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THERMODYNAMICS OF PROTON TRANSFER PROCESSES FOR SOME α-AMINO-ACIDS WITH DIFFERENT SIDE-CHAIN GROUPS

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ABSTRACT

The first and second proton ionization processes of two classes of α-amino-acids have been examined. Variation in the thermodynamic properties of the first and second proton transfer processes for the α-amino-acids were compared, using glycine as a reference compound. The structures of the two classes considered have side-chains formed by hydrophobic groups and polar groups without charge respectively.

The compounds from the second of these classes (with the exception of proline in the first ionization process) ionize more readily than the compounds from the first, in both the processes of ionization.

INTRODUCTION

The behaviour of some 'standard' α-amino-acids in water has been studied in our laboratory [1–4]. These compounds can be classified into four classes, according to the different structures of the side-chains, called R groups. These groups are defined as: (1) apolar or hydrophobic groups; (2) polar groups without charge; (3) polar groups with negative charge and (4) polar groups with positive charge.

Systematic calorimetric and thermodynamic investigations of the proton dissociation processes of the carboxylate group (first proton dissociation) and the amino group (second proton dissociation) for classes 1, 2 and 3 have been carried out [1–4].

The aim of the present work is to compare the transfer proton processes of some α-amino-acids from classes 1 and 2, using glycine as a reference compound. This compound can be considered as belonging to both classes, so it can be used as a common term for a reasonable comparison between the two classes.

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The α-amino-acids selected for study from class 1 were: L-α-alanine (Ala), L-proline (Pro) and L-tryptophan (Trp). The compounds from class 2 were L-tyrosine (Tyr), L-threonine (Thr) and D-serine (Ser). The reference compound, as noted above, was L-glycine (Gly).

The structures of the side-chains of the compounds from class 1 vary greatly: α-alanine bears a short linear side-chain (methyl group), proline bears a cyclic aliphatic side-chain, and tryptophan has two aromatic cycles in its side-chain. The α-amino-acids from class 2 have aromatic and aliphatic side-chains bearing hydroxyl groups. The reference compound, L-glycine, is the shortest α-amino-acid: its side-chain is formed by only one hydrogen atom.

The two classes were compared in terms of variation in the thermodynamic properties of the first and second proton transfer processes for the amino-acids from each and for the glycine compound.

EXPERIMENTAL AND PROCEDURE

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

Values for the thermodynamic properties of tyrosine, serine and threonine have been calculated previously [1–4].

The first and second proton ionization processes for α-alanine, proline, tryptophan and glycine can be represented as

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

and

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

The partial molar enthalpy of dissociation $\Delta\bar{H}_1$ of $\mathrm{RCHNH_3^+COOH}$ was obtained by measuring

(a) the partial molar enthalpy of solution $\Delta\bar{H}_3$ of crystalline $\mathrm{RCHNH_3^+COO^-}$ in water at a pH close to the isoelectric values

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

(b) the partial molar enthalpy of protonation $\Delta\bar{H}_4$ of the same compound in water at $\mathrm{pH} = 0.0$

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

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

Concentrations of about 10^{-3} m were used in processes (3) and (4), so the $\Delta\bar{H}$ values can be considered as being at infinite dilution $\Delta H^{\ominus}$ [5].

These values refer to the proton dissociation of 1 mol of $\mathrm{RCHNH_3^+COOH}$ at infinite dilution in 1000 g of water, yielding 1 mol of $\mathrm{RCHNH_3^+COO^-}$ ions and 1 mol of protons solvated in the same amount of water.

The dissociation process in water, for a compound containing the carboxylate and the amino groups, is complicated by tautomeric equilibrium and zwitterion formation [6,7]. While the α-amino-acids in the acid solution can be represented by the form $RCHNH_3^+COOH$, in solutions approaching pH = 7.00 the principal species are neutral molecules, which may either be in the $RCHNH_2COOH$ or the zwitterion form, $RCHNH_3^+COO^-$.

Therefore only the $RCHNH_3^+COOH$ form is present in eqn. (4) at pH = 0.0, while in eqn. (3) this is not true. However, it has been possible to calculate the isoelectric pH values of the compounds under consideration, by means of the dissociation constant values [8–14]. 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 were calculated.

The partial molar enthalpy of the second proton dissociation process of $RCHNH_3^+COO^-$ is obtained by measuring the partial molar enthalpy of neutralization $\Delta\overline{H}_5$ 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 $\Delta\overline{H}_3$ and the $\Delta\overline{H}_6$ partial molar value in water [15] related to the process

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

are subtracted from the $\Delta\overline{H}_5$ values, then the relation $\Delta\overline{H}_5 - (\Delta\overline{H}_3 + \Delta\overline{H}_6)$ supplies the enthalpy values of process (2). Again, the $\Delta\overline{H}$ values for this process can be considered as being equal to $\Delta H^\ominus$. These values refer to the dissociation process of 1 mol of $RCHNH_3^+COO^-$ at infinite dilution in 1000 g of water, yielding 1 mol of $RCHNH_2COO^-$ ions and 1 mol of protons solvated in the same amount of water. It is noted that process (5) is carried out at pH = 14.00, so that only the $RCHNH_2COO^-$ form is present.

The free-energy values for the compounds under consideration are available in the literature [8–14]

Let us consider process (1) for the glycine compound.

$$HCHNH_3^+COOH(aq) \rightarrow HCHNH_3^+COO^-(aq) + H^+(aq) \quad (1a)$$

Subtracting eqn. (1a) from eqn. (1) gives the following transfer proton process

$$\begin{aligned} &RCHNH_3^+COOH\ (aq) + HCHNH_3^+COO^-(aq) \\ &\quad \rightarrow RCHNH_3^+COO^-(aq) + HCHNH_3^+COOH \end{aligned} \quad (7)$$

Equation (7) is employed 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.

The change of a thermodynamic property $\delta\Delta P_i (P_i = G_i,\ H_i,\ S_i)$ related to this proton transfer process can be written as $\delta\Delta P_1 = \Delta P_1 - \Delta P_{1a}$ where ΔP_1

and ΔP_{1a} are the values of thermodynamic properties, for the first ionization process, of the α-amino-acids belonging to classes 1 and 2 and the corresponding thermodynamic values of the glycine respectively.

Similarly, the proton transfer process related to the second proton dissociation can be written as

$$\mathrm{RCHNH_3^+COO^-(aq) + HCHNH_2COO^-(aq)}$$
$$\rightarrow \mathrm{RCHNH_2COO^-(aq) + HCHNH_3^+COO^-(aq)}$$

and variation in thermodynamic properties for this process is expressed by the equation $\delta\Delta P_2 = \Delta P_2 - \Delta P_{2a}$ where ΔP_2 and ΔP_{2a} are the values of thermodynamic properties, for the second ionization process, of the α-amino-acids under consideration and glycine respectively.

RESULTS AND DISCUSSION

The free-energy, enthalpy and entropy values of processes (1) and (2) for all the α-amino-acids studied are reported in Table 1. The thermodynamic values related to the transfer proton processes of the same compounds are reported in Table 2.

From our experimental evidence as regards the first and second proton transfer processes of the compounds belonging to class 1, the following observations may be made.

The free-energy and enthalpic terms favour the first dissociation process of α-alanine in comparison with that of the glycine compound, while the entropic term hinders it, with a consequently greater order or solvation for the system.

Proline shows an ionization larger than that of α-alanine, as can be seen from the free-energy and enthalpic terms of the first dissociation (both favourable). Enthalpy plays the major role in this process.

TABLE 1

Free-energy, enthalpy and entropy values of the first and 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 mol^{-1} K^{-1})	$\Delta G_2^{\ominus}$ (kcal mol^{-1})	$\Delta H_2^{\ominus}$ (kcal mol^{-1})	$\Delta S_2^{\ominus}$ (cal mol^{-1} K^{-1})
L-α-alanine	3.20	0.58	−8.79	13.45	11.03	−8.12
L-glycine	3.21	0.93	−7.65	13.34	10.59	−9.22
L-proline	2.66	0.07	−8.69	14.51	10.26	−14.25
L-tryptophan	3.25	−0.34	−12.04	12.81	11.93	−2.95
L-tyrosine	3.00	−0.29	−11.03	12.42	8.30	−13.82
D-serine	2.98	0.32	−8.92	12.56	9.71	−9.56
L-threonine	2.86	0.37	−8.35	12.42	9.83	−8.63

TABLE 2

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

Compound	$\delta\Delta G_1^{\ominus}$ (kcal mol^{-1})	$\delta\Delta H_2^{\ominus}$ (kcal mol^{-1})	$\delta\Delta S_2^{\ominus}$ (cal mol^{-1} K^{-1})	$\delta\Delta G_2^{\ominus}$ (kcal mol^{-1})	$\delta\Delta H_2^{\ominus}$ (kcal mol^{-1})	$\delta\Delta S_2^{\ominus}$ (cal mol^{-1} K^{-1})
L-α-alanine	−0.01	−0.35	−1.14	0.11	0.44	1.10
L-proline	−0.55	−0.86	−1.04	1.17	−0.33	−5.03
L-tryptophan	0.04	−1.27	−4.39	−0.53	1.34	6.27
L-glycine	0.00	0.00	0.00	0.00	0.00	0.00
L-tyrosine	−0.21	−1.22	−3.38	−0.92	−2.29	−4.60
D-serine	−0.23	−0.61	−1.27	−0.78	−0.88	−0.33
L-threonine	−0.35	−0.56	−0.70	−0.92	−0.76	0.53

The first dissociation process for tryptophan is hindered by the free-energy and entropic terms and favoured by the enthalpic term; for this system too, dissociation of the compound involves a considerable degree of solvation.

The free-energy scale, going from cyclic aliphatic to linear aliphatic and cyclic aromatic side-chains, is proline > α-alanine > tryptophan. This scale gives a measure of the strength of the first dissociation process. The scales for the contribution to this proton dissociation supplied by the enthalpic and entropic terms are, respectively: tryptophan > proline > α-alanine, and proline > α-alanine > tryptophan.

The second ionization process of α-alanine is hindered by the free-energy and enthalpic terms, while the entropic term is favourable to it.

Proline shows an unfavourable trend for the second proton dissociation process as regards free-energy and entropy, while the enthalpic term is favourable.

The second proton dissociation of tryptophan is favoured by the entropic term, while the enthalpic term hinders it.

The sequences for the second proton dissociation for free-energy, enthalpy and entropy are, respectively: tryptophan > α-alanine > proline; proline > α-alanine > tryptophan; and tryptophan > α-alanine > proline.

A comparison of the two dissociation processes leads us to make the following remarks.

The entropic term for the tryptophan compound is always the driving-force (unfavourable in the first process, favourable in the second).

The first-place position of proline for the first ionization process is due to the enthalpic term, while its position in last place for the second ionization process is due to the entropic term.

The two ionization processes of α-alanine show an opposite trend, the enthalpic term being always the driving force.

A comparison of the proton transfer processes of the compounds from class 1 with those from class 2 (tyrosine, serine and threonine), both having glycine as a reference compound, has also been carried out.

The free-energy strength scale for the first ionization process is threonine > serine > tyrosine. The ionization strength sequences for the enthalpic and the entropic terms are, respectively; tyrosine > serine > threonine and threonine > serine > tyrosine. The tyrosine enthalpic term is favourable to the ionization process, while the entropic term is unfavourable to it.

The first-place position of threonine is due to a small degree of opposition of the entropic term to the dissociation process, and to a favourable enthalpic contribution.

The favourable enthalpic term and the unfavourable entropic term maintain serine in the central position.

The free-energy, enthalpy and entropy strength scales for the second ionization process are, respectively: tyrosine = threonine > serine; tyrosine ≫ serine > threonine; and threonine > serine ≫ tyrosine.

The equal values of free-energy for tyrosine and threonine are due to the large favourable enthalpic term for the former, and the large favourable entropic term for the latter.

The complete free-energy scale for the first ionization process, containing all the compounds from classes 1 and 2 is proline > threonine > serine > tyrosine > α-alanine > tryptophan. The sequence for enthalpy is tryptophan > tyrosine > proline > serine > threonine > α-alanine, while that for entropy is threonine > proline > α-alanine > serine > tyrosine > tryptophan.

The complete free-energy scale for the second ionization process shows the order tyrosine = threonine > serine > tryptophan > α-alanine > proline. The sequences for enthalpy and entropy are, respectively: tyrosine > serine > threonine > proline > α-alanine > tryptophan; and tryptophan > α-alanine > threonine > serine > tyrosine > proline.

It can be seen that the compounds from class 2 (with the exception of proline in the first ionization process) ionize more readily than the compounds of class 1 in both the processes of ionization.

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