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THERMODYNAMICS OF THE "STANDARD" α-AMINO ACIDS IN WATER AT 25°C

F. RODANTE

Dipartimento di Ingegneria Chimica, dei Materiali, delle Materie Prime e Metallurgia, Università di Roma "La Sapienza", Via del Castro Laurenziano 7, 00161 Roma (Italy)

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ABSTRACT

A thermodynamic study of 23 α-amino acids (19 "standard" α-amino acids plus 4 derivatives) is the subject of this work. Thermodynamic quantities of the first, second and third ionization processes are correlated with the features of the four classes into which the "standard" α-amino acids are classified.

A detailed thermodynamic analysis of the following effects influencing the ionization processes is presented.

(i) The solvation effects on a molecule having the same atom in different positions on its skeleton.

(ii) The solvation effects on different classes of α-amino acids.

(iii) The thermodynamic effects on different classes of α-amino acids.

(iv) The thermodynamic effects on α-amino acids having similar structures.

INTRODUCTION

The twenty α-amino acids which are usually found in proteins are called "standard" α-amino acids, and, with the exception of proline, have one carboxyl group and one amidic group, both linked to the α-carbon atom.

"Standard" α-amino acids differ from one another in the structure of their side-chains (called R groups), the polarization of which is the criterion used to classify these compounds into four groups: (1) a class with apolar or hydrophobic chains, (2) a class with polar chains having no charge, (3) a class with polar chains having positive charge, and (4) a class with polar chains having negative charge. There are considerable variations in the sizes and properties of the R groups in the same class.

Because the carboxyl and amino groups linked to the α-carbon atom and the functional groups of the side chains are used to identify each α-amino acid, it is important to know their thermodynamic properties. In the last fifty years, many researchers using various techniques have studied the thermodynamic quantities related to the dissociation processes [1–15] of

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"standard" α-amino acids. The thermodynamic study of 23 α-amino acids (19 "standard" plus 4 derivatives) has been the subject of an extended research programme carried out in this laboratory [16–21].

The first, second and third ionization processes of these compounds were measured in water by calorimetric techniques. The dominant effect influencing the ionization processes was found to be the solvation of the ions and molecules which in turn depends on the different side chains and on the different groups within the same side chain.

The aim of this work was to correlate our experimental data and those in the literature with the features of the four classes into which the "standard" α-amino acids are classified.

The α-amino acids belonging to the first class which were studied are L(−)-α-alanine, *d,l*-valine, L(+)-leucine, *d,l*-isoleucine, L(−)-proline, *d,l*-phenylalanine, L(+)-tryptophan, L(−)-methionine and *o*-, *m*- and *p*-fluorophenylalanine. In the second class L(−)-glycine, D(−)-serine, L(−)-threonine, L(−)-cysteine, L(−)-tyrosine, L(−)-aspargine, L(−)-glutamine and 3-OH-tyrosine were studied. Those studied from the third class were L(−)-lysine and L(−)-arginine, and those of the fourth class were *d,l*-aspartic acid L(−)-glutamic acid.

EXPERIMENTAL AND PROCEDURE

The compounds (Carlo Erba RPE Chemicals, used without purification) were weighed and handled in a nitrogen-filled dry-box. The calorimetric apparatus has been previously described [16–21].

The first and second proton ionization of a generic "standard" α-amino acid $RCHNH_3^+COOH(aq)$ in water can be represented as

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

and

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

The partial molar enthalpy of dissociation $\Delta\overline{H}_1$ of $RCHNH_3^+COOH$ in water is obtained by measuring the following quantities.

a) The partial molar enthalpy of solution $\Delta\overline{H}_3$ of crystalline (cry) $RCHNH_3^+COO^-$, zwitterion form, in water at a pH close to the isoelectric value

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

b) The partial molar enthalpy of protonation $\Delta\overline{H}_4$ of the same compound in water at pH 0.00

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

The partial molar enthalpy of process (1) can be obtained by subtracting $\Delta\bar{H}_4$ from $\Delta\bar{H}_3$ values. Concentrations of about 10^{-3} m were used in processes (3) and (4). Therefore, the $\Delta\bar{H}$ values can be considered to be at infinite dilution $\Delta H^{\ominus}$ [16–21]. These values refer to the proton dissociation of 1 mol of $RCHNH_3^+COOH$ at infinite dilution in 1000 g of water yielding 1 mol of $RCHNH_3^+COO^-$ ions and 1 mol of protons solvated in the same amount of water.

The dissociation processes in water, for a compound containing the carboxyl and amino groups, are complicated by tautomeric equilibrium and zwitterion formation [16–21]. While the α-amino acids, in acid solution, can be represented by the formula $RCHNH_3^+COOH$, in the 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 $RCHNH_3^+COO^-$. Thus, only the $RCHNH_3^+COOH$ form is present in eqn. (4) at pH = 0.00, while in eqn. (3) this is not the case. However, it is possible to calculate the isoelectric pH values for the compounds examined, by means of the dissociation constants [16–21]. 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 are calculated.

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

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

If the solution process enthalpy values $\Delta\bar{H}_3$ and the $\Delta\bar{H}_6$ partial molar value [18–21] in water related to process

$$H^+(aq) + OH^-(aq) \rightleftarrows H_2O \qquad (6)$$

are subtracted from the $\Delta\bar{H}_5$ values, then the relationship $\Delta\bar{H}_5 - (\Delta\bar{H}_3 + \Delta\bar{H}_6)$ supplies the enthalpy values of process (2).

The $\Delta\bar{H}$ values for this process can also be considered equal to $\Delta H^{\ominus}$. These values refer to the dissociation processes of 1 mol of $RCHNH_3^+COO^-$ at infinite dilution in 1000 g of water, yielding 1 mol of $RCHNH_2COO^-$ and 1 mol of protons solvated in the same amount of water. It has been observed that process (5) proceeds at pH = 14.00, so that only the $RCHNH_2COO^-$ form is present.

Finally, for those compounds with a third proton in a functional group $HRCHNH_3^+COOH$, process (5) must be written as

$$HRCHNH_3^+COO^-(cry) + 2OH^-(aq) \rightarrow {}^-RCHNH_2COO^-(aq) + 2H_2O \qquad (5a)$$

Therefore, for these compounds, $\Delta\bar{H}_5 - (\Delta\bar{H}_3 + 2\Delta\bar{H}_6)$ values refer to the sum of the second and third proton dissociation processes. If the enthalpy values of the second and third proton dissociation processes for these α-amino acids are available in the literature, it is possible so enter these and our values into the equations

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

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 are used in these mathematical equations instead of the enthalpy values (thus hypothesizing a linear relationship between them), other enthalpy values for the second and third dissociation processes can be calculated.

RESULTS AND DISCUSSION

The features of the four classes were correlated with the above cited experimental data.

First class

Five of these α-amino acids have aliphatic side chains: α-alanine, leucine, isoleucine, valine and proline. Two show aromatic side-chains: methionine and tryptophan, Methionine includes a sulphur atom. These compounds should be less soluble in water than the α-amino acids which have polar side chains. α-Alanine is the least hydrophobic compound of the first class and lies near to the line which separes polar (without charge) from hydrophobic α-amino acids. Proline differs from all the other “standard” α-amino acids: it is in fact an imino acid.

Second class

The compounds of the second class (serine, threonine, tyrosine, aspargine, glutamine, cysteine and glycine) are more soluble in water than hydrophobic α-amino acids. Their polar (without charge) side chains can form hydrogen bonds with water molecules. The polar character of serine, threonine and tyrosine is due to their hydroxyl groups, that of aspargine and glutamine to their amidic groups and that of cysteine to its sulphidic group. Glycine is sometimes classified as an apolar α-amino acid. Aspargine and glutamine are amides of aspartic and glutamic acids. Cysteine and tyrosine bear the most polar groups of this class, i.e. thiolic and hydrophenolic groups.

Third class

The compounds of this class have six carbon atoms. Lysine has a positively charged aminic group at the ε-position of its side chain and arginine has a guanidinic group with a positive charge.

Fourth class

The compounds of the fourth class, aspartic and glutamic acids, have a second carboxyl group which is completely ionized at pH 6.00–7.00 and so have a negative charge.

SOLUBILITY AND SOLVATION

The enthalpy values of solubility, of protonation and of the neutralization processes [16,21] are reported in Table 1. It can be seen that the enthalpy solubility values are all endothermic with the exception of that of proline. This signifies that the energy required to break the electrostatic bonds among the zwitterions of the α-amino acids is not returned by the interactions between ions and solvent.

As these endothermic values increase from the first to the fourth class, the solute–solvent interactions decrease in the same direction, in contrast to

TABLE 1

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

	$\Delta H_3^{\ominus}$	$\Delta H_4^{\ominus}$	$\Delta H_5^{\ominus}$
L(−)-α-Alanine	1.82	1.24	−0.49
d,l-Valine	1.28	1.11	−4.00
L(+)-Leucine	0.70	0.34	−1.31
d,l-Isoleucine	0.63	0.65	−1.61
L(−)-Proline	−0.74	−0.81	−3.82
L(−)-Methionine	2.68	2.06	−0.12
L(+)-Tryptophan	1.61	1.94	0.25
d,l-Phenylalanine	1.84	1.70	−0.61
o-F-Phenylalanine	2.79	1.92	−0.07
m-F-Phenylalanine	1.79	1.61	−0.27
p-F-Phenylalanine	1.87	2.22	0.34
D(−)-Serine	2.63	2.31	−0.99
L(−)-Threonine	2.42	2.05	−1.09
L(−)-Glycine	3.44	2.50	0.69
L(−)-Cysteine	2.63	2.09	−8.02
L(−)-Glutamine	5.44	4.77	1.85
L(−)-Asparagine	7.53	6.79	4.04
L(−)-Tyrosine	4.77	5.06	−4.39
3-OH-Tyrosine	4.70	4.23	−2.73
L(−)-Lysine	2.88	2.54	0.24
L(−)-Arginine	5.94	4.96	4.74
d,l-Aspartic acid	6.16	5.36	−3.28
L(+)-Glutamic acid	5.91	5.74	−3.40

what one would expect. Indeed the hydrogen bonds of the hydroxyl groups of the compounds of the second class, and the positive and negative charges of the third and fourth classes, ought to interact with water molecules.

In the first class, proline has the highest solute–solvent interaction. In the second class, the lowest solute–solvent interactions are those of glutamine, aspargine, tyrosine and 3-OH-tyrosine, for the last two this being due to the benzene ring effect.

The carboxylate groups of aspartic and glutamic acids in the third class interact slightly with water molecules. In the fourth class, the lowest interaction is that of arginine. Furthermore, the values of $\delta\Delta H_3 = \Delta H_3^\ominus(\mathrm{a}) - \Delta H_3^\ominus(\mathrm{b})$ can give the relative solvation between the zwitterions of compound a with respect to compound b. From the $\Delta H_3^\ominus$ values, it can be seen that there is a decreasing solvation trend from the first to the fourth classes.

The protonation and neutralization enthalpy values can also indicate the interactions of the undissociated molecules and the ionic forms $RCHNH_2COO^-$ with the solvent molecules. The $\delta\Delta H_4^\ominus$ and $\delta\Delta H_5^\ominus$ values indicate the relative solvation (respectively for the undissociated molecules

TABLE 2

Free energy values (kcal mol^{-1}) of the first ($\Delta G_1^\ominus$), second ($\Delta G_2^\ominus$) and third ($\Delta G_3^\ominus$) ionization processes for "standard" α-amino acids in water at 25 °C

	$\Delta G_1^\ominus$	$\Delta G_2^\ominus$	$\Delta G_3^\ominus$
L(−)α-Alanine	3.20	13.45	
d,l-Valine	3.18	13.25	
L(+)-Leucine	3.18	13.30	
d,l-Isoleucine	3.16	13.31	
L(−)-Proline	2.66	14.51	
L(−)-Methionine	3.11	12.57	
L(+)-Tryptophan	3.25	12.81	
d,l-Phenylalanine	3.52	12.67	
o-F-Phenylalanine	2.89	12.29	
m-F-Phenylalanine	2.86	12.25	
p-F-Phenylalanine	2.91	12.35	
D(−)-serine	2.98	12.56	
L(−)-Threonine	2.86	12.42	
L(−)-Glycine	3.21	13.34	
L(−)-Cysteine	2.33	14.71	11.36
L(−)-Glutamine	2.96	12.46	
L(−)-Asparagine	2.76	11.02	
L(−)-Tyrosine	3.00	12.42	13.73
3-OH-Tyrosine	2.32	8.68	9.88
L(−)-Lysine	2.97	12.21	14.37
L(−)-Arginine	2.48	12.57	17.02
d,l-Aspartic acid	2.72	13.65	5.34
L(+)-Glutamic acid	2.95	12.77	5.84

and for the ionic form $RCHNH_2COO^-$) of a series of compounds with respect to a chosen reference compound. For the undissociated molecules, the behaviour is similar to that of the zwitterion forms (a decreasing solvation trend).

For the solvation of the $RCHNH_2COO^-$ ionic forms, it can be noted that there is not a sharp difference in average values between the first and second classes. The compounds of the third class are the least solvated while those of the fourth are the most solvated.

The solvation values show a substantial influence on the first and second ionization processes.

THERMODYNAMICS

The free-energy, enthalpy and entropy values of the first, second and third ionization processes for the twenty-three α-amino acids are reported in Tables 2, 3 and 4 [16–21].

TABLE 3

Enthalpy values (kcal mol^{-1}) of the first ($\Delta H_1^\ominus$), second ($\Delta H_2^\ominus$) and third ($\Delta H_3^\ominus$) ionization processes for "standard" α-amino acids in water at 25 °C

	$\Delta H_1^\ominus$	$\Delta H_2^\ominus$	$\Delta H_3^\ominus$
L(−)α-Alanine	0.58	11.03	
d,l-Valine	0.17	8.06	
L(+)-Leucine	0.36	11.33	
d,l-Isoleucine	−0.02	11.08	
L(−)-Proline	0.07	10.26	
L(−)-Methionine	0.62	10.54	
L(+)-Tryptophan	−0.34	11.93	
d,l-Phenylalanine	0.14	10.89	
o-F-Phenylalanine	0.87	10.48	
m-F-Phenylalanine	0.18	11.29	
p-F-Phenylalanine	−0.35	11.81	
D(−)-Serine	0.32	9.71	
L(−)-Threonine	0.37	9.83	
L(−)-Glycine	0.94	10.59	
L(−)-Cysteine	0.54	9.04 [a]	6.99 [a]
L(−)-Glutamine	0.67	9.75	
L(−)-Asparagine	0.74	9.85	
L(−)-Tyrosine	−0.29	8.32 [a]	9.24 [a]
3-OH-Tyrosine	0.47	14.15	
L(−)-Lysine	0.03	11.04 [a]	12.99 [a]
L(−)-Arginine	0.98	11.82 [b]	13.64 [b]
d,l-Aspartic acid	0.80	15.44 [b]	1.80 [b]
L(+)-Glutamic acid	0.17	16.87 [b]	0.67 [b]

[a] Values obtained using free-energy values from the literature in a mathematical system.
[b] Values obtained using enthalpy values from the literature in a mathematical system.

TABLE 4

Entropy values (cal mol^{-1} °C^{-1} of the first ($\Delta S_1^{\ominus}$), second ($\Delta S_2^{\ominus}$) and third ($\Delta S_3^{\ominus}$) ionization processes for "standard" α-amino acids in water at 25 °C

	$\Delta S_1^{\ominus}$	$\Delta S_2^{\ominus}$	$\Delta S_3^{\ominus}$
L(−)α-Alanine	−8.79	−8.12	
d,l-Valine	−10.10	−17.44	
L(+)-Leucine	−9.40	−6.71	
d,l-Isoleucine	−10.67	−7.80	
L(−)-Proline	−8.69	−14.25	
L(−)-Methionine	−8.35	−6.81	
L(+)-Tryptophan	−12.04	−2.95	
d,l-Phenylalanine	−11.34	−5.97	
o-F-Phenylalanine	−6.77	−6.07	
m-F-Phenylalanine	−8.99	3.22	
p-F-Phenylalanine	−10.93	−1.81	
D(−)-Serine	−8.92	−9.56	
L(−)-Threonine	−8.35	−8.69	
L(−)-Glycine	−7.65	−9.22	
L(−)-Cysteine	−6.00	−19.01	−14.65
L(−)-Glutamine	−7.68	−9.10	
L(−)-Asparagine	−6.77	−3.92	
L(−)-Tyrosine	−11.03	−13.82	−15.06
3-OH-Tyrosine	−6.20	−9.29	
L(−)-Lysine	−8.96	−3.92	−4.63
L(−)-Arginine	−5.03	−1.51	−11.34
d,l-Aspartic acid	−6.43	6.03	−11.67
L(+)-Glutamic acid	−9.32	13.11	−17.84

Table 2 shows that the averages of the free-energy values for the various classes with respect to the first ionization processes are in the order 3rd > 4th > 2nd > 1st. For the second ionization process the order is 3rd > 2nd > 4th > 1st. The third ionization process is in the order 4th > 2nd > 3rd.

The enthalpic thermodynamic property for the first process is 1st > 2nd > 4th ≥ 3rd, for the second 2nd > 1st > 3rd > 4th and for the third 4th > 2nd > 3rd.

Finally, for entropy the following values were found for the various classes: first process, 3rd > 4th > 2nd > 1st; second process, 3rd > 1st > 4th > 2nd; and third process, 3rd > 4th ≥ 2nd.

It can therefore be seen that the free-energy values for the first ionization process in all the classes are mostly influenced by the entropy values. For the second ionization process, the free-energy values depend mostly on enthalpy values in the second class, on entropy values in the third and fourth classes, and on neither in the first class.

Finally, the free-energy values of the third process are mostly influenced by the enthalpy values in the third and fourth classes and by neither enthalpy not entropy in the second class.

A more detailed analysis of the thermodynamic and solvation data leads to the following remarks on the effects influencing the ionization processes.

THE SOLVATION EFFECTS ON A MOLECULE HAVING THE SAME ATOM IN DIFFERENT POSITIONS ON ITS SKELETON

Let us consider phenylalanine and its fluoro-derivatives (*o*-, *m*- and *p*-) and 4-hydroxy- and 3,4-dihydroxyphenylalanine. The differences in enthalpy ionization processes for different compounds can be explained by means of the differences in enthalpy solvation of the respective ions and undissociated molecules. For this purpose, the differences in enthalpy values of processes (3) $\delta\Delta H_3^\ominus$, processes (4) $\delta\Delta H_4^\ominus$ and processes (5) $\delta\Delta H_5^\ominus$ of the various fluoro and hydroxyl derivatives with respect to phenylalanine, chosen as reference compound, can be identified with the differences in solvation of the zwitterions $RCHNH_3^+COO^-$, the undissociated molecules $RCHNH_3^+$-$COOH$ and the ionic forms $RCHNH_2COO^-$ of the same compounds in water.

These values, together with the differences in the first and second ionization processes ($\delta\Delta H_1^\ominus$ and $\delta\Delta H_2^\ominus$), are given in Table 5. With respect to the first proton dissociation order (pF > Phe > mF > oF) it can be observed that *ortho*- and *meta*-fluoro derivatives behave as weaker acids than phenylalanine, while for the *para*-fluoro derivative, the reverse is true.

The orders of solvation of the undissociated molecules and zwitterions are respectively mF > Phe > oF > pF and mF > Phe > pF > oF. For the *meta* and *ortho* derivatives, the solvation process of undissociated molecules prevails, while for the *para* derivative the solvation process of the zwitterion form is prevailing.

The fluorine atom in the *ortho* position in the zwitterion form enhances the positive charge on the amino group, which in turn reinforces the cyclic form (due to the field effect) and increases the desolvation effect. In the *para* derivative, the presence of the fluorine atom mostly increases the

TABLE 5

Differences in enthalpy values (kcal mol^{-1}) for processes (1), (2), (3), (4) and (5) of phenylalanine derivatives with respect to phenylalanine in water at 25 °C

	$\delta\Delta H_1^\ominus$	$\delta\Delta H_2^\ominus$	$\delta\Delta H_3^\ominus$	$\delta\Delta H_4^\ominus$	$\delta\Delta H_5^\ominus$
Phe	0.00	0.00	0.00	0.00	0.00
oF	0.73	−0.41	0.95	0.22	0.54
mF	0.04	0.40	−0.05	−0.09	0.34
pF	−0.49	0.92	0.03	0.52	0.95
4-(OH)	−0.43	−2.57	2.93	3.36	−3.73
3,4-$(OH)_2$	0.33	3.69	2.86	2.53	−2.12

desolvation of the undissociated molecule. The order of the second proton dissociation process is *o*F > Phe > *m*F > *p*F. The influence of the fluorine atom was studied by considering the scales of solvation of the zwitterions and the $RHNH_2COO^-$ ions. For the latter scale the order is Phe > *m*F > *o*F > *p*F.

The first place of the *ortho* derivative in the second ionization process is linked to the desolvation process of the zwitterion forms; and the places of the *meta* and *para* derivatives are related to the desolvation process of the $RCHNH_2COO^-$ forms.

The orders of the first and second proton dissociation processes for the hydroxyl derivatives are the same, namely 4-(OH) > Phe > 3,4-$(OH)_2$.

The solvation scales for the undissociated molecules, the zwitterion forms and the ionic forms $RCHNH_2COO^-$ are respectively Phe > 3,4-$(OH)_2$ > 4-(OH), Phe > 3,4-$(OH)_2 \geq$ 4-(OH) and 4-(OH) > 3,4-$(OH)_2$ > Phe. The large negative values of the last scale show how the solvation of the ionic form $RCHNH_2COO^-$ is the prevailing effect on the second ionization process.

The complete scales of the first and second ionization processes are respectively *p*F > 4-(OH) > Phe > *m*F > 3,4-$(OH)_2$ > *o*F and 4-(OH) > *o*F > Phe > *m*F > *p*F > 3,4-$(OH)_2$.

The greater effect of the hydroxyl group on the dissociation of the amino group (second ionization process) for tyrosine is clearly due to the high solvation of the $RCHNH_2COO^-$ form.

Finally, it is possible to calculate the total process of ionization expressed by the equation

$$RCHNH_3^+COOH(aq) \rightarrow RCHNH_2COO^-(aq) + 2H^+(aq) \qquad (7)$$

obtained by summing eqns. (1) and (2). The $\Delta H_7^\ominus$ values are reported in Table 6.

The order of the total proton dissociation is 4-(OH) > Phe $\geq$ *o*F $\geq$ *m*F $\geq$ *p*F > 3,4-$(OH)_2$. The hydroxyl group of the tyrosine greatly favours the total dissociation process (with respect to that of phenylalanine) while the effect of the fluorine atom seems to be negligible.

TABLE 6

Enthalpy values (kcal mol^{-1}) of process (7) for phenylalanine derivatives in water at 25 °C

	$\Delta H_7^\ominus$
Phe	11.03
*o*F	11.35
*m*F	11.47
*p*F	11.46
4-(OH)	8.01
3,4-$(OH)_2$	14.62

SOLVATION EFFECTS ON DIFFERENT CLASSES OF α-AMINO ACIDS

The first and second ionization processes of some α-amino acids of the first and second classes were considered.

The compounds studied were *d,l*-valine, *d,l*-isoleucine, L(+)-leucine, *d,l*-phenylalanine, D(−)-serine, L(−)-threonine and L(−)-tyrosine.

The calorimetric scale of ionization strength for the first ionization process is Tyr > Ill > Phe > Val > Ser > Leu.

The following solvation sequence for undissociated molecules was considered: Leu > Ill > Val > Phe > Thr > Ser > Tyr. For the zwitterions, the order based on the solvation is Ill > Leu > Val > Phe > Thr > Ser > Tyr.

The experimental evidence supports the following remarks. In contrast with the expected results, both the ions and the undissociated molecules of the α-amino acids containing hydroxyl groups are less solvated than the ions and the undissociated molecules of the α-amino acids with hydrophobic groups. The calorimetric techniques proved sensitive enough to reveal differences with respect to the first ionization process, even between α-amino-acids having apparently similar structures.

The free-energy values were found to be very similar (with the exception of phenylalanine) and the entropic strength scale was similar to the calorimetric scale, the large entropy values of tyrosine and phenylalanine being due to the presence of the benzene ring.

The calorimetric strength scale for the second proton dissociation process is Val > Tyr > Ser > Thr > Phe > Ill > Leu.

The different influences of the same side chains on the first and second proton dissociation processes can be related to the fact that both ionization processes depend on the solvation of the zwitterions, but differ in the second solvation process (solvation of undissociated molecules and solvation of the ionic forms $RCHNH_2COO^-$ respectively for the first and second ionization processes). The sequence of ionic forms is Tyr > Val > Ill > Leu > Thr > Ser > Phe. It was noted that the ionic forms of the α-amino acids containing hydroxyl groups (with the exception of tyrosine) are less solvated than the corresponding forms of the α-amino acids with hydrophobic groups, as occurs in the first ionization process for the zwitterions and the undissociated molecules.

It can also be noted that the α-amino acids under consideration differ mostly in the enthalpy ionization values, while their free-energy values are very similar. Thus it seems that for the second ionization process (as for the first one) the data obtained by calorimetric measurements enhance the differences among the compounds.

THERMODYNAMIC EFFECTS ON DIFFERENT CLASSES OF α-AMINO ACIDS

Some α-amino acids of the first and second classes were compared using transfer proton processes with glycine chosen as a reference compound.

Glycine can be considered as belonging to both classes, therefore it can provide a comparison between the two cited classes.

The compounds of the first class were L(−)-α-alanine, L(−)-proline and L(−)-tryptophan. The compounds of the second class were L(−)-tyrosine, L(−)-threonine and D(−)-serine.

The chosen compounds of the first class differ widely from one another in the structure of their side chains. They show the following features.

(1) α-Alanine has a short linear side chain (methyl group).

(2) Proline has a cyclic aliphatic side chain.

(3) Tryptophan has two aromatic rings in its side chain.

The second class is made up of α-amino acids with 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.

A comparison between the two classes was carried out using the variation in thermodynamic properties of the first and second proton transfer processes as compared to glycine. Considering process (1) for glycine

$$\mathrm{HCHNH_3^+COOH(aq)} \rightarrow \mathrm{HCHNH_3^+COO^-(aq)} + \mathrm{H^+(aq)} \tag{1a}$$

and by subtracting eqn. (1a) from eqn. (1), the following transfer proton process can be written

$$\begin{aligned}&\mathrm{RCHNH_3^+COOH(aq)} + \mathrm{HCHNH_3^+COO^-(aq)}\\&\quad = \mathrm{RCHNH_3^+COO^-(aq)} + \mathrm{HCHNH_3^+COOH(aq)}\end{aligned} \tag{8}$$

Equation (8) is employed to account for the effects of changing the structural side chains on the energy differences for the cited α-amino acids, compared to a reference compound.

The change of some thermodynamic properties $\Delta P_i(P_i = G_i, H_i, S_i)$ related to this proton transfer process can be written as $\delta\Delta P_i = \Delta P_1 - \Delta P_1(\mathrm{g})$ where ΔP_1 and $\Delta P_1(\mathrm{g})$ are respectively the values of thermodynamic properties for the first ionization process of the α-amino acids belonging to the two classes, and the corresponding thermodynamic values of glycine.

The proton transfer process related to the second proton dissociation can be similarly written as

$$\begin{aligned}&\mathrm{RCHNH_3^+COO^-(aq)} + \mathrm{HCHNH_2COO^-(aq)}\\&\quad = \mathrm{RCHNH_2COO^-(aq)} + \mathrm{HCHNH_3^+COO^-(aq)}\end{aligned} \tag{9}$$

and its thermodynamic properties variation is expressed by the equation $\delta\Delta P_2 = \Delta P_2 - \Delta P_2(\mathrm{g})$, ΔP_2 and $\Delta P_2(\mathrm{g})$ being the thermodynamic properties values for the second ionization process of the above cited α-amino acids and of glycine. The thermodynamic values related to the transfer proton processes of these compounds are reported in Table 7.

The free energy scale containing the compounds of the first and second classes for the first ionization process is proline > threonine > serine >

TABLE 7

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

	$\delta\Delta G_1^{\ominus}$	$\delta\Delta H_1^{\ominus}$	$\delta\Delta S_1^{\ominus}$	$\delta\Delta G_2^{\ominus}$	$\delta\Delta H_2^{\ominus}$	$\delta\Delta S_2^{\ominus}$
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

tyrosine > α-alanine > tryptophan. This scale gives a measure of the strength of the first ionization process.

The scales of contribution to this proton dissociation supplied by the enthalpic and entropic terms are respectively tryptophan > tyrosine > proline > serine > threonine > α-alanine and threonine > proline > α-alanine > serine > tyrosine > tryptophan.

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

As can be seen, compounds of the second class prevail (with the exception of proline in the first ionization process) over the compounds of the first for both ionization processes.

THERMODYNAMIC EFFECTS ON α-AMINO ACIDS WITH SIMILAR STRUCTURES

A comparison among α-amino acids showing similar structures can be carried out by considering the different values of their thermodynamic properties related to the first and second ionization processes. The following three pairs of compounds were considered: L-methionine and cysteine; L-glutamine and L-aspargine; L-lysine and L-arginine.

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

Glutamine and aspargine have an amidic group in the γ and β positions of their side chains.

The differences in free-energy values (which give a measure of the proton loss in the ionization process) are more clearly explained by the corresponding differences in enthalpy and entropy values. Indeed the differences of the former can be related to the influence of the side chain groups on the proton loss of the carboxyl and amino groups, while that of the latter can explain the system order change as a function of the formation and decomposition of stabilized ionic forms.

The free energy values for the first ionization process of the methionine/cysteine pair 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 attracts the negative charge from the oxygen atom of the hydroxyl of the carboxyl group. This leads to easier proton dissociation as demonstrated 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^+ group) with a consequent decrease of the negative entropy value, related in turn to the reduction of 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 with respect to that of methionine. The SH group, in the zwitterion form, attracts 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 process of cysteine because of the decomposition of the zwitterion-stabilized form. The consequent increase of 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 ionization processes, which are chiefly influenced by the entropic term. The amidic group, in aspargine, is closer to the NH_3^+ group and greatly activates it. This allows the formation, in the first ionization process, of a zwitterion-stabilized form 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 the negative entropy value favours the dissociation process of aspargine. The enthalpy values show a trend opposite to that expected in both ionization processes.

The first dissociation process of arginine is easier than that of lysine. The higher number of NH_2 groups in the side chain of the former deactivates the NH_3^+ group with a consequent decreased dissociation of the carboxyl group, as indicated by the enthalpy values. However, the stabilization of the arginine zwitterion form is better than that of the lysine, as can be seen from the lower 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 zwitterion form of arginine is greatly deactivated and releases its proton with more difficulty, as shown by the enthalpy value. This is the driving-force of the ionization process.

In conclusion, the entropy values for both ionization processes with the exception of the second ionization processes of the arginine/lysine pair) 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 values of the entropy so that the ionization process is less hindered. On the contrary, the decomposition of the stabilized form in the second ionization process increases the negative entropy values, thus hindering the ionization process.

The above-cited differences are results of a different group (i.e. SH and S), a different position in the side chains of the same groups ($CONH_2$) and the presence of a different number of the same group (NH_2).

REFERENCES

1 E.J. King, J. Am. Chem. Soc., 82 (1960) 3575.
2 E.J. King and G.W. King, J. Am. Chem. Soc., 78 (1956) 1089.
3 P.K. Smith, A.C. Taylor and E.R.B. Smith, J. Biol. Chem., 122 (1937) 109.
4 J.M. Sturtevant, J. Am. Chem. Soc., 64 (1942) 762.
5 M. May and W.A. Felsing, J. Am. Chem. Soc., 73 (1951) 406.
6 L.F. Nims and P.K. Smith, J. Biol. Chem., 101 (1933) 401.
7 E.R.B. Smith and P.K. Smith, J. Biol. Chem., 146 (1942) 187.
8 S.P. Datta and A.K. Grybowski, Trans. Faraday Soc. (1958) 1179, 1188.
9 S.P. Datta, A.K. Grybowski and R.G. Bates, J. Phys. Chem., 68 (1964) 275.
10 J. Llopis and D. Ordonez, J. Electroanal. Chem., 5 (1963) 129.
11 B.B. Owen, J. Am. Chem. Soc., 76 (1934) 24.
12 J.M. Sturtevant, J. Am. Chem. Soc., 63 (1941) 88.
13 E.J. King, J. Am. Chem. Soc., 73 (1957) 6151.
14 K.P. Anderson, W.O. Greenhalgh and R.M. Izzatt, Inorg. Chem., 5 (1966) 2106.
15 R.N. Diebel and D.F. Swinehart, J. Phys. Chem., 61 (1957) 333.
16 F. Rodante and M. Tocci, Thermochim. Acta, 86 (1985) 109.
17 F. Rodante and F. Fantauzzi, Thermochim. Acta, 111 (1987) 233.
18 F. Rodante, F. Fantauzzi and P. Di Girolamo, Thermochim. Acta, 142 (1989) 203.
19 F. Rodante, F. Fantauzzi and G. Marrosu, Thermochim. Acta, 141 (1989) 297.
20 F. Rodante and F. Fantauzzi, Thermochim. Acta, 144 (1989) 75.
21 F. Rodante and F. Fantauzzi, Thermochim. Acta, 144 (1989) 275.