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Phosphorylated Calixarenes as Receptors of L-Amino Acids and Dipeptides: Calorimetric Determination of Gibbs Energy, Enthalpy and Entropy of Complexation

WOJCIECH ZIELENKIEWICZ[a], AGNIASZKA MARCINOWICZ[a], SERGEY CHERENOK[b], VITALY I. KALCHENKO[b] and JAROSLAW POZNAŃSKI[a,*]

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Thermodynamics of 5,17*bis*(dihydroxyphosphorylhydroxymethyl)-25,27-dipropoxy-calix[4]arene in pure *Racemic* form and 1:1 mixture of the *Meso* and *Racemic* forms (host molecule) interacting in methanol solution with free amino acids (guest molecule), and additionally with five dipeptides, has been studied using isothermal titration calorimetry. A moderate variation in the changes of enthalpy, entropy and Gibbs free energy, depending on the nature of the guest molecule as well as the stereomeric form of the host molecule, was observed. The stability constants in the range of 3000–17000 M^{-1} (25000–45000 M^{-1} for dipeptides) and enthalpy changes in the range of −10– − 2 kJmol^{-1} (−10.5– − 5.9 for dipeptides) were evaluated experimentally by ITC. The decreased variation in the estimated Gibbs free energy (−25– − 20 kJmol^{-1} for amino acids, and −26.5– − 25.3 for dipeptides, respectively) was attributed to the effect of enthalpy-entropy compensation. The complexation phenomenon was found driven by electrostatic interactions between protonated N-terminal amino group of the guest and calixarene phosphoryl groups. The complex stability correlates with the hydrophobicity of amino acid residues, indicating significant partition of the solvatophobic interactions.

Keywords: Phosphorylated calix[4]arene; Amino-acids; Complexation; Calorimetry; Entropy-enthalpy compensation

INTRODUCTION

Calixarenes have drawn a remarkable interest not only in chemistry, but also in physics and biochemistry. For the last thirty years, since Gutsche introduced calixarenes, their binding properties have been intensively studied [1–7]. The chemical modifications of calixarenes enable synthesis of the variety of derivatives with different pendant functional groups. Thus, calixarenes are capable of forming host-guest complexes not only with cationic or anionic ligands, but also with neutral molecules. The complexes formed between calixarenes and amino acids [8], basic amino acids (lysine and arginine) [9], amines [10], metal ions [11–15], fullerenes [16], or small neutral organic molecules (methanol, ethanol, n-propanol, acetone, butanone, acetonitrile) [17,18] were reported.

The calix[4]arenes have been investigated as a platform to design the artificial receptors [19]. Similarly to the natural enzymes they are able to recognize a wide range of bioactive guest molecules such as amino acids [20], dipeptides, proteins [21], choline and acetylcholine [22], carbohydrates [23], vitamins B_2 (riboflavin) and B_{12} [24], nucleotides, nucleosides and short DNA fragments [25] or nucleic acid bases [26,27]. Calix[4]arene derivatives were also used for the recognition of protein surfaces [28]. The ability to mimic receptor—substrate interaction with the bio-relevant molecules is the basis of the putative bio-medical applications for calixarene derivatives.

In the presented work, we consider the host—guest complexation of 5,17 *bis*(dihydroxyphosphorylhydroxymethyl)-25,27-dipropoxy-calix[4]arene (pure *Racemic* form, CA1, and mixture of the *Meso* and two *Racemic* forms, CA2) (Scheme 1) with a series of amino acids and dipeptides (Scheme 2) in methanol solution.

Recently we have demonstrated that for aliphatic residues (Gly, Ala, Val, Leu, Ile) the calixarene—amino acid interaction is affected by the size of the hydrophobic side-chain [29]. In the case of isoleucine

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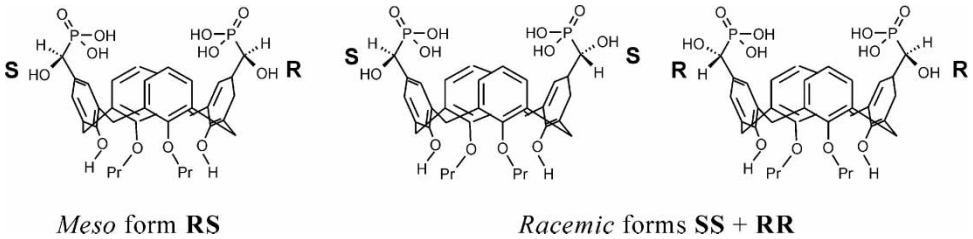
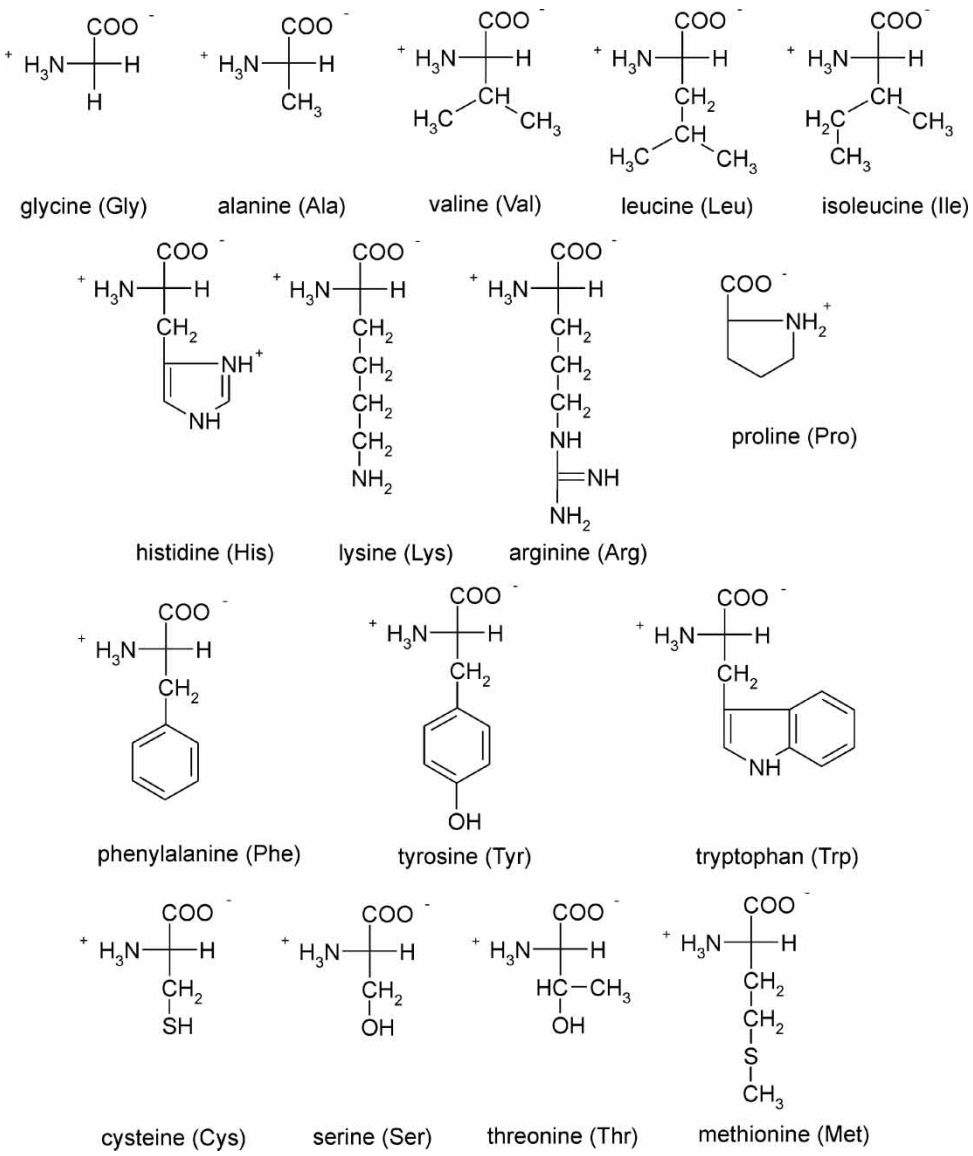


SCHEME 1 5,17-*bis*(dihydroxyphosphoryl-hydroxymethyl)-25,27-dipropoxy-calix[4]arene stereomeric forms (host molecules).

residue the evidence of weak 2:1 complex formation has been also demonstrated [30], while for the other residues 1:1 complex was found to dominate [29]. The complexation of CA1 towards aliphatic amino acid residues has been also analyzed using ^{1}H NMR and UV-Vis titration experiments. Finally, the calorimetric data (ITC), the binding induced ^{1}H NMR chemical shift drifts and UV absorbance changes gave an insight into the nature of the aliphatic amino acid complexation by calix[4]arene *bis*-hydroxymethylphosphoryl acids [29,30].

In the presented work we extend the investigations to hydroxyproline, 15 native amino acids residues and five model dipeptides (Ala-Ala, Ala-Leu, Ala-Glu, Thr-Leu, Gly-Tyr) (Scheme 2). Five amino acids (Glu, Gln, Asp, Asn, Tyr) and four dipeptides (Ala-Ser, Ala-Thr, Ser-Leu, Gly-Gly) exhibited so low solubility in the methanol solution that complexation



SCHEME 2 Guest molecules: Amino acids used in complexation experiments.

phenomena could not be analyzed by ITC. We also consider the influence of the stereomeric form of the calixarene molecule, determined by the configuration of the dihydroxyphosphorylhydroxy-methyl residues, on the host—guest interaction.

RESULTS AND DISCUSSION

Calorimetric Titration At Different PH

In order to verify whether the amino group is involved in complexation under experimental conditions, preliminary titration calorimetry experiments were performed for the CA1-Val, CA1-Phe and CA1-Ile systems at different pH values. The pH-dependence of evaluated thermodynamic parameters describing complex formation is summarized in Table I.

As expected, ΔG^0 values determined for complex formation are the lowest under neutral conditions (Fig. 1). At pH ~6-7 N-terminal amino group is protonated and phosphoryl groups are deprotonated, thereby strong intermolecular electrostatic interactions between the charged centers stabilize the complex. Consequently, the interaction of Val, Ile and Phe with the phosphorylated calixarene is strongly decreased in both acidic and alkaline solutions, which is caused by either the deprotonation of the amino group or the protonation of the phosphoryl group. Finally, no interactions were observed in strong alkaline conditions (pH ~ 13), as the guest molecule does not carry positive charges to bind to the negatively charged host molecule.

Complexation Properties of Phosphorylated Calix[4]arene Derivatives CA1 and CA2 Towards Free Amino Acids

The main aim of the calorimetric experiments performed for phosphorylated calixarene derivatives, CA1 and CA2, was to establish the thermodynamic characterization of the complexation phenomena, including the effect of the stereochemistry change of calixarene carrying two phosphoryl groups in the upper rim. The differences in titration curves observed in Fig. 2 for tryptophan and CA1 or CA2 indicate remarkable variations in heat released caused by the stereo-differentiation of the complex formation.

In both cases the binding phenomena is dominated by the strong exothermic effect. The dilution peaks are small, exothermic, and equal in size (not shown), indicating that for the used concentration ranges no aggregation or other nonspecific intermolecular interactions occurs, which was also confirmed by the analysis of ^{1}H NMR resonances line-shape [29]. The binding constant, K, and enthalpy changes, ΔH^0, were determined directly from calorimetric experiments, whereas Gibbs free energy, ΔG^0, and entropy changes ΔS^0, were calculated according to the formula: $\Delta G^0 = -RTlnK = \Delta H^0 - T\Delta S^0$. Similar results were found for all of the analyzed host-guest systems. The thermodynamic parameters derived from the calorimetric measurements are summarized in Table II.

The complexation of amino acids with CA1 and CA2 is enthalpy stabilized (all ΔH^0 values are negative). The thermodynamic behaviors of both phosphorylated calixarene derivatives are similar. The values of Gibbs free energy do not differ significantly across the series. This indicates that non-specific electrostatic interactions play a major role in the association process.

The process of complexation of amino acids in methanol at 298.15 K is both ligand and host dependent, according to the general order: CA1 > CA2. For the CA2 (*Meso* + *Racemic* forms) the decrease in all the thermodynamic parameters was observed, as compared to those determined for the CA1. Generally speaking, upon complexation process involving phosphorylated calixarenes exist-

TABLE I Thermodynamic parameters characterizing the complexation of valine (Val), phenylalanine (Phe) and isoleucine (Ile) by CA1 at different pH values (298.15 K, stoichiometry 1:1).

Conditions	pH values[§]	Amino acids	log K	ΔH^0, kJmol^{-1}	T ΔS^0, kJmol^{-1}*	ΔG^0, kJmol^{-1}**
Acidic[†]	3.40	Val	3.95[‖]	−5.44[#]	17.13	−22.56
		Phe	3.86	−3.85	18.19	−22.04
		Ile	4.16	−6.05	17.68	−23.72
Moderate acidic	4.52	Val	4.09	−6.49	16.84	−23.33
		Phe	4.01	−6.46	16.40	−22.86
		Ile	4.18	−6.54	17.31	−23.85
Neutral[‡]	7.29	Val	4.14	−7.08	16.53	−23.61
		Phe	4.02	−6.56	16.38	−22.93
		Ile	4.23	−7.05	17.10	−24.15
Moderate Alkaline	9.25	Val	3.82	−6.00	15.82	−21.82
		Phe	3.78	−5.59	15.99	−21.58
		Ile	3.95	−5.40	16.79	−22.19
Strong Alkaline[¶]	12.74	Small effect, almost equal to heat of dilution				

[†] Mixture of methanol with acetic acid (v/v = 99.8/0.2). [‡] Methanol. [¶] Mixture of methanol with sodium hydroxide (v/v = 99.6/0.4). [§] pH determined in methanol using a glass electrode. [‖] Standard deviation less than 0.1. [#] Standard deviation less than and 0.5 kJ mol^{-1}.

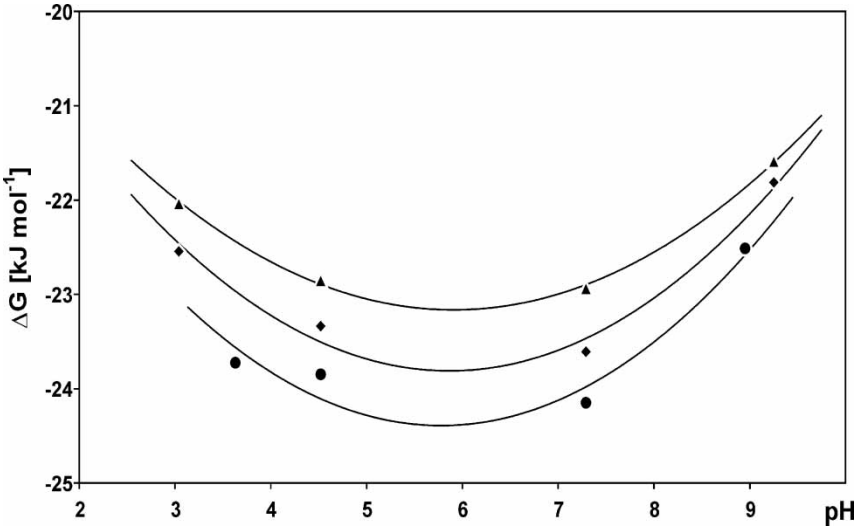


FIGURE 1 pH dependence of the valine (*diamonds*), phenylalanine (*triangles*) and isoleucine (*circles*) binding by CA1. At pH > 12 no interaction was observed.

ing in different forms, the pure stereochemical form of CA1 is favored.

Enthalpy-entropy Compensation Effect

Taking into account the thermodynamic parameters collected in Table II the dependence $T\Delta S^0$ vs $-\Delta H^0$ presented in Fig. 3, should be interpreted as the enthalpy-entropy compensation effect. This effect is well known in the studies of supramolecular chemistry, and was widely analyzed for different types of host molecules including crown ethers and cryptands [31,32], cyclodextrins [33,34], and calixarenes [33–37].

It is evident that although the remarkable changes in enthalpy ($\Delta H = -10 - -2\,kJ{\cdot}mol^{-1}$) and entropy ($T\Delta S = 13$ to $20\,kJ{\cdot}mol^{-1}$) are observed, the Gibbs free energy values do not differ considerably,

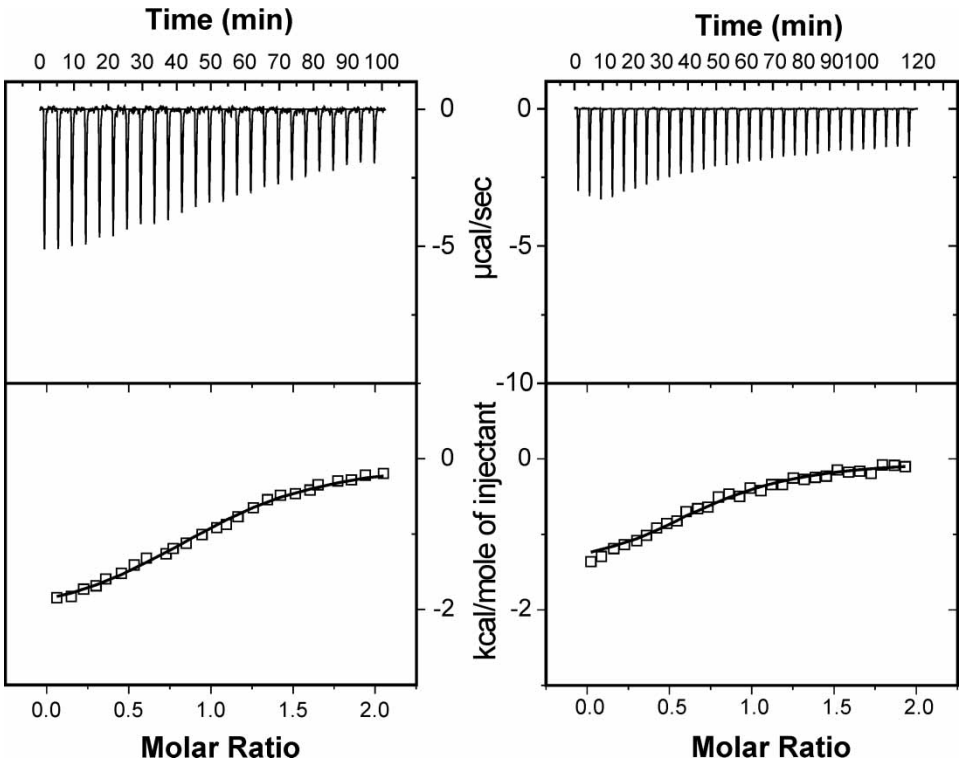


FIGURE 2 Individual titration experiments performed with the aid of MicroCal ITC- the calorimetric response of titrations of phosphorylated calix[4]arenes samples CA1 (*left*) and CA2 (*right*) with tryptophan (Trp) in methanol at 298.15 K. Each peak corresponds to heat effect arising from single injection (*top plots*). The calorimetric titration curve is obtained after the integration of the heat effects arising from individual injections (*bottom plots*). The regression lines were obtained applying 1:1 binding model.

TABLE II Stability constants, free energies, enthalpies and entropies characterizing the complexation of amino acids by phosphorylated calixarenes, CA1 and CA2, in methanol at 298.15 K.

Amino acid	Phosphorylated Calixarene	log K	ΔH^0, kJmol^{-1}	T ΔS^0, kJmol^{-1}	ΔG^0, kJmol^{-1}
Glycine	CA1	3.84 ± 0.15	−9.13 ± 0.32	12.79	−21.19
	CA2	3.46 ± 0.31	−5.22 ± 0.28	14.52	−19.74
L-alanine	CA1	3.89 ± 0.11	−8.19 ± 0.21	14.00	−22.19
	CA2	3.64 ± 0.26	−4.70 ± 0.24	16.08	−20.78
L-valine	CA1	4.12 ± 0.04	−7.11 ± 0.24	16.42	−23.53
	CA2	3.90 ± 0.19	−4.61 ± 0.24	17.35	−21.97
L-leucine	CA1	4.19 ± 0.04	−9.06 ± 0.18	14.82	−23.89
	CA2	3.98 ± 0.21	−5.00 ± 0.18	17.20	−22.20
L-isoleucine	CA1	4.23 ± 0.01	−7.05 ± 0.14	17.10	−24.15
	CA2	4.03 ± 0.09	−4.44 ± 0.11	18.55	−22.99
L-lysine HCl	CA1	3.64 ± 0.07	−7.97 ± 0.39	12.79	−20.78
	CA2	3.57 ± 0.16	3.91 ± 0.59	16.45	−20.36
L-arginine HCl	CA1	3.67 ± 0.07	−7.17 ± 0.38	13.78	−20.95
	CA2	3.60 ± 0.14	3.67 ± 0.36	16.84	−20.51
L-histidine HCl	CA1	3.72 ± 0.08	−6.86 ± 0.41	14.36	−21.21
	CA2	3.67 ± 0.15	−3.26 ± 0.33	17.66	−20.93
L-proline	CA1	4.11 ± 0.10	−5.00 ± 0.26	18.44	−23.44
	CA2	3.90 ± 0.18	−3.51 ± 0.19	18.75	−22.26
L-hydroxyproline	CA1	4.02 ± 0.06	−3.18 ± 0.33	19.72	−22.92
	CA2	3.83 ± 0.14	−1.82 ± 0.27	20.11	−21.83
L-phenylalanine	CA1	4.01 ± 0.05	−6.60 ± 0.27	16.29	−22.89
	CA2	3.90 ± 0.11	−2.49 ± 0.17	19.75	−22.24
L-tryptophan	CA1	3.99 ± 0.17	−7.82 ± 0.22	14.94	−22.76
	CA2	3.75 ± 0.12	−5.08 ± 0.22	16.33	−21.40
L-cysteine	CA1	3.97 ± 0.09	−5.50 ± 0.12	17.15	−22.63
	CA2	3.72 ± 0.17	−3.59 ± 0.21	17.64	−21.23
L-serine	CA1	4.03 ± 0.13	−5.46 ± 0.22	17.51	−22.98
	CA2	3.79 ± 0.21	−3.52 ± 0.20	17.95	−21.47
L-threonine	CA1	4.11 ± 0.08	−5.45 ± 0.24	17.08	−23.47
	CA2	3.85 ± 0.26	−3.37 ± 0.17	18.61	−21.99
L-methionine	CA1	4.21 ± 0.02	−5.38 ± 0.10	18.00	−24.00
	CA2	3.96 ± 0.18	−3.36 ± 0.14	19.25	−22.61

varying in the range of -25– -20 kJ·mol^{-1}. For the example complex of CA1-Ile, the parameters are as follows: enthalpy is equal to -7.05 kJ·mol^{-1} and entropy term corresponds to 17.10 kJ·mol^{-1}. The large entropy of binding suggests that the long aliphatic chain of isoleucine is deeply included inside the cavity, and in consequence the optimal packing of the isoleucine side-chain in the phosphorylated calixarene cavity decrease the electrostatic interaction between the positively charged amino group and phosphoryl group(s) of calixarene. An exactly opposite effect is observed for alanine—the enthalpy change of -8.19 kJ·mol^{-1} indicates stronger electrostatic interactions whereas the entropy term, 14.00 kJ·mol^{-1}, indicates the weak inclusion of a short aliphatic chain–chain inside CA1 cavity.

Moreover, as it has been proposed by Inoue and Hakoushi [33,34] and Tao and Barra [37], the slope, α, and the intercept, $T\Delta S^0$, of $T\Delta S$ vs, ΔH plots can

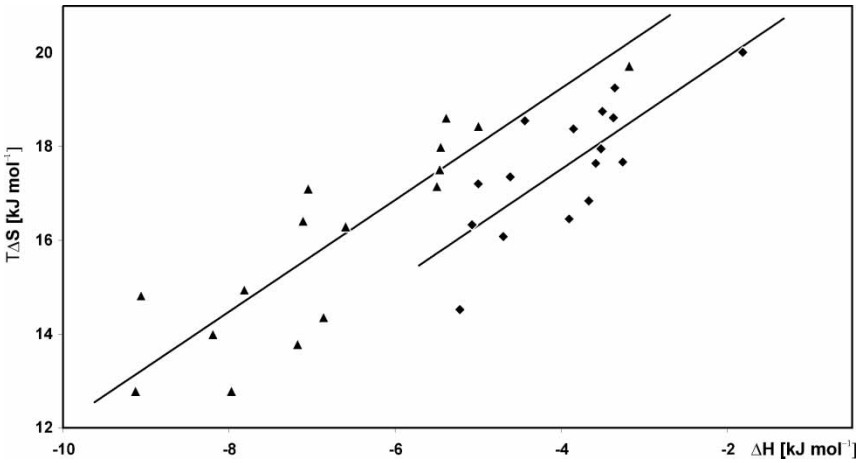


FIGURE 3 Variation of $T\Delta S^0$ with ΔH^0 for the complexation of amino acids by phosphorylated calix[4]arene derivatives: CA1 (triangles) and CA2 (diamonds) in methanol at 298.15 K.

TABLE III Slope, α, and intercept, $T\Delta S^0$, obtained from $T\Delta S$-ΔH plots for 1:1 host-guest complexation in solution.

Host	α	$T\Delta S^0$ [kcalmol^{-1}]	$T\Delta S^0$ [kJmol^{-1}]
CA1	1.2 ± 0.2	–	24.0 ± 1.2
Neutral residues Basic residues	1.08 ± 0.12 1.39 ± 0.13		23.7 ± 0.8 23.8 ± 0.9
CA2	1.2 ± 0.3	–	22.3 ± 1.1
calixarene[†]	1.10	4.60	19.25
calixarene[‡]	0.78	3.40	14.23
Cyclodextrin[b]	0.90	3.10	12.97
modified cyclodextrin[b]	1.07	5.00	20.92

[†] reference [37]. [‡] reference [33,34].

be rationalized. Namely the slope represents the effect of conformational changes of host molecules upon complexation, while the intercept is a measure of solvatophobic effect, respectively (Table III). Detailed analysis of the $T\Delta S$—ΔH relation demonstrated that the correlation pattern determined for the residues carrying positively charged side-chains (Lys, Arg, His) differs from the neutral ones. This fact clearly indicates that the additional basic group localized in residue side-chain, significantly change the pattern of intermolecular interactions, and the same stability of the complex. The intercepts determined for both groups are almost identical, while slopes, α, determined for neutral residues (1.08) significantly differs from that estimated for basic ones (1.39). The latter indicates that in the case of basic residues, the larger conformational adaptation is required for the optimal host-guest interaction.

The values of intercept obtained for analyzed diphosphorylated calixarenes are higher than those presented by Inoue and Hakoushi [33,34] and Tao and Barra [37] for sulphonated calixarenes. Parameters estimated by us are closer to those determined by Tao and Barra [37]. The comparison of parameters determined for cyclodextrin derivatives by Inoue and Hakoushi demonstrates that chemical modification of the host molecule cause considerable changes of both the slope and the intercept values. Thus small distinctions in parameters determined for CA1 and CA2 probably indicates the differences in solvation pattern of these host compounds.

Finally for phosphorylated calixarene CA1 two series of the data can be distinguished. The one mode of interaction corresponds to thirteen neutral amino acids: Gly, Ala, Val, Leu, Ile, Pro, hPro, Phe, Trp, Cys, Ser, Thr, Met, and the second one concerns the amino acids with basic side-chain as Lys, Arg, His (Fig. 4). The latter carry additional charged centers, which could directly interact with the negatively charged phosphoryl groups located on the calixarene upper rim.

Correlation Between Thermodynamic Parameters and Molecular Volume/hydrophobicity of Amino Acids

Changes in the amino acids side-chain size lead to slight differences in binding affinities (Fig. 5). These dependencies can be clearly analyzed in the terms of either molecular volume or hydrophobicity of aliphatic amino acid residues.

Additionally, it is worth adding that a strong correlation is noticed between the hydrophobicity of

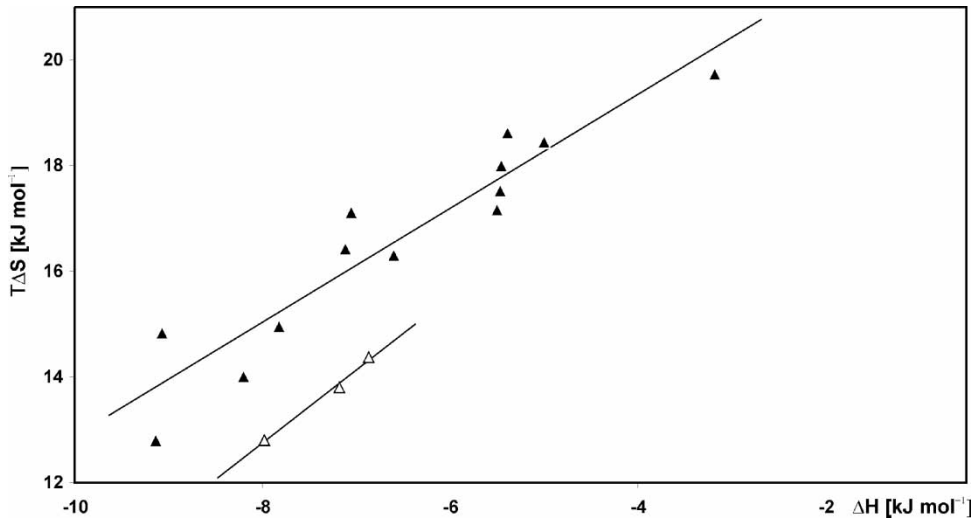


FIGURE 4 Variation of $T\Delta S$ with ΔH for the complexation of CA1 with 13 neutral amino acid residues, filled triangles, and Lys, Arg, His, empty triangles, in methanol at 298.15 K.

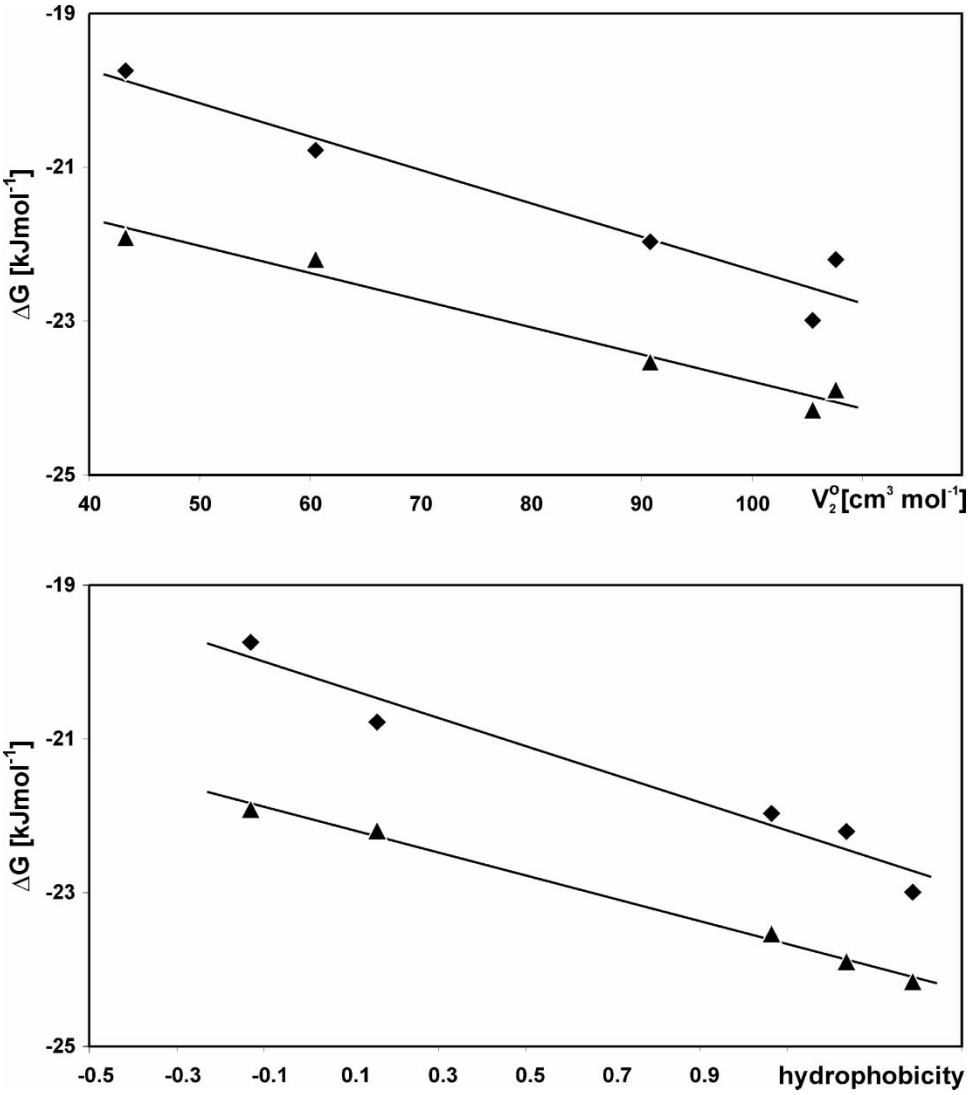


FIGURE 5 Gibbs free energy value ΔG^0 for the complexation of aliphatic residues (Gly, Ala, Val, Leu and Ile) by CA1 (triangles) and CA2 (diamonds) represented as a function of solute molecular volume (*top*) and hydrophobicity [38] (*down*) of amino acids.

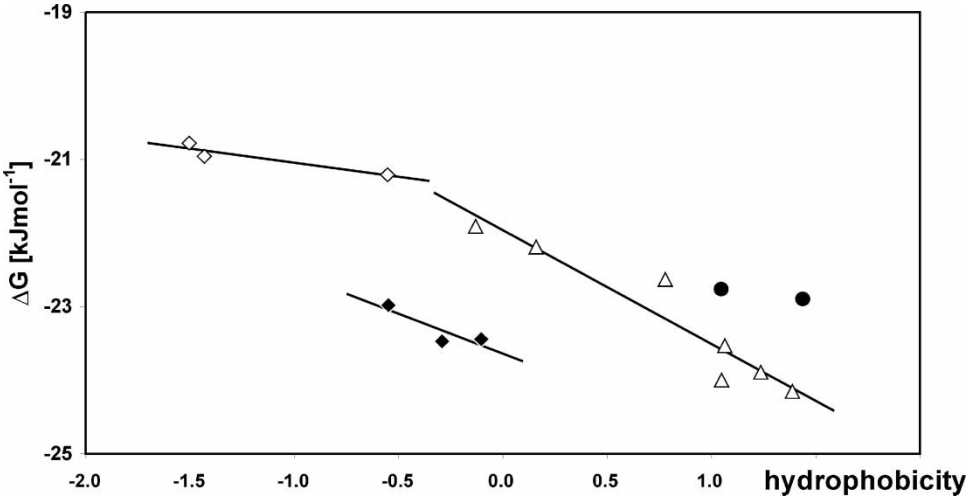


FIGURE 6 ΔG^0 estimated for the complexation of 16 amino by CA1 estimated as a function of the residue hydrophobicity [38]. Apolar, aromatic, polar and charged residues are represented by triangles, circles, solid and empty diamonds, respectively.

all amino acids, and the thermodynamic parameters, as it is presented for Gibbs free energy changes estimated for CA1 host (Fig. 6). For amino acids complexed by CA1 at least four types of residues, exhibiting different complexation patterns, could be distinguished.

The first group consists of the apolar residues, uniformly interacting with the calixarene host. For this group, the complexation abilities strictly depend on residue hydrophobicity. The increase of the latter drives the equilibrium towards the host-guest complex. In the case of aromatic residues (Trp, Phe, circles in Fig. 6), which large side-chains are too large to fit inside the phosphorylated calixarene cavity, the steric hindrances significantly decrease the correlation based on prediction of complex stability. The third group, consisting of charged residues (Lys, Arg, His) exhibits no correlations between the polarity and the complex stability. The explanation of this phenomenon is that the charged side-chains do not penetrate the calixarene cavity, and the total stabilization effect is related only to the direct interaction of the amino group (or equivalently side-chain charge) with the calixarene phosphoryl groups. Thus in complex stabilization, the electrostatic interactions and net effect of desolvation of charged groups dominates. The fourth group (Ser, Thr) exhibits additional stabilization of the complex, arising from the specific interaction of the residue side-chain β-hydroxyl group with the calixarene phosphoryl, which is resulting in the systematic shift towards the more negative values of ΔG^0. Proline, Pro, which is formally imino-acid must experience significantly different organization of the solvating methanol and, as presented in Fig. 6, is moved towards the polar residues (Ser, Thr). Similar observations apply to CA2, demonstrating the existence of universal interactions pattern pointing out the complexes with given residues (partially presented in Fig. 5).

Finally it should be summarized that complexation of free amino acids with analyzed host compounds (phosphorylated calix[4]arenes) is both enthalpically and entropically favored ($\Delta H < 0$, $\Delta S > 0$). The additional calorimetric experiments carried out at different pH proved that the complexation process is electrostatically driven. In contrast to the canonical structure of amines complexed by calix[4]arene, the charged amino group of the amino acid guest is interacting rather with the upper rim phosphoryl groups than with the π electron system bordering the internal cavity, as originally Ikeda and Shinkai proposed [39]. Finally the binding affinity is modulated by the properties of the ligand side-chain—the long apolar/hydrophobic chains stabilize the complex. There can be distinguished few modes by which amino acids may be bound to phosphorylated calixarene. In the most probable model, guest N-terminal amino group is localized within a distance of 3-5 Å from phosphoryl groups of calixarene, while hydrophobic side-chain is placed inside the cavity of the host compound.

Investigation of Complexation CA1 With Dipeptides

The studies were extended to the biologically relevant peptide systems (simple dipeptides), since peptides are known to act as receptors for heparin. The choice of dipeptides was dependent on properties of two joined amino acids, mainly the presence of a different group for comparing the variable effects of complexation. Unfortunately the solubility of some tested dipeptides was not large enough to carry out the calorimetric experiments.

For each of analyzed peptides, both stability constant and enthalpy of complexation were extracted from the calorimetric data. Entropic terms and Gibbs energy were then calculated assuming 1:1 binding model. Analysis of enthalpy and entropy data of complexation of CA1 with dipeptides shows the existence of a 1:1 species, whose stabilization is both enthalpic and entropic in origin. On the basis of these results, and by comparison with the data obtained for free amino acids, the hypothesis that binding undergoes mainly through N-terminal amino group and is modulated by the type of C-terminal residue side-chain could be advanced. In principle the binding affinity is increased, as compared to free residues, due to larger separation between negatively charged phopsphorous groups of calixarene and peptide C-terminal carboxyl.

Taking into consideration the enthalpy-entropy compensation effect for dipeptides and phosphorylated calixarene CA1, it is clearly shown that the slope is quite similar to CA1-amino acids interactions and equal to $\alpha = 1.2 \pm 0.1$, pointing out the same behavior of calixarene molecules. On the other hand the value of $T\Delta S^0 = 27.6 \pm 1.2\,\mathrm{kJmol}^{-1}$ suggests stronger conformational reorganization involving increase of solvatophobic interactions between phosphorylated calixarene and dipeptides in the comparison to amino acids.

Analysis of the changes in the thermodynamic parameters (Table IV) for Ala-Ala and Ala-Leu drives to the conclusion that upon elongation of aliphatic chains, the K value and the entropic term increase remarkably. However, if in Ala-Leu dipeptide Leu is replaced by Glu (Ala-Glu dipeptide), the additional carboxylic group causes significant decrease of the stability constant value and an increase of entropy. For Ala-Leu and Thr-Leu the results are in the same order of magnitude. These findings lead to a conclusion that for the first amino acid of dipeptide the electrostatic interaction

TABLE IV Stability constants log K (K in M^{-1}) and thermodynamic values ΔH^0, $T\Delta S^0$ and ΔG^0 for the complexation of some dipeptides by CA1 in methanol at 298.15 K.

Dipeptide	log K	ΔH^0, $kJmol^{-1}$	$T\ \Delta S^0$, $kJmol^{-1}$	ΔG^0, $kJmol^{-1}$
Ala-Ala	4.43 ± 0.02	−10.46 ± 0.12	14.79	−25.25
Ala-Leu	4.64 ± 0.04	−9.43 ± 0.24	16.99	−26.42
Ala-Glu	4.57 ± 0.05	−8.41 ± 0.16	17.66	−26.07
Gly-Tyr	4.64 ± 0.02	−5.93 ± 0.24	20.55	−26.47
Thr-Leu	4.60 ± 0.01	−9.53 ± 0.24	16.75	−26.28

between the positively charged amino group and the phosphoryl group of calixarene occurs, while the second residue side-chain is preferably inserted into the cavity. It should be noted that in the case of dipeptide Gly-Tyr, which contains aromatic ring amino acid, Tyr, the enthalpy loss is meaningful and the $T\Delta S^0$ achieves the largest value. The explanation can be the π-π interactions (e.g. stacking) between the Tyr side-chain and aromatic cavity of the host compound.

EXPERIMENTAL

Materials

All amino acids and dipeptides 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. All the solutions were prepared by weight/weight in methanol.

Analyzed calixarene hydroxyphosphonic acids, CA1 and CA2, were synthesized by subsequent reaction of their tetraethyl esters with trimethylbromsilane and methanol [40,41]. The tetraethyl ester of CA2 as 1:1 mixture of *Racemic* and *Meso* forms has been synthesized by the interaction of diformylcalixarene with triethylphosphite in accordance with the previously reported method [40,41]. The tertaethyl ester of CA1 in the pure *Racemic* form was isolated from the mixture by crystallization from cyclohexane [40,41]. For NMR identification of the stereochemical forms the *Meso* tetraethyl ester of 5,17-*bis*(dihydroxyphosphoryl-hydroxymethyl)-25,27-dipropoxy-calix[4]arene was isolated from the same mixture with 2% yield by the column chromatography (silicagel, chloroform—acetone 10:1).

RS stereomeric form of molecule has a symmetry plane and generates two overlapping doublets H_a and H_b (6.86, d, 2H, $J = 7.2$ Hz; 6.87, d, 2H, $J = 7.2$ Hz) and two overlapping triplets H_d and H_c (6.64, 6.65, two t, 1H + 1H, $J = 7.2$ Hz), of the para unsubstituted aromatic nuclei in the ^{1}NMR spectrum (Fig. 7) (see [42] for a comparison).

Tetraethyl ester of acid CA1 (either in RR or SS configuration) demonstrates three doublets of doublets of the aromatic protons H_c, H_b, H_a (6.68, dd, 2H, $J = 7.5$ Hz; 6.84, dd, 2H, $J = 7.5$ Hz, $J = 3$ Hz; 6.91 dd, 2H, $J = 7.5$ Hz, $J = 2.3$ Hz) in the NMR spectrum (Fig. 7).

The NMR spectrum of the 1:1 mixture of the *Racemic* and *Meso* forms of the tetraethyl ester of acid CA2 is a superposition of the both spectra presented in Fig. 1 (not shown).

In contrast to the esters, the signals of the aromatic protons in the NMR spectra of free CA1 and CA2 are broadened, probably due to association [40,41], however because stereochemical configuration of the chiral carbon atoms of the starting esters is being

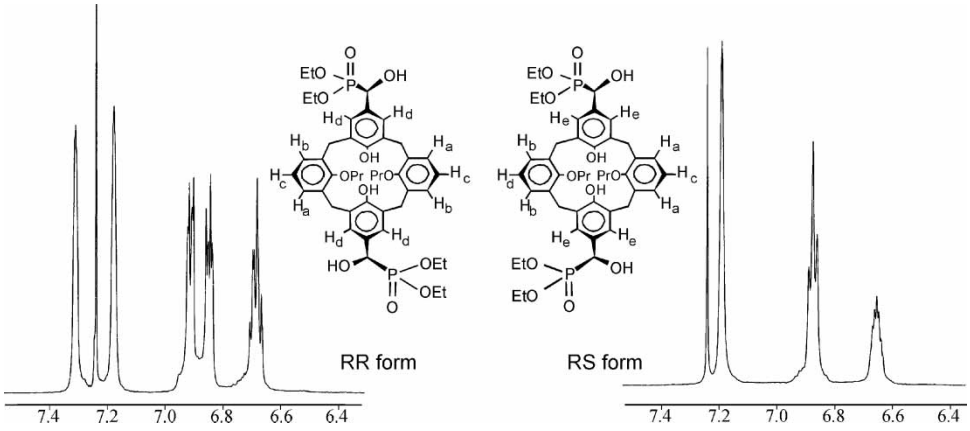


FIGURE 7 A section ^{1}H NMR spectra ($CDCl_3$) of the aromatic nuclei of tetraethyl esters of calixarene phosphonic acid. *Racemic* form, either RR or SS (left), and RS *Meso* form (right).

kept during the reaction with trimethilbromosilane and methanol, the esters and the corresponding free acids have the same stereomeric configuration.

5,17-Bis(dihydroxyphosphonylmethylol)-25,27-dipropoxycalix[4]arene CA1 (RR/SS)

Colorless crystal compound: yield 85%. M.p. 250–260°C (dec.). ^{1}H NMR (CD_3OD-d4), δ: 1.35 (m, 6H, $OCH_2CH_2CH_3$), 2.05 (m, 4H, $OCH_2CH_2CH_3$), 3.35 (d, 4H, J 13 Hz, $ArCH_{2eq}$), 3.90 (m, 4H, $OCH_2CH_2CH_3$), 4.20 (d, 4H, J 13 Hz $ArCH_{2ax}$), 6.58 (m, 2H, ArH), 6.81, 6.86 (two d, 2H + 2H, $J = 7.5$ Hz, Ar*H*), 6.98, 7.05 (two s, 2H + 2H, ArH), ^{31}P NMR (CD_3OD-d4), δ 19.01. MS (ES) m/z; 651[M - $HPO(OH)_2$]$^+$, 693[M-2H_2O]$^+$, 729[M]$^+$. Calculated M 728.0.

ITC CALORIMETRY

The isothermal calorimetric experiments were carried out using the Omega MicroCal Calorimeter. All experiments were performed in methanol at 298.15 K. A syringe containing solution of a given amino acid (4–10 mM) was titrated into a cell filled with a solution of the phosphorylated calixarene (0.4–1 mM). When guest was injected into the cell, as these compounds interact, the heat was released in direct proportion to the amount of binding. As the macromolecule in the cell became saturated with amino acid, the heat signal diminishes until only background heat of dilution was observed. Regarding the latter, separate dilution experiments were done. Typically twenty five injections of guest solution were added to host solution in the ITC cell. The heat released for each injection was equal to the underneath of each single peak. And finally, summing up all the individual effects gave the total heat of reaction. The method of analysis was described in details previously [29,30].

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