ORIGINAL ARTICLE

Inclusion of chemotherapeutic agents in substituted β-cyclodextrin derivatives

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Abstract The stabilities of the inclusion compounds of three chemotherapeutic agents, camptothecin (CPT), docetaxel (DOC) and idarubicin (IDA), plus a model compound 1,4-dihydroxyanthraquinone (DHA) with several β-cyclodextrin (β-CD) derivatives were investigated by solubility measurements, isothermal titration microcalorimetry and fluorescence anisotropy measurements. Ionic heptakis-(6-deoxy-6-thioethers) of β-CD were found to exhibit very high binding potentials for these drugs making them to good candidates for advanced drug delivery.

Abbreviations

CD	Cyclodextrin
CPT	Camptothecin
DHA	1, 4-Dihydroxyanthraquinone
DOC	Docetaxel
HPβCD	Hydroxypropyl-β-cyclodextrin
IDA	Idarubicin
ITC	Isothermal titration calorimetry

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Introduction

Cyclodextrins (CDs) **1** are 1 → 4 α-linked cyclic oligomers of glucopyranose. Those CDs consisting of 6, 7, and 8 glucose units are produced on an industrial scale and called α-, β- and γ-CD, respectively. CDs are able to include hydrophobic or amphiphilic guest molecules in aqueous solution whereby they recognize both their size and polarity patter [1, 2]. Binding data are available for numerous CD complexes, called inclusion compounds. Inclusion of active substances in CDs gives rise to many applications [3], such as formulation of pharmaceutical drugs [4, 5], and cosmetics [6], as well as chromatographic separations [7, 8]. Applications of CDs and CD derivatives in the pharmaceutical field are especially interesting since they allow solubilization of drugs in water [5, 9]. In this way, CDs can increase the plasma-level of the complexed drugs and thus increase the therapeutic effect. The use of native β-CD is limited by the low solubility in water and the nephrotoxicity especially in parenteral drug delivery [10]. Through chemical modification of the hydroxyl groups it is possible to synthesize CD derivatives with better solubility and lower toxicity, for example hydroxypropyl-β-CD (HPβCD, Encapsin®) [11] and sulfobutyl-β-CD [12] (Captisol®). Furthermore both selectivities and affinities of CDs can be increased by chemical modifications of CDs [13, 14].

Since many anti-cancer drugs are not sufficiently soluble in water, complexation by CDs appears to be a very promising strategy to improve bioavailability. For instance, camptothecin (CPT) represents an interesting antineoplastic agent against several types of cancer, including colorectal and ovarian cancer, but administration is problematic because of its low solubility and stability [15]. Therefore inclusion of CPT in CDs and CD ethers was already recommended for diminishing these problems [16]. Recently
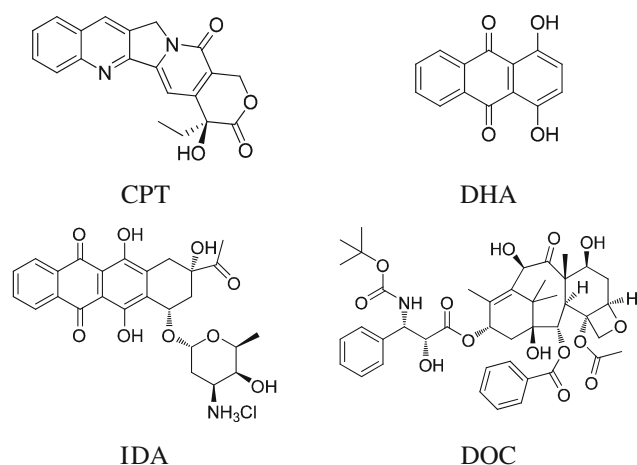
Fig. 1 Structures of cytostatic drugs and the model compound DHA of this investigation

we demonstrated that functional heptakis-(6-deoxy-6-S-thioethers) of β-CD are able to solubilize CPT to a much higher degree [17]. In the following we describe the inclusion and solubilization of other poorly water soluble chemotherapeutics docetaxel (DOC) and idarubicin (IDA), depicted in Fig. 1, by these CD derivatives.

Docetaxel (tradename Taxotére®) is a semi-synthetic analogue of paclitaxel (Taxol). It exerts its cytotoxic efficiency by binding to free tubulin, simultaneously promoting the assembly of tubulin into stable microtubules leading to inhibition of cell division [18, 19]. Because of the extremely low solubility of DOC, its administration requires a formulation with the detergent tween 80 acting as a dispersant. While rather weak solubilization of DOC was observed with native β-CD, already monosubstituted β-CDs and β-CD dimers showed significant solubilization effects [20, 21].

Idarubicin (trade names Zavedos, Idamycin) is a highly toxic anti-neoplastic drug, which is used for the therapy of acute myeloid leukemia. To best of our knowledge, inclusion of IDA in CDs was not previously described. In the following, solubilizations of CPT, DOC and IDA by inclusion in various cationic, neutral and anionic heptakis-(6-S-6-deoxy-thioethers) of β-CD are pointed out in detail. In addition, 1,4-dihydroxy-anthraquinone (DHA) was chosen as a simple model compound for IDA, to investigate whether this moiety is an appropriate binding site for the complexation by β-CD and its derivatives.

Experimental

Materials

The drugs were purchased in pharmaceutical quality from TCI Europe (CPT), AK Scientific (DOC), Sigma–Aldrich

(IDA) and Fluka (DHA). The CD derivatives were synthesized as described previously [14, 17]. Hydroxypropyl-β-CD (HPβCD) and randomly methylated β-CD (RAMEB) were supplied from Wacker Chemie AG, Burghausen, Germany.

Measurements

Isothermal titration calorimetry (ITC) was performed at 25 °C with an AutoITC isothermal titration calorimeter (MicroCal Inc., Northampton, USA) as described before [14].

The solubility measurements were performed according to standard procedures [16]. Solutions of CD derivatives (0–6 mM, 5 mL) in water (20 mM HCl (pH = 1.7) in case of CPT) were stirred with an excess of the drug at 25 °C for 18 h. The resulting solutions were filtered through a Teflon (0.25 μm) syringe filter. The concentration of dissolved drug was determined by UV using the extinction coefficients ε listed in Table 1. The solubility of the drug was plotted versus the concentration of the CD derivative (see Fig. 3). The binding constant K was calculated from the solubility of the drug $[G]_0$ without CD and the slope B according to Eq. 1.

$$K = \frac{B}{(1-B)[G]_0}. \tag{1}$$

Fluorescence intensities I of 15 μM solutions of IDA in 0.1 M saline phosphate buffer pH = 7.4 (PBS) were measured with a Jasco FT 6500 fluorescence spectrometer at an excitation wavelength 480 nm and emission wavelength 570 nm for horizontal (h) and vertical (v) positions of both polarizer and analyzer for various concentration 5–500 μM of the CD derivative at 25 °C as described elsewhere [22]. From the resulting intensity values I_{hh}, I_{vv}, I_{hv}, and I_{vh} the anisotropy r was calculated for each CD concentration according to Eq. 2. The anisotropy r was plotted versus the molar ratio of CD derivative and the drug G. This plot was fitted with the standard equation for a 1:1 complex [23], shown in Eq. 3, with the ratio of the total molar concentrations of CD and drug, $x = [CD]_t/[G]_t$ and the reduced dissociation constant $k = 1/(K[G]_t)$, by non-linear regression using the program OriginPro 7.5G.

Table 1 Extinction coefficients ε and aqueous solubilities of drugs

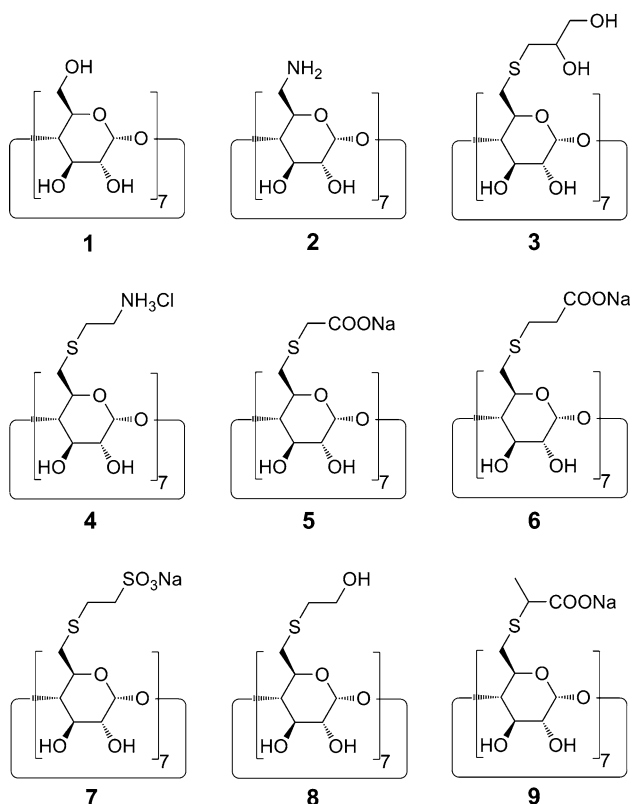| Drug	Solvent	Wavelength (nm)	ε (M^{-1} cm^{-1})	Solubility in water (μM)
CPT	Water/DMSO 1:1 v/v	370	42282	2.1 ± 0.2[a]
DHA	Water/DMSO 1:1 v/v	480	8240	0.24 ± 0.03
DOC	Water/EtOH 1:1 v/v	230	16660	18.9 ± 0.7

[a] 20 mM HCl

$$r = \frac{I_{vv} - I_{vh}\frac{I_{hv}}{I_{hh}}}{I_{vv} + 2I_{vh}\frac{I_{hv}}{I_{hh}}} \quad (2)$$

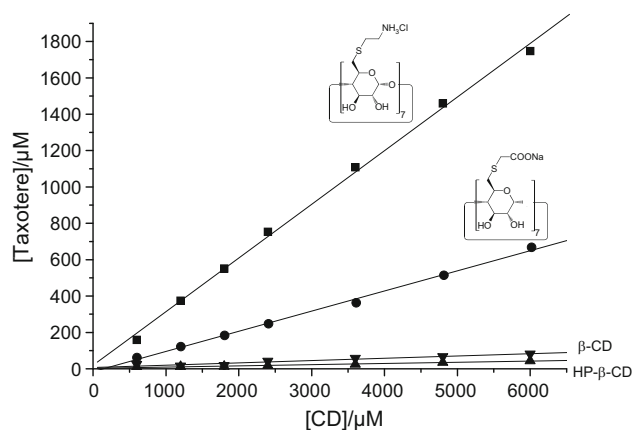$$r = r_0 + (r_\infty - r_0)\frac{x + 1 + k - \sqrt{(x+1+k)^2 - 4x}}{2}. \quad (3)$$

Results and discussion

A series of heptafunctional 6-S-substituted CD derivatives **3–9** (see Fig. 2) was synthesized by nucleophilic displacement reactions of heptakis-(6-iodo-6-deoxy)-β-CD [24] with functional thiols according to published procedures, since these compounds already showed very promising binding constants for other guests and very high aqueous solubilities [14, 17]. The hepta-6-amino derivative **2** and the commercially available statistical derivatives hydroxypropyl-β-CD (HPβCD) and randomly methylated β-CD (RAMEB) were also tested for comparison.

Since compounds DOC and DHA show only a very small solubility in water (see Table 1) the solubility isotherm (phase solubility method) [25] is the best method for the measurement of the binding constants of the corresponding inclusion compounds. A dramatic increase of the solubility of DOC in water was found due to the

Fig. 3 Dependence of the solubility of DOC (Taxotére) in water on the concentrations of β-CD and various β-CD derivatives at 25 °C

complexation of DOC by ionic β-CD derivatives **4** and **5** as shown in Fig. 3. Apparent binding constants K were calculated from the initial straight line portion of the phase solubility diagram (Eq. 1, Fig. 3) assuming that a 1:1 complex is initially formed. Since the concentration of CD extend only up to 6 mM, formation of 1:2 complexes (guest:CD) was rather unlikely.

Since IDA hydrochloride is soluble in water, binding constants K were determined by isothermal titration calorimetry (ITC). In all cases 1:1 stoichiometries and high binding constants, listed in Table 2, were found. Since the ITC data for the complexation of IDA within amino derivative **4** were not sufficiently accurate, the binding constant K was determined by fluorescence anisotropy measurements. Fluorescence anisotropy is a highly sensitive method to determine diffusion coefficients and

Fig. 2 Heptakis-6-substituted CD derivatives for the complexation of anticancer drugs

Table 2 Binding constants K/M^{-1} for the anti-cancer drugs CPT, DOC, IDA and the model compound DHA, with β-CD substituted at all C6-positions by R, as determined by ITC (i), solubility (s), or fluorescence anisotropy (f)

Host	R at β-CD	CPT[b]	DOC	DHA	IDA
1	OH	202[s]	676[s]	1039[s]	<100[i]
2	NH$_2$	397[s]	n.d.	1745[s]	<100[i]
HPβCD	O–CH$_2$–CH(CH$_3$)–OH[a]	223[s]	350[s]	n.d.	<100[i]
RAMEB	O–CH$_3$[a]	186[s]	n.d.	n.d.	<100[i]
3	S–CH$_2$–CH(OH)–CH$_2$OH	4106[s]	n.d.	36226[s]	29600[i]
4	S–CH$_2$–CH$_2$–NH$_3$Cl	4821[s]	22160[s]	453002[s]	9739[f]
5	S–CH$_2$–COONa	1450[s]	6600[s]	40939[s]	316000[i]
6	S–(CH$_2$)$_2$–COONa	3134[s]	n.d.	137041[s]	240000[i]
7	S–CH$_2$–CH$_2$–SO$_3$Na	7496[s]	n.d.	291811[s]	460000[i]
8	S–CH$_2$–CH$_2$–OH	3212[s]	n.d.	112224[s]	60500[i]
9	S–CH(CH$_3$)–COONa	886[s]	n.d.	21552[s]	103000[i]

[a] Statistically substituted; DS(HP) = 0.9; DS(Me) = 1.8

[b] From [17], *n.d.* not determined
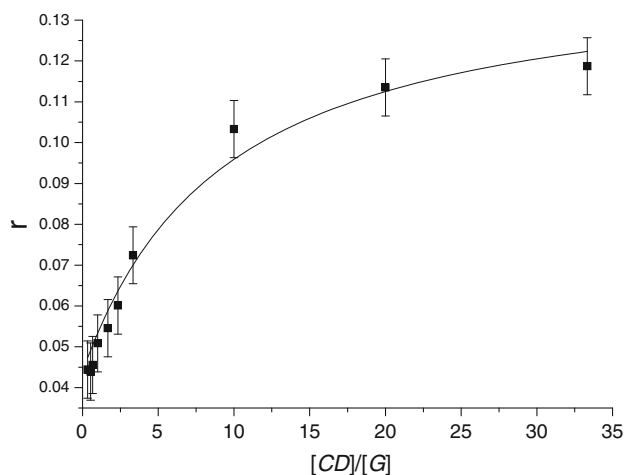
Fig. 4 Fluorescence anisotropy r of IDA as a function of the concentration of the host **4** relative to the constant concentration of IDA in water at 25 °C

hydrodynamic radii of fluorescent guests. It was already used in a few cases for the determination of complex stabilities [22]. Because of its high sensitivity it becomes favorable as soon as the guest is very costly and toxic like IDA. The anisotropy value r is close to zero for unbound fluorophores in solution due to rapid rotational diffusion. Complexation of the fluorophore by CD slows it down leading to an increase of r. We observed, that fluorescence anisotropy of IDA indeed increased with increasing concentration of **4**, as shown in Fig. 4. The dependence of r on the concentration of CD was fitted by Eq. 3, appropriate for 1:1 complex formation [23], leading to a binding constant $K = 9,739$ M^{-1}.

The data in Table 2 clearly demonstrate the superior binding constants of the heptakis-6-thio-ethers **3–9** compared to the ones of native β-CD, and the heptakis-6-amino derivative **2** and the ethers HPβCD and RAMEB. The binding constants were found to be one or two orders of magnitude higher than the binding constants of the reference compounds. This increase was attributed to the higher hydrophobicity of sulfur atoms compared to oxygen and nitrogen atoms. These 7 sulfur atoms elongate the hydrophobic cavity of β-CD. The binding constant K becomes higher the more methylene groups are attached to the CD cavity for the same reason. On the other hand, branching of the attached alkyl chain leads to a reduction of the binding constant, as shown for host **9**. These bulky substituents might hinder the guest entering the cavity from the primary side.

The four guests of this investigation have similar hydrophobic binding sites in common. In all cases benzene moieties are prone for complexation by β-CD rings, since they are only mono-substituted (DOC) or ortho-disubstituted (CPT, DHA, IDA). CPT shows the lowest binding constants

in Table 2 since the highly hydrophilic quinoline nitrogen, protonated under the experimental conditions (pH = 1.7) appears to disturb inclusion compound formation. The exceptionally high binding constants for the model compound DHA are attributed to the absence of any charged group in the molecule. Also the binding constant of DOC in the hepta-cystaminyl derivative **4** is significantly high coming close to the binding constant of a β-CD dimer ($K = 150,000$ M^{-1}) [21].

Since IDA is a cationic guest it behaves differently from its model compound DHA. On one hand, the anionic hosts **5**, **6**, **7** and **9** perform better than for DHA due to Coulomb attraction between host and guest. On the other hand the cationic host **4** performs worse because of Coulomb repulsion. Less pronounced differences between binding of DHA and IDA were found for the neutral hosts **3** and **8**. Similar recognition effects caused by Coulomb interactions between hosts and guests, so-called salt bridges, were already described in the past [14, 26].

Conclusion

Ionic heptakis-(6-deoxy-6-thioethers) of β-CD are well suited for both the complexation and solubilization of anticancer drugs such as camptothecin, docetaxel and idarubicin. The high binding abilities of these β-CD derivatives were attributed to the hydrophobicity of the seven sulfur atoms which elongate the hydrophobic cavity of β-CD. So-called salt bridges between oppositely charged host and guest further stabilize these inclusion compounds significantly.

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