Journal of Thermal Analysis and Calorimetry, Vol. 68 (2002) 775–786

THERMODYNAMICS OF DIPEPTIDES IN WATER
Influence of the hydrophobic forces of the side-chains on the dissociation processes for some series of dipeptides

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(Received July 3, 2001; in revised form November 30, 2001)

Abstract

A calorimetric study has been carried out on the dissociation processes in water of both the free α-amino group belonging to the amino terminal residue and the free α-carboxyl groups belonging to the carboxyl terminal residue for a series having phenylalanine as the first common term.

These thermodynamic quantities have been compared with those of the proton dissociation processes related to the same groups of the corresponding single α-amino acids. The dissociation process values of this series have also been considered in connection with those obtained from others series of dipeptides previously studied, in an effort to compare the alkyl groups and benzene ring hydrophobic effects on the solvation processes of the zwitterions, undissociated molecules and ionic forms.

Keywords: acidity and basicity scales, dipeptides, calorimetry, hydrophobic effects of side-chains

Introduction

Dipeptides which are compounds made up of two α-amino acids are the smallest part of protein chains. For this reason dipeptides allow the mutual influence of different α-amino acids to be easily studied. Calorimetric study of this influence in series of dipeptides having valine, glycine, alanine, leucine and methionine as first common component, was the subject of previous papers [1–5]. This aim was achieved by means of calorimetric determination of enthalpy change values related to the proton transfer processes of the components of these series with respect to valyl–valine, alanyl–alanine, glycil–glycine, leucyl–leucine and methionyl–methionine dipeptides.

On the other hand, in the last 20 years [6–13] an extensive research concerning the thermodynamics of dipeptides in water was carried out. Concluding remarks of many papers stressed on the possibility to assess the role played by two different non-covalent in-

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1418–2874/2002/ $ 5.00

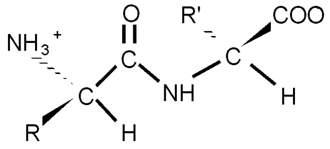
Akadémiai Kiadó, Budapest
Kluwer Academic Publishers, Dordrecht

teractions, namely the electrostatic interaction (between COO^- and NH_3^+ groups) and the solvophobic interaction (between the side chains) on the thermodynamic stereoselectivity in the proton complex formation. This study is based on the calorimetric determination of the enthalpy values of the dissociation processes of the free α-amino group belonging to the amino terminal residue (N-terminal) and the free α-carboxyl group belonging to the carboxyl terminal residuum (C-terminal) for a series having phenylalanylalanine (PhePhe) as the first common term. These values were compared with those of the proton dissociation processes related to the same groups of the phenylalanyl-phenylalanine and of the corresponding single α-amino acids.

The dipeptides studied were *L*-phenylalanine–*L*-alanine (PheAla), *L*-phenylalanine–*L*-glycine (Phe-Gly), *L*-phenylalanine–*L*-isoleucine (Phe-Ile), *L*-phenylalanine–*L*-valine (Phe-Val), *L*-phenylalanine–*L*-methionine (PheMet), *L*-phenylalanine–*L*-phenylalanine (Phe-Phe), *L*-phenylalanine–*L*-tyrosine (Phe-Tyr), *L*-phenylalanine–*L*-proline (Phe-Pro), phenylalanine–*L*-serine (Phe-Ser).

By using some compounds of the series of *L*–*L* dipeptides previously studied [1–5] the influence on the solvation effects of alkyl groups and the benzene ring representing the common side chains of the considered series was studied. Hydroxy group of phenylalanine–tyrosine was converted to ester in order to prevent its reaction with NaOH.

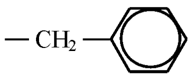
The series is represented by the following structure:

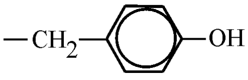


where *R* is Ph-CH_2 and *R′* is:

$—CH_3$; $—H$; $—CH(CH_3)CH_2CH_3$;

$—CH(CH_3)_2$; $—CH_2CH_2SCH_3$;





; $—CH_2CH_2CH_2—$; $—CH_2OH$

To compare the series of dipeptides already studied the pairs considered were:

PheGly/MetGly, PheIle/MetIle, PheLeu/MetLeu, PheMet/MetMet, PheSer/MetSer, PheSer/MetSer, for the series having Phe and Met as the first common term.

PheGly/AlaGly, PheIle/AlaIle, PheLeu/AlaLeu, PheMet/AlaMet, PhePro/AlaPro, PheSer/AlaSer, PheTyr/AlaTyr, for the series having Phe and Ala as the first common term.

PheAla/LeuAla, PheGly/LeuGly, PheIle/LeuIle, PhePro/LeuPro, PheSer/LeuSer, PheTyr/LeuTyr, for the series having Phe and Leu as the first common term.

Experimental and procedure

The compounds (Calbiochem) were weighed and handled in a nitrogen-filled dry box. The purity of all compounds is between 99 and 100% and was checked by means of DSC purity method using Stenton–Redcroft 625 simultaneous TG-DSC (with dynamic purity program supplied by P.L. Thermal Sciences Ltd.) and subsequently by potentiometric titrations.

A Tronac (Model 458) instrument was used to make the measurements. The calorimeter vessel was a rapid response glass vacuum Dewar of capacity 100 cm^3. The thermostat was maintained at 298.1500±0.0004 K during the calorimetric measurement by employing a Tronac P.T.C. 41 precision temperature controller.

Potential *vs.* time measurements were made using a Fluke 88100 model digital voltmeter. To measure the temperature change a thermistor wih a value of 1974 Ω at 298.15 K was used. Its experimental calibration (*T vs.* mV) indicates that it follows the linear equation $T=3.16\cdot10^{-6}$ mV+25. The unbalance (volts) of the bridge of the calorimeter was fed into a Hitachi 561–1000 2/P strip chart recorder and into a digital voltmeter connected to an Olivetti M24 computer.

Data were acquired by the computer via a data-acquisition system and subsequently read and converted into enthalpy values using a basic program run on the Olivetti M24 computer [14]. All the steps of the measurements (calibration curve, cooling curve, and reaction curve and equilibrium temperature) were described elsewhere [14]. Data obtained using the chart recorder were slightly different from those obtained by a computer by virtue of the reading error, and were used only to monitor the shape of the curves temperature *vs.* time.

Standard chemical test system used to check calorimetric system was the standard thermochemical reaction values between solid tris (hydroxymethyl) amino methane (standard reference compound) and 0.1 M HCl at 298.15 K in water. The value obtained (Table 1) is 29.43±0.68 kJ mol^{-1} and was compared with that reported in literature [15] of 29.744 kJ mol^{-1}. The average percentage deviation of the former relative to the latter is 1.1%.

The proton ionisation of the free α-carboxyl group and of the free α-amino group of a generic dipeptide can be represented as:

$$\mathrm{NH_3^+CHRCONHCHR'COOH}(aq)\rightarrow\mathrm{NH_3^+CHRCONHCHR'COO^-}(aq)+\mathrm{H^+}(aq) \quad (1)$$

and

$$\mathrm{NH_3^+CHRCONHCHR'COO^-}(aq)\rightarrow\mathrm{NH_2CHRCONHCHR'COO^-}(aq)+\mathrm{H^+}(aq) \quad (2)$$

Table 1 Partial molar enthalpy of reaction ΔH between solid tris (hydroxymethyl) amino methane (THAM) and 0.1 M $HCl_{(aq)}$ in water at 298.15 K

THAM extent/g	$-\Delta H$/kJ mol^{-1}
0.098	29.40
0.132	29.54
0.144	30.03
0.787	29.92
0.981	27.70
0.775	30.10
1.123	29.30

The molar enthalpy of dissociation at infinite dilution for the free carboxyl groups in water, ΔH_1^0, is obtained by measuring the following quantities:

a) The molar enthalpy of solution of the crystalline (*cr*) $NH_3^+CHRCONHCHR'COO^-$ zwitterion form in water at pH close to the isoelectric value

$$NH_3^+CHRCONHCHR'COO^-(cr) \rightarrow NH_3^+CHRCONHCHR'COO^-(aq) \qquad (3)$$

pH 5.99±0.12 is the mean of the isoelectric pH values; a buffer solution at pH 6.00±0.02 (Carlo Erba RPE at 298.15 K) formed by KH_2PO_4 and Na_2HPO_4 was used. The concentrations of these salts are in the ratio of 1/10 and about fifty times larger than those of dipeptides. So, no variation of pH values for the dissolution of the dipeptides can be hypothesized.

b) The molar enthalpy of protonation of the same compound in 0.02 M of HCl solution according to the following reaction

$$NH_3^+CHRCONHCHR'COO^-(cr)+H^+(aq) \rightarrow NH_3^+CHRCONHCHR'COOH(aq) \qquad (4)$$

Results of at least six determinations of heats of solution for the examined compounds (concentrations from 10^{-4} to 10^{-3} molal) have been extrapolated *vs.* the square root of concentrations at infinite dilution (ΔH_3^0). Moreover, results of at least six determinations of heats of protonation ΔH_4^0 were extrapolated *vs.* the square root of concentrations of the protonated dipeptide.

The molar enthalpy of process (1) ΔH_1^0 at infinite dilution can be obtained by subtracting ΔH_4^0 from ΔH_3^0. These values refer to the proton dissociation of one mole of $NH_3^+CHRCONHCHR'COOH$ at infinite dilution in water, yielding one mole of $NH_3^+CHRCONHCHR'COO^-$ and one mole of protons.

For compounds containing carboxyl and amino groups, the dissociation processes in water become more and more complicated by tautomeric equilibria and zwitterion formation [16–17]. While a generic dipeptide in acid solution can be represented by the form $NH_3^+CHRCONHCHR'COOH$, in a solution approaching pH 7.00 the main species are neutral molecules, which may be either the $NH_2CHRCONHCHR'COOH(aq)$ form or the zwitterion form. Thus, for an acid solution only the $NH_3^+CHRCONHCHR'COOH$ form is represented in Eq. (4) while in Eq. (3) this is not the case. The isoelectric pH values for the compounds examined can be calculated by means of the dissociation constants [18–20]. Therefore, it can be assumed that the zwitterion form in this solution is predominant. In this way, the carboxyl proton dissociation enthalpy values can be calculated.

The molar enthalpy at infinite dilution ΔH_2^0 for the second proton dissociation process of $NH_3^+CHRCONHCHR'COO^-$ is obtained by measuring the molar enthalpy of the neutralisation of the crystalline compound in a $2\cdot10^{-2}$ M NaOH solution.

$$NH_3^+CHRCONHCHR'COO^-(cr)+OH^-(aq)\rightarrow$$
$$NH_2CHRCONHCHR'COO^-(aq)+H_2O \qquad (5)$$

HCl solutions used in Eq. (4) and in the reaction with tris(hydroxymethyl) amino methane was standardized by titration with a solution of standard Na_2CO_3. NaOH solutions used in the Eq. (5) was standardized by titration with a solution of $KHC_8H_4O_4$ (analytical grade). The enthalpy values of ΔH_5^0 process (5) were extrapolated *vs.* the square root of concentrations of the anion form.

If the solution process enthalpy ΔH_3^0 and ΔH_6^0 values for water, (equal to 55.94 kJ mol^{-1} [21]) related to the process (6)

$$H^+(aq)+OH^-(aq)\rightarrow H_2O(l) \qquad (6)$$

are subtracted from ΔH_5^0, then the expression $\Delta H_5^0-(\Delta H_3^0+\Delta H_6^0)$ supplies the enthalpy values of process (2). These values refer to the proton dissociation of one mole of $NH_3^+CHRCONHCHR'COO^-$ at infinite dilution in water, yielding one mole of $NH_2CHRCONHCHR'COO^-$ and one mole of protons.

It has been noted that process (5) occurs in basic solution so that only the $NH_2CHRCONHCHR'COO^-$ form is present. Finally, it was noted that in process (6) the hydroxyl group of alanyl–tyrosine (AlaTyr) was converted into the ester of trifluoroacetate because the heat of neutralisation of process (5) must be referred only to the amino-group.

All the enthalpy values shown in Table 2 are reported with their total uncertainties (calorimetric, chemical and extrapolation).

Let us consider process (1) for *L*-phenylalanyl-phenylalanine compound:

$$NH_3^+CHRCONHCHRCOOH(aq)\rightarrow NH_3^+CHRCONHCHRCOO^-(aq)+H^+(aq) \qquad (1a)$$

By subtracting reaction (1a) from reaction (1) gives the following proton transfer process:

$$NH_3^+CHRCONHCHR'COOH(aq)+NH_3^+CHRCONHCHRCOO^-(aq)\rightarrow$$
$$NH_3^+CHRCONHCHR'COO^-(aq)+NH_3^+CHRCONHCHRCOOH(aq) \qquad (7)$$

Table 2 Enthalpy values (kJ mol^{-1}) of processes (1), (2), (3), (4) and (5) for some dipeptides having phenylalanine as the first common term, in water at 298.15 K

Compounds	ΔH_1^0	ΔH_2^0	ΔH_3^0	ΔH_4^0	ΔH_5^0
PheAla	0.31±0.0	32.35±0.48	–2.58±0.04	–2.89±0.05	–26.18±0.6
PheGly	1.71±0.0	45.59±0.24	–3.41±0.01	–5.12±0.04	–13.76±0.16
PheIle	2.75±0.0	50.55±0.22	–6.61±0.02	–9.36±0.03	–12.00±0.05
PheLeu	–2.13±0.02	35.09±0.23	–1.59±0.02	0.54±0.01	–22.44±0.10
PheMet	3.60±0.06	40.95±0.22	8.24±0.06	4.64±0.01	–6.75±0.02
PhePhe	0.45±0.	43.74±0.23	16.00±0.12	15.55±0.04	3.80±0.01
PhePro	–1.09±0.03	37.24±0.22	4.38±0.03	5.47±0.01	–14.32±0.02
PheSer	1.64±0.09	37.90±0.22	–0.69±0.06	–2.33±0.07	–18.73±0.03
PheTyr	2.54±0.05	62.93±0.24	8.83±0.04	6.29±0.03	–15.82±0.14

Similarly, the proton transfer process related to the second proton dissociation can be written as:

$$\mathrm{NH_3^+CHRCONHCHR'COO^-}(aq)+\mathrm{NH_2CHRCONHCHRCOO^-}(aq)\rightarrow$$
$$\mathrm{NH_2CHRCONHCHR'COO^-}(aq)+\mathrm{NH_3^+CHRCONHCHRCOO^-}(aq) \qquad (8)$$

The enthalpy changes related to processes (7) and (8) can be written as:

$$\delta\Delta H_1^0=\Delta H_1^0(\mathrm{PheSub})-\Delta H_1^0(\mathrm{PhePhe})$$

and

$$\delta\Delta H_2^0=\Delta H_2^0(\mathrm{PheSub})-\Delta H_2^0(\mathrm{PhePhe})$$

where ΔH_1^0(PheSub) and ΔH_1^0(PhePhe) are related to the first ionisation process of dipeptides and of *L*-phenylalanyl-phenylalanine whereas ΔH_2^0(PheSub) and ΔH_2^0(PhePhe) are the values of the second ionisation process for the same compounds.

Results and discussion

The discussion on the enthalpy values which follows is based on the assumption that the investigated dipeptides are in β-type conformation in their acid, neutral and basic species. In such a β-conformation both 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.

For all the compounds studied the enthalpy values of solution ΔH_3^0, protonation ΔH_4^0 and neutralisation ΔH_5^0 are reported in Table 2. In this table the ionization enthalpy values for the free carboxyl groups (ΔH_1^0) and for the free amino groups (ΔH_2^0) of the dipeptides were also given.

L-phenylalanyl–*L*-phenylalanine, which has a symmetrical structure, is chosen as reference compound to study the reciprocal influence of *L*-phenylalanyl and other α-amino acids in this series of dipeptides. The quantities $\delta\Delta H_1^0$ and $\delta\Delta H_2^0$ are reported in Table 3. Thus dissociation processes of the carboxyl and amino groups of the studied dipeptides were compared with those of the corresponding groups of *L*-phenylalanyl-phenylalanine. By using *L*-phenylalanyl–*L*-phenylalanine as reference compound, it is possible to observe that the proton transfer process of the carboxyl group of each α-amino acid components varies as a function of *L*-phenylalanine influence, so that the enthalpy contribution to a relative scale of acidity can be found.

Table 3 Differences in enthalpy values (kJ mol^{-1}) of processes (1), (2), (3), (4) and (5) for the studied dipeptides with respect to the same processes for PhePhe

Compounds	$\delta\Delta H_1^0$	$\delta\Delta H_2^0$	$\delta\Delta H_3^0$	$\delta\Delta H_4^0$	$\delta\Delta H_5^0$
PheAla	–0.14±0.14	–11.39±0.53	–18.58±0.13	–18.44±0.06	–29.98±0.60
PheGly	1.26±0.14	1.85±0.33	–19.41±0.12	–20.67±0.06	–17.56±0.16
PheIle	2.30±0.14	6.81±0.32	–22.61±0.12	–24.91±0.05	–15.80±0.05
PheLeu	–2.58±0.13	–8.65±0.32	–17.59±0.12	–15.01±0.04	–26.24±0.10
PheMet	3.15±0.14	–2.79±0.32	–7.76±0.13	–10.91±0.04	–10.55±0.02
PhePhe	0.00	0.00	0.00	0.00	0.00
PhePro	–1.54±0.13	–6.54±0.32	–11.62±0.12	–10.08±0.04	–18.12±0.02
PheSer	1.19±0.16	–5.84±0.32	–16.69±0.13	–17.88±0.08	–22.53±0.02
PheTyr	2.09±0.14	19.19±0.32	–7.17±0.13	–9.26±0.05	12.02±0.03

The scale for the dissociation processes of the carboxyl groups is: PheLeu>PhePro>PheAla>PhePhe>PheSer>PheGly>PheTyr>PheIle>PheMet. This sequence can be explained by considering the scales for relative solvation of the zwitterions $\delta\Delta H_3^0=\Delta H_3^0$(PheSub)–$\Delta H_3^0$(PhePhe) and of the undissociated molecules $\delta\Delta H_4^0=\Delta H_4^0$(PheSub)–$\Delta H_4^0$(PhePhe).

For the zwitterions the order of solvation is: PheIle>PheGly>PheAla>PheLeu>PheSer>PhePro>PheMet>PheTyr>PhePhe. For the undissociated molecules, the order becomes: PheIle>PheGly>PheAla>PheSer>PheLeu>PheMet>PhePro>PheTyr>PhePhe (Table 3).

By using in this series of dipeptides *L*-phenylalanyl-phenylalanine as reference compound it can be observed that the proton transfer process of the free amino group varies as a function of the second component and supplies the enthalpic contribution to a relative scale of basicity.

The scale for the dissociation of the free amino groups of the dipeptides can be written as: PheAla>PheLeu>PhePro>PheSer>PheMet>PhePhe>PheGly>PheIle> PheTyr.

This can be explained by considering the solvation scales for the zwitterions and the anionic form $NH_2CHRCONHCHR'COO^-$.

The relative solvation scale $\delta\Delta H_5^0=\Delta H_5^0$(PheSub)–$\Delta H_5^0$(PhePhe) shows the following sequence: PheAla>PheLeu>PheSer>PhePro>PheGly>PheIle>PheMet>PhePhe>PheTyr (Table 3).

As far as the first ionisation process is concerned, from the enthalpic point of view it can be observed that PheLeu, PhePro, and PheAla dissociate more easily than *L*-phenylalanyl-*L*-phenylalanine does by virtue of a more intense solvation effect produced by the zwitterions with respect to that of undissociated molecules. For the remaining compounds the solvation of the undissociated molecules prevails with respect to that of the zwitterions.

It is well known that the hydrophobic effects due to amino acids with alkyl groups as side-chains cause reinforcement of the hydrogen bonds between the water molecules surrounding the alkyl groups. For this reason water–water hydrogen bonds in water zones surrounding the alkyl groups of the amino acids are stronger than those in the bulk. This effect is transferred on the hydration layer of the zwitterion, which reinforces the interactions between the water molecules and the amino acid polar portion.

Following this trend for the studied dipeptide series it can be hypothesised that the side-chains of the components reinforce the interactions between the water molecules and the polar portion both for zwitterions and undissociated molecules with respect to the corresponding zwitterion and undissociated molecule of PhePhe. Indeed, it can be noted that all the zwitterions and undissociated molecules of the components of the series are more solvated than those of PhePhe (Table 3). The relative solvation of zwitterions and undissociated molecules are the drawing forces which favours or hinders the dissociation of the components of the series with respect to PhePhe.

In the second ionisation process the amino groups of PheAla, PheLeu, PhePro, PheSer and PheMet are more dissociated than in *L*-phenylalanyl-*L*-phenylalanine. For these compounds the solvation of the ionic form prevails with respect to that of the zwitterions, while for the others the reverse is true.

Ala, Leu, Pro, Ser and Met decrease the enthalpic contribution to the basicity of phenylalanine in the dipeptides with respect to that of the same compound in the reference compound (PhePhe). All the ionic forms of the series with the exception of PheTyr are more solvated than that of PhePhe.

The first ‘relative’ scale gives a measure of the effect of phenylalanine upon the dissociation of other α-amino acids by assuming PhePhe as reference compound, while the scale for the second ionisation process shows how α-amino acids can affect phenylalanine dissociation.

The enthalpy values of the first and second ionisation processes of the corresponding free α-amino acids in aqueous solution have been previously calculated [22]. A further contribution to the comprehension of the reciprocal influence of phenylalanine and other α-amino acids can be supplied from the following relations, which directly compare the two series in absolute scales and supply percentage values of the enthalpy contribution to the acidity and basicity of dipeptides:

$$[\Delta H_1^0(\mathrm{PheSub}) - \Delta H_1^0(\mathrm{Sub})]/\Delta H_1^0(\mathrm{Sub}) \tag{9a}$$

and

$$[\Delta H_2^0(\mathrm{PheSub}) - \Delta H_2^0(\mathrm{Phe})]/\Delta H_2^0(\mathrm{Phe}) \tag{9b}$$

where $\Delta H_1^0(\mathrm{Sub})$ represents carboxyl dissociation process of free amino acids and $\Delta H_2^0(\mathrm{Phe})$ the dissociation process of the amino group of phenylalanine.

Values related to the 'absolute' influence of phenylalanine on the free carboxyl groups of dipeptides and of the α-amino acids upon the amino group of phenylalanine in the dipeptides, obtained using expressions (9a) and (9b) are given in Table 4. These scales supply percentage values of the enthalpic contribution to the acidity and basicity variation in the dipeptides. It can be seen that in the first ionisation process, the influence of phenylalanine favours the dissociation of the free α-carboxyl groups with respect to those of the corresponding dipeptides α-amino acids, for Ala, Gly, Ile, Phe, Pro and Tyr and hinders the same process for the other α-amino acids.

Table 4 Values from Eqs (9a) and (9b)

Compounds	(9a)	(9b)
PheAla	–0.87	–0.29
PheGly	–0.56	0.00
PheIle	–35.31	0.11
PheLeu	2.41	–0.23
PheMet	0.39	–0.10
PhePhe	–0.24	–0.04
PhePro	–4.75	–0.18
PheSer	0.23	–0.17
PheTyr	–3.10	0.38

The influence of other α-amino acids on phenylalanine was also studied from the second relationship (9b). In dipeptides studied the proton dissociation of the free amino group of phenylalanine is favoured by Ala, Leu, Met, Phe, Pro and Ser while Ile and Tyr hinder the proton dissociation of this group.

A further contribution to the comprehension of the reciprocal influence of phenylalanine, methionine, alanine and leucine and the other α-amino acids can be obtained in the following way. Differences in enthalpy ionisation both in the first and second ionisation processes for different series with respect to the series having Phe as first term were calculated (Table 5). As far as the first and second dissociation process are concerned it can be observed that by comparing the two series having Phe and Met as first common terms most of the compounds of the first series dissociate with more difficulty than the second one.

Table 5 Differences in enthalpy values (kJ mol^{-1}) of processes (1), (2), (3), (4) and (5) for various series of dipeptides having Phe, Met, Ala and Leu as first common term.

Compounds	$\delta\Delta H_1^0$	$\delta\Delta H_2^0$	$\delta\Delta H_3^0$	$\delta\Delta H_4^0$	$\delta\Delta H_5^0$
PheGly/MetGly	–0.16±0.09	9.24±0.32	–11.61±0.01	–11.45±0.09	–2.37±0.17
PheIle/MetIle	4.94±0.07	3.72±0.32	4.98±0.03	0.04±0.07	8.70±0.11
PheLeu/MetLeu	0.25±0.05	–8.59±0.32	0.10±0.03	–0.15±0.05	–8.49±0.10
PheMet/MetMet	3.00±0.06	2.14±0.33	–6.33±0.13	–9.33±0.02	–4.19±0.13
PheSer/MetSer	–0.90±0.13	3.64±0.31	–3.71±0.09	–2.81±0.10	–0.07±0.04
PheTyr/MetTyr	7.71±0.23	23.49±0.34	–5.66±0.14	–13.37±0.19	17.83±0.14
PheGly/AlaGly	0.84±0.17	0.35±0.39	–6.80±0.06	–7.63±0.07	–6.45±0.31
PheIle/AlaIle	4.68±0.33	4.99±0.22	4.62 0.04	–0.06 0.04	7.61±0.05
PheLeu/AlaLeu	–0.73±0.53	–10.78±0.46	15.86±0.45	16.59 0.31	5.08±0.10
PheMet/AlaMet	5.96±0.33	–6.67±0.67	3.87±0.24	–2.09 0.14	–1.60±0.67
PhePro/AlaPro	1.87±0.45	–5.34±0.5	23.87±0.26	21.99±0.38	18.53±0.31
PheSer/AlaSer	0.88±0.13	–7.93±0.28	–2.33±0.13	–3.21±0.07	–10.26±0.13
PheTyr/AlaTyr	4.53±0.22	29.60±0.26	–15.80±0.14	–19.83±0.19	13.91±0.14
PheAla/LeuAla	–8.01±0.12	–6.12±0.49	3.08±0.0	11.09±0.09	–3.05±0.60
PheIle/LeuIle	2.21±0.07	1.54±0.24	9.95±0.03	7.74±0.07	11.43±0.10
PhePro/LeuPro	6.22±0.04	43.53±0.22	7.49±0.03	1.27±0.02	51.02±0.04
PheGly/LeuGly	1.70±0.04	1.34±0.32	1.73±0.01	0.01 0.04	3.07±0.25
PheSer/LeuSer	1.97±0.13	–6.94±0.23	6.21±0.09	4.24±0.10	–0.73±0.04
PheTyr/LeuTyr	11.28±0.15	20.18±0.24	18.60±0.15	7.32±0.03	38.98±0.14

By comparing methionyl with phenylalanyl dipeptides the former enables more dissociated the carboxyl groups of amino acids units, which, in turn, make more dissociated the amino group of methionine unit with respect that of phenylalanine. This is due to the fact that for PheSub/MetSub pairs the relative solvation of undissociated molecules prevails over that of zwitterions, which, in turn, show a major solvation towards ionic forms. It can also be noted that most of the zwitterions, undissociated molecules and ionic forms of the first series are more solvated than those of the second one. because of the prevailing hydrophobic effects of of the benzene ring with respect methionine.

By comparing the two series having Phe and Ala as the first common term for as concern the first ionisation processes it can be noted that the former dissociates with more difficulty, while for the second dissociation the reverse is true. This behaviour is due to the fact that the relative solvation of the undissociated molecules in the pairs PheSub/AlaSub prevails with respect to the relative solvation of zwitterions which, in turn, shows a relative less intense solvation effect than that of the ionic form. It was also noted that, the hydrophobic effects of Ala and Phe in the zwitterions and undissociated molecules are equivalent.

Finally, for the pairs of the series having Phe and Leu as the first common terms, the hydrophobic force of Leu is prevailing and allows to the greater difficulty in dissociation both for the first and second ionisation processes of the first series.

Conclusions

Our experimental evidence supports the conclusion that in a series of dipeptides the mutual influence of phenylalanine and the other α-amino acids can be considered from two points of view. This was achieved by using the proton transfer processes of these compounds in the aqueous phase. The enthalpy values for these proton transfer processes (with phenylalanylphenylalanine as reference compound) vary as function of the influence of both phenylalanine and the second component, so that in aqueous solution two relative scales of acidity and basicity are provided.

Two relationships which compare the proton dissociation processes of the carboxyl and the free amino groups of the dipeptides with the same processes of the free α-amino acids supply on absolute scale the enthalpic contribution to the basicity and acidity variation in the dipeptides. In the series having phenylalanylphenylalanine as first common term the hydrophobic forces of the side chains are the drawing forces in the relative solvation of the zwitterions, undissociated molecules and ionic forms of the components with respect the reference compound PhePhe. By comparing two series having methionine and phenylalanine as the first common term it was noted that the latter shows a prevailing hydrophobic forces. In the comparison of the series having Phe, Ala and Leu as the first common term the hydrophobic effect of Leu is prevailing while those of Phe and Ala are equivalent.

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This work was supported by the Consiglio Nazionale delle Ricerche of Italy. The authors are grateful to Mr. Fabio Raimondi for his assistance with computer calculations.

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