycine and the ionization of water are considered to be the significant reactions taking place. Un-ionized NaOH and sodium glycinate ion pairs do not exist in significant amounts at the conditions of the experiments.

The effect of pressure on the reaction enthalpies at a given temperature and mass flow is small. Hence, pressure effects were ignored in the data reduction. The observed small effect of pressure on log K and ΔH may be confirmed by estimating the pressure dependence of K by means of the Marshall-Mesmer equation (20),

$$\ln K_{P,T} - \ln K^\circ = -\frac{\Delta V^\circ}{RT\beta^\circ}\ln\frac{\rho_{P,T}}{\rho^\circ}, \qquad (4)$$

where $K_{P,T}$ is the calculated protonation constant at the P and T of measurement, K° and ρ° are the protonation constant and density at the T and experimental saturation vapor pressure of water at that T, ΔV° is the change in apparent molar volume at infinite dilution for the protonation reaction, β° is the isothermal compressibility of water, and $\rho_{P,T}$ is the density of water at the experimental temperature and pressure. Bhattacharyya and Sengupta (21) reported a value of 4 cm^3/mol for ΔV° for reaction 3 at 303.15 K, which is in agreement

TABLE 1 Calorimetric data for the reaction of glycine with NaOH

NaOH flow A	Glycine flow B	Experimental heat of reaction	Calculated heat of reaction
$g\ H_2O \cdot min^{-1}$	$g\ H_2O \cdot min^{-1}$	$Q/J \cdot min^{-1}$	$Q/J \cdot min^{-1}$
T: 298.15 K /	P: 0.43 MPa /	A: 0.5552 m /	B: 0.5449 m
0.1337	0.4447	−0.8936	−0.8638
0.2005	0.4447	−1.3147	−1.2869
0.2673	0.4447	−1.7341	−1.7077
0.3342	0.4447	−2.1519	−2.1264
0.4010	0.4447	−2.5438	−2.5406
0.4678	0.4447	−2.7020	−2.7623
T: 323.15 K /	P: 0.43 MPa /	A: 0.5552 m /	B: 0.5449 m
0.1337	0.4447	−0.6345	−0.6406
0.2005	0.4447	−0.9103	−0.9507
0.2673	0.4447	−1.1933	−1.2580
0.3342	0.4447	−1.4880	−1.5630
0.4010	0.4447	−1.7852	−1.8633
0.4678	0.4447	−1.9163	−2.0247
0.0336	0.4488	−0.1247	−0.1504
0.0671	0.4488	−0.2635	−0.2961
0.1007	0.4488	−0.3951	−0.4398
0.1342	0.4488	−0.5580	−0.5822
0.2013	0.4488	−0.8427	−0.8642
0.2685	0.4488	−1.1096	−1.1438
0.3356	0.4488	−1.3925	−1.4217
0.4027	0.4488	−1.6600	−1.6974
0.4698	0.4488	−1.8814	−1.9605
0.5369	0.4488	−2.0746	−2.0298
T: 323.15 K /	P: 12.50 MPa /	A: 0.5033 m /	B: 0.5417 m
0.1343	0.4496	−0.5810	−0.5827
0.2015	0.4496	−0.8599	−0.8649
0.2687	0.4496	−1.1166	−1.1448
0.3359	0.4496	−1.3951	−1.4228
0.4030	0.4496	−1.6550	−1.6988
0.4702	0.4496	−1.8289	−1.9621
0.5374	0.4496	−1.8414	−2.0316
T: 348.15 K /	P: 0.43 MPa /	A: 0.5035 m /	B: 0.5449 m
0.1337	0.4447	−0.3358	−0.3736
0.2005	0.4447	−0.5239	−0.5467
0.2673	0.4447	−0.7204	−0.7163
0.3341	0.4447	−0.9125	−0.8835
0.4010	0.4447	−1.1150	−1.0485
0.4678	0.4447	−1.2912	−1.2045
0.6014	0.4447	−1.3444	−1.2538
0.6682	0.4447	−1.4023	−1.2577
0.7351	0.4447	−1.4095	−1.2608
T: 348.15 K /	P: 7.49 MPa /	A: 0.5034 m /	B: 0.5430 m
0.1341	0.4477	−0.3899	−0.3747
0.2011	0.4477	−0.5619	−0.5484
0.2681	0.4477	−0.7097	−0.7185
0.3351	0.4477	−0.9002	−0.8861
0.5362	0.4477	−1.2269	−1.2520
0.6032	0.4477	−1.2371	−1.2579
0.6703	0.4477	−1.2472	−1.2618

with data reported by Kharakoz (22). When this value for $\Delta V°$ and values for $\beta°$, $\rho°$, and $\rho_{P,T}$ calculated from the correlation given by Kell (23) are substituted into Eq. 4, values of −0.0078 and −0.0074 are obtained for $\log K_{P,T} - \log K°$ at 12.50 MPa and 323.15 K, and at 12.50 MPa and 348.15 K, respectively. The effect of pressure on the log K values is $<0.1\%$. Bhattacharyya and Sengupta (21) have explained the small ΔV of protona-

TABLE 1 (continued)

NaOH flow *A*	Glycine flow *B*	Experimental heat of reaction	Calculated heat of reaction
T: 348.15 K /	*P*: 10.49 MPa /	*A*: 0.5033 m /	*B*: 0.5423 m
0.1342	0.4488	−0.3808	−0.3752
0.2013	0.4488	−0.5487	−0.5491
0.2685	0.4488	−0.7103	−0.7194
0.3356	0.4488	−0.8796	−0.8873
0.4027	0.4488	−1.0418	−1.0529
0.4698	0.4488	−1.1795	−1.2096
0.5369	0.4488	−1.2235	−1.2537
0.6040	0.4488	−1.2219	−1.2595
T: 348.15 K /	*P*: 12.50 MPa /	*A*: 0.5033 m /	*B*: 0.5417 m
0.1343	0.4496	−0.4069	−0.3755
0.2015	0.4496	−0.5784	−0.5495
0.2687	0.4496	−0.7356	−0.7200
0.3359	0.4496	−0.8817	−0.8880
0.4030	0.4496	−1.0609	−1.0538
0.4702	0.4496	−1.1286	−1.2106
0.5374	0.4496	−1.1665	−1.2548
0.6045	0.4496	−1.1705	−1.2607
0.6717	0.4496	−1.1965	−1.2646
0.8061	0.4496	−1.1997	−1.2705

tion in terms of the solvation of the zwitterion and glycinate ion, and the electron-withdrawing inductive effect of the carboxylate group on the $^+NH_3$. The small ΔV values determined by Bhattacharyya and Sengupta (21) are in agreement with the small pressure effect reported here.

The data at 298.15 K were measured at only one pressure because the value of log *K* for the protonation reaction at this temperature was already known. Investigations made before 1975 were compiled by Christensen et al. (24). More recently, Kiss et al. (25) carried out a critical survey of equilibrium constants for the formation of complexes of glycine. Values of log *K* for the protonation reaction at 298.15 K are almost coincident and a value of 9.78 may be recommended. Assuming this value for log *K*, a value of $\Delta H = -43.95$ kJ · mol^{-1} was obtained from the data taken at 298.15 K and 0.43 MPa. The three sets of data taken at 323.15 K and the four sets taken at 348.15 K were then used to calculate a satisfactory set of log *K* and ΔH values for the three temperatures involved in this study. A satisfactory set of log *K* and ΔH values is obtained when the following criteria are met. First, at each ionic strength and at each temperature, the experimental heat of reaction data are fitted well by a curve constructed using the calculated log *K* and ΔH values. Second, a knowledge of ΔH as a function of temperature and of log *K* at a given temperature allows one to calculate log *K* as a function of temperature by integration of the Van't Hoff equation. Agreement of these calculated log *K* values with those determined calorimetrically provides a stringent test of consistency. Third, the plot of log *K* vs. the inverse of temperature is approximately linear for the reaction written in its isocoulombic form; i.e., equal numbers of positive and negative charges are present on each side of the reaction.

Table 2 gives the set of log *K*, ΔH, and ΔS values

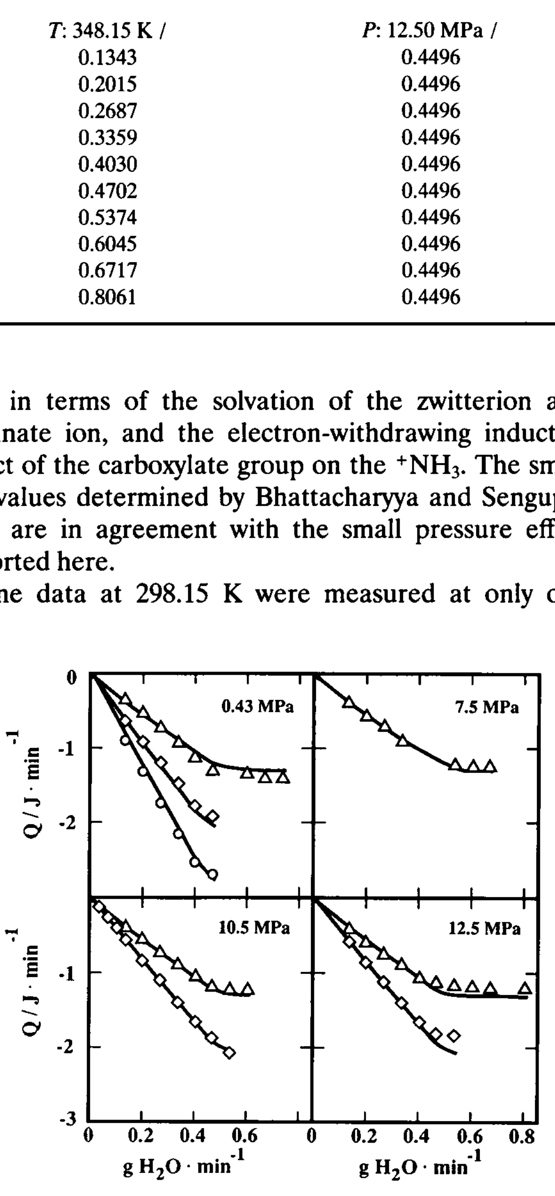

FIGURE 2 Heats of reaction (*Q*) as a function of NaOH flow rates (g H_2O min) and temperature; ○, 298.15 K; ◇, 323.15 K; and △, 348.15 K. The solid lines represent heats of reaction calculated using the best set of thermodynamically consistent ΔH and log *K* values.

TABLE 2 **Data for the aqueous reaction glycinate ion + H^+ = glycine**

T/K	Log *K*	$\Delta H°$/kJ · mol^{-1}	Method*	$\Delta S°$/J · K^{-1} · mol^{-1}	Reference	$\Delta S°$/J · K^{-1} · mol^{-1} (Eq. 7)
278.15	10.34	−45.13	T	36.0	26	36.3
283.15	10.193	−44.92	T	—	27	37.0
	—	−45.43	C	34.8	28	
288.15	10.05	−44.72	T	37.3	26	37.7
298.15	—	−43.95	C	39.1	This study	39.1
	9.779	−44.54	C	37.3	30	
	9.78	−44.38	C	37.7	31	
	9.780	−44.17	C	39.2	28	
	—	−44.09	C	—	29	
	9.768	−44.38	T	38.5	32	
	9.777	−44.17	T	39.4	27	
	9.78	−44.30	T	38.9	33	
	9.780	−45.24	T	35.6	34	
	—	−44.46	C	—	35	
	9.78	—	—	—	36	
	9.78	—	—	—	37	
308.15	9.53	−43.79	T	40.6	33	40.5
313.15	9.412	−43.42	T	—	27	41.1
	—	−43.46	C	41.5	28	
318.15	9.30	−43.29	T	41.9	33	41.8
323.15	9.20	−43.07	C	42.4	This study	42.4
348.15	8.70	−42.37	C	45.5	This study	45.5

*The method of ΔH determination is designated by *C* (calorimetric) or *T* (temperature variation of *K*).

obtained together with literature values. In Fig. 2, the calculated heats of reaction are compared with those determined experimentally. Values of 0.036, 0.068, and 0.057 J · min^{-1} are obtained for the standard deviation between calculated and experimental heats of reaction at 298.15, 323.15, and 348.15 *K*, respectively. The method of ΔH determination is designated by *C* (calorimetric) or *T* (temperature variation of *K*). The ΔH and ΔS values increase, whereas log *K* values decrease, as temperature increases.

Fig. 3 shows the variation of ΔH with temperature. Some of the enthalpy change values reported at 298.15 K differ considerably from each other. The present value (−43.95 kJ/mol) agrees well with some (27–29), but not with other (30–35), values. There is not a correlation between the magnitude of the value and the method of determination used. The observed discrepancies could be due to the extrapolation procedures used to derive the standard state values. With the exception of the values of −45.43 and −45.24 kJ · mol^{-1} reported at 283.15 and 298.15 K, respectively, the ΔH values show a fairly linear variation with temperature and can be fitted to an equation of the form

$$-\Delta H(T) = C_0 + C_1 T, \qquad (5)$$

where *T* is the temperature in Kelvins and C_0 and C_1 are adjustable parameters. Values of 56.45 kJ · mol^{-1} and −41.19 J · K^{-1} · mol^{-1} were obtained for C_0 and C_1. Using appropriate thermodynamic relationships, temperature-dependent equations for ln *K*; ΔS; and the change in the heat capacity of the reaction, ΔC_p, may be

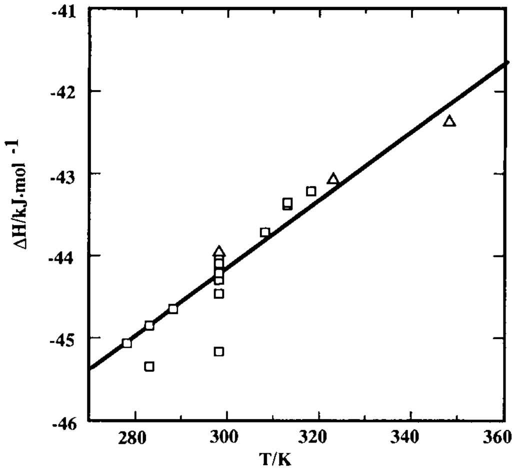

FIGURE 3 Plot of ΔH values for glycine protonation as a function of temperature. The triangles are the results obtained in this study; all other points are taken from references listed in Table 2. The solid line is based on Eq. 5.

derived:

$$\ln K(T) = \ln K_{298} + C_0(RT)^{-1} - C_0(298.15\,R)^{-1} - C_1R^{-1}\ln(T/298.15) \quad (6)$$

$$\Delta S(T) = R\ln K_{298} - C_0(298.15)^{-1} - C_1 - C_1\ln(T/298.15) \quad (7)$$

$$\Delta C_p = -C_1, \quad (8)$$

where K_{298} is the equilibrium constant at 298.15 K. A value of 41.19 J · K^{-1} · mol^{-1} is obtained for ΔC_p. Values of log K obtained from Eq. 6 are compared with those determined experimentally in Fig. 4. The agreement is excellent. Because C_1 is small compared with C_0, the last term on the right hand side of Eq. 6 is small and log K values show a fairly linear variation with the inverse of temperature. Values of ΔS calculated from Eq. 7 are shown in Table 2. In most cases, these values are in good agreement with those reported previously.

Oscarson et al. (7) derived equations for the log K, ΔH, and ΔS values for the protonation of three alkanolamines (Fig. 1): diglycolamine (DGA), diethanolamine (DEA), and methyldiethanolamine (MDEA) as a function of temperature. These equations incorporated the log K values determined using potentiometric data measured at 292.4 K and the ΔH values determined using calorimetric data measured at 299.9, 311.0, 333.2, 361.0, 388.8, and 422.1 K. They found that the ΔH values for the protonation of these amines varied linearly with temperature over the temperature range studied, allowing the thermodynamic values to be fitted using Eqs. 5–8. The fit was found to reproduce the data within experimental error at temperatures between 292.4 and 422.1 K. Plots of log K, ΔH, and ΔS for the protonation of glycine, DGA, DEA, and MDEA as calculated using Eqs. 5–7 are shown in Fig. 5. The trends of the log K

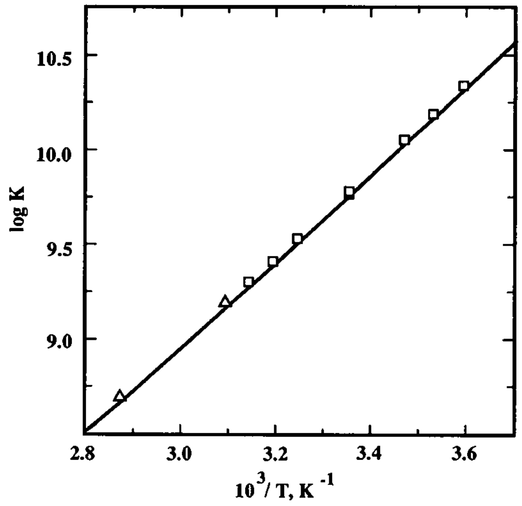

FIGURE 4 Plot of log K for glycine protonation as a function of temperature. The triangles are the results obtained in this study; all of the other points are taken from references listed in Table 2. The solid line is based on Eq. 6.

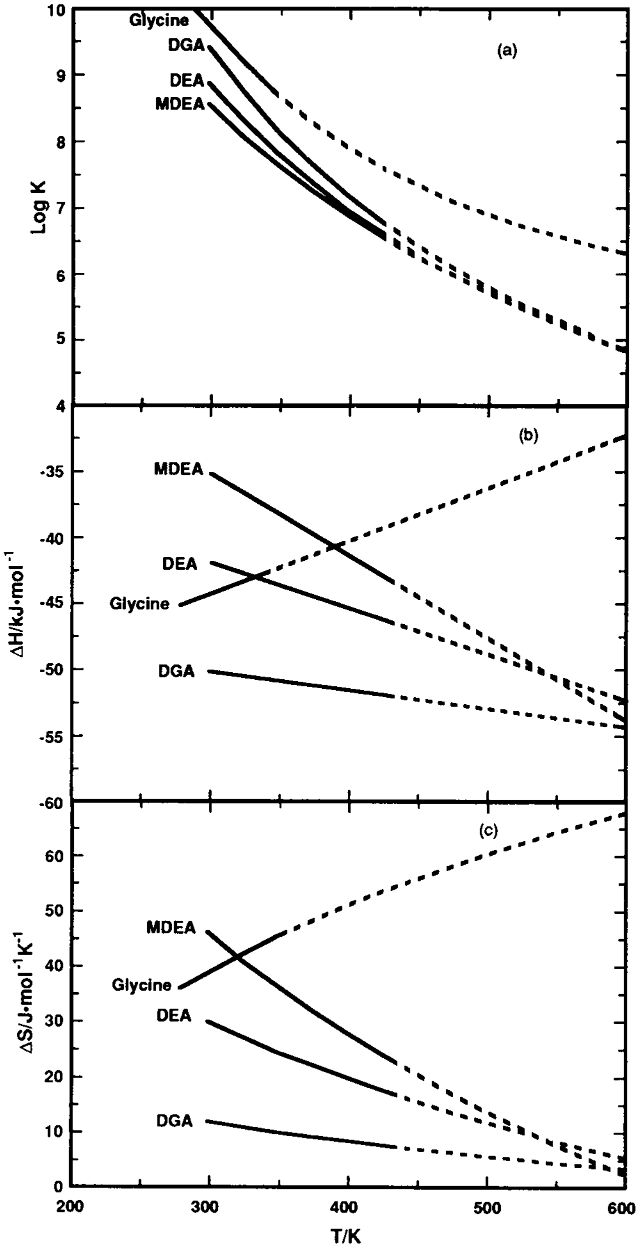

FIGURE 5 Plot of (a) log K, (b) ΔH, and (c) ΔS as a function of temperature for glycine, methyldiethanolamine ($MDEA$), diethanolamine (DEA), and diglycolamine (DGA). Solid lines are based on linear regression calculations using Eq. 5. Dashed lines represent extrapolated values.

values for protonation of glycine and the alkanolamines as a function of temperature are similar. However, the extrapolation with temperature for the log K values for the protonation of glycine should be used with caution since the measured ΔH values are available over a limited temperature range. The ΔH and ΔS values for the protonation of glycine increase with temperature and diverge from the corresponding ΔH and ΔS values for the protonation of the alkanolamines.

The behavior of these amines can be explained qualitatively in terms of solvent–solute interactions. The reaction involves water molecules and can be represented by reaction 9;

$$A(\mathrm{H_2O})_m^{c-} + \mathrm{H(H_2O)}_n{}^{+} = A\mathrm{H(H_2O)}_l{}^{(l-\mathrm{c})+} + (m + n - l)\mathrm{H_2O}, \quad (9)$$

where A is the amine and c is 1 for glycine and 0 for the alkanolamines. The waters of hydration include the water molecules in an inner sphere of influence, which are directly attached to or are close to the solute molecule or ion, and those in an outer sphere of influence, which are some distance away from the solute species but are still influenced by its presence. As the temperature increases, the extent of hydrogen bonding decreases, causing the dielectric constant of water to decrease and the long-range solvation effects become more important relative to short-range effects. The long-range effects are virtually independent of the shape or charge distribution on the solute species and are dependent on the net charge. Hence, the glycine zwitterion appears as a neutral species to water molecules far away from it and the protonated alkanolamines appear as a hydrogen ion. As the temperature increases, the long-range effects become dominant and the protonation of glycine behaves more like the formation of a neutral ion pair. The result is that ΔH and ΔS increase. The reasons for the increase of these quantities when two ions combine to form an ion pair with a net reduced charge have been given elsewhere (38,39). As the extent of hydrogen bonding decreases with increasing temperature, the water molecules in the bulk state have higher enthalpies and entropies. Water molecules released as complexation occurs enter this disordered state and absorb energy from the system to attain the same energy level as that possessed by the bulk water molecules. This effect results in progressively larger positive changes in ΔH and ΔS as temperature increases. It is significant that this effect is found at the relatively low temperatures of this study when the complex formed is a zwitterion.

As the temperature increases, protonated alkanolamines behave as would be expected for a species with a unit positive charge. The ΔH and ΔS values decrease toward a common value with increasing temperature similar to that expected for a gas phase reaction. Among the alkanolamines, the protonated primary alkanolamine, DGA, is structurally most like the hydrogen ion, whereas the protonated tertiary amine, MDEA, is structurally least like the hydrogen ion, so one would expect the short-range effects on the solvent of the protonated DGA to be most similar to the short-range effects of hydrogen ion and the short-range effects on the solvent of the protonated MDEA to be least like the short-range effects of the hydrogen ion. These differences are steric in nature. Thus, at low temperatures, where the short-range effects dominate, the ΔH and the ΔS values for protonation of the alkanol amines are quite different. At high temperatures, where long-range effects dominate, ΔH and ΔS for the three alkanolamines approach a common value.

These results lead to the following prediction. If the behavior of a zwitterion does become like that of a neutral species as temperature increases, the ΔH and ΔS values are expected to increase in an accelerated fashion. Furthermore, log K would be expected to reach a minimum and then increase with temperature. This minimum will be reached when ΔH reaches zero. Thus, the presence of a charged group in the vicinity of the protonation site may result in a higher-than-expected log K value as temperature increases.

These data show that much more information can be obtained about a system when ΔH and ΔS values are available than from log K alone. The log K data in Fig. 4 indicate that glycine behaves like the alkanolamines. However, the ΔH and ΔS values coupled with experience with other high-temperature systems provide a much more complete understanding of proton–ligand interaction at elevated temperatures. It is expected that thermodynamic data as a function of temperature for other systems of biochemical importance will be useful in elucidating the solute–solvent behavior of such systems.

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