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Complexation behavior of cucurbit[6]uril with short polypeptides

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Abstract

The binding properties of cucurbit[6]uril towards various peptides have been investigated in acidic aqueous solution. Stability constants and thermodynamic values of the complex formation between following peptides: glycyl-L-alanine, L-leucyl-L-valine, glycyl-L-asparagine, L-leucyl-L-phenylalanine, L-leucyl-L-tryptophan, glycyl-L-histidine, L-glutathione reduced (γ-L-glutamyl-L-cysteinyl-glycine, GSH), and DL-leucyl-glycyl-DL-phenylalanine) with cucurbit[6]uril in aqueous formic acid (50%, v/v) have been calculated from calorimetric titrations. From these results it can be seen that the peptides form exclusion complexes with cucurbit[6]uril. Due to the polar peptide bond they are not included within the hydrophobic cavity of cucurbit[6]uril. The complex formation is favoured by entropic contributions. The release of water molecules from the polar amino groups of the peptides and the carbonyl groups of cucurbituril are responsible.

Keywords: Cucurbit[6]uril; Peptides; Complexes; Calorimetric titration

1. Introduction

The synthesis of cucurbit[6]uril by condensation reaction of glycoluril and formaldehyde during an acid-catalysed reaction has been reported by Behrend et al. in 1905 [1]. The characterization of this compound concerning the structure and properties was carried out in 1984 by Mock et al. He extensively studied the host-guest binding capacity of cucurbituril towards alkyl- and aryl-substituted ammonium ions in solution establishing that ion–dipole interactions and shape complementarity with guests confer the specificity of cucurbit[6]uril as a molecular receptor [2–6]. Despite its poor solubility in common solvents except for acidic aqueous solution, cucurbit[6]uril was used in many applications among other receptors such as cyclodextrins, crown ethers and calixarenes [7–14]. Cucurbit[*n*]urils are characterized by a hydrophobic cavity and two oxygen-crowned portals. The forces involved in the binding of molecular guests by cucurbit[6]uril are of van der Waals type, hydrophobic interactions, ion–dipole and hydrogen bond interactions. In the meantime, cucurbituril chemistry has been enhanced with several cucurbit[*n*]uril derivatives, They are new synthetic receptors with various cavities and portal sizes having good solubility in common solvents and interesting molecular recognition properties.

One of the most interesting applications of cucurbit[6]uril is as building block in supramolecular architectures. In this respect, Kim and coworkers [15] synthesized a large variety of supramolecular compounds such as rotaxane dendrimers [16–18] with their potential application as gene carriers [19], polyrotaxanes [20,21] and rotaxane-based molecular switches [22] using cucurbit[6]uril as a molecular bead. Lipkowski [23] reported an interesting study of interaction of cucurbit[*n*]uril–water complex and pointed out the importance of this feature in complexation equilibria and transport properties of the complexes, especially in aqueous environment.

The investigation of the nature of interactions involved in ligand–peptide complex formation is of particular relevance for understanding many specific biomolecular interactions which play a key role in regulation of cellular processes [24–28]. Along this topics, the study of factors that contribute to the complex formation are of current interest in peptide chemistry. Peptides contain in their structure one

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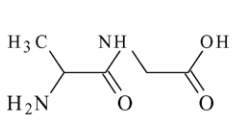
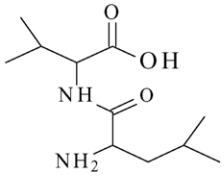
free α-amino group (NH_2− terminal amino group) and at least one free α-carboxyl group (COOH− terminal carboxyl group) at their ends (Fig. 1). These groups which are involving in peptide bonds together with some other groups that are present in molecule can ionize and therefore be responsible of acid–base behavior of peptide. There are biological peptides that are not derived from proteins such as the tripeptide glutathione. In this tripeptide one of the peptide bonds is unusually formed, thus involving an amino group other than that at the α-position [29,30]. The tripeptide, glutathione (GSH) is a sulfhydryl (−SH) antioxidant, antitoxin and enzyme cofactor having a high electron donating capacity given by the sulfhydryl group comes from the cysteine residue. Glutathione with a high reducing power, is found mainly in the cell cytosal and other aqueous phases of living system.

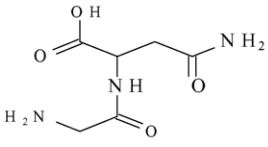
Based on the resembling between the natural receptor α-cyclodextrin and the synthetic receptor cucurbit[6]uril, we have recently reported [31] a comparative thermodynamic study of complex formation of some amino acids and dipeptides with both ligands in aqueous solutions. The hydrophobic interactions are involved in the complex formation of α-cyclodextrin with dipeptides and ion–dipole interactions



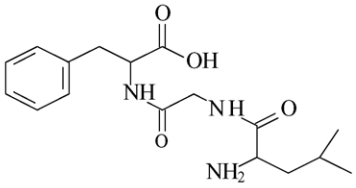
Gly-L-Ala



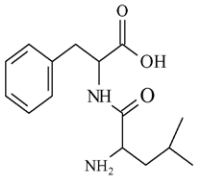
L-Leu-L-Val



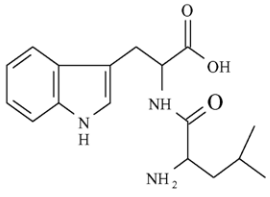
Gly-L-Asn



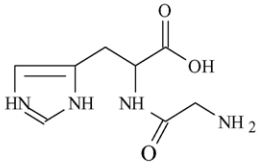
DL-Leu-Gly-DL-Phe



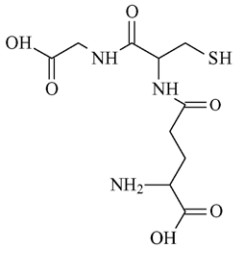
L-Leu-L-Phe



L-Leu-L-Trp



(A) Gly-L-His



(B) L-Glutathione reduced

Fig. 1. The structure of peptides used in experiments.

in the complex formation between dipeptides with cucurbit[6]uril.

Only few studies have been published on this topics so far, so we have extended our investigations concerning the binding ability of cucurbit[6]uril towards biological compounds over the aspects of complexation between various dipeptides and tripeptides and this molecular receptor in aqueous formic solutions.

2. Experimental

The di- and tripeptides glycyl-L-alanine (Gly-L-Ala, ICN), L-leucyl-L-valine (L-Leu-L-Val, ICN), glycyl-L-asparagine (Gly-L-Asn, ICN), L-leucyl-L-phenylalanine (L-Leu-L-Phe, Fluka), L-leucyl-L-tryptophan (L-Leu-L-Trp, ICN), glycyl-L-histidine (Gly-L-His, Fluka), L-glutathione reduced (γ-L-glutamyl-L-cysteinyl-glycine, GSH, Fluka), DL-leucyl-glycyl-DL-phenylalanine (DL-Leu-Gly-DL-Phe, ICN) are of the highest purity commercially available and they are used without any further purification. Their structures are given in Fig. 1. The ligand cucurbit[6]uril (see Fig. 2), is synthesized and purified according to published methods [13]. Reagent grade formic acid is purchased from Merck. The complexation of peptides with cucurbit[6]uril is studied in formic acid (50%, v/v) due to the low solubility of the ligand in water.

All calorimetric titrations are performed using a calorimeter Tronac Model 450 (TRONAC, Orem, UT, USA). The solution of the ligand (0.04–0.06 mol/L) is added continuously to a solution of the peptide (0.002–0.004 mol/L). The heat, Q, produced during the titration is related to the reaction enthalpy ΔH, after correction for all non-chemical heat effects, by the following equation:

$$Q = \Delta n \Delta H \quad (1)$$

with Δn as the number of moles of the complex formed. In the literature the mathematical treatment of the experimental data has been described in detail [32]. The reliability of the calorimeter used is controlled using the reaction of 18-crown-6 with (Merck) with $Ba(ClO_4)_2$ (Merck) in aqueous solution. At a temperature of 298.15 K the following values are obtained: $\log K = 3.54 \pm 0.03$, $\Delta H = -31.5 \pm 0.6\,\text{kJ mol}^{-1}$ and

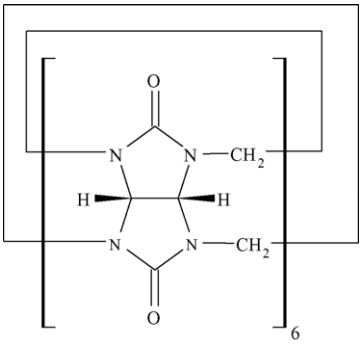


Fig. 2. Structure of cucurbit[6]uril.

Table 1
Stability constants $\log K$ (L/mol) and thermodynamic values ΔH and $T\Delta S$ (kJ mol^{-1}) for the complex formation of peptides with cucurbit[6]uril in formic acid (50%, v/v) at 25 °C

Peptide	$\log K$	$-\Delta H$	$T\Delta S$
Gly-L-Ala	2.80 ± 0.03	2.4 ± 0.1	13.5 ± 0.2
L-Leu-L-Val	2.79 ± 0.01	2.1 ± 0.3	13.7 ± 0.2
Gly-L-Asn	2.82 ± 0.03	3.2 ± 0.5	12.9 ± 0.7
L-Leu-L-Phe	2.78 ± 0.02	3.2 ± 0.3	12.7 ± 0.4
L-Leu-L-Trp	2.92 ± 0.12	3.6 ± 0.4	13.1 ± 0.3
Gly-L-His	2.79 ± 0.01	4.8 ± 0.3	11.2 ± 0.3
L-Glutathione reduced (GSH)	2.74 ± 0.01	2.4 ± 0.1	13.3 ± 0.1
DL-Leu-Gly-DL-Phe	2.80 ± 0.02	4.9 ± 0.1	11.1 ± 0.1

$T\Delta S = -11.4 \pm 0.8\,\text{kJ mol}^{-1}$. These values are in complete accordance with values reported in the literature [33].

3. Results and discussion

The stability constants for the complexation of the peptides with cucurbit[6]uril are summarized in Table 1. The values of the stability constants and of the thermodynamic parameters for all peptides examined are identical within experimental error. The main interactions between cucurbit[6]uril and the peptides result from ion–dipole interactions between the protonated amino groups of the peptides and the carbonyl donor atoms of cucurbit[6]uril. The strength of the interactions should further be influenced by energetic contributions from the inclusion of the guest molecule inside the cavity of host molecule. This effect is known from the complexation studies of the ammonium ion, alkylamines and dialkylamines [9]. Surprisingly the size of the different peptides examined has no influence upon the complex stability. Even the reaction entropies are constant for all peptides. All these results can only be explained by the assumption of the formation of exclusion complexes (see Fig. 3).

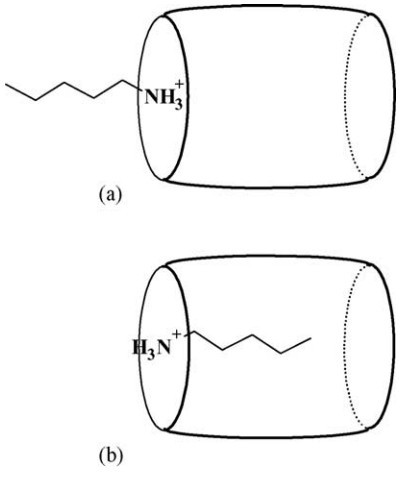


Fig. 3. Schematically presentation of the formation of an exclusion complex (A) and an inclusion complex (B) between cucurbit[6]uril and an protonated alkylamine.

The complex formation between a ligand L and a host molecule H takes place in several reaction steps. During the first reaction step, the separated molecules L and H come in contact and they form an exclusion complex $L \cap H$. In the next reaction step both molecules form an inclusion complex $L \subset H$:

$$L + H \leftrightarrow L \cap H \leftrightarrow L \subset H \quad (2)$$

This two-step mechanism is well established for the formation of cryptand complexes with cations [34]. If the cations are too big to be accommodated within the cavity of the cryptands only exclusive complexes are formed [35]. Obviously cucurbit[6]uril and peptides are not able to form inclusion complexes. Sterical reason cannot solely be responsible due to the fact that also the peptide Gly-L-Ala does not form an inclusion complex. More likely the polar peptide bond is responsible. The inclusion of the this polar part of the peptide molecule in the unpolar cavity of cucurbit[6]uril would lead to a very unfavourable energetic state.

The complex formation between cucurbit[6]uril and peptides is mainly favoured by entropic contributions. Both the polar amino group of the peptides and the carbonyl groups at the portals of cucurbituril are strongly solvated. During the formation of an exclusion complex solvent molecules are set free. The entropy of fusion of water at 25 °C is $T\Delta S = 6.6\,\mathrm{kJ\,mol^{-1}}$ [36]. Neglecting other contributions to the reaction entropy one can easily see from the values in Table 1 that two molecules of water are released during all complex formations with peptides.

In contrast to amino acids [37] the examined peptides do not form inclusion complexes with cucurbit[6]uril. The polarity of the peptide bond seems to be responsible. Thus, one may expect that cucurbit[*n*]urils with larger cavities will be able to form real inclusion complexes with peptides. These ligands are expected to be able to encapsulate peptides together with solvent molecules located at the peptide bonds.

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References

[1] R. Behrend, E. Meyer, F. Rusche, Liebigs Ann. Chem. 339 (1905) 1.
[2] W.A. Freeman, W.L. Mock, N.-Y. Shih, J. Am. Chem. Soc. 103 (1981) 7367.
[3] W.L. Mock, N.-Y. Shih, J. Org. Chem. 48 (1983) 3618.
[4] W.L. Mock, N.-Y. Shih, J. Org. Chem. 51 (1986) 4440.
[5] W.L. Mock, N.-Y. Shih, J. Am. Chem. Soc. 110 (1988) 4706.
[6] W.L. Mock, in: F. Vögtle (Ed.), Comprehensive Supramolecular Chemistry, vol. 2, Pergamon Press, Oxford, 1996, pp. 477–493.
[7] H.-J. Buschmann, Schriftenreihe Biologische Abwasserreinigung, vol. 9, Technische Universität, Berlin, 1997, p. 101.
[8] R. Hoffmann, W. Knoche, C. Fenn, H.-J. Buschmann, J. Chem. Soc., Faraday Trans. 90 (1994) 1507.
[9] C. Meschke, H.-J. Buschmann, E. Schollmeyer, Thermochim. Acta 297 (1997) 43.
[10] K. Jansen, H.-J. Buschmann, A. Wego, D. Döpp, C. Mayer, H.-J. Drexler, H.-J. Holdt, E. Schollmeyer, J. Incl. Phenom. 39 (2001) 357.
[11] H.-J. Buschmann, E. Cleve, K. Jansen, A. Wego, E. Schollmeyer, J. Incl. Phenom. 40 (2001) 117.
[12] H.-J. Buschmann, L. Mutihac, K. Jansen, J. Incl. Phenom. 39 (2001) 1.
[13] K. Jansen, A. Wego, H.-J. Buschmann, E. Schollmeyer, D. Döpp, Des. Monom. Polym. 6 (2003) 43.
[14] H.-J. Buschmann, E. Cleve, E. Schollmeyer, Inorg. Chim. Acta 193 (1992) 93.
[15] J.W. Lee, S. Samal, N. Selvapalam, H.-J. Kim, K. Kim, Acc. Chem. Res. 36 (2003) 621.
[16] J.W. Lee, Y.H. Ko, S.-H. Park, K. Yamaguchi, K. Kim, Angew. Chem. Int. Ed. 40 (2001) 746.
[17] K. Kim, Chem. Soc. Rev. 31 (2002) 96.
[18] J.W. Lee, K. Kim, Top. Curr. Chem. 228 (2003) 111.
[19] Y.-B. Lim, T. Kim, J.W. Lee, S.-M. Kim, H.-J. Kim, K. Kim, Bioconjugate Chem. 13 (2002) 1181.
[20] E. Lee, J. Heo, K. Kim, Angew. Chem. Int. Ed. 39 (2000) 2699.
[21] K.-M. Park, D. Wang, E. Lee, J. Heo, K. Kim, Chem. Eur. J. 8 (2002) 498.
[22] J.W. Lee, K. Kim, K. Kim, Chem. Commun. (2001) 1042.
[23] J. Lipkowski, Book of Abstracts of the 28th International Symposium on Macrocyclic Chemistry, Gdansk, July 13–18, 2003.
[24] M.W. Peczuh, A.D. Hamilton, Chem. Rev. 100 (2000) 2479.
[25] J. Lipkowski, O.V. Kulikov, W. Zielenkiewcz, Supramol. Chem. 1 (1992) 73.
[26] O.V. Kulikov, I.V. Terekhova, Russ. J. Coord. Chem. 24 (1998) 395.
[27] A. Metzger, K. Gloe, H. Stephan, F.P. Schmidtchen, J. Org. Chem. 61 (1996) 2051.
[28] M. Czekalla, H. Stephan, B. Habermann, J. Trepte, K. Gloe, F.P. Schmidtchen, Thermochim. Acta 313 (1998) 137.
[29] A. Meister, J. Biol. Chem. 269 (1994) 9397.
[30] M.E. Anderson, Adv. Pharmacol. 38 (1997) 65.
[31] H.-J. Buschmann, E. Schollmeyer, L. Mutihac, Thermochim. Acta 399 (2003) 203.
[32] H.-J. Buschmann, Inorg. Chim. Acta 195 (1992) 51.
[33] H.-J. Buschmann, E. Schollmeyer, Thermochim. Acta 333 (1999) 49.
[34] E. Mei, A.I. Popov, J.L. Dye, J. Am. Chem. Soc. 99 (1977) 6532.
[35] H.-J. Buschmann, Inorg. Chim. Acta 108 (1985) 241.
[36] A.A. Bondi, Physical Properties of Molecular Crystals: Liquids and Glass, Wiley, New York, 1968.
[37] H.-J. Buschmann, K. Jansen, E. Schollmeyer, Thermochim. Acta 317 (1998) 95.