© 1982 The Chemical Society of Japan Bull. Chem. Soc. Jpn., 55, 3064—3067 (1982) [Vol. 55, No. 10

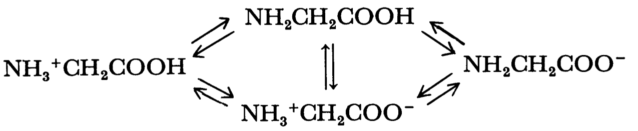
On the Ratio of Zwitterion Form to Uncharged Form of Glycine at Equilibrium in Various Aqueous Media

Goro WADA,* Eiko TAMURA, Mitsuko OKINA, and Mariko NAKAMURA
Department of Chemistry, Nara Women's University, Nara 630

(Received November 19, 1981)

The ratios of the zwitterion form to the uncharged form of glycine, $K_D = [NH_3^+CH_2COO^-]/[NH_2CH_2COOH]$, were determined in mixtures of water with methanol, ethanol, *t*-butyl alcohol, dimethyl sulfoxide, acetonitrile, 1,4-dioxane, *N,N*-dimethylformamide, and tetrahydrofuran. The value of ln K_D decreases and, therefore, ΔG°_{298} increases, with the increase in the mole fractions of the organic solvents. The plots of ΔG° *vs.* reciprocal of the dielectric constants of the alcoholic mixtures are simply linear indifferent to the kind of alcohols, indicating that this relation obeys the Scatchard equation and that the free energy change of the process of the intramolecular protonation in glycine is controlled mainly by electrostatic interaction, especially by the entropy change associated with the preferential solvation around the charged amino and carboxyl groups.

In aqueous solution, glycine (aminoacetic acid) exists in various ionized or neutral forms depending upon the hydrogen-ion concentrations of the solution, as shown in the following scheme.[1)]



The formulas $NH_3^+CH_2COOH$ and $NH_2CH_2COO^-$ are cationic and anionic forms respectively, whereas NH_2CH_2COOH and $NH_3^+CH_2COO^-$ are both neutral, corresponding to the uncharged and zwitterion forms respectively, and establish a chemical equilibrium between themselves with a constant K_D:

$$K_D = \frac{[NH_3^+CH_2COO^-]}{[NH_2CH_2COOH]}. \tag{1}$$

These neutral forms are also at equilibria with other species with equilibrium constants, defined as follows.

$$K_1 = \frac{[NH_2CH_2COOH][H^+]}{[NH_3^+CH_2COOH]} \tag{2}$$

$$K_2 = \frac{[NH_3^+CH_2COO^-][H^+]}{[NH_3^+CH_2COOH]} \tag{3}$$

$$K_{12} = \frac{[NH_2CH_2COO^-][H^+]}{[NH_2CH_2COOH]} \tag{4}$$

$$K_{21} = \frac{[NH_2CH_2COO^-][H^+]}{[NH_3^+CH_2COO^-]} \tag{5}$$

According to the experimental method due to titration, the uncharged and the zwitterion forms can not be discriminated from each other, but only the sum of the concentrations of both species are determined. Thus, the apparent acidity constant of the acid form $NH_3^+CH_2COOH$ found experimentally, K, has a meaning as expressed by

$$K = \frac{([NH_2CH_2COOH] + [NH_3^+CH_2COO^-])[H^+]}{[NH_3^+CH_2COOH]}. \tag{6}$$

Then, the following relationships are derived from Eqs. 1, 2, 3, and 6.

$$K = K_1 + K_2 \tag{7}$$

$$K_D = \frac{K_2}{K_1} \tag{8}$$

Since $[NH_3^+CH_2COO^-]$ is much larger than $[NH_2CH_2COOH]$ in aqueous solution as will be seen later, K_2 is approximately equal to K, and therefore K_D can be considered to be equal to the ratio of K to K_1.

$$K \fallingdotseq K_2 \tag{7'}$$

$$K_D \fallingdotseq \frac{K}{K_1} \tag{8'}$$

For the determination of K_1, the ethyl ester of glycine, $NH_2CH_2COOC_2H_5$, was used in place of glycine, in order to mask the acidity of the carboxyl group, on the basis of an assumption that the basicity of amino group in glycine would not be greatly affected by the esterization of the carboxyl group, because the basicities of the methyl ester and ethyl ester of glycine have been known to be approximately identical.[2—4)] Therefore,

$$K_1 = \frac{[NH_2CH_2COOC_2H_5][H^+]}{[NH_3^+CH_2COOC_2H_5]}. \tag{2'}$$

In the present investigation, the values of K_D were determined in various aqueous solutions mixed with alcohols, such as methanol, ethanol, and *t*-butyl alcohol, and with other organic substances, such as acetonitrile, 1,4-dioxane, *N,N*-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), and tetrahydrofuran (THF), at various mole fractions and various temperatures. The K_D values in water can be well compared with the data appearing in the literature.[4)] Thus, the thermodynamic functions and the dielectric dependences of the equilibrium, the uncharged form⇄the zwitterion form, will be discussed.

Experimental

Materials. *Glycine*: Commercially obtained glycine of guaranteed reagent grade from Wako Pure Chemical Industries, Ltd. was recrystallized from water.

Glycine Ethyl Ester Hydrochloride: It was prepared by leading hydrogen chloride gas into the glycine suspended in ethanol. The crystals of the ester hydrochloride salt were purified by recrystallization from ethanol and its purity was checked by paper-chromatography.

Various Alcohols and Other Solvents: They were purified by ordinary methods.

Procedure and Calculations. Solutions of glycine which had been added by equimolar amount of hydrochloric acid or solutions of glycine ester hydrochloride were titrated with sodium hydroxide and the pH values of the solutions were

TABLE 1. VALUES OF K_1, K_2, AND K_D AT 25 °C IN MIXTURES OF WATER WITH ORGANIC SOLVENTS AT VARIOUS MOLE FRACTIONS x

Organic solvent	x	$\frac{K_1}{\text{M}}$	$\frac{K_2}{\text{M}}$	K_D
None	—	1.65×10^{-8}	3.90×10^{-3}	2.36×10^{5}
Methanol	0.051	1.86×10^{-8}	2.99×10^{-3}	1.61×10^{5}
	0.100	2.14×10^{-8}	3.28×10^{-3}	1.53×10^{5}
	0.199	2.57×10^{-8}	1.79×10^{-3}	6.96×10^{4}
	0.300	3.09×10^{-8}	1.19×10^{-3}	3.84×10^{4}
	0.400	4.17×10^{-8}	6.07×10^{-4}	1.45×10^{4}
	0.496	2.63×10^{-8}	9.95×10^{-5}	3.78×10^{3}
	0.601	3.31×10^{-8}	1.07×10^{-4}	3.23×10^{3}
	0.705	4.00×10^{-8}	3.93×10^{-5}	9.83×10^{2}
Ethanol	0.050	2.54×10^{-8}	2.06×10^{-3}	8.11×10^{4}
	0.098	2.62×10^{-8}	1.65×10^{-3}	6.30×10^{4}
	0.202	3.44×10^{-8}	1.05×10^{-3}	3.05×10^{4}
	0.299	4.32×10^{-8}	5.52×10^{-4}	1.28×10^{4}
	0.397	5.43×10^{-8}	1.63×10^{-4}	3.00×10^{3}
t-Butyl alcohol	0.050	2.97×10^{-8}	2.62×10^{-3}	8.83×10^{4}
	0.100	4.59×10^{-8}	2.29×10^{-3}	5.00×10^{4}
	0.300	9.07×10^{-8}	7.95×10^{-4}	8.77×10^{3}
DMSO	0.050	1.37×10^{-8}	1.51×10^{-3}	1.10×10^{5}
	0.100	1.14×10^{-8}	6.57×10^{-4}	5.76×10^{4}
Acetonitrile	0.300	5.49×10^{-8}	1.25×10^{-3}	2.28×10^{4}
1,4-Dioxane	0.300	7.01×10^{-8}	2.41×10^{-4}	3.44×10^{3}
DMF	0.300	1.53×10^{-8}	8.50×10^{-6}	5.56×10^{2}
THF	0.100	2.72×10^{-8}	1.05×10^{-3}	3.86×10^{4}

measured simultaneously by a pH-meter in a thermostat at given temperature. The hydrolysis of the ester was not observed appreciably during the course of titration. For the calculations of K and K_1, sets of pH values *vs.* volumes of sodium hydroxide solution at nearly middle points of the neutralization titration were used and averaged.

Results and Discussion

Equilibrium Constants and Related Thermodynamic Quantities. The values of K_1, K_2, and K_D at 25°C in mixtures of water with methanol, ethanol, *t*-butyl alcohol, DMSO, acetonitrile, 1,4-dioxane, DMF, and THF at various mole fractions are listed in Table 1. In the table, it is clearly seen that the zwitterion form is far more predominant than the uncharged one approximately by the order of magnitude 10^5 in water, but its ratio decreases with the increase in mole fraction of organic substances, especially in the case of DMF at $x=0.3$, the ratio being the smallest with the order of magnitude 10^2 among the data ever observed. Another feature is that K_D always decreases with temperature at any x in the all media, although the observed temperature dependences are not shown in Table 1.

In Fig. 1 are shown the variations of the free energies associated with the transformation of the uncharged form to the zwitterion form at various mole fractions of the organic solvents. The standard free energy change is calculated by the equation,

$$\Delta G^\circ = -RT \ln K_D. \tag{9}$$

The value of ΔG°_{298} in pure water is equal to -7.27 kcal mol^{-1} (1 cal=4.2 J), which agrees well with -7.3 kcal mol^{-1} obtained by Edsall and Blanchard.[4] Figure 1 indicates that ΔG°_{298} grows up gradually with the increase of x of alcohols, and is in a sequence at $x=0.3$, DMF> 1,4-dioxane> *t*-butyl alcohol> ethanol> acetonitrile>methanol.

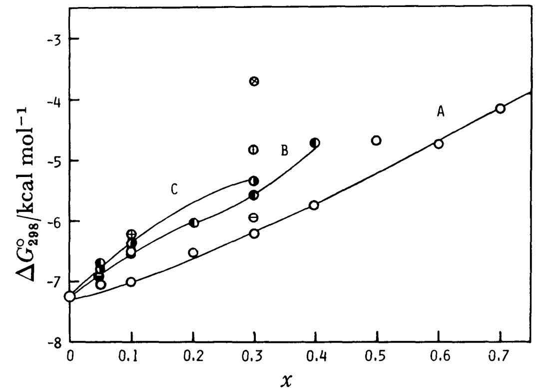


Fig. 1. The free energy $\Delta G_{\circ}^{298}$ for the change of the uncharged form to the zwitterion form at 25 °C in mixtures of water and organic solvents at mole fractions x.

○: Methanol (A), ◐: ethanol (B), ◑: *t*-butyl alcohol (C), ◒: DMSO, ⊖: acetonitrile, ⦶: 1,4-dioxane, ⊗: DMF, ⊕: THF.

Influence of Dielectric Constants of Media upon ΔG°. When ΔG°'s in various aqueous alcohols at diverse temperatures are plotted against reciprocals of the dielectric constants of the media, they come altogether on a single straight line with a positive slope indifferent to the kind of the alcohols, except for the case of *t*-butyl alcohol at $x=0.3$, as are drawn in Fig. 2. (Points for

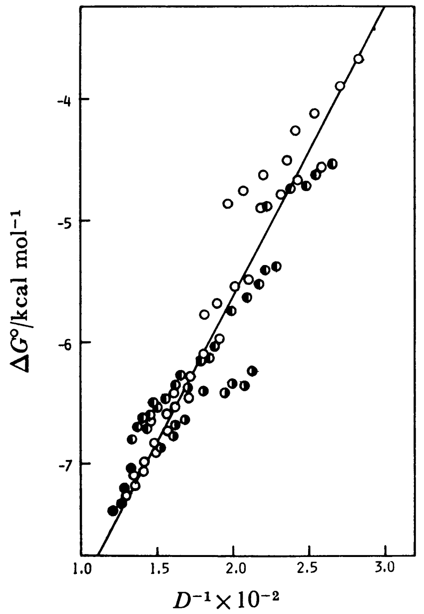


Fig. 2. Dependence of $\Delta G°$ in aqueous alcohols at various temperatures upon reciprocal of dielectric constants D^{-1}. ●: Water, ○: methanol, ◐: ethanol, ◑: *t*-butyl alcohol.

$x=0.3$ of *t*-butyl alcohol are omitted in the figure, which should appear towards the right out of the figure.) The dielectric constants of the media were calculated by numerical interpolations from the data observed by Åkerlöf.[5]

This linear relationship could be applied to the Scatchard equation,[6,7]

$$\Delta G° = \Delta G°_0 - \frac{z_A z_B e^2 N_A}{rD}, \qquad (10)$$

where $\Delta G°$ originally implies a free energy change related to association of two ions, A and B, bearing electric charges $z_A e$ and $z_B e$ respectively at a distance r in media of various dielectric constants D and $\Delta G°_0$ and N_A are a constant and the Avogadro's number, respectively. In the present case, the change is not an approach between two separate ions, but an intramolecular prototropy resulting in occurrence of a zwitterion within an uncharged molecule. The zwitterion can be regarded as two ions which are combined with each other by a carbon chain behind. If the Scatchard's relationship could be valid in the zwitterion formation, the value of r may be estimated to be approximately 2 Å from the slope of the straight line in Fig. 2, assuming that the macroscopic value of D is roughly available in the very close vicinity around the polar groups. Since the value of $r=2$ Å looks like to be shorter than the expected value about 3.3 Å as the probable closest distance between N- and O-atoms ignoring the electric charges on respective atoms, it may be safely said that the positive electricity on the charged group $-NH_3^+$ is partly scattered on the three H-atoms and the centers of the electricities on both $-NH_3^+$ and $\text{-C}{\lessgtr}^{\text{O}}_{\text{O}}\,(-)$ are forced to come closer to each other than the initial distance, although r may not be strictly equal to 2 Å, on account of certain theoretical inaccuracies involved in Eq. 10. Recently, these two functional groups were found to interact with each other at a fairly short distance in both gas phase[8] and solutions, from theoretical[9] and dilatometric[10] investigations.

In the case of *t*-butyl alcohol, the plot of $\Delta G°$ *vs.* D^{-1} is supposed to deviate gradually from the common linearity in Fig. 2 as the mole fraction becomes larger than 0.1. Thus, the deviation found at $x=0.3$ might be only recognized by a consideration that the discrepancy between D values at a local spot around the ion and in the bulk of media can be no longer ignored and that the hydrophobicity of *t*-butyl group in the vicinity of the ion alters the structures not only of the zwitterion itself but also of the solvation sheaths surrounding the zwitterion strongly, in addition to the dielectric effect of the medium.

TABLE 2. THERMODYNAMIC QUANTITIES FOR THE CHEMICAL CHANGE FROM THE UNCHARGED FORM TO THE ZWITTERION FORM, $\Delta G°_{298}$, $\Delta H°$, AND $\Delta S°$, IN AQUEOUS MIXTURES WITH VARIOUS ORGANIC SOLVENTS AT $x=0.3$
1 cal=4.2 J.

Organic solvent	$\Delta G°_{298}$ kcal mol^{-1}	$\Delta H°$ kcal mol^{-1}	$\Delta S°$ cal K^{-1} mol^{-1}
None(water)	−7.27	−10.3	−10.2
Methanol	−6.27	−10.7	−15.0
Ethanol	−5.60	−10.1	−15.0
t-Butyl alcohol	−5.38	−9.1	−11.5
Acetonitrile	−5.94	−9.1	−10.3
1,4-Dioxane	−4.82	−9.6	−15.8
DMF	−3.74	−11.7	−26.5

Thermodynamic Quantities, $\Delta G°_{298}$, $\Delta H°$, and $\Delta S°$ in Mixed Media. Table 2 shows the thermodynamic quantities, $\Delta G°_{298}$, $\Delta H°$, and $\Delta S°$, for the transformation of glycine from the uncharged form to the zwitterion form in pure water and in various mixed media at $x=0.3$. Values of $\Delta H°$ and $\Delta S°$ were obtained from the linear relationship of $\ln K_D$ *vs.* reciprocal of the absolute temperature. On general survey of the table, a trend is seen that the increase in $\Delta G°_{298}$ due to the addition of organic solvents is largely attributed to the decrease in $\Delta S°$, while $\Delta H°$ remains almost unchanged; namely this transformation is rather entropy-controlled.

This fact may indicate that the negative value of $\Delta H°$ is mainly caused by the change in the energy of chemical bondings related to protonation and deprotonation on amino and carboxyl groups respectively, while, on the other hand, the negative value of $\Delta S°$ depends upon the extent of solvation around the charged groups, the circumstances of the preferential solvation being different from solvent to solvent. The fact that $\Delta S°$ is more negative in the mixed solvents than in pure water, may suggest the possibility of the preferential solvation in the mixed solvents which is accompanied by a larger increment in the order than the simple hydration in pure water, in the vicinity of the zwitterions. After all, the gradual depression of K_D as x is increased may be mainly attributed to the growth of the degree of the preferential solvation in the mixed media with alcohols, as shown in Fig. 1.

When the ionization constant K_a of various aliphatic carboxylic acids R–COOH or protonated amines $R'NH_3^+$ is empirically given by

$$-\log K_a = A_1T^{-1} - A_2 + A_3T, \tag{11}$$

where A_1, A_2, and A_3 are constants characteristic of the substance, $\Delta H°$ and $\Delta S°$ are expressed by the following equations.[11]

$$\Delta H° = 2.303\,R(A_1 - A_3T^2) \tag{12}$$

$$\Delta S° = 2.303\,R(A_2 - 2A_3T) \tag{13}$$

The transformation of glycine from the uncharged to zwitterion form consists of two processes; the protonation of amino group and the deprotonation of carboxyl group. If these two processes occur on separately different molecules, the total change is roughly expected as the sum of the following respective changes.

$$R'\text{-}NH_2 + H^+ \longrightarrow R'\text{-}NH_3^+ \tag{14}$$

$$R\text{-}COOH \longrightarrow R\text{-}COO^- + H^+ \tag{15}$$

$$R'\text{-}NH_2 + R\text{-}COOH \longrightarrow R'\text{-}NH_3^+ + R\text{-}COO^- \tag{16}$$

By using the values of A_1, A_2, and A_3,[11] the enthalpies and entropies at 25°C for several alkylamines (ammonium type) and aliphatic carboxylic acids can be calculated, and thus the final $\Delta H°$ and $\Delta S°$ for the Reaction 16 are readily estimated to be approximately -12 ± 2 kcal mol^{-1} and -15 ± 3 cal K^{-1} mol^{-1} respectively. These values are very close to those listed in Table 2, indicating that the thermodynamic quantities associated with a proton transfer in solution is almost unaffected whether it occurs intramolecularly or intermolecularly, although amino and carboxyl groups are located fairly close with each other in α-amino acids, like glycine. The value of $\Delta G°$ at $D=1$ (in gas phase) is assumed to be very large from the extrapolation in Eq. 10. This suggests that the value of K_D may be actually equal to zero in vacuum. In fact, it has been studied energetically and geometrically that the zwitterion is not expected to exist in gas-phase during at least an observable life time.[8,12]

Appendix

Examination of K_1 by Hammett-Taft Rule. For the determination of K_1, we have measured the basicity of ethyl ester of glycine in place of glycine, for the purpose of masking the acidic ionization of carboxyl group in glycine. Although the basicity of the ethyl ester has been found not to differ greatly from that of the methyl ester,[2–4] it is necessary to examine how much it differs from that of glycine itself. According to the Hammett–Taft rule for the substitution effect on pK_a, the pK_a of primary alkyl amines can be estimated by the following expression.[13]

$$pK_a = 13.23 - \Sigma\sigma^* \tag{17}$$

Since $\sigma^*(H)$, $\sigma^*(C_2H_5)$, and $\sigma^*(CH_2COOH)$ are $+0.49$, -0.10, and $+1.05$ respectively, $\sigma^*(CH_2COOC_2H_5)$ is supposed to be $+0.98$, taking into account that σ^* is reduced approximately half per every bond as the substitution group goes apart from N-atom. Therefore, the difference of log K_1, $pK_a(NH_2CH_2COOC_2H_5) - pK_a(NH_2CH_2COOH)$ will be 0.22, which could be considered to have so small an effect upon the order of magnitude in K_D. Furthermore, the values of σ^* should be altered in mixed solvents, which are unknown at the present stage, in order to correct the observed data.

References

1) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," John Wiley & Sons, New York (1961), Vol. 1, Chap. 4, p. 445.

2) R. Wegscheider, *Monatsh.*, **16**, 153 (1895); **23**, 287 (1902).

3) L. Ebert, *Z. Phys. Chem.*, **121**, 385 (1926).

4) J. T. Edsall and M. H. Blanchard, *J. Am. Chem. Soc.*, **55**, 2337 (1933).

5) G. Åkerlöf, *J. Am. Chem. Soc.*, **54**, 4125 (1932).

6) G. Scatchard, *Chem. Rev.*, **10**, 229 (1932).

7) E. S. Amis, "Solvent Effects on Reaction Rates and Mechanisms," Academic Press, New York (1966), Chap. 1.

8) M. J. Locke, R. L. Hunter, and R. T. McIver, Jr., *J. Am. Chem. Soc.*, **101**, 272 (1979).

9) P. Palla, C. Petrongolo, and J. Tomasi, *J. Phys. Chem.*, **84**, 435 (1980).

10) L. Lepori and V. Mollica, *Z. Phys. Chem. Neue Folge*, **123**, 51 (1980).

11) R. A. Robinson and R. H. Stokes, "Electrolyte Solutions," Butterworth, London (1955), p. 496.

12) Y.-C. Tse, M. D. Newton, S. Vishveshwara, and J. A. Pople, *J. Am. Chem. Soc.*, **100**, 4329 (1978).

13) R. W. Taft, Jr., "Steric Effects in Organic Chemistry," ed by M. S. Newman, John Wiley & Sons, New York (1956), pp. 595 and 619.