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Complexation of peptides with crown ethers. Part 1. Composition and thermal behaviour of compounds consisting of oligopeptides and some crown ethers

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Abstract

Crystals of the complexes 18-crown-6/diglycine, 18-crown-6/diglycine/water, 18-crown-6/triglycine, 18-crown-6/L-α-alanyl-L-α-alanine and 1,10-diaza-18-crown-6/diglycine/water were obtained. The thermal behaviour of these compounds was investigated by DSC and TGA methods. It was found that the anhydrous complexes have at least two physicochemical transitions in the range 320–470 K: melting of the complexes at 360–380 K and decomposition above 410 K. Two types of water, i.e. weakly and strongly bound with the complex, were observed on heating the hydrated complexes. The enthalpic and entropic changes of the melting process are discussed in terms of the cationic and anionic binding of the peptide molecule to crown ether.

INTRODUCTION

The study of peptide–crown interactions is very important for understanding many biological processes. Crown ethers can be considered as models of some macrocyclic antibiotics and enzymes. However, it is known that the interaction of antibiotics with charged groups of proteins plays a significant role in their antimicrobial activity [1]. The results of the investigation of peptide–crown compounds give possibilities for an explanation of the mechanism of membrane processes and for the further development of separation processes. The results are also necessary in the selection of a receptor for single dipeptide units in peptide recognition [2–4]. Complexation plays an essential role in enzyme–catalysed reac-

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tions. Therefore the application of this type of reaction helps to discover new ways of peptide synthesis, using the crown ethers as the enzyme model [5, 6].

In the present paper, we describe the composition and thermal behaviour of the compounds composed diglycine, triglycine L-α-alanyl-L-α-alanine and some crown ethers, investigated by differential scanning calorimetry (DSC) and thermogravimetry (TGA) methods.

EXPERIMENTAL

Preparation of complexes

Single crystals of the complexes were prepared by slow evaporation of solvent from their equimolar water–ethanol solutions at room temperature. In the case of 18-crown-6/diglycine, the hydrated form of the complex, crystallized in aqueous solution (m(peptide) = 0.6 mol kg^{-1}, m(crown) = 0.6 mol kg^{-1}) at room temperature, was also obtained.

Chromatographically homogeneous peptides produced by Reanal (Hungary) were used for preparation of the complexes. The purity of the commercially obtained peptides was above 98 mol.% Each peptide was purified by recrystallization from water + ethanol and dried under vacuum for 48 h at 300 K. The purity of the commercially obtained crown ethers was above 99 mol.% and they were used without further purification.

DSC and TGA analyses

The thermal properties of the crystalline complexes were investigated in the range 320–470 K on a Du Pont 1090 installation, equipped with DSC and TGA cells. The runs were performed at a scan rate of 5 K min^{-1} in an atmosphere of dry argon. The sample mass was 10–20 mg. The relative error of the measurements of the thermal effect was 1% and the precision of the temperature measurements was 0.4 K. The anhydrous complexes were prepared by drying at 353 K for 6 h. The hydrated forms of some of the complexes were also studied.

RESULTS

The DSC and TGA data of the complexes 18-crown-6/diglycine (**I**), 18-crown-6/diglycine/water (**II**), 18-crown-6/triglycine (**III**), 18-crown-6/L-α-alanyl-L-α-alanine (**IV**), 12-crown-4/L-α-alanyl-L-α-alanine (**V**) and 1,10-diaza-18-crown-6/diglycine/water (**VI**) are presented on Figs. 1–6 and in Table 1. Table 1 also lists the temperatures, enthalpies and entropies of melting of the 18-crown-6 and 1,10-diaza-18-crown-6. The thermogravimetric curves of the crystalline peptides obtained earlier [7] did not show a decrease in the sample weight up to 470 K. Therefore

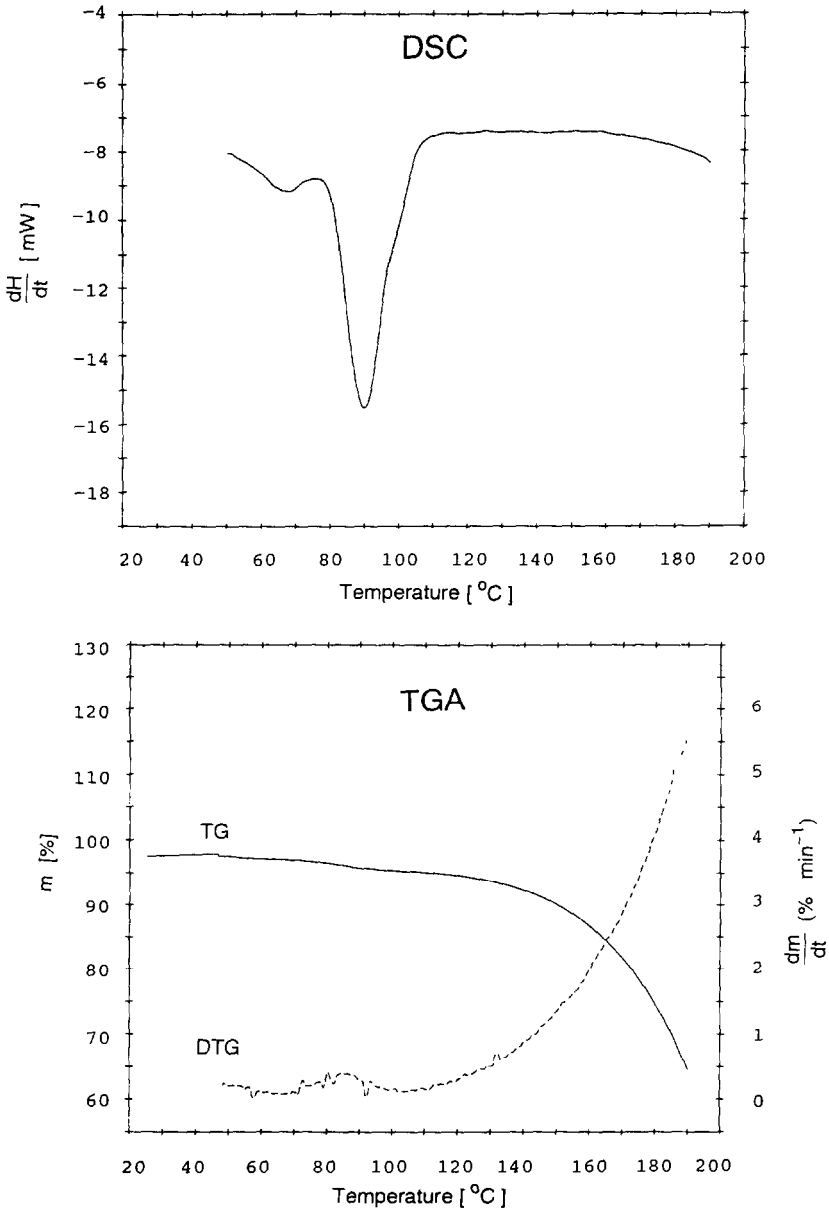

Fig. 1. DSC and TGA thermograms of heating of 18-crown-6/diglycine (I).

all the changes in sample mass observed in the complexes must be caused by the evaporation of water and/or crown ether. From the TGA thermograms of 18-crown-6 and 1,10-diaza-18-crown-6, it was found that about 20% and 40% of the sample mass, respectively, remain in the sample container after heating up to 470 K. These values were taken into account in the calculation of the compositions of the complexes.

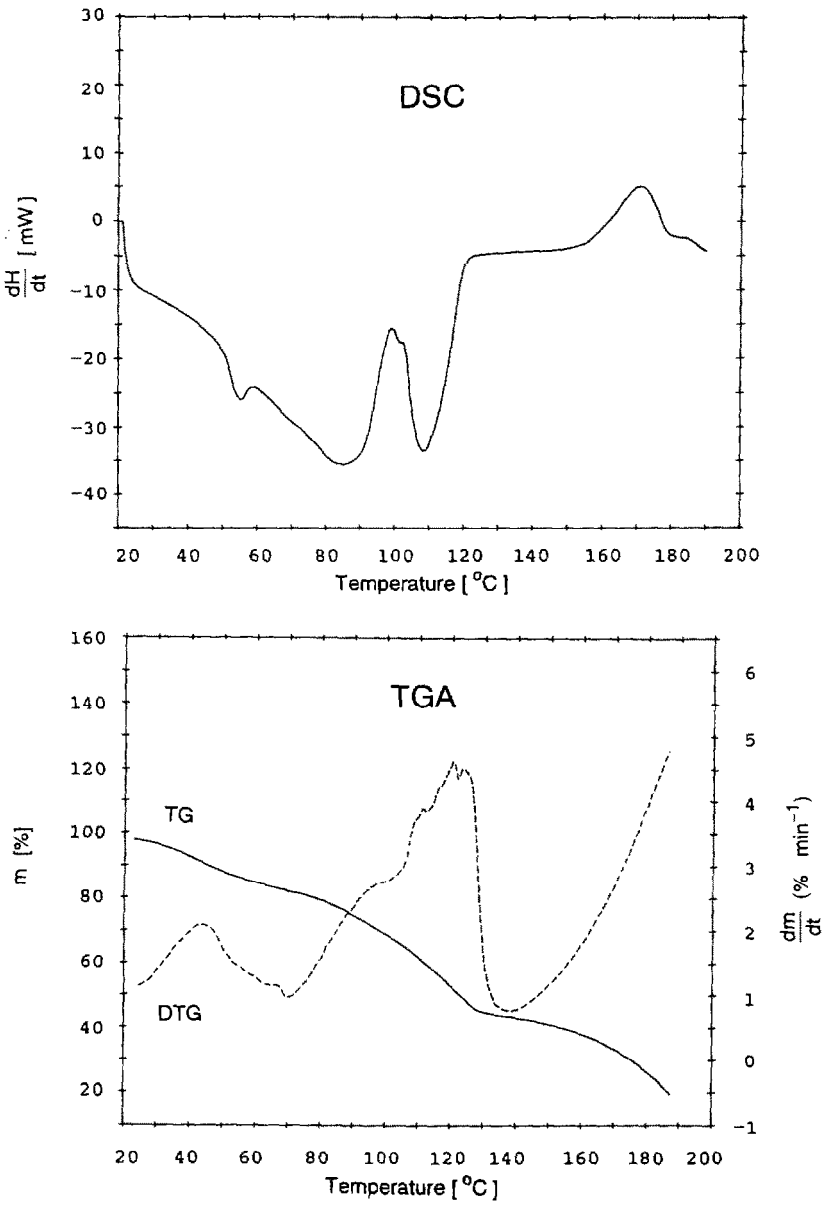

Fig. 2. DSC and TGA thermograms of heating of 18-crown-6/diglycine/water (**II**).

The mass loss was not greater than 2% in the 300–373 K range for complexes **I** and **III**. For complexes **IV** and **VI**, the mass loss in the same temperature range was 3.8%, as can be seen in the TGA thermograms (Figs. 4 and 6). These mass losses are due to the presence of some hydration water in the samples, which were taken into account in the calculations of the thermodynamic characteristics of the physicochemical transformations.

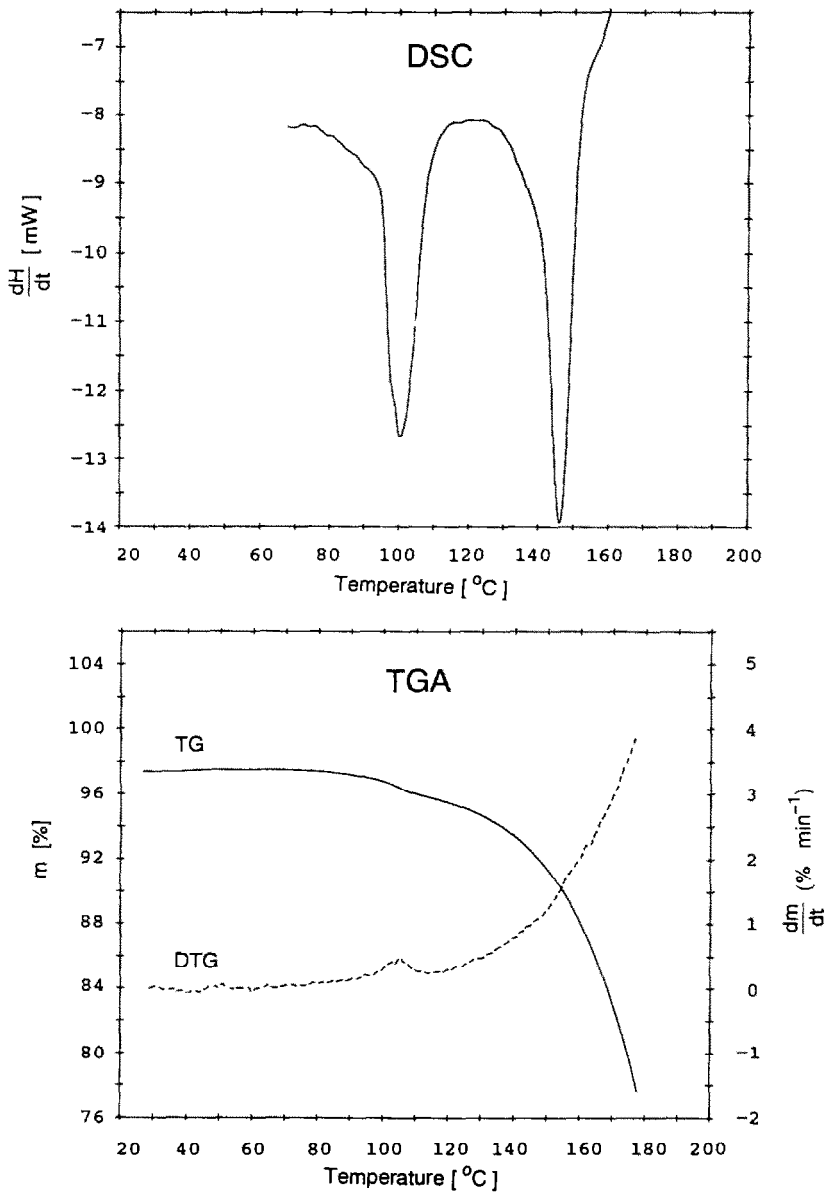

Fig. 3. DSC and TGA thermograms of heating of 18-crown-6/triglycine (**III**).

For 18-crown-6/diglycine, the anhydrous (**I**) as well as the hydrated (**II**) forms of the complex were investigated by DSC and TGA (Figs. 1 and 2). A calculation of the mass decrease for **I** yields an approximate composition of 1:1 for the complex. This compound has three stages of physicochemical transformation: (1) a weakly distinguished peak at 339.7 K, (2) melting of the complex at 363.5 K, and (3) decomposition

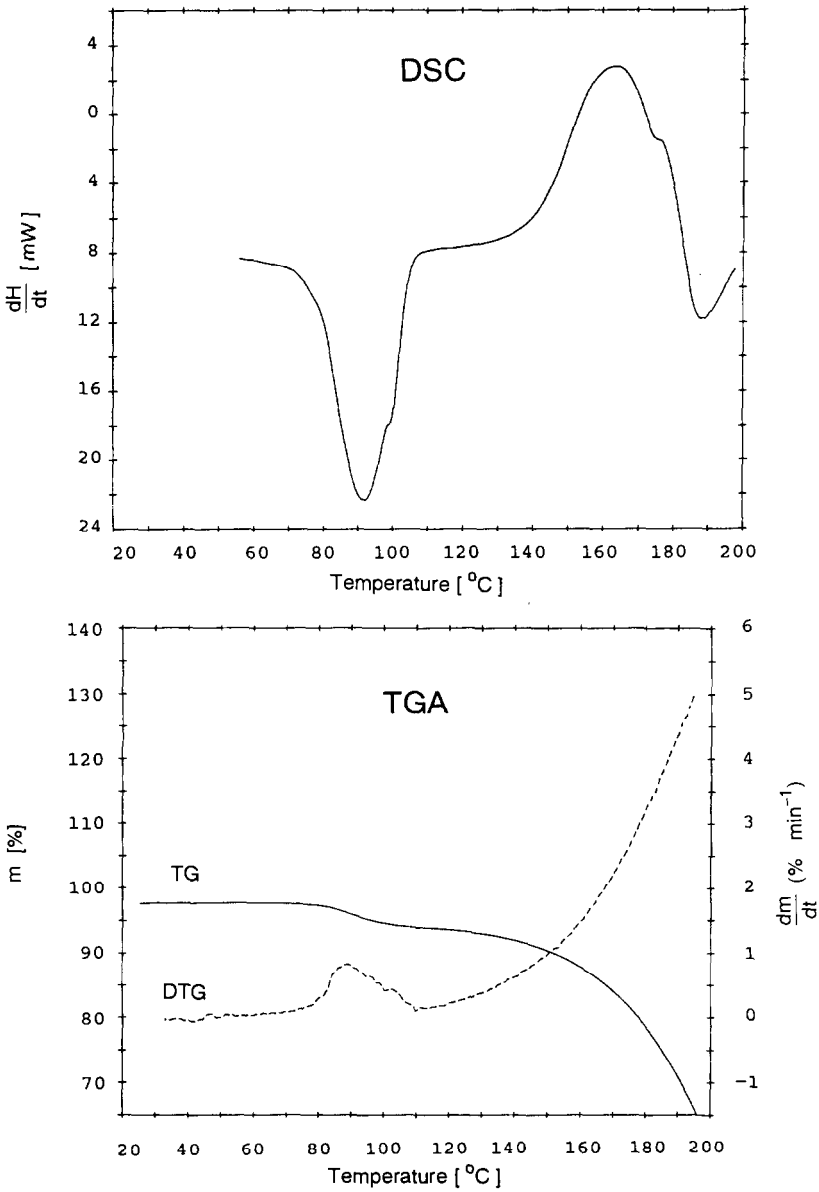

Fig. 4. DSC and TGA thermograms of heating of 18-crown-6/L-α-alanyl-L-α-alanine (**IV**).

above 413 K, as seen in the TGA thermograms. Thermograms of **II** are more complicated (Fig. 2). At least four clearly separated thermal processes can be observed on these curves. These are probably (1) evaporation of weakly bound water at 328.7 K (loss of mass, 15%), (2) melting and evaporation of strongly bound water at 358 K (loss of mass, 13.3%), (3) melting of the complex with endo-effect at 381.8 K (loss of

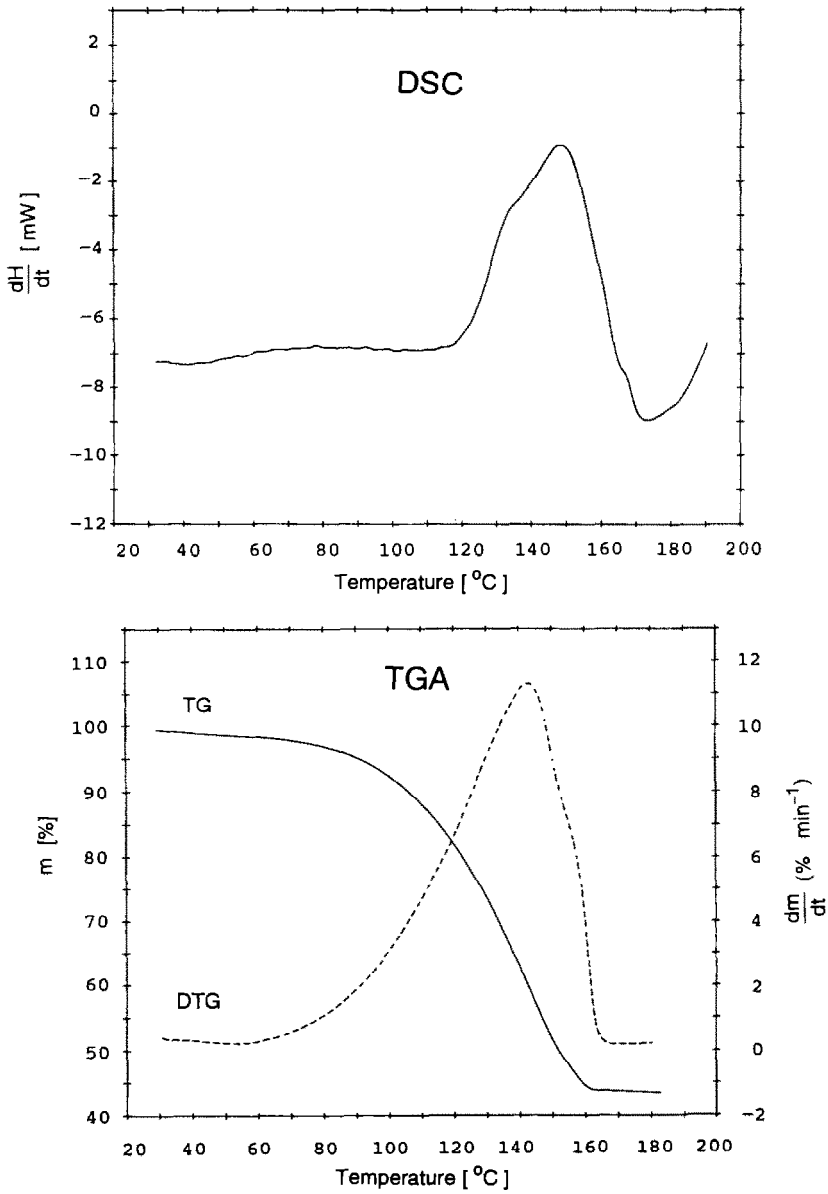

Fig. 5. DSC and TGA thermograms of heating of 12-crown-4/L-α-alanyl-L-α-alanine (V).

mass, 9.7%), and (4) decomposition of the complex with exo-effect at 443.7 K. Therefore, II contains approximately 1 mole of 18-crown-6, 1 mole of diglycine and 16 moles of water, 6 moles of which are strongly bound with the complex.

Figure 3 illustrates the thermograms of 18-crown-6/triglycine (III) which show two peaks at 373.5 and 419.3 K. These represent, perhaps,

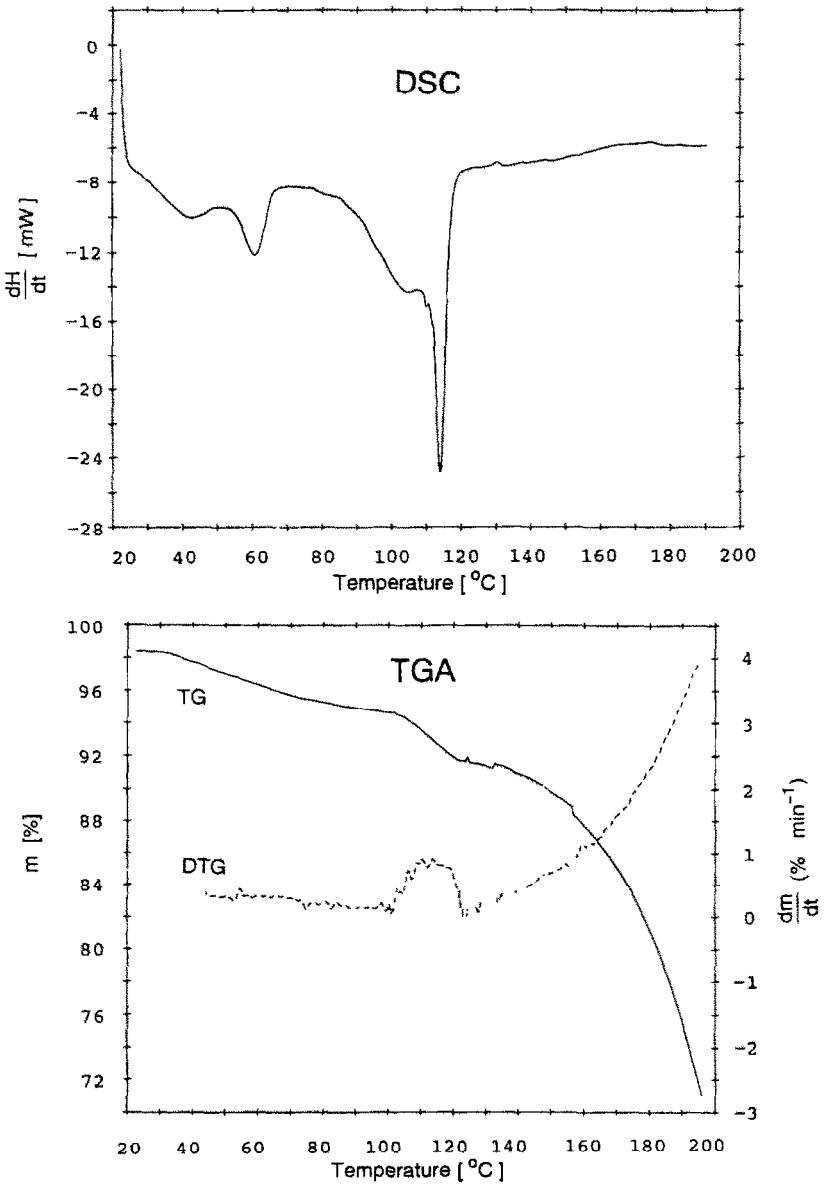

Fig. 6. DSC and TGA thermograms of heating of 1,10-diaza-18-crown-6/diglycine/water (**VI**).

melting of the two different crystalline forms of **III** conventionally named the α- and β-forms, respectively. The β-form, which has the higher melting temperature, is formed by increasing the temperature and time of drying. The α-form corresponds to the 1:1 composition of the complex.

Thermograms of the 18-crown-6/L-α-alanyl-L-α-alanine (**IV**) are presented on Fig. 4. The composition of the complex as calculated from the

TABLE 1

DSC data on the thermodynamic characteristics of the physicochemical transformations of the complexes

Compound	T [a] (K)	ΔH [b] ($kJ\,mol^{-1}$)	ΔS [b] ($J\,mol^{-1}\,K^{-1}$)
18-Crown-6 (melting)	313.5	42.2	135
1,10-Diaza-18-crown-6 (melting)	389.3	61.3	157
18-Crown-6/diglycine (melting)	363.5	180.9	498
18-Crown-6/diglycine/water			
Melting of complex	381.9	163.0	427
Decomposition of complex	443.7	−50.5	
Dehydration of weakly bound water	328.7	15.1 [c]	
Dehydration of strongly bound water	358.0	62.8 [c]	
18-Crown-6/triglycine			
Melting of α-form	373.5	107.7	288
Melting of β-form	419.3	75.7	181
18-Crown-6/L-α-alanyl-L-α-alanine			
Melting of complex	365.1	140.0	383
Decomposition of complex	437.0	−170.1	
12-Crown-4/L-α-alanyl-L-α-alanine			
Decomposition of complex	421.9	−57.5	
1,10-Diaza-18-crown-6/diglycine/water			
Melting of monohydrate	378.5	106.5	281
Dehydration of dihydrate	334.0	33.8	

[a] The temperatures of the peaks are presented. [b] All the values were calculated for 1:1 composition of the complexes without water molecules. [c] These values were calculated taking into account the TGA data on loss of mass and molar weight of water.

TGA data approximates to 1:1. Two types of physicochemical processes take place on heating of this compound: (1) melting at 365.1 K, and (2) decomposition of the complex above 412 K. We also attempted to obtain the DSC and TGA thermograms of diglycine and dialanine complexes with 12-crown-4 and 15-crown-5. However, only for 12-crown-4/L-α-alanyl-L-α-alanine (**V**) was a satisfactory physicochemical transformation peak observed, in the range 310–450 K (Fig. 5). Analysis of the DSC and TGA data shows that this compound decomposes (exo-effect) at 421.9 K without melting.

On the DSC thermogram of the 1,10-diaza-18-crown-6/diglycine/water complex (Fig. 6) (**VI**), it can be seen that its melting at 378.5 K is accompanied by the melting of the 1,10-diaza-18-crown-6 at 387.2 K. Losses of mass in the range 298–373 K (3.8%) and in the range 373–393 K (2.9%), when all the complex was melted, correspond to the evaporation of one + one mole of water. Therefore, the crystalline **VI** that we synthesized exists as a dihydrate, but only the monohydrate of **VI** melts at 378.5

DISCUSSION

The main thermal results are collected in Table 1. As can be seen, the enthalpies and entropies of melting of crown/peptide complexes are greater than those for pure crown ethers. The opposite results were obtained for complexation of KNCS with dibenzo-18-crown-6 [8] and cryptand(222) [9]. This was attributed to a stiffening of the ligand when complexed [8, 9]. It was found by X-ray analysis data [10] that the peptides studied are bound to the 18-crown-6 macrocycle by three $-NH\cdots O$ hydrogen bonds. The charged carboxylic groups at the opposite side of the peptide molecule do not take part in the binding to 18-crown-6. Evidently, the result obtained can be explained by the complexation of crown ethers with the sufficiently flexible peptide molecules. Therefore, reduction of the fusion entropy of the crown ethers due to complexation does not compensate for the increase of the fusion entropy of the whole crown/peptide compound.

The enthalpies of melting of the complexes studied increase in the order **VI** < **III** < **IV** < **II** < **I**. It is interesting to note that the enthalpy of melting of **I** is higher than the enthalpy of melting of **IV**, in approximately the same ratio as the "depth of penetration" for **I** to that of IV. The distance between the nitrogen atoms of $-NH_3^+$ peptide group and the plane of the crown macrocycle has been assumed as the characteristic of the "depth of penetration" [10]. The decrease of this distance or increase of the "depth of penetration" results in the higher enthalpy of melting for this pair. The complexes of the L-α-alanyl-L-α-alanine decompose with considerable exo-effects (Figs. 4 and 5). However, the enthalpy of decomposition of **IV** is much higher than that of **V**, which must be caused by the increased stability of **IV** over **V**.

The considerable difference between the enthalpy of melting of the hydrates **II** and **VI** can be explained by the different types of diglycine binding to crown macrocycles. The presence of two –NH groups in 1,10-diaza-18-crown-6 makes possible the binding by the anionic carboxylic group of the peptide and by the two amino groups of the crown ethers. In this case, two hydrogen bonds must be formed instead of the three formed in the cationic binding of the peptide to the 18-crown-6. The correlation of the enthalpies of melting for **VI** and **II** is also 2:3. However, it is possible that analogous results may be obtained for some other reasons.

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REFERENCES

1 H. Kleinkauf and H. von Dohren (Eds.), Biochemistry of Peptide Antibiotics: Recent Advances in the Biotechnology of β-Lactams and Microbial Bioactive Peptides, de Gruyter, Berlin, New York, 1990.
2 J.-M. Lehn, J. Inclus. Phenom., 6 (1988) 351.
3 A.D. Hamilton, N. Pant and A. Muehldorf, Pure Appl. Chem., 61 (1989) 533.
4 I.O. Sutherland, Pure Appl. Chem., 61 (1989) 1547.
5 K. Koga and S. Sasaki, Pure Appl. Chem., 60 (1988) 539.
6 S. Sasaki and K. Koga, J. Inclus. Phenom. Molec. Recognit. Chem., 7 (1989) 267.
7 V.G. Badelin, O.V. Kulikov, V.S. Vatagin, E. Utzig, A. Zielenkiewicz, W. Zielenkiewicz and G.A. Krestov, Thermochim. Acta, 169 (1990) 81.
8 A. Bianchi, J. Giusti and P. Paoletti, Thermochim. Acta, 90 (1985) 109.
9 J. Giusti, G.G.T. Guarini, P. Orioli and P. Paoletti, Thermochim. Acta, 162 (1990) 99.
10 J. Lipkowski, O.V. Kulikov and W. Zielenkiewicz, J. Supramolec. Chem., (1992) in press.