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# Calorimetric study of proton transfer processes of some dipeptides in water compared with the same processes in the gaseous phase

F. Rodante[a,*], G. Catalani[b]

[a]*Dipartimento di Ingegneria Chimica, dei Materiali, delle Materie Prime e Metallurgia, Università di Roma "La Sapienza", Via del Castro Laurenziano 7, 00161 Rome, Italy*
[b]*Istituto Regina Elena, V.le Regina Elena 293, Rome, Italy*

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**Abstract**

The basicity of the neutral form of a series of dipeptides having valine as common first term has been studied from the calorimetric and statistical points of view. This was achieved by using the proton transfer processes of these compounds in the aqueous and the gaseous phase. The values of the thermodynamic quantities for these proton transfer processes (with valylvaline as reference compound) vary as a function of the structure of the second component, so that two relative scales of basicity, in the aqueous and in the gaseous phase, are found.

A relationship which compares the proton dissociation processes of the free amino groups of the dipeptides with that of the corresponding free first α-amino acid (the first component) supplies, on an absolute scale, the percentage values of the basicity variation in the dipeptides. Finally, a monoparametric linear regression analysis leads (in terms of probability) to the hypothesis that the structure of the second component influences in a different way the proton transfer processes (and thus the basicities) of the dipeptides in aqueous and gaseous phases.

*Keywords:* Absolute scale; Basicity; Calorimetry; Dipeptide; Proton transfer; Relative scale; Statistics; Structural effect

* Corresponding author.


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## 1. Introduction

The thermodynamics of α-amino acids and peptides are of interest because these compounds are the building blocks of proteins. Dipeptides (which are compounds made up of two α-amino acids) are the smallest units of protein chains. For this reason, dipeptides allow the reciprocal influences of the structures of different α-amino acids to be studied. A calorimetric study of the mutual structural influences of α-amino acids in these compounds has been the subject of three papers [1–3] from our laboratory.

In the first [1], the influence of the structure of valine, which was one component of each dipeptide, upon a number of other α-amino acids as the second component, and the reciprocal influence of these other α-amino acids on the structure of valine were investigated using valylvaline as reference structure. In the second paper [2], the influence of a methyl substituent group upon some structures of "standard" α-amino acids was studied. The third [3] reported the reciprocal influence of structures in a series of dipeptides for which the reference structure was glycine.

As solvation factors play an important role in determining the reactions of α-amino acids in the aqueous phase, the thermodynamic quantities related to the solvation processes from the gaseous to the aqueous phase can emphasize the influence of the side chains on some proton transfer processes of α-amino acids.

Recently developed techniques [4–13] such as ion cyclotron resonance (ICR), high pressure mass spectrometry (HPMS) and laser desorption/chemical ionization (LD/CI) have afforded a deeper insight into gaseous proton transfer processes. It has become possible to study acid–base reactivity in terms of molecular structures and bonding in the absence of solvent effects.

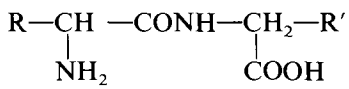
In recent work [14], by using the proton transfer process (related to the amino group) of some α-amino acids in the gaseous and the aqueous phase, a thermodynamic cycle was presented which allows the effects of the solvent to be separated from those intrinsic to neutral and protonated molecules.

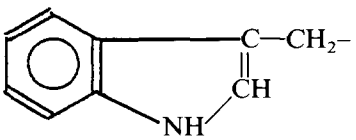
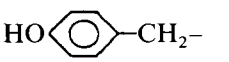
Monoparametric linear regression analysis between thermodynamic properties of the proton transfer processes and the electron charge distribution of the compounds leads to the hypothesis that it is uncertain whether the side chains influence the basicity of the α-amino acids in the same way in the gaseous and in the aqueous phase. It was also noted that molecule–proton interactions at a specific site are not determined by the charge delocalization on the neutral molecules of α-amino acids.

Gas-phase basicities of some dipeptides that contain valine were recently measured by a double bracketing method in a Fourier transformation ion cyclotron resonance spectrometer [15].

The aim of this work is to study, in the aqueous and in the gaseous phase, the basicity values of a series of dipeptides and to compare these values with that of the corresponding free first α-amino acid (the first component).

The following dipeptides (each consisting of two standard α-amino acids) were studied (Scheme 1): glycylvaline (Gly-Val), valylserine (Val-Ser), valylvaline (Val-Val), valyl-leucine (Val-Leu), tyrosylvaline (Tyr-Val), valyltryptophan (Val-Trp), valyltyrosine (Val-Tyr), valylproline (Val-Pro), and valyl-lysine (Val-Lys).



| No. | R | R′ | Notation |
|---|---|---|---|
| 1 | H | $(CH_3)_2CH-$ | Gly-Val |
| 2 | $(CH_3)_2CH-$ | $CH_2(OH)-$ | Val-Ser |
| 3 | $(CH_3)_2CH-$ | $(CH_3)_2CH-$ | Val-Val |
| 4 | $(CH_3)_2CH-$ | $(CH_3)_2CHCH_2-$ | Val-Leu |
| 5 |  | $(CH_3)_2CH-$ | Tyr-Val |
| 6 | $(CH_3)_2CH-$ |  | Val-Trp |
| 7 | $(CH_3)_2CH-$ |  | Val-Tyr |
| 8 | $(CH_3)_2CH-$ | $-CH_2-CH_2-CH_2-$ | Val-Pro |
| 9 | $(CH_3)_2CH-$ | $(NH_3^+)(CH_2)_4-$ | Val-Lys |

Scheme 1.

## 2. Experimental and procedure

The compounds (Carlo Erba RPE Chemicals, used without purification) were weighed and handled in a nitrogen-filled dry-box. The purity of all compounds was between 99% and 100%, and was checked by means of a DSC purity method using a Stanton-Redcroft 625 DSC instrument (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 temperature of 298.15 K was maintained constant to $2 \times 10^{-4}$ K during the calorimetric measurements by employing a Tronac P.C. 41 precision temperature controller.

Potential versus time measurements were made using a Fluka 88100 model digital voltmeter. The imbalance (in V) of the bridge of the calorimeter was fed into a Hitachi 561-10002/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 [16] run on the Olivetti M24 computer.

All the steps of the measurements (calibration, cooling curve, reaction curve and equilibrium temperature) have been described elsewhere [16].

Data obtained using the chart recorder may be slightly different from those obtained using the computer; they also give the shape of the thermograms represented as temperature vs. time curves.

The proton dissociation processes of the free $\alpha$-carboxyl group belonging to the carboxyl terminal residue (C-terminal) and of the free $\alpha$-amino group belonging to the amino terminal residue (N-terminal) of a generic dipeptide can be represented as follows

$$NH_3^+CHRCONHCHR'COOH(aq) \longrightarrow NH_3^+CHRCONHCHR'COO^-(aq) + H^+(aq) \quad (1)$$

and

$$NH_3^+CHRCONHCHR'COO^-(aq) \longrightarrow NH_2CHRCONHCHR'COO^-(aq) + H^+(aq) \quad (2)$$

The partial molar enthalpy of dissociation $\Delta\bar{H}_1$ for the free carboxyl group in water is obtained by measuring the following quantities:

(a) The partial molar enthalpy of solution $\Delta\bar{H}_3$ of the crystalline (cr) $NH_3^+CHRCONHCHR'COO^-$ zwitterionic form in water at a pH close to the isoelectric point

$$NH_3^+CHRCONHCHR'COO^-(cr) \longrightarrow NH_3^+CHRCONHCHR'COO^-(aq) \quad (3)$$

(b) The partial molar enthalpy of protonation $\Delta\bar{H}_4$ of the same compound in water at pH 0.0 obtained using 1.00 m (molal) HCl

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

The partial molar enthalpy of process (1) can be obtained by subtracting $\Delta\bar{H}_4$ from $\Delta\bar{H}_3$. Concentrations ranging from $10^{-4}$ to $10^{-3}$ m were used in processes (3) and (4), and the corresponding $\Delta\bar{H}$ lay within the experimental error limits, so that they can be considered as being at infinite dilution $\Delta\bar{H}^\circ$ [17].

These values refer to the protonation dissociation of one mole of $NH_3^+CHRCONHCHR'COOH$ at infinite dilution in 1000 g of water, yelding one mole of $NH_3^+CHRCONHCHR'COO^-$ ions and one mole of protons solvated in the same amount of water.

For a compound containing carboxyl and amino groups, the dissociation processes in water are complicated by tautomeric equilibria and zwitterion formation [1–3]. Although a generic dipeptide in acid solution can be represented by the form $NH_3^+CHRCONHCHR'COOH$, in a solution approaching pH 7.00 the principal species are neutral molecules, which may be in either the $NH_2CHRCONHR'COOH$ form or the zwitterionic form.

Thus only the $NH_3^+CHRCONHCHR'COOH$ form is represented in Eq. (4) at pH 0.0, whereas in Eq. (3) this is not the case.

The isoelectric pH values for some of the compounds examined can be calculated by means of the dissociation constants [1–3]. If this is not possible, it can be noted that the isoelectric values of dipeptides are close to those of the corresponding free α-amino acids, by virtue of the small differences in $pK_a$ of their carboxyl and amino groups. It can therefore be assumed that in this solution the zwitterionic form is predominant. In this way the carboxyl proton dissociation enthalpy values can be calculated.

The partial molar enthalpy of the second proton dissociation process of $NH_3^+CHRCONHCHR'COO^-$ is obtained by measuring the partial molar enthalpy $\Delta\bar{H}_5$ of the neutralization of the crystalline compound in water at pH 14 (obtained using a solution of 1 m NaOH).

$$NH_3^+CHRCONHCHR'COO^-(cr) + OH^-(aq) \longrightarrow$$
$$NH_2CHRCONHCHR'COO^-(aq) + H_2O(l) \quad (5)$$

If the solution process enthalpy value $\Delta\bar{H}_3$ and the partial molar value $\Delta\bar{H}_6$ in water related to the process [18]

$$H^+(aq) + OH^-(aq) \longrightarrow H_2O(l) \quad (6)$$

are subtracted from $\Delta\bar{H}_5$, then the relation $\Delta\bar{H}_5 - (\Delta\bar{H}_3 + \Delta\bar{H}_6)$ supplies the enthalpy values of process (2). The $\Delta\bar{H}$ values for this process can also be considered as being equal to $\Delta H^{\ominus}$.

These values refer to the dissociation process of one mole of $NH_3^+CHRCONHCHR'COO^-$ at infinite dilution in 1000 g of water, yielding one mole of $NH_2CHRCONHCHR'COO^-$ and one mole of proton solvated in the same amount of water. Process (4) occurs at pH 14.00, so that only the $NH_2CHRCONHCHR'COO^-$ form is present.

## 3. Results and discussion

The enthalpy values of the solution, protonation and neutralization processes of the studied dipeptides are reported in Table 1. The enthalpy values of the first and second ionization processes are reported in Table 2. The standard deviations are indicated next to the values.

As the dipeptides in the gaseous phase are assumed [15] to be the neutral (not the zwitterionic) form, the reaction related to the gaseous phase basicity of dipeptides is

$$NH_3^+CHRCONHCHR'COOH(g) \longrightarrow$$
$$H_2CHRCONHCHR'COOH(g) + H^+(g) \quad (7)$$

As the dipeptides of interest are zwitterions in solution, reaction (7) is not directly observed in the aqueous phase.

Process (7) in water can be obtained by the reactions

Table 1
Enthalpy values (kJ mol$^{-1}$) of solution, protonation and neutralization processes for some dipeptides in water at 298 K

| Compounds | $\Delta H_3^\ominus$ | $\Delta H_4^\ominus$ | $\Delta H_5^\ominus$ |
|---|---|---|---|
| Gly-Val | −8.37 ± 0.05 | −6.40 ± 0.03 | −19.33 ± 0.02 |
| Val-Ser | −10.50 ± 0.08 | −11.97 ± 0.07 | −11.26 ± 0.01 |
| Val-Val | −13.48 ± 0.06 | −11.68 ± 0.06 | −21.76 ± 0.03 |
| Val-Leu | −24.31 ± 0.03 | −22.72 ± 0.03 | −34.36 ± 0.01 |
| Tyr-Val | −7.49 ± 0.05 | −6.32 ± 0.03 | −50.85 ± 0.03 |
| Val-Trp | 16.61 ± 0.04 | 19.04 ± 0.01 | 11.51 ± 0.06 |
| Val-Tyr | 2.68 ± 0.03 | 0.29 ± 0.01 | −41.22 ± 0.07 |
| Val-Pro | −2.20 ± 0.07 | 1.17 ± 0.02 | −63.57 ± 0.08 |
| Val-Lys | 0.21 ± 0.06 | −0.50 ± 0.01 | −9.29 ± 0.01 |

$$NH_3^+CHRCONHCHR'COOH(aq) \longrightarrow NH_3^+CHRCONHCHR'COO^-(aq) + H^+(aq) \quad (1)$$

$$NH_3^+CHRCONHCHR'COO^-(aq) \longrightarrow NH_2CHRCONHCHR'COO^-(aq) + H^+(aq) \quad (2)$$

$$NH_2CHRCONHCHR'COOH(aq) \longrightarrow NH_2CHRCONHCHR'COO^-(aq) + H^+(aq) \quad (8)$$

by using the expression

$$\Delta H^\ominus = \Delta H_1^\ominus + \Delta H_2^\ominus - \Delta H_8^\ominus \quad (9)$$

where $\Delta H_1^\ominus$ and $\Delta H_2^\ominus$ are related to processes (1) and (2) respectively and the $\Delta H_8^\ominus$ are the values for the free carboxyl dissociation of the C-terminal of the neutral dipeptides which are approximated by the dissociation values of the corresponding free acids [19].

Table 2
Enthalpies values (kJ mol$^{-1}$) of the first ($\Delta H_1^\ominus$) and second ($\Delta H_2^\ominus$) ionization processes for some dipeptides in water at 298 K

| Compounds | $\Delta H_1^\ominus$ | $\Delta H_2^\ominus$ |
|---|---|---|
| Gly-Val | −1.97 ± 0.06 | 44.99 ± 0.07 |
| Val-Ser | 1.47 ± 0.10 | 55.19 ± 0.08 |
| Val-Val | −1.80 ± 0.08 | 47.67 ± 0.08 |
| Val-Lue | −1.59 ± 0.04 | 45.91 ± 0.05 |
| Tyr-Val | −1.17 ± 0.06 | 32.48 ± 0.07 |
| Val-Trp | −2.43 ± 0.04 | 50.85 ± 0.09 |
| Val-Tyr | 2.39 ± 0.03 | 32.18 ± 0.09 |
| Val-Pro | −3.43 ± 0.06 | −5.42 ± 0.12 |
| Val-Lys | 0.71 ± 0.05 | 39.21 ± 0.07 |

Table 3
Enthalpy values (kJ mol$^{-1}$) of dissociation process of amine group of neutral dipeptides in water at 298 K

| Compounds | $\Delta H_9^{\ominus}$ |
|---|---|
| Gly-Val | 48.00 ± 0.10 |
| Val-Ser | 57.33 ± 0.11 |
| Val-Val | 50.85 ± 0.12 |
| Val-Leu | 47.24 ± 0.08 |
| Tyr-Val | 36.29 ± 0.10 |
| Val-Trp | 49.09 ± 0.09 |
| Val-Tyr | 35.24 ± 0.08 |
| Val-Pro | −8.85 ± 0.12 |
| Val-Lys | 42.43 ± 0.09 |

$\Delta H_9^{\ominus}$ represents process (7) in water, and the corresponding values are given in Table 3.

Let us consider the following cycle

$$\begin{array}{ccccc} \mathrm{ValH^+A(g)} & + & \mathrm{Val\text{-}Val(g)} & = & \mathrm{ValA(g)} + \mathrm{ValH^+Val(g)} \\ \downarrow \Delta H_s & & \downarrow \Delta H_s & & \downarrow \Delta H_s \quad \downarrow \Delta H_s \\ \mathrm{ValH^+A(aq)} & + & \mathrm{Val\text{-}Val(aq)} & = & \mathrm{ValA(aq)} + \mathrm{ValH^+Val(aq)} \end{array}$$

Scheme 2.

where ValA is a generic dipeptide with valine as common first term (with the exception of Gly-Val and Tyr-Val), Val-Val is the reference compound, and $\mathrm{ValH^+A}$ and $\mathrm{ValH^+Val}$ are the same compounds protonated on the amino terminal residue. So we can write

$$\delta\Delta H(\mathrm{aq}) - \delta\Delta H(\mathrm{g}) = [\Delta H_s(\mathrm{ValA}) - \Delta H_s(\mathrm{ValH^+A})] - [\Delta H_s(\mathrm{Val\text{-}Val}) - \Delta H_s(\mathrm{ValH^+Val})]$$

and again

$$\delta\Delta H_{(\mathrm{aq})} - \delta\Delta H_{(\mathrm{g})} = \delta\Delta H_s \tag{10}$$

where $\delta\Delta H(\mathrm{g})$ is the variation of the enthalpy in the gaseous phase for the proton transfer process from ValA to Val-Val. The corresponding values in water are represented by $\delta\Delta H(\mathrm{aq})$.

The right-hand term of Eq. (10) can be taken as the proton transfer process in water, which refers to a gaseous initial thermodynamic state, i.e. $\delta\Delta H_s = \delta\Delta H^{\mathrm{g}\rightarrow\mathrm{aq}}$.

Hepler and coworkers [19–23] have stressed the usefulness of expressing the variations of thermodynamic functions ($\delta\Delta G$, $\delta\Delta H$, $\delta\Delta S$) related to the proton transfer processes in terms of "internal" and external contributions. Internal effects are those intrinsic to the molecules and ions, whereas external effects are derived from solvent interactions with the molecules and ions and are thus related to the solvation process.

So it can be assumed that the $\delta\Delta H^{g\rightarrow aq}$ term is a measure of the external interactions, $\delta\Delta H^{\ominus}(aq)$ represents the "total" interactions and $\delta\Delta H^{\ominus}(g)$ the internal interactions. These terms can be correlated in Eq. (11)

$$\delta\Delta H^{\ominus}(aq) = \delta\Delta H^{\ominus}(g) + \delta\Delta H^{g\rightarrow aq} \tag{11}$$

As previously seen [9–13] for some "standard" $\alpha$-amino acids, the entropy values of the proton transfer process in the gaseous phase ($A_1H^+ + A_2 = A_1 + A_2H^+$) can be calculated from changes in rotation symmetry number $\sigma$ of the reactants and products according to the equation

$$\Delta S^{\ominus}_{tot} = R \ln[\sigma_{A_1H^+}\sigma_{A_2}/\sigma_{A_1}\sigma_{A_2H^+}]$$

However, the $\alpha$-amino acids used in these studies [4–8] have generally low symmetry numbers. It is thus possible to assume [4–8,14] that $\delta\Delta S_i^{\ominus}(g) \approx 0$ and, consequently, $\delta\Delta G_i^{\ominus}(g)(350\ K) \approx \delta\Delta G_i^{\ominus}(g)(298\ K) \approx \delta\Delta H_i^{\ominus}(g)(298\ K)$.

If the same hypothesis is assumed for dipeptides, then the $\delta\Delta H^{\ominus}$ values at 298 K in the gaseous phase can be calculated from gaseous phase basicity values in the literature [15].

Table 4 lists the $\delta\Delta H^{\ominus}(aq)$ (obtained from Table 3), $\delta\Delta H^{\ominus}(g)$ (obtained from Ref. [15]) and $\delta\Delta H^{g\rightarrow aq}$ (obtained from Eq. (11)) values related to the proton transfer processes of the studied dipeptides.

The scale of the proton transfer process in the aqueous phase can be given as Val-Pro > Val-Tyr > Tyr-Val > Val-Lys > Val-Leu > Gly-Val > Val-Trp > Val-Val > Val-Ser, and in the gaseous phase the scale becomes Gly-Val > Val-Ser > Val-Val > Val-Leu > Tyr-Val > Val-Trp > Val-Tyr > Val-Pro > Val-Lys.

The transfer processes in water, which refer to a gaseous initial thermodynamic state, show the following scale: Val-Pro > Val-Lys > Val-Tyr > Val-Trp > Tyr-Val > Val-Leu > Val-Val > Gly-Val > Val-Ser.

From our experimental evidence on the transfer processes of the protonated molecules of the dipeptides, the following observations may be made.

Table 4
Differences, with respect to valylvaline, in enthalpy values (kJ $mol^{-1}$) of the proton transfer processes of some dipeptides (in neutral form) in the aqueous and the gaseous phase, and in the aqueous phase assuming a gaseous initial thermodynamic state at 298 K

| Compounds | $\delta\Delta H^{\ominus}(aq)$ | $\delta\Delta H^{\ominus}(g)$ | $\delta\Delta H^{g\rightarrow aq}$ |
|---|---|---|---|
| Gly-Val | −2.85 | −10.47 | 7.62 |
| Val-Ser | 6.48 | −10.47 | 16.95 |
| Val-Val | 0.00 | 0.00 | 0.00 |
| Val-Leu | −3.61 | 0.00 | −3.61 |
| Tyr-Val | −14.56 | 10.88 | 25.44 |
| Val-Trp | −1.76 | 29.71 | −31.47 |
| Val-Tyr | −15.61 | 29.71 | −45.52 |
| Val-Pro | −59.70 | 37.24 | −96.94 |
| Val-Lys | −8.42 | 46.84 | −55.29 |

Table 5
Differences, with respect to valine, in enthalpy values (kJ mol$^{-1}$) of the proton transfer processes of some α-amino acids (in neutral form) in the aqueous and the gaseous phase, and in the aqueous phase assuming a gaseous initial thermodynamic state at 298 K

| Compounds | $\delta\Delta H^{\ominus}$(aq) | $\delta\Delta H^{\ominus}$(g) | $\delta\Delta H^{g\rightarrow aq}$ |
|---|---|---|---|
| Gly | 9.38 | −48.54 | 57.92 |
| Val | 0.00 | 0.00 | 0.00 |
| Ser | 3.22 | −8.37 | 11.59 |
| Leu | 12.56 | 10.47 | 2.09 |
| Trp | 9.76 | 40.18 | −30.42 |
| Tyr | −5.14 | 21.35 | −26.49 |
| Pro | 3.80 | 40.18 | −36.38 |
| Lys | 10.72 | 57.37 | −46.62 |

Using valylvaline as a reference compound, it is possible to observe that the proton transfer at the free amino group in this series of dipeptides varies as a function of the structure of the second component, so that a relative scale of basicity can be found.

Val-Pro, Tyr-Val, Val-Tyr, Val-Lys and Gly-Val in the aqueous phase show a proton transfer process larger than that of valylvaline (these compounds are less basic than Val-Val), with external forces prevailing (with the exception of Gly-Val). In contrast, Val-Ser is less dissociated than valylvaline. For the corresponding free α-amino acids, the thermodynamic quantities of the first and second ionization processes in the aqueous phase have been calculated previously [24]. From these values and with the same procedure used for dipeptides, the thermodynamic quantities for dissociation processes of neutral molecules in water has been calculated [14]. The differences, with respect to valine, in enthalpy values of the proton transfer process of these α-amino acids (in neutral form), in the aqueous and gaseous phases (from Ref. [9]) and in the aqueous phase assuming a standard gaseous phase at $p = 1$ atm, are given in Table 5.

For the free α-amino acids in the aqueous phase, only tyrosine shows a favourable proton transfer process with respect to valine: Tyr > Val > Ser > Pro > Gly > Trp > Lys > Leu. The transfer process in the gaseous phase (Table 5) for the same α-amino acids shows the order Gly > Ser > Val > Leu > Tyr > Trp > Pro > Lys.

According to the $\delta\Delta H^{g\rightarrow aq}$ values, the order of solvation of the proton transfer process (Table 5) is Lys > Pro > Trp > Tyr > Val > Leu > Ser > Gly.

So, for the dipeptides and the corresponding free α-amino acids an opposite relative scale of basicity is found in the aqueous phase, and the external forces are dominant for both series.

A further contribution of the structures of α-amino acids to the basicity of dipeptides can be supplied from the relation (12) (Table 6), which directly compares the two series on an absolute scale

$$\frac{\Delta H^{\ominus}_{(\mathrm{ValA})} - \Delta H^{\ominus}_{\mathrm{Val}}}{\Delta H^{\ominus}_{\mathrm{Val}}} \qquad (12)$$

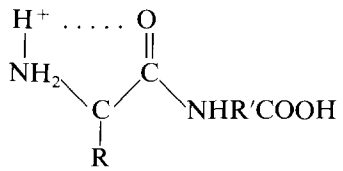
where $\Delta H^{\ominus}_{(\mathrm{ValA})}$ represents the values of proton dissociation for the free amino groups of the dipeptides having (with the exception of Gly-Val and Tyr-Val) valine as first component, and $\Delta H^{\ominus}_{\mathrm{Val}}$ represents the value (with the exception of glycine and tyrosine) of the dissociation process for the free valine amino group.

The influence of the different α-amino acids on the fixed first one was therefore studied by means of relationship (12), which supplies percentage values of the basicity variation in the dipeptides.

In the aqueous phase, the proton dissociation of the free amino group of valine is hindered by the structures of serine, valine, leucine, tryptophan and lysine (the amino group becomes more basic than that of free valine), while for proline and tyrosine the contrary is true (the amino group of the dipeptides becomes less basic).

In the gaseous phase all the dipeptides, with the exception of Val-Ser and Tyr-Val, are more basic than the corresponding free first α-amino acid (the first component).

The trend in the gaseous phase was explained [15] by a reinforced hydrogen bond between the charge of the ammonium group at the N-terminus and the carboxyl oxygen of the amide group (Structure 1) compared with this bond in the corresponding free first α-amino acid (the first component).




Structure 1.

As in the gaseous phase [15] for a fixed α-amino acid at the N-terminus the basicity of dipeptides increases with the increase in the basicity of its C-terminus (second term), an inductive effect due to an enhanced electron density at the amide carboxyl oxygen can be hypothesized. This, in turn, allows a strong hydrogen bond in Structure 1.

Table 6
Values obtained from Eq. (12) in the aqueous and the gaseous phase

| Compounds | Aqueous phase | | Gaseous phase | |
|---|---|---|---|---|
| Val-Ser | 0.45 | 45% | 0 | |
| Val-Val | 0.29 | 29% | 0.01 | 1% |
| Val-Leu | 0.20 | 20% | 0.01 | 1% |
| Val-Trp | 0.24 | 24% | 0.04 | 4% |
| Val-Tyr | −0.11 | 11% | 0.04 | 4% |
| Val-Pro | −1.22 | 122% | 0.05 | 5% |
| Val-Lys | 0.08 | 8% | 0.06 | 6% |
| Gly-Val | −0.02 | 2% | 0.06 | 6% |
| Tyr-Val | −0.06 | 6% | 0 | |

The decreasing effect of proline and tyrosine on the basicity of dipeptides in the aqueous phase could be related to the solvent effect, which hinders formation of the hydrogen bond in Structure 1, whereas for the other compounds this is not the case.

From this evidence it is possible to compare the relative scale of basicity (with respect to valylvaline) with the absolute one. The former shows the prevalence of external or internal forces in determining the relative basicity of dipeptides. The latter shows that, with the exception of Val-Pro and Val-Tyr in the aqueous phase, and Val-Ser and Tyr-Val in the gaseous phase, all dipeptides are more basic than the corresponding free first $\alpha$-amino acid (the first component).

## 4. Statistical correlations of transfer proton processes

If the influence of the structure of the second $\alpha$-amino acid on the free amino groups of the dipeptides is the same in the gaseous and the aqueous phase, then the thermodynamic quantities of the corresponding proton transfer processes (in the two phases) can be related by a linear relationship.

Furthermore, to identify the actual site of the proton transfer processes in both phases, the corresponding thermodynamic quantities are correlated by a linear relationship with the electron density charge distribution of the studied compounds.

For this purpose it is convenient to use a monoparametric linear regression analysis. However, the significance level of these relationships only allows the experimental results to be compared and explained in terms of probabilities. Thus, for this series of compounds, a critical examination of correlations between $\delta\Delta H^{\ominus}_{(aq)}$, $\delta\Delta H^{\ominus}_{(g)}$ and $\delta\Delta H^{g\rightarrow aq}$ allows a further evaluation of the effects of the structures of $\alpha$-amino acids and of the medium on the overall proton transfer process.

Finally, a comparison of the proton transfer process with the electron density of the amino group at the N-terminus of the dipeptides was made. The relationships used were $\delta\Delta H^{\ominus}_{(aq)}$ vs. $\delta q_N$, $\delta\Delta H^{\ominus}_{(g)}$ vs. $\delta q_N$ and $\delta\Delta H^{g\rightarrow aq}$ vs. $\delta q_N$, where $\delta q_N$ is the electron density at the nitrogen atom of the free amino-group of the N-terminus expressed as $\delta q_N = q_{N(ValA)} - q_{N(Val)}$.

The Huckel–McLachlan charge distribution was calculated by a computer program using the values [25] $h_{\dot{N}} = 0.5$, $K_{CC} = 1$, $K_{C-N} = 0.8$, $h_{\dot{O}^{\cdot}} = 2$, $K_{C=O} = 1$, $K_{C-C} = 0.8$, $h_{\dot{O}} = 1$, and $K_{C-O} = 0.8$, where $h$ is the increment of the Coulomb integral (interaction energy between each electron and its respective nucleus) and $K$ is the bond integral, which represents the energy of two atomic orbitals; C–C symbolizes a single bond, C=C a double bond and CC an aromatic bond.

The correlations have to be studied from the statistical viewpoint by using a linear regression analysis, which supplies the precise form of the mathematical function relating the two variables and tests how the experimental results support the theoretical relationship within the limits of the experimental error of the measurements. In this context, the more useful tests are the standard deviations of the slope and of the intercept, the total standard deviation and the Student $t$ test for the intercept, slope and correlation coefficient values of the linear regression [26–31].

It is also worth noting that the degree of significance (highly significant, significant, insignificant) for these correlations allows the experimental results to be compared and explained only in terms of probability. The introduction of subjective data (confidence level, error distribution) is the reason why the statistical analysis cannot supply absolute answers.

For all the correlations the null hypotheses considered were: (1) for the intercept $a = 0$ and (2) for the slope $b = 0$. The null hypotheses were tested by using the Student $t$ test. Indeed, the $t$ values of $a$ and b were compared with those of a set of $t'$ tables.

The $t$ values of $a$ and $b$ were calculated by means of the expressions $t_a = (a - A)/S_a$, $t_b = (b - B)/S_b$, where $A = 0$, $B = 0$ and $S_a$ and $S_b$ are the standard deviations of $a$ and $b$.

If $t > t_{CL,n-2}$, where $(n - 2)$ is the degree of freedom and CL is the confidence level for significance of the regression, then for $CL < 0.95$ the null hypothesis is accepted (chemical hypothesis), while for $CL > 0.999$ its regression is highly significant.

From the above cited values the following correlations were examined critically by means of linear monoparametric analysis: $\delta\Delta H^{\ominus}_{(aq)}$ vs. $\delta\Delta H^{\ominus}_{(g)}$ (Table 7), and $\delta\Delta H^{g\rightarrow aq}$ vs. $\delta\Delta H^{\ominus}_{(g)}$ (Table 8).

It is usually [6–10] hypothesized that for an isodesmic process (ion–molecule reaction) a linear correlation between the transfer process in the aqueous and the gaseous phase is to be expected if the quantity $\delta\Delta H^{g\rightarrow aq}$ is constant within a series of compounds or if there is an approximately linear function between the solvation and gaseous phase proton transfer processes.

For the first relationship, an insignificant function between the two variables is evident, so it can be hypothesized that the structures of $\alpha$-amino acids influence in different ways the basicity of the dipeptides in the aqueous and the gaseous phase. The significant regression of the second relationship shows that there is no differential solvation factor which causes departures from a linear relationship between the thermodynamic properties in the two phases.

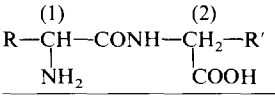
Table 7
Results of the monoparametric regression analysis of $\delta\Delta H^{\ominus}$(aq) vs. $\delta\Delta H^{\ominus}$(g) for some dipeptides in neutral form at 298 K

| $n$ | 9 |
|---|---|
| Intercept | −3.72379 |
| Slope | −0.49847 |
| S.D. of intercept | 7.19215 |
| S.D. of slope | 0.2819 |
| S.D. of regression | 17.421 |
| $r$ | 0.549857 |
| n.h. intercept = 0 | CL < 0.95 |
| n.h. slope = 0 | CL < 0.95 |

Table 8
Results of the monoparametric regression analysis of $\delta\Delta H^{g\rightarrow aq}$ vs. $\delta\Delta H^{\ominus}$(g) for some dipeptides in neutral form at 298 K

| $n$ | 9 |
|---|---|
| Intercept | −3.7228 |
| Slope | −1.49873 |
| S.D. of intercept | 7.19026 |
| S.D. of slope | 0.28612 |
| S.D. of regression | 17.4165 |
| $r$ | 0.89601 |
| n.h. intercept = 0 | CL < 0.95 |
| n.h. slope = 0 | 0.99 < CL < 0.999 |

Table 9
Huckel–McLachlan charge density distributions for some dipeptides in neutral form



| Compounds | CH(1) | $NH_2$ | CONH | CH(2) | COOH |
|---|---|---|---|---|---|
| Val-Pro | 0.66380 | 0.49283 | 0.97389 | 0.45399 | 0.15276 |
| Tyr-Val | 0.63246 | 0.66915 | 0.97320 | 0.63385 | 0.27694 |
| Val-Val | 0.65956 | 0.29752 | 0.79997 | 0.63638 | 0.28010 |
| Val-Leu | 0.64548 | 0.28825 | 0.80028 | 0.58142 | 0.26063 |
| Val-Ser | 0.66437 | 0.30417 | 0.89443 | 0.67825 | 0.34310 |
| Val-Lys | 0.63318 | 0.28315 | 0.86084 | 0.57224 | 0.12934 |
| Val-Tyr | 0.70972 | 0.36817 | 0.90321 | 0.60736 | 0.29440 |
| Val-Trp | 0.67615 | 0.30876 | 0.61491 | 0.73760 | 0.46203 |
| Gly-Val | 0.60146 | 0.46786 | 1.14875 | 0.63638 | 0.30880 |

Furthermore, the results of the Huckel–MacLachlan molecular orbital calculations show (Table 9) that in the molecules the highest charge density value is not found at the nitrogen atom of the amino group linked to the N-terminus, so that it is uncertain, in aqueous and gaseous phase, at which side of the molecules the proton processes occur.

To verify these hypotheses the following correlations were examined by means of linear monoparametric analysis: $\delta\Delta H^{\ominus}_{(aq)}$ vs. $\delta q_N$ (Table 10), $\delta\Delta H^{\ominus}_{(g)}$ vs. $\delta q_N$ (Table 11), and $\delta\Delta H^{g\rightarrow aq}$ vs. $\delta q_N$ (Table 12). All these relationships were found to be insignificant.

The same results were obtained for linear regressions of $\delta\Delta H_{(aq)}$, $\delta\Delta H_{(g)}$ and $\delta\Delta H^{g\rightarrow aq}$ against the electron charge density at the amide group of the dipeptides.

Table 10
Results of the monoparametric regression analysis of $\delta\Delta H^{\ominus}$(aq) vs. $Sq_N$ for some dipeptides in neutral form at 298 K

| *n* | 9 |
|---|---|
| Intercept | −4.9764 |
| Slope | −68.8804 |
| S.D. of intercept | 7.57904 |
| S.D. of slope | 49.61010 |
| S.D. of regression | 18.4685 |
| *r* | 0.464681 |
| n.h. intercept = 0 | CL < 0.95 |
| n.h. slope = 0 | CL < 0.95 |

Table 11
Results of the monoparametric regression analysis of $\delta\Delta H^{\ominus}$(g) vs. $Sq_N$ for some dipeptides in neutral form at 298 K

| *n* | 9 |
|---|---|
| Intercept | 15.28260 |
| Slope | −5.06003 |
| S.D. of intercept | 9.44954 |
| S.D. of slope | 61.74940 |
| S.D. of regression | 23.0021 |
| *r* | 3.09568 $E^{-02}$ |
| n.h. intercept = 0 | CL < 0.95 |
| n.h. slope = 0 | CL < 0.95 |

Table 12
Results of the monoparametric regression analysis of $\delta\Delta H^{g\rightarrow aq}$ vs. $Sq_N$ for some dipeptides in neutral form at 298 K

| $n$ | 9 |
|---|---|
| Intercept | 19.81040 |
| Slope | −65.38830 |
| S.D. of intercept | 15.57580 |
| S.D. of slope | 101.77400 |
| S.D. of regression | 37.9649 |
| $r$ | 0.235978 |
| n.h. intercept = 0 | CL < 0.95 |
| n.h. slope = 0 | CL < 0.95 |

Table 13
Results of the monoparametric regression analysis of

$$\frac{\Delta H^{\ominus}(\text{ValA}) - \Delta H^{\ominus}\text{Val}}{\Delta H^{\ominus}\text{Val}} \text{ vs. } \frac{q_{N(\text{ValA})} - q_{N(\text{Val})}}{q_{N(\text{Val})}}$$

for some dipeptides in neutral form at 298 K

| $n$ | 7 |
|---|---|
| Intercept | 0.78888 |
| Slope | −0.2429 |
| S.D. of intercept | 0.54968 |
| S.D. of slope | 0.1593 |
| S.D. of regression | 0.476569 |
| $r$ | 0.528389 |
| n.h. intercept = 0 | CL < 0.95 |
| n.h. slope = 0 | CL < 0.95 |

Finally, the regression

$$\frac{\Delta H^{\ominus}_{(\text{ValA})} - \Delta H^{\ominus}_{(\text{Val})}}{\Delta H^{\ominus}_{\text{Val}}} \text{ vs. } \frac{q_{N(\text{ValA})} - q_{N(\text{Val})}}{q_{N(\text{Val})}}$$

was found to be insignificant (Table 13).

These relationships confirm that, in both the aqueous and the gaseous phase, the proton transfer processes do not occur at preferred sites for all compounds. For the aqueous phase this can be related to the fact that, according to the short-range donor–acceptor model, the interactions of the protonated dipeptides with water molecules cannot be related to the electron density of the neutral molecules of the dipeptides [32–37].

In summary, the structures of different α-amino acids, influence the proton transfer processes (and thus the basicities) of the studied dipeptides in different ways in the aqueous and the gaseous phase, but it is uncertain whether the side chains influence the proton transfer processes of the α-amino acids in different ways in the aqueous and the gaseous phases [14].

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