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The Activity Coefficients of Amino Acids and Peptides in Aqueous Solutions Containing Guanidinium Chloride

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Six systems of the type amino acid- or peptide-guanidinium chloride-water have been investigated over wide solute molality ranges using vapor pressure osmometry. The amino acids used were glycine and L-leucine, while the peptides were diglycine, triglycine, glycyl-L-leucine and L-leucyl-L-leucine. Equations for the ratios of the activity coefficients of these compounds in the salt solutions and water, respectively, were obtained in terms of the molalities of the solutes. The activity coefficient ratios for glycine are not much below one, whereas those for L-leucine are considerably smaller reflecting the presence of the leucyl side chain. The activity coefficient ratios for the peptides are generally smaller than those for the amino acids which can be attributed to the presence of the peptide group.

INTRODUCTION

Guanidinium chloride is a strong denaturant of proteins, i. e., it produces large conformational changes in protein molecules and ultimately complete unfolding[1]. It can be expected that this activity be reflected in thermodynamic quantities referring to its interaction with amino acids and peptides occurring in proteins. The relevant quantities are the change in Gibbs free energy, enthalpy, and entropy accompanying the transfer of amino acids and peptides from water to aqueous guanidinium chloride solutions. In our previous research, Gibbs free energies for the transfer of several amino acids and peptides were determined from solubility data[2,3]. The values determined thus referred to saturated amino acid and peptide solutions at various guanidinium chloride solutions, and they truly accounted semiquantitatively for the values of the same quantities of transfer observed with proteins. However, since the activity coefficients have not been considered, Gibbs free energies of transfer have been designated as »apparent«.

It therefore appeared worthwhile to do research on the activity coefficients of the same amino acids and peptides in guanidinium chloride solutions. The determination of their values does allow calculation of true Gibbs energies of transfer and thus, if necessary, modification or correction of conclusions based upon the apparent values. The research is also of a general physico-chemical interest. It has to be realized that regardless of the experimental method chosen it may be extremely difficult in many cases to arrive at

accurate values of the activity coefficient considering the high concentration of both, i. e., the amino acid and the denaturant. Similar studies of 3-component systems have been carried out in the past[4–6]. Activity coefficients were determined by using the isopiestic vapor pressure method. We decided to apply vapor pressure osmometry in our research. The method proved valid for a wide variety of electrolytes[7]. Speed and convenience of measurement are distinct advantages of the method in comparison with the isopiestic vapor pressure method, although the accuracy of the results obtained is generally lower. However, if indicated, isopiestic measurements can subsequently be performed.

EXPERIMENTAL

Amino acids and peptides used in this research were supplied by Sigma Chemical Company (St. Louis, Missouri). They were not additionally purified. Guanidinium chloride was a purest grade product from Merck (Darmstadt). Its 10.6 m (6 mol/dm^3) solution had an absorbance below 0.1 at 280 nm.

Osmotic coefficients were determined by vapor pressure osmometry. The Knauer (Berlin) vapor pressure osmometer was used. The principles of its operation have been described elsewhere[7], and need not be repeated here. However, it should be noted that the experimental quantity measured is the resistance change required to rebalance a Wheatstone bridge whose two arms are thermistor beads. The bridge is balanced when solvent drops are on both beads and then on one of the beads the solvent drop is replaced by a solution drop. The resistance change is related to the temperature change of the bead and thus to the activity of water and the molal osmotic coefficient of the solute, see next section. All measurements were made at 25.00 °C. The calibration constant was determined by using sodium chloride.

RESULTS AND DISCUSSION

The molal osmotic coefficient, φ, is given by the relation[7]

$$\Delta R = \nu K \varphi m \tag{1}$$

where ΔR is the resistance change, ν is the number of ions per molecule, K the instrument calibration constant, and m the molality of solute. For 3-component solutions containing nonelectrolyte, electrolyte and water, Robinson and Stokes[8] developed a method of calculating activity coefficients based on the data obtained by the isopiestic vapor pressure method. They defined a quantity Δ by

$$\Delta = 2\, m_{\mathrm{ref}}\, \varphi_{\mathrm{ref}} - m_{\mathrm{A}}\, \varphi_{\mathrm{A}}^{0} - 2\, m_{\mathrm{B}}\, \varphi_{\mathrm{B}}^{0} \tag{2}$$

where m_{ref} is the molality of the reference solution and φ_{ref} is the corresponding osmotic coefficient; m_{A} and m_{B} are the molalities of the nonelectrolyte and electrolyte, respectively, in the aqueous solutions in isopiestic equilibrium with the given reference solution; φ_{A}^{0} and φ_{B}^{0} are the osmotic coefficients of solutions of nonelectrolyte only and of electrolyte only at the molalities m_{A} and m_{B}. Now using vapor pressure osmometry, the first term in equation (2) has to be replaced by $\Delta R/K$, and by measuring ΔR in 3-component systems values of φ can be obtained. Calculation of the activity coefficients of the two solutes requires further an equation for the variation of $\Delta/m_{\mathrm{A}}\, m_{\mathrm{B}}$ with m_{A} and m_{B}. The experimental values of $\Delta/m_{\mathrm{A}}\, m_{\mathrm{B}}$ were fitted into the following equation by the multiple regression analysis program STEPREG 1[9]

$$\Delta/m_{\mathrm{A}}\, m_{\mathrm{B}} = A + B\, m_{\mathrm{A}} + C\, m_{\mathrm{A}}^{2} + D\, m_{\mathrm{A}}^{3} + E\, m_{\mathrm{B}} + F\, m_{\mathrm{B}}^{2} + G\, m_{\mathrm{B}}^{3} \tag{3}$$

The activity coefficients of both solutes in these solutions, γ_{A} and γ_{B}, are related to $\Delta/m_{\mathrm{A}}\, m_{\mathrm{B}}$ by the following equations[8]

$$\Delta/m_A m_B = \left[\frac{\delta \ln \gamma_A}{\delta m_B}\right]_{m_A} = 2 \left[\frac{\delta \ln \gamma_B}{\delta m_A}\right]_{m_B} \quad (4)$$

Integration of equation (4) then leads to polynomials representing dependence of $\ln \gamma_A$ and $\ln \gamma_B$ on m_A and m_B:

$$\ln \frac{\gamma_A}{\gamma_A{}^0} = m_B \left[A + B m_A + C m_A{}^2 + D m_A{}^3 + \frac{1}{2} E m_B + \frac{1}{3} F m_B{}^2 + \frac{1}{4} G m_B{}^3\right] \quad (5)$$

and a similar equation for $\ln \frac{\gamma_B}{\gamma_B{}^0}$. In these equations, $\gamma_A{}^0$ and $\gamma_B{}^0$ refer to the activity coefficients in solutions not containing the other solute.

As we may conclude from equation (2), evaluation of Δ requires the knowledge of the osmotic coefficients of both solutes, i. e., the nonelectrolyte and the electrolyte, in aqueous solutions containing one of them only. Their values were determined by vapor pressure osmometry using equation (1). Comparison of the values for glycine, diglycine, and triglycine with those calculated from the polynomials of m given in the literature and based on the data obtained by the isopiestic vapor pressure method, displays agreement ranging between 0.5 and 2.0 per cent depending on the solute and concentration range[6,10]. We can also arrive at the latter figure by considering the random and systematic errors involved[7]. For glycyl-L-leucine, an equation for the osmotic coefficients, obtained from a least-squares treatment of the data, is $\varphi = 1 + 0.1441\ m - 1.0270\ m^2 + 5.2561\ m^4$. For L-leucine and L-leucyl-L-leucine low solubility does not allow determination of φ by vapor pressure osmometry, and no data are available in the literature. The values of $\gamma_A{}^0$ were therefore put equal to one. For the electrolyte, i. e., guanidinium chloride, the values of φ were taken from the literature[11].

The coefficients to be used in equation (5) for glycine, L-leucine and the peptides in aqueous guanidinium chloride solutions and the concentration range are presented in Table I. In Figures 1 and 2 the logarithms of the activity coefficient ratio of the compounds studied are plotted as a function of guanidinium chloride molality. The standard errors of regression coefficients, cf. Table I, are relatively large. This is due to the random and systematic errors involved. Comparison with similar data obtained by the isopiestic vapor pressure method shows that the errors observed with the latter method are generally smaller[4-6]. Therefore, when more accurate data are needed application of the isopiestic method is mandatory.

Comparison of the data for aqueous guanidinium chloride solutions of glycine, diglycine, and triglycine with similar data for aqueous sodium chloride solutions can now be made. For glycine, the values of $\ln (\gamma_A/\gamma_A{}^0)$ in sodium chloride solutions differ greatly from those in guanidinium chloride solutions, and in both they decrease with increasing electrolyte concentration[5]. However, in 3 m guanidinium chloride a weakly expressed minimum appears, see Figure 1. For sodium chloride, the data do not extend beyond 3 m. For diglycine and triglycine in sodium chloride solutions only the trace activity coefficients, i. e., the limiting values at zero peptide molality, are available. They display well-expressed minima at relatively low electrolyte concentrations, i. e., below 0.25 m, and then they steeply increase. Different behavior is observed

TABLE I

Coefficients for Representation of $\Delta/(m_A m_B)$ *and* $\ln \gamma_A/\gamma_A^0$ *by Equations* 3 *and* 5 *for Each of Systems Studied*

Compound A	Coefficients							Concentration Range (mol kg^{-1})	
	A	*B*	*C*	*D*	*E*	*F*	*G*	Compound A	Guanidinium Chloride
Glycine	7.42±1.9	—17.83±4.4		10.24±2.5	0.074±0.01		—0.0014±0.0006	0.5—1.0	0—4.5
Diglycine	—0.421±0.04			—0.091±0.04	0.197±0.04	—0.021±0.009		0.25—1.0	0—5.0
Triglycine	—0.595±0.03				0.188±0.02		—0.0035±0.001	0.2—0.3	0—4.0
Glycyl-l-leucine	—0.648±0.07				0.471±0.12	—0.151±0.06	0.021±0.008	0.2	0—4.0
L-Leucine	—16.4±0.03	255.4±55		—9986±2000		0.147±0.05	—0.023±0.01	0.08—0.10	0—4.0
L-Leucyl-L-leucine	—1.71±0.16					0.32±0.1	—0.036±0.02	0.03—0.04	0—4.0

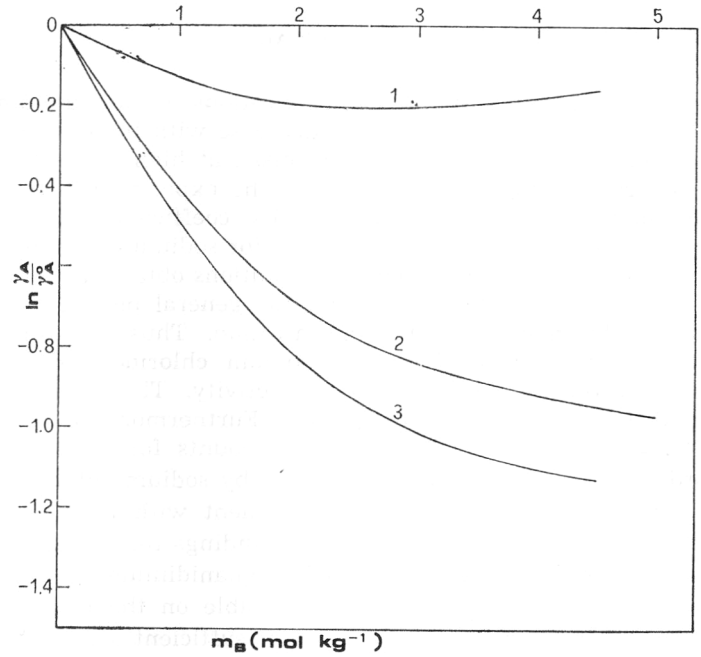

Figure 1. The logarithm of the activity coefficient ratio, ln γ_A/γ_A°, of 1 *m* glycine (Curve 1), 1 *m* diglycine (Curve 2), and 0.25 *m* triglycine (Curve 3) as a function of guanidinium chloride molality.

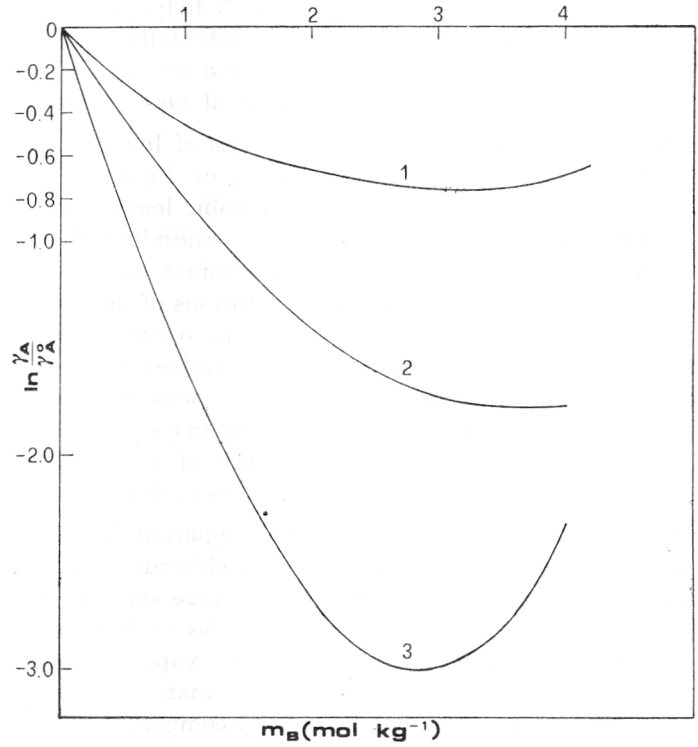

Figure 2. The logarithm fo the activity coefficient ratio, ln γ_A/γ_A°, of 0.2 *m* glycyl-L-leucine (Curve 1), 0.1 *m* L-leucine (Curve 2), and 0.03 *m* L-leucyl-L-leucine (Curve 3) as a function of guanidinium chloride molality.

with diglycine and triglycine at finite concentrations in guanidinium chloride. The values of $\ln(\gamma_A/\gamma_A^0)$ for both peptides decrease with increasing electrolyte concentration, but levelling off may be surmised at high concentrations, i. e., above 5 *m*. Before drawing conclusions from the experimental findings, it is necessary to assess the change of the activity coefficients ratio with nonelectrolyte concentration. Considering the data for sodium chloride solutions[5,6], as well as the data for guanidinium chloride solutions obtained in this research, cf. Table I, it is reasonable to assume that the general picture established is not affected by the nonelectrolyte concentration. Thus the differences in behavior of diglycine and triglycine in sodium chloride and guanidinium chloride solutions reflect their denaturing activity. The former is a non-denaturant and the latter a strong denaturant. Furthermore, there is no doubt that the peptide group, —CH_2—CO—NH—, accounts for different behavior. In other words, the peptide group is salted out by sodium chloride and salted in by guanidinium chloride. This is in agreement with previous findings[12]. Now we will consider the implications of the findings for the apparent Gibbs free energies of transfer from water to aqueous guanidinium chloride solutions. Although a quantitative assessment is not feasible on the basis of the data obtained in this research, since they are not of sufficient accuracy and/or are not available at high denaturant concentrations, it is clear that the true Gibbs free energies of transfer are in general more negative than the apparent ones. Namely, the largest by far correction term is $RT\ln(\gamma_A/\gamma_A^0)$, and for all our 3-component solutions its values are negative[13]. Incidentally, the term represents the Gibbs free energy of transfer of an amino acid or peptide from water to aqueous salt solution at specified concentrations of both solutes.

For L-leucine and L-leucyl-L-leucine the values of $\ln(\gamma_A/\gamma_A^0)$ are distinctly more negative than for diglycine and triglycine, cf. Figure 2. This behavior undoubtedly reflects the presence of the hydrophobic leucyl side chain, $(CH_3)_2$—CH—CH_2—, which however is salted in by guanidinium chloride. For the dipeptide a minimum appears at 2.7 *m* guanidinium chloride, whereas for L-leucine it is only indicated. At higher concentrations of denaturant, levelling off of the activity coefficients ratio is likely to occur, since guanidinium chloride usually produces denaturation at concentrations above 6 *m*. The true Gibbs free energies of transfer are thus also more negative that the apparent ones. For glycyl-L-leucine at 0.2 *m* the values of $\ln(\gamma_A/\gamma_A^0)$ are less negative than could be expected considering the presence of the peptide group and the leucyl side chain for which no immediate explanation is available.

In conclusion, it may be claimed that the apparent Gibbs free energies of transfer from water to aqueous guanidinium chloride solutions for amino acids and peptides are sufficiently close to the true ones to allow a semi-quantitative assessment of the Gibbs free energies of transfer for proteins based on the amino acid composition. Furthermore, vapor pressure osmometry proved very convenient for obtaining fast approximate values of the activity coefficients of amino acids and dipeptides in 3-component systems.

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POVZETEK

Aktivnostni koeficienti aminokislin in peptidov v vodnih raztopinah gvanidinijevega klorida

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Raziskali smo šest sistemov vrste aminokislina- oz. peptid-gvanidinijev klorid--voda, pri čemer smo uporabljali osmometrijo na parni tlak. Aminokisline so bile glicin in L-levcin, peptidi pa diglicin, triglicin, glicil-L-levcin in L-levcil-L-levcin. Na osnovi dobljenih rezultatov smo izpeljali enačbe za razmerja aktivnostnih koeficientov teh spojin v raztopini soli in vodi kot funkcije molalnosti topljencev. Razmerja aktivnostnih koeficientov za glicin imajo vrednosti blizu ena, medtem ko so za L-levcin precej manjše, kar je pripisati prisotnosti levcilove stranske verige. Razmerja aktivnostnih koeficientov za peptide so na splošno manjša kot za aminokisline, kar gre pripisati prisotnosti peptidne skupine. V skladu s prejšnjimi dognanji je tako potrjeno, da gvanidinijev klorid poveča topnost tako peptidne skupine kakor tudi levcilove stranske verige.

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