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Pyrogallol[4]arenes as artificial receptors for L-carnitine

Bjoern Schnatwinkel [a], Mikhail V. Rekharsky [b], Victor V. Borovkov [b], Yoshihisa Inoue [b,*], Jochen Mattay [a,*]

[a] Organic Chemistry I, Department of Chemistry, Bielefeld University, Bielefeld, Germany
[b] Department of Applied Chemistry, Osaka University, 2-1 Yamada-oka, Suita 565-0871, Japan

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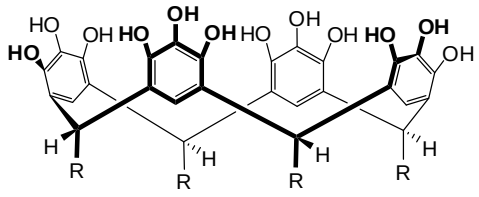
ABSTRACT

Complexation thermodynamics of pyrogallol[4]arenes with L-carnitine and other biologically important ammonium ions were investigated by isothermal titration calorimetry.

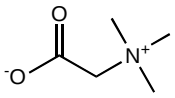
Molecular recognition plays a key role in biological processes. One of the most important phenomena in this relation is the highly precise substrate selectivity of enzymes. This interaction represents an excellent paragon for supramolecular chemistry, which is of highest importance for stereoselective reactions, drug delivery, and catalysis. In this context, calixarenes[1] provide a good host system for the investigation of molecular recognition. The first detailed experimental study on the host–guest interactions between biologically important compounds and calixarenes was reported by Schneider and co-workers for resorcin[4]arenes with choline and acetic acid.[2] Few years later, Inouye et al. reported a method for nondestructive detection of acetylcholine in protic media with resorcin[4]arenes[3] followed by a pioneering work of Rebek Jr. et al. on molecular recognition with similar systems.[4,5]

Betaine **2**, choline **4**, acetylcholine **5**, and L-carnitine **6** (Fig. 1) are biological guest compounds that are specifically difficult to be recognized with artificial hosts.[4] The positive charge and the nearly spherical shape of the trimethylammonium group in these guests require a bowl-shaped cavity for van der Waals contacts, as well as negative charges at the rim of the bowl for electrostatic interactions. A compensation of the positive charge on the nitrogen, for example, by forming hydrogen bond and/or salt bridges, is hindered, because of the shielding effect upon quaternization, whereas cation-π interaction may take place.[6] This strategy can be found in natural receptors, for example, acetylcholine-esterase.[7] In this context, pyrogallol[4]-arenes,[8] possessing an electron-rich, concave cavity, provide an excellent model system for elucidating the factors and mechanisms operating in biological supramolecular systems. Furthermore, a great advantage of pyrogallol[4]arene hosts is the simple, efficient synthetic access, including facile one-pot reaction, high yield, and straightforward workup procedures.[9] The procedure yields in the most thermodynamic stable C_{v4}-symmetrical bowl-shaped product which is stabilized by an intramolecular hydrogen-bonding network between the pyrogallol subunits.

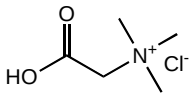


R = $CH_2CH(CH_3)_2$

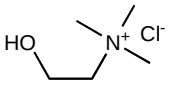
Guest:



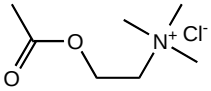
2 (Betaine)



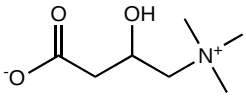
3 (Betaine hydrochloride)



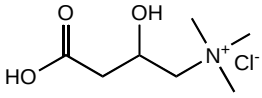
4 (Choline)



5 (Acetylcholine)



6 (L-Carnitine)



7 (L-Carnitine hydrochloride)

Figure 1. Pyrogallol[4]arene (**1**) and biologically important guests (**2**–**7**) used in this study.

* Corresponding authors. Tel.: +49 521 106 2072; fax: +49 521 106 6417 (J.M.).
E-mail addresses: inoue@chem.eng.osaka-u.ac.jp (Y. Inoue), oc1jm@uni-bielefeld.de (J. Mattay).

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We wish now to report the results of our thermodynamic studies on the complexation of pyrogallol[4]arene **1** with biologically important trimethylammonium-containing compounds **2**–**7**, shown in Figure 1, in ethanol. The choice of the spectrometric method and the experimental procedure depends on the spectral properties of the used guest and host molecules. However, calorimetry is the only direct method for determining the reaction enthalpy. For this purpose, we chose the isothermal titration calorimetry (ITC), which enabled us to determine the complex stoichiometry, corresponding stability constants (K), and the thermodynamic parameters, that is, the standard free energy (ΔG°), enthalpy (ΔH°) and entropy changes (ΔS°).[10] The results are summarized in Table 1, along with the experimental conditions employed.

Upon the interaction of **1** with **2**–**7**, only stoichiometric 1:1 complexes were formed with stability constants greater than 10^3 M^{-1}, which is reasonable in view of the results reported for relevant systems.[2–5] The smallest affinity of $K = 1500$ M^{-1} was found for betaine hydrochloride **3**. Betaine **2** and choline **4** gave higher K's of 3200–3400 M^{-1}, while acetylcholine showed a yet better affinity of 6100 M^{-1}, which is comparable to the K of the structurally related trimethylethylammonium cation (Table 1). Although the selectivity is small, the following order can be deduced from these experiments: **5**>**4**>**2**>**3**.

Examination of the crystal structure of a similar host–guest system[2] is useful in elucidating the origin of the observed selectivity and also in designing a better guest. For a higher affinity and selectivity of a specific guest, all available interactions, including the van der Waals, ion-dipole, hydrogen-bonding, and cation-π interactions, should cooperate. In the pyrogallol[4]arene case, the concavity of the 'bowl' is modest and hence a large gain from the hydrophobic interaction is not expected, but there are four electron-rich aromatic rings suitable for cation-π interactions and twelve phenolic hydrogens on the upper rim for ion-dipole and hydrogen-bonding interactions. We therefore chose L-carnitine as a most suitable guest best fitted to pyrogallol[4]arene.

As anticipated from its structure possessing trimethylammonium, hydroxyl, and carboxylate groups, L-carnitine **6** was strongly bound to **1** with $K = 18{,}000$ M^{-1}, which is larger by a factor of 3–12 than those of the other biological guests employed. This clearly indicates that all of the functional groups (i.e., trimethylammonium, hydroxyl, and carboxylate) as well as the extended hydrocarbon chain contribute to the affinity enhancement through the contribution of ion-dipole, cation-π, hydrogen-bonding, and van der Waals interactions, as schematically illustrated in Figure 2 (Spartan '04).

The control ITC experiments with **3** and **7**, which are the protonated forms of **2** and **6**, support our proposal that the carboxylate anion is responsible for the increased affinities of **2** and **6** through the stronger hydrogen-bonding interaction of carboxylate anion, rather than neutral carboxylic acid, with phenolic hydrogen.

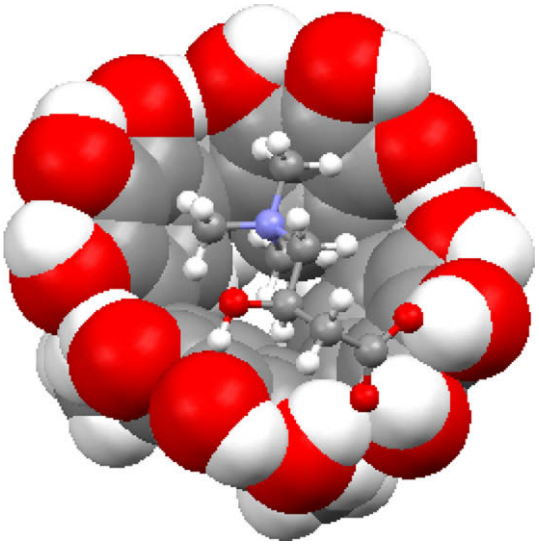

Figure 2. A schematic drawing of complex **6@1.**

From the thermodynamic point of view, the host–guest complexation of pyrogallol[4]arene host **1** with a series of biologically important zwitterionic or cationic guests **2**–**7** is clearly driven by the enthalpic gains of 20–38 kJ mol^{-1}, which are cancelled to some degree by the unfavorable entropic losses ($T\Delta S^\circ$) of 2–18 kJ mol^{-1}. The large enthalpic gains may be attributed to the above-mentioned ion-dipole, cation-π, hydrogen-bonding, and van der Waals interactions, while the relatively small entropic losses may be accounted for the desolvation upon complexation from the charged groups of the guests and also from the pyrogallol[4]arene preaggregated with ethanol, that is, **EtOH@(1)$_2$**, which was recently revealed by the X-ray crystallographic study of Nissinen and coworkers.[11]

We further examined the validity of the compensatory enthalpy–entropy relationship in this particular host–guest system. The enthalpy–entropy compensation effect has widely been observed in essentially all of the natural and artificial supramolecular systems[12,13] with an rare exception of the cucurbituril-ferrocene pairs that were found very recently.[14] Thus, the plot of the entropy change ($T\Delta S^\circ$) as a function of the enthalpy change (ΔH°) gives a good straight line in general, and the slope and intercept of the regression line can be used as quantitative measures of the conformational changes and the degree of desolvation upon complexation, respectively.[12] In Figure 3, the $T\Delta S^\circ$ values are plot-

Table 1
Complex stability constant (K), standard free energy (ΔG°), enthalpy (ΔH°), and entropy changes (ΔS°) for the complexation of biologically-important ammonium cations[a] with pyrogallol[4]arene **1** in ethanolic solution at 298.15 K

Guest	K (M^{-1})	ΔG° (kJ mol^{-1})	ΔH° (kJ mol^{-1})	$T\Delta S^\circ$ (kJ mol^{-1})
2 (Betaine)	3200 ± 100	−20.0 ± 0.1	−26.9 ± 0.3	−6.9 ± 0.3
3 (Betaine hydrochloride)	1500 ± 100	−18.1 ± 0.2	−20.4 ± 0.3	−2.3 ± 0.4
4 (Choline)	3400 ± 200	−20.2 ± 0.2	−38.4 ± 0.4	−18.2 ± 0.4
5 (Acetylcholine chloride)	6100 ± 200	−21.6 ± 0.1	−32.7 ± 0.4	−11.1 ± 0.4
6 (L-Carnitine)	18000 ± 1000	−24.3 ± 0.7	−36.0 ± 0.4	−11.7 ± 0.5
7 (L-Carnitine hydrochloride)	4400 ± 200	−20.8 ± 0.2	−30.7 ± 0.3	−9.9 ± 0.3
$(CH_3)_3(C_2H_5)N^+Cl^-$ (1:1 complex)	5300 ± 1000 (K_1)	−21.3 ± 0.6	−45 ± 4	−24 ± 4
(1:2 Complex)	2100 ± 500 (K_2)	−19.0 ± 0.7	−48 ± 4	−29 ± 4

[a] In ITC experiments, a solution of the corresponding ammonium salt in ethanol (0.2 mM), was placed into the reaction cell of microcalorimeter, titrated by injecting a solution of pyrogallo[4]arene (4 mM) in ethanol. Typical ITC experiment consists of 25–50 injections (10 μL each) of the pyrogallol[4]arene solution into the cell.

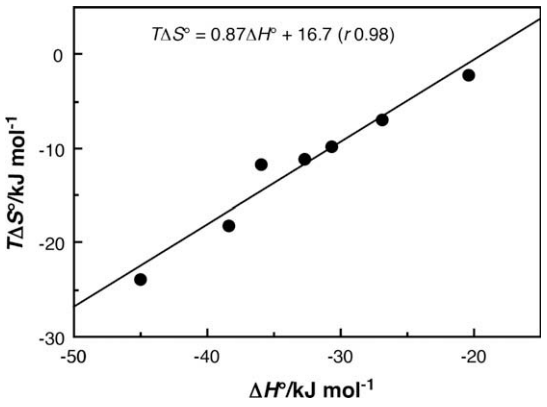

Figure 3. The enthalpy–entropy compensation plot for the complexation of biologically important compounds **2**–**7** and trimethylmethylammonium with pyrogallol[4]arene **1**.

ted against the ΔH° to give a good straight line with a slope (α) of 0.87 and an intercept ($T\Delta S^{0}$) of 17 kJ mol^{-1}. These values are similar to those reported for native cyclodextrins ($\alpha = 0.88$; $T\Delta S^{0} = 12$ kJ mol^{-1}),[13] indicating that the present pyrogallol[4]arene and cyclodextrins share the similarity in complexation behavior as viewed from the conformational change and the degree of solvation upon complexation.

Acknowledgments

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