Solubilities of Glycine and Its Oligopeptides in Aqueous Solutions

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The solubilities at 298.15 K of glycine and its oligopeptides (glycylglycine, triglycine, tetraglycine, pentaglycine, and hexaglycine) in aqueous solution at various pH values and concentrations of NaCl, PEG 6000, and ethanol are reported. At high and low pH, the solubilities increase remarkably. The solubilities generally decrease with an increase of the concentrations of ethanol and PEG 6000 in the experimental range, which indicates that ethanol and PEG 6000 can be used as an antisolvent or precipitant for the precipitation and crystallization of glycine and its oligopeptides. As the NaCl concentration increases, the solubility of glycine first decreases and then increases, while the solubilities of glycylglycine and triglycine increase monotonically with the NaCl concentration.

Introduction

Biochemicals are of importance due to their applications in the chemical, pharmaceutical, and food industries.[1] Knowledge of solubilities of biochemicals under various conditions is useful for rational design of their separation and purification processes. Calculation techniques used to acquire solubility are dependent on the availability of thermodynamic parameters. Because thermodynamic parameters are difficult to obtain, calculation becomes complicated for most systems.[2] Experimental measurement is still the major source of the solubilities of amino acids and peptides. Recently a lot of measurements have been carried out on the solubilities of amino acids. For example, Day and Lahiri[3] measured the solubilities of amino acids in ethanol + water mixtures. Orella and Kirwan[4] measured the solubilities of amino acids in the mixtures of water and 1-propanol. Gude et al.[5] measured the solubilities of amino acids in water + 1-butanol systems. Carta[6] measured the solubilities of four amino acids in aqueous solutions containing another amino acid. Pradhan and Vera[7] studied the effect of anions on the solubilities of amino acids. Khoshkbarchi and Vera[1] studied the effect of cations on the solubilities of amino acids. Gatewood and Rousseau,[8] Pradhan and Vera,[9] and Carta and Tola[10] all investigated the effect of pH on the solubilities of amino acids. Matsuo et al.[11] investigated the effect of pressure on the solubilities of amino acids. Park et al.[12] measured the solubility of glycine (α- and γ-forms) in water at different temperatures by use of a differential scanning calorimeter. As to the solubilities of peptides, the experimental measurements are quite scarce. Castronuovo et al.[13] measured the solubilities of cyclic dipeptides Gly-Gly, Ala-Ala, and Leu-Gly in water via calorimetry. Breil et al.[14] measured the solubilities of glycylglycine and glycyl-L-alanine in aqueous electrolyte solutions at 298.15 K.

The main objective of this work was to measure the solubilities of glycine and its oligopeptides in aqueous solution at various pH values and concentrations of NaCl, PEG 6000, and ethanol. These results will contribute to a better understanding of the thermodynamic behavior of amino acids and oligopeptides in solution and eventually of polypeptides and biomacromolecules. Meanwhile, the experimental results will be useful in the development of their separation and purification processes.

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Materials and Methods

Glycine (241261, α-form), glycylglycine (G7278), triglycine (G1377), tetraglycine (G3882), pentaglycine (76790), and hexaglycine (G5630) were purchased from Sigma-Aldrich (St. Louis, MO) and used without further purification. Ethanol, PEG 6000, sodium chloride, hydrochloric acid, and sodium hydroxide were also obtained from Sigma-Aldrich. All chemicals were of the highest grade available.

Solutions with desired concentrations were prepared by dissolving known amounts of ethanol, PEG 6000, sodium chloride, hydrochloric acid, and sodium hydroxide in deionized water (Millipore, Billerica, MA). A jacketed glass vessel of 30 mL was used to measure the solubilities. Excess amounts of glycine or oligopeptides were added to pure water or the solutions of ethanol, sodium chloride, or PEG 6000. The Teflon-coated magnetic stirring bar ensured proper mixing in the vessel. Temperature was controlled at 298.15 K by a circulator (PolyScience, Niles, IL). The pH was measured by a Mettler Toledo 320 digital meter (Mettler Toledo, Columbus, OH) and adjusted by the addition of NaOH or HCl solution. After 40 h when the sample had reached equilibrium, the agitation was stopped, and the solution was allowed to settle for 6 h. The supernatant in equilibrium with a macroscopically observable solid was then filtered through Millex-VV 0.1 μm filters (Millipore).

The concentration of filtered supernatant was determined by use of the dry mass method. About 3 mL of the filtered supernatant was withdrawn with a pipet and placed in a sample bottle preweighed by use of a Sartorius CP225D analytical balance (Sartorius, Goettingen, Germany) with a resolution of ± 0.01 mg. The sample was placed in a Vacucenter VC 20 oven (Salvis-Lab, Rotkreuz, Switzerland) and vacuum-evaporated to dryness at 303.15 K until the mass was constant. The low evaporation temperature was chosen to eliminate hydrolysis or decomposition. The solid residue masses were determined, and the concentration was then calculated. Meanwhile, the concentrations of filtered supernatants of glycylglycine and triglycine were checked spectroscopically by measuring the absorbance at 210 nm by UV spectroscopy (Shimadzu, Tokyo,

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Table 1. Experimental Solubilities *s* at Various pH Values (298.15 K)

glycine		glycylglycine		triglycine		tetraglycine		pentaglycine		hexaglycine	
pH	s/mg·mL^{-1}	pH	s/mg·mL^{-1}	pH	s/mg·mL^{-1}	pH	s/mg·mL^{-1}	pH	s/mg·mL^{-1}	pH	s/mg·mL^{-1}
2.48	365.76	2.46	306.79	2.22	132.61	2.16	9.61	2.21	3.47	2.23	1.93
2.88	288.18	3.06	248.58	2.75	103.93	2.56	7.58	2.31	2.97	2.53	1.30
3.17	251.23	3.54	228.66	3.33	85.96	3.09	6.29	2.56	2.43	2.82	1.07
3.61	233.99	4.05	215.60	4.23	75.62	3.47	5.81	2.99	1.95	3.37	0.84
4.42	221.67	4.93	203.77	4.77	73.19	3.82	5.49	3.67	1.68	4.13	0.71
5.38	210.45	5.41	201.10	5.20	72.35	4.18	5.34	4.49	1.55	4.76	0.65
6.04	206.41	6.75	206.35	5.93	73.68	4.54	5.25	5.40	1.50	5.22	0.63
6.89	215.92	7.72	218.12	6.75	76.33					6.25	0.60
7.63	231.74	8.79	246.44	7.71	80.54						
8.89	277.87	9.42	276.23	8.63	87.67						
9.78	351.61	9.98	333.42	9.73	105.95						
10.21	444.49	10.28	392.86	10.48	146.89						

Table 2. Solubilities *s* of Glycine and Glycylglycine at 298.15 K in Pure Water

biochemicals	s/mg·mL^{-1}	sources
glycine	206.41	this work
	214.25	ref 16
	214.50	ref 7
	205.77	ref 6
glycylglycine	201.10	this work
	201.43	ref 14

Japan).[14] Extinction coefficients of glycylglycine and triglycine obtained through calibration experiments are 12.12 mL·mg^{-1}·cm^{-1} and 13.42 mL·mg^{-1}·cm^{-1}, respectively. Calibration curves were determined in pure water. All experiments were replicated three times. The data reported in this work are the average of the replicates.

Results and Discussion

The experimental solubilities, *s*, as a function of pH are reported in Table 1. The pH values are the equilibrium values. The solubilities of tetraglycine, pentaglycine, and hexaglycine were measured in pure water and HCl aqueous solutions.

It is well-known that in pure water over 99.9 % of the dissolved amino acid or oligopeptide (A) is present in the form of zwitterions, $^{-}A^{+}$, as overall neutral species carrying a strong dipole.[15] That is, when a divalent solid amino acid or oligopeptide (A) is dissolved in the pure water, the following processes will take place:[10]

$$A(s) \leftrightarrow A(aq) \quad (1)$$

$$A(aq) \leftrightarrow {}^{-}A^{+}(aq) \quad (2)$$

$$H_2O(l) \leftrightarrow {}^{-}OH(aq) + H^{+}(aq) \quad (3)$$

where A(s) and A(aq) are the amino acids or oligopeptides in the solid and aqueous phases, respectively. In the presence of an acid, reaction 4 occurs from right to left:[9]

$$A^{+}(aq) \leftrightarrow H^{+}(aq) + {}^{-}A^{+}(aq) \quad (4)$$

and in the presence of a base, reaction 5 occurs from left to right:

$$^{-}A^{+}(aq) \leftrightarrow H^{+}(aq) + {}^{-}A(aq) \quad (5)$$

where A^{+} represents amino acids or oligopeptides that are positively charged, and ^{-}A represents those that are negatively charged. Generally, the addition of acid or base increases the solubilities of amino acids and oligopeptides in aqueous solutions.

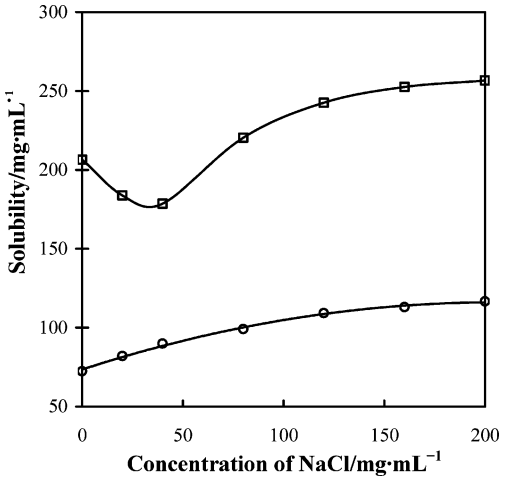

Figure 1. Effect of NaCl on the solubilities of glycine and triglycine: □, glycine; ○, triglycine.

The comparison of the solubilities of glycine and glycylglycine at 298.15 K in pure water with literature data is shown in Table 2. The experimental solubilities of glycine in the HCl solution at pH 2.88 from this work and Carta and Tola[10] are 288.18 mg·mL^{-1} and 263.05 mg·mL^{-1}, respectively. The deviation (less than 10 %) can be attributed to the different measurement methods used.

The measured solubilities of glycine and triglycine, at 298.15 K, at different salt concentrations in neutral solutions are shown in Figure 1. The solubilities of glycylglycine from this work and Breil et al.[14] are compared in Figure 2. In aqueous solution of sodium chloride, the solubilities of glycylglycine and triglycine generally increase with NaCl concentration, while the solubility of glycine first decreases and then increases with an increase in the electrolyte concentration, which is in accord with the results of Pradhan and Vera.[7]

It is known that biomolecule interaction is the sum of different potentials such as electrostatic, van der Waals, hydrophobic, hydration, etc. The effect of electrolytes on the solubilities of biochemicals in aqueous solutions is a result of the interactions between the biomolecule and the electrolyte.[17] As stated above, when a solid amino acid or oligopeptide is dissolved in the aqueous solution of an electrolyte (B^{+} C^{-}), the following reaction may take place besides eqs 1, 2, and 3:

$$^{-}A^{+} + B^{+}C^{-} \leftrightarrow B^{+}(^{-}A^{+})C^{-} \quad (6)$$

The formed complex $B^{+}(^{-}A^{+})C^{-}$ can shield the hydrophobic interactions, and thus the addition of salt can increase the solubilities of amino acids and oligopepetides.[1] However, for such a small biomolecule as glycine at low NaCl concentrations

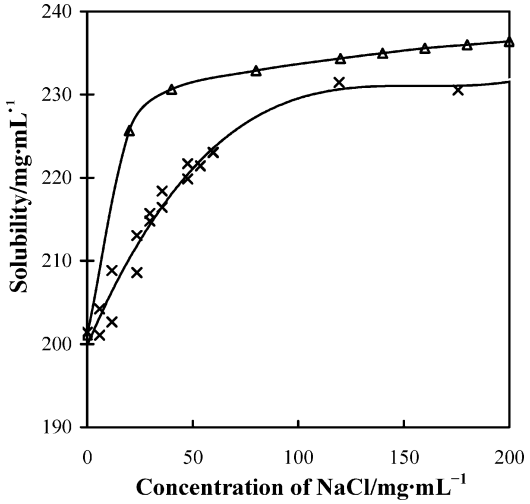

Figure 2. Effect of NaCl on the solubility of glycylglycine: △, this work; ×, ref 14.

Table 3. Experimental Solubilities *s* at 298.15 K in the Presence of Ethanol

	s/mg·mL^{-1}					
x/%	glycine	glycyl-glycine	tri-glycine	tetra-glycine	penta-glycine	hexa-glycine
0	206.41	201.10	72.35	5.25	1.50	0.60
10	162.51	137.34	41.70	3.18	1.07	0.42
20	120.67	83.39	22.31	1.74	0.73	0.30
30	76.58	46.08	12.30	0.98	0.50	0.22
40	46.55	21.37	6.02	0.73	0.32	0.17
50	26.62	11.54	3.12	0.46	0.21	0.13
60	14.12	5.88	1.59	0.36	0.12	0.10

Table 4. Parameters of the Model Equation 7

parameters	glycine	glycyl-glycine	tri-glycine	tetra-glycine	penta-glycine	hexa-glycine
$A \times 10^0$	206.810	201.890	72.194	5.266	1.501	0.599
$B \times 10^1$	−44.159	−74.535	−36.569	−2.593	0.496	−0.210
$C \times 10^2$	−0.92	8.23	6.78	0.49	0.060	0.030
$D \times 10^3$	0.5	−0.2	−0.4	−0.03	−0.003	−0.002

but where the concentration of NaCl increases and repulsive electrostatic or hydration forces decrease, glycine molecules appear more and more attractive, leading to lower solubility. In general, the effect of electrolytes on the solubilities of biochemicals depends strongly on the specific nature of both electrolytes and biomolecules.[18,19]

Amino acids and oligopeptides are generally hydrophilic and often have poor solubility in alcohol. The experimental solubilities *s* in water + ethanol mixtures are presented in Table 3. In all cases, the solubilities are significantly reduced by the presence of ethanol. The experimental solubility data are well-correlated by an expression of the form

$$s = A + Bx + Cx^2 + Dx^3 \quad (7)$$

where *s* is the solubility (mg of solute/mL of solution), and *x* is the volumetric percent of ethanol in the total solvent (water + ethanol). The parameters *A*, *B*, *C*, and *D* are obtained by fitting the experimental data and are presented in Table 4.

The effect of alcohol on the solubility of a biomolecule can be attributed to the hydrophobic and electrostatic interactions of the solute. The promoted hydrophobicity of the solute with an increased amount of alcohol in the mixture destabilizes the intramolecular interaction between the solute and the water, thereby reducing the solubility. Even though the electrostatic repulsion of the solute, induced to enhance the solubility, improves with an increase in the alcohol composition, this is overwhelmed by the promoted hydrophobicity of the solute. Therefore, the solubility of the biomolecule generally decreases with an increased amount of alcohol in the mixture.[20] Fialaire and Postaire[21] applied an 80:20 (v/v) mixture of ethanol and water to precipitate an oligopeptide with a desirable selectivity.

Table 5. Experimental Solubility of Triglycine *s* at 298.15 K in the Presence of PEG 6000

PEG concentration/mg·mL^{-1}	s/mg·mL^{-1}
0	72.35
50	55.52
100	43.23
150	34.65
200	23.85
250	16.02
300	4.47

The measured solubility of triglycine in PEG 6000 aqueous solution is presented in Table 5. The solubility of triglycine decreases with an increase in the concentration of PEG 6000.

Conclusions

We have presented experimental results on the solubilities at 298.15 K of glycine, glycylglycine, triglycine, tetraglycine, pentaglycine, and hexaglycine. In the presence of acid or base, solubility increases as the chemical equilibria move. The addition of ethanol or PEG 6000 generally decreases the solubility. However, the addition of sodium chloride has different effects on the solubilities of the biomolecules studied. The solubilities of glycylglycine and triglycine increase monotonically with NaCl concentration, while the solubility of glycine first decreases and then increases with an increase in the NaCl concentration. These results can contribute to a better understanding of the thermodynamic behavior of glycine and its oligopeptides in aqueous solution. The precipitation and crystallization of amino acids and oligopeptides can be conducted by the addition of alcohol or PEG and the adjustment of the pH values.

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