Available online at www.sciencedirect.com
SCIENCE DIRECT®

Thermochimica Acta 438 (2005) 130–133

thermochimica acta

www.elsevier.com/locate/tca

Influence of isoleucyl dipeptide side-chains on the dissociation and intermolecular interactions in water at 298.15 K

S. Vecchio [a,*], M.L. Antonelli [b], F. Fantauzzi [c], F. Rodante [a], C. Spadaro [b]

[a] *Dipartimento di Ingegneria Chimica dei Materiali delle Materie Prime e Metallurgia, Università La Sapienza, Via del Castro Laurenziano 7, 00161 Roma, Italy*
[b] *Dipartimento di Chimica, Università La Sapienza, P.le Aldo Moro 5, 00185 Roma, Italy*
[c] *Dipartimento di Chimica, Ingegneria Chimica e Materiali, Facoltà di Ingegneria, Università degli Studi de l'Aquila, Monteluco di Roio, L'Aquila, Italy*

Received 1 March 2005; received in revised form 27 June 2005; accepted 6 August 2005
Available online 3 October 2005

Abstract

Enthalpies of dissociation of four isoleucyl dipeptides having alkyl side-chains (L-isoleucine-glycine, L-Ile-Gly; L-isoleucine-L-alanine, L-Ile-L-Ala; L-isoleucine-L-valine, L-Ile-L-Val; L-isoleucine-L-isoleucine, L-Ile-L-Ile) in water at 298.15 K have been determined. Enthalpies of dilution have also been calculated. These results were used to derive the enthalpic coefficients of interactions according to the modified McMillan–Mayer's model.

The results are considered in an effort to establish a possible relation between dissociation, intermolecular interactions and the size of the alkyl side-chains.

Keywords: Isoleucyl dipeptides; Enthalpies of dissociation; Enthalpies of dilution; Enthalpic coefficients of interactions

1. Introduction

The thermodynamic properties of aqueous solutions of dipeptides are particularly useful as simple models for protein chains. Dipeptides allow the mutual influence of different α-amino acids to be studied. Calorimetric studies have been carried out in the past for series of dipeptides having valine, glycine, alanine, leucine and methionine as N-terminal amino acid [1–5], and over the last 20 years [6–15] extensive research on the thermodynamics of dipeptides in water has been done to assess the role played by two different non-covalent interactions, namely the electrostatic interaction (between COO^- and NH_3^+ groups) the solvophobic interaction (between the side-chains). The first part of the present paper represents a continuation of the research on calorimetric determination of the enthalpy change for dissociation of the free α-amino group and the free α-carboxyl group for some isoleucyl dipeptides. The second part of the paper focuses on the determination of the enthalpic coefficients for the interactions between solvated solute molecules. The dipeptides studied were L-isoleucine-glycine (L-Ile-Gly), L-isoleucine-L-alanine (L-Ile-L-Ala), L-isoleucine-L-valine (L-Ile-L-Val) and L-isoleucine-L-isoleucine (L-Ile-L-Ile). Their structural formulas are given in Fig. 1.

2. Experimental

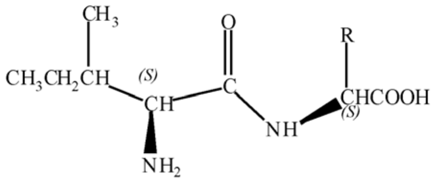
The studied compounds (Calbiochem) were weighed and handled in a nitrogen-filled dry box. The purity of all compounds is over 99% and was checked by means of DSC with a Stanton–Redcroft 625 simultaneous TG/DSC (with dynamic purity program supplied by P.L. Thermal Sciences Ltd.). A Tronac (Model 458) calorimeter with a 100 cm^3 Dewar was used to obtain the enthalpies of dissociation. All the steps of the measurements (calibration curve, cooling curve, and reaction curve at equilibrium temperature) were described elsewhere [16].

Standard chemical test used to check calorimetric system was the standard thermochemical reaction between solid tris-(hydroxymethyl)-amino methane (standard reference compound) and 0.1 M HCl at 298.15 K in water. The obtained mean

---

* Corresponding author. Fax: +39 0 6 49766749.
E-mail address: stefano.vecchio@uniroma1.it (S. Vecchio).

0040-6031/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.tca.2005.08.022



L-Ile-Gly: **R** = H

L-Ile-L-Ala: **R** = CH_3

L-Ile-L-Val: **R** = $CH(CH_3)_2$

L-Ile-L-Ile: **R** = $CH(CH_3)CH_2CH_3$

Fig. 1. Structural formulas of Ile dipeptides studied.

value of $29.427 \pm 0.68\,kJ\,mol^{-1}$ was in agreement with the reported value [17]. HCl solutions used were standardised by titration with a solution of standard Na_2CO_3. NaOH solutions were standardised by titration with a $KHC_3H_4O_4$ solution (analytical grade).

The enthalpimetric measurements of dilution were carried out by means of a batch calorimeter LKB 2107 [18] equipped with two gold vessels of about 7 mL total volume, a multi-temp cooling circulator (LKB 2209), a control unit (LKB 2107-350) and a potentiometric recorder (LKB 2210). Each vessel consists of a chamber partially divided into two compartments of 2.5 and 4.5 mL, respectively, by an interior wall. To start the experiment, the calorimetric unit is rotated, thereby mixing the reactants. All the instrumentation is housed in a thermostatic room at 25 ± 1 °C, all the measurements are performed at 25.00 ± 0.01 °C. The calorimetric accuracy was checked by measuring the sucrose dilution heat [19] and our results were in agreement with the literature values within 0.5%.

The molar enthalpy of dissociation at infinite dilution for the free carboxyl group in water, ΔH_1^0, is obtained by measuring: (a) the molar enthalpy of solution of the crystalline (cr) neutral form in water at pH close to the isoelectric value (pH 5.99 ± 0.12). A buffer solution at pH 6.00 ± 0.02 (Carlo Erba RPE at 298.15 K, KH_2PO_4 and Na_2HPO_4) was used. The concentrations of these salts are in the ratio of 1:10 and about 50 times larger than those of the dipeptides, and (b) the molar enthalpy of protonation of the same compound in 0.02 M HCl solution.

Results of at least six determinations of heats of solution for the examined compounds (concentrations from 10^{-4} to 10^{-3} M) have been extrapolated versus the square root of concentrations to infinite dilution (ΔH_3^0). Moreover, results of at least six determinations of heats of protonation ΔH_4^0 were extrapolated versus the square root of concentrations of the protonated dipeptide. By subtracting ΔH_4^0 from ΔH_3^0 the molar enthalpy at infinite dilution, ΔH_1^0 can be obtained. These values refer to the proton dissociation of 1 mol of $CH_3CH_2CH(CH_3)CHNH_3^+$ CONHCHRCOOH at infinite dilution, yielding 1 mol of $CH_3CH_2CH(CH_3)CHNH_3^+$ $CONHCHRCOO^-$ and 1 mol of protons. The molar enthalpy at infinite dilution ΔH_2^0 for the second proton dissociation process of $CH_3CH_2CH(CH_3)CHNH_3^+$ $ONHCHRCOO^-$ is obtained by measuring the molar enthalpy of dissolution of the crystalline compound in 0.02m NaOH solution. This molar enthalpy is calculated by subtracting the enthalpy for dissolution in water (ΔH_3^0) and the enthalpy of ionisation of water ($\Delta H_w^0 = 55.94\,kJ\,mol^{-1}$ [20]): $\Delta H_5^0 - (\Delta H_3^0 + \Delta H_w^0)$. These values refer to the proton dissociation of 1 mol of $CH_3CH_2CH(CH_3)CHNH_3^+$ $CONHCHRCOO^-$ at infinite dilution, yielding 1 mol of $CH_3CH_2CH(CH_3)CHNH_2CONHCHRCOO^-$ and 1 mol of protons. Since this process occurs in basic solution, only the form $CH_3CH_2CH(CH_3)CHNH_2CONHCHRCOO^-$ is present.

The excess enthalpy, $H^E(m)$, of a solution containing only one solute species can be expressed in terms of m moles of the solute for 1 kg of water as

$$H^E(m) = h_{xx}m + h_{xxx}m^2 \tag{1}$$

where h_{xx} is the enthalpic coefficient, which measures the enthalpic contribution of interactions between pairs of solvated solute molecules and h_{xxx} is related to triplet interactions. For the dilution of a solution of initial molality m_i to give a solution of final molality m_f, the molar enthalpy of dilution, $\Delta_{dil}H_m$, is given by the expression

$$\Delta_{dil}H_m = H^E(m_f) - H^E(m_i) \tag{2}$$

By combining Eqs. (1) and (2) it follows that

$$\Delta_{dil}H_m = h_{xx}(m_f - m_i) + h_{xxx}(m_f - m_i)^2 \tag{3}$$

A non-linear regression based on a least-square treatment of Eq. (3) enables the enthalpic coefficients h_{xx} and h_{xxx} to be determined according to the modified McMillian–Mayer model [21,22]. The results of this second part of the paper have been mainly interpreted in terms of the pair associations between solvated solute molecules.

3. Results and discussion

The discussion on the enthalpy values, which follows, is based on the assumption that the investigated dipeptides are in the β-type conformation in their acid, neutral and basic species. In such a β-conformation, both the α hydrogen bonds lie in the same plane of the amine bond. For L–L dipeptides the large side-chains lie in the different side of the molecules and therefore may not interact with each other, unlike in the D–L isomer where they lie on the same side. In L–L conformation, these (hydrophobic) forces are not effective and the driving forces are the solvation and desolvation processes in amino and carboxyl groups of zwitterions, ions and neutral molecules.

The experimental enthalpy change for solution ΔH_3^0, protonation ΔH_5^0 and neutralisation ΔH_5^0 are reported in Table 1. The ionisation enthalpy changes for the free carboxyl groups (ΔH_1^0) and for the free amino groups (ΔH_2^0) of the dipeptides are given in Table 2 together with the enthalpy changes related to the corresponding free α-amino acids previously reported in ref. [23]. The enthalpy changes obtained from this study are reported with

Table 1
Experimental enthalpy changes (kJ mol^{-1}) of dissolution in water, acid and basic solution at 298.15 K

Compounds	ΔH_3^0	ΔH_4^0	ΔH_5^0
L-Ile-Gly	−6.2	−3.5	−12.6
L-Ile-L-Ala	−15.7	−15.5	−24.9
L-Ile-L-Val	−15.4	−13.6	−12.0
L-Ile-L-Ile	−24.2	−31.2	−33.9

Standard deviations for our reported values are <±0.6 kJ mol^{-1}.

Table 3
Enthalpic coefficients of Eq. (3) and relative standard deviations in water at 298.15 K

Compounds	h_{xx} (J kg mol^{-2})	h_{xxx} (J kg^2 mol^{-3})
L-Ile-Gly	1772 ± 16	−45 ± 15
L-Ile-L-Ala	2088 ± 20	−1360 ± 80
L-Ile-L-Val	3093 ± 35	−1307 ± 75
L-Ile-L-Ile	5018 ± 56	−2743 ± 150

their total uncertainties (calorimetric, chemical and extrapolation). L-Ile-L-Ile, which has a symmetrical structure, is chosen as reference compound to study the reciprocal influence of L-Ile and other α-amino acids in this series of dipeptides. The first “relative” scale gives a measure of the effect of L-Ile upon the dissociation of other α-amino acids by assuming L-Ile-L-Ile as reference compound, while the scale for the second ionisation process shows how α-amino acids may affect L-Ile dissociation.

By comparing the relative enthalpies of both the dipeptides and the free α-amino acids, the influence of the N-terminal L-Ile moiety on the proton transfer process of the carboxyl group of each C-terminal amino acid moiety can be established. The order of ΔH_1^0 for the dissociation of the carboxyl groups is L-Ile-Gly > L-Ile-L-Val > L-Ile-L-Ala ≫ L-Ile-L-Ile (Table 2). A decrease in the exothermicity of ΔH_1^0 with the increasing size of the alkyl side-chain was found instead of the increasing trend observed for the free α-amino acids.

The proton transfer of the free amino group varies as a function of the C-terminal amino acid moiety. The order of $-\Delta H_2^0$ for dissociation of the free amino groups is: L-Ile-L-Ile ≥ L-Ile-L-Ala > L-Ile-Gly > L-Ile-L-Val (Table 2). On the contrary, the dissociation of the free amino group of the N-terminal L-Ile moiety is not remarkably affected by the presence of the C-terminal amino acids. In fact, similar ΔH_2^0 values are found (Table 2) both in the dipeptide series and when the ΔH_2^0 value of each dipeptide is compared with that of its related free α-amino acid.

The experimental enthalpies of dilution along with the initial and final molalities of dipeptide solutions are presented in Supplementary data. These values are represented in Eq. (3) as a second-degree polynomial function obtained by least-squares method. The derived h_{xx} and h_{xxx} coefficients are given in Table 3. Positive h_{xx} values were obtained in this table for all the dipeptides studied. This result agrees quite well with those reported in literature for other dipeptides with apolar side-chains [24]. As the side-chains are in the vicinity of the charged CO_2^- group in the dipeptide, a further positive contribution to h_{xx} term can be due to the pairwise electrostatic interactions between the positively charged amino group of a given dipeptide and the negatively charged carboxyl group of another one close to it [24]. This process is accompanied by overlapping of the solvation co-spheres of the solute molecules, resulting in a partial rearrangement of the solvation co-spheres in the proximity of the apolar side-chains of the dipeptides. When dipeptides have apolar side-chains, the h_{xx} coefficient usually increases linearly as the number of carbon atoms in the side-chains (represented as the number of equivalent methylene groups n_{CH_2}) increases. To determine the n_{CH_2} for each dipeptide it has been assumed that CH_3 and CH groups are equivalent to 1.5 and 0.5 CH_2 groups, respectively [25].

Table 2
Enthalpy changes (kJ mol^{-1}) of proton ionizations for the studied dipeptides and for the relative free α-amino acids in water at 298.15 K

Dipeptides	ΔH_1^0	ΔH_2^0	Free α-amino acids	ΔH_1^{0a}	ΔH_2^{0a}
L-Ile-Gly	−2.7	49.5	Gly	3.93	44.32
L-Ile-L-Ala	−0.2	46.8	L-Ala	2.42	46.16
L-Ile-L-Val	−1.7	59.0	L-Val	0.71	33.73
L-Ile-L-Ile	7.0	46.2	L-Ile	−0.08	46.37

Standard deviations for our reported values are <± 0.8 kJ mol^{-1}.
[a] Taken from ref. [23].

A linear relationship between the h_{xx} values and the corresponding n_{CH_2} of the side-chains is obtained for the dipeptides examined,

$$h_{xx} = (1130 \pm 180) + (529 \pm 55) n_{CH_2}$$

The enthalpic coefficients h_{xxx}, are negative for all the considered dipeptides and are related to multiple interactions that are difficult to interpret [26].

4. Conclusions

According to the obtained experimental results the following conclusions may be drawn. L-Ile-L-Ile dipeptide shows an enthalpy change for the first proton ionisation process less favourable than the ΔH_1^0 values of the other considered dipeptides, while the corresponding free α-amino acids show an opposite ΔH_1^0 trend. By contrast, the comparable ΔH_2^0 values of the considered dipeptides indicate that the ionisation of the terminal amino group is not particularly influenced by the presence of the different C-terminal amino acids. In this study positive h_{xx} coefficients related to pairwise interactions confirm the literature data regarding dipeptides with hydrophobic side-chains and linearly increase with the length of the side-chain. These results have been interpreted either in terms of a partial rearrangement of the solvation co-spheres near the apolar side-chains of the dipeptides and of electrostatic interactions between the positively charged amino group or the negatively charged carboxyl group of two adjacent solute molecules [27].

Acknowledgement

Authors are grateful to the Italian M.I.U.R. for financial support.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tca.2005.08.022.

References

[1] F. Rodante, F. Fantauzzi, Thermochim. Acta 154 (1989) 279.
[2] F. Rodante, F. Fantauzzi, Thermochim. Acta 176 (1991) 277.
[3] F. Rodante, F. Fantauzzi, G. Catalani, Thermochim. Acta 296 (1997) 15.
[4] F. Rodante, F. Fantauzzi, G. Catalani, Thermochim. Acta 311 (1998) 43.
[5] F. Rodante, S. Vecchio, F. Fantauzzi, J. Therm. Anal. Calorim. 66 (2001) 79.
[6] C. Jolicoeur, J. Boileau, Can. J. Chem. 56 (1978) 2707.
[7] S. Cabani, G. Conti, E. Matteoli, M. Tiné, J. Chem. Soc., Faraday Trans. 77 (1981) 2385.
[8] K. Mishra, J.C. Ahluwalia, J. Phys. Chem. 88 (1984) 86.
[9] G.R. Hedwig, J.F. Reading, T.H. Lilley, J. Chem. Soc., Faraday Trans. 87 (1991) 1751.
[10] G.R. Hedwig, J. Chem. Soc., Faraday Trans. 89 (1993) 2761.
[11] G.R. Hedwig, H. Hoiland, Biophys. Chem. 49 (1994) 175.
[12] A.W. Hakin, M.M. Duke, L.L. Groft, J.L. Marty, M.L. Rushfeldt, Can. J. Chem. 73 (1995) 725.
[13] A.W. Hakin, M.M. Duke, J.L. Marty, K.E. Preuss, J. Chem. Soc., Faraday Trans. 90 (1994) 2027.
[14] M.L. Antonelli, V. Carunchio, E. Cernia, R. Purrello, J. Inorg. Biochem. 37 (1989) 201.
[15] A.C. Palacios, M.L. Antonelli, C. La Mesa, Thermochim. Acta 418 (2004) 69.
[16] F. Rodante, A. Onofri, R. Perticaroli, Thermochim. Acta 124 (1988) 185.
[17] J.O. Hill, G. Ojelund, I. Wadso, J. Chem. Thermodyn. 1 (1969) 111.
[18] I. Wadsö, Acta Chem. Scand. 22 (1968) 927.
[19] P. Monk, I. Wadsö, Acta Chem. Scand. 22 (1968) 1842.
[20] F. Rodante, F. Rallo, P. Fiordiponti, Thermochim. Acta 6 (1973) 369.
[21] W.G. McMillan Jr., J.E. Mayer, J. Chem. Phys. 13 (1945) 276.
[22] J.E. Desnoyers, G. Perron, L. Avedikian, J.-P. Morel, J. Solution Chem. 5 (1976) 631.
[23] F. Rodante, Thermochim. Acta 149 (1989) 157.
[24] J.F. Reading, G.R. Hedwig, Thermochim. Acta 242 (1994) 41.
[25] G. Castronuovo, V. Elia, C. Giancola, S. Puzziello, J. Solution Chem. 19 (1990) 855.
[26] G. Barone, C. Giancola, Pure Appl. Chem. 62 (1990) 57.
[27] B. Palecz, J. Am. Chem. Soc. 124 (2002) 6003.