Selective binding and controlled release of anticancer drugs by polyanionic cyclodextrins

Jian-Guang Cheng a, Hua-Jiang Yu a, Yong Chen a, Yu Liu a,b,⇑

a College of Chemistry, State Key Laboratory of Elemento-Organic Chemistry, Nankai University, PR China
b Collaborative Innovation Center of Chemical Science and Engineering (Tianjin), Tianjin 300071, PR China

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The binding stoichiometry, binding constants, and inclusion mode of some water-soluble negatively charged cyclodextrin derivatives, i.e. heptakis-[6-deoxy-6-(3-sulfanylpropanoic acid)]-β-cyclodextrin (H1), heptakis-[6-deoxy-6-(2-sulfanylacetic acid)]-β-cyclodextrin(H2), mono-[6-deoxy-6-(3-sulfanyl-propanoic acid)]-β-cyclodextrin (H3) and mono-[6-deoxy-6-(2-sulfanylacetic acid)]-β-cyclodextrin (H4), with three anticancer drugs, i.e. irinotecan hydrochloride; topotecan hydrochloride; doxorubicin hydrochloride, were investigated by means of 1H NMR, UV–Vis spectroscopy, mass spectra and 2D NMR. Polyanionic cyclodextrins H1-H2 showed the significantly high binding abilities of up to 2.6 × 10^5-2.0 × 10^6 M^-1 towards the selected anticancer drugs, which were nearly 50–1000 times higher than the corresponding Ks values of native β-cyclodextrin. In addition, these polyanionic cyclodextrins also showed the pH-controlled release behaviors. That is, the anticancer drugs could be efficiently encapsulated in the cyclodextrin cavity at a pH value similar to that of serum but sufficiently released at an endosomal pH value of a cancer cell, which would make these cyclodextrin derivatives the potential carriers for anticancer drugs.

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1. Introduction

Recently, numerous effective anticancer drugs have been used for the treatment of various human and animal cancers. Among them, irinotecan hydrochloride (CPT-11), topotecan hydrochloride (TPT) and doxorubicin hydrochloride (DOX) are three prominent leader compounds. CPT-11 and TPT are both water-soluble semisynthetic derivatives of the alkaloid camptothecin. 1,2 CPT-11 exhibits remarkable antitumor activity in clinical trials against a variety of human tumors, 3–5 including colorectal cancer, lung cancer and malignant lymphoma. 6–8 TPT is used clinically in the treatment of relapsed ovarian, lung cancer, and cervical cancer. 9–12 DOX is a chemotherapeutic agent used for the treatment of a wide variety of human malignancies with an anthracryine structure, which consists of an aglycon, adriamycinone, combined with an amino sugar, daunosamine. 13–16 On the other hand, cyclodextrins (CDs), a class of cyclic oligosaccharides linked by 1,4-glucose bonds, are water-soluble, nontoxic, compounds commercially available at low price. 17–23 and their torus-shaped cavity can bind various inorganic/organic/biological molecules. This excellent property enables the wide application of CDs in fields of molecular recognition and molecular assembly. Among the CD family, the most used one is β-CD that contains 7 glucose units. 24–28 Nevertheless, the complex stability constants (Ks) between native β-CD and anticancer drugs (CPT-11, TPT and DOX) are very limited, 29 i.e. 2.6 × 10^5 M^-1 for β-CD/CPT-11 pair, 8.8 × 10^5 M^-1 for β-CD/TPT pair, and 2.1 × 10^5 M^-1 for β-CD/DOX pair, respectively, which greatly restricts the application of β-CD as carriers of anticancer drugs. Recently, the negatively charged CD derivatives have attracted more attention because of their potential applications in drug delivery. For example, Zhang et al. reported a negatively charged CD named ORG25969 as a good acceptor to give an extraordinarily high binding affinity towards rocuronium bromide (Ks up to 10^10 M^-1), and thus can be clinically used as a reversal agent in the post-operative recovery. 30 Wenz and Apostolakis et al. synthesized a series of negatively charged CDs and researched their binding behaviors with camptothecin. The result showed that the stabilities of camptothecin complexes obtained from solubility measurements of negatively charged CD derivatives were significantly higher than those of other reported CD derivatives. 31,32 Herein, we selected four negatively charged CD derivatives, i.e. heptakis-[6-deoxy-6-(3-sulfanylpropanoic acid)]-β-CD (H1), heptakis-[6-deoxy-6-(2-sulfanylacetic acid)]-β-CD (H2), mono-[6-deoxy-6-(3-sulfanylpropanoic acid)]-β-CD (H3) and mono-[6-deoxy-6-(3-sulfanylpropanoic acid)]-β-CD (H4) with three anticancer drugs, i.e. irinotecan hydrochloride; topotecan hydrochloride; doxorubicin hydrochloride, were investigated by means of 1H NMR, UV–Vis spectroscopy, mass spectra and 2D NMR. Polyanionic cyclodextrins H1-H2 showed the significantly high binding abilities of up to 2.6 × 10^5-2.0 × 10^6 M^-1 towards the selected anticancer drugs, which were nearly 50–1000 times higher than the corresponding Ks values of native β-cyclodextrin. In addition, these polyanionic cyclodextrins also showed the pH-controlled release behaviors. That is, the anticancer drugs could be efficiently encapsulated in the cyclodextrin cavity at a pH value similar to that of serum but sufficiently released at an endosomal pH value of a cancer cell, which would make these cyclodextrin derivatives the potential carriers for anticancer drugs.
deoxy-6-(2-sulfanylacetic acid)-β-CD (H4), and investigated their selective binding and controlled release behaviors towards anticancer drugs CPT-11, TPT and DOX (Scheme 1). Significantly, with binding abilities much stronger than those of most previously reported CD derivates, these polyanionic CDs exhibited the pH-responsive release of drug in a cancer cell environment. That is, the polyanionic CD/anticancer drug complex was stable in a biological environment such as serum (pH 7.2), but efficiently released the encapsulated anticancer drug at pH 5.7 (endosomal pH values of a cancer cell).

2. Results and discussion

2.1. Job plots and binding constants of H1-H4 and anticancer drugs

UV–vis spectroscopy was employed to determine the host–guest binding stoichiometry. As shown in Fig. 1, the Job plot of H1/CPT-11 in water gave a maximum at molar fraction of 0.5, indicating that H1 formed stoichiometric 1:1 inclusion complex with CPT-11. Moreover, the mass spectrum measurements (Figs. S31–S33) also demonstrated the formation of 1:1 inclusion complexes between cyclodextrin hosts and anticancer drugs. The quantitative investigation on the molecular binding behavior of H1 with CPT-11 was examined by means of UV–vis spectral titration, wherein the UV–vis spectra of a series of solutions containing the same amounts of CPT-11 and different amounts of H1 were measured to determine the binding constant between CPT-11 and H1. As can be seen from Fig. S20, with the addition of H1, the absorbance maximum of CPT-11 slightly decreased, accompanied by the appreciable red shift of maximum wavelength. By using the nonlinear least-squares method, the stability constants (Ks) values could be calculated as (1.7 ± 0.2) × 10^4 M⁻¹ according to the sequential changes of absorbance intensity of CPT-11 with the different concentrations of H1. Similar 1:1 binding stoichiometry was also found in the association of hosts H1-H4 with anticancer drugs CPT-11, TPT and DOX, and the corresponding stability constants (Ks) were determined (Fig. 2) and listed in Table 1. Moreover, we also tried to use isothermal titration calorimetry to determine the binding constants. However, the isothermal titration calorimetry experiment required the higher concentrations, and the inclusion complex formed precipitate under such a concentration.

Accordingly, the encapsulation and loading efficiency of anticancer drugs by hosts were calculated and listed in Table 1. As seen in Table 1, the native β-CD only showed very poor binding ability towards the selected anticancer drugs. Possessing an anionic side arm on the β-CD rim, host H3 or H4 showed the moderate binding ability (1.02 × 10⁻²–1.7 × 10⁴ M⁻¹) towards anticancer drugs owing to the electrostatic interactions between the anionic side arm of host and the cationic guest. However, host H1 or H2 showed a significantly increased binding ability towards anticancer drugs up to 2.6 × 10⁻²–2.0 × 10⁵ M⁻¹, which was nearly 50–1000 times higher than the corresponding Ks values of native β-CD. A possible reason may be that the seven anionic side arms on H1 or H2 (either of H1 or H2 possesses 7 negative charges) gave the greatly strengthened electrostatic interactions with the cationic guest. Moreover, the extended cavity formed by seven side arms may also provide the additional van der Waals and hydrophobic interactions towards the accommodated drug. As a result, host H1 exhibited the fairly high encapsulation efficiency (>75%) and loading efficiency (>18%) towards the selected anticancer drugs when the concentrations of anticancer drugs and hosts were fixed at 0.1 mM, which enables it as a good candidate of anticancer drug carriers. The anti-
cancer drugs encapsulation efficiency and loading efficiency was calculated by the following formulas: 35

\[
\text{encapsulation efficiency} \left( \% \right) = \left( \frac{m_{\text{loaded}}}{m_{D}} \right) \times 100
\]

\[
\text{loading efficiency} \left( \% \right) = \left( \frac{m_{\text{loaded}}}{m_{cd}} \right) \times 100
\]

\[
m_{\text{loaded}} = \frac{1}{2} M_D v \left( [H]_0 + [G]_0 + \frac{1}{K_S} \right) - \sqrt{\left( [H]_0 + [G]_0 + \frac{1}{K_S} \right)^2 - 4[H]_0[G]_0}
\]

where \( m_{\text{loaded}} \) is the mass of anticancer drugs that formed inclusion complex with hosts, \( m_D \) is the mass of anticancer drugs added, \( m_{cd} \) is the mass of hosts, \( [G]_0 \) is the initial concentration of anticancer drugs added, \( [H]_0 \) is the initial concentration of hosts, and \( M_D, v \) and \( K_S \) are molecular weight of anticancer drugs, volume of the solution, and the stability constants of the inclusion complex, respectively. In addition, the solubility and stability of inclusion complexes in 10% serum solution were also investigated. The results showed that the solubility could reach 0.5 mmol/mL, and the complex could keep stable for at least 24 h.

2.2. Binding mode of H1 with anticancer drugs

2D NMR spectroscopy is an essential method to investigate binding mode between host and guest. As shown in Fig. 3, we could see NOE correlations between protons of CPT-11 and interior protons of H1 from the ROESY spectrum of an equimolar mixture of H1 with CPT-11. The cross peak A was assigned to NOE correlations between H18/H23 protons of CPT-11 and H3 protons of H1, and the cross peak B was assigned to NOE correlations between H28 protons of CPT-11 and H3/H5 protons of H1, and H5 protons gave stronger NOE correlations than H3 protons. Moreover, the cross peak C was assigned to NOE correlations between H11/H12/H13/H14 protons of CPT-11 and H3 protons of H1. Therefore, we deduced that the CPT-11 guest entered the H1 cavity from the wide side. Based on the ROESY and molecular simulation experiments, the geometry of inclusion complex of H3 with CPT-11 was proposed where the CPT-11 guest entered the H1 cavity from the wide side.

2.3. Controlled release of anticancer drugs

In addition to the UV–vis spectral changes, the association with polyanionic CDs also led to the obvious fluorescence and color changes of anticancer drugs, which could be readily monitored by fluorescence spectra or naked eyes. For example, the fluorescence intensity of DOX showed the obvious decrease, and its color turned dark, after the association with H1 (Fig. 4). In the control experiment, the pH dependence of DOX fluorescence changes in the absence of β-CD hosts was relatively limited. Therefore, the flu-
photographs of fluorescence changes of behaviors of polyanionic CD/anticancer drug systems at different fluorescence spectra were used to investigate the controlled release behaviors of negatively charged CDs with some anticancer drugs were investigated by means of 1H NMR, UV–Vis spectroscopy, mass spectra and 2D NMR, and their binding constants (Ks) can reach 10^5 M⁻¹ level, which is higher than most previously reported CD derivatives. Therefore, we deduce that the present polyanionic CD/anticancer drug systems may find their application potential in cancer therapy.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.bmc.2018.03.013.

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