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Medium Effect on the Second-Stage Dissociation Constant of *N*,*N*-Bis(2-hydroxyethyl)glycine

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The second-stage dissociation constants of *N*,*N*-bis(2-hydroxyethyl)glycine (bicine) were determined at 25.00 ± 0.02 °C by potentiometric pH titration in pure water and different mixed solvent mixtures (methanol, ethanol, *N*,*N*-dimethylformamide, dimethyl sulfoxide, acetone, and dioxane). It is observed that the pK_{a_2} value is slightly influenced as the solvent is enriched in methanol or ethanol and remains practically constant in the presence of different amounts of DMF and DMSO. A pronounced change in the pK_{a_2} value is observed as the solvent is enriched in acetone or dioxane. These results are discussed in terms of various solvent characteristics. It is concluded that the electrostatic effect has only a relatively small influence on the dissociation equilibrium of the imino group ($-NH^+$). Other solvent effects such as solvent basicity and different stabilization of the conjugate acid free base by hydrogen-bonding interactions in aquo–organic solvent media relative to pure aqueous media as well as proton–solvent interaction play an important role in the acid dissociation equilibrium.

Introduction

The choice of alternative buffers has increased with the commercial availability of zwitterionic amino acids, mainly N-substituted taurines or N-substituted glycines prepared by Good and co-workers (*1*), so that organic buffers suitable for use in biochemistry now include *N*,*N*-bis(2-hydroxyethyl)glycine (bicine). Zwitterionic buffers—comparable to amino acids—show significant advantages over conventional buffers: insignificant penetration through biological membranes, maximum buffer capacity at the physiological pH range 6.0–8.50, and no enzyme substrate or enzyme inhibitor properties. Though studies on the dissociation constants of acids and bases in mixed and nonaqueous solvents have been extensively investigated, relatively little work has been done to determine the dissociation constants of the biologically important zwitterionic buffers (*2–7*).

Bicine is an organic acid of the class of Good buffers (*1*). It is a potentially useful zwitterionic buffer for use in biochemistry at the physiological pH range (6.0–8.50), because of its low toxicity. In the present investigation the second-stage dissociation constant of bicine has been reported in aqueous systems, including both water and various water + organic mixtures as solvent using the technique of potentiometric pH titration. The organic solvents used are methanol, ethanol, DMF, DMSO, dioxane, and acetone. Methanol and ethanol were chosen as representatives of amphiprotic solvents. We have chosen DMF and DMSO as aprotic polar solvents because their dielectric constants are relatively high, but lower than that of water. Acetone and dioxane have been chosen as a low basic aprotic solvent and an aprotic nonionizing solvent, respectively. All the solvents chosen are of frequent use in biochemical and biological studies.

Experimental Section

Chemicals and Solutions. $(HOCH_2CH_2)_2NCH_2COOH$ (bicine) was analytical grade (BDH) with purity 98%. Further purification of bicine was done by recrystallization from 80% methanol (*8*). This method improved the purity of the commercially available bicine. The organic solvents methanol, ethanol, *N*,*N*-dimethylformamide, dimethyl sulfoxide, acetone, and dioxane were of high purity (A.R. or manufactures spectro grade products). Carbonate-free KOH was prepared and was standardized by titration with standard potassium hydrogen phthalate. The molarity of HNO_3 was determined by titration with standard KOH solution. Generally, dilute solutions were prepared by appropriate dilution of the stock. The accuracy of all solution concentrations in various water + organic mixtures was checked by mass.

Procedure. pH potentiometric measurements were made on solutions in a double-walled glass vessel at 25.00 °C with a commercial Fisher combined electrode. The pH was measured with a Fisher Accumet pH/ion meter, model 750. The instrument was standardized against standard buffers of 4.0 and 9.20 pH values. The instrument was rechecked after each experiment. The temperature was controlled by circulation of water through the jacket, from a VEB Model E3E ultrathermostat bath and maintained within ±0.02 °C. Purified nitrogen was bubbled through the solution in order to maintain an inert atmosphere. Efficient stirring of the solution was achieved with a magnetic stirrer. All test solutions were prepared in 0.1 M KNO_3 by mixing the appropriate amounts of ligand, nitric acid, potassium nitrate, and the proportion of the different organic solvents studied. The concentration of hydrogen ion was decreased by the addition of potassium hydroxide, prepared in the same medium used for the test solution. At each solvent percentage, at least 6 titrations (35 data points) for each were performed.

The concentration of free hydrogen ion, *h*, at each point of the titration was calculated from the measured electromotive force (emf), *E*, of the cell RE/TS/GE (RE and GE denote the reference and glass electrodes, respectively, and TS is the test solution) from the Nernst equation:

$$E = E^\circ + Q \log h \qquad (1)$$

where E° is a constant which includes the standard potential of the glass electrode and Q is the slope of the glass electrode response. It is to be assumed that the activity coefficient is constant, an assumption usually justified by performing the experiments with a medium of high ionic strength (0.1 M KNO_3). The value of E° for the electrode was determined from a separate titration of nitric acid with potassium hydroxide, both of known concentration, under the same temperature and medium conditions as for the test solution titration. The data obtained were analyzed by the program MAGEC (*9*). During the MAGEC calculation the autopro-

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tolysis constant of water, K_w, was refined until the best value for Q was obtained. This refined K_w value is necessary as an adjustable parameter in a pK_{a_2} calculation in pure water. The determination of $E°$ and Q is only to determine K_w, and all the rest of the work is by pH titration with the different electrodes standardized in aqueous buffers. Values for K_w for water in water + organic solvent systems have been taken from the literature (*10–13*).

The pH meter readings have been corrected in accordance with the method described by Douheret (*14, 15*). Thus, if the pH meter is standardized using an aqueous buffer, the meter reading pH(*R*) obtained in a partially aqueous medium differs by an amount δ from the corrected reading pH* which is reffered to the standard state in the partially aqueous solvent, pH* = pH(*R*) − δ.

Initial estimates of pK_{a_2} values were refined with the ESAB2M computer program (*16*) by minimizing the error squares sum

$$U_v = \sum\nolimits_i w_i (v_i - V_{\mathrm{calc},i})^2 \qquad (2)$$

The weight is calculated by

$$1/w_i = S_i^2 = S_v^2 + (\delta v_i/\delta E_i)^2 S_E^2 \qquad (3)$$

We chose the minimization function (*2*), since the titrant volume, V_{calc}, can be calculated from an explicit equation (*17, 18*). The program ESAB2M minimizes eq 2 by using the Gauss–Newton nonlinear least-squares method (*19*) with the very efficient Levenberg–Marquardt algorithm (*20, 21*). In our potentiometric pH titrations we put $E° = 0$ ($E°$ = formal potential of the electrodic couple) in the input instructions, since the program reads in pH in this case. Our calculations have been performed with a Gaussian error in $V{:}S_V = 0.005$.

Results and Discussion

Bicine possesses the following zwitterionic structures:

$$(\mathrm{HOCH_2CH_2})_2\mathrm{NCH_2COOH} \rightleftharpoons (\mathrm{HOCH_2CH_2})_2{}^{+}\,\mathrm{NHCH_2COO^-}$$

The second-stage dissociation constant of bicine is due to the cationic group $\mathrm{NH^+}$. The refined pK_{a_2} values of bicine in the different aquo–organic solvent mixtures are given in Table 1. The value obtained in the present work for pK_{a_2} = 8.27 in pure water agrees with the literature data (*1*). The results reveal three important features: (a) With an increase in the amphiprotic methanol or ethanol solvent concentration in the aqueous medium, the change in pK_{a_2} is quite small; in most cases it is slightly decreased. (b) With an increase in the DMF or DMSO content, the pK_{a_2} remains practically constant. (c) By increasing the acetone or dioxane (nonpolar solvents) concentration in the aqueous medium, the pK_{a_2} value increases.

Table 1. Refined pK_{a_2}*[a] Values of Bicine at Different Mass Fractions *w* for *w* Organic Solvent + (1 − *w*) Water Mixtures at 25.00 °C

organic solvent	w	pK_{a_2}	pK_{a_2}*
methanol	0.00	8.27	8.27 ± 0.02
	0.10	8.02	8.02 ± 0.05
	0.20	8.08	8.07 ± 0.03
	0.30	8.08	8.04 ± 0.05
	0.40	8.05	7.96 ± 0.04
	0.50	8.10	7.98 ± 0.03
ethanol	0.00	8.27	8.27 ± 0.02
	0.10	8.05	8.04 ± 0.06
	0.20	8.02	7.98 ± 0.50
	0.30	8.19	8.11 ± 0.05
	0.40	8.22	8.09 ± 0.05
	0.50	8.22	8.01 ± 0.05
DMF	0.00	8.27	8.27 ± 0.02
	0.10	8.40	8.34 ± 0.06
	0.20	8.47	8.34 ± 0.06
	0.30	8.51	8.28 ± 0.07
	0.40	8.67	8.34 ± 0.05
	0.50	8.82	8.37 ± 0.04
DMSO	0.00	8.27	8.27 ± 0.02
	0.10	8.27	8.24 ± 0.06
	0.20	8.36	8.28 ± 0.05
	0.30	8.44	8.28 ± 0.05
	0.40	8.67	8.39 ± 0.05
	0.50	8.73	8.31 ± 0.04
	0.55	8.84	8.33 ± 0.05
acetone	0.00	8.27	8.27 ± 0.02
	0.10	8.38	8.40 ± 0.06
	0.20	8.59	8.63 ± 0.05
	0.30	8.62	8.67 ± 0.06
	0.40	8.68	8.75 ± 0.06
	0.50	8.83	8.94 ± 0.07
dioxane	0.00	8.27	8.27 ± 0.02
	0.10	8.43	8.45 ± 0.07
	0.20	8.44	8.48 ± 0.07
	0.30	8.57	8.65 ± 0.06
	0.40	8.62	8.74 ± 0.06
	0.50	8.93	9.10 ± 0.05
	0.55	9.06	9.26 ± 0.06

[a] pK_{a_2}* = corrected pK_{a_2} values according to Douheret (*14, 15*) ± uncertainties referring to statistically determined uncertainties at small 95% confidence intervals.

Effect of the Amphiprotic Solvent. The proton activity a_{H}, as a measure of acidity of an acid HA at molality *m* in a mixed amphiprotic solvent (SH) can be expressed (*10*) in terms of K_{HA} and K_{SH}, which are, respectively, the acidity constants of HA and $\mathrm{SH_2^+}$:

$$a_{\mathrm{H}} = (mK_{\mathrm{HA}}K_{\mathrm{SH}}(\nu_{\mathrm{SH_2^+}}\,\nu_{\mathrm{HA}}/\nu_{\mathrm{SH}}\,\nu_{\mathrm{A^-}}))^{1/2} \qquad (4)$$

where $\nu_{\mathrm{SH_2^+}}$, ν_{HA}, ν_{SH} and $\nu_{\mathrm{A^-}}$ are the activity coefficients of the species $\mathrm{SH_2^+}$, HA, SH, and $\mathrm{A^-}$, respectively. According to eq 4, a lowering of the dielectric constant due to addition of methanol or ethanol may have little effect on the acidity of an unbuffered solution of *N,N*-bis(2-hydroxyethyl)glycine. With respect to the solvent basicity effect, the autoprotolysis constant of water is slightly influenced by addition of methanol or ethanol (p*K* of pure water is changed from 14.0 to 14.90 for a 50% mass/mass ethanol + water mixtures). Accordingly, one can deduce that changing the medium basicity by increasing additions of methanol or ethanol to the aqueous medium has little influence on the second-stage dissociation constant of *N,N*-bis(2-hydroxyethyl)glycine. In ethanolic or methanolic solution the values of pK_{a_2}'s are only slightly changed compared with those for an aqueous medium. This can conveniently be discussed in terms of $\Delta G_{\mathrm{ionizn}}$ defined as $2.303RT(\log K^{(w)} - \log K^{(s)})$, i.e., the difference between the standard free energies of ionization in the mixed solvent and in water (*22*). The ionization process can be represented by the general equation

$$\mathrm{HA + SH \rightleftharpoons SH_2^+ + A^-}$$

where HS denotes a molecule of solvent. Since the solutes involved in the ionization are charged, then of the two basic steps in solute–solvent interactions, i.e., charge transfer and charge separation (*22*), only the first will be dominant. Therefore, although the difference in dielectric constnats between pure water and the water + ethanol or the water + methanol mixture is appreciable, it will have little effect on the pK_{a_2} values of bicine. Also the solvation in mixed ethanol + water or methanol + water as solvent should not differ much from that in water. Consequently the two effects will result in relatively small values of $\Delta G_{\mathrm{ionizn}}$.

Effect of Dipolar Aprotic Solvents (DMF and DMSO). The observed constancy in the pK_{a_2} value of bicine with organic solvent (DMF and DMSO) content may be explained in terms of possible variation in microheterogeneity of solvation shells around the solute. Bicine may be strongly and asymmetrically hydrated in water. It could be thus expected to remain hydrated up to a high content of DMF or DMSO. The apparent constancy of pK_{a_2}* values in these hydroorganic media might be due to this effect.

Effect of Acetone or Dioxane Solvents. The observed increase in pK_{a_2}* values of bicine when the concentration of the coorganic solvent acetone or dioxane in the medium is increased can be mainly attributed to the following effects.

(a) Solute–solvent interaction effect: Acetone is considered to be both a poorer acceptor and a poorer donor of hydrogen bonds compared to water (*23*). Thus, one may expect a low stabilization of the free conjugate base of bicine by hydrogen-bonding interactions in the presence of this hydroorganic medium. This leads to high pK_{a_2} values.

(b) Proton–solvent interaction effect: It can be concluded from the literature data (*24*) that also in acetone + water (and probably in dioxane + water mixtures too) the hydrogen ion is solvated by water molecules. Both these solvents have hydrogen-bond-donating and -accepting abilities distinctly lower with comparison to water. Thus, the H$^+$ ion becomes less stabilized in these hydroorganic media; this results in a high transfer activity coefficient of the proton, i.e., high pK_{a_2}* values of bicine.

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