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The Complexation Properties of the Water-Soluble Tetrasulfonatomethylcalix[4]resorcinarene toward α-Aminoacids

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Abstract

The flexible bowl-type water-soluble molecule **1**, consisting of a resorcinol core and four convergent tetrasulfonatomethylene groups, existing as a tetraanion in neutral water solution, was studied as the host molecule for recognition of α-aminoacids. Out of 12 examined guest molecules only those possessing aromatic hydrophobic moieties or a long hydrophobic chain with a second ionogenic group form inclusion complexes with **1**. The complex formation was considered with the help of both ^{1}H NMR and pH-metric titration in a broad range of pH. The role of host and guest geometric complementarity as well as additional π–π and hydrophobic interactions is discussed. The lack of these interactions in aqueous media provides domination of the guest solvation by water over the 1 : 1 complex formation with **1**.

Introduction

A simple, high-yield method of preparation of the novel water-soluble tetrasulfonatomethylcalix[4]resorcinarene tetrasodium salts (Figure 1) was recently presented [1]. Their complexation properties towards targets such as α-aminoacids, a polyfunctional organophosphorus compound – diglycidylmethyl phosphonate and some different sized and shaped metal complexes were also examined in aqueous media of different pH [1, 2].

Here we report a detailed study of α-aminoacid binding by tetrasulfonatomethylcalix-[4]resorcinarene **1**.

Figure 2 shows that recognition of aminoacids is based on two different kinds of interactions: H-bonding and electrostatic interactions with the carboxylic and amino-groups common for all aminoacids and specific interactions with the α-substituent which is crucial for the selectivity of recognition. Variation of pH gives a tool to alter the interactions of the carboxylic and amino-groups, thus, for example, alkylammonium ions (cationic and zwitter-ionic forms) can partake in the cation-π interactions with the aromatic cavity of calixarenes and calixresorcinarenes [3] as well as in the electrostatic attraction with the sulfonatomethyl and hydroxy-groups [4, 5, 6]. The carboxylic group can also experience different kinds of interactions with the binding sites on the upper rim of the host. A ^{1}H NMR titration study of the binding was performed in neutral conditions, while in the acidic and alkaline media a potentiometric titration study was used.

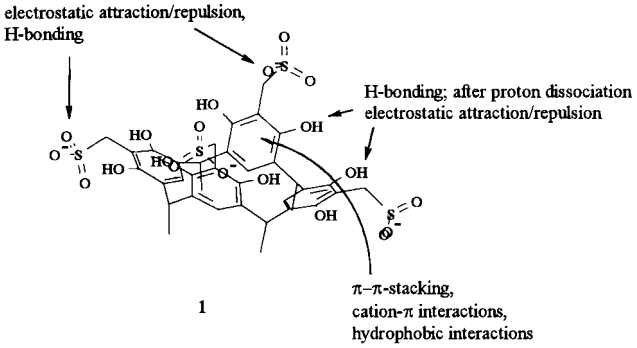

Figure 1. The structure of the host **1** and its potential binding centers.

Experimental

Tetrasulphonatomethylcalix[4]resorcinarene **1** was synthesised as described [1]. The ^{1}H NMR study of the complexation of **1** with α-aminoacids was carried out in D_2O, pD 7.2 (0.1 M phosphate buffer) [7]. The ^{1}H NMR titration experiments were performed at a constant guest concentration of 6×10^{-3} M, the concentration of **1** was varied in the range of 0 to 6×10^{-2} M. ^{1}H NMR spectra were recorded on a Varian Unity 400 spectrometer with a working frequency of 300.13 MHz at 298 K and DSS as internal standard. The

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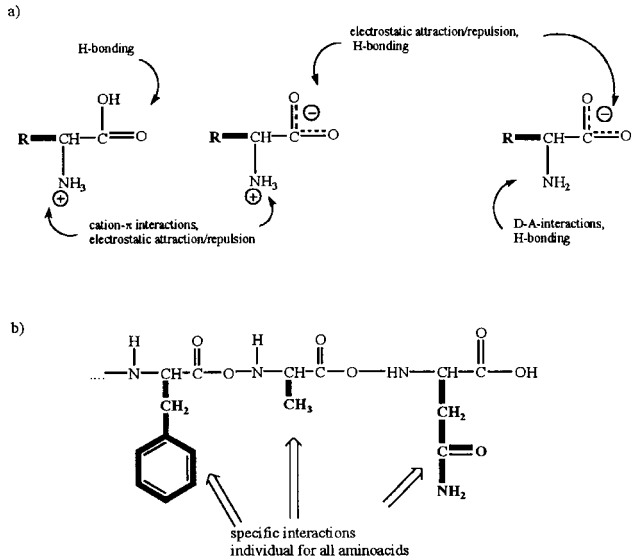


Figure 2. Recognition of α-aminoacids: (a) pH-dependence of charge distribution in the α-aminoacids; (b) α-aminoacids sequence in a protein molecule-specific interaction with α-substituents.

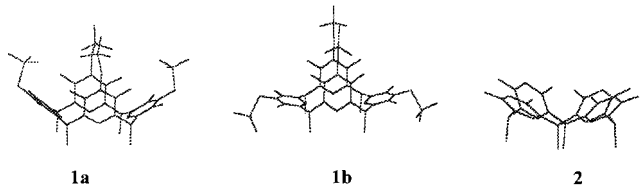


Figure 3. Two low energy conformers of **1** (**1a**, **1b**) and one low energy conformer of calix[4]resorcinarene **2** optimised by molecular mechanics method (MM^+) *in vacuum*.

Table 1. The acid-base properties of **1** and **2**, potentiometric titration study

	1	**2***
pK_{a1}	9.0 ± 0.1	7.9 ± 0.1
pK_{a2}	9.3 ± 0.1	9.4 ± 0.1
pK_{a3}	10.8 ± 0.3	10.2 ± 0.1
pK_{a4}	10.6 ± 0.1	10.6 ± 0.1

* Water-methanol mixture (1 : 4) [14].

stability constants β were calculated according to the non-linear least squares method [8] and checked by Scatchard [9], Scott [10] and Benesi-Hildebrand plots [11].

The pH-metric measurements were carried out at *ca.* 25 °C using an "Ionomer I-160" instrument (Belorussia, Gomel) with a homemade flat pH-electrode and a Ag | AgCl | KCl (saturated) reference electrode. CO_2-free KOH solution (8.6×10^{-3} M) and HCl solution (1×10^{-2} M) were used as titrants. The pH-titration was performed in the range of pH 2–11 with the concentration of **1** being 3.5×10^{-3} M and a host : guest ratio of 1 : 1. About 30–40 experimental points were mathematically treated to calculate both dissociation and complex formation constants from experimental data by means of the CPESSP computer program [12]. It is based on the iteration procedure with the evaluation of the relibility of the mathematical model by Fisher's criterion.

Results and discussion

Calixresorcinarene **1** possesses four negatively charged binding sites (SO_3^- groups) on the upper rim (Figure 1). The computer-aided molecular modelling performed with the help of molecular mechanics, force field MM^+ [13], suggested that **1** exists in the pseudo-cone conformation with C_2-symmetry. The distance between the opposite sulphonatomethyl groups can vary from 5 to 14 Å for conformer **1a** and from 6 to 18 Å for conformer **1b** due to the free rotation around the CH_2–SO_3^- bond (Figure 3).

The acid-base properties of **1** determined with the help of pH-titration are presented in Table 1. The distribution diagram of the ratio of the deprotonated species of H_8L^{4-} as a function of pH is presented in Figure 4. Accumulation of H_5L^{7-} is less than 18% and occurs at the same pH range as the accumulation of H_4L^{8-}, therefore, pK_{a3} is very close to pK_{a4} and its standard deviation value is larger than that of pK_{a4}. Taking this fact into account one can see that in a certain area the value of 10.5–11.3 for pK_{a3} is in agreement with the pK_{a4} vlaue of 10.5–10.7, unfortunately more precise determination of these values was not possible.

The binding properties of **1** toward 12 α-aminoacids (*D*, *L-Phe*, *L-Pro*, *L-Trp*, *L-Arg*, *L-Lys*, *L-His*, *L-Asp*, *L-Asn*, *L-Thr*, *L-Leu*, *L-Met*, *L-Glu*) were studied with the help of both ^{1}H NMR – (pD 7.2) and pH-metric titration.

The ^{1}H NMR titration experiments were performed with fixed concentration of the aminoacid (6×10^{-3} M), while the concentration of **1** was varied in the range of 0 to 6×10^{-2} M. In the case when complex formation occurs the observed chemical shifts of protons of the aminoacid are the average from the fast exchange between the free and bound aminoacid. The results of ^{1}H NMR titration show that the chemical shift of all protons of *D*, *L-Phe*, *L-Pro*, *L-Trp*, *L-Arg*, *L-Lys* are shifted upfield in the presence of **1**. The values obtained of complexation induced shifts, CIS, were used for complex formation constant determination according to the non-linear least squares method [8] under the respective conditions (Figure 5).

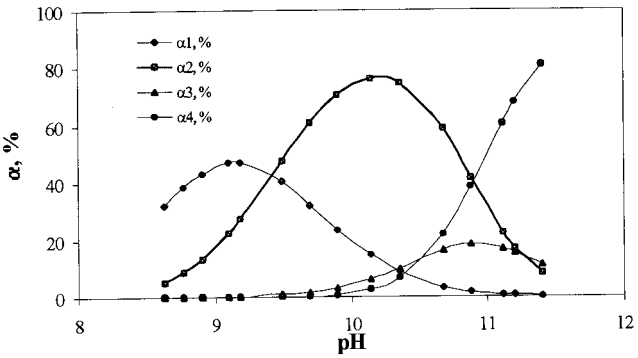


Figure 4. Distribution diagram of the deprotonated species of sulfonatomethylencalix[4]resorcinarene (α1-H_7L^{5-}, α2-H_6L^{6-}, α3-H_5L^{7-}, α4-H_4L^{8-}).

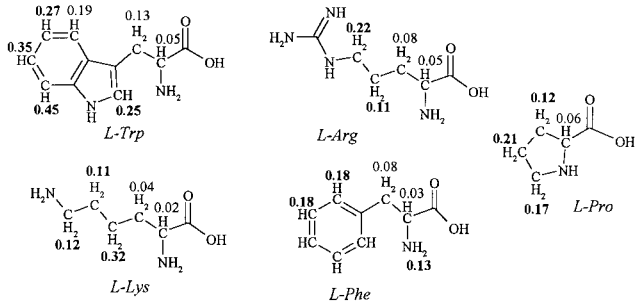


Figure 5. CIS for the aminoacids in the presence of **1** (in the equilibrium, ^{1}H NMR- titration data).

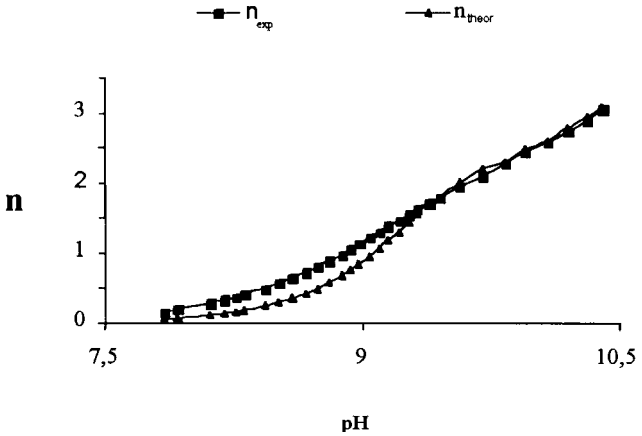


Figure 6. pH-dependence of Bjerrum functions nexp and ntheor for the system **1**-*L*-Lys.

The potentiometric titration was used for the study of the acid-base properties of the respective aminoacids in the presence and absence of host **1**. The following analysis of pH-metric data was carried out with the help of the Bjerrum function [15], indicating the average number of evaluated protons per one mole of titrant, and was used for the determination of the association constants in acidic and basic conditions. (Table 2, Figure 6).

Concerning the properties of the host molecule, it should be emphasized that the eight unsubstituted OH-groups of **1** are less acidic than those of unsubstituted calix[4]resorcinarene **2** (Table 1). As in the case of sulfonatocalix[n]arenes [16] the presence of SO_3^- groups on the upper-rim of **1** increases its solubility in water and provides four additional centres of electrostatic interactions. The conductance method in unbuffered solutions of **1** showed that in the millimolar concentration range used in the present study no aggregation of host molecules takes place. According to the data of [1, 2] and computer molecular modelling **1** ought to bind the potential guests by the interaction of its negatively charged $CH_2SO_3^-$ groups or ionised OH-groups with the polar functional groups of the guest and by the interaction of the apolar host cavity with the hydrophobic moieties of guests. Thus, considering its structure, **1** can be predicted to act as a host for aminoacids with both electrostatic (four SO_3^--groups) and hydrophobic interactions (aromatic cavity) being the driving force of complexation.

The ^{1}H NMR and pH-metric titration data, indicate that the examined aminoacids can be divided into three groups:

(i) those possessing bulky hydrophobic moieties: *D*, *L-Phe*, *L-Trp*, *L-His*, *L-Pro*;

(ii) the long-chain hydrophobic aminoacids with a second ionogenic group (amino- or guanidinium-group): *L-Arg*, *L-Lys*;

(iii) and those including a short alkyl chain or/and bearing a hydrophilic ω-group: *L-Asp*, *L-Asn*, *L-Thr*, *L-Leu*, *L-Met*, *L-Glu*.

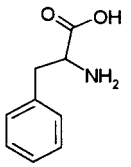
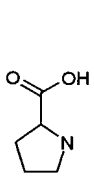
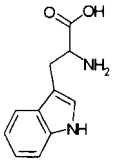
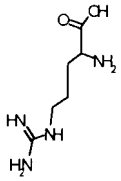
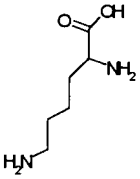
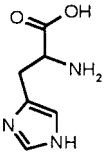
The data of both ^{1}H NMR titration in the phosphate buffer and pH-metric titration demonstrate the complex formation of the first two groups with **1**, while for the third group no complex formation was found.

As shown in the numerous papers [17] dealing with the ^{1}H NMR study of calixarenes toward aminoacids, the observed complex formation constants are typically in the range of log $\beta \geq 2$, while in the present study with a calix[4]resorcinarene derivative in some cases the log β-values are one order of magnitude higher. Ungaro et al. discussed also the importance of the macrocycle cone conformation, provided by lower rim substituents, and anchoring sulfonato-groups [16, 17a]. In the case of **1** OH-groups as well as $CH_2SO_3^-$ groups are positioned on the upper rim, thus a certain difference both in the acid-base properties and binding affinity takes place.

In ^{1}H NMR titration, pD 7.2, all the examined aminoacids are in the zwitter-ionic form. The complex formation constants for *D*, *L-Phe*, *L-Trp*, *L-Pro* (the first group) are relatively close, while for *L-Arg* and *L-Lys* (the second group) they are 15 times higher (Table 2). This is in a good agreement with the geometry of binding. The shielding effect for the protons of *L-Pro*, *D*, *L-Phe* and *L-Trp* is significant for the most remote, hydrophobic protons and is small for the methyl protons of aminocarboxylic groups. It indicates that the hydrophobic moieties of *L-Pro*, *D*, *L-Phe* and *L-Trp* are included into the cavity of the calixresorcinarene, while charged amino- and carboxylic-groups face the hydrophilic upper rim of the calixarene. The almost equal changes in the chemical shifts of the aromatic protons of *L-Trp* indicates that the more bulky hydrophobic moiety of *L-Trp* is included more deep into the cavity of calixarene than it is in the case of *L-Pro* and *D*, *L-Phe*.

The hydrophobic aminoacids of the second group, *L-Lys* and *L-Arg*, at pD 7.2 possess three charged binding sites: two positive ammonium ions and one negative carboxyl ion. Taking into account the most significant upfield shifts observed for the protons situated in the center of the hydrophobic chain it can be suggested that *L-Lys* and *L-Arg* concave into the cavity of **1** by the hydrophobic chain, while the charged groups face the upper-rim of **1**. The highest binding constants obtained for these aminoacids in neutral media are also in agreement with the importance of the additional positively charged binding center. de Rango et al. [4] reported a new type of intercalation within the calixarene bilayer system showing that a chiral cationic organic molecule possessing a flexible aliphatic side chain, in this case *L-Lys*, can span the bilayer. The crystalline title compound also exhibits a chiral hydrophilic layer, containing three other *L-Lys* molecules, which separates the bilayers.

Table 2. The acid-basic properties of complexes of **1** with α-aminoacids

Guest						
	D, L-Phe	*L-Pro*	*L-Trp*	*L-Arg*	*L-Lys*	*L-His*
The association constants, Log β, for complexes of **1** with aminoacids *in the neutral* media (pD = 7.2), ^{1}H NMR-titration.						
Log β	1.44 ± 0.01	1.94 ± 0.02	1.77 ± 0.03	2.15 ± 0.03	2.17 ± 0.11	–
The Log $\beta = pK_a^* - pK_a$ – values for complexes of **1** with aminoacids *in the acidic* media, pH-metric titration						
Log β	–	–	3.1 ± 0.09	3.0 ± 0.07	1.9 ± 0.05	2.9 ± 0.1
K_a – constant of dissociation of the protonated form of aminoacids. $H_2A^+ \rightarrow HA^{\pm} + H^+$.						
K_a^* – constant of equilibrium: $H_2A^+ + H_8L^{4-} \rightarrow [HA{\cdot}H_8L^{4-}]^{\pm} + H^+$.						
The Log $\beta_I = pK_{ai} - pK_{ai}^*$ values for complexes of **1** (i = 1, 2) with aminoacids *in the alkaline* media, pH-metric titration.						
Log $\beta_1 = pK_{a1} - pK_{a1}^*$	[a]	[a]	[a]	3.1 ± 0.1	3.1 ± 0.1	[a]
Log $\beta_2 = pK_{a2} - pK_{a2}^*$	[a]	[a]	[a]	3.1 ± 0.1	3.4 ± 0.1	[a]
K_{a1} and K_{a2} – stepwise deprotonation constants of H_8L^{4-} itself.						
K_{a1}^* – constants of equilibrium: $H_8L^{4-} + HA^{\pm} \rightarrow [H_7L^{5-}{\cdot}HA^{\pm}] + H^+$						
K_{a2}^* – constants of equilibrium: $H_7L^{5-} + HA^{\pm} \rightarrow [H_6L^{6-}{\cdot}HA^{\pm}] + H^+$						

[a] The accurate determination of the complex formation constant from the pH-metric titration data with the fixed "host : guest" ratio is only possible if logβ > 1.9.

For aminoacids of the third group (*L-Asp*, *L-Asn*, *L-Thr*, *L-Leu*, *L-Met*) no change in the chemical shifts in the presence of **1** was observed, therefore it was concluded that they do not form any inclusion complexes with **1** under selected conditions. Here a relatively short chain of the α-substituent bearing a hydrophilic group leads to both the lack of geometrical complementarity of the aminoacid and host binding sites and competition between the solvent (water) and the hydrophilic upper rim for interaction with the respective ω-group.

It should be noted that in contrast to the ^{1}H NMR titration performed in the 0.1 M phosphate buffer, the pH-metric study (pH 2–11) of the binding affinity of **1** toward the chosen aminoacids was performed in the unbuffered aqueous solutions. The relative effect of the buffer and salt influence on the complex formation in the aqueous media was recently described in [18]. It was observed that in the absence of a buffer significant ion pairing leads to the domination of the electrostatic interactions, while buffering of the solution decreases ion pairing and thus, lipophilic interactions gain in energetic importance. Table 2 presents the results of pH-metric titration and shows that complexation with aminoacids of the second group (*L-Lys* and *L-Arg*) was observed under all applied conditions – in acidic, neutral and basic media. It was shown that in the last case complex formation with the respective deprotonated host forms, H_7L^{5-} and H_6L^{6-}, occurs. For the aminoacids of the first group (*D*, *L-Phe*, *L-Pro*) the results of the potentiometric study (Bjerrum function consideration) show the agreement between the experimental n_{exp} and n_{theor}, calculated according to the law of acting masses for independent protonation of the aminoacid in the presence of **1** in acidic media and for independent deprotonation of the aminoacids and **1** in alkaline media.

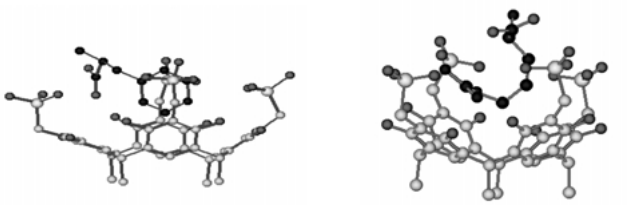


Figure 7. Models of inclusion complexes of **1** with *L-Phe* (a) and *L-Arg* (b) optimised by molecular mechanics method (MM^+) *in vacuum*.

For *L-Arg* and *L-Lys* (the second group), *L-Trp* and *L-His* (the first group) the deviation between n_{exp} and n_{theor} increase with the addition of HCl (Figure 6). This indicates a decrease of the pK_a^*-value of these aminoacids in the presence of **1** in comparison with the pK_a obtained for the solution of the only respective aminoacid (Table 2). Therefore, pH-metric data proves the coordination mode obtained from NMR spectroscopy with a rather shallow inclusion of all aminoacids into the H_8L^{4-} cavity with the carboxylate-groups being rather far from the charged upper rim (Figure 7).

The alkalization of the media leads to the deprotonation of both the host and the guest with pK-values rather close to each other. Bjerrum function dependence on pH in the systems **1**-*L-Lys* (*L-Arg*) deviates from those calculated for their independent deprotonation (n_{theor} on pH) and the phenolic groups of **1** on the first and the second steps in the presence

of *L-Lys* (*L-Arg*) become more acidic with (pK_a^*–pK_a) values presented in Table 2. This indicates that the interaction of both these aminoacids in zwitter-ionic form is rather strong to promote the deprotonation of the host phenolic groups, while further alkalization (pH > 11) leads to the destruction of the complex due to the deprotonation of guest ammonium groups.

In contrast to the ^{1}H NMR titration, where no *L-His* binding by **1** was observed pH-metric titration found log β = 2.9 $\pm$ 0.1. This means that the protonated form $HisH^+$ is bound by **1** stronger than $His^{\pm}$, and is in agreement with the pK_a-values of *His* (1.77, 6.0, 9.3, respectively). At pD 7.2 (^{1}H NMR titration) *His* exists in the zwitter-ion form bearing the only positive charge on the amino-group, while in acidic media the protonated imidazole nitrogen provides increased strength of interaction between the α-substituent of the aminoacid and the aromatic cavity of the host (electrostatic and cation-π interactions).

Conclusions

The detailed study of the interactions of water-soluble tetrasulfonatomethylcalix[4]resorcinarene **1** with 12 aminoacids was performed in aqueous media with the help of ^{1}H NMR (buffered solution, pD 7.2) and pH-metric (pH 2–11) titration. The results of the former correspond to the interactions of the host under study with the zwitter-ionic form, while results of the latter deal with the guests in all possible forms depending on pH. For those examined aminoacids containing either bulky aromatic or long-chain hydrophobic α-substituents, formation of inclusion complexes was observed and complex formation constants were determined. For those examined aminoacids with short alkyl chains bearing hydrophilic ω-groups no complex formation was found. Complex formation constants obtained for different pH do not differ from each other for the same guests, except in the case of hystidine. A significant negative charge delocalized on the upper rim of the host leads to the situation where the host charge state change does not influence charged guest binding, while geometric complementarity and additional π–π and hydrophobic interactions play a crucial role in the complex formation. The lack of these interactions (the third group) in aqueous media provides domination of the guest solvation by water over the 1 : 1 complex formation with **1**.

Acknowledgements

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