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Effect of pH on the solubilities of divalent and trivalent amino acids in water at 298.15 K

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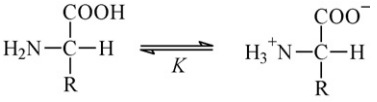
ABSTRACT

The objective of this study is to report the effects of pH on the solubility of amino acids in water at 298.15 K. Two divalent amino acids, asparagine and glutamine, and three trivalent amino acids, tyrosine, aspartic acid and glutamic acid, were examined. The pH range studied was from 2 to 10. The solubility of amino acids in water is affected by the pH of the solution because amino acids dissociate into different ionic forms in aqueous solution: zwitterions, cations and anions. A chemical model was employed to describe the dissociation equilibria of all amino acid species with hydrogen ions in water. Moreover, for asparagine, glutamine and tyrosine, the chemical model indicated that the formation of cations and anions is insignificant in the isoelectric solution, with almost 100% of the amino acids exist as zwitterions. However, in the case of trivalent amino acids with acidic side chain groups, the zwitterion is not the only species present at the isoelectric point. Therefore, for aspartic acid and glutamic acid, at least three different ionic forms must be considered. To correlate the solubility data for amino acids in water, a non-random two liquid (NRTL) model was used to describe the non-idealities in the solution. The results calculated with two adjustable parameters were quite satisfactory, with an overall deviation of 2.21%.

1. Introduction

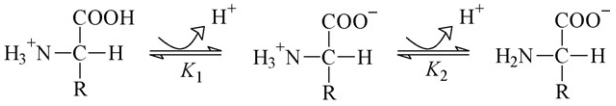
Amino acids, the building blocks of protein, are commonly found in living systems. In an α-standard amino acid, an amino group and a carboxyl group are located around a central carbon atom, called the α carbon. The other two groups bonded to the α carbon are a side chain group and a hydrogen atom. During the dissolution of solid amino acids in water, the amino and carboxyl groups are ionized and the amino acids exist as zwitterions:



(1)

where K is the equilibrium constant and R is the side chain group.

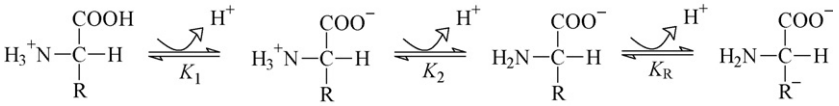
For an amino acid containing a neutral side chain, such as an alkyl group, only the amino and carboxyl groups are ionizable, both of which are protonated at low pH. Thus, the amino acid molecule has a net positive charge. From a chemical viewpoint, this cationic amino acid can be regarded as a weak diprotic acid [1] and can be further characterized as a divalent amino acid [2]. When the divalent amino acid is titrated by a strong base solution, it will yield the neutral zwitterion and eventually the anionic species. Thus, the stepwise dissociation of a divalent amino acid involves two stages:



(2)

where K_1 and K_2 are the dissociation equilibrium constants.

Furthermore, several amino acids contain titratable side chain groups, such as tyrosine, aspartic acid and glutamic acid. Because the α-amino group is protonated at low pH, we can regard this cationic amino acid as a weak triprotic acid [1] or a trivalent amino acid [2]. For tyrosine, the side chain group, a para-hydroxide, is the weakest acid of all titratable groups. Therefore, the dissociation involves three stages:



(3)

where the sequence for the three dissociation equilibrium constants is K_1, K_2 and K_R and R^- represents deprotonated side chain group. However, the side chains for aspartic and glutamic

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Nomenclature

List of symbols

A	amino acid
a	activity
D	average absolute relative deviation
F	objective function
K	equilibrium constant
R	side chain
W	water
x	solubility in mole fraction
m	solubility in molality

Greek letter

γ	activity coefficient

Superscripts

±	zwitterion
+	cation
−	single-charged anion

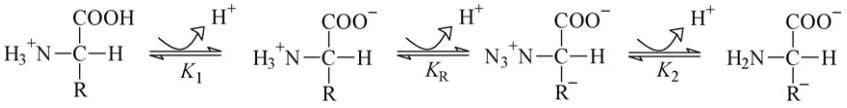
Single-charged anion

=	double-charged anion
∞	infinite dilute
cal	calculated value
*	asymmetric scale

Subscripts

c	molarity basis
x	mole fraction basis

acids contain carboxyl groups. These side chain carboxyl groups are stronger acids than amino groups and are almost fully ionized at neutral pH. The stages of the stepwise dissociation for amino acids with acidic side chain groups are



(4)

where the three dissociation equilibrium constants are K_1, K_R and K_2.

Based on these dissociation equilibria for either divalent or trivalent amino acids with hydrogen ions in water, it is obvious that the solubility of amino acids in water will be affected by the pH of the solution. The literature contains some experimental studies on the solubility of amino acids in water. Hitchcock [3] measured the solubility of tyrosine in hydrochloric acid and sodium hydroxide solutions. By assuming ideal behavior, an equilibrium relation between the tyrosine solubility and pH was proposed. Carta and Tola [4] reported the solubilities of four amino acids in sodium chloride solutions at various pH values, and their work was later continued [5]. These reported solubility data [3–5] were given on a molarity scale under the assumption that the ideal circumstance of all activity coefficients being equal to unity. This approach provided reasonable agreement with the experimental data. Pradhan and Vera [6] determined the effect of acids and bases on the solubility of DL-alanine. The solubility data were well correlated within a pH range of 2–10. Brown and Rousseau [7] reported the solubilities of L-isoleucine, L-leucine and L-valine in aqueous solution containing sodium hydroxide. Their experimental results showed that all three amino acids exhibit an increase in solubility as the pH of the solutions move away from the isoelectric point. Ninni and Meirelles [8] determined the water activity, pH and density of some aqueous amino acid solutions at 298.15 K in three different types of solvents. They used an UNIFAC group contribution model incorporated with a Debye–Hückel term to estimate the solubility of amino acids. In our previous study [9], the solubility of the amino acids in water at various pH values and 298.15 K were measured. Five divalent amino acids were examined: DL-alanine, L-leucine, L-isoleucine, L-serine and DL-phenylalanine. The data were correlated using a chemical model and a non-random two liquid (NRTL) model [10] to describe the dissociation of amino acids in water. The calculated results were quite satisfactory. Reviewing these studies, the solubility data are valuable in developing thermodynamic frameworks and modeling the dissociation of amino acids in water. However, only limited experimental data on the effects of temperature, pH, solvent and salinity on the solubility of amino acids in water are reported in the literature.

In this study, the solubility data for five amino acids in a pH range of 2–10 were measured at 298.15 K. Two divalent amino acids, asparagine and glutamine, and three trivalent amino acids, tyrosine, aspartic acid and glutamic acid, were investigated. Furthermore, the solid–liquid equilibrium and the NRTL activity coefficient model were used to correlate the solubilities of amino acids in water at various pH values.

2. Experimental

2.1. Chemicals

The chemicals used were the following: deionized water, sodium hydroxide (Riedel deHaen, 98 wt%), hydrochloric acid (Riedel deHaen, 37 wt%), ninhydrin (Riedel deHaen, 99 wt%), L-asparagine (Sigma–Aldrich, 99 wt%), L-glutamine (Sigma–Aldrich, 99 wt%), L-aspartic acid (Sigma–Aldrich, 99 wt%), L-glutamic acid (Sigma–Aldrich, 99 wt%) and L-tyrosine (Acros, 99 wt%). All chemicals were of analytical grade and were used without further purification.

2.2. Equipment, procedures and analytical method

The following equipment, operational procedures and analysis methods, essentially following those in our previous work [9], were used to determine the solubility of amino acids in water. To prepare each sample, deionized water was mixed with hydrochloride or sodium hydroxide to achieve the desired pH value. A small excess of solid amino acid was then introduced into an equilibrium tube. The tubes were vigorously shaken for 16 h and then allowed to settle for at least 8 h in a water bath maintained at 298.15 ± 0.05 K. After they reached equilibrium, the final pH values of the saturated solutions were recorded by a calibrated pH meter (Thermo Orion 420Aplus, ±0.01 unit). The amino acid contents in water were determined by ninhydrin assay using a colorimetric method. A detailed description of the use of a ninhydrin assay to analyze amino acid content can be found in our previous study [9] or elsewhere [11]. The measurement accuracy of the solubility of amino acid in water was estimated to be within ±1%.

3. Theory

3.1. Chemical model

As mentioned earlier, the solubility of the divalent amino acids in water can be evaluated by a chemical model that considers the