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ORIGINAL ARTICLE

Selective membrane transport of amino acids by functionalised calix[4]arenes

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Abstract The selective active transport through liquid membrane assisted by the pH gradient of amino acid methylesters by using a series of calix[4]arenes substituted by acid and amido functions, glycolic chains, and hydroxyl groups as carriers has been performed. All these receptors have been found to act as carriers for transport of aromatic amino acid methylesters from the aqueous source phase to the aqueous receiving phase aiming at their separation. The receptors bearing diacid and tetraamido functions have the better ability to transport of amino acids than the other receptors studied. The influence of calixarene and amino acid structures upon transport through liquid membrane is discussed. The obtained results are correlated with those acquired by solvent extraction.

Keywords Calixarenes · Amino acid methylesters · Transport through liquid membrane

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Introduction

The synthesis of ion transporters means an important task for chemistry and supramolecular chemistry with applications in clinical analysis, selective sensor, enzyme assays, and biology.

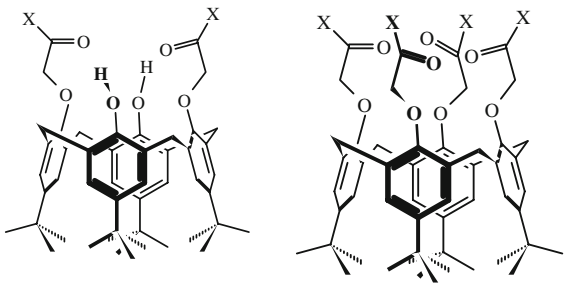
The place of calix[n]arenes in the interesting and challenging field of ion transport through liquid membrane as synthetic ionophores is a topic of current interest in supramolecular chemistry as well as in the field of analytical chemistry [1–7]. Much of the analytical interest in calix[n]arenes comes from their potential as selective complexation agents and useful carriers through liquid membrane. Smith et al. [8, 9] perform partition of transporters onto three mechanistic classes: mobile carriers, monomeric channels [3, 4] and self-assembled pores [10]. Along with crown ethers, steroids, cyclodextrins, and cucurbit[n]urils, calix[n]arenes belong to the family of synthetic transporter compounds. Thus, the contribution of Cragg et al. [11] has pointed out the potential of calix[n]arenes and their derivatives to be incorporated onto ion transporting or channelling systems.

Calix[n]arenes as cyclic oligomers are important receptors of supramolecular hosts involved in host–guest molecular recognition of various compounds as well as in analytical applications such as separation of chemical or biochemical compounds [12–16]. Derivatisation of calix[n]arenes at the upper and lower rim in order to introduce various functional groups has led to new compounds with desired properties [17–23]. Moreover, calix[n]arenes modified with additional binding sites enhance the binding ability of the parent calix[n]arenes. It is well known that the calix[4]arene cavity is not large enough to accommodate some molecules but its functionalization allows the obtaining of external binding sites

appropriate to form inclusion complexes with guest molecules [24]. Li et al. [25] reported that a water-soluble calix[4]arene is able to adjust its conformation to fit the size of aromatic L-tryptophan with the penetration of the benzene ring of amino acid into the hydrophobic cavity of calix[4]arene. Recently, Sirit et al. [26] synthesized chiral calix[4]azacrowns for enantiomeric recognition of amino acid derivatives. It was shown by Sansone et al. [27] that a water soluble peptidocalix[4]arene in a rigid cone structure forms complexes with aromatic amino acids and the complexation occurs through the interactions of the calixarene cavity with the apolar groups of the amino acids.

The ability of calix[6]arene carboxylic acid derivatives to act as carriers through liquid membrane of aromatic amino acids was reported by Oshima et al. [5]. Liquid–liquid extraction and transport through liquid membrane of some amino acids methylesters by *p*-tert-butylcalix[n]arenes ($n = 4, 6, 8$) as extractants or carriers in the presence of picrate [28] or tropaeolin 00 ([4-(4′-anilinophenylazo)benzenesulphonic acid]) [29] as counterions were also investigated. Okada et al. [6] obtained calix[4]arene with chiral pendant groups and used them to selective extraction and transport of some amino acid esters, and Z-amino acid carboxylates into CH_2Cl_2.

In a previous study [30] we have presented the ability of a series of functionalized calix[4]arenes variously substituted by acid or amido functions, glycolic chains and hydroxyl groups as extractants towards amino acid native and ester derivatives. The results have suggested that aromatic amino acid methylesters are extracted from aqueous phase into organic phase and the extractability was essentially controlled by the structure of the calix[4]arene derivative and the nature of the amino acid. Continuing our research in the recognition and separation of biologically relevant molecules such as amino acids by synthetic receptors we report herein the study of the transport through liquid membrane of some amino acid methylesters (Chart 1) by using as carriers the same functionalized calix[4]arenes (Chart 2).



1 X = OH
2 X = $NHCH(CH_2OH)_2$
3 X = $NHC(CH_2OH)_3$
4 X = OH
5 X = $NH(CH_2)_2O(CH_2)_2OH$

Chart 2 Chemical structures of calixarenes **1–5**

Experimental

Materials

All amino acid derivatives used throughout the experiments: L-tryptophane methylester hydrochloride (L-TrpOMe), L-phenylalanine methylester hydrochloride (L-PheOMe) and L-tyrosine methylester hydrochloride (L-TyrOMe) were purchased from Fluka (purity >99.5%) and were employed without further purification (Chart 1). The organic solvent chloroform (dielectric constant $\varepsilon_r = 4.81$) [31] was distilled before use. Distilled (Millipore) water was used throughout the experiments.

Liquid membrane transport

The transport experiments were carried out by using a U-shaped glass tube (Fig. 1). The source phase contains 10 mL of amino acid aqueous solution (the concentrations ranged between 2.5×10^{-4}–1.0×10^{-3} M, depending of the amino acid) at pH = 5.5 present in one arm (left in Fig. 1) whereas the aqueous receiving phase, 10 mL (pH = 1.5) is present in the other arm (right in Fig. 1). The

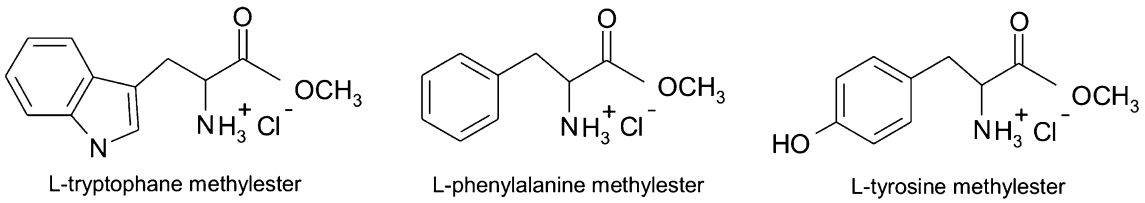


Chart 1 Chemical structures of the methylester amino acids

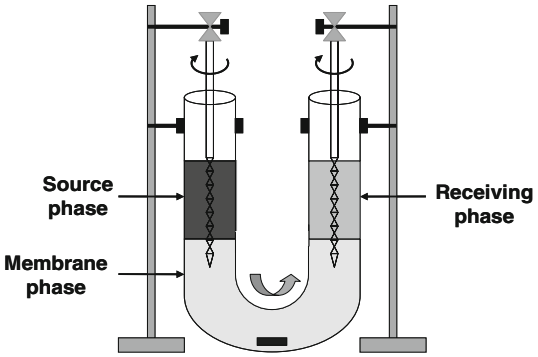


Fig. 1 Schematic device of the transport experiments

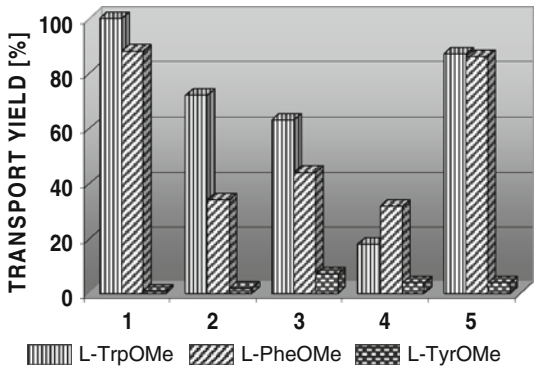


Fig. 2 Transport yields (%) of amino acids through liquid membrane by calix[4]arene derivatives **1**–**5**

membrane phase, 25 mL of functionalized calix[4]arenes of 5.0×10^{-4} M in chloroform was introduced in the tube. Transport experiments were carried out by stirring the aqueous and organic phases at 180 rpm at room temperature for 24 h. The concentration of amino acids in both the aqueous phases (source and receiving phase) was determined by UV–Vis measurements with an UV–Vis Spectrometer JASCO V-530. Each experiment was repeated three times and reproducibility was ±10%. Blank experiments were performed for reference in the absence of carrier.

The pH was measured by a digital MV-870 Pracitronic pH-meter with glass electrode and saturated calomel electrode.

Results and discussion

Calixarenes **1–5** were prepared from the corresponding methyl esters as described in a previous paper [30].

Transport of amino acids by **1**–**5**

The values of the transport yields of L-TrpOMe, L-PheOMe, and L-TyrOMe through chloroform liquid membrane by means of calix[4]arene derivatives **1**–**5** as carriers are given in Fig. 2. All calixarenes perform the transport of amino acid methylesters.

As one can see from Fig. 2, the receptors **1** and **5** exhibit high transport ability towards L-TrpOMe (98% with **1** and 87% with **5**) and L-PheOMe (88% with **1** and 86% with **5**). We can notice the efficiency of receptors **1** and **5** for the both amino acids transport while no selectivity is observed. The whole series of calix[4]arene derivatives shows poor transport behaviour towards L-TyrOMe, none of the transport yields exceeds a value of 7% (case of L-TyrOMe with **3**) and a value of 2% with **1**. As in extraction experiments [30] the sequence of the decreasing transport yields of amino acids using calixarene derivatives **1**–**5** as carriers is the following: L-TrpOMe > L-PheOMe ≫ L-TyrOMe. At a first glance the amino acids hydrophobicity enforces this sequence, along with the structure of calix[4]arene derivatives. For instance, the following sequence of the carriers transport efficiency towards L-TrpOMe holds: **1** > **5** > **2** (72%) > **3** (63%) > **4** (18%) and the following sequence of calix[4]arene derivatives as extractants: **5** (65%) > **3** (61%) > **1** (50%) > **2** (41%) > **4** (5%) [30]. The most efficient extractant in this case was tetraamido calix[4]arene (**5**). The same sequence of carriers transport efficiency towards L-TrpOMe is found in the case of L-PheOMe, except the receptor **2** whose transport ability is lower by an order of magnitude than the receptor **3**: **1** > **5** > **3** (44%) > **2** (36%) > **4** (32%). The most effective extractant for L-PheOMe was proven to be diamido calix[4]arene (**3**) followed by the receptor **5** [30].

One can observe that the values of the transport yield are larger than those of the extraction yields. There are several factors that influence the transport through membrane. As such, the overall transport process consists of a mixture of diffusion steps and complexation/decomplexation reactions at the interfaces. Moreover, solvation and desolvation at the interfaces of compounds involved in transport process play an important role [32, 33]. The distribution equilibrium of the complex at the membrane interfaces rely on the physicochemical characteristic of the solvent, the nature of amino acids, pH of the aqueous phases, the structure of carrier, and the complex stability.

The extraction and transport through liquid membrane of aromatic amino acid methylesters by parent calix[4]arene, namely *p*-tert-butylcalix[4]arene as extractant and carrier [29] led to the values of extractability and transport yields much smaller than those obtained by using functionalized calix[4]arenes **1**-**3** and **5** for extraction and transport of tryptophan, phenylalanine, and tyrosine. Obviously, the functional groups attached to calix[4]arene enhance the recognition properties of calix[4]arene towards amino acids.

Consequently, the calix[4]arene **1** bearing two acidic functions acts much better as transporter through

membrane than the calix[4]arene **4** which bears four acidic functional groups. We still need more experiments to elucidate all the interaction types involved in host–guest properties of calix[4]arene derivatives.

In all our experiments, the values of amino acid esters transport through liquid membrane by using calix[4]arene (**4**) as carrier are smaller than that of calix[4]arenes **1-3** and **5**. Hence, the changes of the functional groups profoundly influence the transport abilities of calix[4]arenes.

High transport of L-TrpOMe and L-PheOMe was also observed by using tetraamido calix[4]arene (**5**) as carrier through membrane. It turns out that a well-defined complex between the amino acid and tetraamido calix[4]arene (**5**) has been formed by specific types of interactions. The results pointed out that the structure of calix[4]arenes is one of the most important parameter for the recognition of L-TrpOMe and L-PheOMe. As in the case of calix[4]arene (**1**), the tetraamido calix[4]arene (**5**) showed lower transport through membrane towards L-TyrOMe (4%). The receptors diamido calix[4]arene (**3**) and diamido calix[4]arene (**2**) also exhibit an efficient transport of L-TrpOMe (63% with **3** and 73% with **2**) and L-PheOMe (44% with **3** and 36% with **2**). Under our experimental conditions, it was noticed that receptor **1** prefers L-TrpOMe and L-PheOMe over L-TyrOMe with $S_{\text{L-TrpOMe/L-TyrOMe}} = 49$ and $S_{\text{L-PheOMe/L-TyrOMe}} = 44$. A similar behaviour was observed for receptor **2** with $S_{\text{L-TrpOMe/L-TyrOMe}} = 36$ and $S_{\text{L-PheOMe/L-TyrOMe}} = 18$. Further experiments are in progress aiming to get optimal separation of amino acids by functionalized calixarenes.

Conclusions

The transport abilities of calix[4]arene derivatives **1–5** were investigated. The results suggested that calix[4]arenic receptors **1–5** are efficient carriers of aromatic amino acid methylesters as well as exhibiting their separation. It was observed from the experimental results that the functional groups introduced on the calix[4]arene structure profoundly influence the transport abilities of calix[4]arenes. The nature of amino acids is also responsible for the effective transport through liquid membrane. There has been found no meaningful correlation between extractability of the amino acids complexes with calix[4]arenic receptors and their transport through liquid membrane.

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