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THERMODYNAMIC STUDY OF SOME α-AMINO ACIDS BEARING DIFFERENT GROUPS IN THEIR SIDE-CHAINS

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ABSTRACT

A study of the behaviour of some α-amino acids bearing different groups in their side-chains is presented. α-Amino acids with similar structures are compared on the basis of the thermodynamic quantities related to the first and second ionization processes. Three pairs of compounds are considered: methionine/cysteine, glutamine/aspargine and lysine/arginine. The entropy values play a major role in explaining the different behaviour of the constituents of the pairs in both the first and second ionization processes.

INTRODUCTION

A thermodynamic study of several α-amino acids in water has been the subject of extensive research in our laboratory [1–5], in which particular attention has been paid to the effects of side-chains on the proton dissociation processes of carboxyl and amino groups in three of the four classes of α-amino acids. These classes are defined by the structures of their side-chains as: (a) class showing apolar or hydrophobic chains; (b) class showing polar chains without charge; (c) class showing polar chains with negative charge. A comparison between the first and second classes has also been carried out using the first and second proton transfer processes of the α-amino acids in each class with respect to those of glycine, which can be placed in both classes [5].

The aim of this work is to study the thermodynamic behaviour of some α-amino acids bearing different groups (NH_3, NH_2, NH_2^+, S, SH, NH_2CO). Some of these belong to the fourth class, and show a positive charge in their side-chains. The α-amino acids considered are L-methionine, L-cysteine, L-glutamine, L-aspargine, L-lysine and L-arginine.

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Methionine and cysteine bear one sulphur atom in the central position and in the last position respectively of their side-chains. The latter compound shows a third dissociation process, i.e. the dissociation of the SH group.

Glutamine and aspargine bear an amidic group in the γ and β positions of their side-chains. It is also of interest to compare these two compounds on the one hand and glutamic and aspartic acid on the other. Indeed, as glutamine and aspargine are the amides of glutamic and aspartic acid, respectively, the dissociation processes of their amino groups will provide interesting information on the second proton dissociation processes of the two acids.

Finally, arginine and lysine belong to the fourth α-amino acid class by virtue of the positive charge of their side-chains. Both of these compounds show three processes of dissociation.

EXPERIMENTAL AND PROCEDURE

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

The first and second proton ionization for methionine, glutamine and aspargine can be represented as

$$\mathrm{RCHNH_3^+COOH(aq)} \rightarrow \mathrm{RCHNH_3^+COO^-(aq)} + \mathrm{H^+(aq)} \qquad (1)$$

and

$$\mathrm{RCHNH_3^+COO^-(aq)} \rightarrow \mathrm{RCHNH_2COO^-(aq)} + \mathrm{H^+(aq)} \qquad (2)$$

The partial molar enthalpy of dissociation $\Delta\overline{H}_1$ of $\mathrm{RCH{-}NH_3^+COOH}$ was obtained by measuring the partial molar enthalpy of solution $\Delta\overline{H}_3$ of crystalline (cry) $\mathrm{RCHNH_3^+COO^-}$ in water at a pH close to the isoelectric pH

$$\mathrm{RCHNH_3^+COO^-(cry)} \rightarrow \mathrm{RCHNH_3^+COO^-(aq)} \qquad (3)$$

and the partial molar enthalpy of protonation $\Delta\overline{H}_4$ of the same compound in water at pH 0.0

$$\mathrm{RCHNH_3^+COO^-(cry)} + \mathrm{H^+(aq)} \rightarrow \mathrm{RCHNH_3^+COOH(aq)} \qquad (4)$$

The partial molar enthalpy of process (1) can be obtained by subtracting $\Delta\overline{H}_4$ from $\Delta\overline{H}_3$. Concentrations of about 10^{-3} m were used in processes (3) and (4). Therefore, the $\Delta\overline{H}$ values can be considered to be at infinite dilution ΔH^0 [6]. These values refer to the proton dissociation of 1 mole of $\mathrm{RCHNH_3^+COOH}$ at infinite dilution in 1000 g of water to yield 1 mole of $\mathrm{RCHNH_3^+{-}COO^-}$ ions and 1 mole of protons solvated in the same amount of water.

The dissociation processes in water are complicated by tautomeric equilibrium and zwitterion formation [1–5] for a compound containing carboxylate and amino groups. Although the α-amino acids in acid solution can be represented by the form $RCHNH_3^+COOH$, in a solution approaching pH 7.00 the principal species are neutral molecules, which may either be in the $RCHNH_2COOH$ form or in the zwitterion form $RCHNH_3^+COO^-$. Therefore, only the $RCHNH_3^+COOH$ form is present in eq. (4) at pH 0.0, whereas in eqn. (3) this is not so. However, it is possible to calculate the isoelectric pH values for the compounds examined using the dissociation constant values [7,8]. It can therefore be assumed that in this solution the $RCHNH_3^+COO^-$ form is predominant. In this way the first proton dissociation enthalpy values can be calculated.

The partial molar enthalpy of the second proton dissociation process of $RCHNH_3^+\text{-}COO^-$ is obtained by measuring the partial molar enthalpy $\Delta \bar{H}_5$ of neutralization of the crystalline compound $RCHNH_3^+COO^-$ in water at pH 14.00

$$RCHNH_3^+COO^-(cry) + OH^-(aq) \rightarrow RCHNH_2COO^-(aq) + H_2O(aq) \quad (5)$$

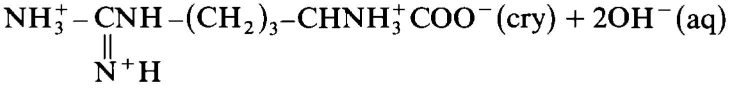
If the solution process enthalpy values $\Delta \bar{H}_3$ and the $\Delta \bar{H}_6$ partial molar value [9] in water related to the process

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

are subtracted from the ΔH_5 values, then the relation $\Delta \bar{H}_5 - (\Delta \bar{H}_3 + \Delta H_6)$ supplies the enthalpy values of process (2). The $\Delta \bar{H}$ values for this process can also be considered to be equal to ΔH^0. These values refer to the dissociation process of 1 mole of $RCHNH_3^+\text{-}COO^-$ at infinite dilution in 1000 g of water to yield 1 mole of $RCHNH_2COO^-$ and 1 mole of protons solvated in the same amount of water. Process (5) is carried out at pH 14.00 so that only the $RCHNH_2COO^-$ form is present. The free-energy and enthalpy values for the various dissociation processes of the above compounds are available in the literature [7–10].

Process (5) for lysine, arginine and cysteine must be written respectively as

$$NH_3^+\text{-}(CH_2)_4\text{-}CHNH_3^+\text{-}COO^-(cry) + 2OH^-(aq)$$
$$\rightarrow NH_2\text{-}(CH_2)_4\text{-}CHNH_2COO^-(aq) + 2H_2O(aq)$$



$$NH_3^+\text{-}\underset{\underset{N^+H}{\|}}{C}NH\text{-}(CH_2)_3\text{-}CHNH_3^+COO^-(cry) + 2OH^-(aq)$$

$$\rightarrow NH_2\underset{\underset{N^+H}{\|}}{C}HNH\text{-}(CH_2)_3CHNH_2COO^-(aq) + 2H_2O(aq)$$

$$HSCH_2CHNH_3^+\text{-}COO^-(cry) + 2OH^-(aq)$$
$$\rightarrow {}^-SCH_2CHNH_2\text{-}COO^-(aq) + 2H_2O(aq)$$

Therefore, for these compounds the $\Delta\overline{H}_5 - (\Delta\overline{H}_3 + 2\Delta\overline{H}_6)$ values refer to the sum of the second and third proton dissociation processes.

As the enthalpy values of the second and third proton dissociation processes for these three α-amino acids are available in the literature [8,10], it is possible to put these and our values in the system

$$\Delta H_2/\Delta H_3 = x/y \qquad x + y = C$$

where ΔH_2 and ΔH_3 are the literature values of the second and third dissociation processes and x and y are our corresponding enthalpy values (C being their sum).

If the corresponding free-energy values [7,8] are used in the mathematical system instead of the enthalpy values (thus assuming a linear relationship between them), another set of enthalpy values for both the second and third dissociation processes can be calculated.

The enthalpy values of cysteine, obtained using the free-energy values in the mathematical system, are close to the most reliable values available in the literature [10]. The latter were experimentally obtained by the proton dissociation processes of the NH_3^+ group contained in *S*-methylcysteine (second ionization process) and of the SH group belonging to mercaptoic acid (third ionization process).

The enthalpy values of lysine obtained from free-energy values ($\Delta H_2 < \Delta H_3$) are different from those available in the literature ($\Delta H_2 > \Delta H_3$) [8]. Nevertheless, it seems reasonable that the ΔH_3 value should be larger than ΔH_2 because the NH_3^+ group in position ϵ is more basic than the same group in position α.

Finally, the enthalpy values of arginine were obtained using the enthalpy values from the literature in the mathematical system.

RESULTS AND DISCUSSION

The free-energy, enthalpy and entropy values of the first and second proton dissociation processes for all the compounds studied are reported in Table 1. The thermodynamic quantities related to the third ionization process for the compounds cysteine, lysine and arginine are also reported in Table 1.

Compounds with similar structures can be compared using the different values of the thermodynamic quantities related to the first and second ionization processes. The differences in free-energy values (which give a measure of the proton loss in the ionization processes) are more clearly explained by the corresponding differences in enthalpy and entropy values. Indeed, the differences in the former can be related to the influence of the side-chain groups on the proton loss of the carboxyl and aminic groups,

TABLE 1

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

Amino acid	ΔG_1^0 (kcal mol^{-1})	ΔH_1^0 (kcal mol^{-1})	ΔS_1^0 (cal mol^{-1})	ΔG_2^0 (kcal mol^{-1})	ΔH_2^0 (kcal mol^{-1})	ΔS_2^0 (cal mol^{-1})	ΔG_3^0 (kcal mol^{-1})	ΔH_3^0 (kcal mol^{-1})	ΔS_3^0 (cal mol^{-1})
L-Methionine	3.11	0.62	−8.35	12.57	10.54	−6.81			
L-Cysteine	2.33	0.54	−6.00	14.71	9.04 [a]	−19.01	11.36	6.99 [a]	−14.65
L-Glutamine	2.96	0.67	−7.68	12.46	9.75	−9.10			
L-Aspargine	2.76	0.74	−6.77	11.02	9.85	−3.92			
L-Lysine	2.97	0.03	−8.96	12.21	11.04 [a]	−3.92	14.37	12.99 [a]	−4.63
L-Arginine	2.48	0.98	−5.03	12.27	11.82 [b]	−1.51	17.02	13.64 [b]	−11.34

[a] Obtained using free-energy values taken from the literature in the system.

[b] Obtained using enthalpy values taken from the literature in the system.

whereas the differences in the latter can explain the change in system order as a function of the formation and decomposition of stabilized ionic forms.

The following three pairs of compounds are considered: methionine/cysteine, glutamine/aspargine and lysine/arginine.

The free-energy values for the first ionization process of the pair methionine/cysteine show how cysteine undergoes a larger dissociation process, this being favoured by both the enthalpic and entropic terms. The SH group activates the NH_3^+ group, which, in turn, withdraws the negative charge from the oxygen atom belonging to the hydroxyl of the carboxyl group. This allows an easier proton dissociation as stressed by the enthalpic term: the enthalpy dissociation value of cysteine is smaller than that of methionine. Moreover, a strong field bond forms in the zwitterion between the carboxylate ion and the NH_3^+ groups. This produces a decrease in the negative entropy value which is related, in turn, to the reduction in solute–solvent interactions. This decrease in the negative entropy value favours the dissociation process.

The second proton dissociation process of cysteine takes place with more difficulty than that of methionine. The SH group in the zwitterion form partially draws the nitrogen lone pair binding the proton and thus decreases the enthalpy value of dissociation. Nevertheless, the entropy term plays a major role in the second dissociation of cysteine by virtue of the decomposition of the zwitterion stabilized form. The consequent increase in solute–solvent interactions increases the negative entropy value, which, in turn, hinders the dissociation process.

Aspargine undergoes a dissociation larger than that of glutamine in both the ionization processes, which are chiefly influenced by the entropic term. The amidic group in the aspargine compound is closer to the NH_3^+ group and greatly activates it. This allows the formation of a zwitterion stabilized form in the first ionization process, which, as previously seen, decreases the negative entropy value and favours the ionization process.

The prevailing factor in the second ionization process is again the entropic term. The proton loss from the NH_3^+ group does not decrease the stabilization of the $RCHNH_2COO^-$ form and thus the small drop in negative entropy value favours the dissociation process of aspargine. The enthalpy values show a trend opposite to that expected in both the ionization processes.

Finally, the first dissociation process of arginine is easier than that of lysine. The larger number of NH_2 groups in the side-chain of the former deactivates the NH_3^+ group with a consequent decrease in the dissociation of the carboxyl group as stressed by the enthalpy values. However, the arginine zwitterion form is more stable than that of lysine as can be seen by the less negative entropy value. This thermodynamic quantity is the prevailing factor in the ionization process.

The second proton dissociation is easier for lysine. The NH_3^+ group in the

TABLE 2

Free-energy, enthalpy and entropy values of the first, second and third ionization processes for aspartic and glutamic acids at 25 ° C in water

	ΔG_1^0 (kcal mol^{-1})	ΔH_1^0 (kcal mol^{-1})	ΔS_1^0 (cal mol^{-1})	ΔG_2^0 (kcal mol^{-1})	ΔH_2^0 (kcal mol^{-1})	ΔS_2^0 (cal mol^{-1})	ΔG_3^0 (kcal mol^{-1})	ΔH_3^0 (kcal mol^{-1})	ΔS_3^0 (cal mol^{-1})
Aspartic	2.72	0.80	−6.43	13.65	15.44 [a]	6.00	5.34	1.80 [a]	−11.87
Glutamic	2.95	0.17	−9.32	12.78	16.87 [a]	13.71	5.84	0.67 [a]	−17.34

[a] Obtained using enthalpy values taken from the literature in the system.

zwitterion form of arginine is greatly deactivated and releases its proton with more difficulty as stressed by the enthalpy value. This is the driving force of the ionization process.

Our experimental evidence supports the following remarks.

The entropy values in both the ionization processes (with the exception of the second ionization process of the pair arginine/lysine) largely account for the different behaviour of the compounds in the pairs. Indeed, the formation of stabilized zwitterion forms in the first ionization process decreases the negative entropy values so that the ionization process is less hindered. In contrast, the decomposition of the stabilized forms in the second ionization process increases the negative entropy values, thus hindering the ionization process. The above differences are due to the following: (i) a different group (i.e. SH and S), (ii) a different position in the side-chains of the same group (i.e. $CONH_2$) and (iii) a different number of the same group (NH_2).

A comparison between the aspargine/glutamine pair and the aspartic acid/glutamic acid pair, which have already been studied [4], is of interest. The components of the first pair are the amides of aspartic and glutamic acid. The free-energy, enthalpy and entropy values of the three ionization processes for the acids are reported in Table 2. The thermodynamic quantities of the first ionization process are very similar for the acids and their corresponding amides, whereas this is not so (mainly for the enthalpy values) in the second ionization process. It is proposed that the amidic group in the zwitterion form of the amides strongly activates the NH_3^+ group, thus favouring proton release.

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