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SHORT COMMUNICATION

The protonation equilibria of selected glycine dipeptides in ethanol–water mixture: solvent composition effect

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Abstract Knowledge of the protonation constants of small dipeptide is important, interesting and necessary for complete understanding of the physiochemical behavior of dipeptide. In this study, the protonation constants of some aliphatic dipeptides (*Gly–Gly, Gly–Val, Gly–Leu, Gly–Thr, Gly–Phe and Gly–Met*) were studied in water and ethanol–water mixtures [20%ethanol–80%water, 40%ethanol–60%water, 60%ethanol–40%water, (v/v)] at 25 ± 0.1°C under nitrogen atmosphere and ionic strength at 0.10 mol dm^{-3} by potentiometry. The constants of the systems were calculated by using BEST computer program, and distribution species diagrams were produced using the SPE computer program. The protonation constants were influenced by changes in solvent composition, and their variations were discussed in terms of solvent and structural properties. The concentration distribution of the various species in ethanol–water mixtures was evaluated.

Keywords Dipeptides · Protonation constants · Ethanol–water mixture · Potentiometry

Introduction

The amino acids and small peptides, as the building blocks of proteins, have special important substances among the other chemical groups for living systems. They are not only components of tissues, but also reactive organic compounds which are important regulators of biological processes. Obviously, the physical properties of peptides have to be known to explain the behaviors and the synthesis of proteins and enzymes in the organisms.

The study of amino acids, peptides or DNA units has been the subject of increasing research efforts, which have revealed the role of hydrogen ion at molecular level. It seems therefore to be of considerable interest to conduct some investigations involving the peptide ligand (Sigel 1975; Lyons and Pettit 1985; Fonteh et al. 2007; Kılyen et al. 2003; Gergely and Farkas 1982; Facchin et al. 2002; Diaz-Cruz et al. 2000; Gooding et al. 2001; Ivanova et al. 2006; Koleva et al. 2006, 2007a, b). Also, small peptides have attracted great attention in the past decades in relation to the bioinorganic chemistry. These compounds are usually considered as good model systems to attain a better insight into the characteristics of naturally occurring copper metalloproteins (Kozlowski et al. 1999; Sigel 1973; Mendieta et al. 1996; Gergely and Nagypal 1977). On the other hand, several copper complexes containing simple ligand groups have displayed diverse pharmacological activities. For instance, copper complexes with amino acids and peptides as ligands show anti-inflammatory and cytostatic activities (Facchin et al. 2006; Farrell 1989).

Therefore, the data related to the protonation constants of the small peptides in various media will be valuable in further understanding of the peptides chemistry in biological systems. The major reasons for the determination of protonation constants can be summarized as follows:

The pH and the ratio of different forms of a certain substance can be calculated by the use of its protonation constants. Due to the fact that different forms of different substances have different UV spectra, choosing a suitable

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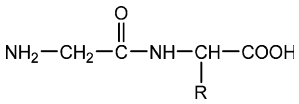
pH value spectrophotometric quantitative analysis can be carried out. The choice of that pH value requires the knowledge of protonation constants. If the protonation constants of a certain substance are known it is possible to isolate it with the maximum yield by finding the pH range where the compounds show minimum ionization. Protonation of a newly synthesized compound can also give supportive information about its structure. If theoretically calculated protonation constants are in good accordance with the experimental values it is possible that the proposed structure can be correct. It is necessary that the protonation constants be known to prepare buffer solutions at different pH values (Rossotti 1978).

In addition, for the calculations of stability constants of the complex formation of dipeptides with metal ions, the protonation constants of dipeptides are used (Sigel and Martin 1982; Brookes and Pettit 1975; Fereeman et al. 1977; Varnagy et al. 2004). It is known that the reactions of peptides with metal ions are of biochemical importance but they are still to be fully elucidated. The explanation of these phenomena in the biological systems is possible only by determining the protonation constants of dipeptides as well as their stability constants.

Vast data are available on the protonation and stability constants of the dipeptides in aqueous solution (Kılyen et al. 2003; Gergely and Farkas 1982; Facchin et al. 2002; Diaz-Cruz et al. 2000; Gooding et al. 2001; Ivanova et al. 2006; Koleva et al. 2007b). Despite this, little is known about the chemistry of dipeptides in non-aqueous media and mixed solvents, in regard to their protonation and stability constants or solvation properties (Lomozik 1984; Aihara et al. 1986). The ethanol–water mixture is a very interesting binary mixture. One reason is that ethanol can dissolve a majority of organic acids and bases more effectively than water. However, it has been suggested that solvents such as ethanol–water mixtures provide a better model for in vivo reactions because the mixtures simultaneously show a low polar character and partially aqueous contents, as do all biological systems. In addition, ethanol–water mixtures are a suitable solvent for the determination of equilibrium constants (Crosby et al. 1970; Arroyo et al. 2000; Doğan et al. 2002a, b; Canel et al. 2006).

As a part of our interest to determine the protonation constants of some compounds such as amino acids and amino acids derivatives, we have extended our investigation (Doğan et al. 2001, 2002a, b; Canel et al. 2006; Köseoğlu and Kılıç Doğan 2000). In this study, the protonation constants of the small peptides (Table 1) in water and ethanol–water mixtures are reported to better understand the interactions of peptides with solvents. Furthermore, the effect of solvent composition and of glycine dipeptides structures on these constants was discussed.

Table 1 The structures of some dipeptides studied



Dipeptide

R	Abbreviation
—H	Gly–Gly
—CH_2—CH(CH_3)$_2$	Gly–Leu
—CH(CH_3)$_2$	Gly–Val
—CH(OH)—CH_3	Gly–Thr
—CH_2—C$_6$H$_5$	Gly–Phe
—CH_2—CH_2—S—CH_3	Gly–Met

Materials and methods

Procedure

All dipeptides (*Gly–Gly, Gly–Met, Gly–Leu, Gly–Val, Gly–Thr, Gly–Phe*) were purchased from Sigma products and used without further purification. Doubly distilled conductivity (Millipore System) water was used as aqueous medium as well as for preparation of ethanol–water mixtures. Ethanol was purified as described in literature (Perrin and Armerega 1991). Doubly distilled water was also used for preparation of the stock solutions of dipeptides. All other chemicals used in this investigation were of reagent grade purity. Hydrochloric acid solution (0.10 mol dm^{-3}) was prepared in water and standardized potentiometrically against sodium carbonate. The base solutions (containing 0.10 mol dm^{-3} NaCl) were standardized via a linear least-squares fit of Gran's plots for end-point determinations obtained by titration with hydrochloric acid (Gran 1952).

Potentiometric titrations

The pH measurements of proton–ligand system of dipepdides were carried out with an Orion E 940 pH meter, equipped with a combined pH electrode (Ingold) and Orion 960 automatic titrator, containing carbonate-free sodium hydroxide at a known ($\sim$0.1 mol dm^{-3}) concentration of 25 $\pm$ 0.1°C, by the circulating water by a thermostatted bath. The potentiometric cell was calibrated before each

experiment to obtain the *p*H (=−log[H^+]) values for the solvent mixture studied (Meloun et al. 1988). The ionic product ($K_{ap} = [H^+][OH^-]\cdot$) was calculated at a constant ionic strength of 0.10 mol dm^{-3} with NaCl in ethanol–water mixture (Canel et al. 2006; Woolley et al. 1970). The values (K_{ap}) are shown in Table 2. For the determination of the protonation constants, stock solutions of hydrochloric acid, dipeptides and sodium chloride were introduced into a volumetric flask, followed by an appropriate amount of ethanol to obtain solutions of the desired concentration and percentage of ethanol. The contents of each flask were diluted up to the mark at the equilibrium temperature. In each solvent mixture, the concentrations of hydrochloric acid, each dipeptide and sodium chloride were kept at 3.0×10^{-3}, 1.5×10^{-3} and 0.10 mol dm^{-3}, respectively. Aliquots of 50 ml were taken from the test solutions and transferred to the potentiometric cell and titrated with standard 0.10 mol dm^{-3} NaOH prepared in ethanol–water mixture.

Data processing

The protonation constants of dipeptides were evaluated by iterative non-linear least squares fit of potentiometric equilibrium curves through mass balance equations for all the components expressed in terms of known and unknown equilibrium constants using a computer program BEST (Martell and Motekaitis 1988). BEST was used to minimize the standard deviation of fit (σ_{fit}) between observed and calculated pH values of the entire titration data.

Species distribution curves

All the species distributions were calculated with the aid of the FORTRAN computer program SPE, which employs the same algorithm as BEST (Martell and Motekaitis 1988).

Results and discussion

The protonation constants of Gly–Gly, Gly–Leu, Gly–Val, Gly–Thr, Gly–Phe and Gly–Met were determined potentiometrically in water and ethanol–water mixtures (20% ethanol–80% water, 40% ethanol–60% water, 60% ethanol–40% water), at 25 ± 0.1°C with an ionic strength of 0.10 mol dm^{-3}. The calculation of the constants has been carried out using a BEST computer program. The values refined for protonation constants are shown in Table 3.

Table 2 The autoprotolysis constants (pK_{ap}) calculated for various ethanol–water mixtures

Medium	pK_{ap}
Water	13.78 ± 0.01
20% ethanol–80% water	14.03 ± 0.02
40% ethanol–60% water	14.30 ± 0.02
60% ethanol–40% water	14.48 ± 0.02

The investigated protonation constants of dipeptides have been determined mostly in aqueous media and rarely in organic solvent–water mixtures (Sigel 1975; Lyons and Pettit 1985; Fonteh et al. 2007; Kılyen et al. 2003; Gergely and Farkas 1982; Facchin et al. 2002; Diaz-Cruz et al. 2000; Gooding et al. 2001; Ivanova et al. 2006; Koleva et al. 2007b). The constants determined in 0.10 mol dm^{-3} NaCl are expected to be close to the physiological ones. The results obtained for dipeptides are given subsequently:

Two protonation equilibria were found for all dipeptides and the first one attributed to the protonation of amino group and the second one was related to the protonation of carboxylate group:

$$L^- + H^+ \rightleftarrows HL \quad K_1 = [HL]/[L^-][H^+] \tag{1}$$

$$HL + H^+ \rightleftarrows H_2L^+ \quad K_2 = [H_2L^+]/[HL][H^+] \tag{2}$$

where L is dipeptide monoanion; HL is total zwitterion and neutral forms of dipeptide.

As briefly outlined for each peptide anion (L), there are two protons, where one can be associated to amino group and the other to carboxylate group (Chakraborty and Bhattacharya 1990).

Chattopadhyay and Lahiri examined the effect of a change of solvent composition on BH^+ ionization and the related Gibbs transfer energies in mixed solvent (Chattopadhyay and Lahiri 1982). They emphasized that the electrostatic charge effects due to changes in the dielectric constants with changes in solvent composition are of minor importance in explaining solvent effects, and that solute–solvent interactions have greater significance in interpretation of solvent effects.

These protonation constants have been considered in some detail to gain more information about the effect of solvent composition on the corresponding equilibria. For this purpose, the plots obtained for gly-met are given in Fig. 1. When the change of these dipeptides $\log K_1$ and $\log K_2$ given in Fig. 1 and in Table 3 with the solvent composition is examined, it is generally observed that these $\log K_1$ values decrease with the increase in the mole fraction of ethanol, whereas $\log K_2$ values increase as the composition of ethanol increases in ethanol–water mixture. The decrease in $\log K_1$ values and increase in $\log K_2$ values of all dipeptides with the increasing ethanol concentration were the same as for the corresponding free amino acids (Doğan et al. 2002).

Table 3 Protonation constants of dipeptides in water and ethanol–water mixture [Temp = 25.0 ± 0.1°C, μ = 0.10 M NaCl]

Protonation constants*										
Dipeptides (Gly-AA)	Water		20% ethanol–80% water (x = 0.072)		40% ethanol–60% water (x = 0.174)		60% ethanol–40%water (x = 0.316)		Literature values in water	
	logK_1	logK_2	logK_1	logK_2	logK_1	logK_2	logK_1	logK_2	logK_1	logK_2
Gly–Gly	8.16	3.40	7.66	3.33	6.92	3.54	6.98	3.81	8.15[c]	3.11[f]
	9.58[a]	2.32[b]	9.30[a]	2.68[b]	9.39[a]	2.89[b]	9.20[a]	3.05[c]	7.99[b]	3.04[f]
Gly–Val	8.34	3.54	7.67	3.52	7.06	3.91	6.92	4.18	8.23[d]	3.15[d]
		2.38[b]		2.76[b]		2.95[b]		3.16[b]		
Gly–Leu	8.29	3.55	7.60	3.38	7.17	3.94	7.02	4.22	8.28[c]	3.13[c]
		2.50[b]		2.75[b]		3.01[b]		3.17[b]	8.07[f]	3.10[f]
Gly–Thr	8.50	3.20	7.74	3.19	7.07	3.37	7.05	3.67		
		2.45[b]								
Gly–Phe	8.29	3.62	7.62	3.33	6.96	3.65	6.98	3.91	8.27[d]	3.12[d]
		2.43[b]		2.57[b]		2.95[b]		3.05[b]		
Gly–Met	8.24	3.27	7.64	3.32	7.02	3.62	6.96	3.91	8.22[e]	3.11[e]
		2.28[g]		2.46[b]		2.85[b]		3.00[b]		

* The relative standard deviations are less than 2%, and σ fit $\leq \pm 0.01$, x = the mole fraction of ethanol

[a] logK_1 of glycine (Doğan et al. 2002; Köseoğlu and Kılıç Doğan 2000)

[b] logK_2 of amino acid, AA (Doğan et al. 2002; Köseoğlu and Kılıç Doğan 2000)

[c] Sigel 1975)

[d] Fonteh et al. (2007)

[e] Lyons and Pettit (1985)

[f] Kılyen et al. (2003)

[g] Martell and Smith (1974)

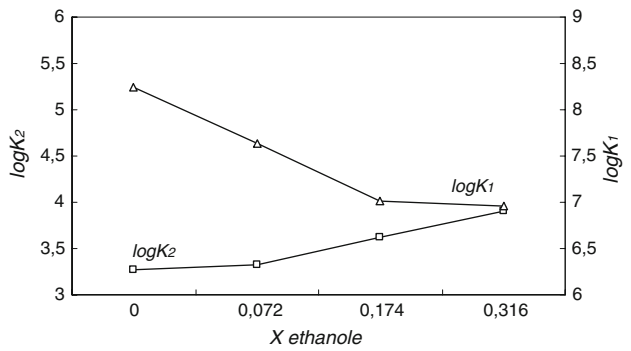


Fig. 1 The variation of protonation constants of gly-met with the mole fraction of ethanol

The fact that the variation of logK_1 and logK_2 of dipeptides with solvent composition is similar to those of the corresponding free amino acids and can be concluded that zwitteionic form of dipeptides dominated in ethanol–water mixtures. In water-rich media, however, the reverse behavior will be the case. This shows that logK_1 values are expected to increase with an increase in the ethanol ratio. This is the only way which explains the decrease in logK_1 as the amount of ethanol in the media is increased because L^- is expected to solvate more than the zwitterionic form of dipeptides in the media rich in ethanol. The fact that logK_2 values are observed to increase as the mole fraction of ethanol is increased in all media investigated supports that dipolar ion is predominant in ethanol–water mixtures as well as in water. This is due to the fact that ethanol solvated H_2L better than zwitterionic form. If the neutral form were the predominant species in ethanol–water mixture, logK_2 values would decrease with increasing amount of ethanol because ethanol solvated HL better than H_2L^+. Thus, we can say that the logK_2 values for these dipeptides increase as the mole fraction of ethanol increases.

Generally, similar variations were' not obtained at the glycine side chains for the protonation constants of all dipeptides examined in ethanol–water mixtures. Because, it is some times extremely difficult to assess how much each effect contributes to the acidity or basicity. Small differences in acidity or basicity between similar molecules are also extremely difficult to interpret, and one must be very careful in deciding which structural effect is the main influence on acidity or basicity.

In order to compare the logK_1 and logK_2 dipeptides with the logK_1 value of glycine and logK_2 value of the other

amino acid forming the dipeptide the protonation constants of the corresponding amino acids are given Table 3. Table 3 shows that the logK_2 values of amino acids (AA) increase and the logK_1 values of glycine decrease due to peptide formation. For instance logK_1 and logK_2 values for glycine in 40% ethanol–60% water medium were 9.39 and 2.89, respectively, while same values were found as 6.92 and 3.54 for gly–gly. Similarly logK_1 for glycine was 9.20 and logK_2 for phe was 3.05 in 60% ethanol–40% water medium while these values were observed to be 6.98 and 3.91 for gly–phe in the same medium. This may be attributed to the fact that the electron density of the amino group in dipeptide is lower than that of glycine, and the electron density of the carboxylate group is higher than that of the corresponding amino acid due to the formation of a peptide bond.

The fact that Table 3 shows that logK_2 values of dipeptides in aqueous medium is higher than those in 20% ethanol–%80 water mixture may be due to preferential solvation of solute by one of the components of the solvent mixture. It is to be expected that in binary solvent mixtures preferential solvation of the species generally takes place, so that their near environment, where the solvent is electrostricted, differs in composition from the bulk. In extreme cases of selective solvation the species may be exclusively surrounded by one of the component of the solvent mixture (Marcus 1984, 2001, 2005; Nishi et al. 1995).

Many alcohols and some non-aqueous solvent such as dimethylsulfoxide or acetonitrile have medium relative dielectric constants of $\varepsilon_r = 35$–46 between high (water) and low (benzene) dielectric solvent, and many electrolytes can be dissolved in these solvents. Their mixtures are macroscopically homogeneous, but it has been reported that the water and organic solvent molecules are not homogeneously dispersed microscopically because of the hydrogen-bonding network formation and hydrophobic interactions (Nishi et al. 1995). Consequently, the molecular composition of the solvation layer around is not the same as that of the bulk mixing ratio of water and organic solvent. The hydrophobic and hydrophilic properties of a solute may be reflected by preferential solvation in such mixed solvent. Similar results found in ethanol–water solvent mixtures also support this conclusion (Kılıç and Köseoğlu Başgut 1994).

Estimation of equilibrium concentrations of proton–ligand complexes as a function of pH provides a useful picture of proton–ligand binding in media (Martell and Motekaitis 1988). Figures 2, 3, 4 and 5 show the change of different species of dipeptides with pH. It is seen from these figures that the most predominant species is HL (total concentration of the zwitterion and the neutral form) between pH 5 and 7 in all ethanol–water media.

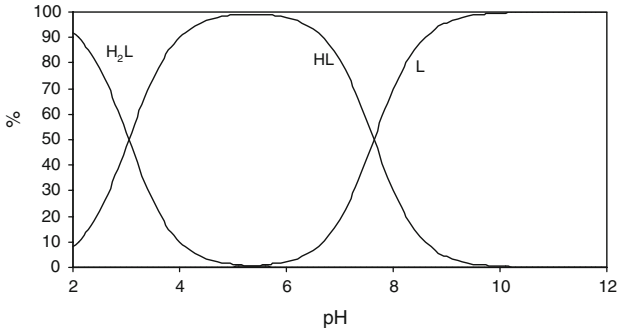


Fig. 2 Species distribution diagram (25.0 ± 0.1°C, $\mu = 0.10$ M NaCl) for gly-met system as a function of pH in 20% ethanol–80% water mixture ($L = 1.5 \times 10^{-3}$ M)

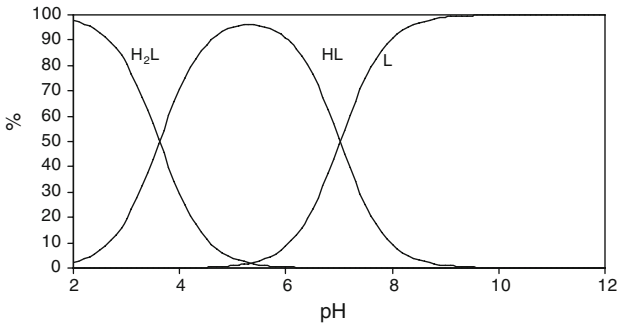


Fig. 3 Species distribution diagram (25.0 ± 0.1°C, $\mu = 0.10$ M NaCl) for gly-met system as a function of pH in 40% ethanol–60% water mixture ($L = 1.5 \times 10^{-3}$ M)

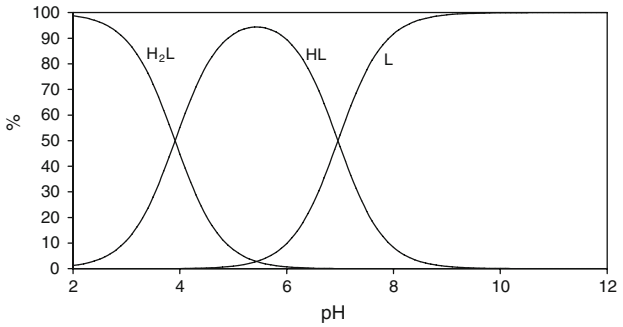


Fig. 4 Species distribution diagram (25.0 ± 0.1°C, $\mu = 0.10$ M NaCl) for gly-met system as a function of pH in 60% ethanol–40% water mixture ($L = 1.5 \times 10^{-3}$ M)

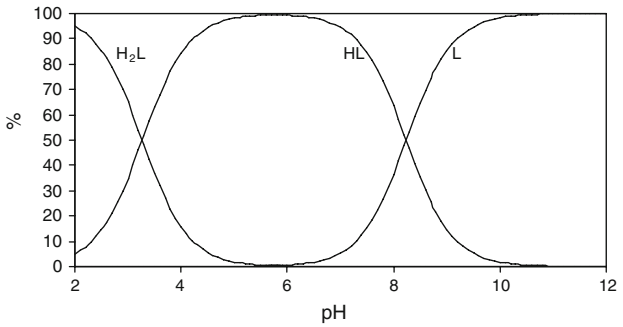


Fig. 5 Species distribution diagram (25.0 ± 0.1°C, $\mu = 0.10$ M NaCl) for gly-met system as a function of pH in water ($L = 1.5 \times 10^{-3}$ M)

Conclusion

The protonation constants of some dipeptides were determined potentiometrically in water and ethanol–water mixtures. Generally, it was observed that the $logK_1$ values of dipeptides decrease and the $logK_2$ values increase with the increase in the mole fraction of ethanol. The chemical and biological activity of these substances would be expected to vary with the degree of ionization. A good knowledge of the constants of dipeptides in ethanol–water media are, therefore, of considerable interest. For this reason, the knowledge of the constants for these substances is a prerequisite to an understanding of their mechanism of action in both chemical and biological processes.

References

Aihara A, Nakanura Y, Nishida Y, Noda K (1986) Electrochemical behavior of copper (II)-dipeptide complexes in mixed solvent. Inorganica Chim Acta 124:169–173

Arroyo M, Torres-Guzman R, Mata I, Castillon MP, Acebal C (2000) Prediction of penicillin V acylase stability in water-organic co-solvent monophasic systems as a function of solvent composition. Enzyme Microb Technol 27:122–126

Brookes G, Pettit D (1975) Thermodynamic of complex formation between hydrogen, copper(II), and nickel(II) ions dipeptides containing non-co-ordinating substituent groups. J Chem Soc Dalton Trans 2106–2111

Canel E, Gültepe A, Doğan A, Kılıç E (2006) The determination of protonation constants of some amino acids and their esters by potentiometry in different media. J Solut Chem 35:5–19

Chakraborty D, Bhattacharya PK (1990) Intramolecular interligand interactions in Cu(II) ternary complexes involving dipeptides and amino acids. J Inorg Biochem 39:1–8

Chattopadhyay AK, Lahiri SC (1982) Studies on the protonation constants and salvation of amino acids in ethanol and water mixtures. Electrochim Acta 27:269–272

Crosby J, Stone R, Lienhard GE (1970) Mechanisms of thiamine-catalyzed reactions. Decarboxylation of 2-(1-carboxy-1-hydroxyetyl)-3,4-dimethylthiazolium chloride. J Am Chem Soc 92:2891–2900

Diaz-Cruz MS, Diaz-Cruz JM, Mendieta J, Tauler R, Esteban M (2000) Soft-and hardmodeling approaches for the determination of stability constants of metal-peptide systems by voltametry. Anal Biochem 279:189–201

Doğan A, Köseoğlu F, Kılıç E (2001) The stability constants of copper(II) complexes with some α-amino acids in dioxan-water mixtures. Anal Biochem 295:237–239

Doğan A, Köseoğlu F, Kılıç E (2002) Studies on the macroscopic protonation constants of some α-amino acids in ethanol–water mixtures. Anal Biochem 309:75–78

Doğan A, Köseoğlu F, Kılıç E (2002) Potentiometric studies on the protonation constants and solvation of some α-amino acid methyl- and ethyl esters in ethanol–water mixtures. Indian J Chem 41A:960–962

Facchin C, Kremer E, Baran EJ, Castellano EE, Piro EO, Ellena J, Costa-Filh AJ, Torre MH (2006) Structral characterization of a series of new Cu–dipeptide complexes in solid state and in solution. Polyhedron 25: 2597–2604

Facchin C, Torre MH, Kremer E, Piro EO, Castellano EE, Baran EJ (2002) Synthesis and characterization of three new dipeptide complexes. J Inorg Biochem 89:174–180

Farrell N (1989) Transition metal complexes as drugs and chemotherapeutic agents. Kluwer, Dordrecht

Fereeman HC, Healy MJ, Scudder ML (1977) A crystallographic study of the structure of glycyl-L-alaninatocopper(II) hydrate and the conformations of copper(II) dipeptide complexes. J Biol Chem 252(24):8840–8847

Fonteh AN, Harrington RJ, Harrington MG (2007) Quantification of free amino acids and dipeptides using isotope dilution liquid chromatography and electrospray ionization tandem mass spectrometry. Amino Acids 32:203–212

Gergely A, Farkas E (1982) Studies on transition-metal–peptide complexes. J Chem Soc Dalton Trans 381–395

Gergely A, Nagypal I (1977) Studies on transition metal–peptide complexes. Part1. Equilibrium and thermodynamical study of the copper-(II)complexes of glycylglycine,glycly-DL-alanine, DL-alanylglycine, and DL-alanyl-DL-alanine. J Chem Soc Dalton Trans 1104–1111

Gooding JJ, Hibbert DB, Yang W (2001) Electrochemical ions sensors. Exploiting amino acids and peptides as recognitions elements. Sensors 1:75–90

Gran G (1952) Determination of the equivalent point in potentiometric titrations. Part II. Analyst 77:661–671

Ivanova BB, Kolev T, Zareva SY (2006) Solid-state IR-LD spectroscopic and theoretical analysis of glycine-containing peptides and their hydrochlorides. Biopolymers 82:587–596

Kılıç E, Köseoğlu F Başgut Ö (1994) Protonation constants of some pyridine derivatives in ethanol–water mixtures. Anal Chem Acta 294:215

Kılyen M, Forgo P, Lakatos A, Dombi G, Kiss T, Kotsakis N, Salifoglou A (2003) Interaction of Al(III) with the peptides AspAsp and AspAspAsp. J Inorg Biochem 94:207–213

Koleva BB, Kolev TS, Spiteller M (2006) Structral and spectroscopic analysis of hydrogensquarates of glycine-containing tripeptides. Biopolymers 83:498–507

Koleva BB, Kolev TM, Spiteller M (2007a) Spectroscopic and structural elucidation of alanyl-containing dipeptides and their hydrogensquarates. J Mol Struct (in press)

Koleva BB, Kolev TS, Zareva SY, Spiteller M (2007b) The dipeptide alanylphenylalanine (H-Ala-Phe-OH)-protonation and coordination ability with Au(III). J Mol Struct 831:165–173

Kozlowski H, Bal W, Dyba M, Kowalil-Jankowska T (1999) Specific structure–stability relations in metallopeptides. Coord Chem Rev 184:319–346

Köseoğlu F, Kılıç E. Doğan A (2000) Studies on the protonation constants and solvation of α-amino acids in dioxan–water mixture. Anal Biochem 277:243–246

Lomozik L (1984) A study of complex equilibria of phenylglycine with nickel(II), copper(II) and zinc(II) in water and water–methanol solution. Monatsh Chem 115:921–926

Lyons AQ, Pettit LD (1985) Formation constants of silver (I) complexes of some sulphur-containing dipeptides and valylvaline. J Chem Soc Dalton Trans 102:2305–2308

Marcus Y (1984) The effectivity of solvents as electron pair donors. J Solut Chem 13:599–624

Marcus Y (2001) Preferential solvation in mixed solvents X. Completely miscible aqueous co-solvent binary mixtures at 298.15K. Monatsh Chem 132:1387–1411

Marcus Y (2005) The standard partial molar volumes of ions in solution. Part 3. volumes in solvent mixtures where preferential solvation takes. J Solut Chem 34:317–331

Martell AE, Motekaitis RJ (1988) The determination and use of stability constants. VCH, Weinheim

Martell AE, Smith RM (1974) Critical stability constants. Plenum, New York

Meloun M, Havel J, Högfelt H (1988) Computation of solution equilibria. Wiley, New York

Mendieta J, Diaz-Cruz MS, Tauler R, Esteban M (1996) Application of multivariate curve resolution to voltametric data. II. Study of metal-binding properties of the peptides. Anal Biochem 240:134–141

Nishi N, Takahashi S, Matsumoto M, Tanaka A, Muraya K, Taramuku T, Yamaguchi T (1995) Hydrogen-bonded cluster formation and hydrophobic solute association in aqueous solutions of ethanol. J Phys Chem 99: 462–468

Perrin DD, Armerega WLF (1991) Purification of laboratory chemicals. Pergamon, Elmsford, New York

Rossotti H (1978) The study of ionic equilibria. Longman, London

Sigel H (1975) Ternary complexes in solution. Inorg Chem 1415:35–1540

Sigel H (ed) (1973) Metal ions in biological systems. Marcel Dekker, New York, vol 2

Sigel H, Martin RB (1982) Coordination properties of the amide bond. Stability and structure of metal ion complexes of peptides and related ligands. Chem Rev 82:385–426

Varnagy K, Süli-Vargha H, Champay A, Sanna D, Micerra G, Sovago I (2004) Acid-base properties and copper(II)complexes of dipeptides containing histidine and additional chelating bis(imidazol-20yl) residues. J Inorg Biochem 98:24–32

Woolley EM, Hukot DG, Hepler LG (1970) Ionization constant for water in aqueous organics mixture. J Phys Chem 74:3908–3913