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Spectroscopic study on the inclusion complexes of β-cyclodextrin with selected metabolites of catecholamines

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ABSTRACT

Inclusion complexes formed between β-cyclodextrin (β-CD) and metabolites of catecholamines, i.e. vanillylmandelic acid (VMA), homovanillic acid (HVA) as well as vanillin (VA) were studied using NMR spectroscopy. Due to the importance of these compounds for the diagnosis tumours of the sympathoadrenal system, hydrogels containing β-CD moieties for enhancing entrapping metabolites of catecholamine from aqueous solutions are located in the area of our interest. Stoichiometry and association constants of the complexes of β-CD with VMA, HVA and VA respectively were determined by using continuous variation and ^{1}H NMR titration methods. Significant discrepancies were pointed out depending on used referencing method. In this study water solution of 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt as an external reference was used to avoid errors in the determination of association constants. β-CD formed the most stable complexes with VA and HVA molecules whilst smallest value of association constant was determined for the VMA/β-CD complex. Two-dimensional rotating-frame Overhauser effect spectroscopy (2D ROESY) allowed to establish definite information on the molecular structures of the complexes formed. Geometry of the latter was proposed basing on contour plots of the 2D ROESY spectra, which also indicated two possibilities of complexed molecule arrangement into β-cyclodextrin interior. The values of determined association constants are in good agreement with postulated geometry of the complexes.

Value of association constant determined for inclusion complexes of β-cyclodextrin with homovanillic acid an vanillin indicates the strongest binding of molecules among investigated complexes, so it was finally concluded that β-CD moiety introduced into hydrogel network could be effective for homovanillic acid and vanillin entrapping.

1. Introduction

Cyclodextrins (CDs) are known to form inclusion complexes with variety of guest compounds, having hydrophobic moieties of appropriate size. Inclusion complexes of cyclodextrins with 1:1 or 1:2 guest-host stoichiometry are the most extensively studied but non-inclusion complexes have also been described [1].

An inclusion phenomenon of CDs originates from their structure. Cyclodextrins are a group of cyclic oligomers, composed of six or more α-D-glucopyranose units. In this study, we have used β-cyclodextrin (β-CD) containing 7 units (Scheme 1). Due to the chair conformation of the glucopyranose units, the CDs have truncated cone shape. The primary hydroxyl groups are oriented towards the narrower cone exterior whilst secondary hydroxyl groups towards the wider edge. Interior of cyclodextrins is somewhat hydrophobic thus water molecules in CD cavity are energetically unfavoured and therefore can be readily substituted by less polar guest molecules.

A wide variety of analytical methods was employed for characterization of inclusion complexes formed between the guest and cyclodextrin molecules both in the solid and solution state. The most direct evidence for the inclusion of a guest into a CD cavity in solution is obtained by NMR spectroscopy [2]. The NMR techniques enable determining stoichiometry, association constants, structural characterization of CDs complexes and allow to analysis of their dynamics (for review, see Refs. [3,4]).

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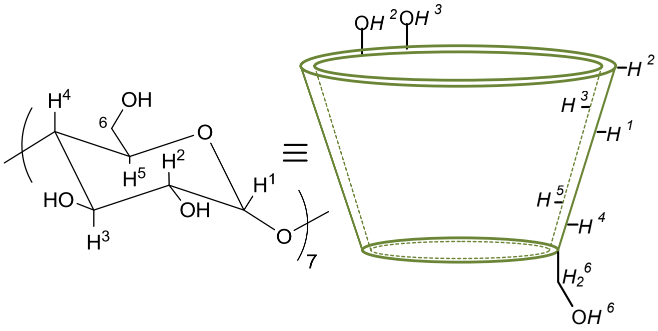


Scheme 1. Structure of ß-CD.

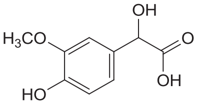
Cyclodextrins, due to their ability to form inclusion complexes with selective molecules, achieved great significance in molecular recognition processes. Moreover, CDs are chiral and exhibit chiral recognition [5]. Native, modified CDs or CDs moieties introduced into polymers have been used as masking agent and stabilizer in food and cosmetics [6], in chromatographic separations, drug delivery systems (as drug solubilizing agent), hydrogels formed *in situ* [7], molecularly imprinted polymers [8].

We have considered the use of hydrogels with β-CD moieties for enhancing entrapping metabolites of catecholamine from aqueous solutions. Catecholamines are biogenic amines which play an important role in the nervous systems. Some catecholamines as well as their metabolites are used as markers of certain types of tumours. Exemplary quantification of catecholamines or their metabolites [dopamine, norepinephrine, epinephrine, vanillylmandelic acid (VMA) (I, Scheme 2) and homovanillic acid (HVA) (II, Scheme 2)] in biological fluids is important in the diagnosis of neuroblastoma as well as the other catecholamine secreting malignancy such as pheochromocytomas. Neuroblastoma is a cancer of the symphathetic nervous system and is the most common solid tumor in childhood. More than 90% of patients afflicted with neuroblastoma exhibit elevated levels of dopamine, VMA and HVA in serum and urine [9]. Pheochromocytomas are endocrine tumours and are characterized by elevated levels of urinal metanephrines (norepinephrine and epinephrine) and vanillylmandelic acid [10]. Moreover, excretion rate of VMA with urine is also informative and of great interest in characterization the effects of many drugs.

Materials composed of *N*-isopropylacrylamide, methacrylic acid or itaconic acid and crosslinking agent were demonstrated recently as materials for dopamine recognition [11]. Designing hydrogels with β-CD moieties for entrapping VMA and HVA is the subject of our present study. In this paper we report a NMR study of the β-CD inclusion complexes with VMA and HVA, formed in water. Additionally, inclusion complexation of vanillin (VA) (III, Scheme 2) by β-CD was studied because of that vanillylmandelic acid readily undergoes oxidation in mild condition to vanillin. In the case of obtaining effective materials for binding vanillin, indirect determination of VMA could be also possible.

The inclusion of vanillin by β-cyclodextrin in water was confirmed by NMR by Divakar [12] and Pîrnău group [13]. Complexes of β-cyclodextrin with homovanillic acid and other natural polyphenols were also investigated by NMR, thermodynamic and molecular modeling studies by A. Rescifina group [14]. Association constants were determined from NMR analysis in all above-mentioned cases. L. A. Kartsova et al. used Capillary Zone Electrophoresis for association constants determination of complexes of i.a. β-CD with VMA and HVA [15].

In this article complexes of β-CD with selected metabolites of catecholamines and vanillin were characterized. Their geometry and stability in water solution were studied in details in order to evaluate the usefulness of the β-CD moieties in binding process of the VMA, HVA and VA molecules in aqueous environment.



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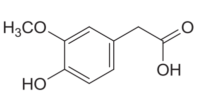

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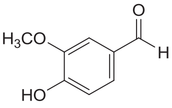

III

Scheme 2. Structures of VMA (I), HVA (II) and VA (III).

2. Experimental

2.1. Materials

Vanillylmandelic acid, homovanillic acid and vanillin were supplied by Acros Organics (Chemiatrade, Gliwice, Poland). Deuterium oxide was obtained from The Radioisotope Production and Distribution Centre (Świerk, Poland). β-cyclodextrin and 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt (TSP) were purchased from Sigma-Aldrich Co. (Poznań, Poland).

All chemicals and solvents were of commercial grade and used as received.

2.2. NMR experiments

NMR spectra were recorded at 25 °C with the aid of UNITY/*INOVA* 300 MHz or 600 MHz spectrometers (Varian). All tested solutions were prepared one day prior the measurements.

2.2.1. Job's plot

Equimolar solutions (10 mM) of the β-CD and one of complexed molecule (CM): VMA, HVA or VA in D_2O were prepared respectively and distributed among 11 NMR tubes in such a way that the molar fractions of β-CD varied between 0:1 and 1:0 (with a constant sample volume of 0.6 mL). Solution of 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt (TSP) in D_2O placed in melt-point capillary was used as an external reference. Standard ^1H NMR spectra were acquired with 48 scans and the relaxation delay of 3s. The widths at half-height of TSP signals were less than 1 Hz. Values of the molar fractions were recalculated from appropriate ^1H NMR signals integration.

2.2.2. Titration studies for evaluation of association constants

Two stock solutions were prepared in D_2O: first containing the β-CD (10 mM), second containing the β-CD (10 mM) and the VMA, HVA or VA respectively (50 mM). These stock solutions were mixed to a constant volume (6 mL) keeping the β-CD concentration equal to 10 mM and ranging the CM/β-CD nominal ratio from 1.0 to 5.0. Solution of TSP in D_2O placed in capillary was used as an external reference. Standard ^1H NMR spectra were acquired with 48 scans and the relaxation delay of 3s. The widths at half-height of TSP signals were less than 1 Hz. Experiments were carried out in triplicate. Values of molar ratio of CM to β-CD were recalculated from appropriate ^1H NMR signals integration.

2.2.3. 2D ROESY

For two-dimensional rotating-frame Overhauser effect spectroscopy (2D ROESY) measurements solutions of β-CD (15 mM) and the VMA, HVA or VA (60 mM) were prepared. No reference was added and chemical shifts were set based on the residue water signal at 4.790 ppm [16]. ROESY experiments were carried out on 600 MHz spectrometer using a mixing time of 500 ms.