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THERMODYNAMICS OF DIPEPTIDES IN WATER
Calorimetric determination of enthalpy change values related to proton transfer processes of a series of dipeptides in water

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Abstract

Calorimetric measurements on a series of dipeptides having methionine as first common term enabled us to determine the thermodynamic quantities related to the proton transfer processes at the free α-amino and α-carboxyl groups of the dipeptides using methionyl-methionine (MetMet) as reference compound.

The drawing forces were found to be the solvation processes of zwitterions, ions and undissociated molecules of dipeptides, hydrophobic effects of side-chains playing an important role. The results were interpreted in terms of the reciprocal influence of the dipeptides components.

Relative and absolute scales related to the enthalpic contribution to the acidity and basicity of the dipeptides studied are supplied.

Keywords: acidity and basicity variations, calorimetry, dipeptides, heat of dissociation, heat of neutralisation, heat of protonation, solvophobic interactions

Introduction

The thermodynamics of α-amino acids and dipeptides are of interest because the compounds are the building blocks of proteins and other biologically important substances.

For this reason mainly in the last 20 years much work concerning the thermodynamics of dipeptides in water was carried out [1–8]. In particular, the stereoselectivity in the proton complex formation of *l–l–* or *l–d–* pairs of dipeptides was investigated in aqueous solution by means of calorimetric technique [9–16]. On the basis of the obtained results it was possible to assess the role played by two different non-covalent interactions, namely the electrostatic interactions (between COO^- and NH_3^+ groups) and the solvophobic interaction (between the side chains) on the thermodynamic stereoselectivity in the proton complex formation. To study these inter-

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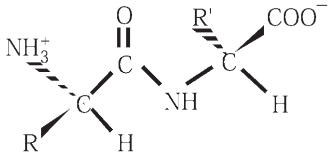
actions, difference in proton dissociation processes both for the carboxyl and amino groups of the components of some series of dipeptides and the corresponding single α-amino acids was the subject of an extended research in our laboratory [17–21]. This study is based on the calorimetric determination of the enthalpy values related to dissociation processes of the free α-amino and the α-carboxyl groups belonging to the amino (N-terminal) and carboxyl (C-terminal) residues respectively, for a series of dipeptides having methionine as first common term. These values were compared with those of the corresponding groups of the methionyl-methionine and of the individual α-amino acids.

The dipeptides studied were: *l*-methionyl-*l*-glycine (MetGly), *l*-methionyl-*l*-glutamic acid (MetGlu), *l*-methionyl-*l*-isoleucine (MetIle), *l*-methionyl-*l*-methionine (MetMet), *l*-methionyl-*l*-aspartic acid (MetAsp), *l*-methionyl-*l*-aspargine (MetAsn), *l*-methionyl-*l*-tyrosine (MetTyr), *l*-methionyl-*l*-tryptophan (MetTrp), *l*-methionyl-*l*-valine (MetVal), *l*-methionyl-*l*-threonine (MetThr), *l*-methionyl-*l*-leucine (MetLeu), *l*-methionyl-*l*-serine (MetSer), *l*-methionyl-*l*-glutamine (MetGln). This series is represented by

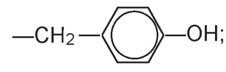


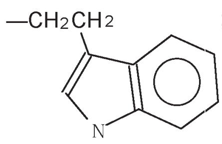
Structure 1

where *R* is $-CH_2CH_2SCH_3$ and *R*' is respectively:

For some enthalpic measurements, the second carboxyl group of both methionyl-aspartic acid and methionyl-glutamic acid and the hydroxyl group of methionyl-tyrosine were converted in ester groups.

$-H$; $-CH_2CH_2COOH$; $-CH(CH_3)CH_2CH_3$; $-CH_2CH_2SCH_3$; $-CH_2COOH$

$-CH_2CONH_2$; 



; $-CH(CH_3)_2$;

$-CH(OH)CH_3$; $-CH_2CH(CH_3)_2$; $-CH_2OH$; $-CH_2CH_2COONH_2$

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 $298.15\pm2\cdot10^{-4}$ 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. The unbalance (volts) of the bridge of the calorimeter was fed into 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 [22]. All the steps of the measurements (calibration curve, cooling curve, and reaction curve and equilibrium temperature) were also described [22]. Data obtained using the chart record are slightly different from those obtained using the computer and they also give the shape of the curves expressed as temperature *vs.* time curves.

Standard chemical test system used to check the calorimetric system was the reaction between solid tris(hydroxymethyl)amino methane and 0.1 M HCl at 298.15 K in water. The value of the partial molar enthalpy of reaction obtained (Table 1) is 29.71±0.31 kJ mol^{-1} and was compared with that obtained in the literature (29.74 kJ mol^{-1} [23]). The average percentage deviation of the former value relative to the latter is 0.11%.

Table 1 Partial molar enthalpy of reaction ΔH between solid tris(hydroxymethyl)amino methane (HMAM) and 0.1 M HCl(*aq*) in water at 298.15 K

HMAM 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
0.123	29.30

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

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

and

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

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

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

$$\mathrm{NH_3^+CHRCONHCHR'COO^-}(cr) \rightarrow$$
$$\mathrm{NH_3^+CHRCONHCHR'COO^-}(aq) \tag{3}$$

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

$$\mathrm{NH_3^+CHRCONHCHR'COO^-}(cr)+\mathrm{H^+}(aq) \rightarrow$$
$$\mathrm{NH_3^+CHRCONHCHR'COOH}(aq) \tag{4}$$

Results of at least six determinations of heats of solution of the various compounds (concentrations from 10^{-4} to 10^{-3} molal) have been extrapolated vs. the square root of concentrations, to infinite dilution ΔH_3^0.

Again, 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 $\mathrm{NH_3^+CHRCONHCHR'COOH}$ at infinite dilution in water, yielding one mole of $\mathrm{NH_3^+CHRCONHCHR'COO^-}$ and one mole of protons.

For compound containing carboxyl and amino groups, the dissociation processes in water are complicated by tautomeric equilibria and zwitterion formation [24–25].

While a generic dipeptide in acid solution can be represented by the form $\mathrm{NH_3^+CHRCONHCHR'COOH}$, in a solution approaching pH 7.00 the principal species are neutral molecules, which may be either the $\mathrm{NH_2CHRCONHCHR'COOH}(aq)$ form or the zwitterion form.

Thus, only the $\mathrm{NH_3^+CHRCONHCHR'COOH}$ form is represented in Eq. (4) for acid solution, 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 [26–27]. Therefore, it can be assumed that in this solution the zwitterion form is predominant. In this way, the enthalpy values related to carboxyl proton dissociation can be calculated.

The molar enthalpy at infinite dilution ΔH_2^0 of 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 $2\cdot10^{-2}$ m NaOH solution

$$\begin{gathered}NH_3^+CHRCONHCHR'COO^-(cr)+OH^-(aq)\rightarrow\\ NH_2CHRCONHCHR'COO^-(aq)+H_2O\end{gathered}\qquad(5)$$

The enthalpy value of ΔH_5^0 process (5) were extrapolated *vs.* the square root of concentrations of the anion form.

If the solution process enthalpy change values ΔH_3^0 and the ΔH_6^0 value for water, (equal to 55.94 kJ mol^{-1} [28]) 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 only the $NH_2CHRCONHCHR'COO^-$ form is present. Finally, it was noted that heat of neutralization of process (5) must be referred only to the protonated amino group since the second carboxyl group of both methionyl-aspartic acid (MetAsp) and methionyl-glutamic acid (MetGlu) was converted to methyl ester while the hydroxyl group of methionyl-tyrosine (MetTyr) was converted to trifluoroacetate ester. In addition, all the values of the total uncertainties (calorimetric, chemical and extrapolation) are given.

Let us consider the process (1) for MetMet compound:

$$\begin{gathered}NH_3^+CHRCONHCHRCOOH(aq)\rightarrow\\ NH_3^+CHRCONHCHRCOO^-(aq)+H^+(aq)\end{gathered}\qquad(1a)$$

subtracting Eq. (1a) from Eq. (1) gives the following transfer proton process:

$$\begin{gathered}NH_3^+CHRCONHCHR'COOH(aq)+NH_3^+CHRCONHCHRCOO^-(aq)\rightarrow\\ NH_3^+CHRCONHCHR'COO^-(aq)+NH_3^+CHRCONHCHRCOOH(aq)\end{gathered}\qquad(7)$$

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

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

The change of enthalpy related to process (7) and (8) can be written as:

$$\delta\Delta H_1^0=\Delta H_1^0(\text{MetSub})-\Delta H_1^0(\text{MetMet})$$

and

$$\delta\Delta H_1^0=\Delta H_2^0(\text{MetSub})-\Delta H_2^0(\text{MetMet})$$

where ΔH_1^0(MetSub) and ΔH_1^0(MetMet) are related to the first ionisation process of dipeptides and of methyl-methionine whereas ΔH_2^0(MetSub) and ΔH_2^0(MetMet) are the values of the second ionisation process for the same compounds.

Results and discussion

The discussion of 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 amide bond. For *L-L* dipeptides the large side chains lie on different sides 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 (solvophobic) forces are not effective and the drawing forces are the solvation and desolvation processes in amino and carboxyl groups of zwitterions, ions and neutral molecules.

The enthalpy values of solution ΔH_3^0, protonation ΔH_4^0 and neutralization ΔH_5^0 processes for all the compounds cited are reported in Table 2. This table also gives ionisation enthalpy values for the free carboxyl groups (ΔH_1^0) and for the free amino group (ΔH_2^0) of the methionine. MetMet (which has a symmetrical structure) is chosen as reference compound to study the reciprocal influence of methionine and other α-amino acids in the series of dipeptides. The quantities $\delta\Delta H_1^0$ and $\delta\Delta H_2^0$ are reported in Table 3.

Table 2 Enthalpy values (kJ mol^{-1}) of processes (1), (2), (3), (4) and (5) for some dipeptides having methionine 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
MetAsn	1.22±0.08	40.83±0.54	4.47±0.06	3.25±0.06	–10.64±0.70
MetAsp	2.38±0.04	36.32±0.22	12.99±0.03	10.61±0.03	–6.63±0.01
MetGln	3.07±0.55	39.53±0.39	17.69±0.45	14.62±0.31	1.10±0.05
MetGlu	–0.68±0.20	35.20±0.26	14.83±0.13	15.51±0.16	–5.91±0.16
MetGly	1.87±0.08	36.35±0.22	8.20±0.01	6.33±0.08	–11.39±0.05
MetIle	–2.19±0.06	46.83±0.23	–11.59±0.02	–9.40±0.06	–20.70±0.10
MetLeu	–2.38±0.05	43.68±0.22	–1.69±0.02	0.69±0.05	–13.95±0.02
MetMet	0.60±0.12	38.81±0.25	14.57±0.12	13.97±0.02	–2.56±0.13
MetSer	2.54±0.10	34.26±0.22	3.02±0.07	0.48±0.07	–18.66±0.02
MetThr	2.23±0.02	39.06±0.26	2.72±0.02	0.49±0.01	–14.16±0.21
MetTrp	1.61±0.06	40.15±0.26	4.54±0.01	2.92±0.06	–11.25±0.19
MetTyr	–5.17±0.23	39.44±0.24	14.49±0.13	19.66±0.19	–2.01±0.01
MetVal	1.45±0.04	42.54±0.22	–2.80±0.01	–4.25±0.04	–16.20±0.04

Thus dissociation processes of the carboxyl and amino groups of the studied dipeptides were compared with the dissociation processes of the corresponding groups of methionyl-methionine. By using MetMet as reference compound it is possible to observe that the proton transfer process at the carboxyl groups of dipeptides vary as a function of the methionine influence, so the enthalpy contribution to a relative scale of acidity can be found.

The scale for the dissociation processes of the carboxyl groups is MetTyr > MetLeu > MetIle > MetGlu > MetMet > MetAsn > MetVal > MetTrp > MetGly > MetThr > MetAsp > MetSer > MetGln (Table 3).

This sequence can be explained by considering the scales for relative salvation of zwitterion $\delta\Delta H_3^0=\Delta H_3^0$(MetSub)–$\Delta H_3^0$(MetMet) and for undissociated molecules $\delta\Delta H_4^0=\Delta H_4^0$(MetSub)–$\Delta H_4^0$(MetMet).

For the zwitterions the order of solvation is: MetIle > MetVal > MetLeu > MetThr > MetSer > MetAsn > MetTrp > MetGly > MetAsp > MetTyr > MetMet > MetGlu > MetGln (Table 3).

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

Compounds	$\delta\Delta H_1^0$	$\delta\Delta H_2^0$	$\delta\Delta H_3^0$	$\delta\Delta H_4^0$	$\delta\Delta H_5^0$
MetAsn	0.62	2.02	–10.10	–10.72	–8.08
MetAsp	1.78	–2.49	–1.58	–3.36	–4.07
MetGln	2.47	0.54	3.12	0.65	3.66
MetGlu	–1.28	–3.61	0.26	1.54	–3.35
MetGly	1.27	–2.46	–6.37	–7.64	–8.83
MetIle	–2.79	8.02	–26.16	–23.37	–18.14
MetLeu	–2.98	4.87	–16.26	–13.28	–11.39
MetMet	0.00	0.00	0.00	0.00	0.00
MetSer	1.94	–4.55	–11.55	–13.49	–16.10
MetThr	1.63	0.25	–11.85	–13.48	–11.60
MetTrp	1.01	1.34	–10.03	–11.04	–8.69
MetTyr	–5.77	0.63	–0.08	5.69	0.55
MetVal	0.85	3.73	–17.37	–18.22	–13.64

For the undissociated molecules, the order becomes: MetIle > MetVal > MetSer > MetThr > MetLeu > MetTrp > MetAsn > MetGly > MetAsp > MetMet > MetGln > MetGlu > MetTyr.

By using again methionyl-methionine as reference compound, it can be observed that the proton transfer process at the free amino group of methionine, in this series of dipeptides, 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: MetSer > MetGlu > MetAsp > MetGly > MetMet > MetThr > MetGln > MetTyr > MetTrp > MetAsn > MetVal > MetLeu > MetIle (Table 3).

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

This last relative solvation scale $\delta\Delta H_5^0=\Delta H_5^0$(MetSub)–$\Delta H_5^0$(MetMet) shows the sequence: MetIle > MetSer > MetVal > MetThr > MetLeu > MetGly > MetTrp > MetAsn > MetAsp > MetGlu > MetMet > MetTyr > MetGln (Table 3).

As regard the first ionisation process it can be observed that MetTyr, MetLeu and MetIle dissociate, from the enthalpic point of view, more easily than methionyl-methionine does, by virtue of the greater relative solvation of the zwitterions with respect to the relative solvation of undissociated molecules (Table 3).

It is well known that, for amino acids with alkyl groups as side-chains, hydrophobic effects causes reinforcement of the hydrogen bonds between the water molecules surrounding the alkyl groups [29]. For this reason water-water hydrogen bonds in water zones surrounding the alkyl groups of the amino acids are stronger than those in bulk water. 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 it can be hypothesized that, for the studied dipeptides series, the side-chains of Leu and Ile reinforce (with respect to that of Met) the interactions between the water molecules and the polar portion of dipeptides.

The hydrophobic effect of benzene ring prevails on the OH effect as regards the greater solvation of MetTyr zwitterion with respect that of MetMet.

For MetGlu the carboxyl group in the side-chain accounts of the decrease of the water-water hydrogen bonds around the polar portion both for the zwitterion and undissociated molecule with respect the corresponding zwitterion and undissociated molecule of MetMet. The relative desolvation of the undissociated molecule is the drawing force, which favours the dissociation of MetGlu with respect MetMet.

For MetAsn, MetVal, MetTrp, MetGly, MetThr, MetSer, MetAsp and MetGln compounds carboxyl groups dissociate with more difficulty than that of methionyl-methionine.

For MetVal, MetTrp, MetGly, MetThr, MetSer and MetAsp both anions and undissociated molecules are more solvated than those of MetMet, the solvation of undissociated molecules being the drawing force. For MetVal and MetTrp hydrophobic effect of Val and Trp allows to a greater solvation of zwitterions and undissociated molecule, while for MetGly, MetThr, MetSer, MetAsn and MetAsp this is not the case: Gly has not alkyl chain, Thr and Ser have a hydroxyl group while Asp, Asn and Gln have carboxyl and amidic groups, so hydrophobic effect is absent.

For MetGln both zwitterion and undissociated molecule are less solvated than those of MetMet, the solvation of zwitterion being the drawing force.

In the second process of ionisation MetSer, MetAsp, MetGly, MetGlu are more dissociated, in the amino group than methionine-methionine. For these compounds the solvation of the ions form prevails with respect to that of zwitterions, while for the others, the reverse is true.

In the first ionisation process, carboxyl groups of Tyr, Leu, Ile and Glu dissociate more easily than that of methionine by virtue of the influence of the same methionine.

In the second dissociation process Asn, Val, Trp, Thr, Ile, Leu, Tyr and Gln decrease the enthalpic contribution to the basicity of methionine in the dipeptides with respect to that of the reference compound.

The first relative scale gives a measure of the effect of methionine upon the other α amino acids, assuming MetMet as reference compound, while the scale for the second ionisation process shows how α-amino acids can affect methionine.

For the corresponding free α-amino acids, the enthalpy values of the first and second ionisation processes, in the aqueous phase were calculated previously [30].

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

Compounds	(9a)	(9b)
MetAsn	–0.61	–0.07
MetAsp	–0.29	–0.18
MetGln	0.10	–0.11
MetGlu	–1.96	–0.17
MetGly	–0.54	–0.17
MetIle	26.37	0.06
MetLeu	–2.58	–0.06
MetMet	–0.77	–0.12
MetSer	0.91	–0.22
MetThr	0.44	–0.11
MetTrp	–2.13	–0.10
MetTyr	3.27	–0.11
MetVal	1.04	–0.04

A further contribution to the comprehension of the reciprocal influence of methionine and other α-amino acids can be supplied from the following relationships, which directly compare the dipeptides and the single α-amino acids series in absolute scale and supply percentage values of the contribution of the enthalpy to the acidity and basicity of the dipeptides:

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

and

$$[\Delta H_2^0(\mathrm{MetSub})–\Delta H_2^0(\mathrm{Sub})]/\Delta H_2^0(\mathrm{Sub}) \tag{9b}$$

where ΔH_1^0(MetSub) represents the ionisation process values for the carboxyl groups of the dipeptides, ΔH_1^0(Sub) represents the values of the dissociation process for carboxyl groups of the corresponding free α-amino acids, ΔH_2^0(MetSub) represents the values of the dissociation for the free amino groups of dipeptides and ΔH_2^0(Sub) the values for the dissociation processes of the amino group of free methionine. Values related to the ‘absolute’ influence of methionine upon the free carboxyl groups of dipeptides and of the α-amino acids upon the amino group of methionine 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.

In the first dissociation process of the dipeptides, the influence of methionine favours the dissociation (from the enthalpic point of view) of the free carboxyl groups of Asn, Asp, Glu, Gly, Leu, Met, Trp, with respect to those of the corresponding single α-amino acid. For these compounds the carboxyl groups of the dipeptides show enthalpy changes for dissociation more exothermic or less endothermic than those of the corresponding free α-amino acids while for the other dipeptides the reverse is true.

Table 5 Hückel–Mclachlan charge density distributions for the studied dipeptides and their free C-terminal α-amino acids in neutral form for comparison

Compounds	NH_2	COOH
MetAsn	0.2813	0.4995
Asn	0.2751	0.6189
MetAsp	0.2813	0.4995
Asp	0.2751	0.6189
MetGln	0.2808	0.4415
Gln	0.2795	0.5144
MetGlu	0.2808	0.4415
Glu	0.2795	0.5144
MetGly	0.2846	0.5459
Gly	0.3211	0.6785
MetIle	0.2567	0.2234
Ile	0.1714	0.2405
MetLeu	0.2454	0.2244
Leu	0.1725	0.2498
MetMet	0.2190	0.3660
Met	0.2567	0.3962
MetSer	0.2755	0.3470
Ser	0.2455	0.3784
MetTyr	0.6107	0.3253
Tyr	1.1043	0.9059
MetTrp	0.0001	0.7786
Trp	1.1099	0.9059
MetThr	0.2634	0.2484
Thr	0.1873	0.2659
MetVal	0.2721	0.2750
Val	0.1978	0.2837

The proton dissociation process of the free amino group of methionine is hindered by Ile while other α-amino acids favour it. The proton dissociation trends could be related to the increase or decrease of electron charge localised on the carboxyl and amino groups of the dipeptides with respect to the electron charge distribution on the same groups of the free α-amino acids. The trend of the first dissociation process of most of the dipeptides agrees with the results of the Hückel–McLachlan molecular orbital calculations. These calculations show the decrease of the electron charge density on the carboxyl groups of dipeptides with respect to the density charge localised on the carboxyl groups of the corresponding α-amino acids (Table 5).

For second proton dissociation only the behaviour of MetIle, MetMet and MetTyr agrees with the electron charge density of α-amino acids.

A computer program using the following values [31]:

$h_{\ddot{N}}=0.5$ $K_{CC}=0.8$ $K_{C-C}=0.8$
$h_{\ddot{O}}=2$ $K_{C=O}=1$
$h_{\dot{O}}=1$ $K_{C-N}=0.8$

calculated the Hückel–McLachlan charge distribution, where h is the Coulomb integral (interaction energy between each electron and his respective nucleus) increment and K is the bond integral which represents the energy of two atomic orbitals; C–C symbolises a single bond, C=C a double bond and CC an aromatic bond.

Conclusions

Our experimental evidence supports the conclusion that the mutual influence of methionine and the other α-amino acids, in a series of dipeptides, 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 methionyl-methionine as reference compound) vary as a function of the reciprocal influence of methionine and of the second component, so that, two relative scales of acidity and basicity, in the aqueous phase, can be found.

The drawing forces are the relative solvation and desolvation processes of zwitterions, undissociated molecules and ions, where the hydrophobic effect of side chains plays an important role.

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 due to the reciprocal influence of methionine and other α-amino acids.

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