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COMPLEX FORMATION OF *D*-MANNONAPHTO-18-CROWN-6-ETHER AND ENANTIOMERS OF PHENYLALANINE IN WATER STUDIED BY DIFFERENT PHYSOCHEMICAL METHODS

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Complex formation of *D*-mannonaphto-18-crown-6-ether **1** with *D*- and *L*-phenylalanine (Phe) and their derivatives was studied using conduction and titration microcalorimetry in aqueous solution, and solvent–solvent (water–chloroform) extraction. The thermal effects accompanying the complexation process were determined, but the chiral recognition effects were very small. The chiral differentiation of amino acid was observed in the experiments of the extraction from water to chloroform phase containing chiral receptor.

Keywords: *amino acid, calorimetry, chiral receptor, crown ethers, molecular recognition*

Introduction

Amino acids and peptides are important as the biological compounds, which can be produced by the synthetic and biotechnological methods. The purification of the received products, particularly enantiomeric purity is rather a difficult task. Equally essential, as far as the environment is concerned, is the removal of biological substances from waste. In the methods used at present in the process of removal of amines, amino acids and peptides an important role is played by compounds, which can bind them, forming intermolecular complexes. Crown ethers have been well known as such compounds [1, 2]. They can bind amino acids by forming hydrogen bonds between oxygen atoms of macrocyclic rings and protonated NH_3^+ group of amino acid. The very useful tool for studying the effects accompanying the binding processes is titration microcalorimetry [3–5]. Our earlier study has been shown the chiral differentiation of the chosen amino acids in the process of the solvent–solvent extraction, the transport through liquid and supported membrane, and HPLC by means of the chiral crown ethers with sugar units [6–9]. The example of such type of compound is crown ether **1**, which was designed and synthesized in order to examine the possibility of chiral recognition of amino acids. The structure of crown ether **1** is shown in Fig. 1.

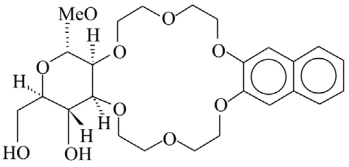

Fig. 1 The structural formula of crown ether **1**

The aim of our study was to examine the complexation process of the enantiomeric phenylalanine in the three forms: zwitterion, hydrochloride and potassium salt by use the calorimetric methods and water–chloroform extraction.

Experimental

Materials

Phenylalanine (Phe) was purchased from Fluka and used as received. Crown ether **1** was synthesized individually by Pietraszkiewicz [6]. Aqueous solutions were prepared with deionized and distilled water (Mili-Q System, Millipore USA).

Methods

TG

The determination of water content in the crystalline crown ether **1** was performed using DuPont TGA 951 thermogravimetric analyser in the range of 298.15–373.15 K.

Conduction calorimetry

Thermal effects accompanying the complexation of several *D*, *L* enantiomers of Phe, PheK and PheHCl with crown ether **1** in aqueous solution were studied using a home-made heat conduction microcalorimeter of sensibility equal to 103 μV mW^{-1} [10]. The vessel was loaded with 2 cm^3 of 0.01 mol dm^{-3} solution of ether **1** and then inserted into the measurement posi-

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tion. After temperature equilibration, using a dosimetric device 0.2 cm^3 of 0.1 mol dm^{-3} amino acid solution was injected into the vessel during 75 s, at a constant rate. The thermal effect of dilution of the amino acid in water was also determined by the same procedure. Stirring (rate 60 rpm) was applied in each measurement. On the basis of the recorded calorimetric signal the heat effects, Q, of reaction were evaluated: total heat, Q_{compl}, and the course of thermal power, $W(t)$. Both quantities were evaluated assuming relation $Q_{compl}=Q_{overall}-Q_{dilution}$.

Titration calorimetry

Titration calorimetry experiments were carried out using isothermal titration calorimeter Omega-ITC (Microcal Inc. Northampton MA). The microcalorimetric titrations were performed at 298.15 K in aqueous solutions.

In each run a constant volume (8 μL/injection) of *D*- and *L*-phenylalanine hydrochloride solution (0.1 or 0.04 mol dm^{-3}) in the 250 μL syringe was injected into 1.3186 cm^3 stirred (400 rpm) sample cell containing crown ether solution (0.01 or 0.004 mol dm^{-3}).

Each titration experiment consists of 30 successive injections.

The Origin software (Microcal Inc.) was used to calculate the thermal effects of complexation. Heat effects of dilution were subtracted from effects of complexation.

Single injection experiments were also performed using Omega-ITC calorimeter at 298.15 K in aqueous solutions. The single injection of 250 μL of *D*- and *L*-PheHCl (0.04 mol dm^{-3}) was added to vessel containing crown ether **1** solution (0.004 mol dm^{-3}) during 185 s.

Extraction experiments

Extraction water–chloroform experiments were carried out at 298.15 K for 3 h. The concentration of crown ether **1** in chloroform phase was equal 10^{-3} mol dm^{-3}. The water phase initially contained 10^{-3} mol dm^{-3} of enantiomers of Phe, and their potassium salts or hydrochlorides, respectively. The equilibrium was reached after 3 h of stirring at 200 rpm of chloroform phase containing crown ether **1** and water phase containing amino acids.

The concentration of amino acids in water phase was determined by UV spectroscopy (Varian Cary 1E) at 260 nm and in water and chloroform phase by HPLC. Blind tests for crown ether **1** by UV spectroscopy at 309 and 312 nm, indicated that the partition coefficient of crown ether between chloroform and water was ca. 1000 indicating that the concentration of the carrier in water was negligible.

Results and discussion

The molecular structure of crown ether **1**, as determined in X-ray measurement by Lipkowski [11], is shown in Fig. 2. There are two conformers of crown ethers and seven molecules of water in the crystallographic unit cell. The same amount of water was confirmed by TG measurements, as shown in Fig. 3. The presence of water molecules (one or two) in the crystal structures of crown ethers was frequently observed [12]. The thermal effects derived from conduction calorimetry are presented in Table 1. On one hand, for the three forms of phenylalanine: zwitterion, potassium salt and hydrochloride the heats of complexation are different as expected, both in the sign and magnitude, and on the other hand for each couple of *D*, *L* isomers these heats are very similar to each other. More detailed information on the reaction under study can be derived from the thermal power courses, shown in Fig. 4. The results presented confirm the very poor differentiation of *D*, *L* isomers in aqueous solution in complexation. The similar character of heat changes of complexation in time for *D* and *L* enantiomers in each form of Phe suggests analogous structure of the complexes. On the other hand, very different values of total heat and thermal power courses for another form of amino acid enantiomers

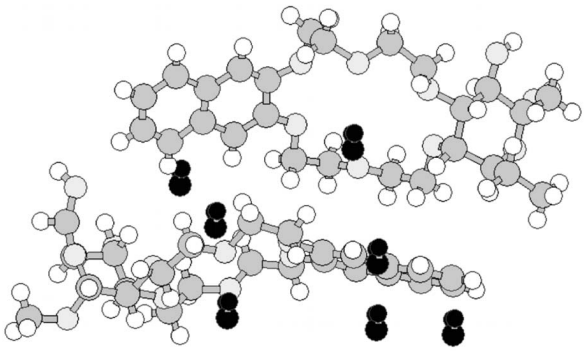

Fig. 2 The molecular structure of crown ether **1**. In black water molecules

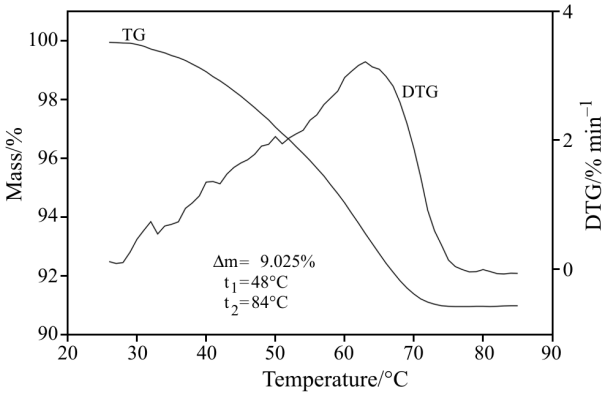

Fig. 3 TG and DTG curves of crystalline crown ether **1**

Table 1 Total heat effects of complex formation of enantiomeric phenylalanine in three forms: zwitterion, potassium salt and hydrochloride with crown ether **1**

Amino acid	Total heat, Q		
	Overall/ J	Dilution/ J	Complexation/ kJ mol^{-1}
L-Phe	–0.0218	0.0037	–1.28
D-Phe	–0.0250	–0.0025	–1.13
L-PheK	0.0512	0.0198	1.57
D-PheK	0.0501	0.0120	1.90
L-PheHCl	0.1081	0.0068	5.07
D-PheHCl	0.1044	0.0022	5.12

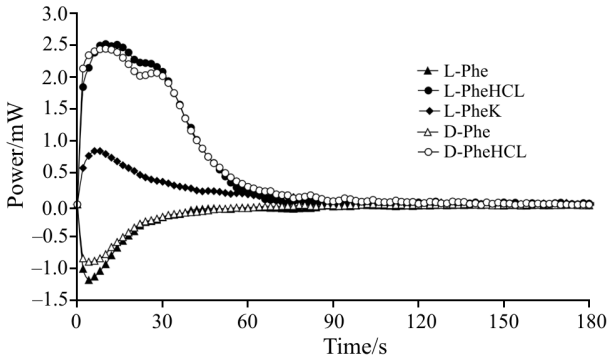

Fig. 4 The changes of power during complexation of three forms of enantiomeric Phe (zwitterion, potassium salt and hydrochloride) using conduction calorimetry at 298.15 K

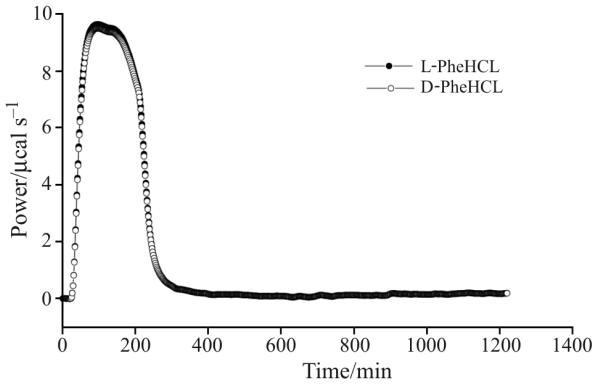

Fig. 5 The changes in heat of complex formation between crown ether **1** and *D*-, *L*-Phe hydrochloride during single injection experiment at 298.15 K using Omega ITC Titration calorimeter

suggest quite different and complicated mechanism of the reaction under study. At the end of the injection the molar ratio of amino acid to ether was 1:1. In all cases the courses of $W(t)$ reveal intensive heat generation at an early stage of the injection. It means that more than one molecule of the ether bond with one molecule of amino acid. The solution of this problem needs the use of more appropriate models of processes taking place there [13].

Table 2 Water–chloroform extraction data for three forms of enantiomeric phenylalanine

Amino acid	Partition coefficient, $k_{o/w}$	α_E
L-Phe	$7.7 \cdot 10^{-2}$	5.5
D-Phe	$1.4 \cdot 10^{-2}$	
L-PheK	$4.9 \cdot 10^{-2}$	7.3
D-PheK	$0.67 \cdot 10^{-2}$	
L-PheHCl	$2.7 \cdot 10^{-2}$	0.43
D-PheHCl	$6.3 \cdot 10^{-2}$	

$$\alpha_E = \frac{k_{o/w}(L)}{k_{o/w}(D)}$$

In order to compare the results obtained by two calorimetric techniques we performed a similar experiment with phenylalanine hydrochloride in aqueous solutions by titration calorimetry. In Fig. 5 the thermal effect of complexation during a single injection experiment is shown. The data obtained during titration experiments confirm earlier results.

In order to check the possibility of achieving chiral discrimination of three forms of Phe enantiomers studied we performed water–chloroform extraction using chiral crown ether **1**.

The values of partition coefficient for three forms of Phe were presented in Table 2. These data show that during the extraction process the chiral recognition takes place. The highest differences were observed for *D* and *L* enantiomers of potassium salt of Phe.

Conclusions

In aqueous solutions low heats of complexation and poor chiral recognition of amino acids enantiomers by crown ether **1** were observed; it could be explained by the presence of water molecules in the structure of ether. In such a case the competition of amino acids with water during a complex formation is possible [14]. The other characteristics in thermal courses for three forms of Phe suggested different architecture of complexes. The positive thermal effects for potassium salt of Phe and its hydrochloride form and negative for zwitterion species may be connected with different interaction characters between amino acid and ether species. The protonated NH_3^+ group of amino acid molecule in zwitterion form may form hydrogen bonds with oxygen atoms in macrocyclic ring. In the case of amino acid salt, potassium from carboxylate group of phenylalanine may be connected with macrocyclic ring. Hydrochloride forms another type of complexes. In extraction chiral recognition takes place for all pairs of enantiomers amino acids studied which suggests the decisive role of the phase transfer phenomena.

The results confirm that both calorimetric techniques may be used for study of complexation of enantiomeric species. The information obtained by these methods can be useful in design of purification methods and ways of removal of biological substances from waste. These can be very important in the problem of environmental protection.

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