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THERMODYNAMICS OF THE SECOND PROTON DISSOCIATION PROCESSES OF NINE α-AMINO-ACIDS AND THE THIRD IONIZATION PROCESSES OF GLUTAMIC ACID, ASPARTIC ACID AND TYROSINE

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ABSTRACT

The thermodynamics of the second proton dissociation processes for nine α-amino-acids were evaluated using electrochemical and calorimetric data. A calorimetric acid strength scale in water is presented. The acidity order is explained as a function of the solvation of the zwitterions and anions.

The differences between the thermodynamics of the first and second ionization processes of the compounds result from the fact that the two processes involve different solvation processes. The anions of the compounds containing a hydroxyl group are less solvated than the anions of the α-amino-acids having a hydrophobic group.

INTRODUCTION

The first and second proton dissociation processes of some α-amino-acids were the subject of a systematic study in our laboratory [1–3].

The first proton ionization processes of six α-amino-acids were studied calorimetrically [1]. The different values of the ionization enthalpy were related to the structure variation of the side-chains (called R groups). The thermodynamics of the first acid proton dissociation processes of seven α-amino-acids were evaluated using electrochemical and calorimetric data [2]. The calorimetric technique proved sensitive enough to reveal differences between the ionization processes, even among α-amino-acids of apparently similar structure. The free-energy values were found to be very similar and the entropic strength scale was found to be similar to the calorimetric scale, the largest enthalpy value (that for tyrosine) being associated with the presence of the benzene ring. Finally, a calorimetric study of the first and second proton dissociation processes for some fluoro and hydroxyl derivatives of phenylalanine was carried out [3]. Calorimetric acid strength scales in water for both these processes were also presented. The effects influencing the acidity scales were found to be the solvation processes of the zwitterions and of the anions. The fluorine atom affects all the processes considered, while the hydroxyl group greatly influences the solvation processes of the anions.

The aim of this work is to study the thermodynamics of the second proton dissociation processes of some α-amino-acids whose first ionization processes have been studied from the thermodynamic point of view [1,2]. For this purpose, a thermodynamic investigation of the second proton dissociation processes of nine α-amino-acids, *d,l*-valine (Val), L(+)-leucine (Leu), *d,l*-isoleucine (Ile), L(–)-threonine (Thr), D(–)-serine (Ser) L(–)-tyrosine (Tyr), *d,l*-phenylalanine (Phe), L(+)-glutamic acid (Glu) and *d,l*-aspartic acid (Asp), has been carried out. The free-energy data for the second proton dissociation processes of these compounds are available in the literature [4–10].

EXPERIMENTAL AND PROCEDURE

The compounds (from Carlo Erba RPE Chemicals, used without purification), were weighed and handled in a nitrogen-filled dry-box.

For valine, leucine, threonine, phenylalanine, isoleucine and serine the second proton dissociation process can be represented as follows

$$\mathrm{NH_3^+CHRCOO^-(aq)} \rightarrow \mathrm{NH_2CHRCOO^-(aq)} + \mathrm{H^+(aq)}. \qquad (1)$$

The molar enthalpy of ionization $\Delta\overline{H}_1$ of $\mathrm{NH_3^+CHCOO^-}$ was obtained by measuring

(a) the molar enthalpy of solution $\Delta\overline{H}_2$ of crystalline $\mathrm{NH_3^+CHROO^-}$ in water at a pH value close to the isoelectric point

$$\mathrm{NH_3^+CHRCOO^-(crys)} \rightarrow \mathrm{NH_3^+CHRCOO^-(aq)} \qquad (2)$$

(b) and the partial molar enthalpy $\Delta\overline{H}_3$ of neutralization of the same compound in water at a pH of 14.00

$$\mathrm{NH_3^+CHRCOO^-(crys)} + \mathrm{OH^-(aq)} \rightarrow \mathrm{NH_2CHRCOO^-(aq)} + \mathrm{H_2O(l)} \qquad (3)$$

If the solution process value $\Delta\overline{H}_2$ and the value of $\Delta\overline{H}_4$ [11] for the process (in water)

$$\mathrm{H^+(aq)} + \mathrm{OH^-(aq)} = \mathrm{H_2O(l)} \qquad (4)$$

are subtracted from the $\Delta\overline{H}_3$ value, i.e. $\Delta\overline{H}_3 - (\Delta\overline{H}_2 + \Delta\overline{H}_4)$, this gives the enthalpy value of process (1).

In processes (2) and (3), concentrations ranging from 10^{-3} to 10^{-4} mol kg^{-1} were used. The $\Delta \overline{H}$ values can therefore be considered to be equal to those at infinite dilution, $\Delta H^{\ominus}$ [12]. These values refer to the dissociation process of 1 mol of zwitterions $NH_3^+CHROO^-$ at infinite dilution in 1000 g of water, yielding 1 mol of $NH_2CHRCOO^-$ anions and 1 mol of protons solvated in the same amount of water.

The following points are noteworthy.

(a) For the α-amino-acids in solution at pH 14 only the α-amino-acids anions $NH_2CHRCOO^-$ are present.

(b) By means of the dissociation constants [10,13] it has been possible to calculate the isoelectric pH values of the various compounds. It could be hypothesized that at these pH values $NH_3^+CHRCOO$ zwitterions are the predominant forms.

For glutamic and aspartic acid, reactions (2) and (3) must be written as

$$COOHRCHNH_3^+COO^-(crys) \rightarrow COO^-RCHNH_3^+COO^-(aq) + H^+(aq).$$

and

$$COOHRCHNH_3^+COO^-(crys) + 2OH^-(aq)$$
$$\rightarrow COO^-RCHNH_2COO^-(aq) + 2H_2O(l)$$

It is well known that at pH 6 the β- and γ-carboxyl groups are completely dissociated [1], so that $\Delta \overline{H}_3 - (\Delta \overline{H}_2 + 2\Delta \overline{H}_4)$ values refer to the sum of the second and third proton dissociation processes.

For tyrosine, the process can be represented as follows

$$HORCHNH_3^+COO^-(crys) \rightarrow HORCHNH_3^+COO^-(aq)$$

and

$$HORCHNH_3^+COO^-(crys) + 2OH^-(aq)$$
$$\rightarrow {}^-ORCHNH_2COO^-(aq) + 2H_2O(l)$$

Again, $\Delta \overline{H}_3 - (\Delta \overline{H}_2 + 2\Delta \overline{H}_4)$ is the sum of the second and third proton dissociation processes.

As the enthalpy values of the second (ΔH_2^*) and third (ΔH_3^*) proton dissociation processes of glutamic and aspartic acid are available in the literature [8,9,14], it is possible to put these values and our values in to the equations $\Delta H_2^*/\Delta H_3^* = x/y$ and $x + y = C$, where x and y are our corresponding enthalpy values and C is their sum.

For tyrosine, enthalpy values are not available from the literature and the corresponding free energy [13] is thus used in the mathematical equations, assuming a linear relationship between enthalpy values and free-energy values.

In these ways the enthalpy values of the third proton dissociation processes of these three compounds can be calculated.

RESULTS AND DISCUSSION

The free-energy, enthalpy and entropy values of processes (1) for the α-amino-acids are reported in Table 1. The enthalpy values of solution and neutralization of the same compounds are reported in Table 2.

The differences in the ionization processes for different compounds are usually explained by means of the different values for the enthalpy of solvation of the ions and undissociated molecules [1–3]. For this purpose, the differences in the enthalpies of the processes (2), $\delta\Delta H_2^{\ominus}$, and (3), $\delta\Delta H_3^{\ominus}$, of the various α-amino-acids can be identified with the differences in solvation among the zwitterions and among the anions $NH_2CHRCOO^-$. These values, together with the differences in the enthalpies of ionization, $\delta\Delta H_1^{\ominus}$, are given in Table 3.

The different influences of the same side-chain groups on the first and second proton dissociation processes are evident from a comparison of the respective enthalpy differences $\delta\Delta H_1$ for the proton dissociation processes [1–3]. These differences are related to the fact that both the first and the second ionization processes depend on the solvation processes of the zwitterions, but differ in the second solvation process (solvation of undissociated molecules and solvation of anions for the first and second ionization processes respectively).

For the couple Ile/Leu, the greater solvation of the isoleucine anion gives rise to a larger value for the enthalpy of the second proton dissociation process for isoleucine. The weaker ionization of isoleucine with respect to valine is due to lesser solvation of its anion. As regards the Ile/Phe couple, the weaker ionization of isoleucine results from greater solvation of the zwitterion.

TABLE 1

Free-energy, enthalpy and entropy values of second proton dissociation processes for some α-amino-acids in water at 25 °C

Compound	$\Delta G_1^{\ominus}$ (kcal mol^{-1})	$\Delta H_1^{\ominus}$ (kcal mol^{-1})	$\Delta S_1^{\ominus}$ (cal K^{-1} mol^{-1})
d,l-valine	13.26	8.06	−17.44
d,l-isoleucine	13.31	11.08	−7.80
L(+)-leucine	13.30	11.33	−6.71
d,l-phenylalanine	12.67	10.89	−6.97
D(−)-serine	12.56	9.71	−9.56
L(−)-threonine	12.42	9.83	−8.69
L(−)-tyrosine	12.42, 13.73 [a]	8.30, 9.22 [a]	−13.82, −15.12 [a]
d,l-aspartic acid	13.65, 5.34 [a]	15.36, 1.87 [a]	5.73, −11.64 [a]
L(+)-glutamic acid	12.78, 5.84 [a]	16.71, 0.66 [a]	13.18, −17.37 [a]

[a] third dissociation proton process.

TABLE 2

Enthalpy values of solution ($\Delta H_2^{\ominus}$) and neutralization ($\Delta H_3^{\ominus}$) for some α-amino-acids in water at 25 °C

Compound	$\Delta H_2^{\ominus}$ (kcal mol^{-1})	$\Delta H_3^{\ominus}$ (kcal mol^{-1})
d,l-valine	1.28	−4.00
d,l-isoleucine	0.63	−1.63
L(+)-leucine	0.70	−1.31
d,l-phenylalanine	1.84	−0.61
D(−)-serine	2.63	−0.99
L(−)-threonine	2.42	−1.09
L(−)-tyrosine	4.77	−4.39
d,l-aspartic acid	6.16	−3.28
L(+)-glutamic acid	5.91	−3.40

Thus, for the couples Ile/Leu and Ile/Val the solvation of the anion of isoleucine is the prevailing process in the second ionization process, whilst the isoleucine zwitterion solvation process prevails in the first ionization process [1,2].

For the α-amino-acids with apolar or hydrophobic side-chains, the order of solvation of the zwitterions is Ile > Leu > Val > Phe whilst for the anions the order becomes Val > Ile > Leu > Phe. Furthermore, the calorimetric acid strength scale can be written in the order Val > Phe > Ile > Leu. It can be argued that the presence of the benzene ring in phenylalanine results in less solvation of the zwitterion and of the anion of phenylalanine. The first position of valine in the calorimetric acid strength scale for the second ionization process is clearly related to the greater solvation of the anion whilst the last position of leucine is a result of the greater solvation of the zwitterion.

It may also be noted that the α-amino-acids of the above-cited class, with the exception of phenylalanine, differ mostly in the ionization enthalpy values, whilst the free-energy values are very similar. It seems that for the

TABLE 3

Differences in the enthalpy ($\delta\Delta H_1$) and free-energy ($\delta\Delta G_1^{\ominus}$) of the second proton dissociation process, and in the zwitterion solvation ($\delta\Delta H_2^{\ominus}$) and anion solvation ($\delta\Delta H_3^{\ominus}$), for some α-amino-acids in water at 25 °C (in kcal mol^{-1})

Couple	$\delta\Delta H_1^{\ominus}$	$\delta\Delta H_2^{\ominus}$	$\delta\Delta H_3^{\ominus}$	$\delta\Delta G_1^{\ominus}$
Ile/Leu	−0.25	−0.070	−0.32	0.01
Ile/Phe.	0.19	−1.21	−1.02	0.64
Ile/Val	3.02	−0.65	2.37	0.05
Phe/Tyr	2.59	−2.93	3.78	0.25
Ser/Thr	−0.12	0.21	0.10	0.14
Ser/Tyr	1.41	−2.14	3.40	0.14

second ionization process (as well as for the first) the data obtained from calorimetric measurements enhance the differences between Leu, Ile and Val. For phenylalanine the free energy is the driving force.

α-Amino-acids bearing a polar group having no charge, such as those α-amino-acids containing a hydroxyl group (serine, threonine and tyrosine), are now considered. The following calorimetric acid strength is found: Tyr > Ser > Thr. The first position of tyrosine in the calorimetric acid strength scale is a result of the greatest solvation and least solvation of the anion and zwitterion respectively.

From the evidence so far collected using calorimetric data it is possible to give a complete calorimetric acid strength scale for the second proton dissociation process: Val > Tyr > Ser > Thr > Phe > Ile > Leu. The complete sequences of the zwitterions and anions are Ile > Leu > Val > Phe > Thr > Ser > Tyr and Tyr > Val > Ile > Leu > Thr > Ser > Phe respectively.

The first and second positions of valine and tyrosine in the calorimetric acid strength scale result from the high degree of solvation of the anions, and the last positions of isoleucine and leucine are connected to the low degree of solvation of the zwitterions. It was noted that the anions of the α-amino-acids containing the hydroxyl group (with the exception of tyrosine) are less solvated than the corresponding anions of the α-amino-acids bearing hydrophobic groups, as is seen in the first ionization process for the zwitterions and the undissociated molecules of the same α-amino-acid [1–3].

For the α-amino-acids bearing polar groups with a negative charge the acidity strengths in the second ionization process are in the order Asp > Glu, and these acids can be put in the last positions in the complete calorimetric acid strength scale as concerns the second proton dissociations process. As regards the third ionization process, the sequence becomes Glu > Asp > Tyr.

It was noted that our enthalpy values for the third dissociation processes are close to those available in the literature [8,9], whilst for the second dissociation process this is not true. This results from the large enthalpy values of the sum of the second and third proton dissociation processes.

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