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THERMODYNAMICS OF DIPEPTIDES. PART II. INFLUENCE OF METHYL GROUPS ON THE α FREE CARBOXYL AND AMINO GROUPS. STUDY OF A SYMMETRICAL SYSTEM

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ABSTRACT

The effect of a methyl group upon the structures of different dipeptides was studied. The methyl group is the key factor in the proton dissociation processes of carboxyl and α-amino free groups in a system of two couples of dipeptides. Each couple shows a common term in dipeptide structures. A study of a symmetrical system (valyl–tyrosine and tyrosyl–valine couple) is also presented. The dissociation-process values of three functional groups (free carboxyl, the amino groups of dipeptides and the hydroxyl group of tyrosine) are equal in different dipeptides by virtue of the symmetry of the systems.

INTRODUCTION

Dipeptides which are compounds made up of two amino acids are the smallest parts of proteic chains. For this reason dipeptides allow the reciprocal influences of the structures of different amino acids to be studied easily.

In our laboratory, a calorimetric study on seven dipeptides (valyl–valine, valyl–leucine, valyl–proline, valyl–tryptophan, valyl–serine, valyl–tyrosine and valyl–lysine) has been carried out [1]. It is noteworthy that the components of these dipeptides are 'standard' α-amino acids and the first term of all the compounds of this series is valine. The effect of the structure of valine upon the other α-amino acids and the effects of the other structures upon valine were studied using valyl–valine as the reference compound [1].

The aim of the present work was to study the influence of a methyl substituent group upon some structures of 'standard' α-amino acids. For this purpose the following series of dipeptides was chosen: alanyl–threonine

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(Ala–Thr), glycyl–threonine (Gly–Thr), seryl–alanine (Ser–Ala) and seryl–glycine (Ser–Gly). This series can be subdivided into two pairs of dipeptides, the features of which support the following remarks.

Each couple shows a common term in the dipeptide structures: the former shows threonine as a common second term, and the latter serine as a common first term. The other α-amino acids in the dipeptide structures are α-alanine and glycine. These two compounds, which are the simplest 'standard' α-amino acids, differ only by one methyl group, to which can be related all the differences found in the reciprocal influence among the same and different structures. Indeed, in this system of couples there are the influences of a same structure (serine of threonine) upon the proton dissociation of the α free carboxyl group of α-alanine and of glycine and upon the α free amino group of the same α-amino acids. Furthermore, the influence of glycine and α-alanine upon the proton dissociation of the carboxyl group of threonine and of the amino group of serine can be observed. Finally, a third couple (Tyr–Val and Val–Tyr) is presented. This couple is made up of two α-amino acids which change their positions in the dipeptides.

The influence of the symmetric structures upon the proton dissociation processes of free carboxyl and free aminic groups can be studied in this second system of dipeptides.

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 described previously [2–7].

The proton ionization of the free α-carboxyl group and of free α-amino group of a generic dipeptide can be represented as

$$\mathrm{NH_3^+\text{-}CHRCONHCHR'\text{-}COOH(aq)} = \mathrm{NH_3^+\text{-}CHRCONHCHR'\text{-}COO^-(aq) + H^+(aq)} \tag{1}$$

and

$$\mathrm{NH_3^+\text{-}CHRCONHCHR'\text{-}COO^-(aq)} = \mathrm{NH_2\text{-}CHRCONHCHR'\text{-}COO^-(aq) + H^+(aq)} \tag{2}$$

For compounds bearing a third proton in a functional group R′H (valyl–tyrosine and tyrosyl–valine) there are the processes

$$\mathrm{NH_2\text{-}CHRCONHCHR'H\text{-}COO^-(aq)} = \mathrm{NH_2\text{-}CHRCONHCHR'^{-}COO^-(aq) + H^+(aq)} \tag{3a}$$

and

$$\mathrm{NH_2\text{-}CHR'HCONHCHR\text{-}COO^-(aq)} = \mathrm{NH_2\text{-}CHR'^{-}\text{-}CONHCHR\text{-}COO^-(aq) + H^+(aq)} \tag{3b}$$

The partial molar enthalpy of dissociation $\Delta\overline{H}_1$ for the free α-carboxyl group in water is obtained by measuring: (i) the partial molar enthalpy of solution $\Delta\overline{H}_4$ of crystalline (cry) NH_3^+-CHRCONHCHR′-COO^- in zwitterion form in water at a pH close to the isoelectric one

$$NH_3^+\text{-CHRCONHCHR}'\text{-COO}^-(\text{cry}) = NH_3^+\text{-CHRCONHR}'\text{-COO}^-(\text{aq}) \tag{4}$$

and (ii) the partial molar enthalpy of protonation $\Delta\overline{H}_5$ of the same compound in water at pH 0.0

$$NH_3^+\text{-CHRCONHCHR}'\text{-COO}^-(\text{cry}) + H^+(\text{aq}) = NH_3^+\text{-CHRCONHCHR}'\text{-COOH(aq)} \tag{5}$$

The partial molar enthalpy of process (1) can be obtained by subtracting $\Delta\overline{H}_5$ from $\Delta\overline{H}_4$. Concentrations of about 10^{-3} mmol were used in processes (4) and (5). Therefore, $\Delta\overline{H}$ values can be considered at infinite dilution, ΔH^0 [8]. These values refer to the proton dissociation of 1 mol of NH_3^+-CHRCONHCHR′-COOH at infinite dilution in 1000 g of water, yielding 1 mol of NH_3^+-CHRCONHR′-COO^- ions and 1 mol of protons solvated in the same amount of water.

The dissociation processes in water, for a compound containing carboxyl and amino groups, are complicated by tautomeric equilibrium and zwitterion formation [9,10]. While a generic dipeptide in acid solution can be represented by the form NH_3^+-CHRCONHCHR′-COOH in the solution approaching pH 7.00, the principal species are neutral molecules which may be in either the NH_2-CHRCONHCHR′-COOH form or the zwitterion form. Therefore, only the NH_3^+-CHRCONHCHR′-COOH form is present in process (5) at pH 0.0, while in process (4) this is not true. The isoelectric pH values for some of the compounds examined can be calculated by means of the dissociation constants [11,12]. If this is not possible, it can be noted that the isoelectric values of dipeptides are close to those of the corresponding free α-amino acids by virtue of the small differences in pK_a of their carboxyl and amino groups. It can therefore be assumed that in this solution the zwitterion form is predominant. In this way the carboxyl proton dissociation enthalpy values were calculated.

The partial molar enthalpy of the second proton dissociation process of NH_3^+-CHRCONHCHR′-COO^- was obtained by measuring the partial molar enthalpy $\Delta\overline{H}_6$ of neutralization of the crystalline compound in water at pH 14.00

$$NH_3^+\text{-CHRCONHCHR}'\text{-COO}^-(\text{cry}) + OH^-(\text{aq}) = NH_2\text{-CHRCONHCHR}'\text{-COO}^-(\text{aq}) + H_2O(\text{aq}) \tag{6}$$

If the solution process enthalpy values $\Delta\overline{H}_4$ and the $\Delta\overline{H}_7$ partial molar value [13] in water that are related to the process

$$H^+(\text{aq}) + OH^+(\text{aq}) = H_2O(\text{l}) \tag{7}$$

are subtracted from the $\Delta\overline{H}_6$ values, then the relation $\Delta\overline{H}_6 - (\Delta\overline{H}_4 + \Delta\overline{H}_7)$ supplies the enthalpy of process (2).

The $\Delta\overline{H}$ values for this process too can be considered equal to ΔH^0. These values refer to the dissociation process of 1 mol of NH_3^+-CHRCONHCHR′-COO$^-$ at infinite dilution in 1000 g of water, yielding 1 mol of NH_2-CHRCONHCHR′-COO$^-$ and 1 mol of protons solvated in the same amount of water. It has been noted that process (5) is carried out at pH 14.00, so that only the NH_2-CHRCONHCHR′-COO$^-$ form is present.

Finally, for the compounds bearing a third proton in a functional hydroxyl group (valyl–tyrosine and tyrosyl–valine), process (6) must be written as

$$\begin{aligned}&\mathrm{NH_3^+\text{-}CHRCONHCHR'H\text{-}COO^-(cry) + 2OH^-(aq)}\\&\qquad = \mathrm{NH_2\text{-}CHRCONHR'^-\text{-}COO^-(aq) + 2H_2O(l)}\end{aligned} \tag{8a}$$

and

$$\begin{aligned}&\mathrm{NH_3^+\text{-}CHR'HCONHCHRCOO^-(cry) + 2OH^-(aq)}\\&\qquad = \mathrm{NH_2\text{-}CHR'^-\text{-}CONHCHR\text{-}COO^-(aq) + 2H_2O(l)}\end{aligned} \tag{8b}$$

Therefore, for these compounds the $\Delta\overline{H}_6 - (\Delta\overline{H}_4 + 2\Delta\overline{H}_7)$ values refer to the sum of the proton dissociation processes for the NH_3^+ group of valine (or tyrosine) and of the OH group of tyrosine.

As the ionization enthalpy values of the free valine NH_3^+ group, of the free tyrosine NH_3^+ group and of the free tyrosine OH group are available in the literature [6,14], it was possible to use these values and our experimental values in the equations $\Delta H_2^\star/\Delta H_3^\star = x/y$ and $x + y = C$ where $\Delta H_2^\star$ and $\Delta H_3^\star$ are the literature values of the free valine NH_3^+ group (or free tyrosine NH_3^+ group) and the free tyrosine OH group, respectively, and x and y are the corresponding enthalpy values for the dipeptides.

RESULTS AND DISCUSSION

The enthalpy values of solution (ΔH_4^0) of protonation (ΔH_5^0) and of neutralization (ΔH_6^0) of the compounds studied are reported in Table 1. The ionization enthalpy values of the free carboxyl groups (ΔH_1^0), of the free amino groups (ΔH_2^0) of the dipeptides and of the OH functional group (ΔH_3^0) of the dipeptides are reported in Table 2. A technique used previously [1] to study the influence of the structure upon free α carboxyl and free α amino groups of dipeptides can be described by the following equations

$$\Delta H^0_{1(\mathrm{a-b})} - \Delta H^0_{1(\mathrm{b})}/\Delta H^0_{1(\mathrm{b})} \tag{9a}$$

$$\Delta H^0_{2(\mathrm{a-b})} - \Delta H^0_{2(\mathrm{a})}/\Delta H^0_{2(\mathrm{a})} \tag{9b}$$

$$\Delta H^0_{3(\mathrm{a-b})} - \Delta H^0_3/\Delta H^0_3 \tag{9c}$$

TABLE 1

The enthalpy values of processes (4), (5) and (6) (kcal mol^{-1}) for some dipeptides in water at 25 °C

Dipeptide	ΔH_4^0	ΔH_5^0	ΔH_6^0
Ala–Thr	−0.88	−1.36	−3.96
Gly–Thr	4.88	4.67	2.22
Ser–Ala	3.44	3.91	−1.02
Ser–Gly	3.97	3.65	−1.21
Tyr–Val	−1.79	−1.51	−12.15
Val–Tyr	0.64	0.07	−9.85

TABLE 2

The enthalpy values of processes (1), (2) and (3) (kcal mol^{-1}) for some dipeptides in water at 25 °C

Dipeptide	ΔH_1^0	ΔH_2^0	ΔH_3^0
Ala–Thr	0.48	10.29	
Gly–Thr	0.21	10.73	
Ser–Ala	−0.46	8.90	
Ser–Gly	0.31	8.20	
Tyr–Val	−0.28	7.76	8.62
Val–Tyr	0.57	7.69	8.82

where $\Delta H^0_{1(a-b)}$ is the ionization process value of the free α carboxyl group of a generic dipeptide (a–b), $\Delta H^0_{1(b)}$ is the value of the dissociation process of the carboxyl group of the free α-amino acid b, $\Delta H^0_{2(a-b)}$ is the ionization process value of the free amino group of the dipeptide a–b and $\Delta H^0_{2(a)}$ is the dissociation process value of the amino group of the free α-amino acid a. The $\Delta H^0_{3(a-b)}$ values are the dissociation process values of the OH group in the dipeptides, and ΔH_3^0 is the dissociation enthalpy value of OH in free tyrosine. The values obtained using eqns. (9a)–(9c) are reported in Table 3.

TABLE 3

The enthalpy values (ΔH^0, kcal mol^{-1}) obtained by using eqns. (9a)–(9c)

	Equation		
	(9a)	(9b)	(9c)
Ala–Thr	0.31	−0.07	
Gly–Thr	−0.44	0.01	
Ser–Ala	1.80	−0.08	
Ser–Gly	−0.86	−0.16	
Tyr–Val	−2.65	−0.07	−0.07
Val–Tyr	−2.98	−0.05	−0.04

It can be observed from the data in Table 3 that for the couple Ala–Thr and Gly–Thr the influence of α-alanine upon the dipeptide structure hinders the dissociation of the free carboxyl group with respect to that of free threonine, whilst glycine favours the dissociation. This fact can be explained by the electron donor effect of the α-alanine methyl group which hinders the proton loss of the carboxyl group. Indeed, in Gly–Thr the electron attractive effect of the NH_3^+ group of glycine activates the dissociation process of the free α-carboxyl group. The electron-donor effect of the α-alanine methyl group towards the NH_3^+ group prevents dissociation from occurring in the Ala–Thr dipeptide. Threonine favours the free α-amino group dissociation in the Ala–Thr compound, while it hinders the same process in the Gly–Thr dipeptide: the negative charge of the carboxylate group (COO^-) stabilizes the proton of NH_3^+ in the latter compound, while in the former this is hindered by the methyl steric effect.

The serine structure in the second couple (Ser–Ala and Ser–Gly) hinders the ionization process of the free α-carboxyl group in the seryl–alanine dipeptide, while it favours the process in Ser–Gly.

The OH (σ-electron attractive effect) and NH_3^+ groups of serine favour the dissociation process of the carboxyl group in the latter compound, while this effect is hindered by the CH_3 group (electron-donor effect) in the former. Both α-alanine and glycine favour the proton dissociation process of the free α-amino group (in the Ser–Ala and Ser–Gly system of pairs) with respect to the dissociation process of the same group in free serine. This behaviour could be due to the electron attractive effect of the OH group of serine, which reduces the stabilization of the NH_3^+ positive charge by the carboxylate group (COO^-). The larger dissociation of the free amino group of Ser–Gly with respect to that of Ser–Ala can be related to the electron-donor effect of the methyl group of alanine. It can be concluded that the methyl group effect is the key factor in the reciprocal influence of the different dipeptide structures.

Finally, the present experimental evidence for the couple Val–Tyr and Tyr–Val allows the following remarks to be made. In this system of dipeptides there are three dissociation processes due to the free carboxyl and amino groups of tyrosine and valine and to the tyrosine hydroxyl group. All these processes are favoured with respect to the same processes in free α-amino acids. The values of the same functional group in different dipeptides are very close and this is obviously related to symmetry of the system.

REFERENCES

1 F. Rodante and F. Fantauzzi, Thermochim. Acta, in press.
2 F. Rodante and M. Tocci, Thermochim. Acta, 86 (1985) 109.

3 F. Rodante and F. Fantauzzi, Thermochim. Acta, 111 (1987) 233.
4 F. Rodante, F. Fantauzzi and P. Di Girolamo, Thermochim. Acta, 142 (1989) 203.
5 F. Rodante, F. Fantauzzi and G. Marrosu, Thermochim. Acta, 141 (1989) 297.
6 F. Rodante and F. Fantauzzi, Thermochim. Acta, 144 (1989) 75.
7 F. Rodante and F. Fantauzzi, Thermochim. Acta, 144 (1989) 275.
8 J. Christensen, D.E. Smith, D. Slade and R.M. Izatt, Thermochim. Acta, 4 (1971) 17.
9 J.C. Halle, R. Schall and A. Di Nallo, Anal. Chim. Acta, 60 (1972) 197.
10 D.P. Wrathall, R.M. Izatt and J. Christensen, J. Am. Chem. Soc., 86 (1964) 4779.
11 A.C. Lehninger, Biochimica, Nicola Zanichelli, Bologna, 2nd edn., 1979.
12 A.A. Sober, Handbook of Biochemistry, Chemical Rubber Co., Cleveland, OH, 1970.
13 F. Rodante, F. Rallo and P. Fiordiponti, Thermochim. Acta, 6 (1973) 374.
14 F. Rodante, Thermochim. Acta, 149 (1989) 157.