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The calorimetric study of some α-amino acids bearing heteroatoms in their side chains

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Abstract

A calorimetic study, in the liquid phase, of the behaviour of some α-amino acids, which differ from each other in only the presence of heteroatom or in the position of the same heteroatom in their aliphatic side chains, is presented.

The influence of the different electronegativity of the heteroatom explains how a small variation in the side chain can lead to large variations in the enthalpy values of the first, second and third ionization processes.

INTRODUCTION

The thermodynamic study of several "standard" α-amino acids and of some their dipeptides in liquid and solid phases has been the subject of extensive research in our laboratory [1–14]. In the liquid phase, 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.

Solution calorimetry can provide a useful contribution to this research [1–7]: the differences in enthalpy ionization processes for various compounds can be explained by means of the differences in enthalpy solvations of the respective anions, zwitterions and undissociated molecules, which, in turn, depend on the different side chains and on the different groups within each side chain.

The mutual structural influences of α-amino acids in dipeptides were also

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studied by calorimetric measurements in the liquid phase [8, 9, 11]. Similar research on α-amino acids and dipeptides in the solid phase was carried out by means of simultaneous TG–DSC measurements [10–12].

It is well known that solid α-amino acids have high melting points because of hydrogen bonding between the COO^- and NH_3^+ groups linked to the same α-carbon atom. This dipolar ion structure is the main influence on the general thermal behaviour. However, the effect of the side chains is sufficient to allow the thermal behaviour of α-amino acids to be differentiated both between and within classes.

The thermodynamic and kinetic data of the thermal decomposition processes have been correlated with the features of the four classes into which these compounds are classified in the liquid phase [14]. The thermal stability of the components of the various classes is mainly linked to the decarboxylation process, which, in turn, is influenced by the side chains. The introduction of a heteroatom into the side chain increases the tendency for cleavage of the side chain with a decrease in the decarboxylation process, which, in turn, could account for the large values of the thermodynamic quantities of some compounds.

Using calorimetric techniques, components with similar structure can be grouped by their similarily shaped thermograms, although this does not provide sufficient information on their thermal structural variation [12].

Thermal analyses of different series of dipeptides were also carried out [13]. The thermal behaviour of these compounds was compared with that of the independent free α-amino acids contained in the dipeptides: the mutual influence of the two α-amino acids makes the dipeptides less stable than the single components.

Some compounds show a similar behaviour in liquid and solid phases. The methyl group plays a fundamental role in the thermal stability [13], just as in the liquid phase [9] it is the key factor in the mutual influence of the different dipeptide structures.

In a symmetrical system (valyl–tyrosine and tyrosil–valine), the thermal stability is equal in the two dipeptides [12], just as the enthalpy values of the dissociation processes in solution of the three functional groups (carboxyl group, amine group and hydroxyl group of tyrosine) are equal in the dipeptides, by virtue of the symmetry of the system [9].

The aim of this work is to study in the liquid phase the behaviour of some α-amino acids which differ from each other only in the presence of a heteroatom or in the position of the same heteroatom in their aliphatic chains. The α-amino acids studied in this work were *d,l*-isoleucine, *d,l*-4-thiaisoleucine, L-*O*-methylisoleucine, L-lysine, *S*-2-aminoethyl-L-cysteine, *S*-ethyl-L-cysteine and L-methionine.

In *d,l*-4-thiaisoleucine and L-*O*-methylisoleucine, a CH_2 group of *d,l*-isoleucine is substituted by a sulphur and an oxygen atom, respectively. *S*-2-Aminoethyl-L-cysteine differs from lysine by the substitution of a

sulphur atom for a CH_2 group. *S*-Ethyl-L-cysteine and methionine have a sulphur atom at different positions in the same aliphatic chain.

EXPERIMENTAL AND PROCEDURE

The compounds (Carlo Erba RPE Chemicals), used without purification, were weighed and handled in a nitrogen-filled dry-box. A Tronac (model 458) instrument was used to make the measurements. The vessel calorimeter was a rapid-response glass vacuum Dewar of maximum capacity 25 cm^3. The thermostat was maintained at 298.15 ± 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 volmeter. 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 [15] 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 dissociation processes can be represented respectively as

$$XRCHNH_3^+COOH(aq) = XRCHNH_3^+COO^-(aq) + H^+(aq) \quad (1)$$

and

$$XRCHNH_3^+COO^- = XRCHNH_2COO^-(aq) + H^+(aq) \quad (2)$$

where X is a heteroatom and R the aliphatic chain.

The partial molar dissociation $\Delta\bar{H}_1$ of $XRCHNH_3^+COOH$ was obtained by measuring the partial molar enthalpy of solution $\Delta\bar{H}_3$ of crystalline $XRCHNH_3^+COO^-$ in water at a pH close to the isoelectric point

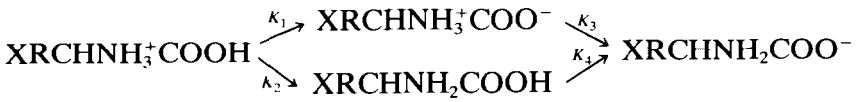
$$XRCHNH_3^+COO^-(cry) = XRCHNH_3^+COO^-(aq) \quad (3)$$

and the partial molar enthalpy of protonation $\Delta\bar{H}_4$ of the same compound in water at pH 0

$$XRCHNH_3^+COO^-(cry) + H^+(aq) = XRCHNH_3^+COOH(aq) \quad (4)$$

The partial molar enthalpy of process (1) can be obtained by subtracting $\Delta\bar{H}_4$ from $\Delta\bar{H}_3$.

In processes (3) and (4), concentrations of about 10^{-3} M were used. Therefore the $\Delta\bar{H}$ values can be considered to be at infinite dilution, $\Delta H^{\ominus}$ [16]. These values refer to the proton dissociation of one mole of $XRCHNH_3^+COOH$ at infinite dilution in 1000 g of water, which yields 1 mole of $XRCHNH_3^+COO^-$ ions and 1 mole of protons solvated in the same amount of water.



Scheme 1. The dissociation process of a compound containing carboxylate and amino groups.

In water, the dissociation process of a compound containing carboxylate and amino groups is complicated by tautomeric equilibrium and by zwitterion formation [17–18]. Scheme 1 shows the equilibrium concerned.

While the α-amino acids in the acid solution can be represented by the form $XRCHNH_3^+COOH$, in solutions approaching pH 7.00, the principal species are neutral molecules which may be either in the $XRCHNH_2COOH$ form or in the zwitterion form $XRCHNH_3^+COO^-$. Therefore, in eqn. (4) at pH 0, only $XRCHNH_3^+COOH$ is present, whereas in eqn. (3) this is not the case. However, it is possible to calculate the isoelectric pH values for the compounds examined using the dissociation constant values [19, 20]. It can therefore be assumed that in this solution the $XRCHNH_3^+COO^-$ 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 the above scheme. The partial molar enthalpy of the second proton dissociation process of $XRCHNH_3^+COO^-$ (K_3 process) was obtained by measuring the partial molar enthalpy of neutralization of the crystalline compound $XRCHNH_3^+COO^-$ in water at pH 14

$$XRCHNH_3^+COO^-(cry) + OH^-(aq) = XRCHNH_2COO^-(aq) + H_2O(l) \quad (5)$$

If the partial molar enthalpy of the solution $\Delta\bar{H}_3$ and the partial molar value $\Delta\bar{H}_6$ related to the process (in water) [21]

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

are subtracted from $\Delta\bar{H}_5$ values, then the enthalphies of process (2) are obtained. These values refer to the dissociation of 1 mole of $XRCHNH_3^+COO^-$ at infinite dilution in 1000 g of water, which yields 1 mole of $XRCHNH_2COO^-$ and 1 mole of protons solvated in the same amount of water. The $\Delta\bar{H}$ values for this process can be also considered equal to $\Delta H^{\ominus}$.

It should be noted that process (5) was carried out at pH 14, so that only the $XRCHNH_2COO^-$ form was present. For *S*-2-aminoethyl-L-cysteine, process (5) must be written as

$$NH_3^+XRCHNH_3^+COO^-(cry) + 2OH^-(aq) = NH_2XRNH_2COO^-(aq) + 2H_2O$$

Therefore, for this compound, the $\Delta\bar{H}_5 - (\Delta\bar{H}_3 + 2\Delta\bar{H}_6)$ value refers to the sum of the second and third proton dissociation processes. These enthalpy values are not available in the literature, while those of lysine have been previously calculated [6]. It is possible, assuming a linear relationship

between the enthalpy values of *S*-2-aminoethyl-L-cysteine and lysine (by virtue of their small structure difference) to put the above cited values in the system

$$\Delta H_2^{\ominus}/\Delta H_{3_a}^{\ominus} = X/Y \qquad X + Y = C$$

where $\Delta H_2^{\ominus}$ and $\Delta H_{3_a}^{\ominus}$ are the values for lysine and X and Y are the corresponding values of *S*-2-aminoethyl-L-cysteine (C being their sum).

RESULTS AND DISCUSSION

The enthalpy values of the solution, protonation and neutralization processes are reported in Table 1. The enthalpy values of the first, second and third ionization processes are reported in Table 2.

The differences in the enthalpy ionization processes for the different compounds are usually explained by means of the differences in the enthalpy solvation of the undissociated molecules, the zwitterions and the anions.

For this purpose, the differences in the enthalpies of process (3) $\delta\Delta H_3^{\ominus}$,

TABLE 1

Enthalpy values (kcal mol^{-1}) of solution $\Delta H_3^{\ominus}$, of protonation $\Delta H_4^{\ominus}$, and of neutralization $\Delta H_5^{\ominus}$ for some α-amino acids in water at 25°C

Compound	$\Delta H_3^{\ominus}$	$\Delta H_4^{\ominus}$	$\Delta H_5^{\ominus}$
d,l-Isoleucine	0.63	0.65	−1.61
d,l-4-Thiaisoleucine	0.65	0.53	−2.57
L-*O*-Methylisoleucine	−1.00	−1.30	−4.38
L-Lysine	2.88	2.54	0.24
S-2-Aminoethyl-L-cysteine	5.72	5.35	1.06
S-Ethyl-L-cysteine	2.81	2.37	−0.25
L-Methionine	2.68	2.06	−0.12

TABLE 2

Enthalpy values (kcal mol^{-1}) of the first $\Delta H_1^{\ominus}$, second $\Delta H_2^{\ominus}$ and third $\Delta H_{3_a}^{\ominus}$ dissociation processes for some α-amino acids in water at 25°C

Compound	$\Delta H_1^{\ominus}$	$\Delta H_2^{\ominus}$	$\Delta H_{3_a}^{\ominus}$
d,l-Isoleucine	−0.02	11.08	
d,l-4-Thiaisoleucine	0.12	10.15	
L-*O*-Methylisoleucine	0.30	10.00	
L-Lysine	0.34	11.04	12.99
S-2-Aminoethyl-L-cysteine	0.37	10.15	11.93
S-Ethyl-L-cysteine	0.44	10.31	
L-Methionine	0.62	10.57	

TABLE 3

Differences (kcal mol^{-1}) in the zwitterion solvation $\delta\Delta H_3^{\ominus}$, in the undissociated molecule solvation $\delta\Delta H_4^{\ominus}$, and in the anion solvation $\delta\Delta H_5^{\ominus}$ for some α-amino acids in water at 25°C

Couples	$\delta\Delta H_3^{\ominus}$	$\delta\Delta H_4^{\ominus}$	$\delta\Delta H_5^{\ominus}$
d,l-4-Thiaisol./*d,l*-isol.	0.02	−0.12	−0.96
L-*O*-Methylisol./*d,l*-isol.	−1.63	−1.95	−2.77
S-2-Aminoethyl-L-cys./lys.	2.84	2.81	0.82
S-Ethyl-L-cys./L-meth.	0.13	0.31	−0.13

process (4) $\delta\Delta H_4^{\ominus}$, and process (5) $\delta\Delta H_5^{\ominus}$ can be identified with the differences in solvation of the zwitterion, the undissociated molecule and the ionic form of the various couples. These values are reported in Table 3. The enthalpies of solution and of protonation are all endothermic with the exception of those of L-*O*-methylisoleucine. This signifies that the energy required to break the electrostatic bonds among the zwitterions in the solid state is not returned by interactions between the zwitterions and the undissociated molecules and the solvent. The σ-acceptor effect of the oxygen atom activates the NH_3^+ group both in the zwitterion and in the undissociated molecule.

In the series comprising *d,l*-isoleucine, *d,l*-4-thiaisoleucine and L-*O*-methylisoleucine, the first component is taken as reference compound. The first ionization process has order *d,l*-isoleucine > *d,l*-4-thiaisoleucine > L-*O*-methylisoleucine, but for the second ionization process the order becomes L-*O*-methylisoleucine > *d,l*-thiaisoleucine > *d,l*-isoleucine.

The different influence of the same heteroatom on the first and second ionization processes is related to the fact that both ionization processes depend on the solvation of the zwitterions but differ in the second solvation (solvation of the undissociated molecule for the first ionization process and of the anion for the second ionization process).

The order of the solvation for the undissociated molecules is L-*O*-methylisoleucine > *d,l*-4-thiaisoleucine > *d,l*-isoleucine, but for the zwitterions the scale of solvation is L-*O*-methylisoleucine $\gg$ *d,l*-isoleucine $\approx$ *d,l*-4-thiaisoleucine.

The greater solvation of the undissociated molecules with respect to that of the zwitterions accounts for the easier first ionization process of *d,l*-isoleucine, while the order of the second ionization process is linked to greater solvation of the anions with respect to the zwitterions (see Table 3). In any case the behaviour of these three compounds can be related to the electronegativity values of the oxygen and sulphur atoms. The presence of a heteroatom hinders the first ionization process and favours the second.

The first dissociation processes of L-lysine and *S*-2-aminoethyl-L-cysteine are very close. This is possibly because of the relative differences in

solvation between the molecules and because the zwitterions of the two compounds are practically equal. In spite of the presence of the sulphur atom in the aliphatic chain, the solvations of the undissociated molecule and of the zwitterion of *S*-2-aminoethyl-L-cysteine are weaker than those of L-lysine. Conversely, the second and third ionization processes of the former are larger than those of the latter, as expected from the presence of the sulphur atom which activates the NH_3^+ group.

In the pair *S*-ethyl-L-cysteine/L-methionine, in the sulphur atom *S*-ethyl-L-cysteine is closer to the NH_3^+ group than is the sulphur atom of L-methionine, which favours the first and second ionization processes. Accordingly, the following solvation order was found: ions > zwitterions > undissociated molecules.

CONCLUSIONS

Comparison of pairs of the studied compounds shows 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. This can be related to the different influence of the electronegativity, due, in turn, to a different heteroatom or to the different position with respect to the functional groups of the same heteroatom.

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