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J. Chem. Thermodynamics 38 (2006) 240–244
JCT
www.elsevier.com/locate/jct

Solubility of proline–leucine dipeptide in water and in aqueous sodium chloride solutions from $T = (288.15 \text{ to } 313.15)$ K

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Received 18 February 2005; received in revised form 5 May 2005; accepted 6 May 2005

Abstract

Solubility of proline–leucine dipeptide, in water and in aqueous sodium chloride solutions, was measured at $T = (288.15, 298.15, 308.15 \text{ and } 313.15)$ K as a function of electrolyte concentration $m = (0.1, 0.25, 0.5, 0.75 \text{ and } 1)\ \text{mol} \cdot \text{kg}^{-1}$ of water. Solubility data has been evaluated from density measurements using a vibrating tube densimeter. It has been observed that sodium chloride renders the dipeptide proline–leucine more soluble in water. Salting-in coefficients and standard free energies of transfer of proline–leucine, from water to aqueous sodium chloride solutions, have been calculated from the solubility data. Standard enthalpies and entropies of transfer have also been estimated and interpreted in terms of electrostatic and hydrophobic perturbed domains in the hydration shells of the dipeptide and of the cation and anion of the salt, as a function of temperature and salt concentration.

Keywords: Density; Solubility; Salt effect; Solute-solvent interactions; Proline–leucine dipeptide; Aqueous solutions

1. Introduction

Intermolecular interactions between water and protein constituent hydrophilic and hydrophobic groups, such as those existing in peptides side chains, have shown to play an important role to clarify the stability conditions of bigger proteins in aqueous media [1–4].

It has been shown that the addition of co-solutes such as electrolytes, surfactants or other biomolecules to aminoacids and small peptides in aqueous solution, have a strong effect on the hydration of these solutes, generating important changes in their ability to bind other molecules [5–9]. Salting-in and salting-out phenomena, related to the observed changes in the solubility of a nonelectrolyte, produced by the addition of electrolytes to aqueous solutions, have been studied and tentatively correlated with the interactions present in solution before and after the addition of the electrolyte [10–16]. The interplay between the solvation of the solute and solute–solute interactions and the knowledge about how solvated species interact with each other are some problems that are not yet very well clarified [17].

The direct correspondence between solubility ratio and the standard chemical potential of transfer of a nonelectrolyte, from water to aqueous electrolyte solution, has been explored in terms of the overall changes in the interactions present in the standard state solutions [12,15]. In most cases, the small values of these standard chemical potentials result from the almost cancellation of the entropy and enthalpy contributions. However, these separate contributions can be calculated provided that solubility data, at different temperatures, are available [15]. Few studies exist in literature concerning the effect of temperature on the solubility of peptides in water and in electrolyte solutions.

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0021-9614/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jct.2005.05.009

In this work we have chosen the dipeptide proline–leucine (P–L) with a significant hydrophobic moiety, to study the interplay of the different interactions in water and in sodium chloride aqueous solutions. Solubility at four temperatures has been obtained using an improved method, based on density determinations. The estimation of standard enthalpies and entropies of transfer of P–L, from water to NaCl aqueous solutions lead us to conclude that the electrostatic interactions between the ions and the charged groups of the zwitterionic form of the dipeptide favors the stabilization of these molecules in aqueous solution.

2. Experimental

Proline–leucine, in the form of a crystalline hydrate, $C_{11}H_{20}N_2O_3 \cdot 0.2H_2O$($M_r$ = 231.891) with a quoted purity of >0.99 mass fraction (determined by CHN-Analysis and TLC) was purchased from Bachem AG and was used without further purification. Water was ion-exchanged (18 MΩ · cm) from a Milli-Q reagent-grade system supplied by Millipore. The sodium chloride used was from Riedel-de Haën p.a. with a quoted purity >0.995 mass fraction. It has been dried at 120 °C in a vacuum oven for several hours. Chlorobenzene (used in the calibration of the densimeter) was a Merck *pro analysis* product (quoted purity >0.99 mass fraction) and was further purified and fractionally distilled under reduced pressure.

NaCl aqueous solutions of approximate molalities of m = (0.1, 0.25, 0.5, 0.75 and 1.0) $\text{mol} \cdot \text{kg}^{-1}$ were prepared in volumetric flasks of 1000 cm^3 and used as solvents. Their exact molalities were determined by density measurements and interpolation on a calibrating curve, built with literature and our own unpublished data [18–21].

Saturated solutions were prepared by equilibrating P–L with water and with the previously prepared NaCl_{aq} solvents in 100 ml volumetric flasks. Flasks were placed in a constant temperature bath controlled to ±0.01 °C, for about 2 days at T = (288.15, 298.15, 308.15 and 313.15) K. Submersible magnetic stirrers were used for agitation. The agitation has been stopped for 12 h after equilibrium had been reached and the solutions were left to rest for about 12 h. Samples of the aqueous phases were then extracted using a hypodermic needle, which had been previously heated and inserted into the aqueous phase. Their densities were then measured, at T = 318.15 K, using an Anton Paar Model 60/602 vibrating tube densimeter. The instrument was calibrated with water, chlorobenzene and NaCl aqueous solutions, of accurately known concentrations, assuring that their densities were higher than those of the working solutions. More details of the procedure used in measuring densities were previously described [22]. The temperature control of the densimeter was made with a circulating water bath, regulated to ±0.005 K. The temperature was measured at the entrance of the cell with a platinum resistance thermometer calibrated against a precision standard platinum resistance thermometer (SPTR) calibrated by the National Physical Laboratory in the international temperature scale ITS-90. The uncertainty in the temperature measurement was ±0.02 K.

TABLE 1
Densities of saturated (sat.) solutions of P–L in water and in NaCl_{aq} solutions, at T = 318.15 K

$m_{\text{NaCl}_{\text{aq}}}$ ($\text{mol} \cdot \text{kg}^{-1}$)	Saturated solutions	d ($\text{kg} \cdot \text{m}^{-3}$)
0	sat(288.15 K)	990.77
0	sat(298.15 K)	990.79
0	sat(308.15 K)	990.84
0	sat(313.15 K)	990.85
0.1004	sat(288.15 K)	994.80
0.1006	sat(298.15 K)	994.83
0.1007	sat(308.15 K)	994.88
0.1004	sat(313.15 K)	994.90
0.2526	sat(288.15 K)	1000.78
0.2523	sat(298.15 K)	1000.80
0.2525	sat(308.15 K)	1000.86
0.2523	sat(313.15 K)	1000.88
0.5063	sat(288.15 K)	1010.51
0.5067	sat(298.15 K)	1010.55
0.5070	sat(308.15 K)	1010.61
0.5063	sat(313.15 K)	1010.63
0.7646	sat(288.15 K)	1020.16
0.7645	sat(298.15 K)	1020.19
0.7651	sat(308.15 K)	1020.28
0.7654	sat(313.15 K)	1020.35
1.0239	sat(288.15 K)	1029.67
1.0218	sat(298.15 K)	1029.63
1.0228	sat(308.15 K)	1029.74
1.0234	sat(313.15 K)	1029.85

The reproducibility and accuracy of the measurements were found to be $5 \cdot 10^{-3}$ $\text{kg} \cdot \text{m}^{-3}$ and $2 \cdot 10^{-2}$ $\text{kg} \cdot \text{m}^{-3}$, respectively.

At least three samples of each flask were measured. In the most part of the cases duplicated flasks were prepared. In some cases different solvents with approximately the same NaCl concentration have been used.

Saturated solution densities were measured at T = 318.15 K assuring that no precipitation occurred during the density measurement. Values obtained are presented in table 1.

3. Results and discussion

3.1. Solubility

Some years ago in our laboratory a density method has been used, for the first time, to determine solubility of non-electrolytes in aqueous electrolyte solutions [14].