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CALORIMETRIC STUDY OF THE FIRST AND SECOND PROTON DISSOCIATION PROCESSES OF FLUORO AND HYDROXYL DERIVATIVES OF PHENYLALANINE

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ABSTRACT

A calorimetric study of the first and second proton dissociation processes of fluoro and hydroxyl derivatives of phenylalanine in water is presented. The calorimetric acid strength scales in water for both of these processes are also presented. The effects which influence the acidity scales are the solvation processes of the undissociated molecules, the zwitterions and the ionic forms. The presence of a fluorine atom affects all the processes considered. The large effect of the hydroxyl groups on the second proton dissociation process is due to the large solvation of the $XPhCHNH_2COO^-$ ionic form. The total proton dissociation process (sum of the two proton dissociation processes) confirms that the hydroxyl group greatly favours this process for tyrosine, while the effect of the fluorine atom is negligible.

INTRODUCTION

The first proton dissociation process of some α-amino acids belonging to various classes was the subject of a recent paper [1]. This dissociation was found to be a function of the side-chain group R.

A subsequent study on the influence of the hydroxyl group in various side-chain groups R (R is aliphatic or aromatic) on the dissociation process of the carboxylate group has been carried out [2].

The aim of this paper is to study the influence of the fluorine atom, at various positions on the benzene ring of phenylalanine, on the first (carboxylate group) and second (amino group) proton dissociation processes. A comparison with monosubstituted and disubstituted hydroxyl derivatives

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of phenylalanine allows a deeper insight into both the proton dissociation processes.

EXPERIMENTAL AND PROCEDURE

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

The first and second proton dissociation processes can be represented respectively as

$$\mathrm{XPhCHNH_3^+COOH(aq) \rightarrow XPhCHNH_3^+COO^-(aq) + H^+(aq)} \tag{1}$$

and

$$\mathrm{XPhCHNH_3^+COO^-(aq) \rightarrow XPhCHNH_2COO^-(aq) + H^+(aq)} \tag{2}$$

where X is the fluorine atom at positions 2, 3 or 4 or the hydroxyl group at positions 4 or 3 and 4.

The partial molar enthalpy of dissociation $\Delta \overline{H}_1$ of $\mathrm{XPhCHNH_3^+COOH}$ was obtained by measuring the partial molar enthalpy of solution $\Delta \overline{H}_3$ of crystalline $\mathrm{XPhCHNH_3^+COO^-}$ in water at a pH value close to the isoelectric point

$$\mathrm{XPhCHNH_3^+COO^-(cry) \rightarrow XPhCHNH_3^+COO^-(aq)} \tag{3}$$

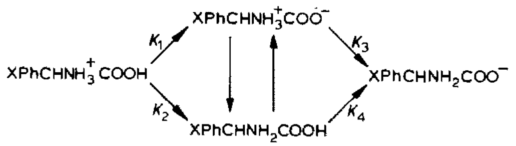
and the partial molar enthalpy of protonation $\Delta \overline{H}_4$ of the same compound in water at pH 0

$$\mathrm{XPhCHNH_3^+COO^-(cry) + H^+(aq) \rightarrow XPhCHNH_3^+COOH(aq)} \tag{4}$$

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

In processes (3) and (4), concentrations of about 10^{-3} m were used. Therefore the $\Delta \overline{H}$ values can be considered to be at infinite dilution ΔH^0 [3]. These values refer to the proton dissociation process of 1 mol of $\mathrm{XPhCHNH_3^+COOH}$ at infinite dilution in 1000 g of water, which yields 1 mol of $\mathrm{XPhCHNH_3^+COO^-}$ ions and 1 mol of protons solvated in the same amount of water.

In water, the dissociation process for a compound containing carboxylate and amino groups is complicated by tautomeric equilibria and zwitterion formation [4,5]. The following scheme shows the equilibria concerned



While the amino acids in the acid solution can be represented by the form $\mathrm{XPhCHNH_3^+COOH}$, in a solution approaching pH 7.00 the principal species are neutral molecules which may either be in the $XPhCHNH_2COOH$ form or in the zwitterion form $XPhCHNH_3^+COO^-$.

Therefore in eqn. (4) at pH 0.0 only $XPhCHNH_3^+COOH$ is present, whereas in eqn. (3) this is not the case. However, using the dissociation constants of phenylalanine [6,7] it is possible to calculate the isoelectric pH value of this compound. This value (pH 5.91) is probably close to the isoelectric pH values of the phenylalanine derivatives. It can therefore be assumed that in this solution the $XPhCHNH_3^+COO^-$ 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 scheme given above.

The partial molar enthalpy of the second proton dissociation process of $XPhCHNH_3^+COO^-$ (K_3 process) was obtained by measuring the partial molar enthalpy $\Delta \overline{H}_5$ of neutralization of the crystalline compound $XPhCHNH_3^+COO^-$ in water at pH 14

$$XPhCHNH_3^+COO^-(cry) + OH^-(aq) \rightarrow XPhCHNH_2COO^-(aq) + H_2O \tag{5}$$

If the partial molar enthalpy of solution $\Delta \overline{H}_3$ and the partial molar value $\Delta \overline{H}_6$ related to the process (in water) [8]

$$H^+(aq) + OH^-(aq) \rightarrow H_2O(aq) \tag{6}$$

are subtracted from the $\Delta \overline{H}_5$ values, then the enthalpies of process (2) are obtained. For this process the $\Delta \overline{H}$ values can also be considered to be equal to ΔH^0. These values refer to the dissociation process of 1 mol of $XPhCHNH_3^+COO^-$ at infinite dilution in 1000 g of water, which yields 1 mol of $XPhCHNH_2COO^-$ ions and 1 mol of protons solvated in the same amount of water.

Finally, it should be noted that process (5) was carried out at pH 14, so that only the $XPhCHNH_2^-COO^-$ form is present.

For the 4-OH and 3,4-$(OH)_2$ derivatives, reaction (5) must be written

$$(OH)PhCHNH_3^+COO^-(cry) + 2OH^-(aq) \rightarrow {}^-OPhCHNH_2COO^-(aq) + 2H_2O(l) \tag{7}$$

and

$$(OH)_2PhCHNH_3^+COO^-(cry) + 3OH^-(aq) \rightarrow {}^{2-}OPhCHNH_2COO^-(aq) + 3H_2O(l) \tag{8}$$

Thus, for tyrosine, $\Delta H_5^0 - (\Delta H_3^0 + 2\Delta H_6^0)$ is the sum of the second and third proton dissociation processes.

As the free energy values of the second (ΔG_2^0) and third (ΔG_3^0) proton dissociation processes of tyrosine are available in the literature [6,7], it is possible, assuming a linear relationship between enthalpy values and free energy values, to insert these values and our values into the equations $\Delta G_2^0/\Delta G_3^0 = x/y$ and $x + y = C$, where x and y are our corresponding enthalpy values and C is their sum. In this way, the enthalpy values related to the amino group (ΔH_2^{0*}) and to the hydroxyl group (ΔH_3^{0*}), can be derived. The enthalpy value related to the amino group of the 3,4-(OH)$_2$ derivative is calculated using the relationship $\Delta H_5^0 - (\Delta H_3 + 3\Delta H_6^0)$ from which the $2\Delta H_3^{0*}$ value is subtracted.

RESULTS AND DISCUSSION

The enthalpies of proton dissociation of the carboxylate and amino groups in *d,l*-phenylalanine, the fluoro derivatives, tyrosine (4-hydroxy derivative) and the 3,4-dihydroxy derivative are reported in Table 1. The enthalpies of solution and protonation of the same compounds are reported in Table 2.

The differences in the enthalpies of the proton dissociation processes for the different compounds can usually be explained by means of the differences in the enthalpies of solvation of the respective ions and undissociated molecules [1,2]. For this purpose, the differences in the enthalpies of solvation of processes (3) ($\delta\Delta H_3^0$), (4) ($\delta\Delta H_4^0$) and (5) ($\delta\Delta H_5^0$) of the

TABLE 1

Enthalpies of the first (ΔH_1^0) and second (ΔH_2^0) proton dissociation processes of phenylalanine derivatives at 25 ° C in water

Compound	ΔH_1^0 (kJ mol^{-1})	ΔH_2^0 (kJ mol^{-1})
d,l-Phe	0.14	10.89
o-F	0.87	10.48
m-F	0.18	11.29
p-F	−0.35	11.81
4-OH	−0.29	8.30
3,4-(OH)$_2$	0.47	14.15

TABLE 2

Enthalpies of solution (ΔH_3^0), protonation (ΔH_4^0) and neutralization (ΔH_5^0) for phenylalanine derivatives in water at 25 ° C

Compound	ΔH_3^0	ΔH_4^0	ΔH_5^0
d,l-Phe	1.84	1.70	−0.61
o-F	2.79	1.92	−0.07
m-F	1.79	1.61	−0.27
p-F	1.87	2.22	0.34
4-OH	4.77	5.06	−4.34
3,4-(OH)$_2$	4.70	4.23	−2.73

TABLE 3

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

Compound	$\delta\Delta H_1^0$	$\delta\Delta H_2^0$	$\delta\Delta H_3^0$	$\delta\Delta H_4^0$	$\delta\Delta H_5^0$
d,l-Phe	0.0	0.0	0.0	0.0	0.0
o-F	0.73	−0.41	0.95	0.22	0.54
m-F	0.04	0.40	−0.05	−0.09	0.34
p-F	−0.49	0.92	0.03	0.52	0.95
4-OH	−0.43	−2.59	2.93	3.36	−3.73
3,4-$(OH)_2$	0.33	3.26	2.86	2.53	−2.12

various fluoro and hydroxyl derivatives with respect to phenylalanine (chosen as the reference compound) can be identified with the differences in solvation of the zwitterions $XPhCHNH_3^+COO^-$, the undissociated molecules $XPhCHNH_3^+COOH$ and the $XPhCHNH_2COO^-$ ions of the compounds in water. These values, together with the differences in the first and second ionization processes ($\delta\Delta H_1$ and $\delta\Delta H_2$), are given in Table 3.

It is worth noting that the dissociation value of *d,l*-phenylalanine differs from that previously found [1,2] by virtue of a different value of the protonation process. This is probably caused by the use of a different method of calculation for the determination of the heats of reaction. Calculation methods and computer programs (in BASIC) for the determination of heats of solution and reaction in isothermal and quasi-adiabatic calorimeters have been the subject of recent papers [9–11]. In the constant-environment calorimeter method, the end-point for a very "slow" reaction is hardly detected [9,10]. Indeed, for the longest points, the mathematical errors introduced by the temperature correction term are larger than the corresponding calculated heats [9]. Furthermore, in the long run the thermal equilibrium condition would be changed so that the condition $\Delta T_{corr} = -K/C \int_{T_\infty}^{t}(T - T_\infty)\,dt$ (where ΔT_{corr} is a correction temperature term, K is the calorimeter leakage constant and C is the heat capacity of the system) would no longer be effective [9]. For the adiabatic calorimeter, this does not occur; this is because the larger temperature variation due to a smaller K/C value supplies a greater sensitivity to the enthalpy readings [11]. Therefore it can be inferred that the different values found for the phenylalanine protonation may be caused by the "slowness" of the reaction and by the different type of calorimeter used. The adiabatic value seems to be the most reliable. The protonation and solution values of tyrosine are equal for the different techniques and calorimeters.

As regards the order of the first proton dissociation (*p*-F > *d,l*-Phe > *m*-F > *o*-F), it can be observed that the *ortho* and *meta* fluoro derivatives behave as weaker acids than phenylalanine, whereas the *p*-fluoro derivative behaves as a stronger acid. This behaviour can be explained by means of the differences in solvation enthalpy of the zwitterions $XPhCHNH_3^+COO^-$ and the undissociated molecules $XPhCHNH_3COOH$ (with respect to the corresponding forms of phenylalanine). The orders of solvation of the undissociated molecules and the zwitterions are *m*-F > *d*,*l*-Phe > *o*-F > *p*-F and *m*-F > *d*,*l*-Phe > *p*-F > *o*-F respectively. For the *meta* and *ortho* derivatives the solvation processes of the undissociated molecules prevail, whereas for the *para* derivative the solvation process of the zwitterion predominates. 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 desolvation of the undissociated molecule.

As regards the order of the second proton dissociation process (*o*-F > *d*,*l*-Phe > *m*-F > *p*-F) it can be observed that the places of the *ortho* and *para* derivatives are exchanged with respect to the first proton dissociation process. The influence of the fluorine atom on the second proton dissociation process was studied by considering the solvation of the zwitterions and the $XPhCHNH_2COO^-$ ions. For the latter the order is *d*,*l*-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 form; the places of the *meta* and *para* derivatives are related to the desolvation processes of the $XPhCHNH_2COO^-$ forms.

The orders of the first and second proton dissociation processes for the hydroxyl derivatives are both 4-OH > *d*,*l*-Phe > 3,4-$(OH)_2$. The order of solvation of the undissociated molecules $XPhCHNH_3^+COOH$, the zwitterion forms $XPhCHNH_3^+COO^-$ and the ionic forms $XPhCHNH_2COO^-$ are *d*,*l*-Phe > 3,4-$(OH)_2$ > 4-OH, *d*,*l*-Phe > 4-OH > 3,4-$(OH)_2$ and 4-OH > 3,4-$(OH)_2$ > *d*,*l*-Phe respectively. The large values of the latter (see Table 3) show how the solvation of the ionic forms prevails in the second ionization process.

The orders of the first and second ionization processes for all the compounds are *p*-F > 4-OH > *m*-F > 3,4-$(OH)_2$ > *o*-F and 4-OH > *o*-F >

TABLE 4

Enthalpies of process (9) for phenylalanine derivatives at 25 °C in water

Compound	ΔH_9^0 (kcal mol^{-1})
d,*l*-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

m-F > *p*-F > 3,4-$(OH)_2$ respectively. The greater effect of the hydroxyl group on the dissociation of the amino group is clearly due to the large solvation of the $XPhCHNH_2COO^-$ form.

Finally, it is possible to calculate the total process ($K_1 + K_3$) expressed by the equation

$$XPhCHNH_3^+COOH(aq) \rightarrow XPhCHNH_2COO^-(aq) + 2H^+(aq) \qquad (9)$$

obtained by summing eqns. (1) and (2). The ΔH_9^0 values are reported in Table 4. The order of the total proton dissociation is 4-OH > *d*,*l*-Phe > *o*-F = *m*-F = *p*-F > 3,4-$(OH)_2$. The hydroxyl group greatly favours the total dissociation process (with respect to that of phenylalanine), while the effect of the fluorine atom seems to be negligible.

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