UDC 547.03+547.562

COMPLEXATION OF CALIX[4]ARENE HYDROXYMETHYL-PHOSPHONIC ACID WITH TRYPTOPHAN AND N-ACETYL-TRYPTOPHAN AMIDE

O.I.Kalchenko, S.O.Cherenok, R.V.Rodik, Y.Mely*, A.S.Klymchenko*, V.V.Gorbatchuk**, V.I.Kalchenko

Institute of Organic Chemistry at the National Academy of Sciences of Ukraine
5, Murmanska str., Kyiv-94, 02660, Ukraine. E-mail: oik@ioch.kiev.ua
* Université de Strasbourg, Laboratoire de Biophotonique et Pharmacologie UMR, France
** Kazan Federal University, Russian Federation

Key words: calixarene hydroxymethylphosphonic acid; tryptophan; N-acetyltryptophan amide; reversed-phase high performance liquid chromatography; supramolecular complexes; binding constants

The Host-Guest complexation of calixarene hydroxymethylphosphonic acid with tryptophan and N-acetyltryptophan amide has been investigated by the RP HPLC method in H_2O/MeCN (99/1) solution (column support Hypersil CN, UV-detector, λ = 254 nm). Adsorption of calixarene hydroxymethylphosphonic acid on the Hypersil CN surface has been studied. It has been found that hydroxymethylphosphonic acid is characterized by reversible sorption on the Hypersil CN surface. The binding constants (K_A = 23000 M^{-1} and 39000 M^{-1} for tryptophan and N-acetyltryptophan amide, respectively) of the supramolecular complexes have been calculated from the ratio between the capacity factors k' of the Guest and the calixarene hydroxymethylphosphonic acid Host concentration in the mobile phase. The Gibbs free energies of the tryptophan and N-acetyltryptophan amide complexes are -24.84 and -26.15 kJ/mol, respectively. The molecular modelling of calixarene hydroxymethylphosphonic acid and its complexes with tryptophan and N-acetyltryptophan amide (Hyper Chem, version 8, force field PM3) has indicated that the complexes are stabilized by hydrogen bonds, electrostatic, π-π, and solvatophobic interactions. The geometric parameters of the energy minimized calixarene macrocycle and its complexes with tryptophan and N-acetyltryptophan amide have been calculated. According to the calculations it has been shown that the Host-Guest complexation does not change the flattened-cone conformation of calixarene. Finally, the inverse correlation has been found between the K_A values of the complexes and the Log P values of the guest molecules.

КОМПЛЕКСОУТВОРЕННЯ КАЛІКС[4]АРЕНГІДРОКСИМЕТИЛ-ФОСФОНОВОЇ КИСЛОТИ З ТРИПТОФАНОМ ТА N-АЦЕТИЛ-ТРИПТОФАНАМІДОМ

О.І.Кальченко, С.О.Черенок, Р.В.Родік, І.Мелі, А.С.Климченко, В.В.Горбачук, В.І.Кальченко

Ключові слова: *каліксаренгідроксиметилфосфонова кислота; триптофан; N-ацетилтриптофанамід; обернено-фазна високоефективна рідинна хроматографія; супрамолекулярні комплекси; константи зв'язування*

Методом ОФ ВЕРХ досліджено процес комплексоутворення типу Господар-Гість каліксаренгідроксиметилфосфонової кислоти з триптофаном та N-ацетилтриптофанамідом у розчині H_2O/MeCN (99/1) (насадка Hypersil CN, УФ-детектор, λ = 254 нм). Досліджено взаємодію каліксаренгідроксиметилфосфонової кислоти з поверхнею хроматографічної насадки Hypersil CN. Встановлено, що каліксаренгідроксиметилфосфонова кислота характеризується оберненою сорбцією на поверхні Hypersil CN. Константи зв'язування супрамолекулярних комплексів (23000 M^{-1} і 39000 M^{-1} для триптофану і N-ацетилтриптофанаміду, відповідно) були розраховані із співвідношення між коефіцієнтом ємкості k' молекули Гостя і концентрацією каліксаренгідроксиметилфосфонової кислоти Господаря в рухомій фазі. Значення вільних енергій Гіббса комплексів каліксаренгідроксиметилфосфонової кислоти з триптофаном і N-ацетилтриптофанамідом складає -24.84 і -26.15 кДж/моль, відповідно. Здійснено молекулярне моделювання каліксаренгідроксиметилфосфонової кислоти і її комплексів з триптофаном і N-ацетилтриптофанамідом (Hyper Chem, версія 8, силове поле PM3). Супрамолекулярні комплекси можуть стабілізуватись водневими зв'язками, а також електростатичними, π-π, і сольватофобними взаємодіями. Розраховані геометричні параметри енергетично мінімізованих структур каліксаренгідроксиметилфосфонової кислоти та її комплексів з триптофаном і N-ацетилтриптофанамідом. Показано, що значення K_A зростають зі зниженням Log P молекул субстратів, а процес комплексоутворення не змінює конформації макроциклічного кістяка каліксарену.

КОМПЛЕКСООБРАЗОВАНИЕ КАЛИКС[4]АРЕНГИДРОКСИМЕТИЛ-ФОСФОНОВОЙ КИСЛОТЫ С ТРИПТОФАНОМ И N-АЦЕТИЛ-ТРИПТОФАНАМИДОМ

О.И.Кальченко, С.А.Черенок, Р.В.Родик, И.Мели, А.С.Климченко, В.В.Горбачук, В.И.Кальченко

Ключевые слова: *каликсаренгидроксиметилфосфоновая кислота; триптофан; N-ацетилтриптофанамид; обратно-фазная высокоэффективная жидкостная хроматография; супрамолекулярные комплексы; константы связывания*

Методом ОФ ВЭЖХ исследован процесс комплексообразования типа Хозяин-Гость каликсаренгидроксиметилфосфоновой кислоты с триптофаном и N-ацетил-триптофанамидом в растворе H_2O/MeCN (99/1) (насадка Hypersil CN, УФ-детектор, λ = 254 нм). Исследовано взаимодействие каликсаренгидроксиметилфосфоновой кислоты с поверхностью хроматографической насадки Hypersil CN. Установлено,

что каликсаренгидроксиметилфосфоновая кислота характеризуется обратимой сорбцией на поверхности Hypersil CN. Константы связывания супрамолекулярных комплексов (23000 М$^{-1}$ и 39000 М$^{-1}$ для триптофана и N-ацетилтриптофанамида, соответственно) были рассчитаны из соотношения между коэффициентом емкости k' молекулы Гостя и концентрацией каликсаренгидроксиметилфосфоновой кислоты Хозяина в подвижной фазе. Значения свободных энергий Гиббса комплексов каликсаренгидроксиметилфосфоновой кислоты с триптофаном и N-ацетил-триптофанамидом составили -24.84 и -26.15 кДж/моль, соответственно. Проведено молекулярное моделирование каликсаренгидроксиметилфосфоновой кислоты и ее комплексов с триптофаном и N-ацетилтриптофанамидом (Hyper Chem, версия 8, силовое поле PM3). Отмечается, что супрамолекулярные комплексы могут стабилизироваться водородными связями, а также электростатическими, π-π, и сольватофобными взаимодействиями. Рассчитаны геометрические параметры энергетически минимизированных структур каликсаренгидроксиметилфосфоновой кислоты и ее комплексов с триптофаном и N-ацетилтриптофанамидом. Согласно расчетам показано, что процесс комплексообразования не меняет конформацию макроциклического остова каликсарена. Установлено, что значения K_A повышаются со снижением Log P молекул Гостей.

L-Tryptophan is an essential amino acid that is low abundant in proteins (1.4% only). As a consequence, Trp residues frequently play a key role in studying the protein structure and functions. For instance, soluble Trp residues in proteins have been shown to be critical for the specific recognition of nucleic acid sequences [1, 2, 3, 4]. Moreover, it is worth mentioning that Trp has the peculiar property to exhibit a significant intrinsic fluorescence that is environment sensitive, and therefore, can be used to investigate the properties and interactions of proteins with ligands [5].

To characterize the role of the given Trp residue in the protein properties and functions, the common strategy is to site-selectively mutate this residue into another one. To disturb the protein structure minimally the aromatic Phe or Tyr residues are frequently selected as a substitute. Nevertheless, due to the key role of Trp residues in protein folding, these mutations can result in improperly folded proteins, and it does not allow characterizing the specific role of the Trp residues mutated.

To characterize the role of soluble Trp residues in proteins it would be interesting to use complexing agents that can selectively bind these Trp residues as an alternative strategy, and therefore, promote the interaction of the target proteins with their ligands.

Calixarenes [6] contain preorganized bio-affine groups that are able to recognize different biological molecules such as amino acids, dipeptides, proteins, choline and acetylcholine, carbohydrates, riboflavin, vitamin B_{12}, nucleotides, nucleosides and short DNA fragments [7, 8, 9, 10]. Calixarene derivatives can also bind amino acids on the surface of proteins [11, 12].

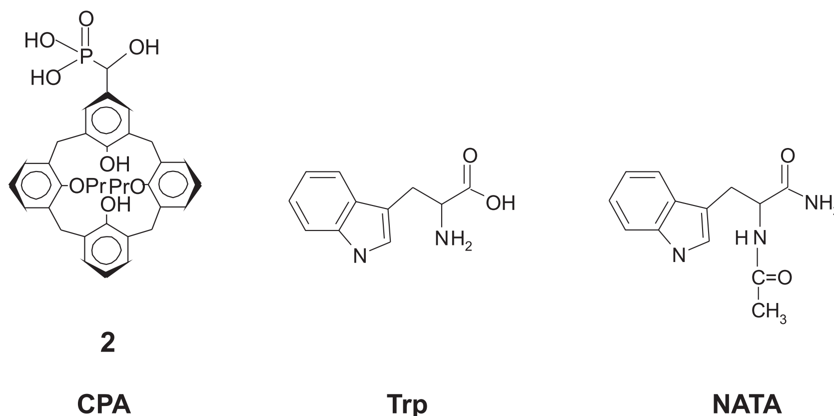
In this respect, calix[4]arene hydroxymethylphosphonic acids [13, 14, 15] which have been shown to form selectively Host-Guest supramolecular complexes with amino acids [16, 17], appear to be good candidates to bind soluble Trp residues. To test this possibility the Host-Guest complexation of calixarene hydroxymethylphosphonic acid (**CPA**) with Trp and N-acetyltryptophan amide (NATA) used as models of Trp residues in proteins has been investigated by RP HPLC and molecular modelling (Scheme).

Experimental Part

CPA was synthesized by the reaction of formylcalixarene with Na salt of ethylphosphite followed by dealkylation of the ester formed by the consecutive action of trimethylbromosilane and methanol in accordance with [13]. Because of its poor solubility in water **CPA** was analysed as a monosodium salt obtained by addition of one equivalent of sodium methylate to **CPA** solution in methanol. Trp and NATA were obtained from Sigma-Aldrich (St. Louis, MO, USA), acetonitrile was obtained from Acros Organics (Janssen Pharmaceuticalaan 3A 2440 Geel Belgium).

HPLC analysis

Chromatographic analysis was performed in isocratic conditions using a Hitachi liquid chromatogra-



Scheme

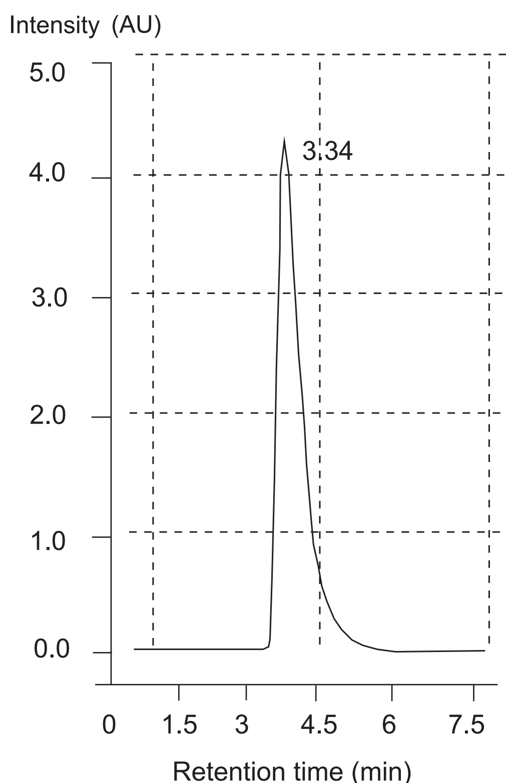


Fig. 1. Chromatogram of calixarene **CPA.**

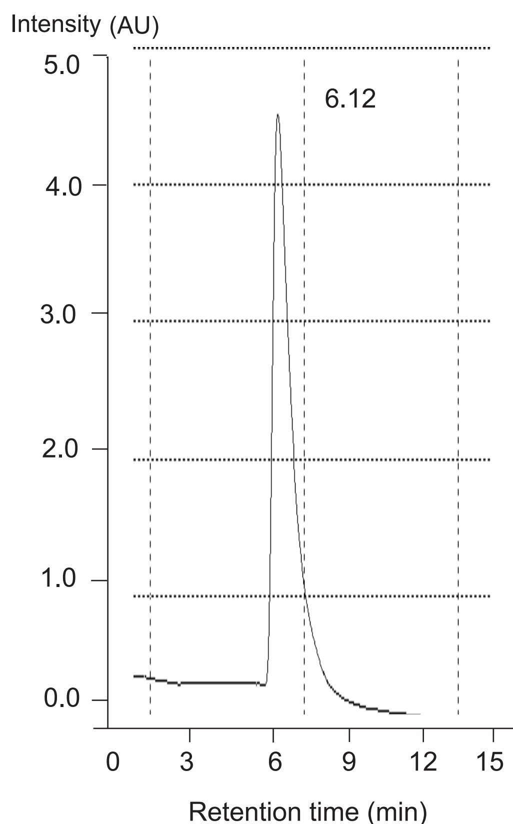
phy system (Hitachi, Ltd, Tokyo, Japan) equipped with a high-pressure pump, a Rheodyne Model Sample 7120 injector (20 μl) and an UV-detector. The column (250×4.6 mm i.d.) was packed with Hypersil CN (Merck, Germany, Darmstadt). The samples of **CPA** for RP HPLC analysis were prepared by dissolution in the mobile phase (H_2O/MeCN, 99/1 v/v). The choice of the solvent was dictated by solubility of **CPA**, Trp and NATA under the same conditions. The flow rate of the mobile phase was 0.8 ml/min. The final **CPA** concentrations were in the range of 0.10-0.70×10^{-4} M. The ultraviolet detector was operated at 254 nm. The Trp and NATA samples for HPLC analysis were prepared in the same solvent (C = 0.05×10^{-4} M). The amount of the sample injected was 20 μL. Each of the samples was analyzed five times. The mobile phase that contained the **CPA** as an additive was equilibrated for 3 h before analysis. Under these conditions the column was saturated with the **CPA** additive. All chromatograms were obtained at 32°C.

Molecular modelling

The initial molecular modelling of **CPA** and its complexes with Trp or NATA was carried out by the molecular mechanics MM+ method (the force field PM3). The structures obtained were optimized by the semi-empirical method (the HyperChem software package, version 8) [http://www.hyper.com/Download/AllDownloads/tabid/470/Default.aspx].

Results and Discussion

Calixarene **CPA**, Trp and NATA in the given analysis conditions were registered on the chromatograms as sharp peaks (Fig. 1-3).



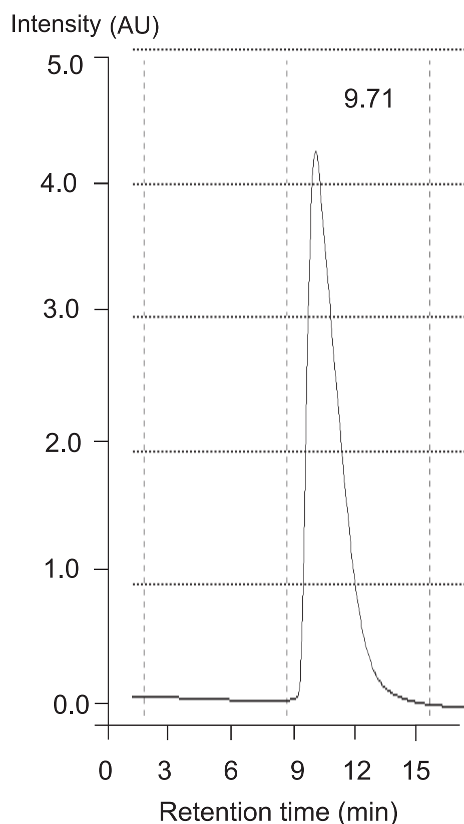


Fig. 2. Chromatograms of NATA (a) and Trp (b) obtained before **CPA** addition in the mobile phase.

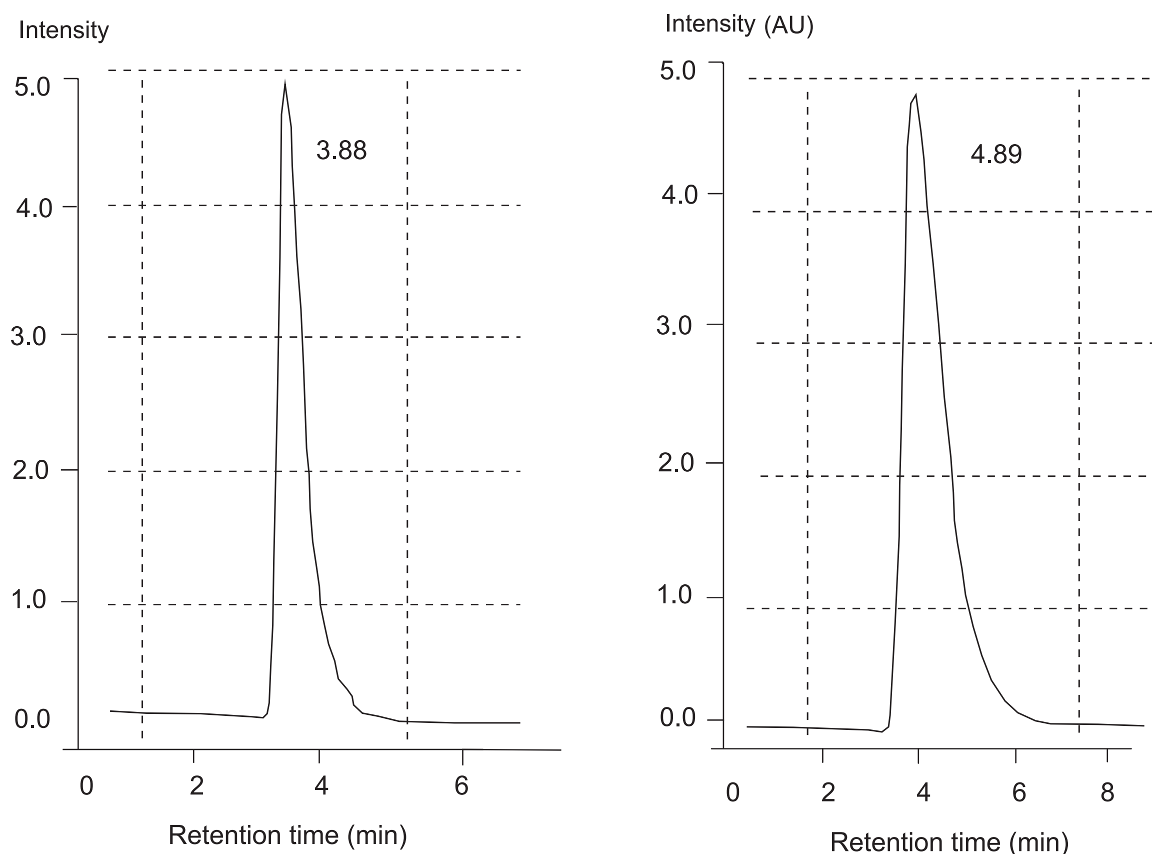


Fig. 3. Chromatograms of NATA (a) and Trp (b) obtained after **CPA** addition in the mobile phase.

The binding constants of Host-Guest complexes of **CPA** with Trp or NATA were determined by the RP HPLC method as previously described [18, 19]. The method is based on determination of the Guest retention time, t_R, and the capacity factor, k', before and after **CPA** addition to the mobile phase. The binding constants K_A of the **CPA** complexes with the Guest molecules were calculated by equation (1):

$$1/k' = 1/k_0' + K_A \times [CA]/k_0' \qquad (1)$$

where k_0' and k' – are the capacity factors of the Guest molecule determined in the absence and the presence of **CPA** in the mobile phase; [CA] is the concentration of **CPA** in the mobile phase.

CPA (monosodium salt), Trp and NATA appear on the chromatograms as sharp symmetrical peaks (Fig. 1, 2) with the chromatographic characteristics given in Table 1.

The linear character of the adsorption isotherm of **CPA** (R^2 = 0.99) indicates its reversible sorption on the Hypersil CN surface. Addition of **CPA** to the mobile phase decreases the capacity factor values of Trp and NATA. The linear plots of their k' values *vs* the calixarene concentration (Tab. 2, Fig. 4) clearly show the formation of Host-Guest supramolecular complexes with 1:1 stoichiometry and allows calculating the K_A values of the complexes by equation (1).

The binding constants K_A and free Gibbs energies ΔG ($\Delta G = -RT \ln K_A$) for the **CPA** complexes are given in Tab. 2.

The binding constant K_A of the **CPA-**NATA complex (39000 M^{-1}) is 1.66-fold higher than the K_A value of the **CPA-**Trp complex (23000 M^{-1}). The binding constant K_A values of the complexes increase with decrease of *Log P* values of the Guest (0.93 for Trp and -0.11 for NATA).

It should be noted that the **CPA-**NATA complex is more stable than complexes of **CPA** with such aminoacids as Ala (21200 M^{-1}), Phe (26600 M^{-1}), Arg (27500 M^{-1}), Asp (28800 M^{-1}), His (31200 M^{-1}), Lys (32500 M^{-1}) [17]. Moreover, comparison of Trp and NATA indicates that changing the -OH group of Trp to the $-NH_2$ group and acylation of its alpha-amino group significantly increase the interaction with **CPA**.

To clarify the nature of the Host-Guest interaction, the molecular modelling study was carried out. The conformational search of the optimum geometry of **CPA**, Trp and NATA was performed by the method of molecular mechanics and the semi-empirical method.

Table 1

Retention times t_R, capacity factors k' and asymmetry coefficient K_S of CPA, Trp and NATA

Compound	t_R, min	k'	K_S
CPA	3.34	0.67	1.00
Trp	9.71	3.86	1.27
NATA	6.12	2.06	1.20

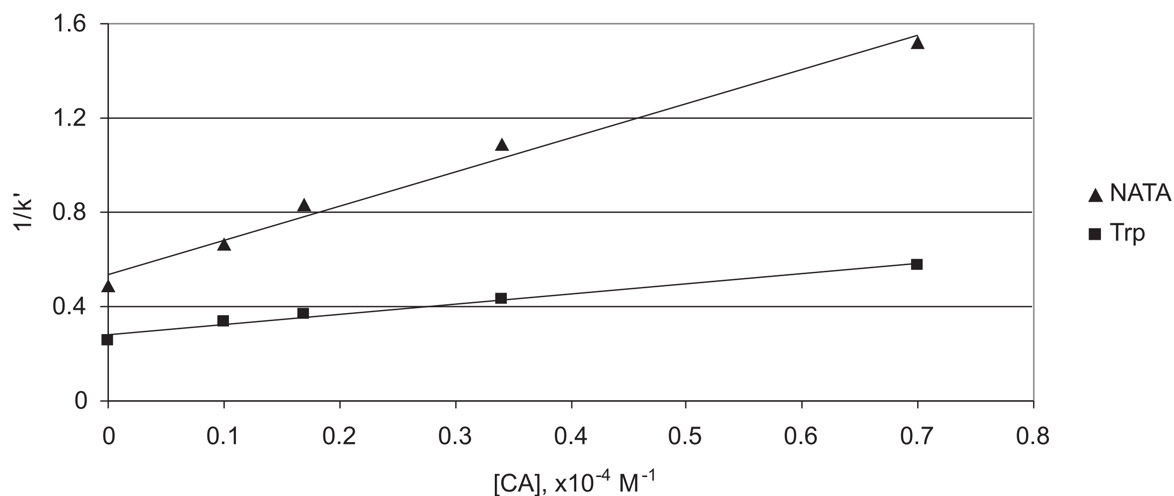


Fig. 4. Plots of the Trp and NATA 1/*k'* values *vs* **CPA** concentration in the mobile phase (R^2= 0.98).

Table 2

CPA concentration, 1/*k'*, KA (M^{-1}) and ΔG (kJ/mol) values of CPA complexes with Trp and NATA

CPA conc. x 10^{-4} M	Trp 1/*k'*	**CPA-**Trp K_A [a]	**CPA-**Trp ΔG	NATA 1/*k'*	**CPA-**NATA K_A [a]	**CPA-**NATA ΔG
0	0.259	23000±200	-24.84	0.485	39000±400	-26.15
0.10	0.336			0.664		
0.17	0.369			0.834		
0.34	0.432			1.090		
0.70	0.577			1.517		

[a] (RSD = 8-12%).

Then the structures of the **CPA** complexes with the least energies were calculated (Fig. 5).

Inclination (dihedral angles) of the calixarene benzene rings A, B, C, D in relation to the main macrocycle plane formed by CH_2 links for **CPA** and its complexes in the structures calculated is presented in Tab. 3.

The macrocyclic skeleton of **CPA** shows a *flattened-cone* conformation. The aromatic rings with phenolic OH groups are almost "coplanar" with the main macrocycle plane, but the propylated rings are "perpendicular" to the plane. The dihedral angle between the "coplanar" rings A and C is 110°, while the angle between "perpendicular" rings B and D is 3°. As seen from Table 3, the Host-Guest complexation does not almost change the *flattened cone* conformation of the calixarene skeleton.

For the structure of the Trp complex calculated an electrostatic contact of the negatively charged oxygen atom of the calixarene phosphonic group with the positively charged nitrogen atom of the amino acid is obvious (P-O···H-N distance is 2.3 Å). Additionally, the complex is stabilized by an intermolecular hydrogen bond between the indole NH group and the oxygen atom at the calixarene lower rim (NH···O distance is 3.0 Å).

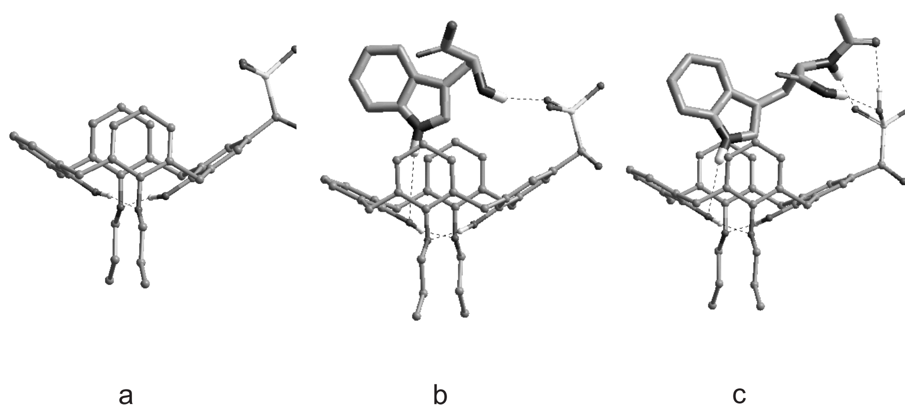
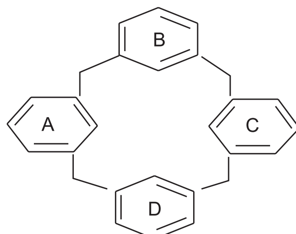


Fig. 5. Energy minimized structures of **CPA** (a) and the complexes with Trp (b) and NATA (c).

Table 3

Inclination of benzene rings A, B, C, D in respect to the main macrocycle plane in CPA and its complexes



Compound	Dihedral angles, °			
	A	B	C	D
CPA	147	92	143	91
CPA-Trp	154	98	147	98
CPA-NATA	152	99	153	97

Similar to the Trp complex an intermolecular hydrogen bond (2.604 Å) between the indole NH group and the oxygen atom at the calixarene lower rim is also observed. However, in contrast to the Trp complex, three intermolecular hydrogen bonds with the phosphonic group are formed in the NATA complex calculated: $POH\cdots O=CCH_3$ (2.247 Å), $CH_3C(O)NH\cdots O=P$ (1.753 Å) and $C(O)NH\cdots O=P$ (1.957 Å). It is possible to predict that under experimental conditions hydrophobic and π-π interactions are also involved in stabilization of both NATA and Trp complexes. As is evidenced by the calculation data with the help of the molecular modelling, the structure of the **CPA** – NATA complex (the relative **CPA** – NATA complex energy ΔE = -14.9 kcal/mol) is more stable comparatively with the structure **CPA** – Trp complex (ΔE = -11.7 kcal/mol). These data are in a good agreement with the data obtained in the chromatographic calculations of the binding constants of the **CPA** – NATA complex (K_A = = 39000 M^{-1}, ΔG = -6.25 kJ/mol) and the **CPA** – Trp complex (K_A = 23000 M^{-1} and ΔG = -5.94 kJ/mol).

Conclusions

Summarizing all above-mentioned information it should be noted that experimental measurements of the complex stabilities show that **CPA** binds more effectively NATA than Trp or other aminoacids, such as Ala < Phe < Arg < Asp < His < Lys in the aqueous solution. It can be explained by formation of three intermolecular hydrogen bonds between the phosphonic group of the **CPA**-Host and the $CNHC(O)CH_3$-$C(O)NH_2$ fragment of the NATA-Guest. The investigation of the molecular recognition and binding of calixarene phosphonic acids to Trp residues in proteins is in progress.

References

1. *Kaneda Y., Tabata Y. Cancer Sci., 2006, Vol. 97, pp.348-354.*
2. *De Guzman R.N., Wu Z.R., Stalling C.C., Pappalardo L., Borer P.N., Summers M.F. Science, 1998, Vol. 279, pp.384-388.*
3. *Amarasinghe G.K., De Guzman R.N., Turner R.B., Chancellor K.J., Wu Z.R., Summers M.F. J. Mol. Biol., 2000, Vol. 301, pp.491-511.*
4. *Bourbigot S., Ramalanjaona N., Boudier C., Gilmar F.J., Salgado R.B.P., Mely Y., Bouaziz S., Morellet N. J. Mol. Biol., 2008, Vol. 383, pp.1112-1128.*
5. *Huili C., Pin Y. Progress in Chemistry, 2002, Vol. 14, pp.239-243.*
6. *Gutsche C.D. Calixarenes Revisited, Cambridge, RSC, 1998.*
7. *Sansone F., Segura M., Ungaro R. Calixarenes in bioorganic and biomimetic chemistry. In: Calixarenes 2001, Asfari M.-Z., Böhmer V., Harrowfield J., Vicens J. (eds.), Kluwer Academic Publishers, Dordrecht, 2001, pp.496-512.*
8. *Casnati A., Sansone F., Ungaro R. Acc. Chem. Res., 2003, Vol. 36, pp.246-254.*
9. *Perret F., Lazar A.N., Coleman A.W. Chem. Commun, 2006, pp.2425-2438.*
10. *Coleman A.W., Perret F., Moussa A., Dupin M., Guo Y., Perron H. Top. Curr. Chem., 2007, Vol. 277, pp.31-88.*
11. *Zadmard R., Schrader T. J. Am. Chem. Soc., 2005, Vol. 127, pp.904-915.*
12. *Park H.S., Lin Q., Hamilton A.D. J. Am. Chem. Soc., 1999, Vol. 121, pp.8-13.*
13. *Markovsky L.N., Kalchenko V.I., Solovyov A.V., Finocchiaro P., Failla S., Atamas L., Consiglio G., Tsymbal I.F. Anales de Quimica, 1998, Vol. 94, pp.164-170.*
14. *Solovyov A.V., Cherenok S., Tsymbal I., Failla S., Consiglio G., Finocchiaro P., Kalchenko V.I. Heteroatom Chemistry, 2001, Vol. 12, pp.58-67.*
15. *Cherenok S.O., Yushchenko O.A., Tanchuk V.Yu., Mischenko I.M., Samus N.V., Ruban, O.V., Matvieiev Yu.I., Karpenko J.A., Kukhar V.P., Vovk A.I., Kalchenko V.I. ARKIVOC, 2012, Vol. 15, pp.278-298.*
16. *Zielenkiewicz W., Marcinowicz A., Poznanski J., Cherenok S., Kalchenko V. J. Incl. Phenom., 2006, Vol. 55, pp.11-19.*
17. *Kalchenko O., Cherenok S., Yushchenko O., Kalchenko V. J. Incl. Phenom., 2013, Vol. 76, pp.29-36.*
18. *Kalchenko O.I., Lipkowski J., Nowakowski R., Kalchenko V.I., Vysotsky M.A., Markovsky L.N. J. Incl. Phenom., 1998, Vol. 23, pp.377-380.*
19. *Kalchenko O.I., Da Silva E., Coleman A.W. J. Incl. Phenom., 2002, Vol. 43, pp.305-310.*

Надійшла до редакції 15.05.2014 р.

Acknowledgements
The authors thank Dr. A.V. Lukin for fruitful discussion.
The work is supported by the NASU-CNRS PICS grant.