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THERMODYNAMICS OF DIPEPTIDES IN WATER. I. CALORIMETRIC STUDY OF THE INFLUENCE OF α-AMINO ACID STRUCTURES ON THE FREE α-AMINIC AND FREE α-CARBOXYL GROUPS

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ABSTRACT

A calorimetric study has been made of the dissociation processes in water of the free α-aminic groups of the amino terminal residuum and the free α-carboxyl groups of the carboxy terminal residuum of various dipeptides.

The influence of the structure of valine, which was one of the components of each of the dipeptides, upon a number of other α-amino acids as second component, and the influence of these other α-amino acids upon the structure of valine were investigated using valil–valine and the individual free α-amino acids as reference structures.

INTRODUCTION

Much work concerning the thermodynamics of the "standard" α-amino acids in water has been carried out in the last 50 years [1–15]. In our own laboratory, 23 α-amino acids (19 "standard" and four derivatives) have been the subject of an extended thermodynamic study [16–22]. The thermodynamics of dipeptides (compounds made up of two α-amino acids) are of interest because these compounds represent the smallest units of proteic chains.

The present work is a calorimetric study of the enthalpy values of proton dissociation processes, for various dipeptides, of the free α-aminic group belonging to the amino terminal residuum (N-terminal) and the free α-carboxyl group belonging to the carboxy terminal residuum (C-terminal). These values are subsequently compared with those of proton dissociation processes related to the corresponding groups of the free α-amino acids.

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The dipeptides studied (each comprising two standard α-amino acids) were: valil–valine (Val–Val), valil–leucine (Val–Leu), valil–proline (Val–Pro), valil–tryptophan (Val–Trp), valil–serine (Val–Ser), (valil–tyrosine (Val–Tyr) and valil–lysine (Val–Lys).

Using valil–valine as a reference compound, it was possible to observe the way in which the proton dissociation value for the free α-aminic group in a dipeptide varies as a function of the structure of the second component. The variation of the proton dissociation values for the free α-carboxyl groups of the same compounds was also studied as a function of valine structure, again using valil–valine as a reference structure.

Of the dipeptides studied, three had second components belonging to the first class of the standard α-amino acids (valil–leucine, valil–proline, valil–tryptophan), two had second components belonging to the second class (valil–serine, valil–tyrosine), and one had a second component belonging to the third class (valil–lysine) [22].

EXPERIMENTAL AND PROCEDURE

The compounds (Carlo Erba RPE Chemicals, used without purification) were weighed and handled in a nitrogen-filled dry-box. The calorimeter apparatus used has been described previously [16–22].

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

$$NH_3^+\text{–CHR–CO–NH–CHR′–COOH (aq)} = NH_3^+\text{–CHR–CO–NH–CHR′–}COO^-\text{(aq)} + H^+\text{(aq)} \quad (1)$$

and

$$NH_3^+\text{–CHR–CO–NH–CHR′–}COO^-\text{(aq)} = NH_2\text{–CHR–CO–NH–CHR′–}COO^-\text{(aq)} + H^+\text{(aq)} \quad (2)$$

The partial molar enthalpy of dissociation $\Delta\overline{H}_1$ for the free carboxyl in water is obtained by measuring the following quantities.

(a) The partial molar enthalpy of solution $\Delta\overline{H}_3$ of the crystalline (cry) NH_3^+–CHR–CO–NH–CHR′–COO^- zwitterion form in water at a pH close to the isoelectric value

$$NH_3^+\text{–CHR–CO–NH–CHR′–}COO^-\text{(cry)} = NH_3^+\text{–CHR–CO–NH–CHR′–}COO^-\text{(aq)} \quad (3)$$

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

$$NH_3^+\text{–CHR–CO–NH–CHR′–}COO^-\text{(cry)} + H^+\text{(aq)} = NH_3^+\text{–CHR–CO–NH–CHR′–COOH(aq)} \quad (4)$$

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 (molal) were used in processes (3) and (4), so the $\Delta\overline{H}$ values can be considered as being at infinite dilution ΔH^0 [16–22]. These values refer to the proton dissociation of one mole of NH_3^+–CHR–CO–NH–CHR′–COOH at infinite dilution in 1000 g of water, yielding one mole of NH_3^+–CHR–CO–NH–CHR′–COO^- ions and one mole of protons solvated in the same amount of water.

For a compound containing carboxyl and aminic groups, the dissociation processes in water are complicated by tautomeric equilibrium and zwitterion formation [16–22]. While a generic dipeptide in acid solution can be represented by the form NH_3^+–CHR–CO–NH–CHR′–COOH, in a solution approaching pH 7.00 the principal species are neutral molecules, which may be of either the NH_2–CHR–CO–NH–CHR′–COOH or the zwitterion form. Thus, only the NH_3^+–CHR–CO–NH–CHR′–COOH form is present in eqn. (4) at pH 0.0, while in eqn. (3) this is not the case. The isoelectric pH values for some of the compounds examined can be calculated by means of the dissociation constants [16–22]. If this is not possible, it can be noted that 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 aminic groups. It can therefore be assumed that in this solution the zwitterion form is predominant. In this way, the carboxyl proton dissociation enthalpy values can be calculated.

The partial molar enthalpy of the second proton dissociation process of NH_3^+–CHR–CO–NH–CHR′–COO^- is obtained by measuring the partial molar enthalpy $\Delta\overline{H}_5$ of the neutralization of the crystalline compound in water at pH 14.

$$NH_3^+\text{–CHR–CO–NH–CHR′–}COO^-\text{(cry)} + OH^-(aq) = NH_2\text{CHR–CO–NH–CHR′–}COO^-(aq) + H_2O\ (l) \quad (5)$$

If the solution process enthalpy value $\Delta\overline{H}_3$ and the $\Delta\overline{H}_6$ partial molar value in water related to the process

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

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

The $\Delta\overline{H}$ values for this process can also be considered as being equal to ΔH^0. These values refer to the dissociation process of one mole of NH_3^+–CHR–CO–NH–CHR′–COO^- at infinite dilution in 1000 g of water, yielding one mole of NH_2–CHR–CO–NH–CHR′–COO^- and one mole of protons solvated in the same amount of water. It has been noted that process (4) occurs at pH = 14.00, so that only the NH_2–CHR–CO–NH–CHR′–COO^- form is present.

Finally, for compounds bearing a third proton in a functional group RH (e.g. valil–tyrosine) or in another NH_3^+ group (e.g. valil–lysine), process (5)

must be written as

$$NH_3^+\text{–CHR–CO–NH–CHR'H–COO}^-\text{(cry)} + 2OH^-\text{(aq)}$$
$$= NH_2\text{–CHR–CO–NH–CHR'}^-\text{–COO}^-\text{(aq)} + 2H_2O\ \text{(l)}$$

and

$$NH_3^+\text{–CHR–CO–NH–CHR'}NH_3^+\text{–COO}^-\text{(cry)} + 2OH^-\text{(aq)}$$
$$= NH_2\text{–CHR–CO–NH–CHR'}NH_2\text{–COO}^-\text{(aq)} + 2H_2O\ \text{(l)}$$

Thus, for these compounds $\Delta\overline{H}_5 - (\Delta\overline{H}_3 + 2\Delta\overline{H}_6)$ values refer to the sum of the proton dissociation processes for the NH_3^+ group of the valine and the RH group (or the other NH_3^+ group).

As the ionization enthalpy values of the free valine NH_3^+ group, the free tyrosine RH group, and the other NH_3^+ group of free lysine are available in the literature [22], it is possible to put these values and our experimental enthalpy values into the equations

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

where $\Delta H_2^\star$ and $\Delta H_3^\star$ are the literature values of the valine NH_3^+ group and the RH (or NH_3^+) group, respectively, x and y are the corresponding enthalpy values in the dipeptides, and C is their sum.

RESULTS AND DISCUSSION

The enthalpic values of solution ΔH_3^0, protonation ΔH_4^0 and neutralization ΔH_5^0 of all the compounds cited above are reported in Table 1. This table also gives ionization enthalpy values for the free carboxyl groups (ΔH_1^0) and the free α-aminic groups (ΔH_2^0) of the dipeptides. Valil–valine, which has a symmetrical structure, is the reference compound.

The quantities $\delta\Delta H_1^0 = \Delta H_{1(\text{Val–Sub})}^0 - \Delta H_{1(\text{Val–Val})}^0$ and $\delta\Delta H_2^0 = \Delta H_{2(\text{Val–Sub})}^0 - \Delta H_{2(\text{Val–Val})}^0$ are reported in Table 2. $\Delta H_{1(\text{Val–Sub})}^0$ and

TABLE 1

Enthalpy values of processes (1), (2), (3), (4) and (5) (kcal mol^{-1}) for some dipeptides in water at 25 °C

Dipeptide	ΔH_1^0	ΔH_2^0	ΔH_3^0	ΔH_4^0	ΔH_5^0
Val–Val	−0.43	11.39	−3.22	−2.79	−5.20
Val–Leu	−0.38	10.97	−5.81	−5.43	−8.21
Val–Ser	0.35	13.19	−2.51	−2.86	−2.69
Val–Pro	−0.82	−1.27	−0.54	0.28	−15.19
Val–Trp	−0.58	12.15	3.97	4.55	2.75
Val–Lys	0.17	9.37	0.05	−0.12	−2.22
Val–Tyr	0.57	7.69	0.64	0.07	−9.85

TABLE 2

Differences in enthalpy values (kcal mol^{-1}) of processes (1), (2), (3), (4) and (5) for some dipeptides with respect to the same processes for valil–valine

Dipeptide	$\delta\Delta H_1^0$	$\delta\Delta H_2^0$	$\delta\Delta H_3^0$	$\delta\Delta H_4^0$	$\delta\Delta H_5^0$
Val–Val	0	0	0	0	0
Val–Leu	0.05	−0.42	−2.60	−2.63	−3.01
Val–Pro	−0.39	−12.66	2.67	3.06	−9.99
Val–Trp	−0.15	0.76	7.19	7.34	7.97
Val–Ser	0.78	1.80	0.71	−0.07	2.51
Val–Tyr	1.00	−3.70	3.86	2.86	−4.66
Val–Lys	0.60	−2.01	3.27	2.67	2.98

$\Delta H^0_{1(\text{Val-Val})}$ are the first ionization processes for dipeptides and for valil–valine, whereas $\Delta H^0_{2(\text{Sub-Val})}$ and $\Delta H^0_{2(\text{Val-Val})}$ are the values of the second ionization process for the same compounds.

Thus, the dissociation processes of the carboxyl and aminic groups of the various dipeptides were compared with the dissociation processes of the corresponding groups of valil–valine. The scale for dissociation of the carboxyl group is: valil–proline > valil–tryptophan > valil–valine > valil–leucine > valil–lysine > valil–serine > valil–tyrosine. This sequence can be explained by considering the scales for solvation of undissociated molecules $\delta\Delta H_4^0 = \Delta H^0_{4(\text{Sub-Val})} - \Delta H^0_{4(\text{Val-Val})}$ and zwitterions $\delta\Delta H_3^0 = \Delta H^0_{3(\text{Sub-Val})} - \Delta H^0_{3(\text{Val-Val})}$ (Table 2). For undissociated molecules, the order of solvation is: valil–leucine > valil–serine > valil–valine > valil–lysine > valil–tyrosine > valil–proline > valil–tryptophan. For zwitterions, the order becomes valil–leucine > valil–valine > valil–serine > valil–proline > valil–lysine > valil–tyrosine > valil–tryptophan. The scale for dissociation of the aminic group can be written as: valil–proline > valil–tyrosine > valil–lysine > valil–leucine > valil–valine > valil–tryptophan > valil–serine. This can be explained by considering the solvation scales for the zwitterions and the anion forms NH_2–CHR–CO–NH–CH–R′–COO^-. This last solvation scale $\delta\Delta H_5^0 = \Delta H^0_{5(\text{Sub-Val})} - \Delta H^0_{5(\text{Val-Val})}$ shows the sequence: valil–proline > valil–tyrosine > valil–leucine > valil–valine > valil–serine > valil–lysine > valil–tryptophan.

It can be observed that, as regards the first ionization process, valil–proline and valil–tryptophan dissociate more easily than does valil–valine, by virtue of the greater solvation of zwitterions. For the remaining compounds, the solvation of the undissociated molecules prevails.

In the second process, valil–proline, valil–tyrosine, valil–lysine and valil–leucine are more dissociated than valil–valine. The solvation of the ionic form prevails with respect to that of the zwitterions. The scale for the first ionization process gives a measure of the effect of the valine structure upon the structures of other α-amino acids, whilst the scale for the second

TABLE 3

Values from eqns. (7a) and (7b)

Dipeptide	(7a)	(7b)
Val–Leu	−2.06 (206%)	0.361 (36.1%)
Val–Pro	−12.68 (1268%)	−1.16 (116%)
Val–Trp	−0.7 (70%)	0.51 (51%)
Val–Ser	0.09 (9%)	0.64 (64%)
Val–Tyr	2.98 (298%)	−0.05 (5%)
Val–Lys	4.80 (480%)	0.16 (16%)
Val–Val	−3.50 (350%)	0.410 (41%)

ionization process shows how different structures can affect the same structure (valine).

A further contribution to the comprehension of the influence of structure upon the free α-carboxyl and free α-aminic groups of dipeptides can be supplied from the equations

$$\Delta H^0_{1(\text{Val}-\text{Sub})} - \Delta H^0_{1(\text{Amin})} / | \Delta H^0_{1(\text{Amin})} | \tag{7a}$$

and

$$\Delta H^0_{2(\text{Val}-\text{Sub})} - \Delta H^0_{2(\text{Val})} / | \Delta H^0_{2(\text{Val})} | \tag{7b}$$

where $\Delta H^0_{1(\text{Val}-\text{Sub})}$ represents the ionization process values for the free α-carboxyl groups of various dipeptides, $\Delta H^0_{1(\text{Amin})}$ represents the values of the dissociation processes for the carboxyl groups of the corresponding free α-amino acids, $\Delta H^0_{2(\text{Val}-\text{Sub})}$ represents the values of dissociation for the free α-aminic groups of various dipeptides, and $\Delta H^0_{2(\text{Val})}$ represents the value for the dissociation process of the aminic group of free valine.

Values related to the effect of the structure of valine upon the free carboxyl groups of the dipeptides, and to the effects of the various structures upon the free α-aminic group of valine obtained using expressions (7a) and (7b) are given in Table 3.

It can be seen that in the first ionization process, the influence of valine (7a) upon the dipeptide structures favours the dissociation of the free α-carboxyl groups with respect to those of the corresponding free α-amino acids for proline, valine, leucine and tryptophan, and hinders the same process for the other compounds. There is a sharp separation between the compounds containing a second α-amino acid belonging to the first class and those containing a second α-amino acid belonging to the second and third classes. In the former, the carboxyl groups become more acid; in the latter, they become less acid.

The influence of the structures of different amino acids on that of valine was also studied for the second dissociation process. The proton dissociation of the free α-aminic group of valine is hindered, in the dipeptides, by the

structures of valine, leucine, tryptophan, serine and lysine (the aminic group becomes more basic). The first four values are close, indicating that the influences of the various structures are similar. Proline and tyrosine favour the proton dissociation of the aminic α group (the aminic group becomes less basic).

CONCLUSION

Our experimental evidence supports the conclusion that the mutual influence of valine and of other α-amino acid structures can be considered from two points of view. The effect of the structure of valine upon the other α-amino acids and the effects of the other structures upon valine can be considered using valil–valine as reference structure. The same effects can also be studied using the individual α-amino acids as reference structures.

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