Solubility of Amino Acids in Mixed Solvent Systems

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Abstract □ The effects of various solvents and pH on the solubility characteristics of glycine, L-alanine, L-valine, L-phenylalanine, and DL-amino octanoic acid were studied in a series of hydroalcoholic solvent systems. The solubility properties of the amino acids studied were found to be dominated by the α-amino carboxylic acid portion of the molecule but also depended to some extent on the nonpolar portion of the molecule and its interaction with each specific solvent system. These interactions gave rise to a rank order of solubility which was characteristic of the specific amino acid rather than the decreasing polarity of the solvent system. Similar solubility profiles were found throughout the concentration spectrum and were defined by using a polynomial regression analysis. As the percent of alcohol was increased in the solvent systems studied, similar increments of acid or base added to the system produced a proportionally greater increase in the magnitude of total amino acid solubility.

Keyphrases □ Amino acid solubility—hydroalcoholic solvent systems □ Hydroalcoholic solvent systems—amino acid solubility □ α-Amino carboxylic acid group domination—amino acid solubility □ Dielectric constant effect—amino acid solubility

In other work the authors studied the solubility of a series of amino acids in pure solvent systems at pH and pH′ above, at, and below the isoelectric point (1). The solubility of the amino acids studied was at a maximum in water and was inversely related to the length of the nonpolar portion of the molecule. Solubility in the series of pure alcoholic solvents was reported to be of the same order of magnitude for the amino acids studied but substantially lower than that found in water. pH variation above and below the isoelectric point was found to increase solubility as a function of acid or base added.

Since this work was originally designed to elucidate the solubility of selected amino acids as a function of pH in various solvents and to correlate this behavior to those properties seen *in vivo*, it was desirable to determine the effect of mixed solvent systems on amino acid solubility.

EXPERIMENTAL

Equipment—A rotating apparatus[1] capable of holding multiple samples was immersed in a water bath, which was kept at constant temperature by a controlled-temperature circulating pump.[2] The apparent pH (pH′) of nonaqueous systems was measured using a combination electrode.

Systems Employed—The amino acids used were glycine, L-alanine, L-valine, L-phenylalanine, and DL-amino octanoic acid. The solubility of each of the amino acids was found in the pure and mixed solvent systems at 0, 10, 30, 50, 70, 90, and 100% (v/v) of methanol–water, ethanol–water, *n*-propanol–water, isopropanol–water, and tertiary butanol–water. The label purity of the manufacturer was accepted, and no pretreatment of the chemicals was deemed necessary. The water was distilled and deionized to minimize the possibility of complex formation. Hydrochloric acid and sodium hydroxide were used to adjust pH. All work was performed at a temperature of 25 ± 0.2°.

Procedure—For each concentration of a solvent system evaluated, six samples of equal volume were prepared from a stock solution, an excess amount of the selected amino acid was added, and the sample bottles were rotated in the constant-temperature bath for 24 hr. or until equilibrium was attained. Either 1- or 5-ml. samples were withdrawn from the sample bottles, using a pipet with a pledget of glass wool as a filtering medium. The size of the sample to be withdrawn was predetermined to minimize error during gravimetric analysis. The sample was immediately transferred to a tared vial, weighed to determine density, placed in an oven, and dried to constant weight. All drying was done at a temperature of 95° or less to avoid decomposition of the amino acids (2). Immediately after the sampling process, the pH or pH′ of each solution was taken. Incremental amounts of acid were added to half of the samples in successive 24-hr. periods, the pH was taken, and a gravimetric analysis was

Table I—Polynomial Regression Data Generated from the Mola Solubility of Glycine as a Function of the Percent Strength of the Solvent System

Solvent System[a]	*Y*-Intercept[b]	Coefficients	
		X	X^2
Methanol	2.6520	−0.0743	0.0005
Ethanol	2.7823	−0.0738	0.0005
n-Propanol	2.7591	−0.0664	0.0004
Isopropanol	2.7915	−0.0721	0.0005
Tertiary butanol	2.7303	−0.0676	0.0004

[a] Molar solubility values of glycine in 0–100% (v/v) of each solvent system were used to generate the polynomial equation. [b] Solubility of glycine (moles/l.) in water.

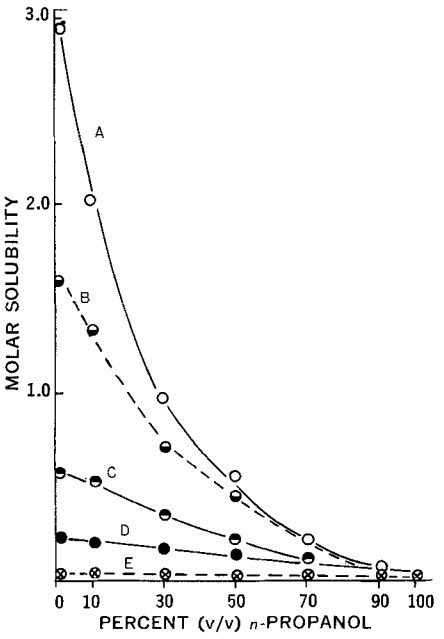

Figure 1—*Molar solubility of amino acid in* n-*propanol–water solvent systems. Key: Curve A, glycine; Curve B,* L-*alanine; Curve C,* L-*valine; Curve D,* L-*phenylalanine; and Curve E,* DL-*amino-octanoic acid.*

[1] Menold rotating apparatus, Lester, Pa.
[2] Porto-Temp, Precision Scientific Co., Chicago, Ill.

Table II—Polynomial Regression Data Generated from the Molar Solubility of L-Alanine as a Function of the Percent Strength of the Solvent System

Solvent System[a]	Y-Intercept[b]	Coefficients	
		X	X^2
Methanol	1.6640	−0.0354	0.0002
Ethanol	1.6238	−0.0366	0.0002
n-Propanol	1.5881	−0.0347	0.0002
Isopropanol	1.6019	−0.0378	0.0002
Tertiary butanol	1.5794	−0.0363	0.0002

[a] Molar solubility values of L-alanine in 0–100% (v/v) of each solvent system were used to generate the polynomial equation. [b] Solubility of L-alanine (moles/l.) in water.

made on the aliquots. Alkali was added to the remaining samples, and a similar analysis was performed.

RESULTS AND DISCUSSION

It has been reported that in pure solvent systems the solubility of certain amino acids was dominated by the α-amino carboxylic acid group, thus producing a high solubility in water and a substantially lower solubility in the alcohols (1). If the attraction that causes the solubility of the amino acid dipoles is defined as being due to the highly polar water molecules interacting with the charged portion of the amino acid, and the semipolar solvents are assumed to have too little polarity to interact with the charged portion of the amino acids, then it would be expected that the addition of a semipolar liquid to the polar aqueous solvent would be reflected by a proportionate decrease in the solubility of the amino acids.

Figure 1 is representative of the variation in molar solubility of the amino acids as the concentration of the semipolar solvents is increased. The decrease in solubility can be seen to be a nonlinear curve rather than the proportional linear decrease expected if the polarity of the solvent system was solely responsible for the solubility behavior. In addition, as the nonpolar–polar ratio of the amino acids is increased, the difference in slope throughout the range of decreasing polarity becomes less, until almost no change is seen for the DL-amino octanoic acid. It would seem that the high charge on the dipolar portion of the molecule is balanced by the nonpolar six-carbon *n*-alkyl chain to produce an invariant solubility. This would seem to correlate with the findings of Cohn (3) in which most α-amino acids were found to be less soluble in ethanol or ethanol–water mixtures than in water, and as the side chain lengthened, a point was reached where the ethanolic solubility was greater than the aqueous solubility.

The overall similarity between the solubility profiles of the amino acids can be illustrated by generating the polynomial regression for each solvent. The equation of the line can be computed using a standard IBM program for an orthogonal polynomial regression (4). By designating the molar solubility as the dependent variable and the percent strength of the alcoholic solution as the independent variable, the polynomial equation of the standard from $Y = A_0 + A_1X + A_2X^2 + A_3X^3 + \cdots A_nX^n$ can be computed.

Table III—Polynomial Regression Data Generated from the Molar Solubility of L-Valine as a Function of the Percent Strength of the Solvent System

Solvent System[a]	Y-Intercept[b]	Coefficients	
		X	X^2
Methanol	0.4581	−0.0089	0.00005
Ethanol	0.4629	−0.0104	0.00006
n-Propanol	0.4732	−0.0080	0.00003
Isopropanol	0.4592	−0.0104	0.00006
Tertiary butanol	0.4578	−0.0104	0.00006

[a] Molar solubility values of L-valine in 0–100% (v/v) of each solvent system were used to generate the polynomial equation. [b] Solubility of L-valine (moles/l.) in water.

Table IV—Polynomial Regression Data Generated from the Molar Solubility of L-Phenylalanine as a Function of the Percent Strength of the Solvent System

Solvent System[a]	Y-Intercept[b]	Coefficients	
		X	X^2
Methanol	0.1646	−0.0019	0.00001
Ethanol	0.1659	−0.0022	0.00001
n-Propanol	0.1602	−0.0021	0.00001
Isopropanol	0.1581	−0.0020	0.00001
Tertiary butanol	0.1609	−0.0022	0.00001

[a] Molar solubility values of L-phenylalanine in 0–100% (v/v) of each solvent system were used to generate the polynomial equation. [b] Solubility of L-phenylalanine (moles/l.) in water.

Tables I–IV show the effect on each of the amino acids in the five solvent systems used in the form of a second-degree equation. The second-degree polynomial equation was considered most representative, since the sum of the squares of the deviation about the regression in the analysis of variants showed little improvement when the third-degree or higher polynomial equation was calculated. The *Y*-intercept in each table is representative of the average solubility in a pure aqueous solvent. The coefficients of the X and X^2 terms define the curve of the line. In Table I, the proximity of the *Y*-intercept values, which range from 2.65204 to 2.79148 with a true solubility of 2.9 *M* in pure water, can be seen. The X and X^2 values also show a similarity. In Table II, L-alanine shows a range of 1.57939–1.66396 in the *Y*-intercepts with a true aqueous solubility of 1.63 *M*. This is a range of only 0.08455 as compared to the range of 0.13944 for glycine, thereby showing a closer approximation of the true solubility. This is expected since the average of the sum of the squares of the deviation about the regression for L-alanine in all solvents is only 0.00538 as compared to 0.11104 for glycine. In Tables III and IV, the computed polynomial equation for L-valine and L-phenylalanine shows an even closer approximation to the experimental points. L-Valine shows a range of only 0.01548 for a solubility of 0.474 *M* and an average sum of squares of the deviation about the regression of 0.00135. L-Phenylalanine has a range of only 0.00788 between its high and low *Y*-intercepts, with a true solubility of 0.172 *M* and an average sum of the squares of the deviation about the regression of only 0.00055.

The closeness of the *Y*-intercepts and the similarity between the coefficients of the X and X^2 terms seem to indicate the existence of a

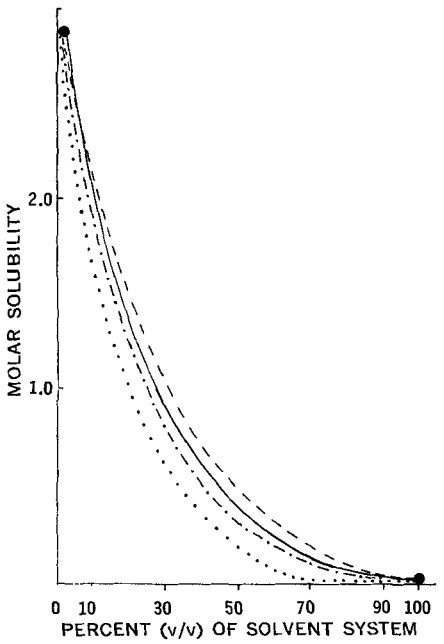

Figure 2—*Molar solubility of glycine in the solvent systems. Key:* ——, *n-propanol;* - - -, *tertiary butanol;* ·-·-, *ethanol; and* ···, *methanol.*

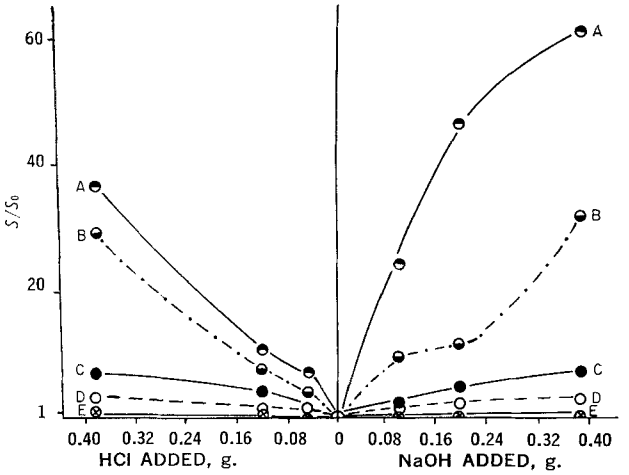

Figure 3—*Ratio of total solubility* (S) *to solubility at the neutral point* (S_0) *for glycine as a function of HCl and NaOH added in an ethanol–water system. Key: Curve A, 100%; Curve B, 90%; Curve C, 70%; Curve D, 50%; and Curve E, 0, 10, and 30%* (v/v) *of ethanol.*

closely related family of curves when the solubilities of each amino acid in the five solvents are compared. Further, it would be expected that the polar amino acids would be the most soluble in the more polar of the mixed solvent systems. By using the dielectric constant as a measure of polarity, the pure solvents can be arranged in this order of decreasing polarity: methanol, ethanol, *n*-propanol, and tertiary butanol. The semipolar solvents in combination with water produce a series of solvent systems with the same order of decreasing polarity. For example, at 30% (v/v) of semipolar solvent–water, the order of decreasing polarity remains methanol–water, ethanol–water, *n*-propanol–water, and tertiary butanol–water. However, it can be seen in Fig. 2 that glycine shows a decreasing solubility in the order of *n*-propanol, tertiary butanol, ethanol, and methanol throughout the concentration scale. The addition of the methyl group to form L-alanine changes the order of binary solubility to what was originally expected; L-valine, with an added isopropyl group, shows its greatest solubility in *n*-propanol followed in decreasing order by methanol, ethanol, and tertiary butanol. The order of solubility of L-phenylalanine does not seem to change appreciably with different solvent systems.

If the solubility of glycine illustrates the behavior of the charged α-amino carboxylic acid portion of the molecule, the variation in order of solubility as methyl, isopropyl, and methyl phenyl groups are added to the α-amino carboxylic acid group serves to illustrate the dependence of solubility on the independent interactions of each nonpolar chain. This appears contrary to the theory that solubility is only a function of the polarity of the solvent system and is not affected by structural variation between systems (5).

The solubility variation when acid or base was added to separate samples of each amino acid in the series of decreasing polarity for 10, 30, 50, 70, 90, and 100% (v/v) of methanol, ethanol, *n*-propanol, isopropanol, and tertiary butanol was similar to that seen previously in pure solvent systems (1). Minimum solubility is seen at the isoelectric point, with a distinct increase in total solubility for each change in pH′ away from the neutral solubility point. However, the increase in total solubility in the semipolar solvents seems to reach a higher magnitude per amount of acid or base added as the polarity of the solvent system is decreased. Figure 3 illustrates this behavior using glycine in seven different ethanol–water solvent systems. The ratio of the total solubility (S) to the solubility at the neutral point (S_0) was plotted as a function of grams of acid or base added. In all cases, the three most polar strengths: 0, 10, and 30% (v/v), merge as one straight line. At 50 and 70% (v/v), a slight increase in salt formation is seen, as evidenced by an increase in the slope of the line. The 90 and 100% (v/v) solutions show a distinct increase in the efficiency of salt formation.

Each amino acid shows this behavior pattern, but there is a difference in the slope of the line for each amino acid which would seem to be due to differences in solute–solvent interaction. It would also seem that these differences in salt formation can be correlated with mole fraction or essentially the number of molecules of water present as compared to the number of molecules of semipolar solvent. In the low percent strengths, where little difference in slope is seen, the water molecules outnumber the ethanol molecules in solution. However, between 70 and 90%, the semipolar solvent molecules begin to outnumber the water molecules and this, in turn, corresponds to the increased efficiency of salt formation for a given solvent system. This can be explained by the increasing importance of interionic forces which make up a greater portion of the attractive charge between oppositely charged ions as the polarity of the solvent system is decreased. Further, since the activity coefficients of the pure HCl and pure NaOH were approximately 0.85 at the molar concentrations in which they were added, the Cl^- and Na^+ ions provided an increasingly greater attractive force for the charged amino acids as the polarity of the solvent system was reduced. Comparison of the ratio of S to S_0 with the quantity of acid or base added seems to indicate reduction of the isoelectric band. Since, by definition, the species present within the isoelectric band are predominately dipolar and have an invariant solubility, an increase in total solubility can probably be attributed to salt formation. The increase in S/S_0 ratio as the concentration of alcohol is increased indicates a reduction in the isoelectric band as a function of decreasing polarity. This reduction is due to an increased affinity of the amino acids for the Na^+ and Cl^- ions.

SUMMARY

1. In the hydroalcoholic solvent systems studied, the solubility of glycine, L-alanine, L-valine, and L-phenylalanine is dominated by the α-amino carboxylic acid portion of the molecule.

2. Each amino acid studied demonstrated an ability to differentiate between the hydroalcoholic solvents used throughout the concentration spectrum, but a similar solubility profile was produced for all molecules.

3. The quantitative order of solubility of each amino acid varied with the change in solvent system. The order of solubility seems to be a function of specific solute–solvent interactions with nonpolar portions of the amino acid molecules.

4. As the concentration of alcohol was increased in the solvent systems, similar increments of acid or base added to the system produced a proportionally greater increase in solubility of the amino acids. This was attributed to an increase in the contribution of the charged species as the polarity of the solvent system was decreased, as well as to an increased affinity of the amino acids for the Na^+ and Cl^- ions present.

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