Carbohydrate Research 344 (2009) 304–310

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

The influence of selected *O*-alkyl derivatives of cyclodextrins on the enzymatic decomposition of L-tryptophan by L-tryptophan indole-lyase

Tomasz Gubica, Elżbieta Winnicka, Andrzej Temeriusz *, Marianna Kańska

Department of Chemistry, University of Warsaw, Pasteura 1, PL-02093 Warsaw, Poland

ARTICLE INFO

Article history:
Received 18 August 2008
Received in revised form 27 October 2008
Accepted 4 November 2008
Available online 13 November 2008

Keywords:
Cyclodextrins
L-Tryptophan
L-Tryptophan indole-lyase
Inhibition
Inclusion complexes

ABSTRACT

A series of *O*-alkyl derivatives of cyclodextrin: *heksakis*[2,3,6-tri-*O*-(2′-methoxyethyl)]-α-cyclodextrin; *heksakis*(2,3-di-*O*-methyl)-α-cyclodextrin; *heptakis*(2,3-di-*O*-methyl)-β-cyclodextrin; *heksakis*[2,3-di-*O*-methyl-6-*O*-(2′-methoxyethyl)]-α-cyclodextrin; *heptakis*[2,3-di-*O*-methyl-6-*O*-(2′-methoxyethyl)]-β-cyclodextrin; *heksakis*[2,3-di-*O*-(2′-methoxyethyl)]-α-cyclodextrin and *heptakis*[2,3-di-*O*-(2′-methoxyethyl)]-β-cyclodextrin have been synthesized. Purity and composition of the obtained substances were examined. The cyclodextrin derivatives listed above as well as (2-hydroxypropyl)-α-cyclodextrin and (2-hydroxypropyl)-β-cyclodextrin, the two commercially available ones, have been investigated as the additives in the course of enzymatic decomposition of L-tryptophan by L-tryptophan indole-lyase. It has been found that each of cyclodextrin derivatives causes the inhibition of enzymatic process, both competitive and non-competitive. The competitive inhibition is connected with the formation of inclusion complexes between cyclodextrins and L-tryptophan, related to the geometry of these complexes. The mechanism of the non-competitive inhibition is not so evident; it could be related to the formation of the cyclodextrin complexes on the surface of the enzyme, leading to the change in the flexibility of the enzyme molecule.

1. Introduction

Cyclodextrins (cyclomaltohexaose, cyclomaltoheptaose or cyclomaltooctaose) are a group of cyclic oligosaccharides composed of 6, 7 or 8 α-D-glucopyranosidic units, called α-, β- and γ-CDs, respectively.[1] The molecular structure of CDs resembles a truncated cone with a centrally situated cavity, which is relatively hydrophobic. In contrast to the cavity, the rims of a particular CD are hydrophilic. These properties of CDs are responsible for their specific complexation properties. The CDs can form non-covalent inclusion complexes with a wide range of guest molecules, including compounds with aromatic moieties. Due to such unique ability of host–guest complexes formation, the CDs have found a wide range of applications, for example, in pharmaceutics, chromatography and enzyme mimicking.[2] Therefore, an increased interest in applications of CDs in enzymology has been observed in the past few years.[3–8]

The CDs are used in enzyme studies as additives[3] (which alter the rate of the enzymatic reactions) and also as covalent modifiers of the enzymes[4] (the modified enzyme was more catalytically active and thermally stable than the native one). Another example of the applications of modified CDs in enzymology has been the co-lyophilization of CDs with the enzymes.[5,6]

In our previous work, we studied the effect of the addition of permethylated cyclodextrins on the enzymatic reaction of tyrosine phenol-lyase.[7] For both permethylated α- and β-CDs, we have noticed the inhibition of the enzymatic reaction. On the other hand, the permethylated γ-CD has caused the activation of tyrosine phenol-lyase.

We have also investigated the influence of the addition of native and permethylated α-, β- and γ-CDs on the decomposition of L-tryptophan by the enzyme L-tryptophan indole-lyase.[8] It has been found that each of the studied CDs caused the inhibition of the enzymatic process. The observed inhibition has been of a mixed type, both competitive and non-competitive, in each case. The competitive inhibition was connected with the formation of inclusion complexes between L-tryptophan, the substrate and the CDs under investigation. The non-competitive inhibition was also most likely connected with the formation of inclusion complexes, but, this time, between the enzyme and the CDs. Such complexes could be formed on the surface of the enzyme between its aromatic amino acid residues and the CDs, possibly by changing the conformational flexibility of the tryptophanase, thus inhibiting the enzymatic reaction.

Interestingly, it is worth mentioning that for the competitive inhibition of the enzymatic decomposition of L-tryptophan, we

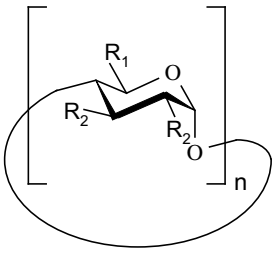
* Corresponding author. Tel.: +48 22 822 2325; fax: +48 22 822 5996.
E-mail address: atemer@chem.uw.edu.pl (A. Temeriusz).

0008-6215/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2008.11.004

did not find the proportionality between the stability constants of the inclusion complexes between the molecules of L-tryptophan and CDs, and the inhibitory effect on the enzymatic reaction. According to the simplest assumption that the stronger inclusion complexes between the substrate of the enzymatic reaction, the lower concentration of the substrate and then the stronger competitive inhibition should be observed did not work. We explained this contradictory result assuming that some L-tryptophan and the CD inclusion complexes, particularly the one with the native β-CD, the stability constant of which was the highest among all investigated inhibitors, should also take part in the enzymatic reaction. Supporting literature[9] indicates that the aliphatic chain of L-tryptophan in its complex with native β-CD is exposed outside the molecular cavity of the CD, while the indole moiety is entrapped inside the CD molecule. So, the simplest assumption leads to the statement that such a complex is still reactive because the aliphatic chain of L-tryptophan is exposed on the attack of the enzyme. And contrary to relatively high stability constant of this complex, the addition of native β-CD caused rather weak inhibition. Although the stability constant between native α-CD and L-tryptophan was definitely lower in comparison to the corresponding complex with native β-CD, the stronger inhibition of the enzymatic reaction was observed for native α-CD contrary to native β-CD. Such and an observation could be explained assuming that, in contrast to β-CD, L-tryptophan accommodates differently in the molecular whole of native α-CD. This time, the aliphatic chain of this amino acid penetrates the CD cavity[10] and therefore such complexes must be unreactive, because the aliphatic fragment of L-tryptophan is not available for the enzyme. Although the amount of these complexes in the solution is lower than that in the case of much stronger complexes with native β-CD, the inhibition in the case of native α-CD is much stronger than that of the latter CD.

The observed phenomenon connected with the competitive inhibition related to the stability constants and the geometry of the formed complexes (between L-tryptophan and CDs) has encouraged us for further investigations. In this paper, we report the effects of a large group of alkylated CDs on the catalytic activity of L-tryptophan indole-lyase (EC 4.1.99.1, TPase). The selected group of *O*-alkyl derivatives of CDs includes *heksakis*[2,3,6-tri-*O*-(2′-methoxyethyl)]-α-cyclodextrin (**1**, TME-α-CD); *heksakis*(2,3-di-*O*-methyl)-α-cyclodextrin (**2**, DM-α-CD); *heptakis*(2,3-di-*O*-methyl)-β-cyclodextrin (**3**, DM-β-CD); *heksakis*[2,3-di-*O*-methyl-6-*O*-(2′-methoxyethyl)]-α-cyclodextrin (**4**, MEDM-α-CD); *heptakis*[2,3-di-*O*-methyl-6-*O*-(2′-methoxyethyl)]-β-cyclodextrin (**5**, MEDM-β-CD); *heksakis*[2,3-di-*O*-(2′-methoxyethyl)]-α-cyclodextrin (**7**, DME-α-CD); *heptakis*[2,3-di-*O*-(2′-methoxyethyl)]-β-cyclodextrin (**9**, DME-β-CD); (2-hydroxypropyl)-α-cyclodextrin (**10**, THP-α-CD) and (2-hydroxypropyl)-β-cyclodextrin (**11**, THP-β-CD).

The structural formulas of above listed CDs are depicted in Scheme 1. These CDs have been chosen in order to evaluate the relationship between the geometry of inclusion complexes and the values of competitive inhibition. Since the variety of chosen substituents of the protons at primary (at C-6) and secondary (at C-2,3) hydroxyl groups of CDs plays the important role on the size and shape of host molecules. For example, CH_3OCH_2– groups in permethylated α-, β- and γ-CDs cause that shallower CD rims are closed, and a molecule of these CDs is resembled to a bowl.[11]



	Compound	n	R_1	R_2
1	TME-α-CD	6	$CH_2O(CH_2)_2OCH_3$	$O(CH_2)_2OCH_3$
2	DM-α-CD	6	CH_2OH	OCH_3
3	DM-β-CD	7	CH_2OH	OCH_3
4	MEDM-α-CD	6	$CH_2O(CH_2)_2OCH_3$	OCH_3
5	MEDM-β-CD	7	$CH_2O(CH_2)_2OCH_3$	OCH_3
7	DME-α-CD	6	CH_2OH	$O(CH_2)_2OCH_3$
9	DME-β-CD	7	CH_2OH	$O(CH_2)_2OCH_3$
10	THP-α-CD	6	$CH_2OCH_2CH(OH)CH_3$	$OCH_2CH(OH)CH_3$
11	THP-β-CD	7	$CH_2OCH_2CH(OH)CH_3$	$OCH_2CH(OH)CH_3$

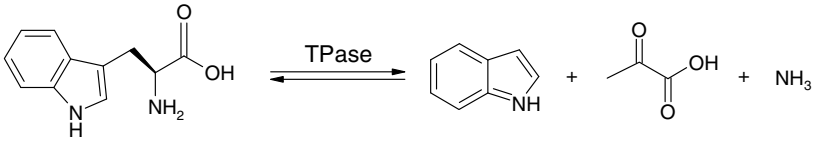
Scheme 1. Structural formulas of selected *O*-alkyl derivatives of CDs added in the course of decomposition of L-tryptophan by L-tryptophan indole-lyase.

2. Results and discussion

In the course of the catalytic reaction of tryptophanase, TPase selected *O*-alkyl derivatives of CDs (Scheme 1) have been added in order to assess their potential inhibitory effects on this enzyme. TPase catalyzes the decomposition of L-tryptophan to the corresponding indole, pyruvic acid and ammonia[12] (Scheme 2). According to the reaction depicted in Scheme 2, there was a possibility of observing either the inhibition or the activation of the enzymatic process in the presence of CDs. Since the CD molecules can include either the substrate (L-tryptophan) or the product (indole) of the reaction under investigations. If the CD molecules form the inclusion complexes with the substrate, the inhibition should be observed, while if the product of enzymatic reaction is entrapped into cavity of a particular CD, the activation of the reaction can be expected.

Figures 1 and 2 show Lineweaver–Burk plots for the TPase catalytic decomposition of L-tryptophan in the presence and absence of α-CDs and β-CDs, respectively. Figures 3 and 4 depict the inverse maximal velocities ($1/V'_{max}$) versus the concentrations of a particular α-CD and β-CD, respectively. The dependences of the Michaelis constants (K'_m) on the concentrations of either α-CDs (Fig. 5) or β-CDs (Fig. 6) are also shown. Figure 7 and 8 combine the inhibition constants, both competitive (K_i^1) and non-competitive (K_i^2) for α-CDs and β-CDs, respectively. Table 1 summarizes all the $1/V'_{max}$ as well as K'_m, K_i^1 and K_i^2 values.

According to the obtained results (Figs. 1–8 and Table 1), the inhibition of the TPase enzymatic reaction in the presence of CDs



Scheme 2. Decomposition of L-tryptophan catalyzed by TPase enzyme.

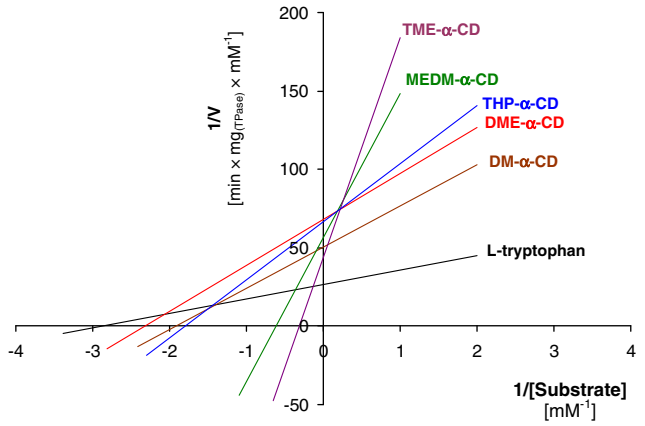


Figure 1. Lineweaver–Burk plot of TPase-catalyzed decomposition of L-tryptophan in the presence and absence of particular α-CD derivative. The obtained plot represents average results for all investigated concentrations of CDs.

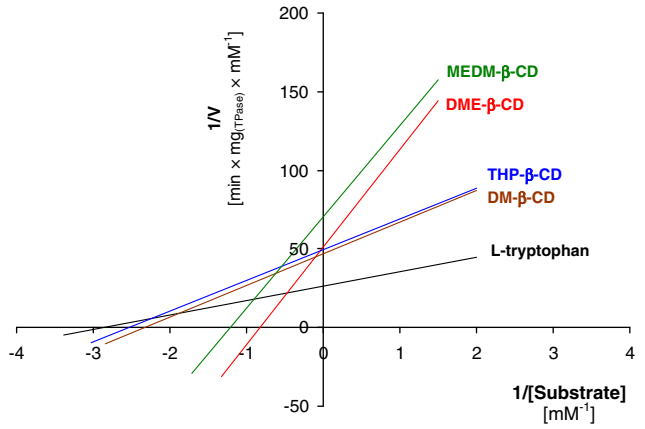


Figure 2. Lineweaver–Burk plot of TPase-catalyzed decomposition of L-tryptophan in the presence and absence of particular β-CD derivative. The obtained plot represents average results for all investigated concentrations of CDs

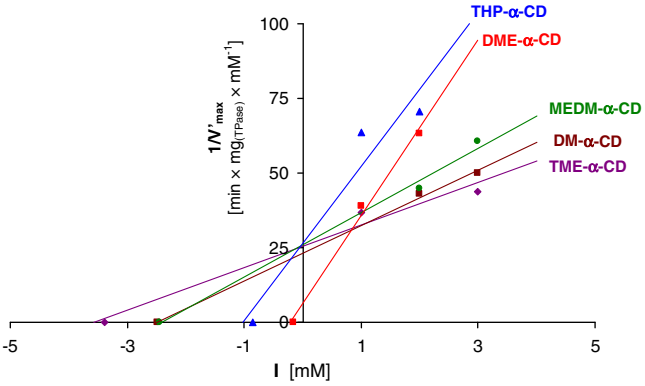


Figure 3. The dependence of the inverse maximal velocities $(1/V'_{max})$ on the concentrations of particular α-CD derivative. The non-competitive inhibition constants are depicted as the points on the *x* axis.

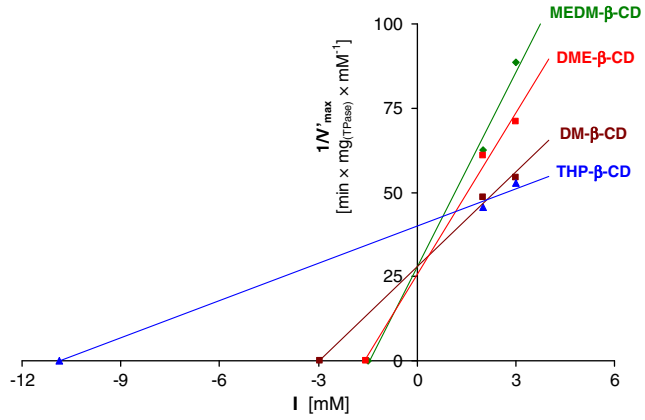


Figure 4. The dependence of the inverse maximal velocities $(1/V'_{max})$ on the concentrations of particular β-CD derivative. The non-competitive inhibition constants are depicted as the points on the *x* axis.

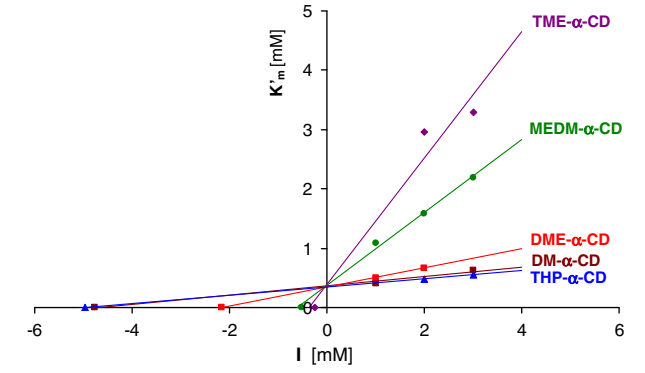


Figure 5. The dependence of the Michaelis constants (K'_m) on the concentrations of particular α-CD derivative. The competitive inhibition constants are depicted as the points on the *x* axis.

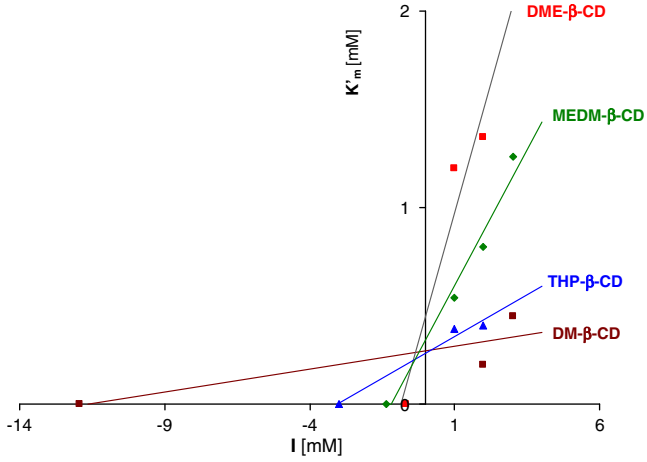


Figure 6. The dependence of the Michaelis constants (K'_m) on the concentrations of particular β-CD derivative. The competitive inhibition constants are depicted as the points on the *x* axis.

has been found in each case. Moreover for each CD derivative under investigation, an inhibition of mixed type has been observed, i.e. competitive and non-competitive. While the competitive inhibition is likely to be connected with formation of inclusion complexes between L-tryptophan and CDs, the cause of observed non-competitive inhibition is not so evident. But this type of inhibition could be explained as a result of disadvantageous influence of CDs on the conformational flexibility of TPase. Such influence could be followed by formation of inclusion complexes between aromatic amino acid residues on the surface of TPase and CDs.

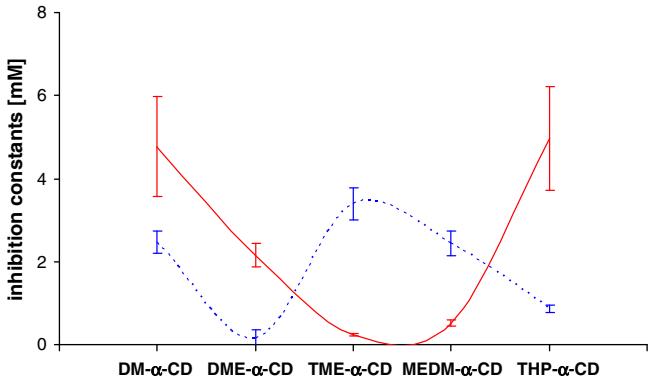


Figure 7. Graphical diagram of inhibition constants versus the type of α-CD derivative. Solid and dashed lines represent competitive (K_i^1) and non-competitive (K_i^2) inhibition constants, respectively.

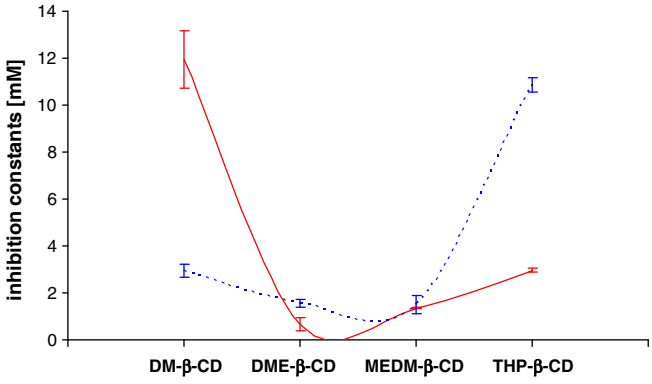


Figure 8. Graphical diagram of inhibition constants versus the type of β-CD derivative. Solid and dashed lines represent competitive (K_i^1) and non-competitive (K_i^2) inhibition constants, respectively.

The values of competitive inhibition constants (K_i^1), listed in Table 1, are in the following order: THP-α-CD > DM-α-CD > DME-α-CD > MEDM-α-CD > TME-α-CD and DM-β-CD > THP-β-CD > MEDM-β-CD > DME-β-CD for α-CD and β-CD derivatives, respectively.

In order to assess the relation between the values K_i^1, obtained in this work, to the geometry of formed complexes it is worth to compare these results with our previous data,[8] which are shown

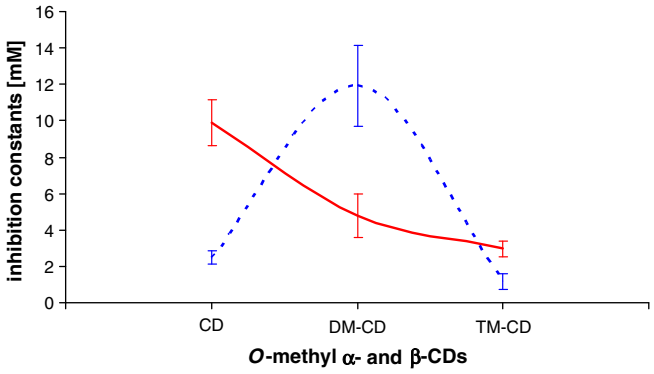


Figure 9. The dependence of the competitive inhibition constants K_i^1 on the type of selectively O-methylated CDs. CD, DM-CD and TM-CD stand for native; *per*(2,3-di-*O*-methyl)-; and *per*(2,3,6-tri-*O*-methyl)-cyclodextrin, respectively. Solid and dashed lines represent α-CDs and β-CDs, respectively.

in Figure 9 as the graphical comparison of K_i^1 values versus the type of selectively O-methylated α- and β-CDs. For the α-CD derivatives, the strongest competitive inhibition is observed for the native one, whereas the weakest inhibition of this type is observed for TM-α-CD. As it was explained previously[8], the complexes with native α-CD are weak and also unreactive during the enzymatic decomposition. Also, it had been suggested previously[10] that L-tryptophan, in its complex with α-CD, is associated with this host molecule by the supporting hydrogen bonds between ammonium, as well as carboxylic groups of the amino acid, and the secondary hydroxyl groups of α-CD. Since the secondary hydroxyl groups in DM-α-CD are replaced by the methoxy groups, which can only play a role of hydrogen bonds acceptors, not donors, such a replacement should result in a more difficult formation of hydrogen bonding with DM-α-CD as compared to the native one. The replacement of all hydroxyl groups of host molecules by the methoxy groups, as in TM-α-CD, disturbs the formation of hydrogen bonding more efficiently than in the case of DM-α-CD. So, the less efficient formation of hydrogen bonds between L-tryptophan and the selectively O-methylated α-CDs, the less probability of the formation of stable complexes between these compounds. Therefore, as shown in Figure 9, lower K_i^1 values have been observed for DM-α-CD and TM-α-CD than for the native α-CD.

Contrary to the effects observed for the α-CD derivatives, in the case of β-CD derivatives, the strongest competitive inhibition is observed for DM-β-CD (Fig. 9), and comparable low values of K_i^1 are observed for native β-CD and permethylated β-CD. The order of

Table 1
Values of inhibition constants (K_i) for all investigated CD derivatives studied in TPase-catalyzed reaction as well as Michaelis constants and inverse maximal velocities in the presence (K'_m and $1/V'_{max}$, respectively) and absence (K_m and $1/V_{max}$, respectively) of particular CD derivative

	K'_m (μM)	$1/V'_{max}$ (min × $mg_{(TPase)}$ × mM^{-1})	K_i^1 (mM)	K_i^2 (mM)
THP-α-CD	557 ± 53	66.67 ± 4.00	4.97 ± 1.25	0.86 ± 0.09
DM-α-CD	521 ± 60	50.25 ± 2.51	4.77 ± 1.20	2.48 ± 0.27
DME-α-CD	431 ± 45	68.02 ± 3.25	2.15 ± 0.29	0.16 ± 0.19
MEDM-α-CD	1620 ± 200	56.17 ± 8.98	0.52 ± 0.06	2.45 ± 0.30
TME-α-CD	3212 ± 333	43.47 ± 10.43	0.24 ± 0.03	3.39 ± 0.40
DM-β-CD	425 ± 129	47.16 ± 4.24	11.94 ± 2.22	2.94 ± 0.51
THP-β-CD	394 ± 58	49.50 ± 4.45	2.97 ± 0.33	10.88 ± 3.00
MEDM-β-CD	832 ± 230	70.42 ± 9.85	1.36 ± 0.21	1.49 ± 0.21
DME-β-CD	1206 ± 120	51.28 ± 11.28	0.68 ± 0.07	1.56 ± 0.16
	K_m (μM)	$1/V_{max}$ (min × $mg_{(TPase)}$ × mM^{-1})		
L-tryptophan	347 ± 27	26.5 ± 3.4	–	–

K_i^1 indicates competitive inhibition; value obtained from the equation: $K_i^1 = [I]/(K'_m/K_m - 1)$; where $[I]$ is inhibitor concentration; K_m is the Michaelis constant; K'_m is the Michaelis constant with presence of inhibitor.
K_i^2 indicates non-competitive inhibition; value obtained from the equation: $K_i^2 = [I]/(V'_{max}/V_{max} - 1)$; where V_{max} is the maximal velocity of the reaction; V'_{max} is the maximal velocity of the reaction with presence of inhibitor.

competitive inhibition, observed for these β-CD derivatives, can be explained as follows. It is probable that the stability constants of the complexes between L-tryptophan and β-CD as well as DM-β-CD are similar because of the unchanged sizes of their molecular cavities. Despite the fact that the molecular cavities of native β-CD and DM-β-CD are the same, the difference between the inhibition constants for these two CDs is seen. It seems probable that the secondary *O*-methyl groups in the latter compound block the availability of aliphatic chain of L-tryptophan. The TM-β-CD cavity is smaller in comparison to the cavities of β-CD and DM-β-CD, because, according to the evidence cited above,[11] the primary *O*-methyl groups penetrate TM-β-CD and the molecular structure resembles a bowl. For this reason, L-tryptophan does not penetrate the molecular cavity of TM-β-CD as deeply as it takes place in its complexes with β-CD and DM-β-CD and, therefore, the aliphatic chain of L-tryptophan exposed outside the molecular cavity of TM-β-CD can still react with the TPase.

According to present studies, the inhibition constants for α-CD derivatives are graphically depicted in Figure 7. Among these α-CD derivatives are the compounds with unsubstituted primary hydroxyl groups (DM-α-CD and DME-α-CD) and the ones with all hydroxyl groups modified (TME-α-CD, MEDM-α-CD and THP-α-CD). In the case of DM-α-CD and DME-α-CD, which show strong competitive inhibition constants, their primary hydroxyl groups could probably form hydrogen bond system with amino and carboxylic groups of L-tryptophan. Therefore, the concentration of the substrate is lowered due to the association of the substrate with the host molecules. Additionally, the aliphatic chain with functional groups of L-tryptophan is probably entrapped into the molecular cavity of these α-CDs, leading to strong competitive inhibition. In contrast, for TME-α-CD and MEDM-α-CD, lower K_i^1 values are observed. And it is worth to notice that in the case of these α-CDs, all their hydroxyl groups are replaced by *O*-methoxyethyl or *O*-methyl groups, which can only play the role of hydrogen bonds acceptors, and therefore the association of L-tryptophan with these host molecules must be very weak. In THP-α-CD, all hydroxyl groups are replaced by *O*-2-hydroxypropyl ones, which can be both acceptors and donors of hydrogen bonds. For this reason, the association of the molecules of L-tryptophan with THP-α-CD is expected to be more efficient, and therefore the K_i^1 value for THP-α-CD is the highest for all α-CDs under investigations.

The graphical representation of the inhibition constants for β-CDs is presented in Figure 8. According to this diagram, the highest K_i^1 value is observed for DM-β-CD. The phenomenon of high competitive inhibition for DM-β-CD has been discussed above. In order to explain the rather weak competitive inhibition for DME-β-CD, it is worth noticing that, although this molecule is similar to DM-β-CD, there is one important difference between them. In the case of DME-β-CD, its secondary hydroxyl groups are replaced by *O*-methoxyethyl groups, and in the case of DM-β-CD its hydroxyl groups are replaced by *O*-methyl groups. *O*-Methoxyethyl groups could possibly block the availability of a cavity of DME-β-CD for accommodation of L-tryptophan more efficiently than *O*-methyl groups. Therefore, the value of competitive inhibition constant for DME-β-CD is definitely lower than that of DM-β-CD.

The difference in molecular structures between DM-β-CD and MEDM-β-CD is only due to the presence and absence of *O*-methoxyethyl groups at narrower rims of these CDs. But this difference in their molecular structures influences strongly the competitive inhibition of these compounds. This effect can be similar to the one observed for TM-β-CD (Fig. 9). As previously mentioned, *O*-methyl groups in the TM-β-CD include the molecular cavity and are close to the narrower rim of this CD. The *O*-methoxyethyl groups possibly also include a cavity of MEDM-β-CD, similarly to the TM-β-CD case. Taking into account these explanations we can conclude that the complexes between L-tryptophan and MEDM-β-CD must be still reactive, because the aliphatic chain of L-tryptophan could be pushed away from the CD cavity by the *O*-methoxyethyl groups.

For THP-β-CD, a little stronger competitive inhibition is observed in comparison to DME-β-CD and MEDM-β-CD. Also, it is important to note that K_i^1 values are comparable for both THP-β-CD and THP-α-CD (Figs. 7 and 8). This effect can be expected, since the *O*-2-hydroxypropyl groups in THP-β-CD can be both acceptors and donors of hydrogen bonding. In contrast to *O*-2-hydroxypropyl groups in THP-β-CD, the *O*-methoxyethyl groups in MEDM-β-CD can be only acceptors for hydrogen bonds. Although the *O*-2-hydroxypropyl groups disturb the entry of L-tryptohan into CD cavity, they cause the association of guest molecule via hydrogen bond formation. Therefore, the similarity in the inhibition effect of both THP-β-CD and THP-α-CD seems to be related to the same mechanism of the association of these CDs and L-tryptophan.

3. Experimental

3.1. Materials

Native α-cyclodextrin (α-CD) and β-cyclodextrin (β-CD); (2-hydroxypropyl)-α-cyclodextrin (**10**, THP-α-CD) (molecular substitution 0.6) and (2-hydroxypropyl)-β-cyclodextrin (**11**, THP-β-CD) (molecular substitution 1.0); 2-methoxyethyl bromide; tetrabutylammonium fluoride; TPase (L-tryptophan indole-lyase, EC 4.1.99.1) from *Escherichia coli*; L-tryptophan; 5′-pyridoxal phosphate (PLP); dithiothreitol; L-lactic dehydrogenase (LDH, EC 1.1.1.27) from *Rabbit muscle*; NADH were purchased from Sigma.

3.2. Synthesis of CD derivatives

3.2.1. *heksakis*[2,3,6-Tri-*O*-(2′-methoxyethyl)]-α-cyclodextrin (1)

NaH (72 equiv) was added to a solution of anhydrous α-cyclodextrin (1 equiv) in freshly distilled DMF. After an hour, 2-methoxyethyl bromide (126 equiv) was added dropwise and the reaction mixture was stirred and heated to 70 °C for 48 h. The rest of unreacted NaH was decomposed by addition of MeOH. Solid residue obtained after evaporation of solvents was dissolved in methylene chloride and washed twice with water. Solution in organic solvent was dried over anhydrous $MgSO_4$ and evaporated to dryness. Crude product was purified by means of column chromatography on silica gel using chloroform/MeOH 10:1 as eluent. It was obtained as yellowish oil; yield 84%; $[\alpha]_D^{25}$ +88.2 (*c* 1, $CHCl_3$); R_f 0.48 (chloroform/MeOH 10:1); ESIMS: *m/z* 2041.4 [**1**+Na], 1032.3 [**1**+2Na]; calcd for $C_{90}H_{168}O_{48}$ 2018.3. ^{1}H NMR: δ (ppm) 5.22 (d, 6H, $J_{H\text{-}1,H\text{-}2}$ = 3.0 Hz, 6H-1); 4.15 (ddd, 6H, $J_{H\text{-}4,H\text{-}5}$ = 1.0 Hz, $J_{H\text{-}5,H\text{-}6a}$ = 4.5 Hz, $J_{H\text{-}5,H\text{-}6b}$ = 5.5 Hz, 6H-5); 4.04 (dd, 6H, $J_{H\text{-}6a,H\text{-}6b}$ = 11.5 Hz, 6H-6a); 3.92–3.87 (m, 12H, 6H-4, 6H-6b); 3.82–3.80 (m, 6H, 6H-3); 3.71–3.67 (m, 24H, C-6–OC*H*$_2$C*H*$_2$OCH$_3$); 3.57–3.48 (m, 48H, C-2,C-3–OC*H*$_2$C*H*$_2$OCH$_3$); 3.36, 3.35, 3.33 (3s, 54H, C-2,C-3,C-6–OCH$_2$CH$_2$OC*H*$_3$); 3.33–3.31 (m, 6H, 6H-2). ^{13}C NMR: δ (ppm) 98.08 (C-1); 81.31, 80.54, 78.52 (C-2, C-3, C-4); 72.44, 72.24, 71.89, 70.83, 70.36 (C-2,C-3,C-6–OC*H*$_2$C*H*$_2$OCH$_3$); 71.38 (C-5); 69.86 (C-6); 58.90, 58.82, 58.77 (C-2,C-3,C-6–OCH$_2$CH$_2$OC*H*$_3$).

3.2.2. *heksakis*(2,3-Di-*O*-methyl)-α-cyclodextrin (2)

This compound was obtained as white bubbles according to the procedure of Yi et al.[13] Yield 82%; $[\alpha]_D^{25}$ +141.5 (*c* 1.5, $CHCl_3$); R_f 0.62 (chloroform/MeOH 2:1); ESIMS: *m/z* 1164.8 [**2**+Na], calcd for $C_{48}H_{84}O_{30}$ 1141.2. ^{1}H NMR: δ (ppm) 5.02 (d, 6H, $J_{H\text{-}1,H\text{-}2}$ = 4.0 Hz, 6H-1); 4.89 (s, 6H, 6H-OH); 3.86–3.81 (m, 6H, 6H-6a); 3.75–3.32 (m, 24H, 6H-3, 6H-4, 6H-5, 6H-6b); 3.64 (s, 18H, C-3–OC*H*$_3$); 3.49 (s, 18H, C-2–OC*H*$_3$); 3.15 (dd, 6H, $J_{H\text{-}2,H\text{-}3}$ = 8.0 Hz, 6H-2). ^{13}C

NMR: δ (ppm) 99.15 (C-1); 82.43, 81.91, 81.64 (C-2, C-3, C-4); 72.81 (C-5); 62.02 (C-6); 61.70, 59.77 (C-2,C-3–OCH_3).

3.2.3. *heptakis*(2,3-Di-O-methyl)-β-cyclodextrin (3)

This compound was obtained as white bubbles according to the procedure of Yi et al.[13] Yield 76%; $[\alpha]_D^{25}$ +136.4 (*c* 1.4, $CHCl_3$); R_f 0.56 (chloroform/MeOH 2:1); ESIMS: *m/z* 1354.6 [**3**+Na], calcd for $C_{56}H_{98}O_{35}$ 1331.4. ^{1}H NMR: δ (ppm) 5.09 (d, 7H, $J_{H\text{-}1,H\text{-}2}$ = 5.0 Hz, 7H-1); 4.42 (s, 7H, 7H-OH); 4.08–3.38 (m, 35H, 7H-3, 7H-4, 7H-5, 7H-6a, 7H-6b); 3.63 (s, 21H, C-3–OCH_3); 3.51 (s, 21H, C-2–OCH_3); 3.21 (dd, 7H, $J_{H\text{-}2,H\text{-}3}$ = 8.0 Hz, 7H-2). ^{13}C NMR: δ (ppm) 98.84 (C-1); 81.94, 81.65, 80.37 (C-2, C-3, C-4); 72.48 (C-5); 61.32 (C-6); 61.52, 58.60 (C-2,C-3–OCH_3).

3.2.4. *heksakis*[2,3-Di-*O*-methyl-6-*O*-(2′-methoxyethyl)]-α-cyclodextrin (4)

Solution of *heksakis*(2,3-di-*O*-methyl)-α-cyclodextrin (**2**) (1 equiv) in dry DMF was added dropwise to a mixture of NaH (120 equiv) and freshly distilled DMF. After an hour, 2-methoxyethyl bromide (120 equiv) was added dropwise and the reaction mixture was stirred and heated to 40 °C for 30 h. The rest of unreacted NaH was decomposed by addition of MeOH. Solid residue obtained after evaporation of solvents was dissolved in methylene chloride and washed twice with water. Solution in organic solvent was dried over anhydrous $MgSO_4$ and evaporated to dryness. Crude product was purified by means of column chromatography on silica gel using chloroform/MeOH/water 40:10:1 as eluent. It was obtained as yellowish oil; yield 85%; $[\alpha]_D^{20}$ +118.2 (*c* 1, $CHCl_3$); R_f 0.72 (chloroform/MeOH/water 40:10:1); ESIMS: *m/z* 1511.9 [**4**+Na], 767.6 [**4**+2Na]; calcd for $C_{66}H_{120}O_{36}$ 1489.7. ^{1}H NMR: δ (ppm) 5.10 (d, 6H, $J_{H\text{-}1,H\text{-}2}$ = 3.0 Hz, 6H-1); 3.91 (dd, 6H, $J_{H\text{-}5,H\text{-}6a}$ = 3.5 Hz, $J_{H\text{-}6a,H\text{-}6b}$ = 11.0 Hz, 6H-6a); 3.80–3.76 (m, 12H, 6H-5, 6H-6b); 3.70–3.64 (m, 12H, 6H-3, 6H-4); 3.56–3.52 (m, 24H, C-6–OCH_2-CH_2OCH_3); 3.65, 3.50, 3.36 (3s, 54H, C-2,C-3–OCH_3, C-6–OCH_2-CH_2OCH_3), 3.16 (dd, 6H, $J_{H\text{-}2,H\text{-}3}$ = 10.0 Hz, 6H-2). ^{13}C NMR: δ (ppm) 100.03 (C-1); 82.22, 81.22 (C-2, C-3, C-4); 71.91, 70.56 (C-6–OCH_2CH_2OCH_3); 71.39 (C-5); 69.97 (C-6); 61.81, 58.92, 57.79 (C-2,C-3–OCH_3, C-6–OCH_2CH_2OCH_3).

3.2.5. *heptakis*[2,3-Di-*O*-methyl-6-*O*-(2′-methoxyethyl)]-β-cyclodextrin (5)

This compound was obtained as yellowish oil according to the same procedure as for *heksakis*[2,3-di-*O*-methyl-6-*O*-(2′-methoxyethyl)]-α-cyclodextrin (**4**). Yield 80%; $[\alpha]_D^{20}$ +113.8 (*c* 1, $CHCl_3$); R_f 0.85 (chloroform/MeOH/water 40:10:1); ESIMS: *m/z* 1760.1 [**5**+Na], 891.7 [**5**+2Na]; calcd for $C_{77}H_{140}O_{42}$ 1737.9. ^{1}H NMR: δ (ppm) 5.20 (d, 7H, $J_{H\text{-}1,H\text{-}2}$ = 3.5 Hz, 7H-1); 3.94 (dd, 7H, $J_{H\text{-}5,H\text{-}6a}$ = 3.5 Hz, $J_{H\text{-}6a,H\text{-}6b}$ = 11.0 Hz, 7H-6a); 3.80–3.76 (m, 14H, 7H-5, 7H-6b); 3.70–3.62 (m, 14H, 7H-3, 7H-4); 3.54–3.50 (m, 28H, C-6–OCH_2CH_2OCH_3); 3.65, 3.52, 3.35 (3s, 63H, C-2,C-3–OCH_3, C-6–OCH_2CH_2OCH_3), 3.17 (dd, 7H, $J_{H\text{-}2,H\text{-}3}$ = 10.0 Hz, 7H-2). ^{13}C NMR: δ (ppm) 98.71 (C-1); 82.05, 81.74, 80.04 (C-2, C-3, C-4); 71.80, 70.43 (C-6–OCH_2CH_2OCH_3); 71.08 (C-5); 69.85 (C-6); 61.45, 58.84, 58.45 (C-2,C-3–OCH_3, C-6–OCH_2CH_2OCH_3).

3.2.6. *heksakis*[6-*O*-*tert*-Butyldimethylsilyl-2,3-di-*O*-(2′-methoxyethyl)]-α-cyclodextrin (6)

Solution of *heksakis*(6-*O*-*tert*-butyldimethylsilyl)-α-cyclodextrin[14] (1 equiv) in dry DMF was added dropwise to a mixture of NaH (120 equiv) and freshly distilled DMF. After an hour 2-methoxyethyl bromide (180 equiv) was added dropwise and the reaction mixture was stirred and heated to 40 °C for 40 h. The rest of unreacted NaH was decomposed by addition of MeOH. Oil obtained after evaporation of solvents was dissolved in methylene chloride and washed twice with water. Solution in organic solvent was dried over anhydrous $MgSO_4$ and evaporated to dryness. Crude product was purified by means of column chromatography on silica gel using hexane/acetone 2:1 as eluent. It was obtained as colourless oil; yield 56%; $[\alpha]_D^{20}$ +68.2 (*c* 1, $CHCl_3$); R_f 0.51 (hexane/acetone 1:1); ESIMS: *m/z* 2377.2 [**6**+Na], 1200.4 [**6**+2Na]; calcd for $C_{108}H_{216}O_{42}Si_6$ 2355.4. ^{1}H NMR: δ (ppm) 5.21 (d, 6H, $J_{H\text{-}1,H\text{-}2}$ = 3.5 Hz, 6H-1); 3.97 (t, 6H, $J_{H\text{-}3,H\text{-}4}$ = 9.0 Hz, $J_{H\text{-}4,H\text{-}5}$ = 9.0 Hz, 6H-4); 3.91 (ddd, 6H, $J_{H\text{-}5,H\text{-}6a}$ = 4.0 Hz, $J_{H\text{-}5,H\text{-}6b}$ = 6.0 Hz, 6H-5); 3.83 (dd, 6H, $J_{H\text{-}6a,H\text{-}6b}$ = 10.5 Hz, 6H-6a); 3.79 (dd, 6H, 6H-6b); 3.73 (dd, 6H, 6H-3); 3.63–3.52 (m, 48H, C-2,C-3–OCH_2CH_2OCH$_3$); 3.37, 3.34 (2s, 36H, C-2,C-3–OCH$_2$CH$_2$OCH_3); 3.26 (dd, 6H, $J_{H\text{-}2,H\text{-}3}$ = 10.0 Hz, 6H-2); 0.87 (s, 54H, C-(CH_3)$_3$); 0.02, 0.02 (2s, 36H, Si–(CH_3)$_2$). ^{13}C NMR: δ (ppm) 97.75 (C-1); 81.72, 80.30 (C-2, C-3, C-4); 72.62, 72.53, 72.48, 72.43 (C-2,C-3–OCH_2CH$_2$OCH$_3$); 70.42 (C-5); 62.45 (C-6); 58.95, 58.86 (C-2,C-3–OCH$_2$CH$_2$OCH$_3$); 26.10 (C-(CH_3)$_3$); 18.47 (C-(CH$_3$)$_3$); −4.57, −4.91 (Si-(CH_3)$_2$).

3.2.7. *heksakis*[2,3-Di-*O*-(2′-methoxyethyl)]-α-cyclodextrin (7)

Solution of *heksakis*[6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-(2′-methoxyethyl)]-α-cyclodextrin (**6**) (1 equiv) and tetrabutylammonium fluoride (10 equiv) in dry THF was refluxed for 24 h. Then reaction mixture was concentrated and diluted in ethyl acetate and filtered through silica gel. Obtained supernatant was evaporated to dryness and dissolved in methylene chloride and washed with saturated solution of NaCl. Solution in organic solvent was dried over anhydrous $MgSO_4$ and evaporated to dryness. Crude product was purified by means of column chromatography on silica gel using chloroform/MeOH/water 40:10:1 as eluent. It was obtained as white bubbles; yield 81%; $[\alpha]_D^{25}$ +148.8 (*c* 2, $CHCl_3$); R_f 0.29 (chloroform/MeOH/water 40:10:1); ESIMS: *m/z* 1691.8 [**7**+Na], 857.6 [**7**+2Na]; calcd for $C_{72}H_{132}O_{42}$ 1669.8. ^{1}H NMR: δ (ppm) 5.16 (d, 6H, $J_{H\text{-}1,H\text{-}2}$ = 3.5 Hz, 6H-1); 4.20–4.14 (m, 6H, 6H-5); 4.04–3.98 (m, 6H, 6H-6a); 3.92–3.84 (m, 12H, 6H-6b, 6H-4); 3.81–3.76 (m, 6H, 6H-3); 3.72–3.51 (m, 48H, C-2,C-3–OCH_2-CH_2OCH_3); 3.36, 3.35 (2s, 36H, C-2,C-3–OCH$_2$CH$_2$OCH_3); 3.34–3.31 (m, 6H, 6H-2). ^{13}C NMR: δ (ppm) 97.69 (C-1); 81.52, 80.11, 79.11 (C-2, C-3, C-4); 72.88, 72.52, 72.49, 72.39 (C-2,C-3–OCH_2-CH_2OCH$_3$); 70.75 (C-5); 62.21 (C-6); 58.89, 58.87 (C-2,C-3–OCH$_2$CH$_2$OCH$_3$).

3.2.8. *heptakis*[6-*O*-*tert*-Butyldimethylsilyl-2,3-di-*O*-(2′-methoxyethyl)]-β-cyclodextrin (8)

This compound was obtained according to the same procedure as for *heksakis*[6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-(2′-methoxyethyl)]-α-cyclodextrin (**6**). It was obtained as colourless oil; yield 64%; $[\alpha]_D^{25}$ +61.2 (*c* 1, $CHCl_3$); R_f 0.49 (hexane/acetone 1:1); ESIMS: *m/z* 2770.5 [**8**+Na], 1396.7 [**8**+2Na]; calcd for $C_{126}H_{252}O_{49}Si_7$ 2748.0. ^{1}H NMR: δ (ppm) 5.26 (d, 7H, $J_{H\text{-}1,H\text{-}2}$ = 3.0 Hz, 7H-1); 4.19–4.16 (m, 7H, 7H-4); 3.89–3.79 (m, 28H, 7H-5, 7H-6a, 7H-6b, 7H-3); 3.62–3.50 (m, 56H, C-2,C-3–OCH_2CH_2OCH$_3$); 3.36, 3.34 (2s, 42H, C-2,C-3–OCH$_2$CH$_2$OCH_3); 3.24 (dd, 7H, $J_{H\text{-}2,H\text{-}3}$ = 9.5 Hz, 7H-2); 0.86 (s, 63H, C-(CH_3)$_3$); 0.01, 0.00 (2s, 42H, Si-(CH_3)$_2$). ^{13}C NMR: δ (ppm) 97.87 (C-1); 81.24, 80.57 (C-2, C-3, C-4); 72.46, 72.34, 72.16 (C-2,C-3–OCH$_2$CH$_2$OCH$_3$); 70.21 (C-5); 62.23 (C-6); 58.78, 58.65 (C-2,C-3–OCH$_2$CH$_2$OCH$_3$); 25.94 (C-(CH$_3$)$_3$); 18.30 (C-(CH$_3$)$_3$); −4.75, −5.18 (Si–(CH$_3$)$_2$).

3.2.9. *heptakis*[2,3-Di-O-(2′-methoxyethyl)]-β-cyclodextrin (9)

This compound was obtained according to the same procedure as for *heksakis*[2,3-di-*O*-(2′-methoxyethyl)]-α-cyclodextrin (**7**). It was obtained as white bubbles; yield 74%; $[\alpha]_D^{25}$ +88.9 (*c* 1, $CHCl_3$); R_f 0.36 (chloroform/MeOH/water 40:10:1); ESIMS: *m/z* 1970.9 [**8**+Na], 996.9 [**8**+2Na]; calcd for $C_{84}H_{154}O_{49}$ 1948.1. ^{1}H NMR: δ (ppm) 5.19 (d, 7H, $J_{H\text{-}1,H\text{-}2}$ = 3.5 Hz, 7H-1); 4.10–4.05 (m, 7H, 7H-5); 4.01–3.99 (m, 7H, 7H-6a); 3.90–3.72 (m, 21H, 7H-6b, 7H-4, 7H-3); 3.65–3.51 (m, 56H, C-2,C-3–OCH_2CH_2OCH$_3$); 3.35, 3.35 (2s, 42H, C-2,C-3–OCH$_2$CH$_2$OCH_3); 3.35–3.33 (m, 7H, 7H-2). ^{13}C

NMR: δ (ppm) 97.76 (C-1); 81.19, 80.28, 78.54 (C-2, C-3, C-4); 72.55, 72.37, 72.34, 72.24 (C-2,C-3–$OCH_2CH_2OCH_3$); 70.56 (C-5); 61.85 (C-6); 58.78, 58.75 (C-2,C-3–$OCH_2CH_2OCH_3$).

3.3. Analyses

^1H and ^{13}C NMR spectra were recorded at 200 or 500 MHz in [$^2H_1$]chloroform ($CDCl_3$) on a Varian UNITY plus-200 or plus-500 spectrometers. Optical rotation values were measured on Perkin–Elmer 241 polarimeter at a wavelength of 589 nm. Molecular peaks in Mass Spectrometry were collected on AMD 604 Intectra spectrometer.

3.4. Methods of kinetic data determination

L-Tryptophan for measurements was preincubated with proper excess of each CD derivative. Incubation was carried out overnight at room temperature. A typical assay contained 0.1 M potassium phosphate buffer, pH 8, 0.2 M dithiothreitol, 50 μM 5′-pyridoxal phosphate, 20.3 U/mL L-lactic dehydrogenase, 0.1 mM NADH, 0.01 U/mL TPase, various amounts of the particular CD derivative (from 1 to 3 mM) and various amounts of L-tryptophan (from 0.4 mM to 0.9 mM). Six assays of different concentrations of L-tryptophan were applied per one experiment. The substrate concentration was different among the experiments in one series, whereas the CD total concentration remained unchanged. The concentrations of CDs have been varied among different experimental series. The concentration of CDs was always in proper excess in comparison to substrate. The decrease of absorbance was measured at a wavelength of 340 nm on a 1202 Shimadzu UV–Vis spectrophotometer during the time of reaction (the kinetics was determined from the change in absorption of NADH at this wavelength). The experiments were performed at room temperature (18 °C). The Lineweaver–Burk plots were used to determine the intercepts (inverse maximal velocity), and slopes (ratio of Michaelis constant per maximal velocity) using the least-squares method. The correction for the decomposition of NADH was added.

4. Conclusions

This article refers to the synthesis of several new CD derivatives and also to their influence on the catalytic activity of L-tryptophan indole-lyase which decomposes L-tryptophan to indole, pyruvic acid and ammonia. All the synthesized CDs, and also the commercial ones, have been found to be inhibitors of the TPase enzymatic reaction. The observed inhibition has been of mixed type, that is, both competitive and non-competitive ones. The phenomenon of competitive inhibition has been related to the formation of inclusion complexes between L-tryptophan and CDs. The order of calculated competitive inhibition constants has been explained by the formation of the inclusion complexes, additional hydrogen bonding and the effect of some CD substituents on the availability of the aliphatic chain of L-tryptophan for the reaction with the enzyme. In the case of non-competitive inhibition, its possible mechanism is connected with formation also inclusion complexes with CDs, but this time on the surface of the enzyme molecules. Such complexation probably changes the flexibility of L-tryptophan indole-lyase, which inhibits its enzymatic properties.

Acknowledgement

Financial support from the University of Warsaw (BST-123 652 and BST-132 623) is gratefully acknowledged.

References

1. Szejtli, J. *Chem. Rev.* **1998**, *98*, 1743–1754.
2. Szejtli, J.; Osa, T. In *Comprehensive Supramolecular Chemistry*; Pergamon: Oxford, 1996; Vol. 3.
3. Orenes-Piñero, E.; García-Carmona, F.; Sánchez-Ferrer, A. *J. Mol. Catal. B* **2007**, *47*, 143–148.
4. Fernández, M.; Fragoso, A.; Cao, R.; Villalonga, R. *J. Mol. Catal. B* **2003**, *21*, 133–141.
5. Fasoli, E.; Castillo, B.; Santos, A.; Silva, E.; Ferrer, A.; Rosario, E.; Griebenow, K.; Secundo, F.; Barletta, G. L. *J. Mol. Catal. B* **2006**, *42*, 20–26.
6. Ghanem, A.; Schurig, V. *Tetrahedron: Asymmetry* **2001**, *12*, 2761–2766.
7. Koralewska, A.; Augustyniak, W.; Temeriusz, A.; Kańska, M. *J. Incl. Phenom. Macrocycle* **2004**, *49*, 193–197.
8. Gubica, T.; Boroda, E.; Temeriusz, A.; Kańska, M. *J. Incl. Phenom. Macrocycle* **2006**, *54*, 283–288.
9. Yang, H.; Bohne, C. *J. Photochem. Photobiol. A: Chem.* **1995**, *86*, 209–217.
10. Lipkowitz, K. B.; Raghothama, S.; Yang, J. *J. Am. Chem. Soc.* **1992**, *114*, 1554–1562.
11. Steiner, T.; Saenger, W. *Angew. Chem., Int. Ed.* **1998**, *37*, 3404–3407.
12. Morino, Y.; Snell, E. E. *Methods Enzymol.* **1970**, *17a*, 439–446.
13. Yi, G.; Bradshaw, J. S.; Rossiter, B. E.; Reese, S. L.; Petersson, P.; Markides, K. E.; Lee, M. L. *J. Org. Chem.* **1993**, *58*, 2561–2565.
14. Fügedi, P. *Carbohydr. Res.* **1989**, *192*, 366–369.