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Complexation of peptides with crown ethers. Part 2. Thermokinetic behaviour of hydrated compounds consisting of α-amino acids, peptides and 18-crown-6

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Abstract

Crystals of 11 hydrated complexes consisting of α-amino acids, dipeptides, tripeptides and 18-crown-6 were isolated. The composition and thermal behaviour of these compounds were investigated and the temperatures, heat effects and activation energies of different stages of their decomposition were determined using DSC and TG. After preliminary dehydration at 300–350 K, the compounds undergo decomposition in one or two stages. It was found that the thermokinetic characteristics of the complexes are very sensitive to their structural features.

INTRODUCTION

Crown ethers are macrocyclic ligands widely used in supramolecular chemistry [1]. Because these compounds can bind selectively to different cations, the thermal properties of their crystalline complexes have been intensively studied [2–5]. Moreover, crown ethers have some similar features with natural cyclic antibiotics and enzymes. Therefore, the investigation of crown ether complexes with model biomolecules, such as α-amino acids and peptides, is important for regulation of the biological activity of the many antibiotics and enzymes. Of special interest are hydrated complexes with high water contents which simulate the surroundings in vitro.

In the previous studies of this series [6, 7], the crystalline structures and thermal behaviour of the complexes consisting of some crown ethers and dipeptides were investigated by X-ray diffractometry, DSC and TG methods. As was shown [6], the dipeptide molecules are bound to 18-crown-6 through three hydrogen bonds between the peptide NH_3^+ group and the oxygen atoms of the macrocycle, resulting in an approximately perpendicu-

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lar orientation of the peptide backbone to the macrocycle plane. In the present paper, we describe the mechanism and thermokinetic parameters of the decomposition of the hydrated complexes composed of some α-amino acids, peptides and 18-crown-6, determined by differential scanning calorimetry (DSC) and thermogravimetry (TG).

EXPERIMENTAL

Preparation of the complexes

Chromatographically homogeneous peptides produced by Reanal (Hungary) were used to prepare the complexes. The purity of the purchased amino acids and peptides was above 98 mol%. Each peptide was purified by recrystallization from a water/ethanol mixture and dried under vacuum for 72 h at 330 K. Research-grade L-α-alanylglycine, glycyl-L-α-alanine, glycyl-β-alanine and trialanine from Serva were used without further purification. The purity of the purchased 18-crown-6 was 99.1 mol%, as confirmed by DSC analysis of the melting peak profiles.

The crystalline complexes were prepared by slow evaporation of the solvent from equimolar aqueous solutions of amino acids, peptides and 18-crown-6 at room temperature. In the case of 18-crown-6/glycyl-L-α-alanine/water, single crystals of the complexes were isolated from aqueous solution, also at room temperature.

DSC and TG analyses

The thermal properties of the crystalline complexes were investigated in the range 300–470 K using a Du Pont Thermal Analyst 2100, equipped with DSC 910 and TG 951 cells. The DSC device was calibrated using the indium sample from the Polish Committee for Standardization, Measures and Quality Control (Warsaw, Poland). The relative error of the measurements of the heat effect was 1% and the reproducibility of the peak temperature measurements was 0.4 K. The determined temperature of melting was 156.3 ± 0.1°C; the extrapolated onset of the melting curve should be 156.6°C. The value of the determined heat of fusion was 28.5 J g^{-1} (recommended 28.4 J g^{-1}). The determinations were started automatically after the preliminary period of time necessary for obtaining equilibrium in the device. In all the TG and DSC measurements in the first period, monotonically diminishing TG and DSC signals were observed in the range 290 K (or below) to 320 K.

Calibration of the TGA cell was made with a sample of calcium oxalate monohydrate in argon atmosphere. The inflection temperature of the first transition (dehydration) corresponds to 189.9 ± 1°C and the loss of bound water was 12.5% (theoretical 12.3%) in flow rate conditions of 100 ml min^{-1}

TABLE 1

The mass losses of the complexes from TG data recorded at a scan rate of 5 K min^{-1}

Compound	Temperature in °C			Mass loss in %
	Start	Midpoint	End	
18-Crown-6/glycine/$2H_2O$ (**I**)	32.2	79.3	105.5	10.62
18-Crown-6/diglycine/$2H_2O$ (**II**)	40.1	60.8	72.3	2.88
	72.1	89.1	102.1	4.82
18-Crown-6/triglycine/$4H_2O$ (**III**)	28.5	64.7	85.6	11.66
	86.5	98.6	115.5	1.83
18-Crown-6/alanine/$2H_2O$ (**IV**)	30.6	72.4	98.4	8.43
18-Crown-6/dialanine/$2H_2O$ (**V**)	42.8	91.7	104.2	7.41
18-Crown-6/trialanine/$2H_2O$ (**VI**)	31.4	101.7	117.5	6.7
18-Crown-6/glycyl-L-α-alanine/H_2O (**VII**)	90.6	111.5	123.0	4.47
18-Crown-6/glycyl-L-α-alanine/$12H_2O$ (**VIII**)	33.9	–	68.0	13.25
	68.0	116.9	138.5	20.54
18-Crown-6/L-α-alanylglycine/$3H_2O$ (**IX**)	28.8	61.0	76.2	8.38
	76.2	87.9	99.6	3.01
18-Crown-6/glycyl-β-alanine/$3H_2O$ (**X**)	32.3	49.8	53.3	2.36
	53.3	70.8	89.0	10.0
18-Crown-6/β-alanylglycine/$3H_2O$ (**XI**)	36.5	60.0	97.3	11.14

and a scan rate of 5 K min^{-1}. The accuracy of the weight measurements was 1%. The DSC runs were performed at a scan rate of 5 K min^{-1} in an atmosphere of flowing dry argon (100 ml min^{-1}) using open standard aluminium sample pans. On the basis of the TG curves obtained for various heating rates (1, 2, 5, 10 and 20 K min^{-1}), the activation energies of the thermal processes were obtained at the 50% conversion level [8, 9]. The TGA Decomposition Kinetics Data Analysis Program, Version 4.0 Du Pont Analyst 2000/2100, was applied for these calculations.

RESULTS

Crystals of the complexes 18-crown-6/glycine/$2H_2O$ (**I**), 18-crown-6/diglycine/$2H_2O$ (**II**), 18-crown-6/triglycine/$4H_2O$ (**III**), 18-crown-6/alanine/$2H_2O$ (**IV**), 18-crown-6/dialanine/$2H_2O$ (**V**), 18-crown-6/trialanine/$2H_2O$ (**VI**), 18-crown-6/glycyl-L-α-alanine/H_2O (**VII**), 18-crown-6/glycyl-L-α-alanine/$12H_2O$ (**VIII**), 18-crown-6/L-α-alanylglycine/$3H_2O$ (**IX**), 18-crown-6/glycyl-β-alanine/$3H_2O$ (**X**) and 18-crown-6/β-alanylglycine/$3H_2O$ (**XI**) were prepared. The results of the thermogravimetric analysis are collected in Table 1. The observed sample mass losses in the range 300–400 K are caused by the dehydration of the complexes [7, 10]. The compositions of the complexes are calculated from TGA data using the procedure described previously [7]. The water content in the complexes studied varies from 1 to 12 moles, while the crown–peptide relation was constant and approximates to 1 : 1.

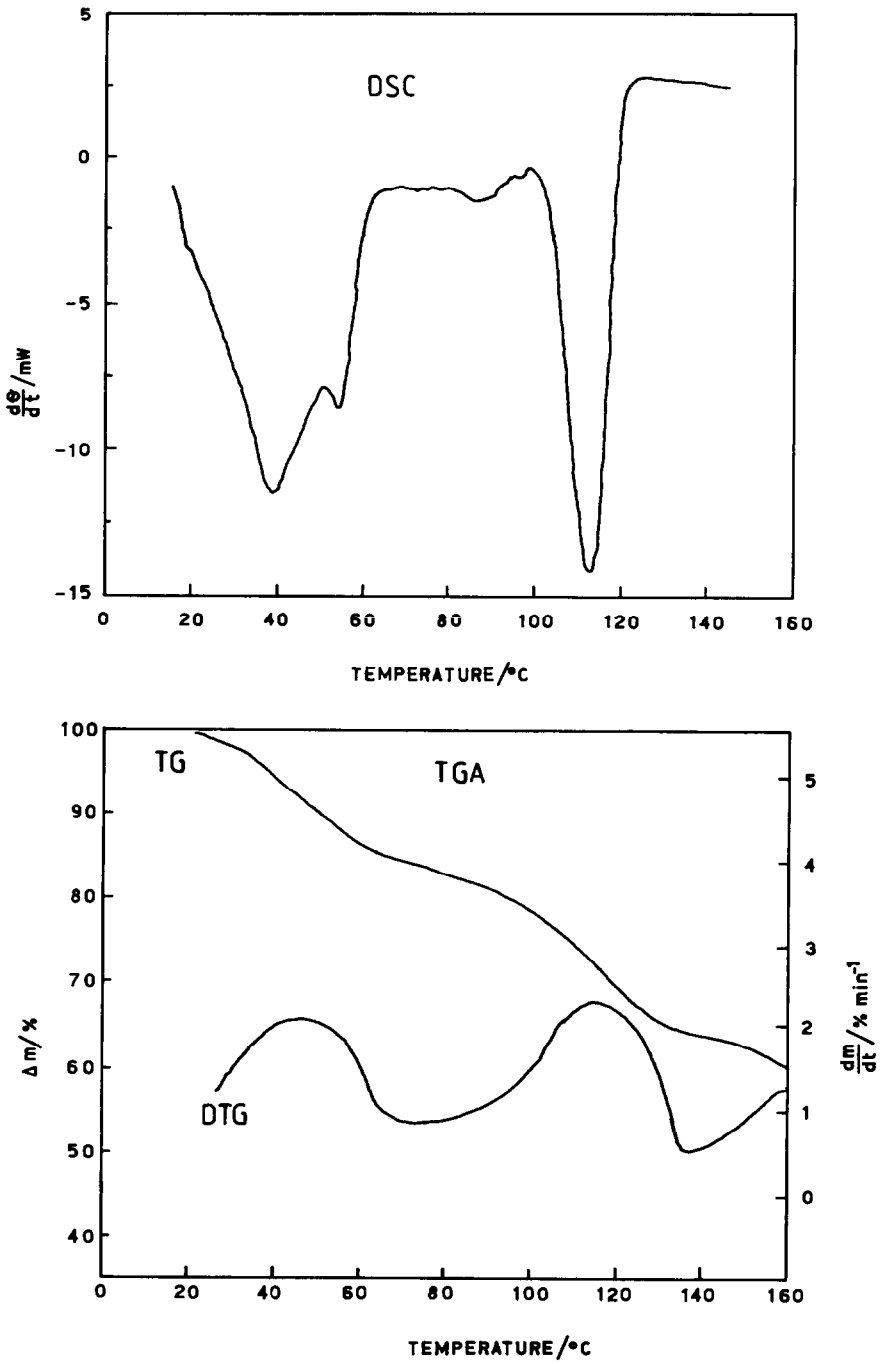

Fig. 1. The DSC and TG thermograms of heating 18-crown-6/glycyl-L-α-alanine/12H_2O (VIII).

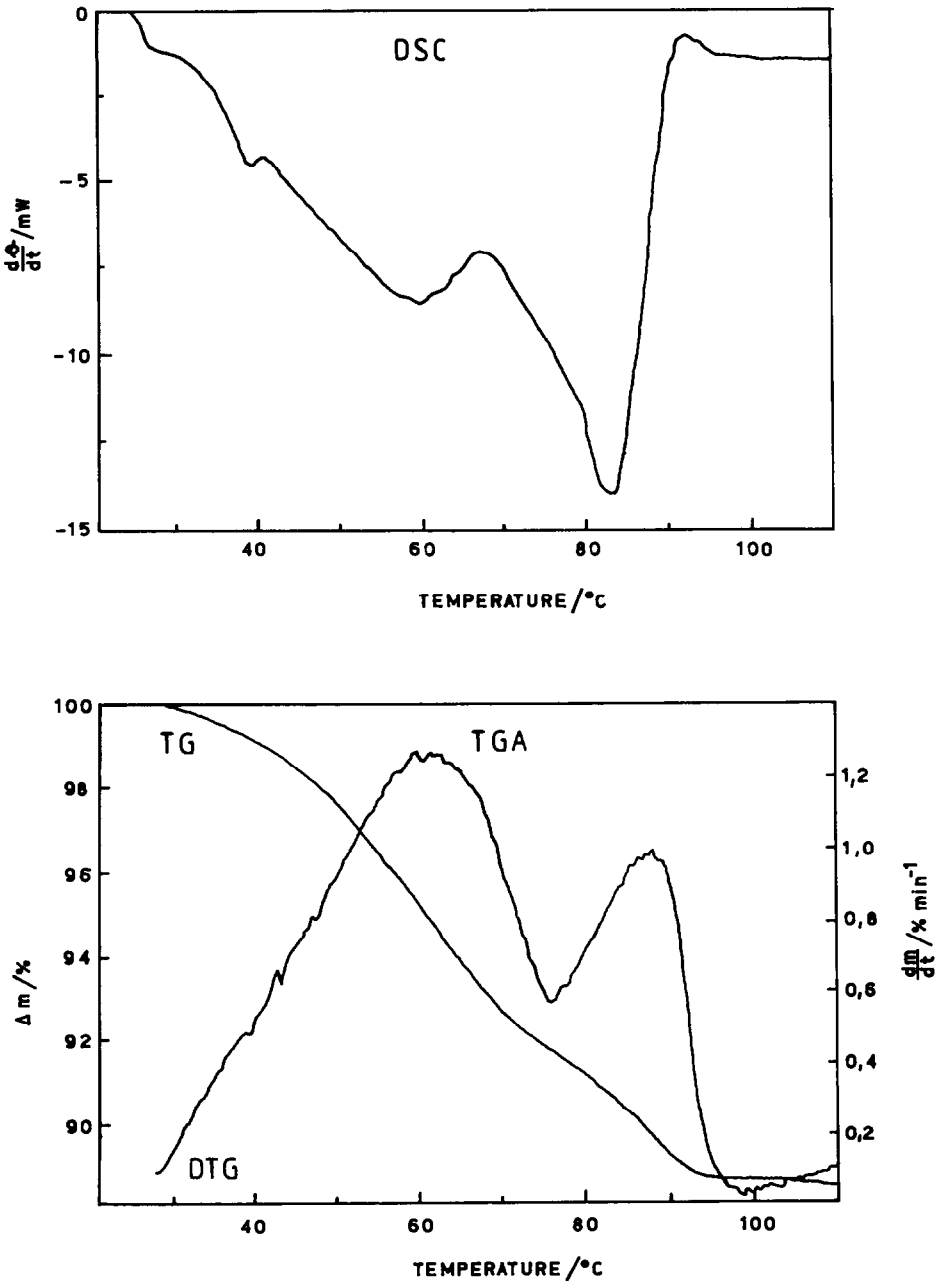

Fig. 2. The DSC and TG thermograms of heating 18-crown-6/L-α-alanylglycine/$3H_2O$ (**IX**).

The DSC and TG curves of some compounds rich in water are presented in Figs. 1–4. The observed endothermic DSC peaks correspond to the dehydration at 300–350 K, the simultaneous dehydration–dissociation at 350–390 K, and the separate ligand dissociation processes (second or third

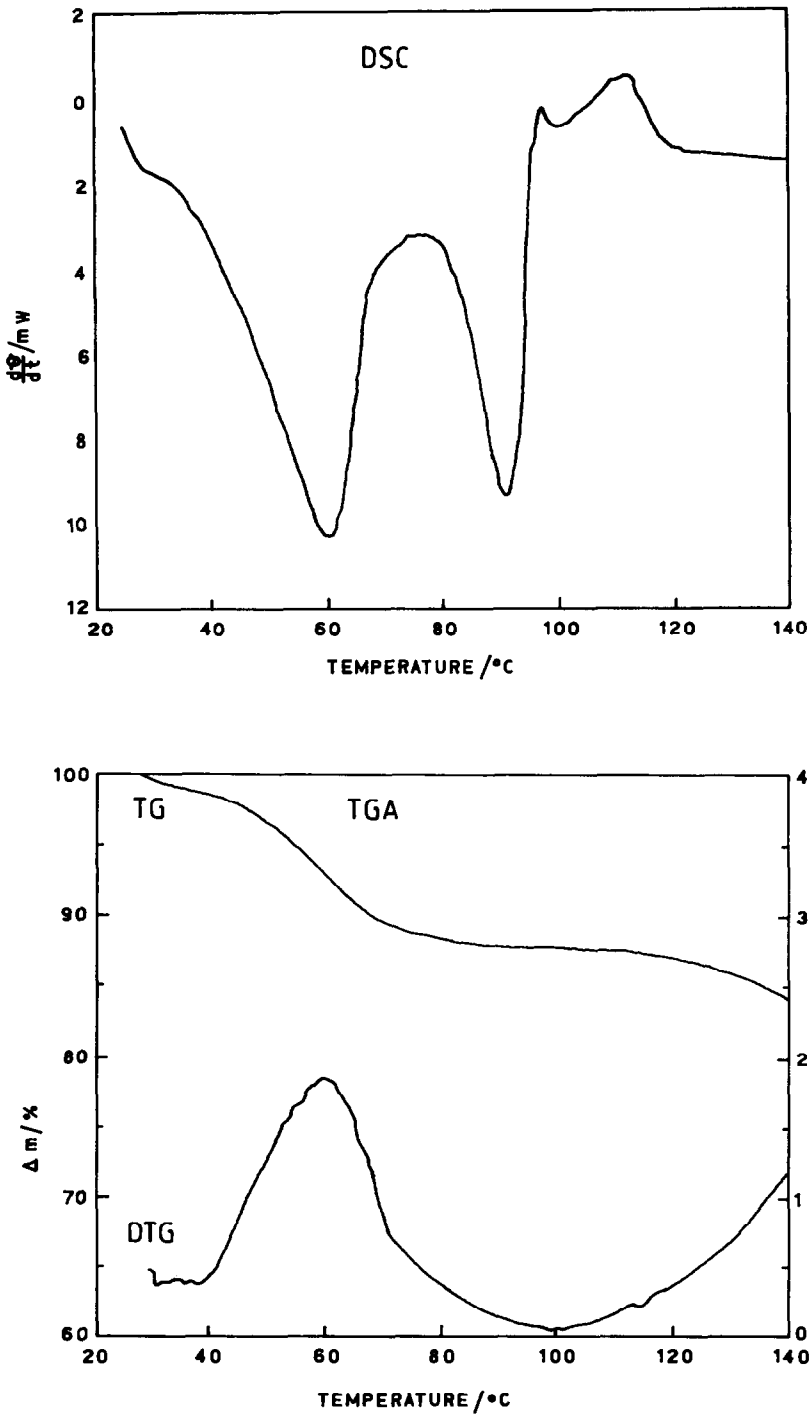

Fig. 3. The DSC and TG thermograms of heating 18-crown-6/β-alanylglycine/$3H_2O$ (**XI**).

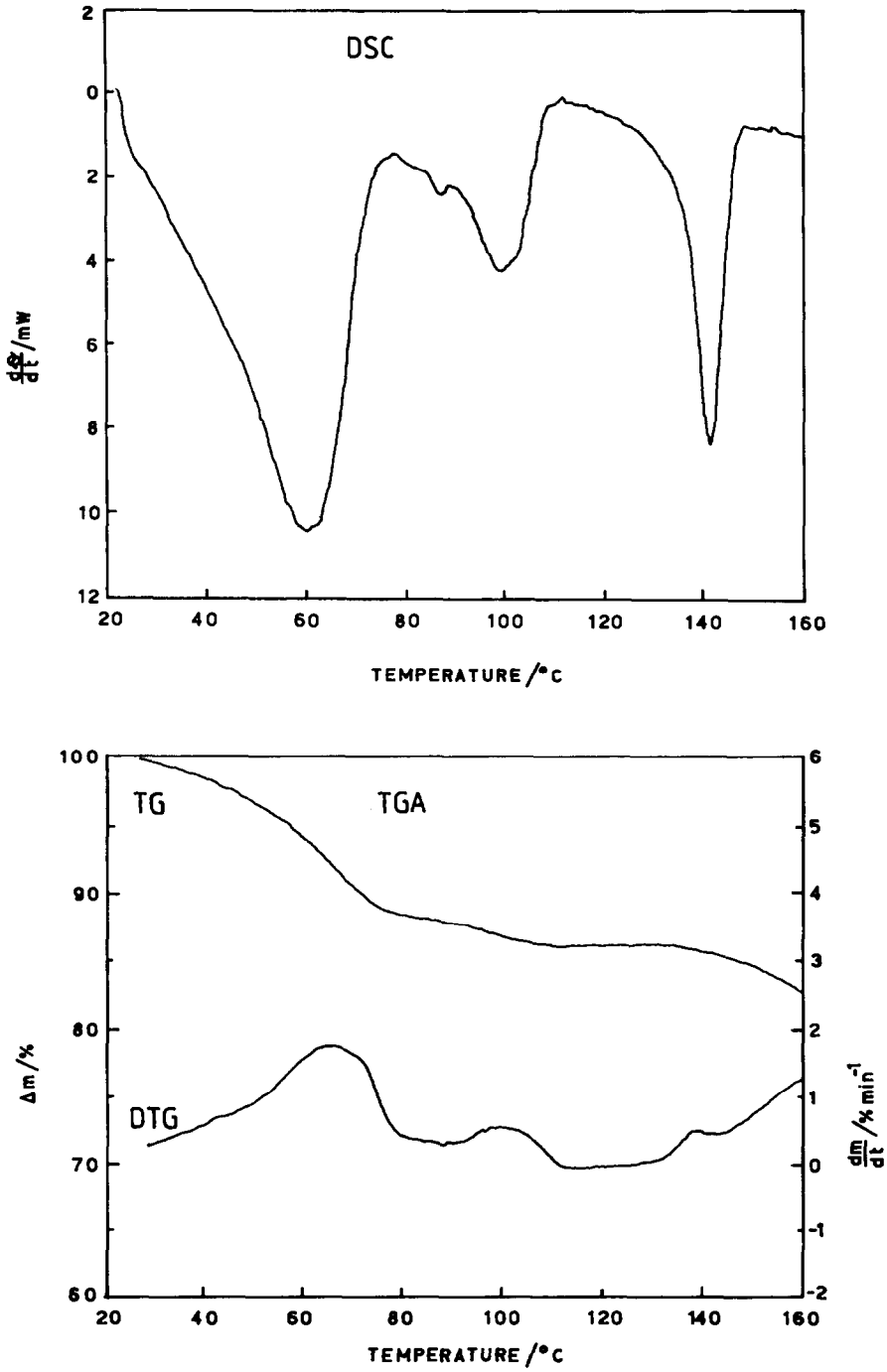

Fig. 4. The DSC and TG thermograms of heating 18-crown-6/triglycine/$4H_2O$ (**III**).

peaks). However, discernible exothermic decomposition peaks are also found for the complexes **IX** and **XI** (Figs. 2, 3) and previously for 18-crown-6/diglycine/16H_2O and 18-crown-6/dialanine [7]. The endothermic process occurring during heating of all the compounds studied is similar to that observed in complexes of 18-crown-6 with potassium thiocyanate [2] and to those of rare earth nitrates [5]: it is termed ligand dissociation. However, for the 18-crown-6/peptide/water complexes this is an irreversible process followed by the dehydration of complexes. In many complexes (**I, IV, V, VI, VII**), the beginning of the water evaporation during the heating induces simultaneous melting and dissociation and the further decomposition of the complexes. Separate dehydration and thermal dissociation processes are observed for only two compounds (**III** and **XI**) (Figs. 3, 4). The remaining complexes undergo stepwise dehydration (Figs. 1, 2, 4). This demonstrates the important role of water molecules in the formation of the crystalline structure of the crown–peptide complexes. Moreover, the high water content simulates the solvent surroundings of complexes in solution.

DISCUSSION

Mechanism of the thermal reactions of the complexes

The possible thermal reaction mechanisms of the 18-crown-6/peptide/water complexes can be represented in the following ways

(A) 18-crown-6/peptide/$nH_2O \rightarrow$ 18-crown-6 + peptide + $nH_2O\uparrow$

(B) 18-crown-6/peptide/$nH_2O \rightarrow$ 18-crown-6/peptide/$(n-i)H_2O$ + $iH_2O\uparrow \xrightarrow[-iH_2O]{}$ 18-crown-6 + peptide + $(n-i)H_2O\uparrow$

(C) 18-crown-6/peptide/$nH_2O \rightarrow$ 18-crown-6/peptide + $nH_2O\uparrow \xrightarrow[-nH_2O]{}$ 18-crown-6 + peptide

(D) 18-crown-6/peptide/$nH_2O \rightarrow$ 18-crown-6/peptide/$(n-i)H_2O$ + $iH_2O\uparrow \xrightarrow[-iH_2O]{}$ 18-crown-6/peptide + $(n-i)H_2O\uparrow \xrightarrow[-(n-i)H_2O]{}$ 18-crown-6 + peptide

Thermal decomposition according to mechanism (A) occurs in complexes **I, IV, V, VI,** and **VII**, and proceeds in one stage of simultaneous dehydration and thermal dissociation of the complex. It is very characteristic for complexes consisting of aliphatic α-amino acids and alanyl-containing oligopeptides. The complexes with glycyl-containing dipeptides, **II, VIII, IX,** and **X**, follow the three-stage decomposition according to mechanism (B). These complexes lose from 1 to 11 moles of water during the preliminary dehydration. The two-step decomposition according to mechanism (C) is

TABLE 2

DTA and TG data of dehydration of 18-crown-6/peptide complexes

Compound	T_p in K	ΔQ in kJ mol^{-1}	E_a in kJ mol^{-1}
18-Crown-6/diglycine/2H_2O	342.3	54.1	147 (4)
18-Crown-6/triglycine/4H_2O	332.4	30.9	75 (2)
18-Crown-6/glycyl-L-α-alanine/12H_2O	312.6	34.8	
	328.6		
18-Crown-6/L-α-alanylglycine/3H_2O	332.6	38.8	73 (3)
18-Crown-6/glycyl-β-alanine/3H_2O	324.2	52.9	
18-Crown-6/β-alanylglycine/3H_2O	333.4	40.8	57 (2)

observed on heating complex **XI** (Fig. 3), which first undergoes full dehydration and then ligand dissociation. The complex **III** decomposes by a similar mechanism, (D), proceeding in three stages with an additional preliminary dehydration step at 332 K, when three of the four moles of water are released from the complex (Fig. 4). The dehydration–dissociation of monohydrated complex **III** takes place at 371.5 K and the decomposition of the unhydrated complex **III** is completed at 426.0 K. Depending on the temperature of transition, water molecules effused in a liquid or vapour state.

Dehydration of the complexes

As mentioned above, the dehydration can be realized in a two-step process. The thermokinetic characteristics of dehydration are presented in Table 2 and the preliminary dehydration processes in the range of 300–350 K are illustrated in Figs. 1–4. Higher values of the temperature, heat effect and activation energy are found for **II**, indicating the strong specific hydration of this complex which can hold about 16 water molecules, four of which are strongly bound by hydrogen bonds [6, 7]. The water-rich crystallohydrate **VIII**, however, is not so stable and loses its water at a lower temperature, accompanied by a lower heat of dehydration. The complex **X**, composed of rigid dipeptide molecules similar to diglycine, also has a high heat of dehydration. The activation energy of dehydration of the complexes studied is contained within the range of values found for complexes of rare earth nitrates with 18-crown-6 [5], whereas the heat effects of dehydration in the latter case are higher. Therefore it can be concluded that the dehydration heat effects of both types of complex are determined by the specificity of the interaction of water with the guest molecule (peptide or nitrate). This conclusion is in accordance with the X-ray data [6], indicating that stable hydrogen bonds of water are only formed with the dipeptide molecules. The activation energy of dehydration decreases over the series **II** > **III** > **IX** > **XI**.

TABLE 3

DSC and TG data of decomposition of 18-crown-6/peptide complexes

Compound	M_c [a]	T_p in K [b]	ΔQ in kJ mol^{-1} [c]	E_a in kJ mol^{-1}
18-Crown-6/glycine/2H_2O	375.4	343.1	129	69 (2)
18-Crown-6/diglycine/H_2O	414.5	364.9	107	106 (4)
18-Crown-6/diglycine/2H_2O	432.5	377.7	139	–
18-Crown-6/triglycine/H_2O	471.5	372.5	24	126 (6)
18-Crown-6/triglycine	453.5	414.4	28	–
18-Crown-6/alanine/2H_2O	389.4	333.8	111	53 (1)
18-Crown-6/dialanine/2H_2O	460.5	361.2	133	100 (3)
18-Crown-6/trialanine/2H_2O	531.6	371.1	117	109 (3)
18-Crown-6/glycyl-L-α-alanine/H_2O	428.5	380.4	91	94 (3)
18-Crown-6/L-α-alanylglycine/H_2O	428.5	355.6	94	105 (1)
18-Crown-6/glycyl-β-alanine/2H_2O	446.5	345.6	139	57 (2)
18-Crown-6/β-alanylglycine	410.5	363.8	54	–

[a] Molecular mass of complexes. [b] Peak temperatures. [c] All values were calculated using the molecular mass in the first column.

Decomposition of the complexes

As can be seen in Table 3, the peak temperatures and the activation energies of decomposition vary within a wide range of values. They are lowest for complexes of α-amino acids and higher for the complexes of triglycine and trialanine (**VI**). The correlation between the peak temperatures and the activation energies of decomposition is presented in Fig. 5. These are the main characteristics of the thermal stability of the crystalline complexes. The molecular packing of 18-crown-6/dipeptide consists of zones of lower polarity formed by double layers of 18-crown-6 and zones of higher polarity formed by the peptides and water. The oxygen atoms from the free carboxylic group of the peptide are involved with the water molecules in the formation of a three-dimensional network of hydrogen bonds [6]. These water molecules are important for bonding the layered structures. In the case of dipeptide complexes, hydrogen bonds are also formed between the amide groups of the anti-parallel peptide backbones of the neighbouring complexes. This also stabilizes the crystalline structures in comparison with α-amino acid complexes, which are not able to form direct hydrogen bonds between the complexes. Such a possibility is only realized through water molecules. Thus, complexes **I** and **IV** decompose according to mechanism (A) with the lowest temperatures and activation energies of decomposition. In contrast, bonding between two pairs of amide groups in tripeptides can be formed if the steric arrangement is favourable. This probably takes place in anhydrous 18-crown-6/triglycine because it has a higher decomposition temperature than that of complex **II**. The linear

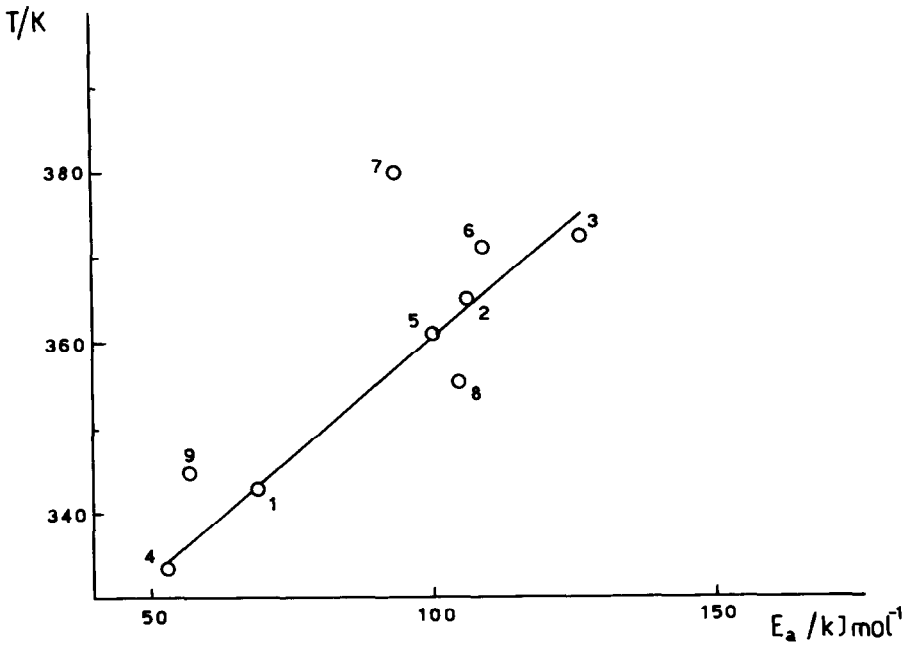

Fig. 5. Correlation between the temperatures of the decomposition peak and the activation energies of decomposition of the complexes: 1, complex **I**; 2, 18-crown-6/diglycine/H_2O; 3, 18-crown-6/triglycine/H_2O; 4, complex **IV**; 5, complex **V**; 6, complex **VI**; 7, complex **VII**; 18-crown-6/L-α-alanylglycine/H_2O; 9, 18-crown-6/glycyl-β-alanyl/$2H_2O$.

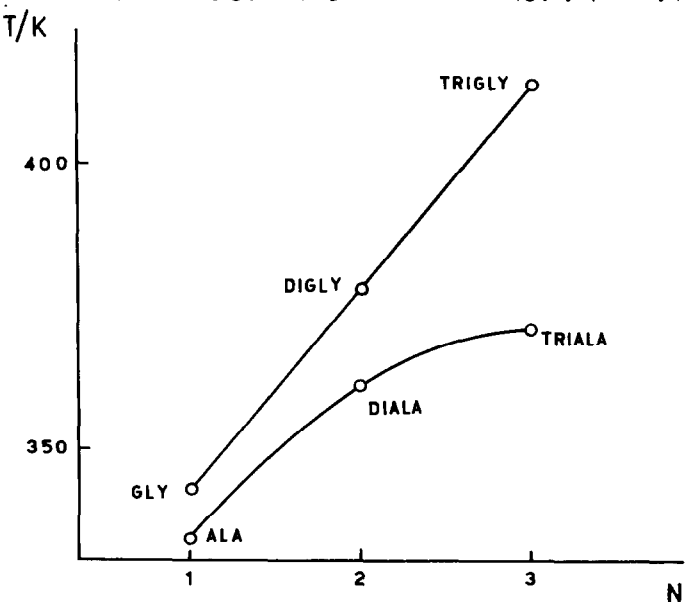

Fig. 6. The temperatures of the decomposition of the peptide complexes with 18-crown-6 versus the number of amino acid residues N in the peptide: Gly, 18-crown-6/glycine/$2H_2O$ (**I**); Digly, 18-crown-6/diglycine/$2H_2O$ (**II**); Trigly, 18-crown-6/triglycine; Ala, complex **IV**; Diala, complex **V**; Triala, complex **VI**.

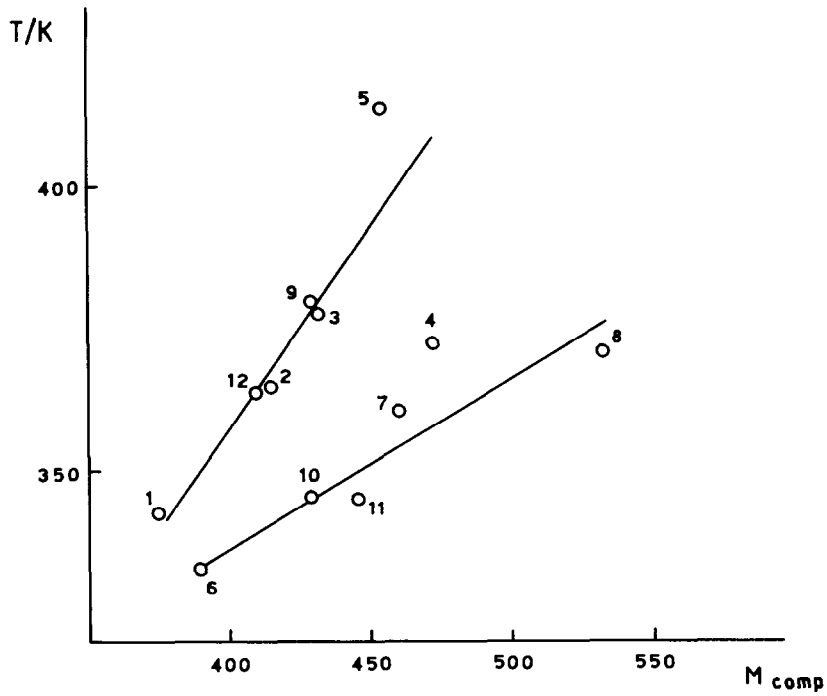

Fig. 7. The temperatures of the decomposition peak versus the molecular mass of the complexes: 1, complex **I**; 2, 18-crown-6/diglycine/H_2O; 3, complex **II**; 4, 18-crown-6/triglycine/H_2O; 5, 18-crown-6/triglycine; 6, complex **IV**; 7, complex **V**; 8, complex **VI**; 9, complex **VII**; 10, 18-crown-6/L-α-alanylglycine/H_2O; 11, 18-crown-6/glycyl-β-alanine/$2H_2O$; 12, 18-crown-6/β-alanylglycine.

dependence of the peak temperatures versus the number of glycyl residues can be observed in Fig. 6; this relationship was not observed for the alanyl-containing complexes **IV**, **V**, **VI** (Fig. 6).

The same regularities are also found for the dependence of the decomposition temperature on the molecular mass of the complexes. As can be seen in Fig. 7, the decomposition temperatures of complexes **I**, **II**, **VII**, **XI**, 18-crown-6/diglycine/H_2O and 18-crown-6/triglycine increase rapidly with increasing molecular mass of the complex, while those of **IV**, **V**, **VI**, **IX**, and **X** increase more slowly, resulting in two straight lines of different slopes. The first group, except **XI**, includes compounds in which the peptides are bound to 18-crown-6 through the glycyl group. The behaviour of the β-alanyl group of **XI** can be considered as very similar to that of the glycyl group on bonding with 18-crown-6. The second group, except **X**, consists of the compounds complexed with 18-crown-6 by the alanyl group. These two types of bonding essentially differ from each other in the "depth of penetration" of peptide into the macrocycle cavity [6]. The closer approach of the glycyl group to the macrocycle plane evidently determines the greater

stability of these complexes in comparison with that of complexes bound to 18-crown-6 through the alanyl group. Thus, the molecular recognition of α-amino acids and peptides by 18-crown-6 in the crystalline state is significantly reflected in the thermokinetic characteristics of their complexes.

We assume that the important information about the specificity of the peptide–crown bonding may be obtained from the heat effects of the decomposition. However, because of the numerous factors (the dehydration of the complexes, the vaporization of the water released, the melting of 18-crown-6, the dissociation of the complexes, etc.) contributing to these values, it is difficult to perform their correct analysis.

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