Complexation of Calix[4]arene bis-Hydroxymethylene-di-phosphonic Acid with Amino Acids

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Host-Guest complexation of calixarene bis-hydroxymethylene-di-phosphonic acid with 17 amino acids in water solution had been studied by the RP HPLC and molecular modelling methods. It had been shown the binding constants of the complexes are depended on the nature of the amino acid residue, *log P* and *pKa* of the acid. The complexation is mainly determined by the electrostatic interactions between the positively charged nitrogen atom of the amino acid and the negatively charged oxygen atom of phosphonic acid residue of the calixarene, the Host-Guest π-π, CH-π and hydrophobic interactions.

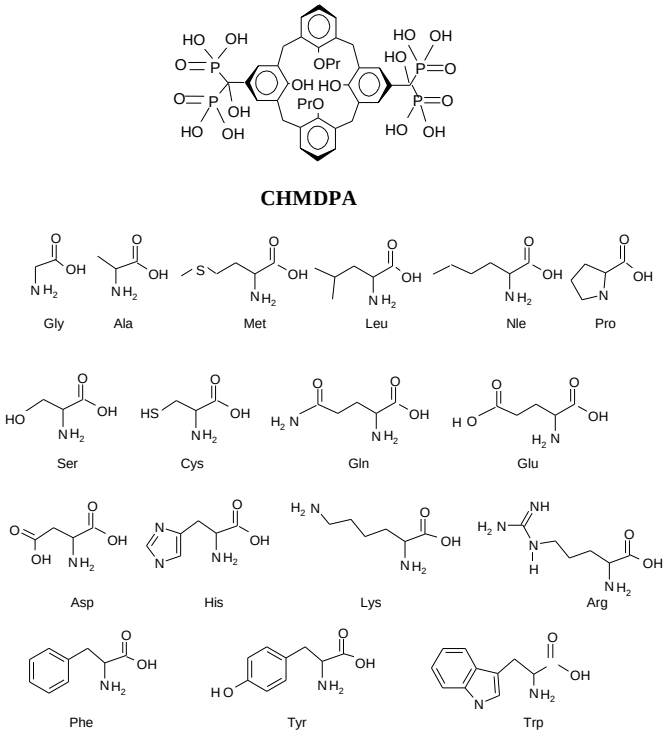
Introduction

Calixarenes [1] contained preorganized bio-affine groups are able to recognize different biologically active molecules such as amino acids, dipeptides, proteins, choline and acetylcholine, carbohydrates, riboflavin, vitamin B_{12}, nucleotides, nucleosides and short DNA fragments [2,3,4,5,6]. Calix[4]arene derivatives can also be fastened on the surface of proteins [7,8]. Due to the ability to simulate the substrate-receptor interactions with biomolecules, calixarenes are objects of bio-medical researches [9,10].

Formerly it was shown that calix[4]arenes functionalized by residues of phosphonic, aminophosphonic or methylenediphosphonic acids are effective inhibitors of alkaline phosphatases [11,12,13], calcium channel modulators [14,15], antithrombotics [16]. They inhibit the ability of P-glycoprotein to remove anticancer drug Doxorubicin from the cells [17]. The base of these biochemical effects are supramolecular (substrate-receptor) interactions of the calixarenes with amino acid fragments in the active sites of the respective protein structures. As a result of the macrocyclic effect of the calixarene platform, the biological activity such phosphonic acids is much higher comparatively with model acyclic compounds [11,12,13,14]. The biological activity is essentially depended

from the number of phosphonic groups, their stereochemical configuration and geometrical parameters of the macrocyclic platform as well.

The aim of this work was to study the complexation of calix[4]arene-bishydroxymethylene-bis-phosphonic acid (**CHMDPA**) (**Scheme**) with a series of 17 amino acids in water solutions. Reversible-phase high-performance liquid chromatography method (RP HPLC) was used for determination of the binding constants of supramolecular complexes of the calixarene with amino acids.



Scheme

Results and discussion

Usually, for the determination of the binding constants of calixarene Host-Guest complexes with organic molecules, the NMR, calorimetry [4,18,19,20], UV, fluorescent spectroscopy and other methods are widely used [14,21]. However in some cases due to poor solubility of the calix[4]arenes or the Guest molecules or absence of a corresponding instrumental response on the complexation process the above menthioned methods are ineffective. To solve this problem we have developed RP HPLC method the determination binding constants of the calixarene complexes with organic compounds in water or water-organic solutions [16,22,23,24,25]. The method includes the determination of retention time t_R and retention factor k' of the Guest molecule before and after the calixarene addition to the mobile phase. The binding constant K_A of the calixarene complex with the Guest molecule (the ratio 1:1) can be calculated by equation (1) [26]:

$$1/k' = 1/k_0' + K_A \times [\mathrm{CA}]/k_0' \quad (1)$$

where k_0' and k' are capacity factors of the Guest molecule determined in the absence and the presence of the calixarene in the mobile phase.

CHMDPA and the amino acids were registered on the chromatograms by the sharp peaks. Linear adsorption (r=0.99) indicated on its reversible sorption on the Zorbax ODS surface.

An addition of the **CHMDPA** to the mobile phase decreases the retention factor k'

of the amino acids. The linear character plots of *k'vs* the **CHMDPA** concentration (**Figure 1**) testifies the formation of the Host-Guest supramolecular complexes with 1:1 stoichiometry and allows the correct calculation K_A values by equation (1) [26].

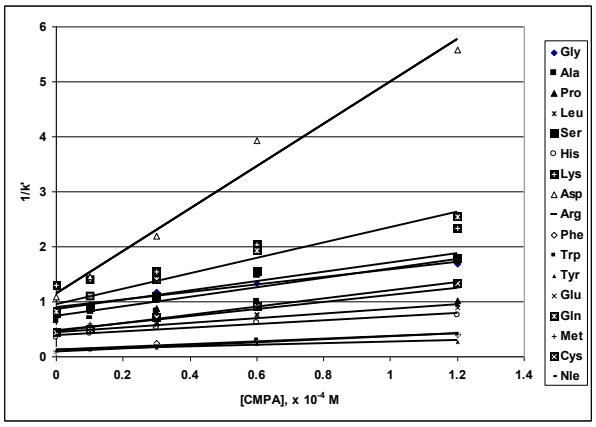


Figure 1. Dependence of $1/k'$ *vs* **CHMDPA** concentration in the mobile phase for Gly, Ala, Pro, Leu, Ser, His, Lys, Asp, Arg, Phe, Trp, Tyr, Glu, Gln, Met, Cys, Nle (r=0.96-0.99).

The binding constants K_A and free Gibbs energy ΔG ($\Delta G = -RT\ lnK_A$) for **CHMDPA** complexes with the amino acids are presented in **Table 1.**

Table 1. Values of the binding constants K_A and free Gibbs energy ΔG (kJ/моль) of **CHMDPA** complexes with the amino acids

№	Amino acid	K_A*	ΔG
Nonpolar amino acids			
1	Gly	11800±2100	-23.19
2	Ala	13500±3900	-23.52
3	Met	14200±1600	-23.64
4	Leu	15800800	-23.92
5	Nle	16100±1600	-23.95
6	Pro	4800±1700	-20.95
	Uncharged polar amino acids		
7	Cys	18900±1800	-24.36
8	Ser	12600±4300	-23.36
9	Gln	32300±6400	-25.68
	Charged amino acids		
10	Asp	31100±4000	-25.59
11	Glu	13300±2100	-23.49
12	His	11800±3410	-23.20
13	Lys	7800±1400	-22.15
14	Arg	13100±3800	-23.45
Aromatic amino acids			
15	Tyr	15796±2800	-23.91
16	Phe	30892±8700	-25.57
17	Trp	45700±6300	-26.54

*relative standard deviation (*RSD*) is 5-34 %

The binding constants are vary from 4800 M^{-1} (ΔG = -20.95 kJ/mol) to 45700 M^{-1} (ΔG = -26.54 kJ/mol) (**Table 1)**. The highest K_A value among the uncharged polar amino acids is observed for Gln (K_A = 32300 M^{-1}), charged amino acids - for Asp (K_A = 31100 M^{-1}), aromatic amino acids – for Trp (K_A = 45700 M^{-1}).

It is evident, that efficiency of the **CHMDPA** complexation depends on the nature of the side chain of amino acids (**Figure 2**). Hydrophobic interactions between the Host and Guest molecules influence the complex stability in water solutions. This is clear to see from the dependence of **CHMDPA** log K_A values from *log P* of amino acids [27] **(Figure 2**).

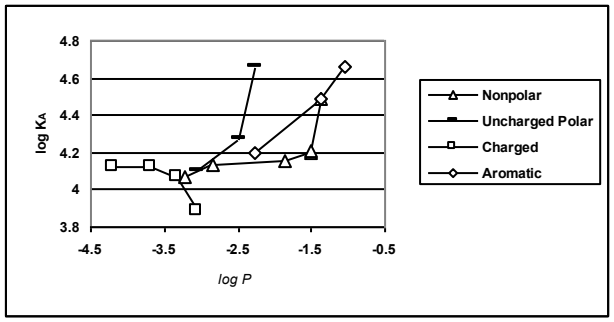
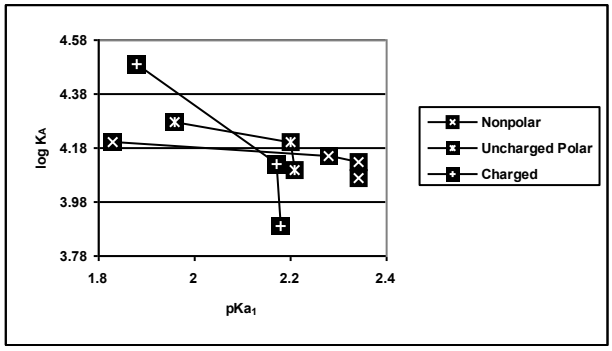


Figure 2. Influence *log P* nonpolar (Gly, Ala, Met, Leu, Nle, Phe), uncharged polar (Ser, Cys, Tyr), charged (Arg, Glu, His, Lys) and aromatic (Tyr, Phe, Trp) amino acids on **CHMDPA** log K_A complexes.

As is shown on **Figure 2** K_A for charged amino acids (Arg, Glu, His, Lys) decreases with the *log P* increasing. At the same time K_A values for nonpolar (Gly, Ala, Met, Leu, Nle, Phe), uncharged polar (Ser, Cys, Tyr) and aromatic (Tyr, Phe, Trp) amino acids increases with the *log P* increasing.

Figure 3 demonstrates the correlation of pKa_1 amino acids complexes of nonpolar (Gly, Ala, Met, Leu, Nle, Phe), uncharged polar (Ser, Cys, Tyr), charged (Arg, Glu, His, Lys) and aromatic (Tyr, Phe, Trp) amino acids with



CHMDPA log K_A.

Figure 3. Correlation of pKa_1 of nonpolar (Gly, Ala, Met, Phe), uncharged polar (Cys, Tyr, Ser,) and charged (Asp, Arg, Lys) amino acids with the log K_A complexes.

CHMDPA adopts the *cone*-conformation stabilized by intramolecular hydrogen bonds OH···OPr at the lower rim of the macrocycle [28]. To clarify the nature of the Host-Guest interaction the molecular modelling of the complexes of **CHMDPA** with Asp (K_A=31100 M^{-1}) and Gln (K_A=32300 M^{-1}) was performed (**Figure 4**).

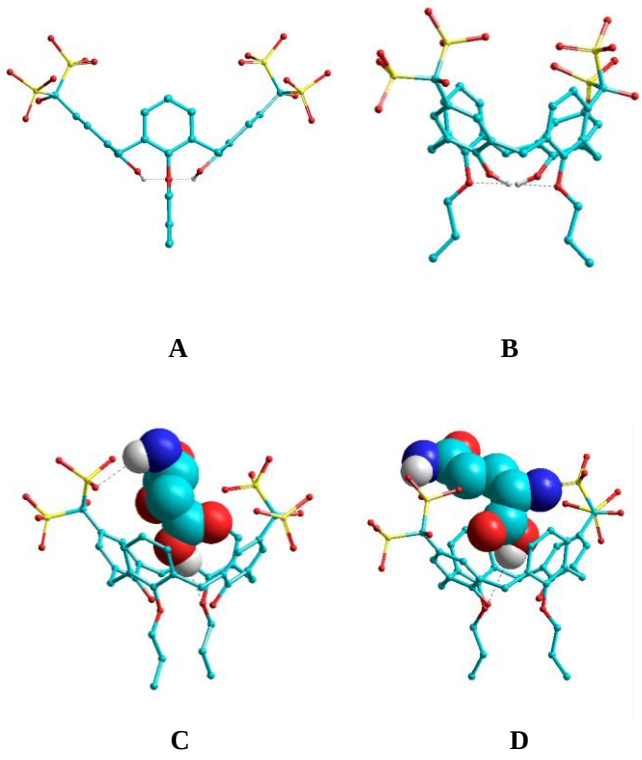


Figure 4. Energy minimized structures of **CHMDPA** (**A** top view), (**B** side view) and its complexes with Asp (**C**) and Gln (**D**). Hyrogen bonds are presented by dotted lines.

In both cases the electrostatic contacts of positively charged nitrogen atoms of the amino acid with the negatively charged oxygen atoms of phosphonic acid residue of the Host molecule are observed (N···O distance ~3.62 Å). **CHMDPA** complex is additionally stabilized by hydrogen bonds P-O-H···O=C between the Host and Guest molecules (O···O distance is 3.93 Å). In both cases the amino acids are deeply included into the calixarene cavity (**Figure 4 C,D**).

Conclusions

CHMDPA forms stable supramolecular Host-Guest complexes with different amino acids in water solution. Binding constants of the complexes were determined by RP HPLC method. Molecular modelling data show that the complex stability is strongly depended on the nature of the amino acid residue. The complexation efficiency is determined by electrostatic contacts of positively charged nitrogen of the amino acid with the negatively charged oxygen atom of phosphonic group at the calixarene upper rim.

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Experimental part

Reagents

Calix[4]arene-bis-hydroxymethylene-bis-phosphonic acid (**Scheme 1**) was synthesised by the method described in [29]. With respect to its

poor solubility in the water solutions, the calixarene was analysed as disodium salt. The disodium salt was obtained by addition of equivalent quantity of sodium methylate to the calixarene solution in methanol.

Methods and Equipment

RP HPLC analysis

RP HPLC analysis was performed on the using high pressure liquid chromatograph Hitachi (Hitachi, Ltd., Tokyo, Japan). Chromatographic experiments were performed with isocratic conditions on the chromatographic column Zorbax ODS (Merck, Darmstadt, Germany). and mobile phase H_2O/MeCN (98/2, v/v). UV detector was operated at 254 nm and the flow rate was 0.8 ml/min. Solutions of the amino acids with concentration 10^{-3}-10^{-4} M in H_2O were used for RP HPLC. All chromatograms of the amino acids were obtained at 34 ^oC. The mobile phases contained **CHMDPA** in the concentrations 0.1 x 10^{-4} M – 1.20 x 10^{-4} M.

Molecular modelling

The molecular modelling of **CHMDPA** and its complexes with the amino acids was carried out by molecular mechanics MM+ method, force field PM3 (software package Hyper Chem, version 8) [30]. RMS gradient was 0.01 kcal/mol.

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