Suppression of Malachite Green-Induced Toxicity to Human Liver Cells Utilizing Host-Guest Chemistry of Cucurbit[7]uril

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We investigated a host-guest complex between cucurbit[7]uril and malachite green, and its effect on the toxicity to human liver cells. The host-guest complexation was evaluated by a UV/vis titration and electrospray-ionization mass spectrometry. Interestingly, the host-guest complex result in remarkable suppression of the toxicity of malachite green in its practical concentration range (ca. –6 μM). This study is one step forward to the active control of the biological effects of potent toxicants utilizing host-guest chemistry.

Keywords: Active control, biological effect, host-guest chemistry, cucurbit[7]uril, malachite green

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Introduction

Macrocyclic compounds are among the prized components for functional materials in the field of host-guest chemistry.1 To date, various types of macrocyclic host molecules, such as crownethers,2,3 cyclodextrins,4,5 calix[n]arenes,6,7 pillar[n]-arenes,8,9 cyclophanes,10,11 and cucurbit[n]urils12-14 (CB[n], n = 5, 6, 7, 8, 10, 12, 13, 14, 15), have been synthesized and their functions have been widely investigated. Especially, CB[n]s have attracted intensive attention for biochemical applications due to their ease of kilogram-scale synthesis,15 synthetic variety,16-19 water-solubility, and unique inclusion phenomena in aqueous media.13,20 The major driving forces for the host-guest complexation are ion-dipole interaction between the ureidyl oxygen of CB[7] and the cationic part of the guest molecule, and hydrophobic interaction between the inner CB[n] cavity and the guest molecule.13 Thus, CB[n]s form host-guest complexes with hydrophobic and/or positively charged13 compounds with binding constants up to ca. ~10¹⁸ M⁻¹.21,22 Furthermore, fundamental biochemical studies demonstrated that CB[n]s can be taken up into cells by multiple internalization mechanisms.23,24 and the CB[n]s were thus shown to be effective in enhancing the pharmacological effects of bioactive compounds, including steroids and anticancer drugs.18,25-28 In other words, CB[n]s can be utilized to control the biological effects of exogenous compounds in vitro and in vivo.29-32 For example, Cutts et al. demonstrated the enhanced survival of the mouse model of cancer by encapsulation of mitoxantrone inside CB[8].27 CB[8] contributed to the increase in uptake of mitoxantrone in cancer cells. Wang et al. reported that the neurotoxicant MPTP (N-methyl-4-phenyl-1,2,3,6-tetrahydro pyrididine) and MPP⁺ (N-methyl-4-phenylpyridinium) were encapsulated in CB[7], which suppressed the onset of the mechanism of neurotoxicity.29

To obtain comprehensive and fundamental insights on the active control of biological effect utilizing host-guest chemistry, more compounds should be investigated. In this regard, we have focused on the host-guest interaction between malachite green (MG) and CB[7] (Fig. 1). MG is a low molecular-weight compound that is used in aquaculture as a parasiticide.31 MG reduces fungal attacks, protozoan infections and some diseases caused by helminths on a wide variety of aquatic organisms such as fish. However, MG has generated much concern due to its toxic effects on mammals.31 Although MG is not registered for use in food production, it can be detected from raw and processed foods.32 Thus, the suppression of MG-induced toxicity to mammals by host-guest chemistry would be an effective approach to alleviate health concerns.

To this end, we investigated the inclusion phenomena of CB[7] for MG, and the effect of the encapsulation on the toxicity to human cells. We selected a combination of human hepatocyte HepG2 cells with MG and CB[7] because 1) there is no previous report on the effect of the formation of MG⊂ CB[7] on cell viability, 2) MG is considered for its toxicity to mammals,31 3) MG is accumulated in the liver11 and 4) HepG2 cells are one of the conventional liver cell lines. The host-guest interaction was evaluated by UV/vis spectrophotometry and mass spectrometry. Importantly, the potent toxicity of MG was remarkably suppressed by the encapsulation in CB[7] as evident from the restored viability of the HepG2 cells. This study serves as an important example for controlling the biological effects of potent toxicants utilizing the host-guest chemistry of CB[n]s.

Experimental

Experimental details are summarized in Supporting Information. The effect of the encapsulation of MG in CB[7] on the cell viability of HepG2 cells was evaluated by AlamarBlue assay using absorbance at 570 and 600 nm.33

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Results and Discussion

The encapsulation of amine-containing guests into CB[7] is conventionally monitored under acidic or weakly acidic conditions to provide a further driving force for the host-guest complexation. In this study, we have performed the UV/vis titration for motoring the encapsulation of MG into CB[7] under neutral conditions (phosphate buffer (50 mM) at pH 7.4) to expand the encapsulation phenomena for biochemical analysis. As shown in Fig. 2, the addition of CB[7] resulted in a decrease in the absorbance of MG at 424 and 621 nm, while very slight spectral shifts were observed. The spectral change was saturated in the presence of 10 eq. CB[7] (Fig. 2(a) inset). The titration isotherm matched well with the fitting curve for the 1:1 binding model and afforded the association constant \( K_{\text{assoc}} \) for MG \( \subset \) CB[7] to be \( K_{\text{assoc}} = 7.0 \times 10^5 \) M\(^{-1}\). The formation of the 1:1 complex was also supported by electrospray-ionization mass spectrometry (ESI-MS) (Fig. 2(b)).

The effect of CB[7] on the cytotoxicity of MG was investigated using HepG2 cells. MG exhibits potent toxicity to the cells at a concentration range higher than 2 \( \mu \)M. In a similar concentration range, we investigated whether the inclusion suppresses the MG-induced cell death. Various concentrations of MG (2 – 10 \( \mu \)M) in the presence or absence of CB[7] were incubated in the cell media, where the concentration of CB[7] was fixed at 250 \( \mu \)M. CB[7] did not cause significant cytotoxicity to HepG2 cells after the co-incubation, while MG was cytotoxic at the concentration range higher than 2 \( \mu \)M under the experimental conditions (Fig. 3). Interestingly, CB[7] remarkably restored the cell viability in the presence of up to 6 \( \mu \)M MG, suggesting that the inclusion of MG into CB[7]

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**Fig. 1** Schematic illustration of this study.

**Fig. 2** (a) UV/vis spectral changes of MG upon the addition of CB[7] in a phosphate buffer (50 mM) at pH 7.4 at 25 °C. [MG] = 5 \( \mu \)M. [CB[7]] = 0 – 60 \( \mu \)M. (b) ESI-MS spectrum of MG \( \subset \) CB[7]. Inset: Theoretical isotopic pattern for MG \( \subset \) CB[7] + H\(^+\) (C\(_{65}\)H\(_{68}\)N\(_{30}\)O\(_{14}\)H\(^{+}\)).
Effect of MG, CB[7] and the encapsulation of MG in CB[7]

**Conclusions**

We investigated the host-guest complex between CB[7] and MG, and its effect on the toxicity to human liver cells. We have demonstrated that MG is encapsulated into CB[7] under neutral conditions, which will be helpful for further biochemical analyses of the host-guest complex. Interestingly, the resulting MG \(\subset\) CB[7] complex restored the cell viability of the HepG2 cells in the practical concentration range of MG (ca. 2 – 6 \(\mu\)M). Although the mechanism of action of MG \(\subset\) CB[7] still needs to be elucidated, this study gives the first example of enhanced cell viability against MG-induced cytotoxicity. This study will help pave the way to regulate the biological effects of exogenous compounds utilizing host-guest chemistry.

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**Supporting Information**

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**References**

1. J. M. Lehn, “Supramolecular Chemistry: Concepts and Perspectives”, 1995, VCH, Weinheim.
2. C. J. Pedersen, *J. Am. Chem. Soc.*, 1967, 89, 2495.
3. G. W. Gokel, W. M. Leevy, and M. E. Weber, *Chem. Rev.*, 2004, 104, 2723.
4. G. Crini, *Chem. Rev.*, 2014, 114, 10940.
5. R. Ozawa and T. Hayashita, *J. Ion Exch.*, 2007, 18, 124.
6. C. D. Gutsche, *Calixarenes: An Introduction 2nd ed.*, 2008, Royal Society of Chemistry.
7. Z. Lai, T. Zhao, J. L. Sessler, and Q. He, *Coord. Chem. Rev.*, 2020, 425, 213528.
8. N. Song, T. Kakuta, T. Yamagishi, Y.-W. Yang, and T. Ogoshi, *Chem.*, 2018, 4, 2029.
9. T. Kakuta, T. Yamagishi, and T. Ogoshi, *Acc. Chem. Res.*, 2018, 51, 1656.
10. F. Diederich, *Angew. Chem. Int. Ed. Engl.*, 1988, 27, 362.
11. Z. Liu, S. K. M. Nalluri, and J. F. Stoddart, *Chem. Soc. Rev.*, 2017, 46, 2459.
12. J. Lagona, P. Mukhopadhyay, S. Chakrabarti, and L. Isaacs, *Angew. Chem. Int. Ed.*, 2005, 44, 4844.
13. S. J. Barrow, S. Kasera, M. J. Rowland, J. del Barrio, and O. A. Scherman, *Chem. Rev.*, 2015, 115, 12320.
14. K. I. Assaf and W. M. Nau, *Chem. Soc. Rev.*, 2015, 44, 394.
15. A. Day, A. P. Arnold, R. J. Blanch, and B. Smushall, *J. Org. Chem.*, 2001, 66, 8094.
16. D. Lucas, T. Minami, G. Iannuzzu, L. Cao, J. B. Wittenberg, P. Anzenbacher, and L. Isaacs, *J. Am. Chem. Soc.*, 2011, 133, 17966.
17. R. Kubota, T. Takabe, K. Arima, H. Taniguchi, S. Asayama, and H. Kawakami, *J. Mater. Chem. B*, 2018, 6, 7050.
18. L. Cao, G. Hettiarachchi, V. Brienek, and L. Isaacs, *Angew. Chem. Int. Ed.*, 2013, 52, 12033.
19. S. K. Ghosh, A. Dhamija, Y. H. Ko, J. An, M. Y. Hur, D. R.
Boraste, J. Seo, E. Lee, K. M. Park, and K. Kim, *J. Am. Chem. Soc.*, **2019**, *141*, 17503.

20. G. V. Oshovsky, D. N. Reinhoudt, and W. Verboom, *Angew. Chem. Int. Ed.*, **2007**, *46*, 2366.

21. L. Cao, M. Šekutor, P. Y. Zavalij, K. Minarić-Majerski, R. Glaser, and L. Isaacs, *Angew. Chem. Int. Ed.*, **2014**, *53*, 988.

22. D. Shetty, J. K. Khedkar, K. M. Park, and K. Kim, *Chem. Soc. Rev.*, **2015**, *44*, 8747.

23. X. Miao, Y. Li, I. Wyman, S. M. Y. Lee, D. H. Macartney, Y. Zheng, and R. Wang, *MedChemComm*, **2015**, *6*, 1370.

24. H. Bai, J. Wang, Z. Li, and G. Tang, *Int. J. Mol. Sci.*, **2019**, *20*, 2097.

25. N. Saleh, I. Ghosh, and W. M. Nau, in *Supramolecular Systems in Biomedical Fields*, ed. H. J. Schneider, 1st ed., The Royal Society of Chemistry, **2013**, pp. 164–212.

26. A. I. Lazar, F. Biedermann, K. R. Mustafina, K. I. Assaf, A. Hennig, and W. M. Nau, *J. Am. Chem. Soc.*, **2016**, *138*, 13022.

27. S. K. Konda, R. Maliki, S. McGrath, B. S. Parker, T. Robinson, A. Spurling, A. Cheong, P. Lock, P. J. Pigram, D. R. Phillips, L. Wallace, A. I. Day, J. G. Collins, and S. M. Cutts, *ACS Med. Chem. Lett.*, **2017**, *8*, 538.

28. Q. Huang, Q. Cheng, X. Zhang, H. Yin, L.-H. Wang, and R. Wang, *ACS Appl. Biol. Mater.*, **2018**, *1*, 544.

29. S. Li, H. Chen, X. Yang, D. Bardelang, I. W. Wyman, J. Wan, S. M. Y. Lee, and R. Wang, *ACS Med. Chem. Lett.*, **2015**, *6*, 1174.

30. Y. Chen, Z. Huang, J.-F. Xu, Z. Sun, and X. Zhang, *ACS Appl. Mater. Interfaces*, **2016**, *8*, 22780.

31. S. Srivastava, R. Sinha, and D. Roy, *Aquat. Toxicol.*, **2004**, *66*, 319.

32. E. Panel o. C. i. t. F. Chain, *EFSA Journal*, **2016**, *14*, e04530.

33. BioRad, “General Method for Measuring Cytotoxicity or Proliferation Using alamarBlue”, https://www.bio-rad-antibodies.com/measuring-cytotoxicity-proliferation-spectrofluorometry-fluorescence-alarablue.html (accessed on Sep. 25th, 2020).

34. S. S. Thomas, H. Tang, and C. Bohne, *J. Am. Chem. Soc.*, **2019**, *141*, 9645.

35. C. P. Carvalho, V. D. Uzunova, J. P. Da Silva, W. M. Nau, and U. Pischel, *Chem. Commun.*, **2011**, *47*, 8793.

36. D. Tang, J. Sun, K. Wu, T. Li, and Y. Zhou, *Adv. Anal. Chem.*, **2012**, *2*, 7.

37. Y.-J. Kim, M. Song, and J.-C. Ryu, *Mol. Cell. Toxicol.*, **2008**, *4*, 22.

38. Koi & Aquarium Fish Diseases, https://fishdoc.co.uk/malachite-green-and-formalin-a-good-general-purpose-anti-parasite-treatment/ (accessed on Sep. 25th, 2020).

39. T. Minami, N. A. Esipenko, B. Zhang, L. Isaacs, and P. Anzenbacher, *Chem. Commun.*, **2014**, *50*, 61.

40. J. W. Lee, H. H. L. Lee, Y. H. Ko, K. Kim, and H. I. Kim, *J. Phys. Chem. B*, **2015**, *119*, 4628.

41. A. C. Bhaskuttan, J. Mohanty, and H. Pal, *Angew. Chem. Int. Ed.*, **2007**, *46*, 9305.