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THERMODYNAMICS OF DIPEPTIDES IN WATER.
Part 3. Strengthening and weakening effects of different side chains on the free α-amino 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-amino group of the amino terminal residue and the free α-carboxyl group(s) of the carboxyl terminal residue in various dipeptides. The influence of the structure of glycine, which was one of the residues in each of the dipeptides, upon a number of other α-amino acids as second components, and the influence of these other α-amino acids upon the structure of glycine were investigated using glycyl glycine and individual free α-amino acids as reference structures. A comparison was made with the reciprocal influence of valine structure on some α-amino acids in a series of valyl dipeptides. Differences in behaviour were found, according to the different lengths of the side-chains of the two reference structures. The aliphatic chain of valine deactivates the carboxyl groups of most of the α-amino acids considered in a series of dipeptides, while the positive charge of the NH_3^+ group of glycine activates them in another series. The α-amino acids deactivate the free α-amino group of valine in the first series of dipeptides, although this does not happen for the amino group of glycine in the second series of dipeptides.

INTRODUCTION

Calorimetric study of the mutual structural influences of α-amino acids in dipeptides has been the subject of two previous papers [1,2]. In the first, the influence of the structure of valine, which was one component of each dipeptide, upon a number of other α-amino acids as the second component, and the reciprocal influence of these other α-amino acids upon the structure of valine, were investigated using valine–valine as a reference structure. Valine is a member of the first class of α-amino acids in our scheme because its side chain is an apolar and hydrophobic group [1].

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The aim of the present work was to study the reciprocal influence of structures in a series of dipeptides where the reference structure was glycine, the simplest α-amino acid. It belongs to the second class of α-amino acids because the side chain, a single hydrogen atom, cannot neutralize the charge of amino and carboxyl groups. The dipeptides studied were glycyl glycine (gly–gly), glycyl α-alanine (gly–ala), glycyl serine (gly–ser), glycyl aspartic acid (gly–asp), glycyl valine (gly–val) and glycyl threonine (gly–thr).

In this way a comparison of the effects of the influence of two different reference structures upon a number of α-amino acids was carried out.

EXPERIMENTAL

The compounds (Carlo Erba RPE, used without purification) were weighed and handled in a nitrogen-filled dry-box. The calorimetric apparatus used has been described previously [1,2]. The proton ionization of the free α-carboxyl group and free α-amino group of a generalized dipeptide can be represented as

$$\begin{aligned}&NH_3^+\text{–CHR–CO–NH–CHR}'\text{–COOH(aq)}\\&\quad\rightarrow NH_3^+\text{–CHR–CO–NH–CHR}'\text{–COO}^-\text{(aq)} + H^+\text{(aq)}\end{aligned}\qquad(1)$$

and

$$\begin{aligned}&NH_3^+\text{–CHR–CO–NH–CHR}'\text{–COO}^-\text{(aq)}\\&\quad\rightarrow NH_2\text{–CHR–CO–NH–CHR}'\text{–COO}^- + H^+\text{(aq)}\end{aligned}\qquad(2)$$

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^- zwitterionic form in water at a pH close to the isoelectric value

$$\begin{aligned}&NH_3^+\text{–CHR–CO–NH–CHR}'\text{–COO}^-\text{(cry)}\\&\quad\rightarrow NH_3^+\text{–CHR–CO–NH–CHR}'\text{–COO}^-\text{(aq)}\end{aligned}\qquad(3)$$

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

$$\begin{aligned}&NH_3^+\text{–CHR–CO–NH–CHR}'\text{–COO}^-\text{(cry)} + H^+\text{(aq)}\\&\quad\rightarrow NH_3^+\text{–CHR–CO–NH–CHR}'\text{–COOH(aq)}\end{aligned}\qquad(4)$$

The partial molar enthalpy of process can be obtained by subtracting $\Delta\overline{H}_4$ from $\Delta\overline{H}_3$. Concentrations of about 10^{-3} M were used in process 3 and 4, so the $\Delta\overline{H}$ values can be considered as being at infinite dilution, ΔH° [3]. 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 amino groups, the dissociation processes in water are complicated by a tautomeric equilibrium and by zwitterion formation [4,5].

While a generalized dipeptide in acidic solution can be represented by the form NH_3^+–CHR–CO–NH–CHR′–COOH, in an approximately neutral solution the principal species are neutral molecules, which may be either NH_2–CHR–CO–NH–CHR′–COOH or the zwitterionic form. Thus only the NH_3^+–CHR–CO–NH–CHR′–COOH form is present in eqn. 4 at pH 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 [6,7]. If this is not possible, it can be noted that the isoelectric points of dipeptides are close to those of the corresponding free α-amino acids [8], 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 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 crystalline compound in water at pH 14

$$\mathrm{NH_3^+{-}CHR{-}CO{-}NH{-}CHR'{-}COO^-(cry) + OH^-(aq)} \\ \rightarrow \mathrm{NH_2{-}CHR{-}CO{-}NH{-}CHR'{-}COO^-(aq) + H_2O(l)} \qquad (5)$$

If the enthalpy of solution value $\Delta\overline{H}_3$ and the partial molar value in water $\Delta\overline{H}_6$ [9], which is related to the process

$$\mathrm{H^+(aq) + OH^-(aq) \rightarrow H_2O(l)}, \qquad (6)$$

are subtracted from the $\Delta\overline{H}_5$ value, then the relation $\Delta\overline{H}_5 - (\Delta\overline{H}_3 + \Delta\overline{H}_6)$ supplies the enthalpy value of process 2. The enthalpy value for this process can be considered as being equal to $\Delta H°$. This value refers to the dissociation 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, so that only the NH_2–CHR–CO–NH–CHR′–COO^- form is present.

Finally, for glycine aspartic acid eqns. 3, 4 and 5 must be written as

$$\mathrm{NH_3^+{-}CH_2{-}CO{-}NH{-}CH{-}COO^-{-}CH_2{-}COOH(cry)} \\ \rightarrow \mathrm{NH_3^+{-}CH_2{-}CO{-}NH{-}CH{-}COO^-{-}CH_2{-}COO^-(aq) + H^+(aq)} \qquad (3')$$

$$\mathrm{NH_3^+{-}CH_2{-}CO{-}NH{-}CH{-}COO^-{-}CH_2{-}COOH(cry) + H^+(aq)} \\ \rightarrow \mathrm{NH_3^+{-}CH_2{-}CO{-}NH{-}CH{-}COOH{-}CH_2{-}COOH(aq)} \qquad (4')$$

$$\text{NH}_3^+\text{-CH}_2\text{-CO-NH-CH-COO}^-\text{-CH}_2\text{-COOH(cry)} + 20\text{H}^-\text{(aq)}$$
$$\rightarrow \text{NH}_2\text{-CH}_2\text{-CO-NH-CH-COO}^-\text{-CH}_2\text{-COO}^-\text{(aq)} + 2\text{H}_2\text{O(l)} \qquad (5')$$

so that ΔH_1° is related to the dissociation of both carboxyl groups:

$$\text{NH}_3^+\text{-CH}_2\text{-CO-NH-CH-COOH-CH}_2\text{-COOH(aq)}$$
$$\rightarrow \text{NH}_3^+\text{-CH}_2\text{-CO-NH-CH-COO}^-\text{-CH}_2\text{-COO}^-\text{(aq)} + 2\text{H}^+\text{(aq)}$$

and the ΔH_2° value refers to the sum of the proton dissociation processes for NH_3^+ and the second carboxyl group:

$$\text{NH}_3^+\text{-CH}_2\text{-CO-NH-CH}_2\text{-COO}^-\text{-CH}_2\text{-COOH(aq)}$$
$$\rightarrow \text{NH}_2\text{-CH}_2\text{-CO-NH-CH}_2\text{-COO}^-\text{-COO}^-\text{(aq)} + 2\text{H}^+\text{(aq)}$$

As the ionization enthalpy values of the two carboxyl groups of aspartic acid are available in the literature [10], it is possible to put these values and our experimental enthalpy values into the equations $\Delta H_1^* / \Delta H_2^* = x/y$ and $x + y = C$ where ΔH_1^* and ΔH_2^* are the literature values for the enthalpy of ionization of the first and second carboxyl groups of aspartic acid, x and y the corresponding enthalpy values in the dipeptides, and C their sum, in process 1.

As regards process 2, one can write $\Delta H_{2'}^\circ + \Delta H_2^\circ = C_1$, where $\Delta H_{2'}^\circ$ is the enthalpy value (previously found) for the second carboxyl group, ΔH_2° the enthalpy value of NH_3^+ dissociation, and C_1 their sum.

RESULTS AND DISCUSSION

The enthalpy values of solution (ΔH_3°), protonation (ΔH_4°) and neutralization (ΔH_5°) of all compounds are reported in Table 1. The table also gives ionization enthalpy values for the free carboxyl groups (ΔH_1° and the free α-amino group (ΔH_2°) of the dipeptides. Glycyl glycine, which has a symmetrical structure, is the reference compound. The quantities $\delta\Delta H_1^\circ =$

TABLE 1

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

Dipeptides	ΔH_1°	ΔH_2°	ΔH_3°	ΔH_4°	ΔH_5°
gly–gly	0.05	10.26	2.81	2.76	−0.26
gly–ala	−0.49	10.77	−1.35	−0.88	−3.91
gly–ser	0.19	9.31	2.90	2.71	−1.12
gly–asp	0.46 [a]	11.53	6.86	6.12	5.06
gly–val	−0.47	10.71	−2.00	−1.53	−4.62
gly–thr	0.21	10.67	4.88	4.67	2.22

[a] 0.28 is the value found for the second carboxyl group.

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 of glycyl glycine

Dipeptides	$\delta\Delta H_1^\circ$	$\delta\Delta H_2^\circ$	$\delta\Delta H_3^\circ$	$\delta\Delta H_4^\circ$	$\delta\Delta H_5^\circ$
gly–gly	0.00	0.00	0.00	0.00	0.00
gly–ala	−0.54	0.51	−4.16	−3.62	−3.65
gly–asp	0.41	1.27	4.05	3.36	5.32
gly–ser	0.14	−0.95	0.09	−0.05	−0.86
gly–val	−0.52	0.45	−4.81	−4.89	−4.36
gly–thr	0.16	0.41	2.07	1.91	2.48

ΔH_1° (gly–sub) − ΔH_1° (gly–gly) and $\delta\Delta H_2 = \Delta H_2^\circ$ (gly–sub) − ΔH_2° (gly–gly), where sub is some other amino acid residue, are reported in Table 2. ΔH_1° (gly–sub) and ΔH_1° (gly–gly) refer to the first ionization process for dipeptides and for glycine glycine, whereas ΔH_2° (sub–gly) and ΔH_2° (gly–gly) are values for the second ionization process for the same compounds.

Comparing the dissociation processes of the carboxyl and amino groups of the various dipeptides with the dissociation processes of the corresponding groups of glycine-glycine, the order of dissociation of the carboxyl groups is found to be glycyl alanine > glycyl valine > glycyl glycine > glycyl serine > glycyl threonine > glycyl aspartic acid. This sequence can be explained by considering the scale for solvation of undissociated molecules, $\delta\Delta H^\circ = \Delta H_4^\circ$ (sub–gly) − ΔH_4° (gly–gly), and zwitterions, $\delta\Delta H_3^\circ = \Delta H_3^\circ$ (sub–gly) − ΔH_3° (gly–gly) (Table 2).

For undissociated molecules, the order of solvation is: glycyl valine > glycyl alanine > glycyl serine > glycyl glycine > glycyl threonine > glycyl aspartic acid. For zwitterions the order changes to glycyl valine > glycyl alanine > glycyl glycine > glycyl serine > glycyl threonine > glycyl aspartic acid.

The order of dissociation of the amino groups is glycyl serine > glycyl glycine > glycyl threonine > glycyl valine > glycyl alanine > glycyl aspartic acid. This can be explained by considering the solvation scales for zwitterions and the anionic forms NH_2–CHR–CO–NH–CH–R′–COO^-. This last solvation scale $\delta\Delta H_5^\circ = \Delta H_5^\circ$ (sub–gly) − ΔH_5° (gly–gly) (Table 2) reveals the sequence glycyl valine > glycyl alanine > glycyl serine > glycyl glycine > glycyl threonine > glycyl aspartic acid. It can be observed that, as regards the first ionization process, glycyl alanine and glycyl valine dissociate more easily than glycyl glycine by virtue of the greater solvation of the zwitterions. For the remaining compounds, the solvation of the undissociated molecules prevails. In the second process, only glycyl serine is more dissociated than glycyl glycine because the solvation of the ionic form prevails with respect to the zwitterion.

TABLE 3

Values from eqns. 7a and 7b

Dipeptides	7a	7b
gly-gly	−0.95	−0.031
gly-ala	−1.845	0.017
gly-asp	−0.061	0.092
gly-ser	−0.41	−0.121
gly-val	−3.765	0.011

The scale for the first ionization process gives a measure of the effect of glycine structure upon the structure of other α-amino acids, while the scale for the second ionization process shows how different amino acid structures can affect the structure of glycine.

Further information on the influence of structure upon the free α-carboxyl and free α-amino groups of dipeptides can be derived from the equations

$$\Delta H_1^\circ(\text{gly–sub}) - \Delta H_1^\circ(\text{amino})/\Delta H_1^\circ(\text{amino}) \tag{7a}$$

and

$$\Delta H_2^\circ(\text{gly–sub}) - \Delta H_2^\circ(\text{gly})/\Delta H_2^\circ(\text{gly}) \tag{7b}$$

where ΔH_1° (gly–sub) represents the ionization values for the free α-carboxyl groups of various dipeptides, ΔH_1° (amino) represents the values of the dissociation processes for the carboxyl groups of the corresponding free α-amino acids, ΔH_2° (gly–sub) represents the values of dissociation for the free α-amino groups of various dipeptides, and ΔH_2° (gly) represents the values for the dissociation process of amino group of free glycine. Values related to the effect of the structure of glycine upon the free carboxyl groups of dipeptides, and to the effects of the various structures upon the free α-amino group of glycine, obtained using expressions 7a and 7b, are given in Table 3.

It can be seen that in the first ionization process, the influence of glycine (7a) upon the dipeptides structures favours the dissociation of the free α-carboxyl groups with respect to those of the corresponding free α-amino acids. (i.e. the carboxyl groups of the dipeptides become more acidic with respect to those of free α-amino acids). The influence of the structures of different α-amino acids on the structure of glycine was also studied for the second dissociation process. The proton dissociation of the free α-amino group of glycine is hindered in the dipeptides by the structures of α-alanine, valine and aspartic acid (i.e. the amino group becomes more basic). Serine favours the proton dissociation of the α-amino groups (the amino group becomes less basic).

CONCLUSION

A comparison of the influence of valine and glycine on the structures of some α-amino acids in two series of dipeptides shows that glycine activates the free α-carboxyl groups of α-amino acids in the first, second and third classes, while valine activates one α-amino acid belonging to the first class and deactivates α-amino acids of second and third classes. This can be explained in terms of the large electron withdrawing effect of the NH_3^+ group, that cannot be neutralized by the side-chain hydrogen atom. Conversely valine has an electron-donating aliphatic side-chain. α-amino acids of the first, second and third classes deactivate the free α-amino group of valine, whereas only serine shows this effect on the free α-amino group of glycine.

In conclusion, the short side-chain of glycine makes the free α-carboxyl groups more acidic and the free α-amino group of glycine less basic in a series of dipeptides, while for the long aliphatic side-chain of valine, in a second series of dipeptides, the reverse is true.

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