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# Calorimetric, NMR, and UV Investigations of Aliphatic L-Amino Acids Complexation by Calix[4]arene *Bis*-hydroxymethylphosphous Acid

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*Key words:* amino acid, calixarene, complexation, titration calorimetry, NMR and UV spectroscopy

**Abstract**

The stability constants, enthalpy $\Delta H^0$, entropy $\Delta S^0$, and Gibbs energy $\Delta G^0$ were determined for the host–guest complexes (1:1) of calix[4]arene *bis*-hydroxymethylphosphous acid with glycine, L-alanine, L-valine, L-leucine, L-isoleucine residues in methanol solution with the aid of the titration experiments followed by calorimetric and spectroscopic ($^1$H NMR, UV) methods. The experimental data indicated that the host–guest complexation was under control of the direct electrostatic interaction between negatively charged calixarene phosphoryl group and amino acid residue $NH_3^+$ group, modulated by the hydrophobic interaction, which drive the inclusion of the residue alkyl side-chain into the calixarene cavity. The stability of the inclusion complexes was found correlated with the size of the aliphatic amino acid's side-chain. The experimental data were additionally analyzed in the terms of the three state model corresponding to coexistence of 2:1 and 1:1 complexation equilibria.

## Introduction

Molecular recognition, separation and membrane transport of bio-relevant amino acids by artificial receptors constitute an important problem in chemistry and biology [1]. Calix[4]arenes [2], the macrocycles composed of four phenolic units linked *via* methylene groups, are of the interest as cavity-shaped molecules forming host–guest complexes with amino acids [3]. In spite of their interesting structural properties, the kinetic and thermodynamic parameters of the host–guest complexation have been carried out to only a limited extent, probably due to the reduced solubility of calixarenes in polar solvents [4].

Complexation of sulphocalixarenes [5–9] and peptidocalixarenes [10–13] with amino acids was investigated by NMR spectroscopy, microcalorimetry, RP-HPLC, and ESI-MS methods, enabling determination of the association constants as well as other thermodynamic parameters. These studies revealed the possibility of the formation of stable complexes between calixarenes and some amino acids residues or short peptides. The general schemes of the interaction were studied with the aid of different experimental techniques. Douteau-Guevel and co-workers used NMR spectroscopic technique to follow the formation of 1:1 complexes of *p*-sulphonatocalixarenes with lysine, arginine, and their di- and tripeptides [6, 7]. The binding constants were determined in the range of 200–30,000 $M^{-1}$, and were higher for the longer peptides. In all cases, the complexation was driven by a favorable enthalpy change. Similarly, in Arena group the water soluble calixarenes were used for the inclusion of aliphatic and aromatic amino acids [5]. This research showed that the most efficient receptors for all the analyzed amino acids were sulphonatocalix[4]arenes, and the binding free energy varied in the range of $-5$ to $-10$ kJ $mol^{-1}$. Analogously to sulphonates at the upper rim of the calixarene, the peptidocalix[4]arenes were designed [11]. The latter complexed the amino acids with the association constants of 20–700 $M^{-1}$, and the aromatic amino acids were included even more strongly than the aliphatic ones.

Recently we have communicated detailed analysis of the complexation of calix[4]arene *bis*-hydroxymethylphosphous acid with isoleucine [14], demonstrating the existence of two types of complex stoichiometry, the dominating and favored 1:1, and existing only for the high calixarene:amino acid molar ratio 2:1. The complexation data determined for the same calixarene with an extended series of aliphatic amino acids including glycine (Gly), L-alanine (Ala), L-valine (Val), L-leucine (Leu) and L-isoleucine (Ile) (Chart 1) in methanol solution are presented (Scheme 1).

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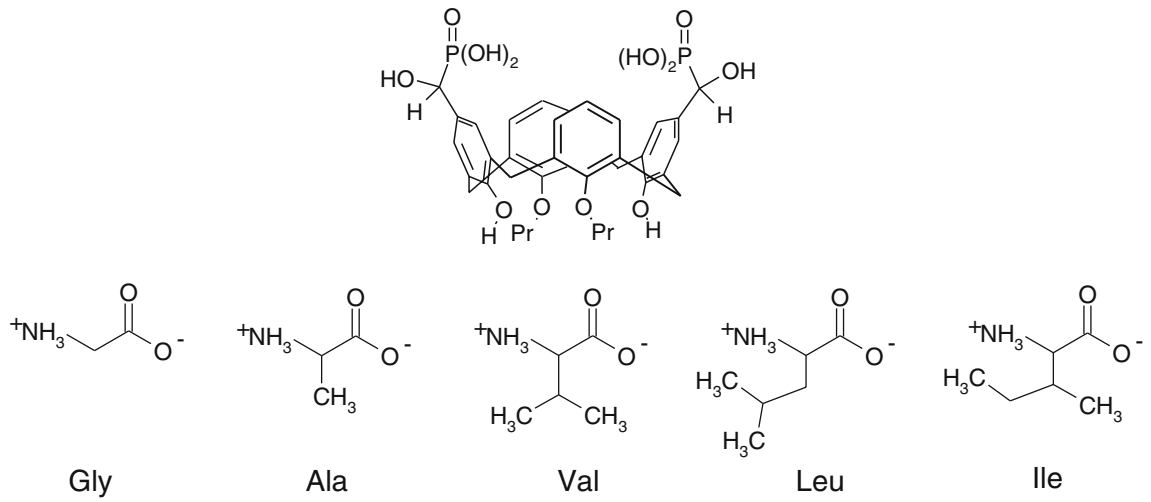


*Scheme 1.* Calix[4]arene *bis*-hydroxymethylphosphous acid and amino acids: Gly, Ala, Val, Leu, and Ile.

## Experimental

Energetic measurements of the host–guest interactions of calixarene with amino acids residues were carried out. Stoichiometry of the host–guest complexes, association constants $K$, and thermodynamic parameters characterizing the complexation process were determined by the titration experiments followed by calorimetry, $^1$H NMR and UV–Vis methods. Additionally the calorimetric experiments were conducted at 298.15 K, 308.15 K and 318.15 K to evaluate $\Delta H$ and $\Delta S$.

### *Materials*

5,17-*bis*(Dihydroxyphosphorylhydroxymethyl)-25,27-dipropoxycalix[4]arene (Kx, 728 g mol$^{-1}$) was synthesized by previously presented method [15] and one diastereomeric form was isolated by crystallization from cyclohexane solution. The stereochemical purity was confirmed by the existence of only one set of signals in the $^1$H and $^{31}$P NMR spectra.

Amino acids: glycine (Gly), L-alanine (Ala), L-valine (Val), L-leucine (Leu) and L-isoleucine (Ile) were purchased from Sigma Aldrich Co. and used without further purification.

Methanol (HPLC grade) (Chemipan R&D Laboratories) was refluxed before experiments. The water content of the solvent was checked by Karl Fischer titration and found to be less than 0.02%. For NMR experiments Fluka's deuterated methanol ($CD_3OD$) was used.

All the solutions were prepared by weight in methanol.

### *Methods*

*Calorimetry*

The isothermal microcalorimetric experiments were carried out using titration ITC Omega MicroCal Calorimeter equipped with a stainless steel titration vessel of 1.8 ml volume. The experiments were done at 298.15 K, 308.15 K and 318.15 K in methanol solutions. The vessel was charged with 0.5 mM calixarene methanol solution and 5 mM amino acids methanol solutions were injected in 10 $\mu$l steps using a syringe pump equipped with a 250 $\mu$l Hamilton syringe. Twenty-five injections were made for each titration experiment. The measured heat effects were corrected for dilution phenomena of both host and guest species estimated from the separate experiments. All the titration experiments were repeated three times for reproducibility.

The corrected heat power pulses corresponding to every injection were integrated to yield the total injection heat effect as the function of variable host and guest concentrations. The method used enabled determination of both the binding constant and the corresponding enthalpy change. The associated changes of Gibbs energy and entropy upon complexation were estimated using van't Hoff equations.

$$\Delta G^0 = -RT\ln K \tag{1}$$

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 \tag{2}$$

*Nmr Spectroscopy*

$^1$H NMR measurements were performed at 298 K in $CD_3OD$ solution using Varian 500 MHz spectrometer. For the titration experiments 4.0 mM base stock solutions of calixarene and amino acids in methanol-$d_4$ were prepared. Then the 20 NMR tubes were filled with variable amounts of the two species to the total volume of 800 $\mu$l. The ratios of amino acid to calixarene concentration ranged from 0 up to 15.

All the spectra were processed with the aid of Mestre-C program [16]. Prior Fourier transformation the Gaussian filtering resulting in $\sim$1 Hz line-width broadening followed by zero filling up to 32 K points was applied.

*UV–Vis Spectroscopy*

The UV–Vis titrations were studied using Shimadzu UV–Vis Spectrophotometer UV-2401 PC. The spectra of methanol solution containing calixarene and amino acid were recorded in the range of 200–400 nm using 2-mm path length quartz cell. The base stocks of 2.0 mM methanol solutions of calixarene and amino acids were used. The exact samples were obtained for amino acid: calixarene concentration ratios varied in the range of 0–20.

*Complexation model*

*Modeling of the equilibrium of 1:1 and 2:1 host–guest complexes*

The host–guest system was described in the terms of three state model corresponding to the two complex formation equilibria:

$$\begin{aligned}[KxI] &= K_1{*}[Kx][I] \\ [Kx_2I] &= K_2{*}[KxI][Kx]\end{aligned} \tag{3}$$

where $[I]$, $[Kx]$, $[KxI]$, $[Kx_2I]$ are the concentrations of free guest, free host, 1:1 and 2:1 complexes, respectively. The $K_1$ and $K_2$ correspond to binding constants for the 1:1 and 2:1 complexes. The mass balance of guest ($I_{\mathrm{tot}}$) and host ($Kx_{\mathrm{tot}}$) molecules leads to the equations:

$$\begin{aligned}[Kx] + [KxI] + 2^{*}[Kx_2I] &= Kx_{\mathrm{tot}} \\ [I] + [KxI] + [Kx_2I] &= I_{\mathrm{tot}}\end{aligned} \tag{4}$$

The combination of Equations (3) and (4) leads to the third order equation in the form:

$$\begin{aligned}\{K_1K_2\}[Kx]^3 + \{K_1 + K_1K_2(2^{*}I_{\mathrm{tot}} - Kx_{\mathrm{tot}})\}[Kx]^2 \\ + \{1 + K_1(I_{\mathrm{tot}} - Kx_{\mathrm{tot}})\}[Kx] = Kx_{\mathrm{tot}}\end{aligned} \tag{5}$$

which could be analytically resolved against $[Kx]$ using Cardano approach [17]. Finally, the concentration of the free guest is expressed in the form:

$$[I] = \frac{I_{\mathrm{tot}}}{1 + K_1[Kx] + K_1K_2[Kx]^2} \tag{6}$$

The concentration of the two complexes is described by Equation (3).

The above complexation model was used to fit the data from spectroscopic methods and to determine the binding constants values.

## Results and discussion

*Calorimetric investigation*

The observed heat effects upon complexation of the amino acid by calixarene were analyzed as the function of both host and guest concentrations. Representative injection experiments for different calixarene–amino acid systems, reporting the heat effect per mole of calixarene, are shown in Figure 1. For the most cases, after the first few injections the recorded heat effects changed rapidly and at the end were almost undetectable. The control experiment performed for *N*-acetyl-L-phenylanine, showed only very small exothermic heat changes, indicating the lack of the significant interaction between this amino acid residue and calixarene.

Calorimetric titration experiments were analyzed in the terms of 1:1; 1:2; 2:1 complex stoichiometries with the aid of nonlinear regression method from the standard software of MicroCal ITC [18]. However no further improvement was obtained when using 1:2 or 2:1 binding models, as related to 1:1 stoichiometry model. Therefore the binding constant $K$ and $\Delta H^0$ values were determined assuming the 1:1 stoichiometry. All the obtained results are reported in Table 1. The estimated values of the corresponding $\Delta S^0$ and $\Delta G^0$ changes are also included.

The presented thermodynamic data demonstrates that the complexation of amino acid molecule by calixarene is both enthalpically and entropically favored ($\Delta H^0 < 0, \Delta S^0 > 0$). The estimated binding parameters are much higher, as compared with that determined for complexation of amino acids with sulphocalix[4]arene in aqueous solutions [9a]. Very small heat effects obtained for the N-blocked amino acids (data not presented) points that the $NH_3^+$ amino group is the main participant of the complexation process and drives the formation of the calixarene–amino acid complexes. The tight ion pair formed between $NH_3^+$ group of the guest and $PO_2^-$ group of the host in methanol solution stabilizing the complex structure is well documented in the literature [19].

The large negative $\Delta H^0$ values indicate the dominance of electrostatic interactions between the deprotonated, negatively charged phosphoryl group of the calixarene, and the $NH_3^+$ group of amino acids residues in the zwitterionic form [20].

The elongation of amino acid aliphatic side-chain causes the increase of $K$ values. This indicates that the calixarene is more efficient in the complexation of Val, Leu and Ile than Gly or Ala, which is pointing out the effect of the length of the hydrophobic side-chain. The increase of entropy term $\Delta S^0$ accompanying the elongation of the amino acid aliphatic chain could be described as the favored solvatophobic effect of inclusion the hydrophobic group into the calixarene hydrophobic cavity, enabling the release of solvent molecules from the solvating sphere of the aliphatic side-chain. For all the analyzed residues, the $\Delta G$ becomes more negative when the side-chain size is increased (Figure 2).

Since the insertion of aliphatic chain into the cavity of calixarene is one of a driving force in the binding process, the increase of alkyl chain length is expected to influence $\Delta G$ values (Figure 3). The entropy terms are smaller for the amino acids with shorter alkyl chain, and the smallest value was obtained for Gly, where the side-chain is reduced to the hydrogen atom.

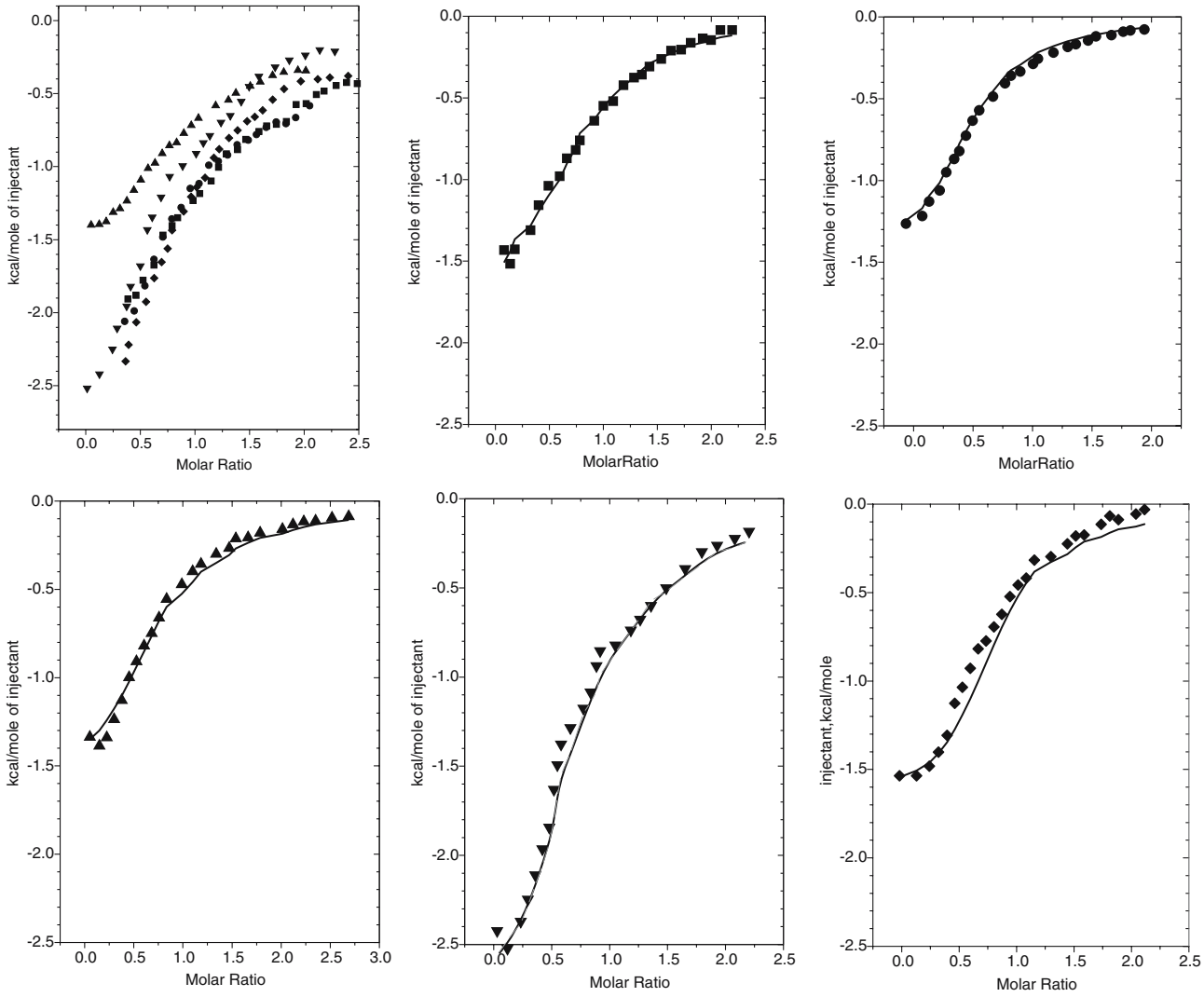


*Figure 1.* Microcal ITC titration run of amino acids: Gly (■), Ala (●), Val (▲), Leu (▼) and Ile (♦) by calixarene (*uper left*), and the 1:1 binding model fitted to the corrected for dilution subsequent data. Experimental error of integrated injection heat is within markers boundaries.

*Table 1.* Stability constants, $K$, and associated thermodynamic values $\Delta H^0$, $\Delta S^0$ and $\Delta G^0$ determined for the 1:1 complexation of amino acids with calixarene in methanol solution

| Residue | Temp, K | Log $K$, $M^{-1}$ | $\Delta H^0$, kJ $mol^{-1}$ | $\Delta Cp$, J $mol^{-1}$ $K^{-1}$ | $\Delta S^0$, J $mol^{-1}$ K | $\Delta G^0$, kJ $mol^{-1}$ |
|---|---|---|---|---|---|---|
| Gly | 298.15 | 3.84 ± 0.15 | −9.13 ± 0.32 | −32.4 ± 0.6 | 42.89 | −21.91 |
| | 308.15 | 3.63 ± 0.17 | −9.46 ± 0.45 | | 38.71 | −21.00 |
| | 318.15 | 3.62 ± 0.20 | −9.77 ± 0.46 | | 38.34 | −21.20 |
| Ala | 298.15 | 3.89 ± 0.11 | −8.19 ± 0.21 | −43.3 ± 0.3 | 46.94 | −22.19 |
| | 308.15 | 3.73 ± 0.10 | −8.62 ± 0.28 | | 43.60 | −21.62 |
| | 318.15 | 3.71 ± 0.13 | −9.06 ± 0.26 | | 42.47 | −21.72 |
| Val | 298.15 | 4.12 ± 0.04 | −7.11 ± 0.24 | −53.4 ± 0.3 | 55.06 | −23.53 |
| | 308.15 | 3.83 ± 0.14 | −7.64 ± 0.34 | | 47.66 | −21.85 |
| | 318.15 | 3.81 ± 0.12 | −8.18 ± 0.33 | | 47.28 | −22.27 |
| Leu | 298.15 | 4.19 ± 0.04 | −9.06 ± 0.18 | −52.2 ± 1.4 | 49.71 | −23.89 |
| | 308.15 | 3.92 ± 0.09 | −9.57 ± 0.20 | | 46.57 | −22.95 |
| | 318.15 | 3.90 ± 0.11 | −10.14 ± 0.19 | | 44.89 | −23.53 |
| Ile | 298.15 | 4.23 ± 0.01 | −7.05 ± 0.14 | −69.9 ± 1.7 | 57.36 | −24.15 |
| | 308.15 | 3.93 ± 0.07 | −7.72 ± 0.15 | | 50.25 | −22.70 |
| | 318.15 | 3.92 ± 0.07 | −8.44 ± 0.14 | | 47.70 | −22.66 |

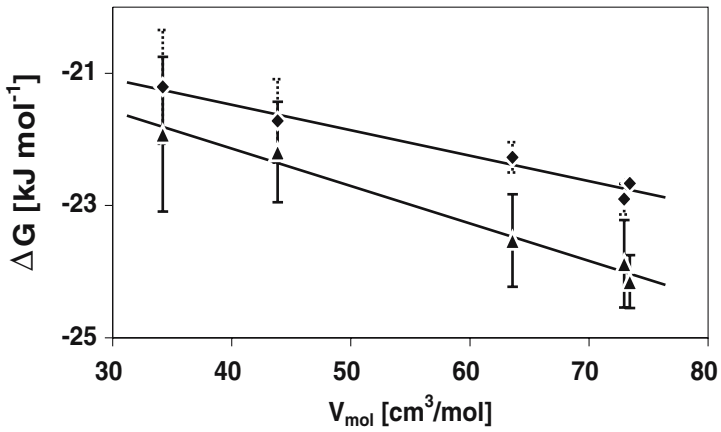


*Figure 2.* Experimental $\Delta G$ value determined at 298.15 K (triangles), and 308.15 K (diamonds) represented as the function of molecular volume of amino acid residue. Bars represent estimated experimental error.

The increment in $\Delta C_p$ accompanying the binding of amino acids by calixarene was found in the range of $-30$ to $-70$ J mol$^{-1}$ K$^{-1}$ (Figure 4). The almost linear relation between *C*p change and residues' side-chain size is characteristic for the hydrophobic interaction [21].

### *$^1$H-NMR investigations*

Based on the concentration dependence of $^1$H-NMR spectra of amino acid residues in the presence of calixarene in methanolic solution the stoichiometry and binding constant of the complexes were estimated. The most remarkable changes were observed for aliphatic protons of amino acid's side chains as well as for the methylene groups bridging the calixarene macrocycle. According to the Job's plot [22] one can determine the mixed 2:1 and 1:1 stoichiometries (Figure 5), which is indicated by the maximum of the resulting plot centered at 0.5, pointing out the 1:1 binding model, and the significant curve distortions in the range of concentration ratios of 0.6–0.7 complying the requirements for the 2:1 complex.

The obtained results suggested that for small amount of amino acids in solution, two calixarene molecules are involved in the binding. When the concentration of amino acid increases the equilibrium is directed towards 1:1 complex formation. Moreover, amino acids aliphatic protons experience the upfield shift due to the inclusion of the alkyl chain into the calixarene cavity. Calixarene's methylene ($ArCH_2Ar$ protons – $\delta = 4.3$ ppm) demonstrate strong downfield shift, due to electrostatic interactions with the proximal charged amino group of amino acid residue. Chemical shifts of Val $H^{\gamma}$ protons were not significantly perturbed ($\Delta\delta$ below 0.1 ppm) upon complexation, while $H^{\alpha}$ protons experience a remarkable upfield shift ($\Delta\delta$ up to 0.5 ppm). The similar correlations were observed for other amino acids.

The observed concentration dependence of proton chemical shifts was initially modeled using proposed 1:1 complex stoichiometry (Figure 6, *left panels*). In the analysis all protons resonances were simultaneously treated in the model fitting, which significantly increased the stability of the optimization algorithm, as well as

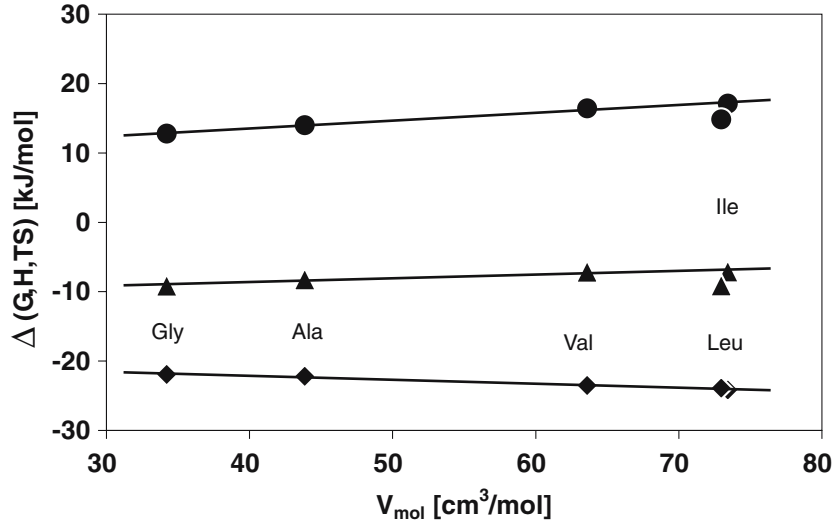


*Figure 3.* Decomposition of $\Delta$G (diamonds) determined at 298.15 K for enthalpy ($\Delta H$, triangles) and entropy ($T\Delta S$, circles) terms as a function of the residue molecular volume, $V_{mol}$. The experimental error, varying in the range of 0.5–1.3 kJ mol$^{-1}$, is within markers boundaries.

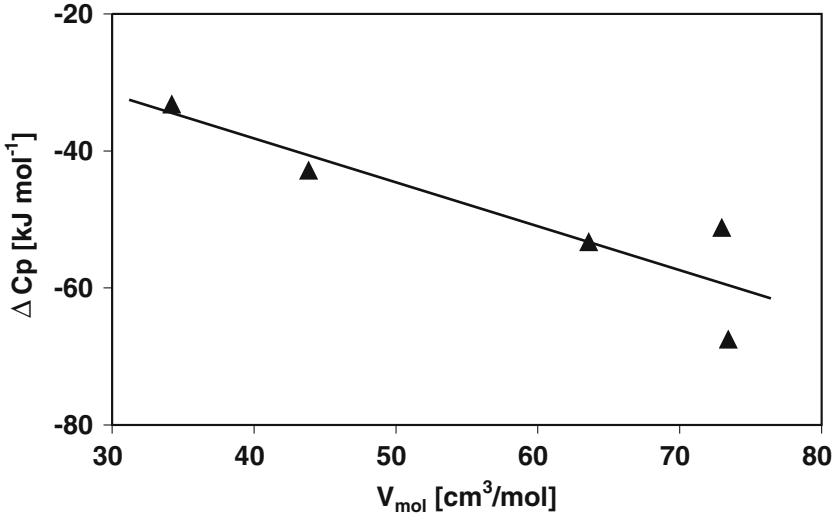


*Figure 4*. $\Delta C$p vs. the volume of amino acids upon complexation determined at 308.15 K. The error in the range of 0.3–1.7 J $mol^{-1}$ $K^{-1}$ is within the markers boundaries.

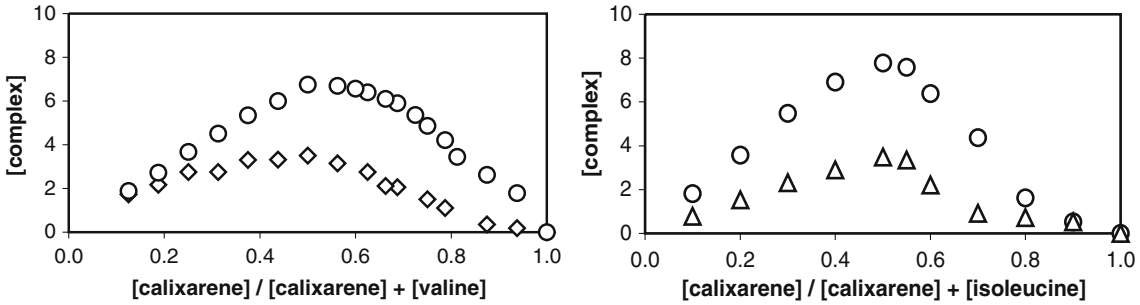


*Figure 5*. Job's plot for the NMR experiment for protons resonances of valine (*left*) $H^{\alpha}$(◇, 3.8 ppm), $H^{\gamma}$(○, 1.0 ppm), and isoleucine (*right*) $H^{\alpha}$(△, 4.3 ppm), $H^{\delta}$(○, 0.9 ppm).

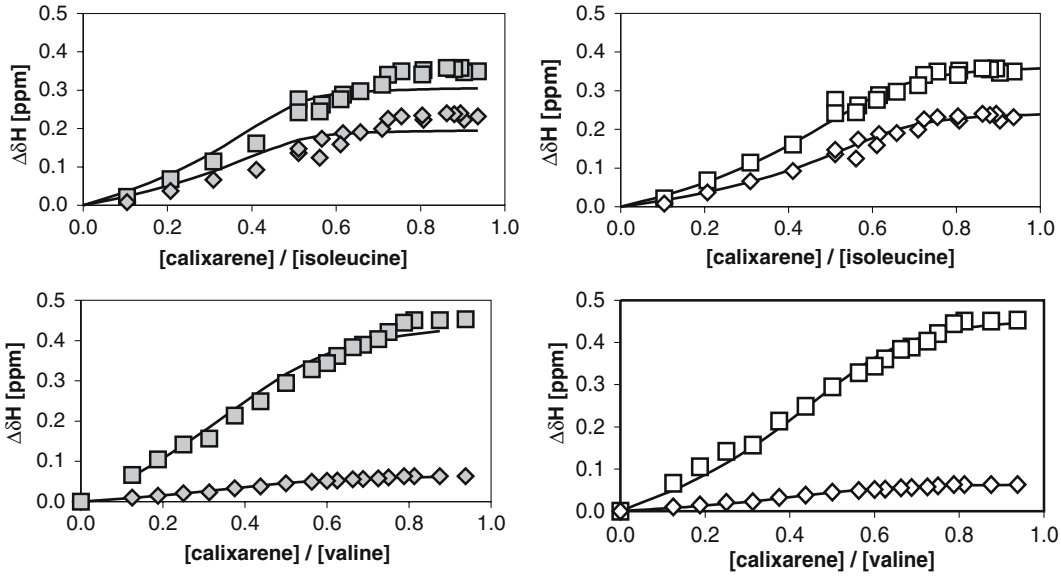


*Figure 6*. Chemical shift values obtained from titration experiments performed for isoleucine (up): □ – 1.95 ppm ($H^{\beta}$ proton); ◇ – 1.03 ppm ($H^{\delta}$), and valine (down): □ – 3.8 ppm ($H^{\alpha}$), ◇ – 1.0 ppm ($H^{\gamma}$) residues represented as a function of calixarene/isoleucine ratio. Left panels correspond to the 1:1 complesxation model while the right ones were obtained under assumption of the coexistence of 2:1 and 1:1 complexes.

decreased the standard deviations of the estimated parameters. Detailed data analysis proved for the long-chain residues the existence of the significant deviations of the model from experimental data, especially in the low concentration amino acid region. The latter strongly suggested the existence of the minor 2:1 equilibrium, in

agreement with stoichiometry analysis. The final model was built assuming the coexistence of 2:1 and 1:1 complexation equilibria, both in the fast exchange regime (Figure 6, right panels). Upon the model fitting $^1$H NMR titration curves were obtained simultaneously for all analyzed protons of amino acid and calixarene partners. The NMR chemical shifts were calculated according to the postulated complexation equilibria (Equation (3)) using $\Delta\delta_1$ and $\Delta\delta_2$ parameters as follows:

$$\Delta\delta = ([KxI] \times \Delta\delta_1 + [Kx_2I] \times \Delta\delta_2)/[Kx_{tot}] \quad \text{for Kx molecule} \tag{7}$$

or

$$\Delta\delta = ([KxI] \times \Delta\delta_1 + [Kx_2I] \times \Delta\delta_2)/[I_{tot}] \quad \text{for amino-acid residue} \tag{8}$$

where $\Delta\delta$ – is the estimated changes of chemical shift; $\Delta\delta_1$ and $\Delta\delta_2$ are the chemical shift changes for 2:1 and 1:1 complexes; $[KxI]$ is the concentration of 1:1 calixarene: amino acid complex; $[Kx_2I]$ – the concentration of 2:1 calixarene: amino acid complex; $[Kx_{tot}]$ and $[I_{tot}]$ are the total concentrations of calixarene and amino acid, respectively.

The estimated binding constants are presented in the second column of the Table 2. For the short side chain amino acids residues the 2:1 complex was found very low populated. For the comparison, the 1:1 and 1:1 + 2:1 complexation models are presented for Ile and Val complexes. The improvement caused by the introduction of the second complexation equilibrium is evident, especially in the high *Kx* concentration region.

*UV–Vis investigations*

The host–guest complexes ratio and the stability constant of the complexes were determined basing on the spectral changes of calixarene absorption of aromatic rings of the macrocyclic skeleton [23] in the presence of variable concentration of amino acid in methanol solutions. Analysis of the Job's plot indicated, analogously to the results of NMR titration experiments, the coexistence of 2:1 and 1:1 host guest complex. In the data analysis the same model of interactions between calixarene and amino acids was used. The UV–Vis absorbance changes was estimated using $\Delta A_1$ and $\Delta A_2$ (changes of the absorbances for both complexes) as follows:

$$\Delta A = ([KxI] \times \Delta A_1 + [Kx_2I] \times \Delta A_2)/[Kx_{tot}] \tag{9}$$

The obtained model parameters were found close to that determined from NMR titration experiments (Table 3).

Both NMR and UV–Vis measurements confirmed the coexistence of 2:1 and 1:1 calixarene–amino acid complexes. According to the obtained data, the calorimetric experiments were reanalyzed using MicroCal software, in order to determine the thermodynamic parameters for the equilibrium of 1:1 and 2:1 complexation. The initial values of binding constants in the model were adopted from NMR titration data. The calculated data for calorimetric titration using $\Delta H_1$ and $\Delta H_2$ parameters (changes of heat effects for complexes 1:1 and 2:1) were equal:

$$\Delta H = ([KxI] \times \Delta H_1 + [Kx_2I] \times \Delta H_2)/[Kx_{tot}] \tag{10}$$

The two types of complexes differ from each other by the dominant interactions, one is enthalpically favorable and the other one (2:1) is described mainly by the entropic term. The major contribution to the association process comes from electrostatic interactions driving the formation of 1:1 complex. On the other hand the inclusion of the hydrophobic side-chain within the host cavity play a minor, but detectable role in the complexation processes studied, modulating the $K_1$ values. The fact that $K_1$ is remarkably higher than $K_2$ indicates that two calixarene molecules bind the amino

*Table 2.* Association constants for calixarene: amino acid 1:1 and 2:1 complexes calculated from the $^1$H NMR and UV–Vis titration experiments

| Method | Host–guest ratio | Log *K* | | |
|---|---|---|---|---|
| | | Residue | | |
| | | Ala | Val | Ile |
| $^1$H-NMR | 1:1 | 3.95 ± 0.77 | 4.18 ± 0.73 | 4.40 ± 1.20 |
| | 2:1 | 2.95 ± 0.39 | 3.08 ± 0.42 | 3.23 ± 1.76 |
| UV–Vis | 1:1 | 3.89 ± 0.82 | 4.12 ± 0.75 | 4.37 ± 0.88 |
| | 2:1 | 2.90 ± 0.32 | 3.03 ± 0.41 | 3.18 ± 0.54 |

*Table 3.* Thermodynamic parameters evaluated for the 1:1 and 2:1 guest–host complexation of amino acids with calixarene in methanol at 298.15 K

| Host–guest Ratio | Log *K* | $\Delta H^0$, kJ mol$^{-1}$ | $T\Delta S^0$, kJ mol$^{-1}$ | $\Delta G^0$, kJ mol$^{-1}$ |
|---|---|---|---|---|
| Gly* | | | | |
| 1:1 | 3.84 | −10.55 | 11.39 | −21.94 |
| 2:1 | 2.87 | 2.24 | 18.69 | −16.45 |
| Ala | | | | |
| 1:1 | 3.89 | −8.54 | 14.02 | −22.56 |
| 2:1 | 2.93 | 2.13 | 19.00 | −16.87 |
| Val | | | | |
| 1:1 | 4.12 | −7.53 | 16.29 | −23.82 |
| 2:1 | 3.00 | 1.67 | 19.25 | −17.58 |
| Leu* | | | | |
| 1:1 | 4.19 | −8.87 | 15.41 | −24.28 |
| 2:1 | 3.15 | 1.67 | 19.80 | −18.12 |
| Ile | | | | |
| 1:1 | 4.23 | −7.36 | 17.74 | −25.10 |
| 2:1 | 3.20 | 2.73 | 21.17 | −18.44 |

*The initial values for Gly and Leu were estimated on the basis of binding constant values for 1:1 stoichiometry and values for Ala and Ile, respectively.

acid residue in a non-cooperative manner. The detailed analysis demonstrate, that both $\Delta G$ values are strongly correlated with the hydrophobicity of the amino acid residue, as it is presented in Figure 7 in the comparison with recently proposed consensus derived amino acid hydrophobicity scale [24].

## Conclusions

Calix[4]arene *bis*-hydroxymethylphosphonic acid is effective binder for aliphatic amino acids. The agreement between the stability constants of the 1:1 and 2:1 calixarene–amino acid complexes determined by different measurements as calorimetry, $^1$H-NMR and UV–Vis spectroscopy is reasonably good, with log $K$ values not differing by more than 0.2. The 2:1 complex, driven by hydrophobic interactions leading to the preferable protection of aliphatic side-chain from solvent accessibility of the entropic origin, while the 1:1 complex is both enthalpically and entropically favorable. The most probable interpretation of the amino acid complexation by calixarene could be described as follows. The 1:1 complex is driven mainly by almost universal electrostatic interaction pattern, which is modulated by the additional effect of aliphatic side-chain protection from the solvent accessibility. Thus for 1:1 complexation the binding affinity is high, and increases with the elongation of the side-chain. When the concentration of amino acid is relatively low, there is a competition for the amino acid residue between two calixarene molecules. In consequence, one complex is enthalpically stabilized by electrostatic interaction between the charged $NH_3^+$ and $PO_2^-$ groups, while the other calixarene molecule tends to protect the residue side chain from the solvent. The additional complex is relatively heatless, stabilized mainly by solvatophobic interactions, and could be hardly determined from direct analysis of the calorimetric data without support of other techniques. The small positive heat arises from the unfavorable electrostatic interaction of two proximal calixarene molecules.

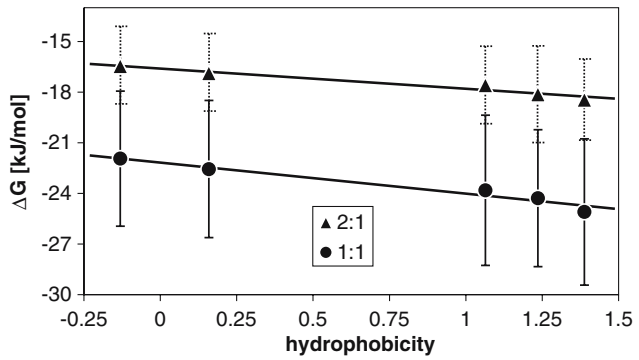


*Figure 7*. Experimentally determined free energies corresponding to 1:1 (circles) and 2:1 (triangles) complex formation represented as a function of amino acid residue hydrophobicity [24]. Experimental error is represented by solid and chopped bars for 1:1 and 2:1 complexes, respectively.

The thermochemical investigations performed at different temperatures show that the binding mode of calixarene vary with the chain length of amino acids. The complexation of amino acids by calixarene is finally driven by a favorable change in enthalpy ($\Delta H^0 < 0$).

The model of host–guest interaction is proposed. Dissociated in methanol solution negatively charged calixarene phosphoryl groups appear to serve as anchoring points for the positively charged ammonium group of amino acids. The electrostatic forces play the key role in the complex formation. On the other hand, in addition to electrostatic forces, the solvatophobic interactions, follow the insertion of the amino acid aliphatic moiety into the calixarene cavity, stabilizing the complex.

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