Thermodynamics of proton transfer processes of standard α-amino acids compared with the same processes in the gaseous phase

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Abstract

The basicity of the neutral form of some "standard" α-amino acids in gaseous and liquid phases is studied from the thermodynamic and statistical points of view.

By using the proton transfer process (related to the amino group) in the gaseous and liquid phases, it is possible to carry out a thermodynamic cycle which allows the solvent effects to be separated from those intrinsic to neutral and protonated molecules.

The basicity of these compounds seems to be mostly influenced by solute–solvent interactions (solvent effects).

A monoparametric linear regression analysis leads (in terms of probability) to the hypothesis that it is uncertain if the side chains influence in the same way the basicity of the α-amino acids in liquid and in gaseous phases.

Keywords: Amino acid; Calorimetry; Heat of ionization; Heat of neutralization; Heat of protonation; Heat of solution; Optical activity; Phase; Protonation; Proton transfer; Thermodynamics; Zwitterion

1. Introduction

The twenty α-amino acids which are usually found in proteins are called "standard" α-amino acids, and, with the exception of proline, have one carboxyl

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group and one aminic group, both linked to the α-carbon atom. "Standard" α-amino acids differ from one another in the structure of their side-chains (called R groups), the polarization of which is the criterion used to classify the compounds into four groups: (1) a class with apolar or hydrophobic chains, (2) a class with polar chains having no charge, (3) a class with polar chains having positive charge, and (4) a class with polar chains having negative charge. There are considerable variations in the size and properties of the R groups within the same class.

The thermodynamic study of these compounds in the liquid phase has been the subject of extensive research in our laboratory [1–8]. This study was carried out with reference to the thermodynamic quantities $\Delta G^{\ominus}$, $\Delta H^{\ominus}$ and $\Delta S^{\ominus}$, as related to the proton dissociation of the carboxyl and aminic groups linked to the α-carbon atom and of some other functional groups contained in the side chains. The first, second and third ionization processes of these compounds were measured in water by calorimetric techniques.

The dominant effect influencing the ionization processes was found to be the solvation of ions and molecules, which in turn depends on the different side chains and on the different groups within the same side chains.

The mutual structural influences of α-amino acids in dipeptides have also been studied by calorimetric measurement in the liquid phase [9–11].

Similar research on standard α-amino acids, and dipeptides in the solid phase has been carried out by simultaneous TG–DSC measurements [12–15].

It was usually observed (both in liquid and solid phases) that small variations in the side chains lead to large variations in the enthalpy values linked to the dissociation processes of the functional groups which characterize these α-amino acids and to the thermal decomposition of the molecules.

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

Recently developed techniques [16–25], such as ion cyclotron resonance (ICR), high-pressure spectrometery (HPMS) and laser desorption/chemical ionization LD/CI, have afforded a deeper insight into the gaseous proton transfer processes. In this way it is possible to study acid–base reactivity in terms of molecule structures and bonding, in the absence of solvent effects.

Furthermore, by using the same proton transfer processes in the gas phase and in the liquid phase, it is possible to carry out a thermodynamic cycle which allows the solvent effects to be separated from those intrinsic to the molecules and ions [16–25].

In this way the proton transfer processes from substituted benzoic acids to benzoic acid in H_2O–DMSO mixtures, compared with the same processes in the gaseous phase, were presented [26–29].

Electron transfer processes for any series of organic compounds, in aprotic solvents and gas phase, were also compared with the proton transfer processes in the same solvents [30–35].

All organic compounds are potential proton acceptors and/or proton donors and may be converted into reactive intermediates by proton transfer processes.

Proton transfer processes such as
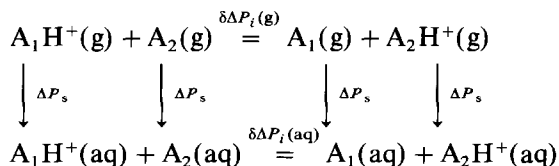
$$A_1H^+ + A_2 = A_1 + A_2H^+ \tag{1}$$

were usually employed, in the gas phase, to measure the gas-phase basicities ($-\Delta G$ of protonation) and the proton affinities ($-\Delta H$ of protonation) of all the 20 common α-amino acids [21–25], where A_1H^+ and A_2H^+ are protonated α-amino acids.

It is well known that in aqueous solution and in the crystalline state α-amino acids have the structure of a dipolar ion or zwitterion. It would be interesting to know if the zwitterion also exists in the gas phase. Evidence indicating that gas-phase amino acid molecules are not zwitterions has been summarized by some authors [21–25].

Hepler and coworkers [36–39] 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, i.e. $\delta\Delta P = \delta\Delta P_{in} + \delta\Delta P_{ex}$.

Internal effects are those intrinsic to the molecules and the ions, i.e. the effects related to the gaseous isodesmic processes, whereas external effects are derived from solvent interactions with the molecules and ions and are thus related to the solvation process.

The following thermodynamic cycle is used to calculate the external interactions

$$A_1H^+(g) + A_2(g) \overset{\delta\Delta P_i(g)}{=} A_1(g) + A_2H^+(g)$$

$$\downarrow \Delta P_s \qquad \downarrow \Delta P_s \qquad \downarrow \Delta P_s \qquad \downarrow \Delta P_s$$

$$A_1H^+(aq) + A_2(aq) \overset{\delta\Delta P_i(aq)}{=} A_1(aq) + A_2H^+(aq)$$

So we can write

$$\delta\Delta P_i(aq) - \delta\Delta P_i(g) = [\Delta P_s(A_1) - \Delta P_s(A_1H^+)]$$
$$- [\Delta P_s(A_2) - \Delta P_s(A_2H^+)] \tag{2}$$

and again

$$\delta\Delta P_i(aq) - \delta\Delta P_i(g) = \delta\Delta P_s \tag{3}$$

where $\delta\Delta P_i(g)$ is the gaseous phase change of any thermodynamic property ($P_i = G, H, S$) from A_1 to A_2. The corresponding value in water is $\delta\Delta P_i(aq)$. The right-hand term of Eq. (3) can be assumed to be the proton transfer process in water, which refers to a gaseous initial thermodynamic state, i.e. $\delta\Delta P_s = \delta\Delta P_i^{g\rightarrow aq}$.
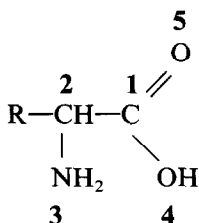
Moreover, it can be assumed that the $\delta\Delta P_i^{g\rightarrow aq}$ term is a measure of external interactions, so that it is reasonable to assume that $\delta\Delta P_i(aq)$, $\delta\Delta P_i(g)$ and $\delta\Delta P_i^{g\rightarrow aq}$, which represent the "total", the internal and the external interactions, respectively, can be correlated

$$\delta\Delta P_i(aq) = \delta\Delta P_i(g) + \delta\Delta P_i^{g\rightarrow aq} \tag{4}$$

Little is known about the acid/base properties of α-amino acids in the gas phase, yet comparison of the gas and solution phases is of fundamental importance.

The aim of this work is to ascertain if the gas-phase substituent effect of the side chains and of the COOH group for some α-amino acids (1–15, below) on the basicity of the amine function may be compared with the analogous substituent effect in solution.

The α-amino acids studied in this work were α-alanine, leucine, isoleucine, valine, phenylalanine, methyonine, serine, threonine, glycine, asparagine, glutamine, cysteine, lysine, aspartic acid and glutamic acid.

$$
\begin{array}{c}
\mathbf{5} \\
\mathrm{O} \\
\mathbf{2}\quad\mathbf{1}\;/\!\!/ \\
\mathrm{R-CH-C} \\
|\qquad\backslash \\
\mathrm{NH_2}\quad\mathrm{OH} \\
\mathbf{3}\qquad\mathbf{4}
\end{array}
$$

1, R is CH_3; 2, R is $(CH_3)_2CHCH_2-$; 3, R is $C_2H_5CH(CH_3)-$; 4, R is $(CH_3)_2CH-$; 5, R is ⬡$-CH_2-$; 6, R is $CH_3-S-(CH_2)_2-$; 7, R is $CH_2(OH)-$; 8, R is $CH_3-CH(OH)-$; 9, R is H; 10, R is NH_2COCH_2-; 11, R is $NH_2CO(CH_2)_2-$; 12, R is $SH-CH_2-$; 13, R is $NH_3^+(CH_2)_4-$; 14, R is $COOH-CH_2-$; 15 R is $COOH(CH_2)_2-$.
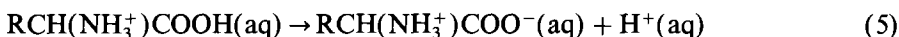
2. Experimental and procedures

All the compounds (Carlo Erba, RPE chemicals, used without purification) were weighed and handled in a nitrogen-filled dry box.
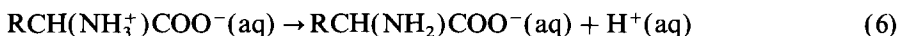
The purity of all compounds was between 99% and 100% and was checked by a DSC purity method using a Stanton-Redcroft 625 DSC and a dynamic purity program supplied by P.L. Thermal Sciences Ltd.

A Tronac (model 458) instrument was used to make the measurements. The calorimetric vessel was a rapid-response glass vacuum Dewar of maximum capacity $100\ cm^3$. The thermostat was maintained at $298.15 \pm 0.0002\ K$ by employing a Tronac P.T.C. 41 precision temperature-controller. Potential versus time measurements were made using a Fluke 88100 model digital voltmeter. The imbalance (volts) 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 [40] run on the Olivetti M24 computer. Data obtained using the chart recorder may be slightly different from those obtained using the computer and they also give the shape of the reaction.

The first and second ionization processes of a generic "standard" α-amino acid $RCH(NH_3^+)COOH$ in water can be represented as

$$RCH(NH_3^+)COOH(aq) \rightarrow RCH(NH_3^+)COO^-(aq) + H^+(aq) \qquad (5)$$

and

$$RCH(NH_3^+)COO^-(aq) \rightarrow RCH(NH_2)COO^-(aq) + H^+(aq) \qquad (6)$$
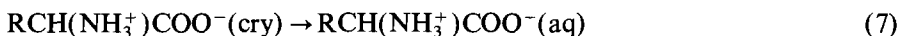
The partial molar enthalpy of dissociation $\Delta\bar{H}_5$ of $RCH(NH_3^+)COOH(aq)$ in water is obtained by measuring the following quantities.

(i) The partial molar enthalpy of solution $\Delta\bar{H}_7$ of crystalline (cry) $RCH(NH_3^+)COO^-$ zwitterion form, in water at pH close to the isoelectric point

$$RCH(NH_3^+)COO^-(cry) \rightarrow RCH(NH_3^+)COO^-(aq) \qquad (7)$$

(ii) The partial molar enthalpy of protonation $\Delta\bar{H}_8$ of the same compound in water at pH 0.0

$$RCH(NH_3^+)COO^-(cry) + H^+(aq) \rightarrow RCH(NH_3^+)COOH(aq) \qquad (8)$$

The partial molar enthalpy of process (5) can be obtained by subtracting $\Delta\bar{H}_8$ from $\Delta\bar{H}_7$ values. Concentrations of about 10^{-3} M were used in processes (7) and (8), so that the $\Delta\bar{H}$ values can be considered as being at infinite dilution $\Delta H^{\ominus}$ [41].

These values refer to the proton dissociation of 1 mol of $RCH(NH_3^+)COOH$ at infinite dilution in 1000 g of water, yielding 1 mol of $RCH(NH_3^+)COO^-$ ions and 1 mol of protons solvated in the same amount of water.

The dissociation processes in water, for a compound containing the carboxylate and amino groups, is complicated by tautomeric equilibria and zwitterion formation [1–7].

Scheme 1 shows the equilibria in question.
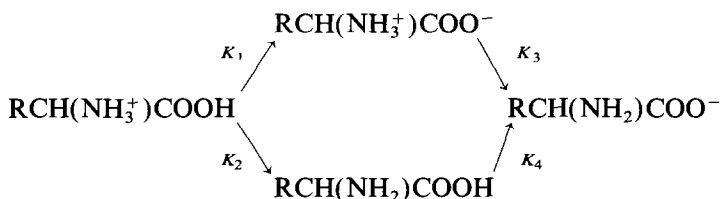
Scheme 1. The dissociation processes of a compound containing carboxylate and amino groups.

While the α-amino acids in the acid solution can be represented by the form $RCH(NH_3^+)COOH$, in solution approaching pH 7.0, the principal species are neutral molecules which may either by in the $RCH(NH_2)COOH$ form or in the zwitterion form $RCH(NH_3^+)COO^-$. Therefore only the $RCH(NH_3^+)COOH$ form is present in Eq. (8) at pH 0.0; in Eq. (7) this is not the case. However, it is possible to calculate the isoelectric pH values of the compounds investigated, by means of the constant values of dissociation [42–48]. It can therefore be assumed that in solution the $RCH(NH_3^+)COO^-$ form is predominant. In this way, it is possible to calculate the first proton dissociation enthalpies, i.e. the processes related to K_1 in Scheme 1.

The partial molar enthalpy of the second proton dissociation process of $RCH(NH_3^+)COO^-$ (K_3 process) was obtained by measuring the partial molar enthalpy of neutralization of the crystalline compound $RCH(NH_3^+)COO^-$ in water at pH 14

$$RCH(NH_3^+)COO^-(cry) + OH^-(aq) = RCH(NH_2)COO^-(aq) + H_2O(liq) \quad (9)$$
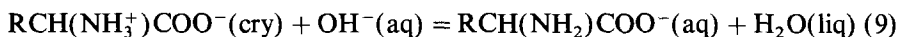
If the partial molar enthalpy values of solution $\Delta\bar{H}_7$ and the partial molar value $\Delta\bar{H}_{10}$ related to the process (in water) [49]

$$H^+(aq) + OH^-(aq) = H_2O(liq) \quad (10)$$

are subtracted from the $\Delta\bar{H}_9$ values, then the enthalpies of process (6) are obtained.

These values refer to the dissociation of 1 mol $RCH(NH_3^+)COO^-$ at infinite dilution in 1000 g water which yields 1 mol of $RCH(NH_2)COO^-$ ions and 1 mol protons solvated in the same amount of water. The $\Delta\bar{H}$ values for this process can also be considered as equal to $\Delta H^{\ominus}$. Process (9) is carried out at pH 14 so that only the $RCH(NH_2)COO^-$ form is present.

3. Results and discussion

The enthalpy values of the solution, protonation and neutralization processes, for the studied α-amino acids, are reported in Table 1. The enthalpy, free energy [7] and entropy values of the first and second ionization processes are reported in Tables 2, 3 and 4.

As the α-amino acids in gas phase are assumed be to in the neutral, i.e. not in the zwitterion, form, the reaction of interest is

$$RCH(NH_3^+)COOH(g) \rightarrow RCH(NH_2)COOH(g) + H^+(g) \quad (11)$$

Table 1
Enthalpy values (kJ mol^{-1}) of solution ($\Delta H_7^{\ominus}$), protonation ($\Delta H_8^{\ominus}$) and neutralization ($\Delta H_9^{\ominus}$) for "standard" α-amino acids in water at 298 K

Compounds	$\Delta H_7^{\ominus}$	$\Delta H_8^{\ominus}$	$\Delta H_9^{\ominus}$
L(−)α-alanine	7.62	5.19	−2.05
(±)-valine	5.36	4.64	−16.74
L(+)leucine	2.93	1.42	−5.48
(±)-isoleucine	2.64	2.72	−6.73
L-(−)methionine	11.22	8.62	−0.50
(±)-phenylalanine	7.70	7.11	−2.51
D(−)serine	11.01	9.67	−4.14
L(−)threonine	10.13	8.58	−4.56
L(−)glycine	14.40	10.46	2.89
L(−)cysteine	11.01	8.75	−33.56
L(−)glutamine	22.77	19.96	7.74
L(−)aspargine	31.51	28.42	16.91
L(−)lysine	12.05	10.62	1.00
(±)-aspartic acid	25.78	22.43	−13.73
L(+)glutamic acid	24.74	24.02	−14.23

Table 2
Enthalpy values (kJ mol^{-1}) of the first ($\Delta H_5^{\ominus}$) and second ($\Delta H_6^{\ominus}$) ionization processes for "standard" α-amino acids in water at 298 K

Compounds	$\Delta H_5^{\ominus}$	$\Delta H_6^{\ominus}$
L(−)α-alanine	2.43	46.16
(±)-valine	0.72	33.85
L(+)leucine	1.51	47.54
(±)-isoleucine	−0.08	46.58
L-(−)methionine	2.60	44.23
(±)-phenylalanine	0.59	45.74
D(−)serine	1.34	40.80
L(−)threonine	1.55	41.26
L(−)glycine	3.94	44.44
L(−)cysteine	2.26	38.08
L(−)glutamine	2.81	40.92
L(−)aspargine	3.09	41.35
L(−)lysine	1.43	46.49
(±)-aspartic acid	3.35	64.86
L(+)glutamic acid	0.72	70.13

Table 3
Free energy values (kJ mol^{-1}) of the first ($\Delta G_5^{\ominus}$) and second ($\Delta G_6^{\ominus}$) ionization processes for "standard" α-amino acids in water at 298 K

Compounds	$\Delta G_5^{\ominus}$	$\Delta G_6^{\ominus}$
L(−)α-alanine	13.39	56.29
(±)-valine	13.31	55.45
I(+)leucine	13.31	55.66
(±)-isoleucine	13.22	55.70
L-(−)methionine	13.01	52.60
(±)-phenylalanine	14.73	53.02
D(−)serine	12.47	52.56
L(−)threonine	11.97	51.98
L(−)glycine	13.43	55.81
L(−)cysteine	9.75	61.56
L(−)glutamine	12.39	52.14
L(−)aspargine	11.55	46.12
L(−)lysine	12.43	51.10
(±)-aspartic acid	11.38	57.12
L(+)glutamic acid	12.35	53.44

Because the α-amino acids of interest are zwitterions in solution, reaction (11) is not directly observed in the liquid phase. Process (11) in water (K_2 process) can be obtained by the reactions
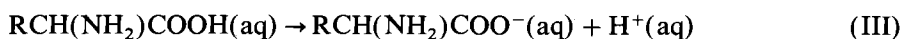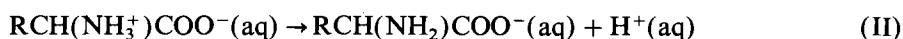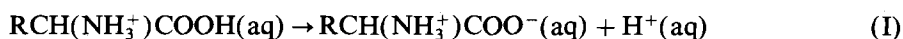
$$RCH(NH_3^+)COOH(aq) \rightarrow RCH(NH_3^+)COO^-(aq) + H^+(aq) \qquad (I)$$

$$RCH(NH_3^+)COO^-(aq) \rightarrow RCH(NH_2)COO^-(aq) + H^+(aq) \qquad (II)$$

$$RCH(NH_2)COOH(aq) \rightarrow RCH(NH_2)COO^-(aq) + H^+(aq) \qquad (III)$$

Table 4
Entropy values ($J\ mol^{-1}\ K^{-1}$) of the first ($\Delta S_5^{\ominus}$) and second ($\Delta S_6^{\ominus}$) ionization processes for "standard" α-amino acids in water at 298 K

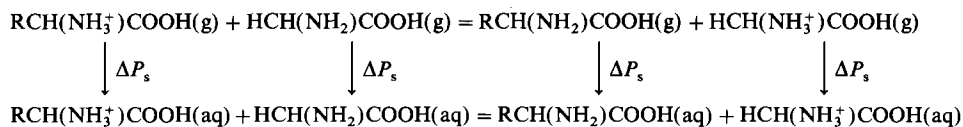| Compounds	$\Delta S_5^{\ominus}$	$\Delta S_6^{\ominus}$
L(−)α-alanine	−36.76	−33.98
(±)-valine	−42.23	−72.45
L(+)leucine	−39.58	−27.23
(±)-isoleucine	−44.61	−30.59
L-(−)methionine	−34.91	−28.07
(±)-phenylalanine	−47.43	−24.42
D(−)serine	−37.33	−39.44
L(−)threonine	−34.95	−35.96
L(−)glycine	−31.83	−38.20
L(−)cysteine	−25.12	−78.75
L(−)glutamine	−32.13	−37.63
L(−)aspargine	−28.37	−16.00
L(−)lysine	−36.89	−15.46
(±)-aspartic acid	−26.93	25.96
L(+)glutamic acid	−39.00	55.98

Table 5
Free energy, enthalpy and entropy values of process (11) in water

Compounds	$\Delta G^{11}/$ (kJ mol^{-1})	$\Delta H^{11}/$ (kJ mol^{-1})	$\Delta S^{11}/$ (J mol^{-1} K^{-1})
L(−)glycine	42.16	48.80	22.27
L(−)α-alanine	41.85	49.26	24.85
L(−)threonine	36.41	45.49	30.45
L(−)cysteine	43.48	41.01	−8.28
(±)-aspartic acid	44.48	65.03	68.93
L(+)glutamic acid	41.01	71.35	101.76
(±)-valine	41.47	39.44	−6.80
L(+)leucine	41.31	51.98	35.95
(±)-isoleucine	41.26	49.43	27.40
L(−)lysine	35.78	50.14	48.17
L(−)-methionine	38.07	49.51	38.37
(±)-phenylalanine	39.92	47.00	23.74
L(−)asparagine	33.65	41.26	25.52
L(−)glutamine	39.75	44.23	15.02
D(−)serine	37.20	42.81	18.81

by using the expression $\Delta P_i = \Delta P_I + \Delta P_{II} - \Delta P_{III}$, where ΔP_I and ΔP_{II} are processes (5) and (6) respectively, and the quantities ΔP_{III} for the acid dissociation of the neutral α-amino acids are approximated by the quantities of dissociation of the corresponding carboxylic acids [50]. Table 5 shows the thermodynamic quantities of process (11), i.e. K_2 process in water.

Let us consider the cycle

$$RCH(NH_3^+)COOH(g) + HCH(NH_2)COOH(g) = RCH(NH_2)COOH(g) + HCH(NH_3^+)COOH(g)$$

$$\Big\downarrow \Delta P_s \qquad\qquad \Big\downarrow \Delta P_s \qquad\qquad \Big\downarrow \Delta P_s \qquad\qquad \Big\downarrow \Delta P_s$$

$$RCH(NH_3^+)COOH(aq) + HCH(NH_2)COOH(aq) = RCH(NH_2)COOH(aq) + HCH(NH_3^+)COOH(aq)$$

to account for the effects of structural side-chain changes on energy differences for the α-amino acids under consideration compared to a reference compound (glycine) in liquid and gas phases.

For the α-amino acids, the entropy of reaction (1) can be calculated for changes in rotation symmetry numbers (σ) of the reactants and products according to

$$\Delta S_{tot} = R \ln[\sigma_{A_1 H^+} \sigma_{A_2}/\sigma_{A_1} \sigma_{A_2 H^+}] \tag{12}$$

It is well known [16–20] that both the external and internal rotations in the half-process $A_1 H^+ \rightarrow A_1 + H^+$, occurring in th gaseous phase, are responsible for the loss of entropy in Eq. (1), so that $\delta \Delta S_i(g) \neq 0$.

However, the α-amino acids used in this study have generally [21–25] low symmetry numbers, so that it is possible to assume $\delta \Delta P_i(g)(350 \text{ K}) \approx \delta \Delta P_i(298 \text{ K})$ [16–20] and to calculate $\delta \Delta P_i^{g \rightarrow a}$ values at 298 K.

3.1. Thermodynamics of proton transfer processes

To separate the solvent effects from the intrinsic effects for the proton transfer process

$$RCH(NH_3^+)COOH(aq) + HCH(NH_2)COOH(aq)$$

$$= RCH(NH_2)COOH(aq) + HCH(NH_3^+)COOH(aq)$$
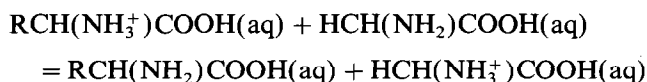
the $\delta \Delta P_i(aq)$ (from Table 5), $\delta \Delta P_i(g)$ (from Ref. 21) and $\delta \Delta P_i^{g \rightarrow aq}$ (from Eq. (4)) values were calculated and are given in Tables 6, 7 and 8. From our experimental evidence on the proton transfer processes of the protonated molecules belonging to first class, the following observation may be made.

The free energy and entropy, in the liquid phase, favour the proton transfer process of α-alanine, leucine, isoleucine and methionine in comparison with that of the glycine compound, while the enthalpy term hinders it. The internal interactions prevail over the external ones.

Valine and phenylalanine undergo a larger proton transfer process because the free energy and enthalpy terms are mostly influenced by external interactions. A free-energy scale of proton transfer processes of the first class can be given as methionine > phenylalanine > isoleucine > leucine > valine > α-alanine, where the external factors play a major role.

This scale could be explained by considering the proximity of the methyl groups to the aminic groups linked to α-carbon atoms where the protonation process is likely to occur.

The increase in the distance between the methyl and aminic groups favours the proton transfer process.

Table 6
Differences, with respect to glycine, in the thermodynamic properties of the proton transfer processes of some α-amino acids in neutral form $RCH(NH_2)COOH$ in water at 298 K

Compounds	$\delta \Delta G^{\oplus}/$ (kJ mol^{-1})	$\delta \Delta H^{\oplus}/$ (kJ mol^{-1})	$\delta \Delta S^{\oplus}/$ (J mol^{-1} K^{-1})
L(−)glycine	0.00	0.00	0.00
L(−)α-alanine	−0.31	0.46	2.58
L(−)threonine	−5.75	−3.31	8.18
L(−)cysteine	1.32	−7.79	−30.55
(±)-aspartic acid	2.32	16.25	46.66
L(+)glutamic acid	−1.15	22.55	79.49
(±)-valine	−0.69	−9.36	−29.07
L(+)leucine	−0.85	3.18	13.68
(±)-isoleucine	−0.90	0.63	5.13
L(−)lysine	−6.38	1.34	25.90
L(−)-methionine	−4.09	0.71	16.10
(±)-phenylalanine	−2.24	−1.80	1.47
L(−)asparagine	−8.51	−7.54	3.25
L(−)glutamine	−2.41	−4.57	−7.25
D(−)serine	−4.96	−5.99	−3.46

Table 7
Differences, with respect to glycine, in the thermodynamic properties of the proton transfer processes of some α-amino acids in neutral form $RCH(NH_2)COOH$ in the gas phase at 298 K

Compounds	$\delta \Delta G^{\oplus}/$ (kJ mol^{-1})	$\delta \Delta H^{\oplus}/$ (kJ mol^{-1})	$\delta \Delta S^{\oplus}/$ (J mol^{-1} K^{-1})
L(−)glycine	0.00	0.00	0.00
L(−)α-alanine	48.55	50.64	7.03
L(−)threonine	78.68	77.42	−4.18
L(−)cysteine	19.67	19.25	−1.41
(±)-aspartic acid	40.18	42.69	8.42
L(+)glutamic acid	96.26	128.06	106.42
(±)-valine	48.55	50.64	7.03
L(+)leucine	59.01	62.77	12.56
(±)-isoleucine	59.01	62.77	12.56
L(−)lysine	105.88	136.43	102.45
L(−)-methionine	78.68	77.42	−4.18
(±)-phenylalanine	69.89	71.98	7.03
L(−)asparagine	69.89	71.98	7.03
L(−)glutamine	78.68	77.42	−4.18
D(−)serine	40.18	42.69	8.41

The phenyl group in phenylaline and the heteroatom in methionine activate the NH_3^+ and also favour the proton transfer process.

With the exception of cysteine, all the compounds of the second class (serine, threonine, asparagine, glutamine and cysteine) undergo a proton transfer process larger than those of the first class, this being due mostly to external interactions.

Table 8

Differences, with respect to glycine, in the thermodynamic properties of the proton transfer processes of some α-amino acids in neutral form $RCH(NH_2)COOH$ at 298 K

Compounds	$\delta\Delta G^\circ/$ (kJ mol^{-1})	$\delta\Delta H^\circ/$ (kJ mol^{-1})	$\delta\Delta S^\circ/$ (J mol^{-1} K^{-1})
L(−)glycine	0.00	0.00	0.00
L(−)α-alanine	− 48.86	− 50.18	− 4.45
L(−)threonine	− 84.43	− 80.79	12.36
L(−)cysteine	− 18.35	− 27.04	− 29.14
(±)-aspartic acid	− 37.86	− 26.46	+ 38.24
L(+)glutamic acid	− 97.41	− 105.51	− 27.16
(±)-valine	− 49.24	− 60.00	− 36.10
L(+)leucine	− 59.86	− 59.59	0.91
(±)-isoleucine	− 59.91	− 62.14	− 7.43
L(−)lysine	− 112.26	− 135.09	− 76.55
L(−)methionine	− 82.77	− 76.71	20.28
(±)-phenylalanine	− 72.13	− 73.78	− 5.56
L(−)asparagine	− 78.40	− 79.52	− 3.78
L(−)glutamine	− 81.09	− 81.99	− 3.07
D(−)serine	− 45.14	− 48.68	− 11.87

Thus it can be concluded that compounds of this class are less basic than those of the first.

The hydroxyl groups of serine and threonine deactivate the NH_2 group (partially drawing the nitrogen lone pair and thus decreasing the bond with the proton) and favour the proton transfer process.

Asparagine undergoes a larger proton transfer process than glutamine because the second amidic group is closer to the NH_2 group and deactivates it considerably, mostly in the gas phase.

The SH group in the protonated form of cysteine also partially draws the nitrogen lone pair bonding the proton and thus decreases the enthalpy value of the proton loss process.

Nevertheless, the entropy term plays a major role in this process: its increase in the solute–solvent interactions hinders the deprotonation process.

Because of the long chain of lysine, cyclization in the gas phase of the protonated form allows high external and internal contributions to the proton transfer process.

The large difference between the external and internal interactions of aspartic and glutamic acids probably derives from the cyclization of the protonated form of glutamic acid.

3.2. Statistical correlations of transfer proton processes

If the side-chains affect the basicity of the amino group of the α-amino acids in the liquid and gas phases in the same way, then the thermodynamic quantities of the corresponding proton transfer proceses will have a linear relationship.

Furthermore to identify the actual site of the proton transfer processes in both phases, the corresponding thermodynamic quantities are linearly related with the electron density charge distribution of the studied compounds.

For this purpose it is convenient to use monoparametric linear regression analysis. However, the significance level of these relations only allows the experimental results to be compared and explained in terms of the probabilities.

A rule may be applied to solvation energies: for an isodesmic proton process (ion–molecule reaction), the difference between thermodynamic properties in solution and in the gas phase lie in the different effects of the molecule's structure (charge distribution through resonance, hydrogen bonding, polarization) on the solvation properties $\delta \Delta P_i^{g \to a}$ of the uncharged molecules and ions. Thus, the differential solvation factors are assumed to be those which cause departures from a linear relationship of gas phase versus solution thermodynamics properties.

Thus, for a series of compounds, a critical examination of correlations between $\delta \Delta P_i$(aq), $\delta \Delta P_i$(g) and $\delta \Delta P_i^{g \to a}$ allows an evaluation of the effects of molecular structure of the side-chains and of the medium on the overall proton transfer process.

Finally, a comparison was made between the proton transfer process and the electron density of the molecules.

The relations used were $\delta \Delta P_i$(aq) vs. δq_N, $\delta \Delta P_i$(g) vs. δq_N, and $\delta \Delta P_i^{g \to a}$ vs. δq_N, where δq_N is the electron density on the nitrogen atom of the amino group linked to the α-carbon atom, expressed as $\delta q_N = q_{NR} - q_{NH}$.

The Hückel–McLachlan charge distribution was calculated by a computer program using the values [51]: $h_N = 0.5$, $h_O^{\cdot\cdot} = 2$, $h_O^{\cdot} = 1$, $h_S = 0$, $K_{CC} = 1$, $K_{C-O} = 1$, $K_{C-O} = 0.8$, $K_{C-S} = 1$, $K_{C-N} = 0.8$, and $K_{C-C} = 0.8$, where h is the Coulomb integral (interaction energy between each electron and its respective nucleus) increment and K is the bond integral which represents the energy of interaction of two atomic orbitals; C–C symbolizes a single bond and CC an aromatic bond.

In every case the correlations have to be studied from a 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, 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 [52–57].

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 (i) for the intercept $a = 0$; and (ii) for the slope $b = 0$. The null hypotheses were tested using the Student t-test.

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 $t_a = (a - A)/S_a$ and $t_b = (b - B)/S_b$ 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 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 rejection is highly significant.

From the values cited above, the following correlations were examined critically by means of linear monoparametric analysis: (i) $\delta \Delta P_i$(aq) vs. $\delta \Delta P_i$(g); (ii) $\delta \Delta P_f^{g \to aq}$ vs. $\delta \Delta P_i$(g).

It is usually hypothesized, as previously seen, that a linear correlation between the transfer proton process in the liquid and gaseous phase is to be expected if the quantity $\delta \Delta P_f^{g \to aq}$ is constant within a series of compounds or if there is an approximately linear function between solvation and gas phase proton transfer processes.

$\delta \Delta G(aq)$ vs. $\delta \Delta G(g)$ (Table 9) and $\delta \Delta H(aq)$ vs. $\delta \Delta H(g)$ (Table 10)

For these relations a function of uncertain significance between the two variables is evident. Therefore, it is uncertain if the side chains influence the basicity of α-amino acids in the liquid and gaseous phases in the same way.

$\delta \Delta G^{g \to a}$ vs. $\delta \Delta G(g)$ (Table 11) and $\delta \Delta H^{g \to a}$ vs. $\delta \Delta H(g)$ (Table 12)

The highly significant regression for the two correlations shows that there are no differential solvation factors which cause departures from energy correlations of gas vs. thermodynamic properties in the liquid phase. Furthermore, the results of the Hückel–MacLachlan molecular orbital calculations show (Table 13) that in the molecules the highest charge density value is not found at the nitrogen atom of the amino group linked to the α-carbon atom, so that it is uncertain if (in the liquid or gas or in both phases) at which side the proton process occurs.

Table 9

Results of the monoparametric regression analysis of $\delta \Delta G$(aq) vs. $\delta \Delta G$(g) for some α-amino acids in neutral form at 298 K

n	15
Intercept	0.37699
Slope	−0.07085
SD of intercept	0.43278
SD of slope	0.02770
SD of regression	0.692265
r	0.578556
n.h. Intercept = 0	CL < 0.95
n.h. Slope = 0	0.95 < CL < 0.99

Table 10
Results of the monoparametric regression analysis of $\delta\Delta H(aq)$ vs. $\delta\Delta H(g)$ for some α-amino acids in neutral form at 298 K

n	15
Intercept	−0.81460
Slope	0.04620
SD of intercept	1.32981
SD of slope	0.07341
SD of regression	2.33468
r	0.171938
n.h. Intercept $= 0$	CL < 0.95
n.h. Slope $= 0$	$0.95 <$ CL < 0.99

Table 11
Results of the monoparametric regression analysis of $\delta\Delta G^{g\to aq}$ vs. $\delta\Delta G(g)$ for some α-amino acids in neutral form at 298 K

n	15
Intercept	0.46014
Slope	−0.92779
SD of intercept	0.39040
SD of slope	0.02384
SD of regression	0.6391
r	0.995735
n.h. Intercept $= 0$	CL < 0.95
n.h. Slope $= 0$	$0.95 <$ CL < 0.99

Table 12
Results of the monoparametric regression analysis of $\delta\Delta H^{g\to aq}$ vs. $\delta\Delta H(g)$ for some α-amino acids in neutral form at 298 K

n	15
Intercept	0.51748
Slope	−1.00268
SD of intercept	0.51748
SD of slope	0.08882
SD of regression	2.68379
r	0.952591
n.h. Intercept $= 0$	CL < 0.95
n.h. Slope $= 0$	$0.95 <$ CL < 0.99

To verify this hypothesis the following correlations were examined by means of linear monoparametric analysis: $\delta\Delta G(g)$ vs. δq_N, $\delta\Delta G(aq)$ vs. δq_N, $\delta\Delta H(g)$ vs. δq_N, and $\delta\Delta H(aq)$ vs. δq_N. All these relations were found to be insignificant.

The same results were obtained for: $\delta\Delta P_i(g)$ vs. $\Sigma\,\delta q$, $\delta\Delta P_i(aq)$ vs. $\Sigma\,\delta q$, and $\delta\Delta P_i^{g\to aq}$ vs. $\Sigma\,\delta q$, where $\Sigma\,\delta q$ is the sum of charge densities at C_1, C_2, N_3, O_4 and O_5, expressed as $\Sigma\,q_R - \Sigma\,q_H$.

Table 13
Hückel–McLachlan charge density distributions for some α-amino acids in neutral form

Compound	C_1	C_2	N_3	O_4	O_5	Σq [a]
L(−)α-alanine	0.41960	0.06797	0.01034	0.11168	1.38372	1.99331
(±)-valine	0.41956	0.07216	0.01096	0.11136	1.37284	1.98688
L(+)leucine	0.41952	0.07030	0.01069	0.11148	1.37740	1.98939
(±)-isoleucine	0.41948	0.07244	0.01100	0.11132	1.37183	1.98607
L-(−)methionine	0.41952	0.07025	0.01068	0.11149	1.37752	1.97752
(±)-phenylalanine	0.31610	0.02971	0.00280	0.97894	0.66865	1.99620
D(−)serine	0.42015	0.07619	0.01155	0.11123	1.36504	1.98416
L(−)threonine	0.41910	0.07400	0.01123	0.11100	1.36652	1.98195
L(−)glycine	0.41600	0.05355	0.00821	0.11181	1.41043	2.00000
L(−)cysteine	0.41961	0.07105	0.01080	0.11146	1.37585	1.98877
L(−)glutamine	0.01320	0.00110	0.00017	0.00037	0.00486	0.00782
L(−)aspargine	0.31790	0.05359	0.00000	0.08446	1.04293	1.49888
L(−)lysine	0.41954	0.07001	0.01065	0.11151	1.37806	1.98983
(±)-aspartic acid	0.41775	0.07140	0.01085	0.11091	1.36805	1.97896
L(+)glutamic acid	0.32212	0.05992	0.00907	0.08518	1.04291	1.51920

[a] Σq is the sum of the charge density at C_1, C_2, N_3, O_4 and O_5.

This could signify that in liquid and gas phases the protonation process does not occur at a preferred site for all the compounds.

This can be related (for the liquid phase) to the fact that according to the short-range donor–acceptor model, the interactions of the neutral and protonated molecules with water molecules cannot be related to the electron density of the neutral molecules.

In summary, the side-chains influence the proton transfer processes of the neutral form of some α-amino acids in different ways in the gaseous and liquid phases, external interactions being the pervailing factors for most of the compounds.

However, the transfer proton process in organic compounds (benzoic acid derivatives, indole derivatives, 3-aryliminoindolenine derivatives, 3-arylimino-3*H*-indole-*N*-oxides and *N,N*-dioxides), are influenced by the substituent groups in the same way in the gas and liquid phases so that a preferred site for the protonation process can be hypothesized [26–35].

This behaviour is related to the fact that there is a localized charge at the carboxylate group of the benzoic acids or at definite sites of the polycyclic molecules, while the charge delocalization on the neutral molecules of the α-amino acids hinders a protonation process at a preferred site.

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