Cooperative Binding and Stepwise Encapsulation of Drug Molecules by Sulfonylcalixarene-Based Metal-Organic Supercontainers

Tian-Pu Sheng 1,2, Xin-Xia Fan 1, Guo-Zong Zheng 1, Feng-Rong Dai 1,* and Zhong-Ning Chen 1,*

1 State Key Laboratory of Structural Chemistry, Fujian Institute of Research on the Structure of Matter, Chinese Academy of Sciences, Fuzhou 350002, Fujian, China; shengtianpu@fjirsm.ac.cn (T.-P.S.); czngroup@fjirsm.ac.cn (X.-X.F.); zhengguozong@fjirsm.ac.cn (G.-Z.Z.)
2 University of Chinese Academy of Sciences, Beijing 100049, China
* Correspondence: dfr@fjirsm.ac.cn (F.-R.D.); czn@fjirsm.ac.cn (Z.-N.C.); Tel.: +86-591-6317-3171 (Z.-N.C.)

Received: 30 April 2020; Accepted: 27 May 2020; Published: 8 June 2020

Abstract: The cooperative binding behavior of a face-directed octahedral metal-organic supercontainer featuring one endo cavity and six exo cavities was thoroughly examined in chloroform solution through ultraviolet-visible (UV-Vis) titration technique using two representative drug molecules as the guests. The titration curves and their nonlinear fit to Hill equation strongly suggest the efficient encapsulation of the guest molecules by the synthetic host, which exhibit interesting cooperative and stepwise binding behavior. Based on the control experiments using tetranuclear complex as a reference, it is clear that two equivalents of the guest molecules are initially encapsulated inside the endo cavity, followed by the trapping of six additional equivalents of the drug molecules through six exo cavities (1 eq. per exo cavity), and the remaining guests are entrapped by the external pockets. The results provide an in-depth understanding of the cooperative binding behavior of metal-organic supercontainers, which opens up new opportunities for designing synthetic receptors for truly biomimetic functional applications.

Keywords: host–guest chemistry; binding cooperativity; stepwise encapsulation; Hill equation; drug delivery

1. Introduction

Binding cooperativity effects are a well-documented phenomenon and an essential principle prevalent in the fields of biology and supramolecular chemistry [1–7]. Well-known examples include allosteric binding of oxygen by hemoglobin in biology and metal ions chelation process by ethylenediaminetetraacetic acid (EDTA) in chemistry. Cooperative binding has been proven to be a very important mechanism that regulates molecular recognition behavior [2,3] and self-assembly processes [4,8,9] in biology and supramolecular chemistry. To date, a cooperative effect has been widely explored in biologic systems, and different basic principles have been developed to understand a wide range of biological phenomena [2]. However, cooperative modulation of macromolecular assemblies and guest binding behaviors in synthetic supramolecular systems remains poorly illustrated and understood.

Over the years, considerable attention has been paid to coordination containers possessing a well-defined hollow space that is suitable for guest encapsulation and allows them to be widely applied in a number of applications, such as guest recognition [10–12], drug delivery [13,14], and supramolecular catalysis [15,16]. A new class of coordination containers, namely, metal-organic supercontainers (MOSCs), incorporating macrocyclic container thia- or sulfonyl-calix[4]arenes as precursors [17], have been well demonstrated by us and others [18–28]. The MOSCs are demonstrated to possess several unique features such as their modular synthesis, robust architecture, and multiple binding
2. Results and Discussion

The metal-organic supercontainer MOSC-1-Co¹⁸, labelled here as H₁, is constructed from six tetranuclear complex units [³⁰] connected by eight units of the 1,3,5-benzenetricarboxylate (BTC) linker (Scheme 1). It features a dual-pore architecture containing two types of well-defined cavities: an endo cavity (with an inner diameter of ca. 1.4 nm and an estimated internal volume of 0.55 nm³) and six open-ended exo cavities (with an opening of ca. 0.8 nm × 0.8 nm and a depth of ca. 0.7 nm) (Figure 1). There are also eight external pockets defined by the BTC linker and three adjacent TBSC units. The multiple binding domains make the H₁ an intriguing and effective synthetic receptor for investigating efficient guest regulation and/or encapsulation, as well as binding cooperativity. To elucidate the guest binding behavior of supercontainer H₁, the host–guest interaction profiles in solutions were carefully examined using the UV-Vis titration technique [³¹]. Two drug molecules, namely, (R)-(+)-rabeprazole sodium (D₁) and (S)-(−)-pantoprazole sodium (D₂) (Figure 1), were selected as the guests. The synthetic precursor of H₁, that is, a tetranuclear complex, labelled as H₂, which mimics the structure of the exo cavities of H₁, was chosen as a control host molecule to simulate the binding behavior of the exo cavities of the H₁.

The absorption spectrum of H₁ in CHCl₃ features an intense and characteristic absorption band centered at 350 nm (Figure 2), ascribable to the combination of π→π* transitions of organic ligands and intramolecular charge transfer (ICT) involving the tetranuclear units [¹⁸,²⁰,²¹]. As shown in Figure 3a, upon gradual addition of D₁ to H₁ solution in CHCl₃, the intensity of the maximum absorption at 350 nm increases gradually along with the appearance of a shoulder centered at 370 nm. The changes observed in the absorption spectra during the titration indicated the encapsulation of D₁ guest molecules within the H₁.

Scheme 1. The self-assembly of host molecules H₁¹⁸ and H₂³⁰ from p-tert-butylsulfonylcalix[4]arene (H₄TBSC); the TBSC units in H₁ molecule are omitted for clarity.
Figure 1. The crystal structures of host molecules (H1 and H2) based on the CIF files reported in the original literature [18,30] and chemical structure diagrams of guest molecules (D1 and D2) used in this study. The spheres serve to guide the eyes representing the endo cavity (purple), six exo cavities (yellow), and one of the eight external pockets (gray).

Figure 2. The ultraviolet-visible (UV-Vis) spectra of free hosts (H1 and H2) and guests (D1 and D2) in chloroform solution.
Figure 3. (a) UV-Vis spectra of host H1 upon titration with D1, and plots of D1/H1 molar ratio vs. absorbance at (b) 350 nm and (c) 370 nm based on the titration experiment. (d) UV-Vis spectra of host H1 upon titration with D2, and plots of D2/H1 molar ratio vs. absorbance at (e) 350 nm and (f) 370 nm based on the titration experiment.

A plot of absorbance at 350 nm vs. D1 equivalents (Figure 3b) exhibits a characteristic sigmoidal kinetic profile typically observed in the binding of substrates by enzymes or receptors containing multiple binding sites [20,32]. At low guest concentrations, the D1 molecules added bind strongly to the H1, causing an initially flat and then sudden increase of the absorbance intensity. As the D1 equivalents continue to increase, binding of D1 guest to H1 appears to become weaker, leading to a deviation of the titration curve from the initial tangent and a much more slow and gradual increase of the absorbance until reaching a saturation plateau. The capability of D1 binding with H1 can be estimated to be ~8 equivalents according to the intersection point of the initial tangent and the asymptote.

Notably, a plot of the absorbance at 370 nm vs [D1]/[H1] ratio displays a rare profile of well-defined multiple incremental steps, indicating sequential and cooperative host–guest interactions (Figure 3c). The stepwise increases in absorbance are observed in the [D1]/[H1] ranges of 0–2, 2–3, 3–8, and 8–23,
respectively, which is ascribable to the stepwise encapsulation of D1 guest molecules within different binding domains of the H1 host, including the endo-cavity, exo-cavities, and the external pockets.

The binding behavior of the D2 guest with H1 was similarly investigated by the UV-Vis titration (Figure 3d–f). Similar sigmoidal kinetic profile was observed via a plot of absorbance at 350 nm vs. [D2]/[H1], but with a faster increase in the absorbance as the D2 concentration increases at lower guest loadings. In the meanwhile, only three incremental steps in the [D2]/[H1] ranges of 0–2, 2–8, and 8–23 were observed. It is plausible that the smaller size of guest molecule (for example, the D2 molecule bears a shorter side chain relative to D1) is beneficial to enhance the binding kinetic of guest encapsulation.

In order to understand how the guest molecules bind to the H1 host, control experiments employing the tetranuclear complex H2 as the host were carried out under similar UV-Vis titration conditions (Figure 4). The binding of the D1 guest molecules with H2 is confirmed by the red shift of the H2 absorption maxima from 347 nm to 360 nm upon gradual increase of the guest equivalents. The binding stoichiometry of D1 or D2 with H2 is estimated to be ~1 as evidenced by the plots of absorbance vs. guest equivalents. This suggests that each exo cavity of the H1 host tends to accommodate one molecule of the D1 or D2 guest. Therefore, it is plausible that the H1 encapsulates six molecules of D1 or D2 through its six exo cavities, and two molecules of D1 or D2 within its endo cavity, while the remaining guest molecules are likely aggregating around the external triangular pockets.

Finally, the UV-Vis titration data was further analyzed using the well-known Hill equation in order to quantitatively understand the guest binding cooperativity. The binding association constants and the Hill coefficient values based on the nonlinear fit are listed in Table 1. The Hill equation is widely utilized for estimating the degree of cooperativity of the guest(s) binding to the receptor. The value of
Hill coefficient provides a means to quantify the extent of cooperativity among multiple ligand binding sites. A Hill coefficient of one suggests independent binding, while the value different from one indicates multiple ligand binding corresponding to negatively (n < 1) or positively (n > 1) cooperative binding. As shown in Figure 5, the overall apparent association constants of guest molecules binding with H1 were calculated to be $3.58 \times 10^4$ M$^{-1}$ and $6.69 \times 10^4$ M$^{-1}$ for D1 and D2, respectively, compared with the corresponding values ($4.11 \times 10^4$ M$^{-1}$ for D1 and $5.55 \times 10^4$ M$^{-1}$ for D2) observed in the tetranuclear host H2 (Figure 6). Taking into account the given Hill coefficient value (n > 1), the results suggested the positively cooperative and relatively strong overall binding between the host and guest [20,23].

**Figure 5.** The nonlinear fits to Hill equation of UV-Vis titration experiments based on the absorption band centered at 350 nm for (a) D1 ≡ H1 and (b) D2 ≡ H1, and at 370 nm in the [D1]/[H1] ranges of (c) 0–2, (d) 2–3, (e) 3–8, and (f) 8–23.
1.69 × 10^4 M^−1 for D2) was established in the first stage of the guest binding process involving two molecules of the guest, attributed to the encapsulation of the guest molecules inside the circumscribed *endo* cavity of H1 through π···π interaction and hydrogen bonding between the host and guest molecules. A medium association constant found in the range of 5.15 × 10^4–9.60 × 10^4 M^−1 in the following stage clearly suggested the entrapment of six molecules of guest by the six open, bowl-shaped *exo* cavities of H1 through hydrogen bonding and hydrophobic interaction. Additional binding of the guest by the external pockets was supported by the weakest binding constant (i.e., 0.84 × 10^4 M^−1 for D1 or 1.69 × 10^4 M^−1 for D2); however, the exact binding stoichiometry could not be determined, likely due to the fast exchange with the free guests.

### 3. Materials and Methods

#### 3.1. General Information

Starting materials and solvents were obtained from commercial suppliers (Fisher Scientific, TCI, Alfa Aesar, etc.) and used without further purification. The metal-organic supercontainer H1 [18] and a related reference compound, known as tetrannuclear complex H2 [30], were synthesized as described in the literature. The host materials were dried on a Schlenk line under vacuum at 120 °C for 4 h. UV-Vis absorption spectra were collected on a Perkin–Elmer Lambda 35 UV-Vis spectrophotometer at room temperature.
3.2. Solution UV-Vis Titration Experiments

Stock solutions of the hosts (H1 and H2), called the titrand, were prepared in CHCl₃ at a concentration of ~5 × 10⁻⁶ M. 25.00 mL of the host’s stock solution was then used to dissolve an accurately known mass of guest molecule, (R)-(+)−raebeprozole sodium (D1) or (S)(−)−pantoprazole sodium (D2), called the titrant, wherein the guest concentration was 50~100 times greater than that of the host. 2.00 mL of the host solution (the titrand) was placed in a 10.0 mm quartz cell, upon which 0.01 to 2 mL of the titrant was added gradually using a syringe. After each addition, the cell was stoppered and inverted, and the UV-Vis spectrum was collected (at 25 °C) after 3 min to ensure complete mixing and reaching equilibration.

3.3. Calculation of Binding Constants from UV-Vis Titration Data

In order to evaluate the overall binding strength, the titration results were fitted to the nonlinear form of Hill equation [33,34].

\[
\alpha = \frac{\Delta A}{\Delta A_{\text{max}}} = \frac{K_a[L]_0^n}{1 + K_a[L]_0^n} = \frac{[L]_0^n}{(1/K_a) + [L]_0^n}
\]

where \(\Delta A (=A_{\text{obs}} - A_0)\) is the change in absorbance, \(\Delta A_{\text{max}}\) is the maximum change of absorbance, \([L]_0\) is initial guest concentration, \(n\) is the Hill coefficient, and \(K_a\) is the association constant. A plot of \(\Delta A\) against \([L]_0\) can be used to estimate \(\Delta A_{\text{max}}\) and \(K_a\). The titration data were fit to this model using the nonlinear regression method within the Origin 9 software.

4. Conclusions

The guest binding behaviour of metal-organic supercontainer H1 with two drug molecules, D1 and D2, was investigated in chloroform solution using the UV-Vis titration technique. The results revealed highly intriguing cooperative and stepwise binding of the drug molecules with the multiple binding domains of H1. Taking into account the control experiments with the H1 replaced by the tetranuclear complex H2, which represents the exo cavities of H1, it is suggested that the guest molecules were likely encapsulated sequentially by the different cavities of H1: two equivalents of the guest molecule were encapsulated inside the endo cavity of H1, followed by the subsequent entrapment of six molecules of the guest in the six exo cavities of H1, and finally, immobilization of additional guest molecules by the external pockets of H1 was evidenced. The present study affords a thorough understanding of the cooperative binding behavior of metal-organic supercontainers featuring multiple binding domains, thus facilitating their potential biological applications such as drug delivery. We are currently addressing exciting opportunities along these lines.

Author Contributions: Data curation, T.-P.S.; Formal analysis, G.-Z.Z. and F.-R.D.; Funding acquisition, F.-R.D. and Z.-N.C.; Investigation, T.-P.S. and X.-X.F.; Project administration, G.-Z.Z., F.-R.D., and Z.-N.C.; Supervision, F.-R.D. and Z.-N.C.; Validation, T.-P.S.; Visualization, F.-R.D.; Writing—original draft, T.-P.S. and F.-R.D.; Writing—review and editing, F.-R.D. and Z.-N.C. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the National Natural Science Foundation of China (21673239, 21501179 and 21531008) and Natural Science Foundation of Fujian Province (2017J06008), and the Strategic Priority Research Program of the Chinese Academy of Sciences (Grant XDB20000000).

Acknowledgments: We thank Zhenqiang Wang (USD) for helpful comments and suggestions.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Fersht, A. Structure and Mechanism in Protein Science; W. H. Freeman and Company: New York, NY, USA, 1999.
2. Whitty, A. Cooperativity and biological complexity. Nat. Chem. Biol. 2008, 4, 435−439. [CrossRef]
3. Williamson, J.R. Cooperativity in macromolecular assembly. Nat. Chem. Biol. 2008, 4, 458−465. [CrossRef]
23. Bhuvaneswari, N.; Dai, F.-R.; Chen, Z.N. Sensitive and Specific Guest Recognition through Pyridinium- 
22. Dai, F.-R.; Qiao, Y.; Wang, Z. Designing structurally tunable and functionally versatile synthetic 
21. Dai, F.-R.; Becht, D.C.; Wang, Z. Modulating guest binding in sulfonylcalixarene-based metal-organic 
20. Dai, F.-R.; Sambasivam, U.; Hammerstrom, A.J.; Wang, Z. Synthetic Supercontainers Exhibit Distinct Solution 
19. Netzer, N.L.; Dai, F.-R.; Wang, Z.; Jiang, C. pH-Modulated Molecular Assemblies and Surface Properties of 
18. Dai, F.-R.; Wang, Z. Modular Assembly of Metal–Organic Supercontainers Incorporating Sulfonylcalixarenes. 
17. Morohashi, N.; Narumi, F.; Iki, N.; Hattori, T.; Miyano, S. Thiacalixarenes. 
16. Yoshizawa, M.; Tamura, M.; Fujita, M. Diels-alder in aqueous molecular hosts: Unusual regioselectivity and 
15. Deraedt, C.; Astruc, D. Supramolecular nanoreactors for catalysis. 
14. Du, S.; Yu, T.-Q.; Liao, W.; Hu, C. Structure modeling, synthesis and X-ray di 
13. Therrien, B. Drug Delivery by Water-Soluble Organometallic Cages. Top. Curr. Chem. 2012, 319, 35–55. 
12. Ronson, T.K.; Giri, C.; Beyeh, N.K.; Minkkinen, A.; Topic, F.; Holstein, J.J.; Rissanen, K.; Nitschke, J.R. 
11. Mirtschin, S.; Slabon-Turski, A.; Scopelliti, R.; Severin, K. A Coordination Cage with an 
10. Mendez-Arroyo, J.; Barroso-Flores, J.; Liščík, A.M.; Sarjeant, A.A.; Stern, C.L.; Mirkin, C.A. A multi-state, 
9. Hunter, C.A.; Anderson, H.L. What is Cooperativity? Angew. Chem. Int. Ed. 2009, 48, 7488–7499. [CrossRef] 
8. Badjic, J.D.; Nelson, A.; Cantrill, S.J.; Turnbull, W.B.; Stoddart, J.F. Multivalency and cooperativity in 
7. Cui, Q.; Karplus, M. Allostery and cooperativity revisited. 
6. Hunter, C.A.; Anderson, H.L. What is Cooperativity? Angew. Chem. Int. Ed. 2009, 48, 7488–7499. [CrossRef] 
5. Ercolani, G.; Schiaffino, L. Allosteric, Chelate, and Interannular Cooperativity: A Mise au Point. Angew. Chem. Int. Ed. 2011, 50, 1762–1768. [CrossRef] 
4. Stephan, D.W.; Erker, G. Frustrated Lewis Pair Chemistry: Development and Perspectives. Angew. Chem. Int. Ed. 2015, 6400–6441. [CrossRef] 
3. Stephan, D.W.; Erker, G. Frustrated Lewis Pair Chemistry: Development and Perspectives. Angew. Chem. Int. Ed. 2015, 6400–6441. [CrossRef] 
2. Dai, F.-R.; Qiao, Y.; Wang, Z. Designing structurally tunable and functionally versatile synthetic 
1. Hunter, C.A.; Anderson, H.L. What is Cooperativity? Angew. Chem. Int. Ed. 2009, 48, 7488–7499. [CrossRef]
28. Xiong, K.; Jiang, F.; Gai, Y.; Yuan, D.; Chen, L.; Wu, M.; Su, K.; Hong, M. Truncated octahedral coordination cage incorporating six tetranuclear-metal building blocks and twelve linear edges. *Chem. Sci.* **2012**, *3*, 2321–2325. [CrossRef]

29. Wang, S.; Gao, X.; Hang, X.; Zhu, X.; Han, H.; Liao, W.; Chen, W. Ultrafine Pt Nanoclusters Confined in a Calixarene-Based [Ni24] Coordination Cage for High-Efficient Hydrogen Evolution Reaction. *J. Am. Chem. Soc.* **2016**, *138*, 16236–16239. [CrossRef]

30. Kajiwara, T.; Kobashi, T.; Shinagawa, R.; Ito, T.; Takaishi, S.; Yamashita, M.; Iki, N. Highly symmetrical tetranuclear cluster complexes supported by p-tert-butylsulfonylcalix[4]arene as a cluster-forming ligand. *Eur. J. Inorg. Chem.* **2006**, *9*, 1765–1770. [CrossRef]

31. Schalley, C.A. *Analytical Methods in Supramolecular Chemistry*; Wiley: Hoboken, NJ, USA, 2012.

32. Bisswanger, H. *Enzyme Kinetics: Principles and Methods*, 2nd ed.; Wiley: Hoboken, NJ, USA, 2008.

33. Hill, A.V. The possible effects of the aggregation of the molecules of haemoglobin on its dissociation curves. *J. Physiol.* **1910**, *40*, iv–vii.

34. Chenprakhon, P.; Sucharitakul, J.; Panijpan, B.; Chaiyen, P. Measuring Binding Affinity of Protein-Ligand Interaction Using Spectrophotometry: Binding of Neutral Red to Riboflavin-Binding Protein. *J. Chem. Educ.* **2010**, *87*, 829–831. [CrossRef]

**Sample Availability:** Samples of the compounds H1 and H2 are available from the authors.