

# Interactions of amino acids with $\beta$ -cyclodextrin and with potato starch studied by thermogravimetric measurements

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**Abstract** The paper discusses the interactions of selected aromatic  $\alpha$ -amino acids (phenylalanine (P), histidine (H) and tyrosine (T)) with  $\beta$ -CD and potato starch in solid state. Thermal analysis of CD mixtures with P, H and T was performed using two procedures, 1 (with EtOH) and 2 (without EtOH); the simultaneous TG-DSC measurements were made. The thermal behavior of investigated systems indicates the formation of inclusion complexes. The theoretical AM1 studies confirm the experimental results. Moreover the described studies show that  $\beta$ -cyclodextrin can be a convenient model of starch. This resemblance can be useful for examination of starch–amino acids interactions.

**Keywords**  $\beta$ -cyclodextrin · Amino acids · Complexes · TG-DSC measurements

## Introduction

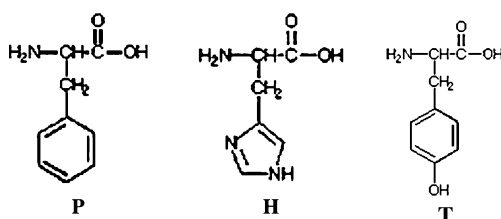
Cyclodextrins, which are torus-shaped cyclic oligosaccharides consisting of six or more  $\alpha$ -(1, 4)-linked D-glucopyranose units are well known receptors capable of forming inclusion complexes (host–guest complexes) with a wide variety of organic molecules having a complementary shape. The complexation can result in changes of the physical, chemical and biological properties of the guest molecule and might have a considerable

pharmaceutical potential [1, 2]. Besides pharmaceutical applications for drug release, cyclodextrins can be employed in environmental protection, since they effectively immobilise toxic or harmful compounds inside their cavities. Cyclodextrins are excellent models of enzymes, these properties have led to their use as catalysts, both in enzymatic and nonenzymatic reactions. For instance cyclodextrin complex with two imidazole residues is a convenient model of ribonucleases [14]. The complexation of  $\alpha$ -cyclodextrin with *o*-imidazolylbenzoic acid leads to a model of chymotrypsin [15].

Formation of inclusion complexes was investigated mainly by  $^1\text{H}$  NMR [3, 11],  $^{13}\text{C}$  NMR [1] HPLC [4], UV spectroscopy [5], X-ray diffraction analysis, IR spectroscopy [1] and thermal analysis [2, 5, 12, 13]. Many scientists have applied theoretical methods such as molecular mechanics, molecular dynamics and CNDO with fixed geometry to cyclodextrin inclusion complexes. However, there are very few quantum mechanical calculations on cyclodextrin using more advanced semiempirical methods, i.e. AM1 with full geometry optimisation [6].

In this paper, the interactions of the aromatic  $\alpha$ -amino acids P, H, T with  $\beta$ -cyclodextrin, denoted as CD were investigated in the solid state by Simultaneous TG-DSC measurements and semiempirical AM1 calculations. These investigations follow our interest in the thermogravimetric and DSC studies of binary solid state systems: starch–amino acids and amylopectin–amino acids [16, 17]. Because of the complex nature of starch thermolysis, a precise measurement of the accompanying weight loss and heat changes process cannot be made, therefore the usefulness of the cyclodextrin as a model of starch was discussed. In experiments, three amino acids, i.e. P, H, T were used, for every experiment two procedures, with ethanol (procedure 1) and without ethanol (procedure 2) were tested.

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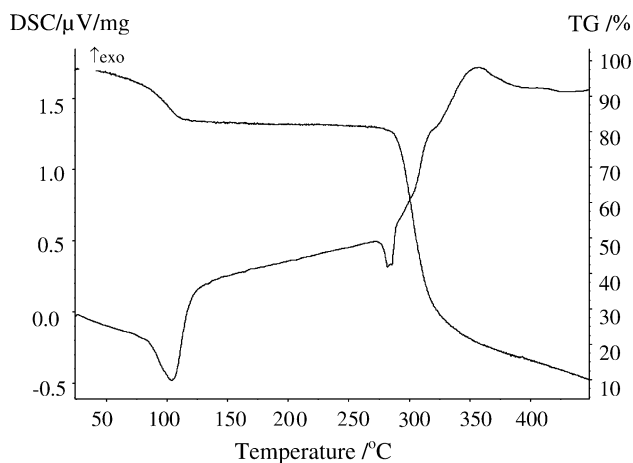
## Experimental

The  $\beta$ -cyclodextrin ( $\beta$ -CD)–amino acid (P, H, T) complexes were prepared according to Szafran and Pawlaczyk [5]. These complexes were available from blending CD with 10% (w/w) admixture of any of the amino acids, using procedures 1 or 2, i.e. with ethanol (10 ml) or without ethanol, respectively. The mixtures were ground in an agate mortar for 1 h. The experimental measurements were carried out by means of the NETZSCH STA 409 simultaneous thermal analyser under argon flow in the range from room temperature to 500 °C at a heating rate of 5 °C/min. Recorded thermograms were analysed with the NETZSCH-TA-ANALYSIS and NETZSCH SEPARATION OF PEAKS programmes.

The AM1 method was applied for geometry optimisation and heat of formation calculation of CD and its complexes with amino acids by using MOPAC 6.0. The visualisation of these complexes was obtained with HyperChem 5.02.

## Results and discussion

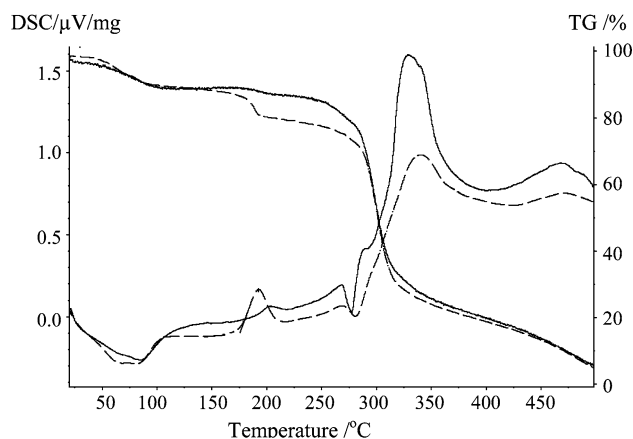
The trends in thermal behaviour for the compounds examined and the values of the thermogravimetric and thermodynamic parameters relating to the TG and DSC measurements are given below.



**Fig. 1** TG-DSC curves of  $\beta$ -cyclodextrin

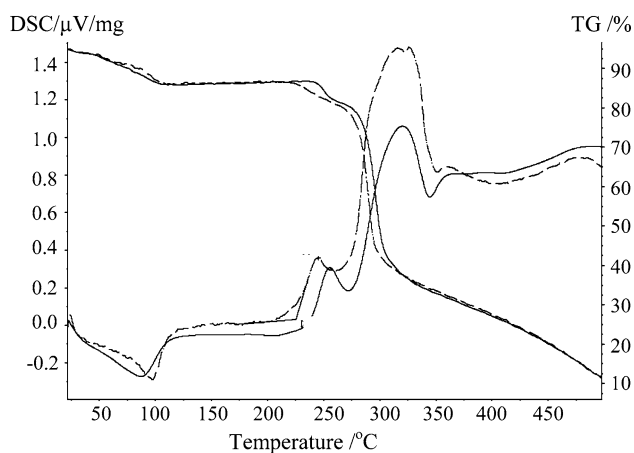
**Table 1** Thermodynamic parameters of thermal decomposition of  $\beta$ -CD/P, H, T mixtures obtained from TG – DSC measurements

Compound	TG		DSC	
	$T_i/^\circ\text{C}$	W/ %	$T_o/^\circ\text{C}$	$T_p/^\circ\text{C}$
$\beta$ -CD	69.8	14.6	76.9	103.8 (endo)
	268.4	56.4	277.6	281.7 (endo)
	335.1	14.0	298.0	360.0 (exo)
$\beta$ -CD + P (physical mixture)	50.7	7.0	38.8	85.2 (endo)
	178.9	1.6	267.7	277.6 (endo)
	244.6	60.4	291.7	328.5 (exo)
$\beta$ -CD + P (ethanolic mixture)	64.4	8.4	45.3; 74.2	64.4 (endo); 72.4 (endo)
	173.8	6.1	<b>175.2</b>	<b>191.8 (exo)</b>
	267.3	45.5	273.7	284.4 (endo)
	319.1	25.1	292.8	343.0 (exo)
	323.4	22.5	292.8	338.4 (exo)
$\beta$ -CD + H (physical mixture)	48.27	7.4	37.8	84.2 (endo)
	181.3	2.2	254.7	272.5 (endo)
	240.2	54.2	293.4	319.4 (exo)
$\beta$ -CD + H (ethanolic mixture)	54.4	5.4	70.8	59.3 (endo);
	172.8	3.1	<b>240.2</b>	<b>213.5 (exo)</b>
	249.3	37.3	273.7	282.3 (endo)
	323.4	22.5	292.8	338.4 (exo)
$\beta$ -CD + T (physical mixture)	53.5	3.0	33.2	82.1 (endo)
	169.7	4.8	254.6	268.3 (endo)
	242.9	54.3	301.3	331.4 (exo)
$\beta$ -CD + T (ethanolic mixture)	61.3	3.7	42.5	62.1 (endo)
	177.5	6.9	<b>225.4</b>	<b>207.6 (exo)</b>
	257.2	43.2	259.6	283.2 (endo)
	322.6	13.4	297.5	316.7 (exo)

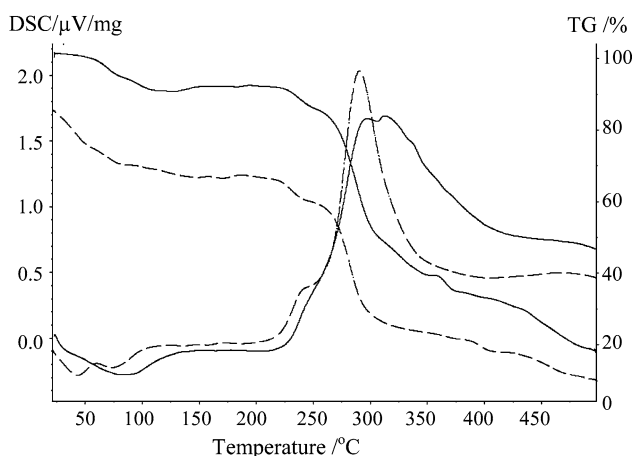


**Fig. 2** TG-DSC curves of  $\beta$ -CD/P without (solid line) and with (dashed line) EtOH

The TG-DSC-gram for CD shows three thermal effects (Fig. 1, Table 1). The first endothermic peak between 70 and 125 °C with 15% weight loss corresponds to the dehydration of CD. The second small endothermic peak



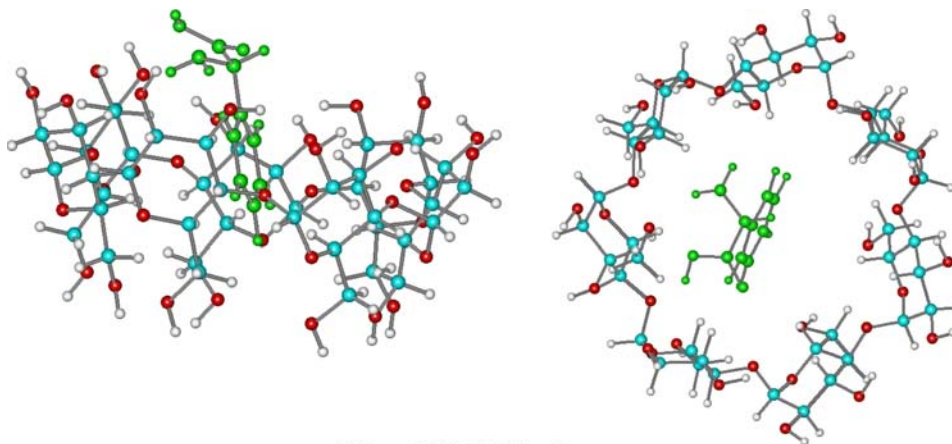
**Fig. 3** TG-DSC curves of  $\beta$ -CD/T without (solid line) and with (dashed line) EtOH



**Fig. 4** TG-DSC curves of  $\beta$ -CD/H without (solid line) and with (dashed line) EtOH

represents a process corresponding to a molecular reorganisation of CD [7]. The third step is related to the decomposition of the CD structure. These three effects can be observed in TG-DSC-gram of CD with P, H, T.

**Fig. 5** Calculated geometry and heat of formation of CD/P inclusion complex



$$\Delta H_f = -1536.39 \text{ kcal}$$

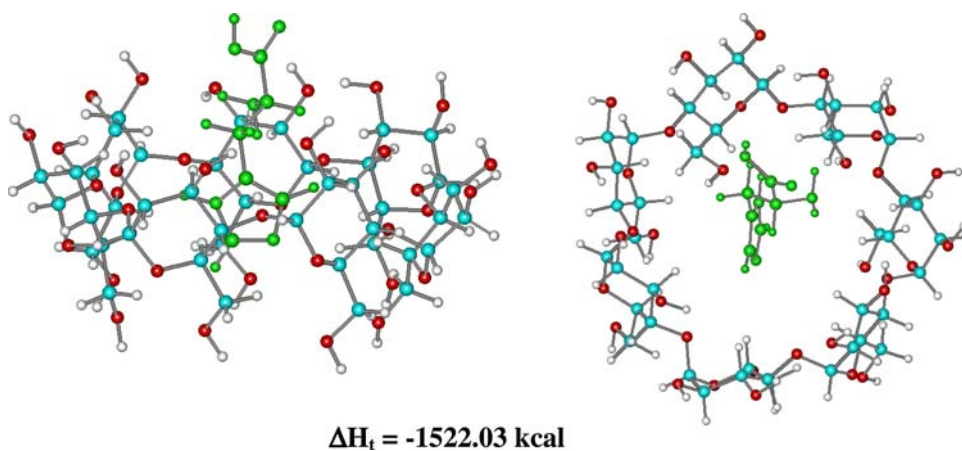
It was found, that for P and for H the CD/amino acid complexes are formed only in the procedure 1, however for T the formation of CD/T complex occurs during both procedures.

In the TG-DSC-gram of CD/P in procedure 1 (Fig. 2, Table 1) the first, small exothermic peak at  $T_o = 175^\circ\text{C}$  is attributed to the formation of an inclusion complex. The TG-DSC-gram for a mixture of CD with T (Fig. 3) resembles that for P with CD. In TG-DSC-gram of CD/T mixture (Fig. 3), in procedures 1 and 2, the peak indicating the inclusion complex appears at  $225^\circ\text{C}$ . It should be mentioned that such peak is not observed in TG-DSC-grams of CD/H mixture in both, 1 and 2, procedures (Fig. 4). The TG-DSC-grams of CD mixtures with P, H and T, in both 1 and 2, procedures, show that the formation of an inclusion complex is accompanied by decomposition of the CD structure and by other processes occurring with the participation of atmospheric oxygen (broad exothermic DSC peak at  $T_o = 253^\circ\text{C}$ ).

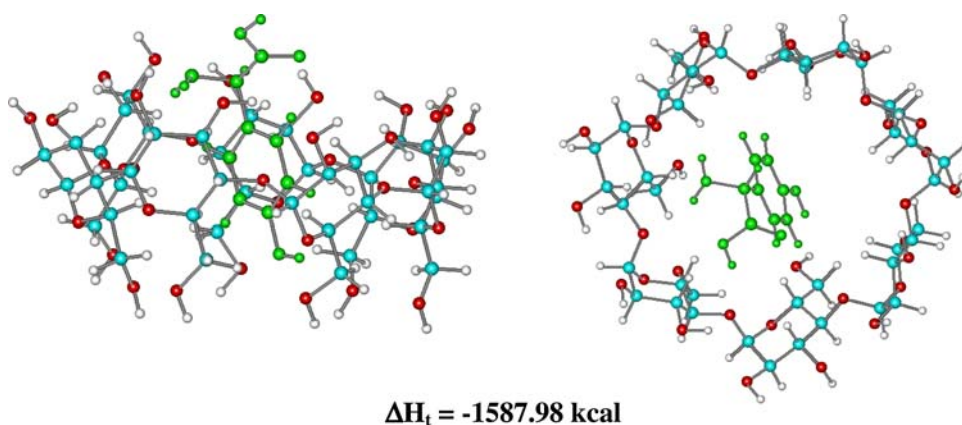
On the basis of our calculations, the attractive interactions reflected in the appearance of an exothermic peak in DSC curves of the three amino acids P, H, T with CD result from their insertion into CD cavity.

The above results are confirmed by the theoretical AM1 studies. These calculations show structures of complexes of CD with P, H, T (Figs. 5, 6, 7). Stability of them decreasing in the order CD/T, CD/P and CD/H, i.e. with smaller size of aromatic moiety of amino acids and lower degree of their substitution. The CD/T inclusion complex (Fig. 7) is favoured ( $-1587.98 \text{ kcal/mol}$ ) in comparison to CD/P complex (Fig. 5) and  $\beta$ -CD/H complex (Fig. 6) for which the heats of formation are  $-1536.39$  and  $-1522.03 \text{ kcal/mol}$ , respectively. The calculations indicate that the aromatic ring of amino acids P, H and T is included in CD cavity and that the hydrophilic part of these amino acids is situated outside the CD torus. The calculations also show the formation of three  $\beta$ -CD/amino acid complexes is an endothermal process. The results of theoretical studies are in good agreement with experimental data.

**Fig. 6** Calculated geometry and heat of formation of CD/H inclusion complex

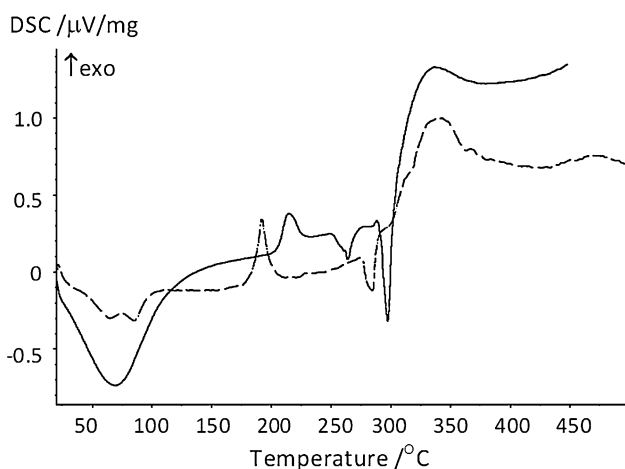


**Fig. 7** Calculated geometry and heat of formation of CD/T inclusion complex

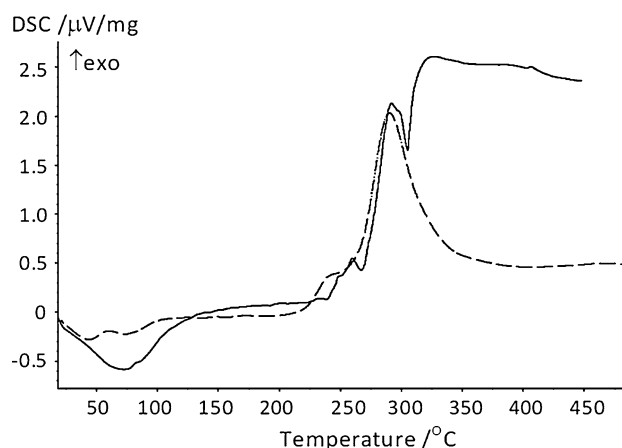


Reactions of nitrogen containing compounds, mainly amino acids, with mono-, di- and oligosaccharides deserve an attention. Thermal reactions between biogenic amino acids and saccharides result in the formation of nitrogenous heterocyclic compounds such as pyrrole, pyrazine and pyridine derivatives which provide so-called secondary food aromas.

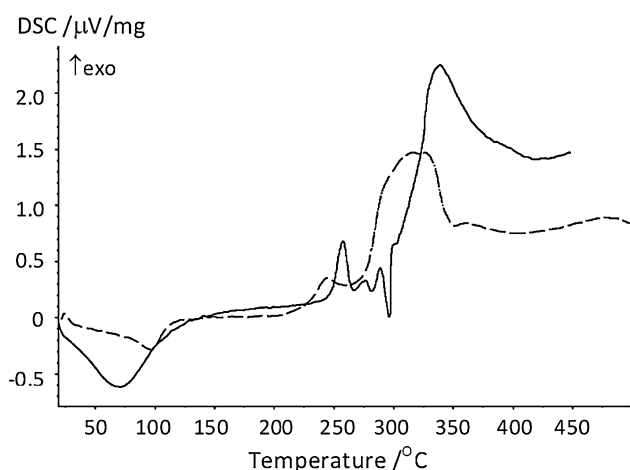
Recently, considerable attention has been paid to the thermal generation of fragrances from systems of biogenic  $\alpha$ -amino acids with polysaccharides such as potato starch. These reactions are difficult to control because of the complex nature of starch thermolysis. Therefore, knowledge of the processes taking place during thermolysis of these systems as well a precise choice of reaction



**Fig. 8** DSC-grams of starch/P mixture (*solid line*) and CD/P mixture in EtOH (*dashed line*)



**Fig. 9** DSC-grams of starch/H mixture (*solid line*) and CD/H mixture in EtOH (*dashed line*)

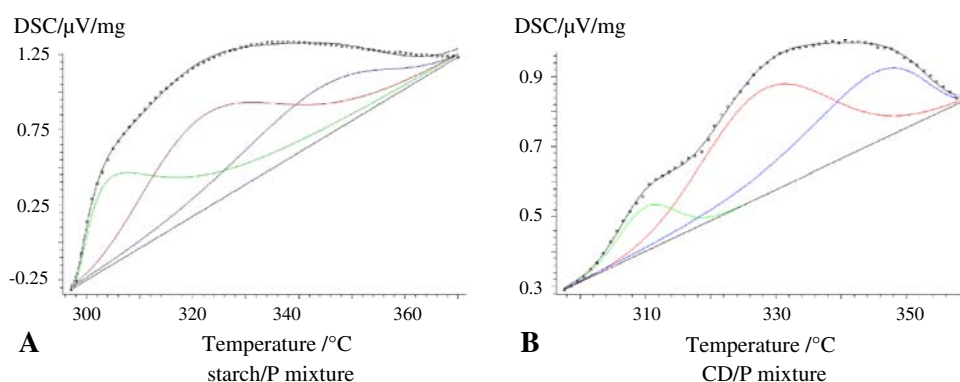


**Fig. 10** DSC-grams of starch/T mixture (solid line) and CD/T mixture in EtOH (dashed line)

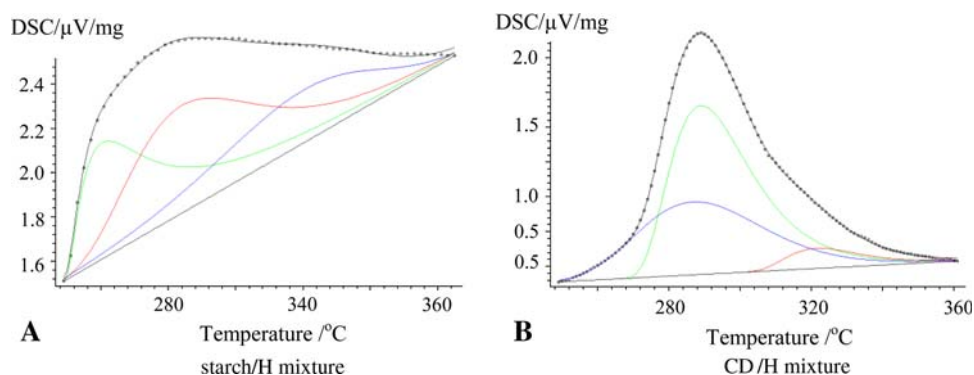
parameters would be beneficial for food, pharmaceutical and cosmetic industries. In our investigations we propose  $\beta$ -cyclodextrin as a good model of starch. For this purpose we carried out additional comparative DSC measurements replacing CD by potato starch (Figs. 8, 9, 10).

In the temperature range of 20–500 °C the mixtures of potato starch with amino acids P, H and T decompose similarly as ethanolic mixtures of CD with P, H and T. For DSC-gram of P, H and T with starch, the first peak is endothermic. It corresponds to the dehydration of polysaccharides. The second, exothermic peak indicates an interaction between starch and amino acids. Under the conditions applied, the amino acids either solubilize and develop as a thin film on the granule surface or penetrate into the granule interiors forming amino acids—amylase inclusion complexes [9]. This effect is similar as in the case

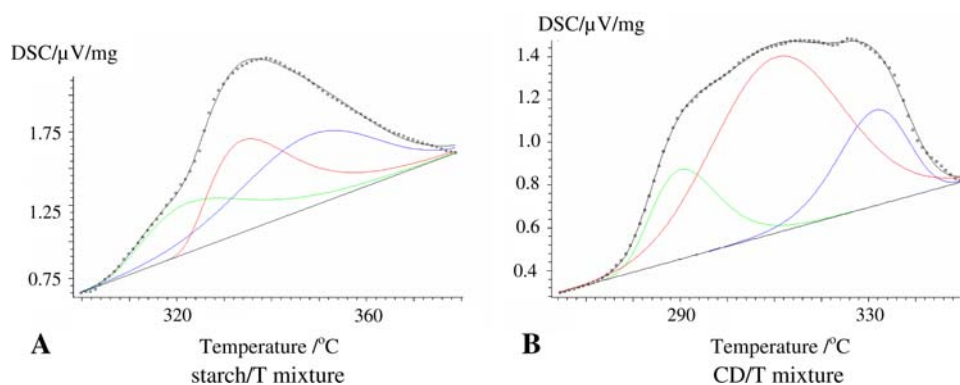
**Fig. 11** The peaks position determined from separation of broad exothermic peak appeared in the DSC-gram of starch mixture with P and CD mixture with P and EtOH



**Fig. 12** The peaks position determined from separation of broad exothermic peak appeared in the DSC-gram of starch mixture with H and CD mixture with H and EtOH



**Fig. 13** The peaks position determined from separation of broad exothermic peak appeared in the DSC-gram of starch mixture with T and CD mixture with T and EtOH



**Table 2** The peaks position (in °C) determined from separation of broad exothermic peak appeared in the DSC-gram of starch/P, H, T mixtures and CD/P, H, T mixtures

	Starch			$\beta$ -cyclodextrin		
	310.2	332.3	355.3	314.9	345.4	369.2
P	305.1	322.8	343.7	310.2	328.4	345.2
H	310.1	322.0	340.1	288.1	288.5	314.1
T	319.7	333.9	350.5	290.1	310.6	331.5

of mixtures of CD with P, H and T in EtOH. It is commonly known that the broad, exothermic peak observed in the DSC curve of mixture starch/amino acids in the temperature range at 300–400 °C corresponds to the evolution of low molecular weight products formed with or without the participation of atmospheric oxygen [10]. This peak determines the thermal stability of starch mixtures with P, H and T. We compared the transformations taking place during thermolysis of mixtures CD/P, H and T with those observed for mixtures starch/P, H, T above 300 °C. For this purpose we performed a separation of discussed peaks using Fraser-Suzuki Profile. Results of these mathematical operations are given in Figs. 11, 12, 13.

The DSC-gram of CD/H mixture shows, that the main peak of the decomposition of CD/H is strong and distinct in the contrary to starch/P, H and T mixtures and CD/P and T mixtures (Table 2). Comparison of the DSC peak positions reveals that CD/amino acids ethanolic mixtures decompose like as mixture starch/amino acids. Particularly, the CD/P ethanolic mixture shows similar thermal properties to starch/P mixture.

## Conclusions

This work shows formation of inclusion complexes of  $\beta$ -CD with three amino acids P, H and T. The above complexes were obtained with and without ethanol, i.e. using procedures 1 and 2, respectively. T was observed, that P and H form inclusion complexes only in procedure 1, and not in procedure 2. However T forms the inclusion complex in both, 1 and 2, procedures. TG-DSC measurements results have shown a similarity of interactions of CD and starch with P, H and T. The general trend stability determined by the theoretical calculations is in agreement with the experimental results.

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