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Effects of Cyclodextrin Derivatives on the Catalytic Activity of Tyrosine Phenol-Lyase

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Abstract

The impact of permethylated α-, β-, γ-cyclodextrin (TMα-CD, TMβ-CD, TMγ–CD, respectively) on TPL-catalysed decomposition of L-tyrosine was investigated by means of spectrophotometric measurements. The inhibitory effects of TM-CDs on the catalytic activity of the enzyme were shown. This phenomenon is supposed to be connected with the host–guest complex formation. CDs were found to have impact on maximal velocity and on Michaelis constant of described catalysis.

Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides that are among the most frequently used host molecules in supramolecular chemistry [1, 2]. Despite of having a hydrophilic surface, they contain a hydrophobic cavity that enables them to entrap hydrophobic molecules. Entrapment/inclusion occurs without the formation of formal chemical bonds [3].

CDs and their substituted derivatives are known to form inclusion complexes with a variety of aromatic compounds including aromatic amino acids. There are examples of such complexes between native CDs and L-tyrosine, L-phenylalanine, or L-tryptophan in the literature [4–6]. Cycloamylose inclusion complexes are utilised in a large variety of fields [7]. They can serve as models for studying catalytic reactions of enzymes, but they can also be additives altering enzymatic reactions [8, 9]. CDs can, e.g., increase the availability of insoluble substrates, reduce substrate inhibition, and limit product inhibition. In each case they enhance the efficiency of the catalysis (by enhancing the enantiomeric ratio E and reaction rate) [10–12]. The inclusion of product in the cavity of CDs may also shift the equilibrium in the desired direction [8]. Griebenow *et al.* have successfully used methylated β-CD to enhance the activity and enantioselectivity E of dehydrated subtilisin *Carlsberg* in organic solvents simultaneously [10]. Similar effects were observed with addition of methylated CDs to (PSL)-catalysed transesterification of l-(2-furyl)-ethanol [13]. Both reaction rate and enantioselectivity E were significantly enhanced, when co-lyophilised lipase was used in the presence of CDs that activated the enzyme.

In the described cases, the permethylated forms were chosen as they have increased solubility in water comparing to the corresponding native species with simultaneously retention of complexing abilities.

In this paper we report the impact of CD derivatives (per(2,3,6-tri-*O*-methyl)-α-, β-, γ-cyclodextrin (TMα-CD, TMβ-CD, TMγ-CD, respectively) Figure 1) on the catalytic activity of tyrosine phenol-lyase (TPL).

Tyrosine phenol-lyase (TPL; E.C. 4.1.99.2) is a bacterial enzyme, which catalyses reversible decomposition of L-tyrosine (Figure 1) to phenol, pyruvate and ammonia (Scheme 1) using 5′-pyridoxal phosphate (PLP) as a cofactor [14].

This enzyme catalyses a series of α, β-elimination, β-replacement and racemization reactions. It decomposes L-serine and L-cysteine, as well as their *O*(S)-derivatives to pyruvate. The enzyme is a very useful tool for syntheses of a wide range of L-tyrosine derivatives starting from phenol (and its *o*- and *m*-derivatives), pyruvate (also from its equivalents, e.g., alanine, cysteine, serine) and ammonia [15].

TPL has the ability to modify and to manufacture natural and non-natural amino acids, which shows its immense biotechnological potential. This fact is due to broad substrate specificity for the α, β-elimination reaction, e.g., L-tyrosine or L-tryptophan and some related analogues [16]. These aspects along with the mentioned ability of CDs to improve enzyme activity prompted us to study their impact on the activity of TPL.

The impact of three selected CD derivatives (TMα-CD, TMβ-CD, TMγ-CD) on TPL-catalysed reaction was studied on the model β-elimination reaction of

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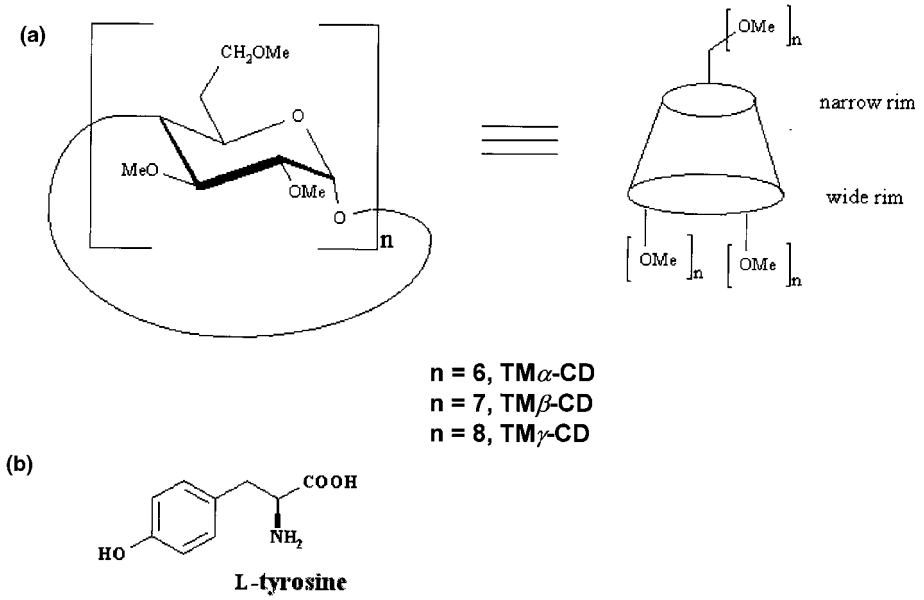


Figure 1. Schematic structures of permethylated CDs (a) and L-tyrosine (b).

L-tyrosine (Scheme 1). CDs were expected to inhibit this process due to host–guest complex formation with amino acid. Consequently, it would allow to find new applications of use of modified CDs in organic synthesis. Effects of these compounds on the TPL-catalysed syntheses of L-tyrosine and its derivatives need further investigations.

Experimental section

Materials

TMα-, β-, γ-CD were prepared using reported procedures [17], and crude products recrystallised twice from *n*-hexane. TPL (TPL, E.C. 4.1.) from *Citobacter freundii*, was donated by Prof. Robert S. Phillips, University of Georgia, Athens, GA, USA. The enzyme activity was 3.8 U/μL.

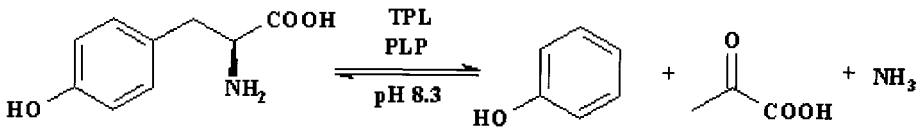
The rest of reagents (L-tyrosine, 5′-pyridoxal phosphate (PLP), dithiothreitol, L-lactic dehydrogenase) were purchased from Sigma. Deuterated water was obtained from Polatom (Swierk, Poland).

Methods of the determination of kinetic data

L-Tyrosine for measurements was preincubated with proper excess of each CD derivative. Incubation was carried out at room temperature overnight. Typical assay contained 0.1 M potassium phosphate buffer, pH 8.3, 0.2 M ditiothreitol, 50 μM 5′-pyridoxal phosphate, 13.4 U/mL L-lactic dehydrogenase (E.C. 1.) from rabbit muscle, 0.1 mM NADH, 0.25 U/mL of TPL, 1.2 mM of the particular TM-CD (previously incubated with L-tyrosine) and various amounts of L-tyrosine (from 0.3 to 0.8 mM). Six assays of different concentrations of tyrosine were applied per one experiment. The decrease of absorbance was measured at wavelength 340 nm in 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). Lineweaver–Burk plots were used to determine intercepts (inverse maximal velocity), and slopes (ratio of Michaelis constant per maximal velocity) using the least square method. The correction for the decomposition of NADH was added.

Results and discussion

The effects of permethylated CDs on TPL-catalysed L-tyrosine decomposition were studied in presence and absence of potential inhibitors (CDs) (Figure 3). Per-substituted CDs are relatively easy to prepare, and the methyl group is the simplest substituent used to modify CDs. The mentioned group is introduced by an alkylation reaction. The methyl groups seem to increase the



Scheme 1. Reaction catalysed by TPL.

hydrophobicity of the CD cavity, possibly by providing its 'extension' by introducing the non-polar groups. Besides, as it was described before, the methylation dramatically improves the water solubility of its derivative [18–21]. These advantages of permethylated CDs allowed us to apply them as additives in the enzymatic reaction.

To prevent CD binding to the enzyme before the peculiar enzymatic reaction, the substrate of this reaction (L-tyrosine) was preincubated with particular permethylated derivative to form the complex TM-CD-L-tyrosine. Its supposed structure demonstrates Figure 2.

Methylation of CD affects the orientation of the guest in its cavity [22, 23]. Molecular modelling calculations [24] performed with natural amino acids indicated that the ammonium group interacts primarily with the narrower rim of CD molecule. This interaction is favourable, as the C-6 methoxyl groups are more flexible to allow better coordination with ammonium group.

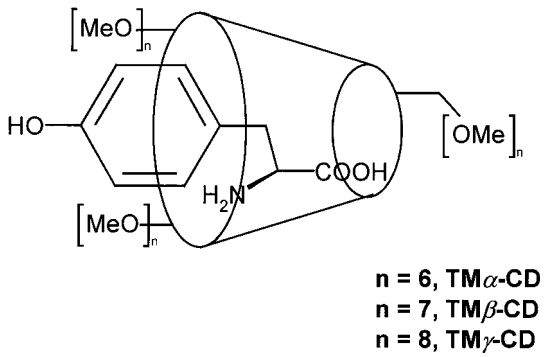


Figure 2. Proposed conformation of the TM-CD-L-tyrosine host–guest complex.

The carboxylic end may also act with the narrower rim, whereas the hydroxyl phenyl group bonds with the oxygen atoms in the wider rim *via* hydrogen bonds. The structure of inclusion complexes between permethylated CDs and natural amino acids in gas-phase was studied by Ramirez *et al.* [25]. In this model the aromatic moiety of L-tyrosine is forced to be placed through the wider rim driving the amino and carboxyl termini to be included in the cavity and the hydroxyl group is situated close to the methylated oxygens on carbons C-2 and C-3 at the wider rim.

The results of number of measurements concerning the influence of above mentioned CDs on the enzymatic β-elimination demonstrate the possibility of not only TM-CD-L-tyrosine complex formation, but also of binding of relevant CD to TPL. These two effects lead to the inhibition of the decomposition of L-tyrosine. The results obtained from kinetic data indicate that this inhibition is of mixed type (competitive and non-competitive) for each CD, as it is shown on Figure 3.

The Lineweaver–Burk plots for each of them differ from this obtained with L-tyrosine (without the addition of CDs). TMα-, β-, γ-CD all inhibit the studied catalysis. Each CD behaves like a non-competitive inhibitor. Two kinds of complexes are present: the L-tyrosine-appropriate CD complex in which amino acid is entrapped in the cavity of CD and TPL-CD one resulting from the binding of CD to a surface residue of the enzyme. These complexes may be interpreted in terms of inclusion forms, which cause the inhibition of investigated reaction.

According to the definition of the inhibition the lower value of K_i (inhibition constant) the stronger inhibition and in consequence the stronger binding of the inhibitor to the enzyme. Table 1 shows that values of

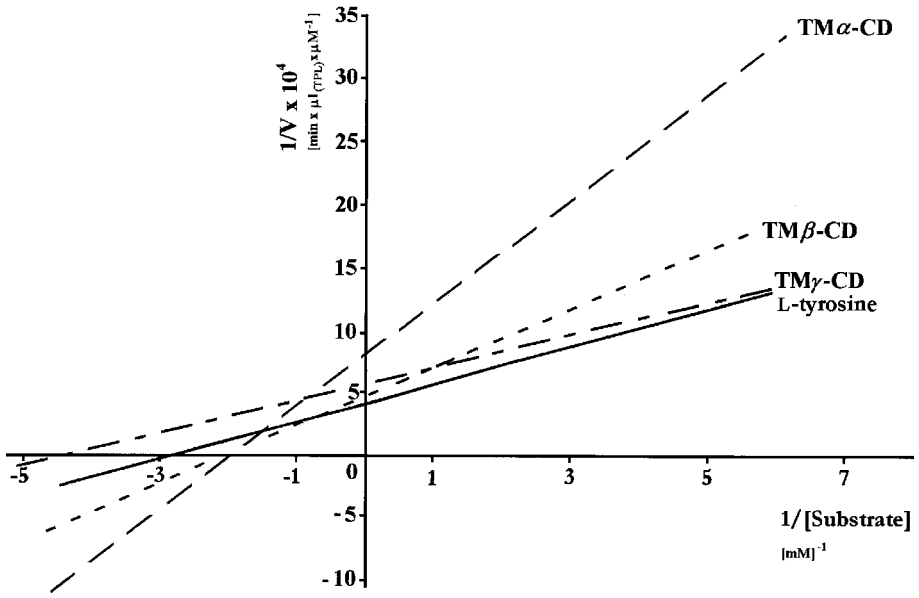


Figure 3. Lineweaver–Burk plot of TPL-catalysed decomposition of L-tyrosine at variable concentration of particular TM-CD.

Table 1. Values of inhibition constants (K_i) for all permethylated CDs studied in TPL-catalysed reaction as well as Michaelis constants and inverse maximal velocities in the presence (K'_m and V'^{-1}_{max}, respectively) and absence (K_m and V^{-1}_{max}, respectively) of particular TM-CD

	K'_m (μm)	$V'^{-1}_{max} \times 10^4$ (min $\times$ μL $_{(TPL)}$ $\times$ μM^{-1})	K_i^1 (mM)	K_i^2 (mM)
TMα-CD	550 ± 37	7.91 ± 0.39	3.38 ± 0.24	1.22 ± 0.06
TMβ-CD	514 ± 50	4.71 ± 0.40	4.51 ± 0.46	6.34 ± 0.55
TMγ-CD	229 ± 33	5.92 ± 0.55	2.75 ± 0.40[a]	2.42 ± 0.23
	K_m (μM)	$V^{-1}_{max} \times 10^4$ (min $\times$ μL $_{(TPL)}$ $\times$ μM^{-1})		
L-tyrosin	406 ± 10	3.96 ± 0.07	–	–

K_i^1 – indicates competitive inhibition; value obtained from equation.
$K'_m = K_m(1 + [I]/K_i)$ where [I] is inhibitor concentration.
K_i^2 – indicates non-competitive inhibition; value obtained from equation.
$V'_{max} = V_{max}/(1 + [I]/K_i)$ where [I] is inhibitor concentration.
[a] K_i^1 for TMγ-CD means here activation constant (K_a).

non-competitive inhibition constants for TM-CDs are in sequence $K_{i\beta} > K_{i\gamma} > K_{i\alpha}$, which suggests that the strength of cyclodextrin binding to the enzyme for TMγ-CD is higher than for TMβ-CD, but lower than for TMα-CD.

In the case of this study the designation 'pseudo-competitive' means the competition for the substrate between the particular CD and TPL. Therefore lower value of K_i indicates stronger binding of L-tyrosine to the host compound. Accordingly the inhibitory effects of CDs signify the competitive strength that decreases in sequence TMα-CD > TMβ-CD (Table 1). This factor indicates that permethylated α-CD stronger binds L-tyrosine than permethylated β-CD. It was established, like described above, that CD does not chemically interact with amino acid and their 'binding' to each other means the host-guest complex formation.

On the contrary, TPL with permethylated γ-CD can better than the native enzyme recognise the substrate what means that TMγ-CD binding to the enzyme gives better substrate recognition. It could be estimated from the reduction of the K_m (Michaelis constant) value for this cyclodextrin. This phenomenon can be described by K_a (activation constant) and calculated from Equation (1):

$$K'_m = K_m(1 - [A]\backslash K_a), \qquad (1)$$

where K'_m is the Michaelis constant with the addition of CD, K_m is the Michaelis constant without CD, $[A]$ is activator concentration, and K_a means the desired activation constant (Table 1).

Permethylated γ-CD weakly entraps L-tyrosine, probably due to larger then two other CDs diameter of the cavity [24]. This may lead to the advantage of the 'activation' effect of the binding domain of TPL. In fact, binding of CDs to TPL may cause conformational changes in the enzyme, which inhibits the catalytic activity of the studied enzyme as seen for the non-competitive inhibition. The same phenomenon may also cause activation of binding domain. This effect can be neutralised by binding the substrate to CD, which inhibits the reaction. We can only observe final result of both effects.

Conclusions

Some conclusions can be derived from our experiments. At first, CD interacts with the enzyme in a specific unknown way, influencing its behaviour. The excess of particular CD that did not bind to L-tyrosine remains in solution, and impacts the enzyme activity. This causes the non-competitive inhibition, when using CD in excess.

On the other hand, the effect of CD on the enzymatic decomposition of L-tyrosine depends on the cavity dimension. For TMα-CD and TMβ-CD, with smaller cavities, the inhibition can be connected with entrapping the substrate into the cavity. In the case of TMγ-CD, with the largest cavity, the binding to the aromatic residues on the enzyme surface is supposed, which facilitates the recognition of the substrate.

To sum up, the ability of CDs to form host-guest complexes may induce various phenomena e.g., inhibition, or activation of enzymes.

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