
Calixarene Based Artificial Selective Sites for the Specific Binding Some Mycotoxins

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Abstract: Number of calix[4]rezortsynarenes with the different structures: R(H)-CH₃, R(H)-C₃H₇, R(H)-C₇H₁₅, R(H)-C₁₁H₂₃ and R(OH)-C₁₁H₂₃ were selected by the computer modeling for the investigation of their absorption properties in respect of such mycotoxins as patulin, T2, aflatoxin B1 and zearalenon. It was found that all used calix[4]rezortsynarenes interact with the mentioned mycotoxins but the structure type R(H)-C₁₁H₂₃ was characterized by the highest level of sorption activity. Based on the level of the concentration of the tested mycotoxins and on the determined association coefficient in the reaction of certain mycotoxins with the calix[4]rezortsynarenes it should be considered that the level of specificity or too low, or nonexistent. Application of the calix[4]rezortsynarenes as selective sites can be recommended only for general screening the presence of mycotoxins in the environment.

Keywords: sensors, selective sites, calyx [4]rezorcinarenas, mycotoxins, determination.

1. INTRODUCTION

In recent decades, there is a considerable interest in the finding ways for the developing effective approaches to create the artificial sites which are able for the molecular recognition and the registration as they play an important functional role in biological, medical, environmental and chemical sciences. However, the synthesis of the artificial sites that could provide high binding affinity, selectivity and sensitivity to keep analyzing substance and maintain stability over time analysis so far is a big issue for the scientific community. Construction of such types of sites is an important task for modern biosensorics [1,2].

Among the objects of the supramolecular chemistry research the special place belongs to calixarenes [2, 3] which are a class of macrocyclic compounds formed by the condensation of phenol typically para-substituted with formaldehyde. The name "Calix [n] arena" from the Greek calix - cup was offered Hyutshe [4], and the number in brackets indicates the number of benzene rings, which are the basis of the macrocycle. Calixarenes have several attractive properties, enabling their use in creating molecular receptors, self-organized systems and nano-objects, such as: the possibility macrocycle forming by the single process, no toxicity, possibility including small hydrophobic organic molecules or metal ions and formation together with them stable centers on a principle of "guest-host", as well as the ability to different conformations of the macrocycle, the ability to capture the desired orientation binding sites, unique opportunity to modify the upper and lower rim macrocycle necessary heteroatomic groups and possibility of forming a molecular system that has multiple binding sites [5]. The most accessible in terms of synthesis is calyx [n] arenes with n, which is four, six and eight although currently derived compounds with n, equal up to fourteen [6].

The main purpose of this article is searching artificial effective selective sites for the binding some mycotoxins and at this case having the hope of using them further in sensory devices for express control of such connections in environmental objects.

2. MATERIAL AND METHODS

The investigations were fulfilled with using patulin, T2 mycotoxin, zearalenone and aflatoxin B1 obtained from the company Sigma-Oldridgh, USA. The structural peculiarities of above mentioned mycotoxins are presented in Fig.1. Patulin is polyketide lactone with a molecular weight of 154.12, zearalenone - phenolic resorcylic acid lactone with a molecular weight - 318.4. T-2- trichothecene mycotoxin that belongs to a series of seskvy terpenoids compounds, molecular weight- 466.5. and aflatoxin B1-polycarbonyl compounds, molecular weight 312, 28.

The used mycotoxins are white, crystalline, chemically stable, heat-resistant compounds which are poorly soluble in water and moderately - in polar liquids. Therefore, their solutions were prepared in ethanol (50% concentration).

Investigated tetra-alkylcalix-rezortsynolarenes are macrocyclic compounds cupped containing different length alkyl groups at the lower crown and hydroxyl groups on the upper macrocycle crown. These calixarenes obtained by acid catalyzed condensation rezortsynoles or pyrogallol with the corresponding aldehydes [7].

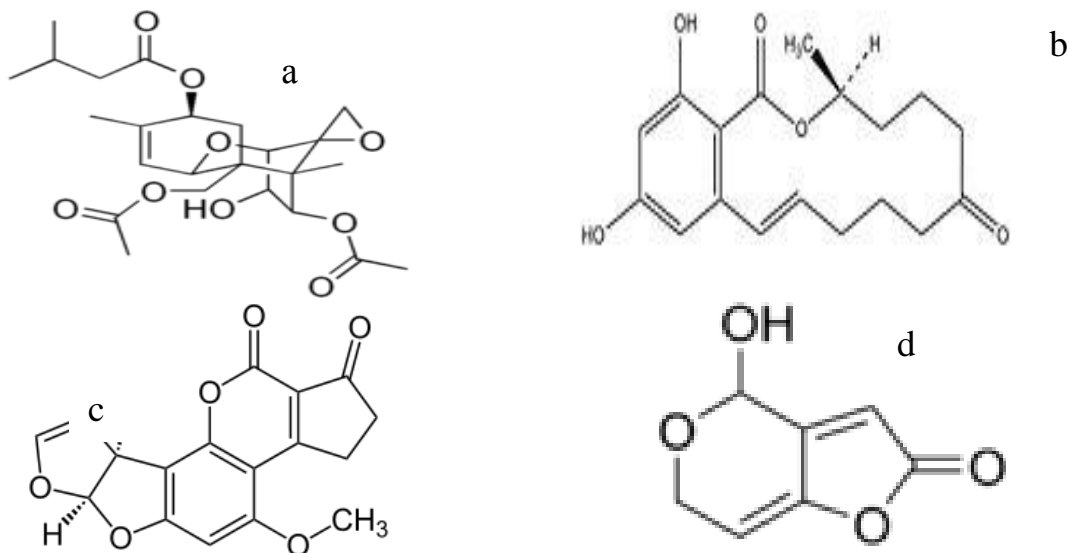


Figure1. Spatial structure of T2 mycotoxin (a), aflatoxin B1 (b), zearalenone (c) and patulin (d).

Before the synthesis of the calixarenes for the respective mycotoxins it was selected so that the upper, central and lower annular rim formed tert-butyl substituents in the para-position of arene aromatic residues and hydroxy- or alkoxy ones in the lower position of the macrocycle, respectively, having the ability to bind to this mycotoxin. Taken together, these structural fragments forming the inner cavity of the molecule. the volume of which is about 10 cubic angstroms on average (Fig. 2). Large-calix [n] arenes with $n > 4$ can have a high degree of conformational freedom. Consequently there was an attempt to apply this type of calix [n] arenes as hosts for specific molecular recognition.

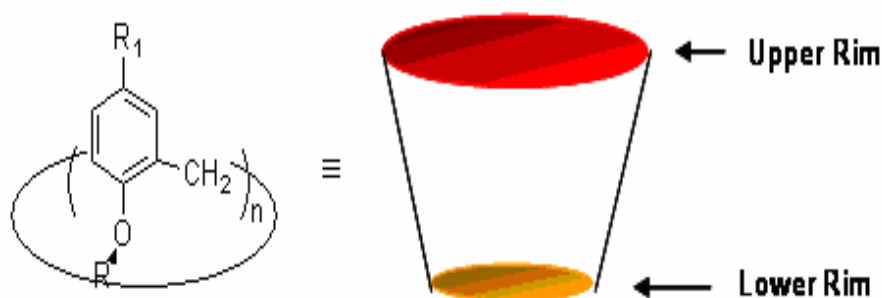


Figure2. Schematic structure of calixarenes.

Investigation of specific molecular interactions on the transducer surface carried by the angular spectroscopy with the help of the automated device of the surface plasmon resonance (SPR) - "Plazmonotest", developed by the Institute of Cybernetics. Glushkov NAS of Ukraine. To ensure contact between the optical prism and the metal film deposited on a glass base it was used liquid immersion - polyphenolether ($n = 1,62$). The modification of the transducer surface of SPR sensor was performed in two stages. In the first phase a glass plate covered with a layer of gold, exhibited 98% solution n-dodekanethyol during 18 hours. Then the surface of the plates were washed by 50% ethanol solution. In the second stage the layer of calixarenes was formed directly in the cell-SPR instrument by the introducing them in measuring cell about 100 $\mu\text{g/ml}$ in 50% alcohol solution to termination of the suspension adsorption, which was monitored by SPR response. To remove excess calixarenes which was not sorbet on the surface, the cell was washed with 50% alcohol solution and record the shift of the SPR resonance angle. Then the cell was filled with a solution of one of the above mentioned mycotoxins in

sequentially increasing concentrations, and kept for 10 min. After the measurement, each cell was washed with 50% alcohol solution to remove analytes that are not contacted, and record feedback sensor.

3. RESULTS AND DISCUSSION

Mycotoxins were analyzed by the five types of biosensor transducer surfaces obtained using calix-rezortsynolarenes (1-5), having intermolecular cavity, a conical conformation stabilized by four hydrogen bonds between the nearest adjacent hydroxyl groups rezortsynol rings. Using X-ray analysis it was revealed that in the crystalline state long aliphatic radicals calix-rezortsynolarenes interact with each other through many weak non-covalent bonds forming tightly packed hydrophobic bylayer [8]. This may lead to steric inaccessibility of molecular cavity of calixarenes for interacting with the studied analyte. Indeed, previous studies have shown that the direct application of calix[4] rezortsynolarenes on gold surface covered with a layer transducers practically does not change the value of the sensor response when exposed to mycotoxin T2 [9]. With a view to optimal spatial orientation of the molecular cavities calix-rezortsynolarenes, the sensor surface was modified by dodecanthiol, sulfur atoms which have a high affinity for gold. As a result, the surface becomes covered by the dodecyl chains [8]. In a number of investigated compounds over 10 types, among them a special attention was given such as: R(H)-CH₃, R(H)-C₃H₇, R(H)-C₇H₁₅, R(H) -C₁₁H₂₃ and R (OH)-C₁₁H₂₃. Among other similar types calixarenes such as: R(H)-CH₃, R(H)C₁₁H₂₃ the best binding mycotoxins was provided by R(H)-C₇H₁₅ and R(H)-C₃H₇ tetrapropilcalix-rezortsynolarenes. The nature of the outage response upon binding patulin, zearalenone and T2 toxin calix-rezortsynolarenes at the studied concentrations was almost identical to response as in case of R (H)-C₃H₇ and R(H)-C₇H₁₅ tetrapropilcalixarenes. However, the deviation of the resonance angle for other calixrezortsynolarenes was significantly lower (Fig. 3, 4). This sample demonstrates the selectivity of calixrezortsynolarenes to mycotoxin molecules.

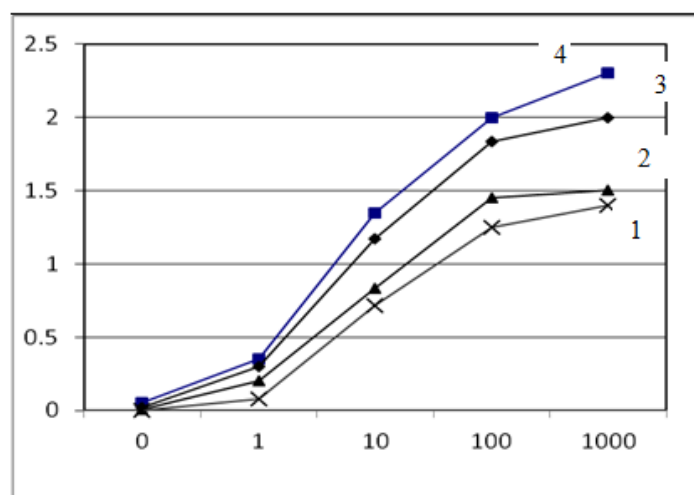


Figure3. Changes of reflex SPR angle (ordinate) at the interaction of different patulin concentrations (ng/ml, ordinate) with a number of calix-resorcinolarenes: 1-R(H)-C₁₁H₂₃, 2- R(H)-CH₃, 3-R(H)-C₇H₁₅, 4-R (H)-C₃H₇, pre-immobilized on the surface transducer.

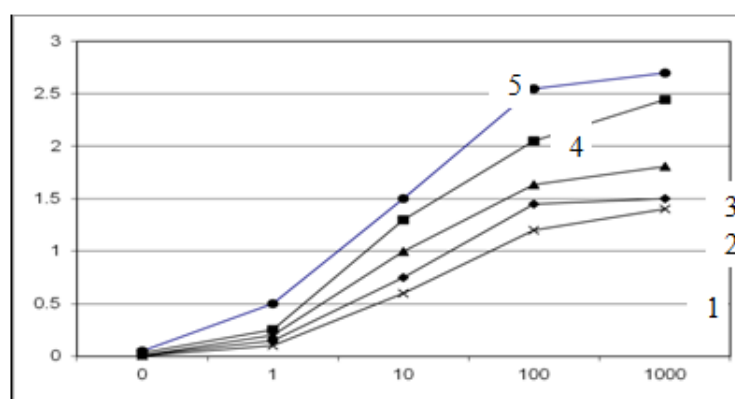


Figure4. Changes of reflex SPR angle (ordinate) at the interaction of different aflatoxin B1 concentrations (ng/ml, ordinate) with a number of calix-resorcinolarenes (1-R(OH)-C₁₁H₂₃, 2- R(H)-C₁₁H₂₃, 3- R(H) -CH₃, 4-R(H)-C₇H₁₅ and 5-R(H)-C₃H₇), pre-immobilized on the transducer surface.

Overall, the efficacy of mycotoxins binding by the calixarenes may depend on their geometric conformation, mobility, rational spatial distribution of hydroxyl groups on the upper crown, and acid-base properties of mycotoxins. The binding of the studied analytes with the surface covered with a layer of (RH)-C3H7 and (RH)-C7H15 calix-rezortsynarenes probably is due to the complexation of the type "guest-host" between mycotoxin and macrocycle. Some fluctuations refractory angle changes observed for certain mycotoxins in their interaction with the above calix-rezortsynarenes likely due to their small difference in molecular weight. To assess the spatial structure of these complexes, we conducted simulations include toxins into the cavity of (RH)-C3H7 and (RH)-C7H15 calix[4]rezortsynarenes using the program "HyperChem 6.03". For this, the first phase, semi-empirical method calculated the structure of the calixarenes and mycotoxins [8]. Then the structures of calixarenes and mycotoxins was compared together by arbitrary ways. The optimal geometry of the formed complexes were calculated on the basis of molecular mechanics. Among the variety of simulated structures of each complex the structure with the lowest total energy (E) was selected. During the chelating process calixarenes changes conformation and form a cavity extending in the direction of flattened rings of the macrocycle, i.e, there is an adjustment molecule "Master" geometry "guests". The virtually flat bicyclic molecule mycotoxin is included into this cavity. As a result of it the supramolecular complex stabilizes interactions between CH₂CH group and mycotoxin near orthogonal macro cycle ring (corresponding to a distance ~ 3.9) and solvatophobic and dispersion interactions.

In special studies the association constants of patulin and aflatoxin B1 with a number of the above-mentioned calix-rezortsynarenes were identified (Table). It turned out that their values are within 2100-2540 M⁻¹. Moreover, their difference for patulin and aflatoxin B1 varies around 10%, indicating a relatively low specificity determining individual mycotoxins.

Table1. Values Of the association constant of the interaction of calix-resortinolarene (R=C7H15) with some mycotoxins, M⁻¹.

Mycotoxin	Value of association constant, M ⁻¹
Patulin	2540
Aflatoxin B1	2390
T2	2300
Zearelenone	2100

Based on the entire complex of the obtained results it is hoped that the use of calix- rezortsynarenes as selective sites can be recommended only for general screening the presence of mycotoxins in the environment.

SUMMARY

- [1] With the help of the computer modeling it was selected number calix-rezortsynarenes different structures, among them special attention paid to the fact that had the following structural features: R(H)-CH₃, R(H)-C₃H₇, R(H)-C₇H₁₅, R(H)-C₁₁H₂₃ and R(OH)-C₁₁H₂₃ and investigated their absorption properties in respect of such mycotoxins as patulin, T2, aflatoxin B1 and zearelenon.
- [2] It was established that all used calixrezortsynarenes interact with the mentioned above mycotoxins and the structures of types: R(H)-C₃H₇, R(H)-C₁₁H₂₃ were characterized by the highest level of sorption activity.
- [3] Based on the level of the concentration of the tested mycotoxins and according to the determined association coefficient in the reaction of certain mycotoxins with calix- rezortsynarenes should be considered that the level of specificity is very low. Using calix-rezortsynarenes, as selective sites, can be recommended only for general screening the presence of mycotoxins in the environment.

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