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THERMODYNAMICS OF ACID PROTON DISSOCIATION OF SOME α-AMINO ACIDS. EFFECT OF THE HYDROXYL SUBSTITUENT GROUP ON ALIPHATIC AND AROMATIC SIDE-CHAINS

F. RODANTE

Dipartimento di Ingegneria Chimica dei Materiali, delle Materie prime e Metallurgia, Università di Roma, Via del Castro Laurenziano 7, Roma (Italy)

F. FANTAUZZI

Dipartimento di Scienze Biomediche, Università degli Studi dell'Aquila, L'Aquila (Italy)

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ABSTRACT

The thermodynamics of the acid proton dissociation for seven α-amino acids were evaluated by electrochemical and calorimetric data. The calorimetric data were sensitive enough to reveal differences between even apparently similar α-amino acids structures.

A calorimetric acid strength scale in water is also presented. The effects influencing the acidity order were found to be ions and molecules of solvation. This scale emphasizes the prevalence of either effect considered. Both the ions and indissociate molecules of compounds containing the hydroxyl group are less solvated than the ions and indissociate molecule of α-amino acids showing an hydrophobic group.

INTRODUCTION

It is well known that α-amino acids, from the chemical view-point, can be classified by means of the structure of the side-chain groups (R). These groups are defined as: (1) apolar or hydrophobic groups, (2) polar groups without charge, (3) polar groups with negative charge, (4) polar groups with positive charge.

In a previous paper [1], six α-amino acids were studied calorimetrically. These compounds belong to three of the four classes presented above and their different values of the enthalpy of ionization were related to the R-structure variation.

The difference in the enthalpy ionization between valine and leucine was related to the additional CH_3 group, which diminishes both the electron-withdrawing and field effects of the NH_3^+ group. These effects tend to dissociate the proton of the 1-carboxyl group.

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In the phenylalanine–tyrosine couple, the difference in the ionization values was related to the effect of the OH substituent group (σ-acceptor and π-donor). The σ-acceptor effect tends to increase the electron-withdrawing effect of the NH_3^+ group on the carboxyl, while the π-electron releasing resonance effect decreases it. The large differences between the two solution and protonation enthalpy values of this couple, were explained by the medium structure-breaking effect displayed by the OH group which forms hydrogen bonds with water. Thus a large difference was found between the two compounds, whose R differ only for the hydroxyl group.

With this premise, a thermodynamic investigation of other α-amino acids such D(−) serine (Se) and L(−) threonine (Thr) (which have OH as a substituent group in the aliphatic side-chain) and such d,l-isoleucine (Il) (which has a hydrophobic side-chain) seemed quite reasonable. Indeed, the free-energy data related to the acid proton dissociation for these three and for the other four α-amino acids, that is, d,l-valine (Val), L(+)-leucine (Leu), d,l-phenylalanine (Phe), L(−) tyrosine (Ty) (which have been studied previously by a calorimetric technique [1]) are available in the literature [2–5]. This allows a deeper insight into the acid proton ionization processes for compounds belonging to the first and second class. It is also possible to study the different effect of the hydroxyl group on the aromatic and aliphatic side-chains.

EXPERIMENTAL AND PROCEDURE

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

The acid proton ionization process can be represented as

$$H_3^+NCHRCOOH_{(cry)} \rightarrow H_3^+NCHRCOO^-_{(aq)} + H^+_{(aq)} \tag{1}$$

The molar enthalpy of ionization, $\Delta \overline{H}_1$, of $H_3^+NCHRCOOH$ was obtained by measuring:

(a) The molar enthalpy of solution, $\Delta \overline{H}_2$, of crystalline $H_3^+NCHRCOO^-$ in water at pH = 5.98

$$H_3^+NCHRCOO^-_{(cry)} \rightarrow H_3^+NCHRCOO^-_{(aq)} \tag{2}$$

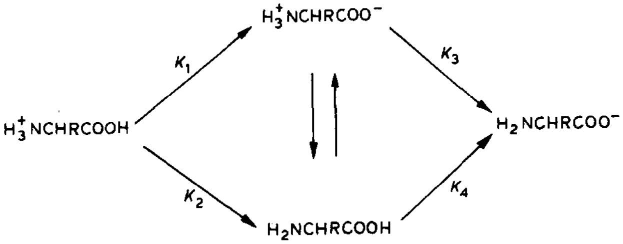
(b) The molar enthalpy of protonation, $\Delta \overline{H}_3$, of the same compound in water at pH = 0.0

$$H_3^+NCHRCOO^-_{(cry)} + H^+_{(aq)} \rightarrow H_3^+NCHRCOOH_{(aq)} \tag{3}$$

The molar enthalpy, $\Delta \overline{H}_1$ of process (1) can be obtained by subtracting the two values $\Delta \overline{H}_2$ and $\Delta \overline{H}_3$. In processes (2) and (3) concentrations about 10^{-3} M were used. Therefore the $\Delta \overline{H}_1$ values can be considered at infinite dilution ΔH_1^0 [6]. These values refer to the ionization process of one mole of

$H_3^+NCHRCOOH$ at infinite dilution in 1000 g of water, yielding 1 mole of $H_3^+NCHRCOO^-$ ions and 1 mole of protons solvated in the same amount of water.

In water, the ionization process, for a compound containing the carboxyl and amino groups, is complicated by tautomeric equilibria and zwitterion formation [7,8]. The following scheme may be constructed to display the equilibria concerned:



While the amino acids, in acid solution can be represented by H_3^+NCHR-COOH, in solution approaching pH 7, the principal species are neutral and may either be in the $H_2NCHRCOOH$ or in the zwitterion form H_3^+NCHR-COO^-.

Therefore, in eqn. (3) at pH 0.0, only the $H_3^+NCHRCOOH$ is present, while in eqn. (2) this is not true. However, by means of the dissociation constant values [9], it has been possible to calculate the isoelectric pH values of the various compounds considered. The pH of eqn. (2) is very close to the isoelectric pH values of our compounds. It can therefore be assumed that in this solution the $H_3^+NCHRCOO^-$ form is predominant. In this way it is possible to calculate the first ionization enthalpy value, that is the process related to K_1 in the above scheme.

DISCUSSION

The free-energy, the enthalpy and the entropy values of process (1) for seven α-amino acids are reported in Table 1. The enthalpy values of solution and protonation of the same compounds are reported in Table 2. The differences in enthalpy ionization processes for different compounds are usually explained by means of the differences in the enthalpy solvation of the respective ions and indissociate molecules [10,11]. For this purpose, the differences in the enthalpies of the processes (2) $\delta\,\Delta H_2$, and (3) $\delta\,\Delta H_3$, of the various α-amino acids, can be identified with the differences in the solvation between the ions and the indissociate molecules of the same compounds in water. These values, together with the $\delta\,\Delta H_1$ differences in

TABLE 1

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

Compound	ΔG_1^0 (kcal mol^{-1})	ΔH_1^0 (kcal mol^{-1})	ΔS_1^0 (cal mol^{-1})
d,l-Valine	3.18	0.17	−10.1
d,l-Isoleucine	3.16	−0.02	−10.67
L(+)Leucine	3.18	0.36	−9.40
d,l-Phenylalanine		−1.00	
D(−)Serine	2.98	0.32	−8.92
L(−)Threonine	2.86	0.37	−8.35
L(−)Tyrosine	3.00	−0.29	−11.03

enthalpy ionization are given in Table 3.

From the ionization enthalpy values of leucine and isoleucine, it can be noted that there is a different sign for the two ionization processes. It can also be seen that the greater desolvation of the indissociate molecule and the greater solvation of the ion cause the larger ionization of isoleucine with respect to leucine.

The enthalpy values of ionization for the isoleucine–valine couple show again a different sign and again the larger ionization of isoleucine is due to its greater ion solvation.

As regards the isoleucine–phenylalanine couple, the lesser ionization of isoleucine depends on the difference in the solvation of the indissociate molecules.

As one would expect, the presence of the benzene ring is mostly responsible for the large desolvation of the phenylalanine. Thus for the α-amino acids belonging to the first class, the order of solvation for the indissociate molecules is Leu > Il > Val > Phe, while for the ions the order becomes

TABLE 2

Enthalpy values of solution (ΔH_2^0) and protonation (ΔH_3^0) for some α-amino acids in water at 25°C (kcal mol^{-1})

Compound	ΔH_2^0	ΔH_3^0
d,l-Valine	1.28	1.11
d,l-Isoleucine	0.63	0.65
L(+)Leucine	0.70	0.34
d,l-Phenylalanine	1.96	2.96
D(−)Serine	2.63	2.31
L(−)Threonine	2.42	2.05
L(−)Tyrosine	4.77	5.06

TABLE 3

Differences in the ionization $\delta \Delta H_1^0$, in the ion solvation $\delta \Delta H_2^0$, and in molecule solvation $\delta \Delta H_3^0$, for some α-amino acids in water at 25°C (kcal mol^{-1})

Couple	$\delta \Delta H_1^0$	$\delta \Delta H_2^0$	$\delta \Delta H_3^0$
Il/Leu	−0.38	−0.07	0.31
Il/Phe	0.98	−1.33	−2.31
Il/Val	−0.19	−0.65	−0.46
Phe/Ty	−0.71	−2.81	−2.10
Se/Thr	−0.05	0.21	0.26
Se/Ty	0.61	−2.14	−2.75

Il > Leu > Val > Phe. Furthermore, the following calorimetric acid strength scale can be written: Phe > Il > Val > Leu.

While for phenylalanine the molecule desolvation prevails, for isoleucine the ion solvation plays the key factor. The latter position of the leucine in the calorimetric strength order scale is clearly related to the greatest solvation of the indissociate molecule. Finally, for valine neither the ion solvation nor the molecule solvation is prevalent.

It can also be noted that the α-amino acids of the first class differ mostly in the enthalpy ionization values while the free-energy values are very similar. So it seems that the thermodynamic data obtained by calorimetric measurements enhance the differences between the three compounds as far as the ionization processes are concerned.

For the α-amino acids which show a polar group without charge, such as those containing the hydroxyl group, three compounds (serine, threonine and tyrosine) are considered. This calorimetric acid strength scale is found to be Ty > Se > Thr.

Again the first position of the tyrosine in the calorimetric scale is due to the largest desolvation of the indissociate molecule, while the largest solvation of the indissociate molecule is the cause of the latter position of threonine (see Table 3). From the evidence so far collected by calorimetric data, it is possible to give a complete calorimetric acid strength scale: Ph > Ty > Il > Val > Se > Leu > Thr.

As the differences in ionization enthalpies were explained by means of the differences in ions and indissociate molecules solvation, thus the following complete sequence for indissociate molecule is considered: Leu > Il > Val > Thr > Ser > Phe > Ty. For the ions the order based on the solvation is found: Il > Leu > Val > Phe > Thr > Ser > Ty. Our experimental evidence also supports the following remarks.

Contrary to expectations, both the ions and the indissociate molecules of the α-amino acids containing the hydroxyl group are less solvated than the ions and the indissociate molecules of the α-amino acids showing a hydrophobic group. For the compounds containing the benzene ring, such as phenylälanine and tyrosine, the desolvation of the indissociate molecule plays a major role. It is also noteworthy that these two compounds can be set in the first and second places in the calorimetric acidity scale.

The calorimetric technique proved sensitive enough to reveal differences, with respect to ionization process, even between apparently similar structures of α-amino acids.

Finally it was noted that, while the free-energy values are very similar, the entropic strength scale is equal to the calorimetric scale, the largest entropy value of the tyrosine being due to the presence of the benzene ring.

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