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PHYSICO-CHEMICAL PROPERTIES OF PEPTIDES AND THEIR SOLUTIONS *

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ABSTRACT

Complex investigations of a series of dipeptides and their solutions in H_2O were conducted by the methods of differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), densimetry, and IR and Raman spectroscopy. The results of investigations by DSC and TGA show that the differences in the spatial structure of solid dipeptides of a homologous series sharply influences the character of phase transformations. Linear correlations between the values of the heat capacity of crystalline amino acids and peptides and the number of atoms, number and length of interatomic bonds.

The dipeptides studied are classified into three groups: those with predominantly hydrophilic, those with predominantly hydrophobic and those with hydrophobic–hydrophilic (at different concentrations) characters of hydration in aqueous solutions. The ratio between hydrophilic and hydrophobic contributions to the hydration of dipeptides, and role of separate fragments of the molecules is considered.

INTRODUCTION

Studies on peptides of small size and their solutions from the point of view of thermodynamics, structure and conformations are necessary for consequent simulation of the properties of more complex polypeptide and protein compounds. Hitherto, properties of some aminoacids [1–9] and their solutions in water [10] have been studied thoroughly, mainly at constant

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temperature and infinite dilution, i.e. under conditions that are far from reality.

A complex approach combining various investigation methods is of great significance when studying such important biological objects. Dipeptides and their solutions were investigated by DSC, TGA, densimetry, and IR spectroscopy methods in the present work.

EXPERIMENTAL

We used chromatographically homogeneous dipeptides supplied by Reanal (Hungary). Before the experiment they were dried in vacuo for 6 h at 320–350 K. Doubly distilled and purified dimethylsulfoxide was used to prepare the solutions (by weight). DSC and TGA measurements were conducted on a Du Pont 1090 (U.S.A.) installation. The values of the heat capacities of solids and solutions were determined with a calorimeter DCM-2M (U.S.S.R.) with a relative error of 0.01 J g^{-1} K^{-1}. The density of solutions was measured using original equipment designed in our laboratory. The equipment was calibrated with water with 2.2×10^{-5} g cm^{-3} precision. Spectroscopic measurements were performed on Specord M80 (D.D.R.) units.

RESULTS

We used DSC to examine the behaviour of substances in the absence of phase transitions, i.e. when the heat capacity depends linearly on temperature. Also investigated were the phase transformations of peptides in the regions of their melting and decomposition points. The first way of using DSC is less traditional for this method. However, even with careful preparation and performance of the experiment, the data on heat capacity, as it will be shown below, only allow us to obtain sufficient information for a qualitative description of the picture of intermolecular interactions and conformational changes in peptide solutions.

The heat capacities of a series of crystalline amino acids and peptides at 298–348 K were measured by DSC to reveal the sensibility of the heat capacity to the molecular structure and for its further usage when interpreting the properties of solutions. The analysis of experimental data represented in Table 1 shows that the molar heat capacities of the substances studied depend linearly on the number of atoms, N_A, and total length of bonds, $\Sigma_n(n_i l_i)$, in a molecule (Fig. 1).

$$C_p = A_1 + B_1 N_A \tag{1}$$

$$C_p = A_2 + B_2 \sum_n (n_i l_i) \tag{2}$$

TABLE 1

Molar heat capacities of crystalline amino acids and peptides

Substance	Molar heat capacity, (J mol^{-1} K^{-1})			
	298 K	313 K	333 K	348 K
Glycine	95	99	104	109
L-α-Alanine	115	119	125	129
DL-α-Alanine	114	117	123	128
β-Alanine	109	113	119	124
DL-Valine	165	171	182	189
D-Valine	158	164	173	179
L-Leucine	191	202	222	242
Glycyl-glycine	149	155	163	168
β-Alanyl-glycine	168	175	185	192
L-α-Alanyl-L-α-alanine	195	202	212	221
DL-α-Alanyl-DL-α-alanine	189	196	205	212
β-Alanyl-β-alanine	196	204	216	226
DL-α-Alanyl-DL-valine	240	249	262	272
DL-α-Alanyl-DL-norleucine	276	287	305	318
DL-α-Alanyl-DL-asparagine·H_2O	256	265	283	297
Diglycyl-glycine	216	225	237	245
DL-α-Alanyl-glycyl-glycine	236	247	262	274

where C_p is the molar heat capacity; A_1, A_2, B_1, B_2 are the empirical coefficients characteristic of the series of substances studied, N_A is the number of atoms in a molecule, and l_i is the bond length. Fulfilment of the correlations noted testifies to the additivity of the contributions from atomic

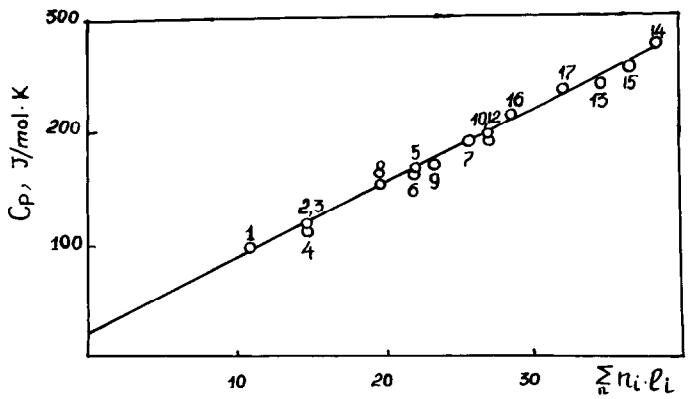

Fig. 1. Dependence of molar heat capacities of crystalline aminoacids and peptides on $\Sigma_n n_i l_i$ at 298.15 K. 1, Glycine; 2, L-α-alanine; 3, DL-α-alanine; 4, β-alanine; 5, DL-valine; 6, D-valine; 7, L-leucine; 8, glycyl-glycine; 9, β-alanyl-glycine; 10, L-α-ananyl-L-α-alanine; 11, DL-α-alanyl-DL-α-alanine; 12, β-alanyl-β-alanine; 13, DL-α-alanyl-DL-valine; 14, DL-α-alanyl-DL-norleucine; 15, triglycine; 16, DL-α-alanyl-glycyl-glycine; 17, DL-α-alanyl-DL-asparagine·H_2O.

heat capacities as well as contributions from separate bonds to the total value of the heat capacity of crystalline substances. Since rotational and translational heat capacities do not depend on the nature of molecules in the crystalline state, the dependence of C_p on the total length of bonds [eqn. (2)] will be mainly determined by the oscillatory heat capacity. Dependences (1) and (2) also have their own meaning for calculation of the heat capacity of the crystalline amino acids and peptides which were not studied.

We used complex investigations of the physico-chemical transformations of dipeptides: β-alanyl-glycine, β-alanyl-β-alanine, DL-α-alanyl-DL-α-alanine, DL-α-alanyl-DL-valine by DSC and TGA.

Data on the thermodynamic characteristics of the physico-chemical transformations in dipeptides are practically absent in the literature (except for some amino acids) [1–9]; however, they are necessary for information on the energy of formation of polypeptides and more complex protein compounds.

Earlier, DSC measurements were performed on DSM-2M (U.S.S.R.) and TGA on derivatograph Q-1000 (Hungary) in the Institute of Non-aqueous Solution Chemistry of the U.S.S.R. Academy of Sciences [5]. DSC and TGA measurements were conducted with the help of a Du Pont 1090 instrument (U.S.A.) at 473–613 K (relative error of the measurements of the thermal effect was 1% and the precision of the temperature measurements was 0.4 K).

The results of the measurements are shown in Table 2 and Figs. 2–4.

Analysis of the data obtained shows that for two dipeptides, β-alanyl-glycine and β-alanyl-β-alanine, the physical process of melting is accompanied by chemical decomposition. The dependences typical of them are

TABLE 2

Thermodynamic characteristics of physico-chemical transformations of dipeptides

Substance	DSC		TGA	DTG
	T (°C)	ΔH (kJ mol^{-1})	T (°C)	T (°C)
β-Alanyl-glycine	234.8	56.6	275.1	281.9
	Melting–decomposition		Melting–decomposition glycine β-alanine	
β-Alanyl-β--alanine	206.9	58.3	234.2	244.4 250
	Melting–decomposition		Melting–decomposition of β-alanine	
DL-α-Alanyl-	159.9–210	33.2	Extremes	
-DL-α-alanine	Melting		179.4	196.8 210 294.2 320
	210–248.5	113.6		
	Decomposition			
DL-α-Alanyl	243–260	79.2	Extremes	
-DL-valine	Decomposition of peptide		266.9	280.6 315.2 319.7
	260—330	54.8		
	Melting–decomposition of DL-alanine and DL-valine			

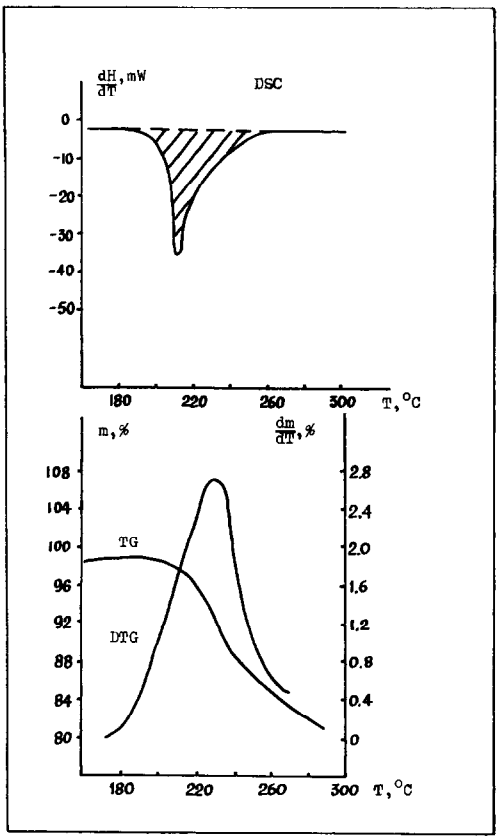

Fig. 2. DSC and TGA thermograms of melting–decomposition of β-alanyl-β-alanine.

shown in Fig. 2. A common structure of these substances in the form of an extended molecular chain which forms β-forms in space does not lead to the conformational changes that energetically are more profitable at a temperature increase. The two other dipeptides, DL-α-alanyl-DL-α-alanine and DL-α-alanyl-DL-valine, differ with respect to the character of the temperature dependence in the process of the physico-chemical transformations. Comparison of the literature data on amino acids [1–7] with our results for dipeptides shows that the amino acid groups in dipeptides preserve their characteristic peculiarities.

Figure 3 shows that, proceeding from DSC data, DL-α-alanyl-DL-α-alanine has two clearly separated stages of the physico-chemical transformation: (a) melting of the dipeptide molecule with partial decomposition into two amino acids and (b) physico-chemical transformations of the amino acid of DL-alanine.

Melting of the dipeptide does not occur on heating DL-α-alanyl-DL-valine, since proceeding from DSC and TGA data there are no phase transitions below 240 °C. The hydrogen peptide bond between two apolar

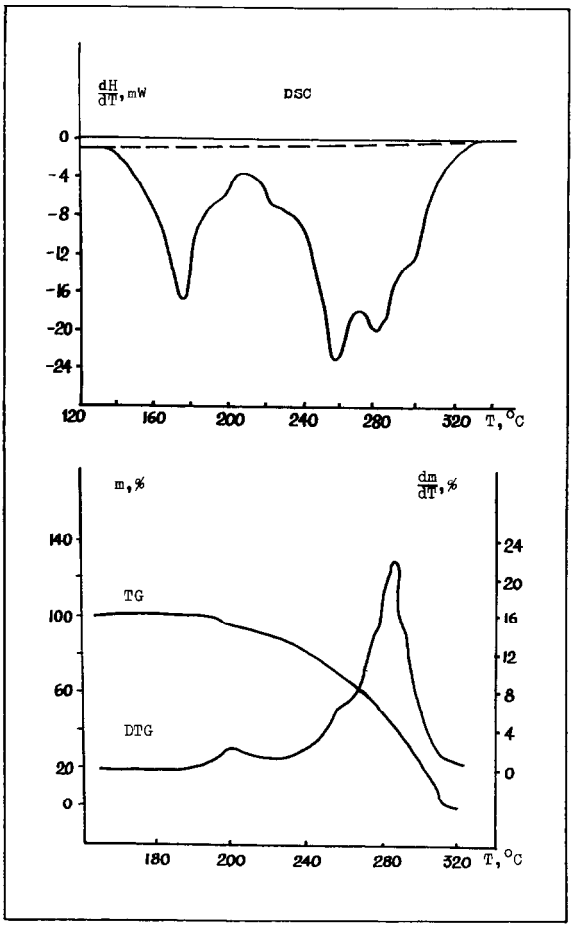

Fig. 3. DSC and TGA thermograms of melting–decomposition of DL-α-alanyl-DL-α-alanine.

amino acid residues of DL-alanine and DL-valine breaks easily and on increasing the temperature the consessive sublimation–decomposition of DL-alanine and DL-valine takes place. The thermal characteristics in Fig. 4 and Table 2 are similar for the two latter amino acids which were investigated earlier.

It is known that the character of solute–solvent interaction has a strong influence on the magnitude and sign of the excess heat capacity characteristics of solutions. Hydrophobic hydration is characterized by a positive heat capacity of solution ΔC_{p2}° and second derivative of the limiting partial molar volume on temperature $(\partial^2 \overline{V}_2^0/\partial T^2)_p$ (the so-called Hepler's criterion) [11].

Analysis of the excess heat capacities made it possible to divide the substances studied into three groups (Figs. 5 and 6): those substances with predominantly hydrophilic ($\Delta C_{p2}^0 < 0$) and those with predominantly hydrophobic ($\Delta C_{p2}^0 > 0$) characters of hydration and the third group which in-

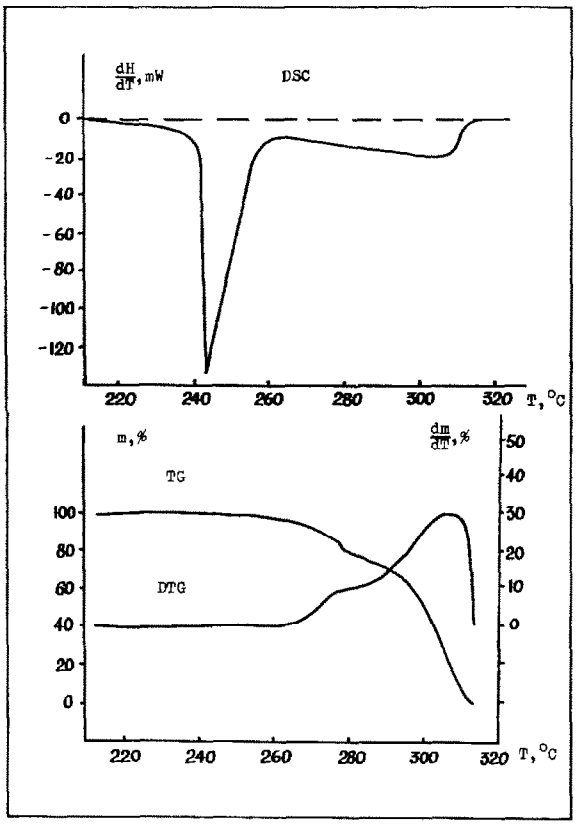

Fig. 4. DSC and TGA thermograms of melting–decomposition of DL-α-alanyl-DL-valine.

cludes β-alanine and β-alanine-containing dipeptides (β-alanyl-glycine, β-alanyl-β-alanine) which have negative excess heat capacities, C_p^E, at high concentrations. For this group of substances, the heat capacity of solution, ΔC_{p2}^0, and Hepler's criterion are close to zero (Table 3), which is determined by comparable magnitudes of the contributions from the hydrophobic and hydrophilic constituents to the total effect of hydration.

Of the initial substances only five dipeptides were chosen to be studied in detail; three of the five have linear structures (glycyl-glycine, β-alanyl-glycine and β-alanyl-β-alanine) and two have side alkyl radicals (DL-α-alanyl-DL-α-alanine and DL-α-alanyl-DL-valine). The heat capacities and volumetric characteristics of aqueous solutions of these dipeptides are shown in Table 3.

The data on the thermodynamic properties of amino acids and peptides for a wide variety of temperatures and concentrations are few. However, such investigations can provide a fuller picture of the structural peculiarities of solutions and help to avoid incorrect conclusions. The concentration and temperature dependences can also reflect the conformational changes of flexible dipeptide molecules.

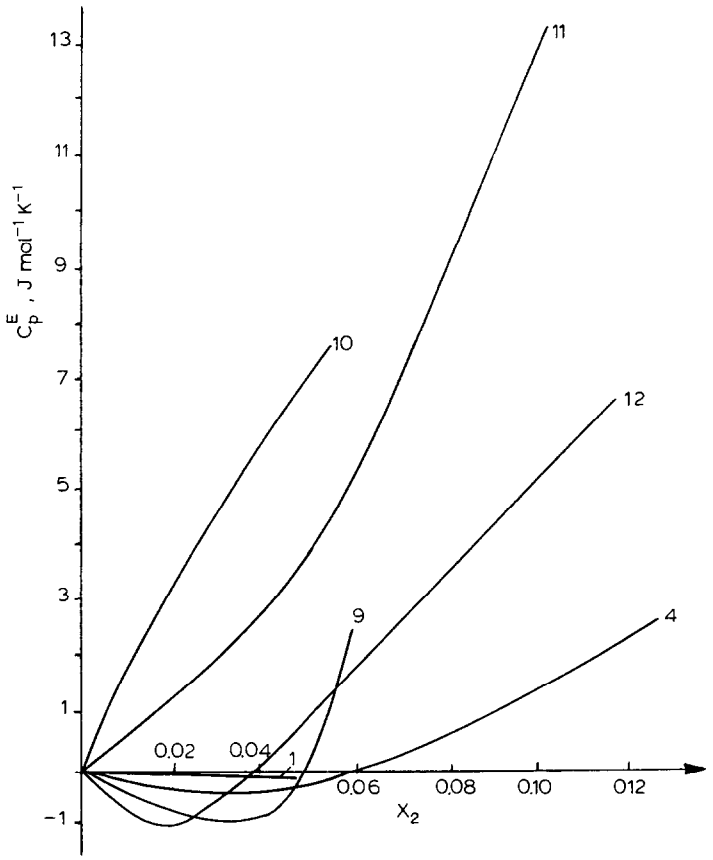

Fig. 5. Excess heat capacities of aqueous solutions of amino acids and peptides at 298 K. See Fig. 1 for key to numbering.

The importance of these effects can be demonstrated by the temperature dependences of the partial molar heat capacities at infinite dilution, $\overline{C}_{p2}^0$, of dipeptides in water at 283–348 K (Fig. 7).

There is a non-linearity in the temperature dependences of $\overline{C}_{p2}^0$ for solutions of glycyl-glycine and β-alanyl-glycine, while for β-alanyl-β-alanine, the structure of which is analogous to the previous ones, the temperature dependence of $\overline{C}_{p2}^0$ is linear. Lowering of the vibration frequency in IR and Raman spectra of the carboxylic groups of glycyl-glycine, β-alanyl-glycine is connected with the formation of an intramolecular hydrogen bond which favours the formation of the spherical form of dipeptide molecules. Due to the destruction of the intramolecular hydrogen bond by increasing temperature the conformational equilibrium will be displaced towards completely extended conformation. The distance between the charged groups is larger for the extended conformation than for a rolled one and, consequently, the overlap of the solvation regions of the end groups is also smaller. The value

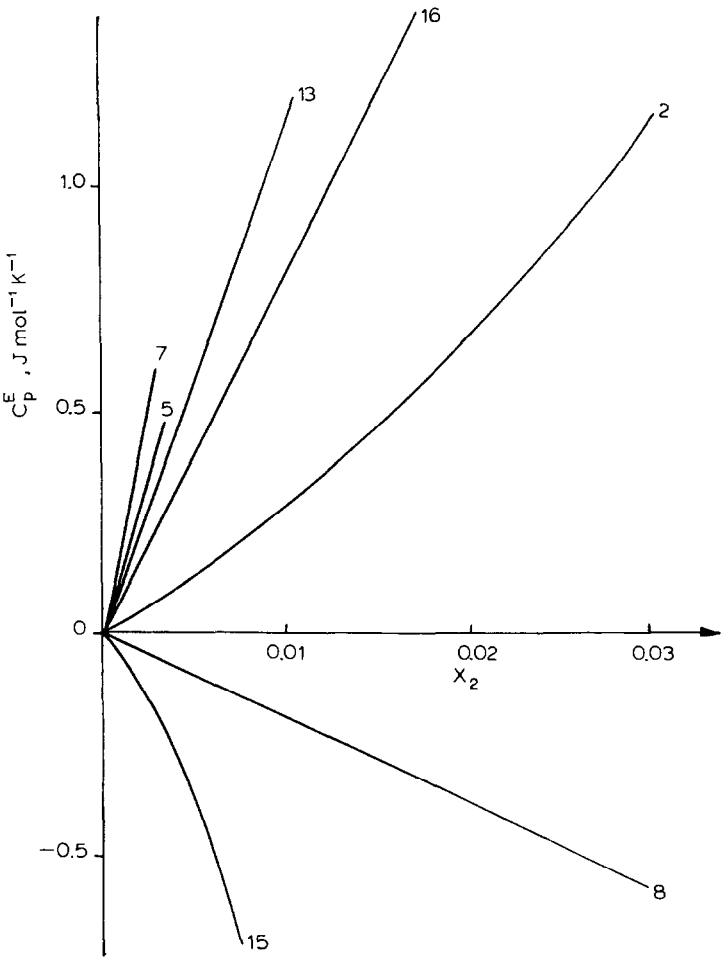

Fig. 6. Excess heat capacities of aqueous solutions of amino acids and peptides at 298 K. Fig. 1 for key to numbering.

of electrostriction will increase and this will result in the decrease in heat capacity. This effect explains the appearance of the maximum in the temperature dependence of the partial molar heat capacity at 333 K.

For DL-α-alanyl-DL-α-alanine and DL-α-alanyl-DL-valine, which have side alkyl radicals and the number of separating atoms is $n = 4$, the temperature dependence of $\overline{C}_{p2}^{0}$ also has a maximum but it is less prominent. The conformational equilibrium for these compounds will be determined together with the electrostatic by the interaction of the side alkyl radicals with each other, the latter contribution evidently playing the leading role. This conclusion is confirmed by the calculations of Go and Scheraga [12] for polyalanine and polyvaline molecules.

TABLE 3

Heat capacity and volumetric characteristics of aqueous solutions of dipeptides at 298.15 K

Substance	$\bar{C}^0_{p2}$ (J mol^{-1} K^{-1})	ΔC^0_{p2} (J mol^{-1} K^{-1})	$\bar{V}^0_2$ (cm^3 mol^{-1})	$(\frac{\partial^2 \bar{V}^0_2}{\partial T^2})_p$	E [a] (cm^3 mol^{-1})	S_V
1. Glycyl-glycine	105	−44	76.24	−0.0205	13.56	1.56
2. β-Alanyl--glycine	185	17	92.34	0.00185	13.46	2.20
3. β-Alanyl--β-alanine	229	33	107.2	−0.0156	14.6	2.70
4. DL-α-Alanyl--DL-α-alanine	323	127	102.63	−0.00254	17.17	1.56
5. DL-α-Alanyl--DL-valine	462	222	144.03	0.0153	6.73	−7.84

[a] Electrostriction.

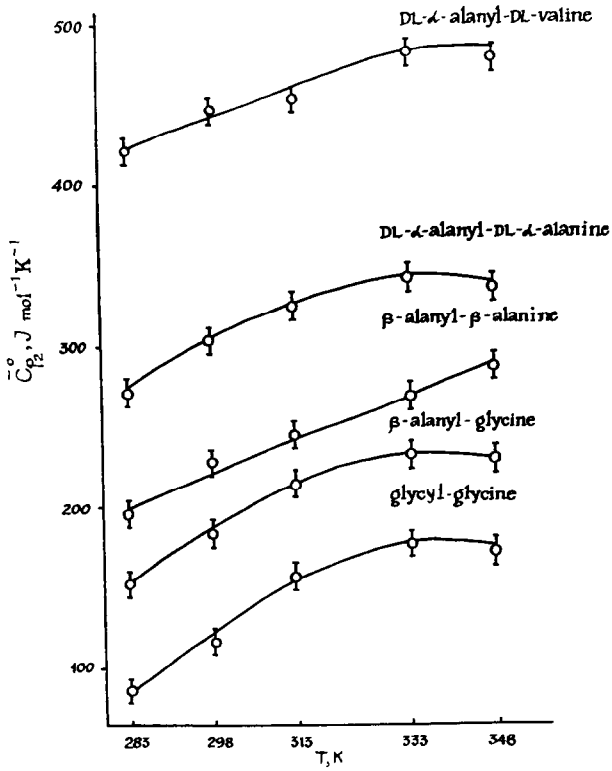

Fig. 7. Temperature dependence of the limiting partial molar heat capacities $\bar{C}^0_{p2}$ of dipeptides in water.

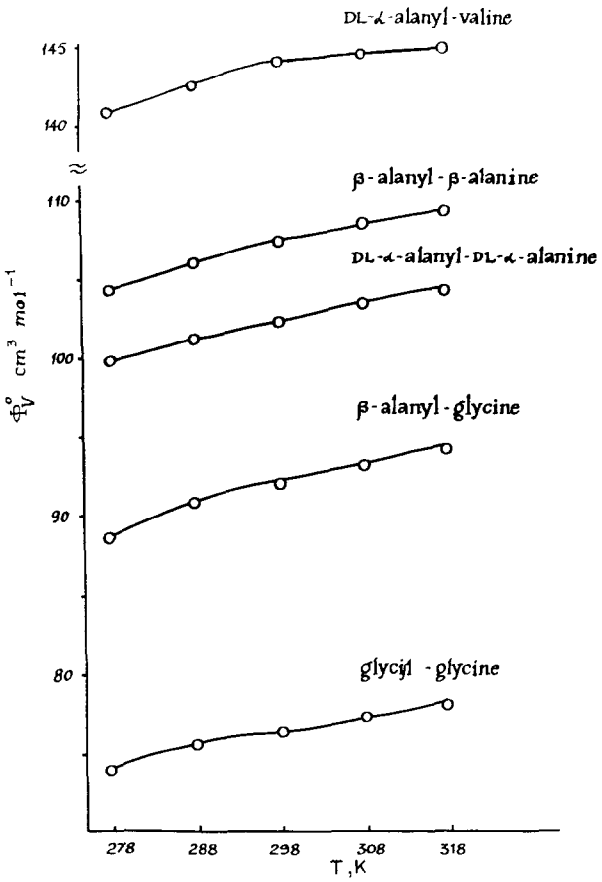

Fig. 8. Temperature dependence of the limiting partial molar volumes $\overline{V}_2^0$ of dipeptides in water.

The temperature dependence of the partial molar volumes of dipeptides was studied at 278–318 K (Fig. 8). It can be seen that, compared with the temperature dependences of amino acids, those for dipeptides have greater deviations from linearity. The effect of screening of the charged groups by the side radicals determines low electrostriction values ($E = 6.73$ cm^3 mol^{-1}) for DL-α-alanyl-DL-valine.

When considering the dependence on concentration of the apparent molar volumes of the dipeptides studied, which are characterized by the S_V coefficient in the equation

$$\Phi_V = \overline{V}_2^0 + S_V m$$

it can be seen that, for four dipeptides, the slope of S_V is positive and for DL-α-alanyl-DL-valine it is strongly negative. Basing the discussion of this fact on the model of the overlapping solvation shells [10], one should expect positive S_V values for the case in which solute–solute interaction is due to

hydrophilic centres (zwitterion and peptide bond) and negative S_V values if the interaction is due to hydrophobic side radicals. The hydrophilic interaction between dissolved molecules is characteristic of glycyl-glycine, β-alanyl-glycine, β-alanyl-β-alanine, DL-α-alanyl-DL-α-alanine, and hydrophobic for DL-α-alanyl-DL-valine.

Here, the increase in the distance between the charged groups for the first four dipeptides is accompanied by a linear increase in the coefficient S_V at 298 K (Table 3) which is connected with the increase in the dipole moment of the zwitterionic molecule and with the strengthening of the dipole–dipole interaction.

CONCLUSIONS

Thermal processes in the region of the physico-chemical transformations of the dipeptides investigated are determined by the nature of the amino acid groups of which they are formed.

The physico-chemical processes have an irreversible character on heating. Melting is accompanied by the chemical decomposition of the dipeptide molecules having a linear structure.

For the dipeptides whose molecules have a spatial structure (with side CH_2 groups), the thermal processes are divided in the heating process into the melting–decomposition of dipeptide and then the sublimation–decomposition of amino acids (the case with dialanine). The phases of dipeptide melting are absent on heating dipeptides with molecules having various apolar amino acid groups. There is a decomposition of dipeptide into the constituent amino acids followed by melting, sublimation and decomposition, which are characteristic of amino acids.

We have discovered linear dependences of the heat capacities of crystalline amino acids and peptides

$$C_p = a + bN_A$$

$$C_p = a + b\left(\sum_n n_i l_i\right)$$

which testify to the additivity of the contributions from the atomic heat capacities as well as the contributions from separate bonds to the total value of the heat capacity. These dependences can be used for an approximate calculation of the heat capacities of amino acids and peptides which have not been studied.

The dipeptides studied are classified into three groups: those with predominantly hydrophilic, those with predominantly hydrophobic and those with hydrophilic–hydrophobic (at various concentrations) characters of hydration in aqueous solutions.

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