THE JOURNAL OF PHYSICAL CHEMISTRY B

Article

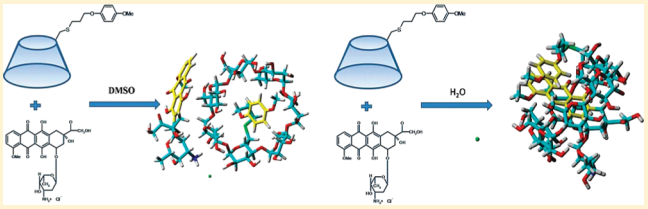
pubs.acs.org/JPCB

Intermolecular Interactions between Doxorubicin and β-Cyclodextrin 4-Methoxyphenol Conjugates

Olga Swiech, Anna Mieczkowska, Kazimierz Chmurski, and Renata Bilewicz*

Department of Chemistry, University of Warsaw, Pasteura 1, 02-093 Warsaw, Poland

ABSTRACT: Newly synthesized derivatives of β-cyclodextrin, mono(6-deoxy-6-(1−1,2,3-triazo-4-yl)-1-propane-3-O-(4-methoxyphenyl))β-cyclodextrin (1) and mono(6-deoxy-6thio-(1-propane-3-O-(4-methoxyphenyl))) β-cyclodextrin (2) were designed to be receptors of the anticancer drug doxorubicin, which could potentially decrease the adverse effects of the drug during treatment. In both aqueous and aqueous dimethyl sulfoxide (DMSO) solutions, doxorubicin forms an inclusion complex with the new cyclodextrin derivatives with formation constants of K_s = 2.3 × 10^4 and K_s = 3.2 × 10^5 M^{-1} for cyclodextrins 1 and 2, respectively. The stabilities of the complexes are 2−3 orders of magnitude greater than those with native β-cyclodextrin, and the flexibility of the linker of the side group of the cyclodextrins contributes to this stability. In a hydrogen-bond-accepting solvent, such as pure DMSO, an association that includes hydrogen bonding and chloride ions is favored over the binding of doxorubicin in the cavity of the cyclodextrin derivative. This contrasts with an aqueous medium in which a strong inclusion complex is formed. Cyclic voltammetry, UV−vis, ^{1}H NMR, and molecular modeling studies of solutions in DMSO and of solutions in water/DMSO demonstrated that the two different modes of intermolecular interaction between doxorubicin and the cyclodextrin derivative depended on the solvent system being utilized.



INTRODUCTION

Anthracycline drugs have been used for nearly fifty years for the treatment of many malignancies, and hundreds of analogs of the first anthracycline antibiotics, doxorubicin and daunorubicin, have been synthesized and evaluated. The clinical effects are associated with modification of the DNA structure primarily through intercalating complexes and covalent bonding.[1,2] Multiple molecular mechanisms have been proposed to explain the cytostatic and cytotoxic effects induced by these drugs and the disturbances caused in processes such as replication, transcription, or immobilization of DNA, repair mechanisms, and apoptosis. The specific toxicity induced by anthracyclines is caused by the formation of reactive oxygen species from the redox reactions of these drugs. A semiquinone is formed as a result of an electron transfer to the quinone group of the drug. When the semiquinone is transformed back to a quinone, oxygen is reduced to a reactive oxygen species with a different level of toxicity: superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and the especially toxic hydroxyl radical ($HO^{\bullet}$) (Scheme 1).

An equally dangerous reaction is the formation of hydroxyl radicals by free iron cations in the Fenton reaction[3] (Scheme 1). In addition, the semiquinone can break the glycosidic bond in the drug molecule, leading to the formation of an aglycone molecule, which penetrates the membrane and releases an even larger amount of reactive oxygen species.[4,5]

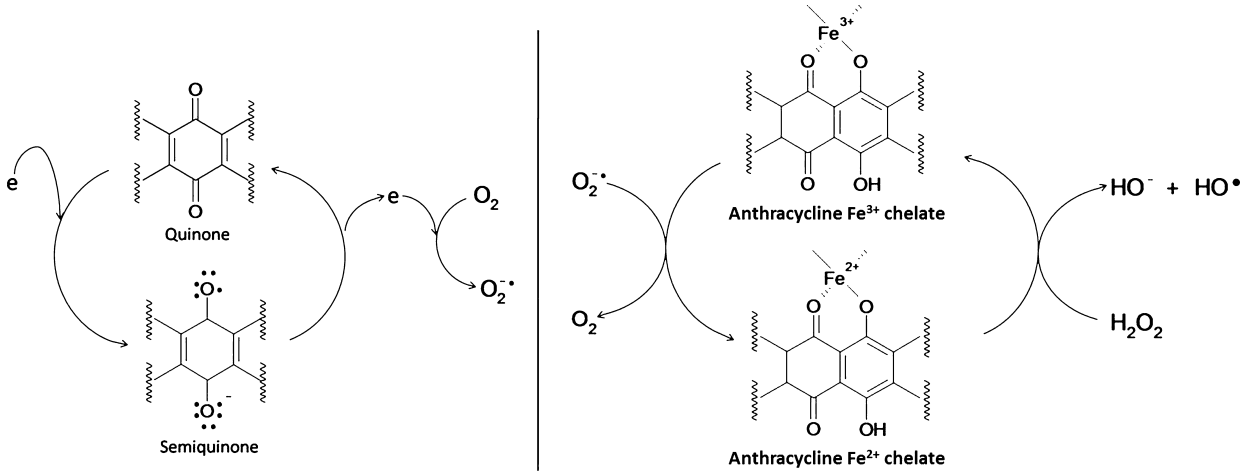
A serious complication associated with the treatment of cancer patients is the extravasation of anthracycline agents during administration.[6,7] Recently, the topical application of dimethyl sulfoxide (DMSO) has been proposed to decrease the effects of doxorubicin on subcutaneous tissue. DMSO penetrates easily into tissues, and the transfer of DMSO across the dermal barrier is rapidly accomplished without irreversible tissue damage. DMSO enhances the return of tissue-entrapped drug to the circulation and is able to scavenge anthracycline-generated oxygen radicals to some extent.

To prevent the adverse action of active oxygen species, the quinone group responsible for their production can be blocked until the delivery of drug molecules to the pathologically changed cells occurs. Such blockage can be achieved by complexing the anthracycline molecule with appropriate nanoscale carriers. Common drug nanoparticulate carriers include pegylated liposomes and anionic polymers. The cationic drug forms complexes with polyelectrolytes, e.g., polyglutamates, polyacrylates, polyaspartates, or dextran sulfate. Electrostatic interactions, aromatic stacking, and hydrogen bonding play a role in such complexes.[8]

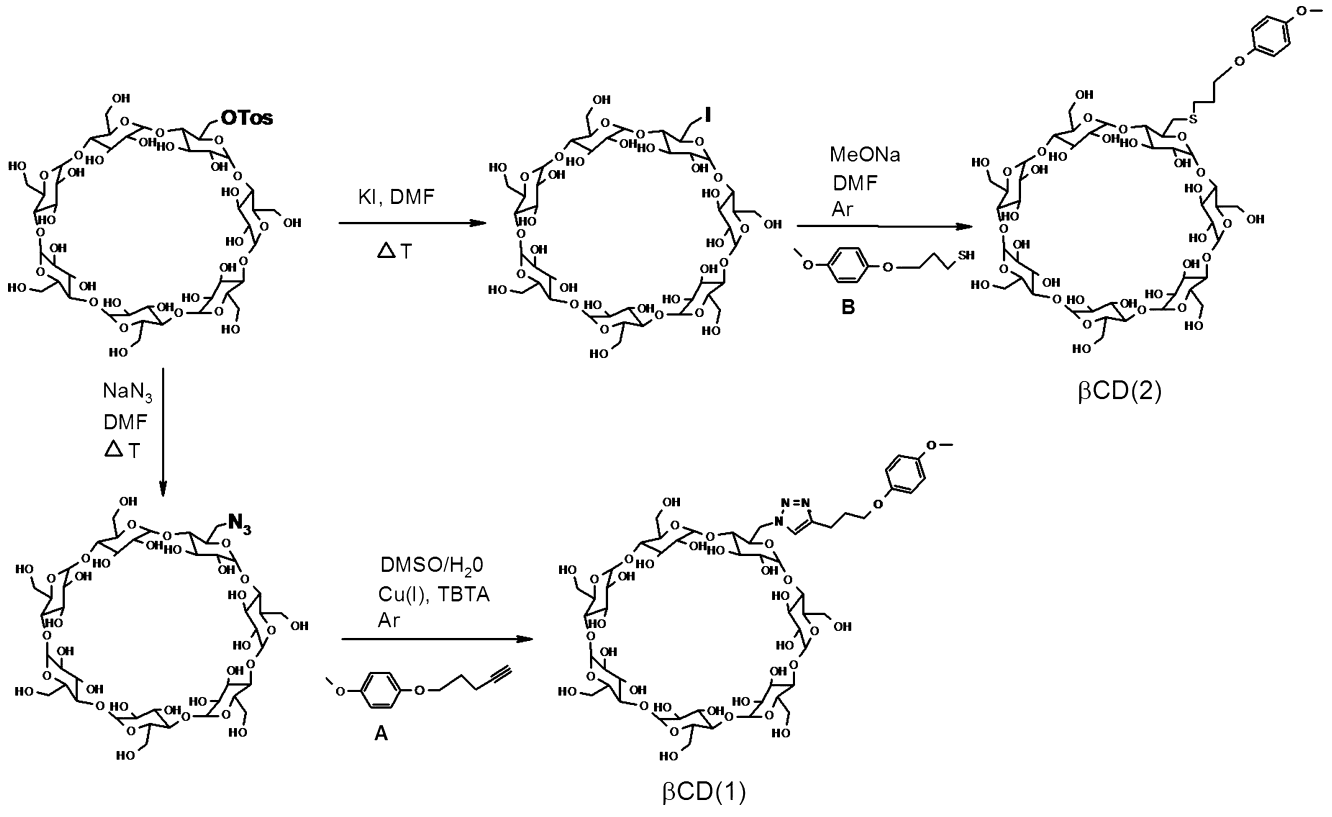
Blockage can also be achieved by complexing the anthracycline molecule using cyclic oligosaccharides, such as cyclodextrins (CDs), as receptors.[9] Previous studies of anthracycline−CD complexes have shown that the doxorubicin molecule fits into the cavity of the CD from the quinone side.[10] The limitation with using CD as the carrier for anthracycline drugs is the low stability of the complex. The stability constants are many orders less than those of the drug−DNA complexes, e.g., 2.1 × 10^2 M^{-1} and 5.4 × 10^5 M^{-1} for β-cyclodextrin (βCD)−Dox and Dox−DNA, respectively.[11,12] Modification of CD with an appropriate functional group can increase the stability of the CD−drug complex, and stability constants that

Received: September 21, 2011
Revised: January 26, 2012
Published: January 27, 2012

Scheme 1. Toxic Reaction of Anthracyclines



Scheme 2. Syntheses of βCD(1) and βCD(2)



are an order of magnitude greater than that of the drug–DNA complex have been reported by Thiele et al. These authors reported increased stabilities for the inclusion compounds of three chemotherapeutic agents, camptothecin (CPT), docetaxel (DOC), and idarubicin (IDA), and a model compound 1,4-dihydroxyanthraquinone (DHA) with heptakis-6-substituted βCD derivatives.[13] Yamanoi et al. prepared a βCD-conjugated with two arbutin moieties and reported, based on surface plasmon resonance measurements, an extremely high association constant of 1.4×10^8 M^{-1} with doxorubicin, which was caused by the stacking effect between the substituent and doxorubicin.[14]

Our goal was to prepare novel βCD derivatives that contained electron-rich aromatic substituents with two different linkers (Scheme 2) and to evaluate their potential as ligands for drug complexation. Using cyclic voltammetry, UV–vis, ^{1}H NMR, and molecular modeling methods, we investigated the modes of interaction of the CD with the drug and the factors that affect the stability of the complex. Because DMSO is often employed in the drug treatment and is useful for the solubilization of CD derivatives, we selected mixed water–DMSO solutions and pure DMSO as the solvents.

MATERIALS AND METHODS

Chemicals and Reagents. βCD hydrate, 4-butyn-1-ol, 1,3-dibromopropane, and 4-methoxyphenol were purchased from Aldrich and used as supplied. *p*-Toluenesulfonic anhydride was synthesized according to a literature procedure.[15] Tris[(1-

dx.doi.org/10.1021/jp2091363 | J. Phys. Chem. B 2012, 116, 1765–1771

benzyl-1*H*-1,2,3-triazole-4-yl)methyl]amine (TBTA) was prepared using the method of Sharpless.[16] Doxorubicin hydrochloride salt was purchased from LC Laboratories (Woburn, USA). The remainder of the compounds used in this work for the syntheses were purchased from Aldrich and Fluka. The buffers were prepared using water from a Milli-Q ultrapure water system. Britton-Robinson buffer (BR, pH = 7) was prepared in the usual way by the addition of appropriate amounts of 0.2 M sodium hydroxide to orthophosphoric acid, acetic acid and boric acid (0.04 M solutions). The ionic strength was increased to 0.2 M by adding an appropriate amount of potassium perchlorate. The pH was measured using a PHM240 MeterLab pH meter (Radiometer Copenhagen).

Preparation of βCD 4-Methoxyphenol Conjugates (1) and (2). Mono(6-O-tosyl) βCD was prepared from βCD hydrate according to the procedure of Bitmann et al.[15] using *p*-toluenesulfonic acid anhydride and an aqueous solution of sodium hydroxide. The βCD monotosylate was used to prepare the mono(6-azido-6-deoxy-) and mono(6-deoxy-6-iodo)βCD derivatives by the nucleophilic displacement of the tosylate with an azide or iodide anion, respectively, at elevated temperature in dimethylformamide (Scheme 2).

4-Butyn-1-ol was coupled with *p*-methoxyphenol under Mitsunobu conditions to afford alkyne derivative **A**.[17] The latter was used for azide−alkyne coupling with the monoazido βCD derivative in the presence of Cu(I) and TBTA, which was used as a Cu(I) stabilizer, in a DMSO:water mixture under an atmosphere of argon.

Mono(6-deoxy-6-(1−1,2,3-triazo-4-yl)-1-propane-3-O-(4-methoxyphenyl)) βCD (**1**) was isolated in 96% yield. TOF MS ES+ *m*/*z* 1350.51 $[M+Na]^+$; ^{1}H NMR (200 MHz, CD_3SOCD_3) δ 7.82 (s 1H (1,2,3 triazoyl H)), 6.85 (s 4H aryl), 5.76 (bs 20H OH), 4.86−4.83 (7H 2 br d H-1), 3.95 (t 2H O−C$\underline{H}_2$), 3.68 (s 3H O−C$\underline{H}_3$), 3.63−3.31 (2 m 36 H remaining sugar H), 2.74(t 2H C$\underline{H}_2$-4(1,2,3 triazole)), 2.00 (t 2H C$\underline{H}_2$-CH$_2$-4(1,2,3 triazole)); ^{13}C NMR (50.28 MHz, CD_3SOCD_3) δ 153.01, 152.48, 115.19, 114.43 (phe) 101.81 (C1), 81.31 (C4) 72.58−70.18 (C2,C3,C5), 55.18 (O-C$\underline{H}_3$) 67.12(O-C$\underline{H}_2$), 59.66 (C-6), 28.47 (CH$_2$-C$\underline{H}_2$−CH$_2$), 21.47 (C$\underline{H}_2$-4(1,2,3 triazole)),

3-O-(4-Methoxyphenyl)-propane-1-thiol (**B**) was prepared from 4-methoxyphenol and 1,3-dibromopropane followed by the exchange of bromine with thiol utilizing thiourea. Thiol **B** was treated with sodium methoxide under an argon atmosphere, and subsequently, S-alkylated was treated with the mono(6-deoxy-6-iodo)-βCD derivative in DMF to produce mono(6-deoxy-6thio(1-propane-3-O-(4-methoxyphenyl)))-βCD (**2**) in 83,6% yield. TOF MS ES+ *m*/*z* 1338.4 $[M+Na]^+$; ^{1}H NMR (500 MHz CD_3SOCD_3) δ6.84 (s, 4H phe), 5.76 (bs 20H OH), 4.86−4.83 (7H 2d H-1), 3.95 (t 2H O−C$\underline{H}_2$), 3.69 (s 3H O−C$\underline{H}_3$), 3.65−3.2 2 (m 36 H sugar H), 2.67 (t 2H C$\underline{H}_2$-S), 2.50 (t 2H C-6$\underline{H}_2$-S), 1.89 (m 2H CH$_2$−C$\underline{H}_2$-CH$_2$); ^{13}C NMR (125.8 MHz, CD_3SOCD_3) δ 154.14, 152.29, 115.26, 114.42 (phe) 102.12−101.46 (C1) 81.30−81.22 (C4) 78.88−70.96 (C2, C3, C5), 55.18 (O-C$\underline{H}_3$) 66.13(O-C$\underline{H}_2$), 33.06 (CH$_2$-C$\underline{H}_2$−CH$_2$), 20.61 (C$\underline{H}_2$−S), 28.78−28.69 (C-6)

Solubility of the Compounds. The solubility of doxorubicin was determined by LC Laboratory (Woburn, USA) at 10 mg/mL and 100 mg/mL in water and DMSO, respectively. The solubility of native βCD in water is 18.5 mg/mL, and its solubility increases in an irregular manner after the addition of DMSO. At less than 30% DMSO, the solubility remains constant (20 mg/mL). Between 30 and 40% DMSO, the solubility rapidly increases to 770 mg/mL. From 40 to 86%, the solubility is constant but then decreases to 500 mg/mL in 100% DMSO.[18] The solubilities of βCD(1) and βCD(2) were determined using UV−vis spectroscopy. The values for the solubility in pure water are less than that of native βCD and equal to 2.18 mg/mL and 0.28 mg/mL for βCD(1) and βCD(2), respectively. In a mixture of water/DMSO (2:1), the solubility increases to 107.9 mg/mL for βCD(1) and 6.10 mg/mL for βCD(2).

Electrochemical Measurements. Electrochemical measurements were performed using a PGSTAT Autolab (Eco Chemie BV, Utrecht, Netherlands). All electrochemical experiments were performed in a three-electrode arrangement with a silver/silver chloride (Ag/AgCl) electrode in a saturated solution of KCl as the reference, platinum foil as the counter and an Au electrode (BAS, 2 mm diameter) or GC electrode (BAS, 3 mm in diameter) as the working electrodes. The working electrodes were polished mechanically with 1.0, 0.3, and 0.05 μm of alumina powder on a Buehler polishing cloth. Prior to measurements, the buffer solutions were purged with purified argon for 30 min, and all experiments were performed at room temperature. Milli-Q ultrapure water (resistivity 18.2 MΩ/cm) was used. Experiments in DMSO were performed using a 0.5 M solution of tetrabutylammonium hexafluorophosphate.

Spectroscopic Measurements. UV−vis spectroscopic measurements were performed using a UV−vis EVOLUTION60 spectrophotometer with a 1-cm acryl cell. ^{1}H NMR spectra were obtained with a Bruker Avance 500 MHz (^{1}H frequency) spectrometer. All spectroscopic analyses were conducted at room temperature. Experiments in DMSO were performed in the absence of daylight.

Molecular Modeling. All theoretical calculations were performed with YASARA[19] using force field AMBER03[20] with periodic boundary conditions. The system consisted of ca. 2700 atoms including CD, the hydrochloride salt of doxorubicin (Dox), and solvent (water or DMSO). Several system configurations were considered including Dox placed inside CD and Dox at the entrance of CD. During the preparation stage, the models of molecules were parametrized and partial charges were obtained using semiempirical methods.[21] Each simulation lasted 100 ns and was preceded by energy minimization.

Calculation of Formation Constants from Spectroscopy and Voltammetric Data. The formation constants of the donor−acceptor associate were calculated using the Benesi−Hildebrand method from the UV−vis data assuming one-to-one associates[22]

$$\frac{A_0}{A - A_0} = \frac{\varepsilon_D}{\varepsilon_{D-A} - \varepsilon_D} + \frac{\varepsilon_D}{\varepsilon_{D-A} - \varepsilon_D}\frac{1}{[CD]\cdot K_s} \qquad (1)$$

where A is the observed absorption and A_0 the absorption of free doxorubicin. K_s is the formation constant. According to eq 1, the ratio of the slope and the intersection from the $A_0/(A-A_0)$ vs $1/[CD]$ plot provides the value of the formation constant.

For the calculation of the formation constant of CD:Dox complexes from cyclic voltammetry experiments, the Osa equation was used[23]

$$I^2_{obs} = \frac{(I^2_{Dox} - I^2_{obs})}{K_s\cdot[CD]} + I^2_{Dox:CD} \qquad (2)$$

dx.doi.org/10.1021/jp2091363 | J. Phys. Chem. B 2012, 116, 1765−1771

where I_{obs} is the observed reduction peak current and I_{Dox} and $I_{Dox:CD}$ are the reduction peak currents for the free doxorubicin and the inclusion complex, respectively. K_s is the complex formation constant, and [CD] is the concentration of CD. The value of K_s was calculated from the slope of the linear plot of I^2_{obs} vs $(I^2_{Dox} - I^2_{obs})/[CD]$.

RESULTS AND DISCUSSION

The Interaction of βCD Derivatives with Doxorubicin in DMSO Solutions. Because DMSO is often used to decrease the adverse effects of doxorubicin treatment, we initially studied the intermolecular interactions of Dox and CDs using pure DMSO as the solvent. The UV–vis spectra of doxorubicin in the presence of increasing concentrations of βCD(1) in DMSO solution are shown in Figure 1. The absorption peaks at

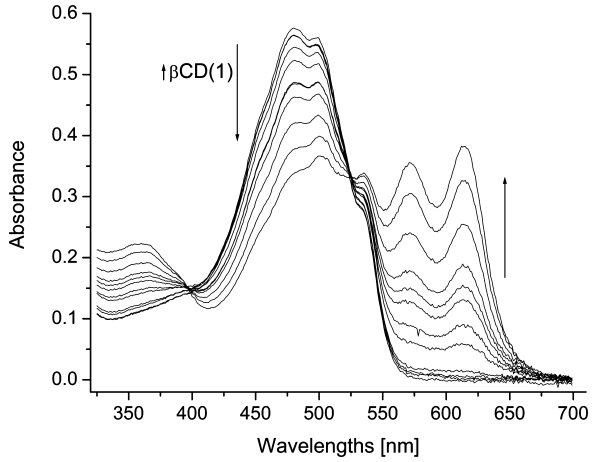


Figure 1. (A) UV–vis spectra of 5×10^{-5} M doxorubicin solution in DMSO with increasing concentrations of βCD(1): 5.0×10^{-5}, 1.0×10^{-4}, 2.5×10^{-4}, 5×10^{-4}, 6×10^{-4}, 7.5×10^{-4}, 8.5×10^{-4}, 1.0×10^{-3}, 1.25×10^{-3}, 1.5×10^{-3}, 1.8×10^{-3}, 1.9×10^{-3}, 2.1×10^{-3}, and 2.8×10^{-3} M.

wavelengths 479 and 496 nm decrease after the addition of βCD(1). However, there are two new peaks at wavelengths 594 and 642 nm, which appear and increase upon the addition of βCD(1).

Similar changes in the spectra were observed for βCD(2). The shift for βCD(1) was observed only when the concentration ratio of Dox:βCD(1) was 1:10 or higher. A change in the color of the solution from orange to dark blue occurred (Figure 2) but only in hydrogen-bond-accepting solvents, such as DMSO. Even a small addition of water leads to the disappearance of the blue color. With native CD, the blue-colored species is not formed (Figure 2). Similar spectral changes have been observed when anthracyclines were reacted with KO_2 crown ether complex in DMSO[24] and have been ascribed to the formation of the anthracycline phenolate anion. Anthracyclines are known to produce blue species in basic solutions that is caused by ionization of the phenolic proton. It has been postulated that similar reactions of superoxide with anthracyclines in vivo may play a role in the antitumor activity of these drugs.[25]

The proton acceptor nature of DMSO, which promotes the dissociation of the compounds and the generation of naked anionic forms, is well described in the literature.[26,27] Proton donor properties of the two CDs 1 and 2 were clearly different, with βCD(2) as the better proton donor.

Figure 2. Photograph of the DMSO solutions of Dox, βCD(2), the mixture of native βCD with Dox, and the mixture of βCD(2) with Dox.

To better understand the interactions of CDs with doxorubicin in DMSO, ^{1}H NMR experiments were performed for βCD(2) in DMSO-d_6 (Figure 3C).

The peak in the proton spectrum of the hydroxyl groups at carbons C2 and C3 of CD change from a broad singlet at 5.55 ppm to a group of at least three multiplets as the concentration of doxorubicin is increased. The same changes were observed for the protons of the hydroxyl groups at the C6 carbon of CD and reflect hydrogen-bonding interaction between the hydroxyl groups of CD and Dox. The aromatic protons of doxorubicin at the C1 and C2 positions of ring A do not show a change in chemical shifts (Figure 3B).

The ^{1}H NMR spectrum of pure Dox in DMSO-d_6 shows two peaks downfield with chemical shifts of 13.28 ppm and 14.06 ppm. These downfield chemical shifts indicate the formation of intramolecular hydrogen bonds between the hydroxyl groups on the C ring of doxorubicin and its quinone groups in ring B (Figure 3A). Even the small addition of a CD derivative removes the intramolecular hydrogen bonding in the Dox molecule because of the intermolecular binding with the C2, C3, and C6 hydroxyl groups of βCD (Figure 3B).

The absence of changes in the chemical shift of the protons of the methoxy group on the phenyl in the side chain of CD (6.85 ppm) when the molar ratio of Dox to βCD is altered indicates that the phenyl substituent resides in the cavity of the CD both in the absence and presence of doxorubicin (Figure 4). Taking the resonance structures of 4-methoxyphenyl units into consideration, the oxygen atoms of the substituent would be expected to donate electron density into the phenyl ring and gain a partial positive charge. Their interactions with the neighboring hydroxyl groups at the C2, C3, and C6 carbons of CD (Figure 3B) increase the acidity of these hydroxyl groups. Such changes in the CD favor the breaking of the intramolecular hydrogen bond at the B and C rings (Figure 3A) of doxorubicin. The free phenolic OH groups of ring C may lose their protons when reacting with proton acceptors that are present in the solution, with naked chloride ions or with the solvent itself. This change in the chromophore structure results in the transition to a blue color.

The spectrum of free doxorubicin showed two proton peaks from the amino group of doxorubicin, a doublet at 7.81 ppm, which corresponds to the NH_3^+ group, and a broad singlet at 4.57 ppm corresponding to the NH_2 group. The presence of these two signals indicates an equilibrium between NH_3^+ and

dx.doi.org/10.1021/jp2091363 | J. Phys. Chem. B 2012, 116, 1765–1771

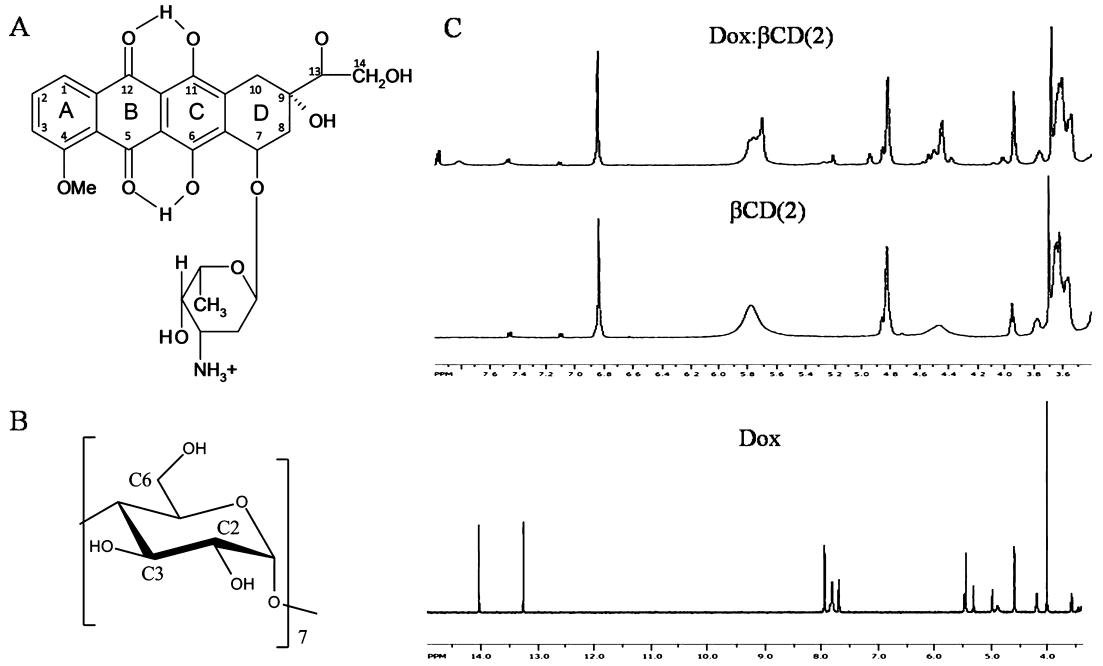


Figure 3. (A) The structure of doxorubicin showing the intramolecular hydrogen bonds. (B) The structure of βCD and (C) NMR spectra for Dox, βCD(2), and the βCD(2) + Dox mixture.

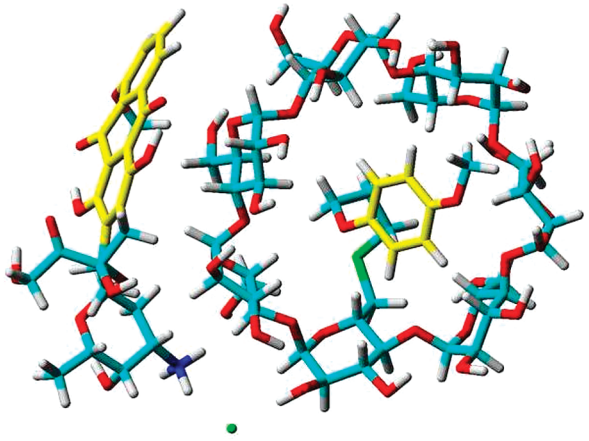


Figure 4. Molecular modeling of the βCD(2) interaction with doxorubicin in DMSO solution in the absence of water.

NH_2. The ratio of these signals changes from 2:1 to 1:2 for free Dox and Dox:βCD(2) (1:1), respectively. These changes reflect the interaction of the doxorubicin amino group with βCD(1) and βCD (2) in aprotic solvents. Simultaneously, the chemical shifts of the protons on the sugar of doxorubicin were observed.

These ^{1}H NMR observations confirm that with pure DMSO-d_6 as the solvent doxorubicin does not reside in the cavity of CD but interacts with CD solely by means of hydrogen bonds because the hydroxyl groups at the C2, C3, and C6 positions of CD are good hydrogen bond donors in DMSO.

On the basis of the spectroscopic results, the formation constants of doxorubicin phenolate in the presence of βCD(1) and βCD(2) were calculated using eq 1. The values obtained for K_s were 121.1 ± 6.2 M^{-1} and 6768 ± 41 M^{-1} for βCD(1) and βCD(2), respectively. The formation constant in the case of βCD(2) that has a more flexible linker is more than an order of magnitude greater than that of the CD with a triazole in the linker.

Molecular modeling confirmed that the substituent, and not doxorubicin, is self-included in the cavity of the CD in DMSO (Figure 4). The trajectories for this system in DMSO also suggest that the naked chloride ion can interact with the hydroxyl groups of the sugars in the CD ring and with the amine group of doxorubicin to form hydrogen-bonded complexes. These complexes in DMSO were favored for βCD(2), while those for βCD(1) with the less flexible side chains dissociated after the complexes were formed. For comparison, modeling was also performed for the native CDs and doxorubicin and showed only sporadic and short-lived complexes in DMSO. This result confirms the inclusion of the side arm substituents of the CD derivatives in DMSO. In the case with the more flexible side arm (βCD(2)), the aromatic moiety enters the cavity more easily than with βCD(1). Thus, the hydrogen atoms of the βCD(2) hydroxyl groups become more acidic, and their tendency to form hydrogen-bonded complexes increases.

Interaction of βCD Derivatives with Doxorubicin in a Mixed Solution of Water:DMSO. Because of the limited solubility of CD complexes in water, experiments were conducted in a mixture of Britton−Robinson buffer and DMSO (2:1 ratio). After the addition of βCD(1) or βCD(2) to the solution of doxorubicin in the mixed solvent, a decrease of the voltammetric peak current was observed that indicates formation of the complex. The dependence of the reduction peak current of Dox on the concentration of βCD(1) is shown in Figure 5A.

The formation constants of 1:1 CD complexes calculated using eq 2 were found to be 2.3 × 10^4 ± 0.2 and 3.2 ± 0.2 × 10^5 $M^{-1}$for βCD(1) and βCD(2), respectively (Figure 5).

Molecular modeling confirmed the formation of the inclusion complex between doxorubicin and βCD(1) or βCD(2) (Figure 6). The systems with water have lower energy, and the average distance between the aromatic groups (phenyl or 1,2,3-triazole) of the side chain of the modified CD

dx.doi.org/10.1021/jp2091363 | J. Phys. Chem. B 2012, 116, 1765−1771

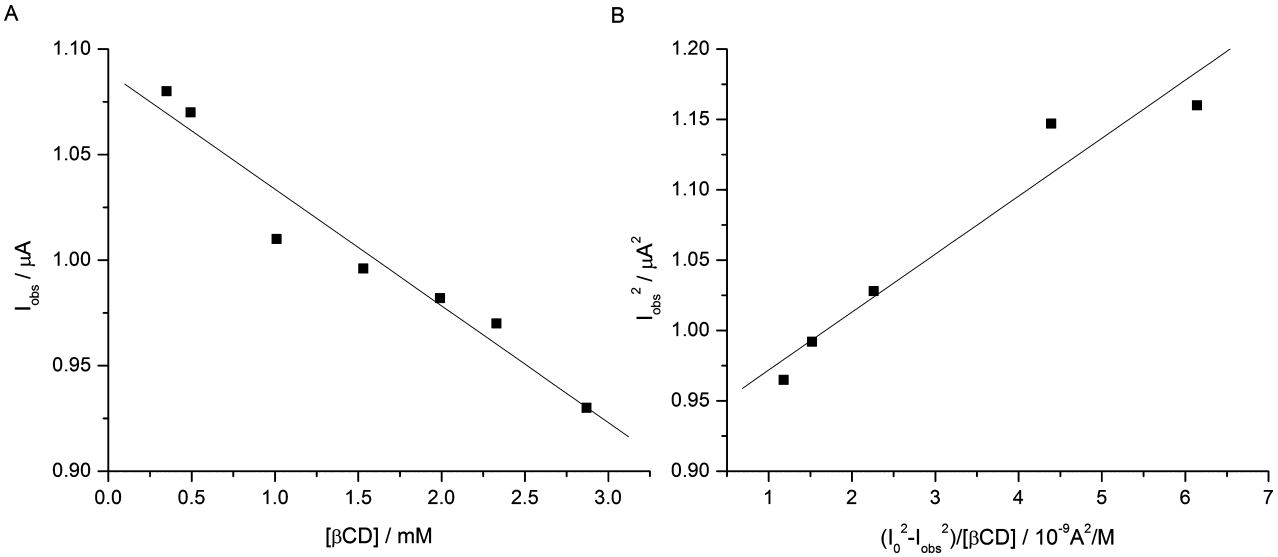


Figure 5. (A) Dependence of reduction peak current for 5×10^{-5} M Dox on the concentration of βCD(1) recorded in Britton–Robinson buffer:DMSO (2:1). ν = 50 mV/s. (B) The plot of the Osa eq 2 for Dox in the presence of βCD(1).

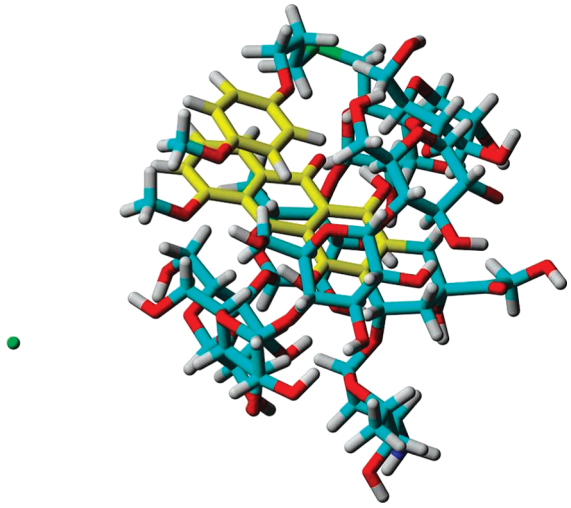


Figure 6. Molecular modeling of the structure of the βCD(2) doxorubicin complex in water.

and the aromatic ring A of doxorubicin (Figure 4) is smaller than that in pure DMSO.

In the case of βCD(1), interactions between both the aromatic triazole and phenyl groups with ring A of doxorubicin may occur. The electron deficient ring A of doxorubicin can interact with the aromatic groups of the side chain in βCD(1). The 1,2,3-triazole moieties, which reside close to the inclusion cavity, should be more prone to interactions. In contrast, the larger stability constant for βCD(2) indicates that the flexibility of the linker plays a dominant role in these interactions. Molecular modeling of βCD(2) revealed the possibility of strong $\pi-\pi$ interactions between the aromatic phenyl ring of the CD side group and ring A of doxorubicin as shown in Figure 6.

CONCLUSIONS

In this study, we have shown that newly designed and synthesized derivatives of βCDs form strong inclusion complexes with doxorubicin in mixed water:DMSO solutions with high formation constants, 2.3×10^4 M^{-1}and 3.2×10^5 M^{-1} for βCD(1) and βCD(2), respectively. The stability constants of the doxorubicin complexes with the CD derivatives that have a single pendant 4-methoxyphenyl-terminated arm are 2–3 orders of magnitude greater than those of the complexes with native βCD. The formation of the inclusion complex requires the presence of water. In dry DMSO, formation of the complex does not occur because doxorubicin cannot compete with the side chain of CD for space in the cavity. The blue species detected in DMSO are the phenolate anions that are formed as a result of proton abstraction from one of the weakly acidic phenolic groups at C6 or C11 of anthracycline. Interestingly, in DMSO some interactions of doxorubicin with modified CD also exist, and they involve the more acidic hydrogen atoms of the CD hydroxyl groups and chloride anions. Modeling performed for the native CDs and doxorubicin showed only sporadic and short-lived complexes in DMSO, which confirms the importance of the inclusion of the side arm substituent of the CD derivative in the mechanism in DMSO. On the basis of the ^{1}H NMR, UV–vis spectroscopy, and molecular modeling results, the formation of a hydrogen-bonded associate with the self-included aromatic group of the CD side-chain is proposed to explain the behavior of the doxorubicin–CD derivative system in dry DMSO.

AUTHOR INFORMATION

Corresponding Author

*Phone/Fax: +48 22 8220211/+48 22 8225996. E-mail: bilewicz@chem.uw.edu.pl.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by the Polish Ministry of Sciences and Higher Education and the National Center for Research and Development (NCBiR), Grant No. NR05-0017-10/2010 (PBR-11), and by the Faculty of Chemistry, University of Warsaw. We would like to thank Alexander Debinski for help with the molecular modeling and discussion of the results.

REFERENCES

(1) Chaires, J. B.; Herrera, J. E.; Waring, M. J. *Biochemistry* **1990**, *29*, 6145−6153.
(2) Quigley, G. J.; Wang, A. H.; Ughetto, G.; Van Der Marel, G.; Van Boom, J. H.; Rich, A. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 7204−7208.
(3) Šimùnek, T.; Štìrba, M.; Popelová, O.; Adamcová, M.; Hrdina, R.; Geršl, V. *Pharm. Rep.* **2009**, *61*, 154−171.
(4) Green, P. S.; Leeuwenburgh, C. *Biochim. Biophys. Acta* **2002**, *1588*, 94−101.
(5) Licata, S.; Saponiero, A.; Mordente, A.; Minotti, G. *Chem. Res. Toxicol.* **2000**, *13*, 414−420.
(6) Rospond, R. M. *J. Oncol. Pharm. Pract.* **1995**, *1*, 33−39.
(7) Laufman, L. R.; Sickle-Santanello, B.; Paquelet, J. *Commun. Oncol.* **2007**, *4*, 464−465.
(8) Yousefpour, P.; Atyabi, F.; Farahani, E. V.; Sakhtianchi, R.; Dinarvand, R. *Int J. Nanomed.* **2011**, *6*, 1487−1496.
(9) Uekama, K.; Hirayama, F.; Irie, T. *Chem. Rev.* **1998**, *98*, 2045−2076.
(10) Bekers, O.; Kettenes-Van Der Bosch, J. J.; Van Helden, S. P.; Seijkens, D.; Beijnen, J.; Bult, A.; Underberg, W. J. M. *J. Incl. Phenom. Mol. Recog. Chem.* **1991**, *11*, 185−193.
(11) Husain, N.; Ndou, T. T.; Munoz De La Pena, A.; Warner, I. M. *Appl. Spectrosc.* **1992**, *46*, 652−658.
(12) Schneider, Y. J.; Baurain, R.; Zenebergh, A.; Trouet, A. *Cancer Chemother. Pharmacol.* **1979**, *2*, 7−10.
(13) Thiele, C.; Auerbach, D.; Joung, G.; Wenz, G. J. *Incl. Phenom. Macrocycl. Chem.* **2011**, *69*, 303−307.
(14) Yamanoi, T.; Kobayashia, N.; Takahashib, K.; Hattorib, K. *Lett. Drug Des. Discov.* **2006**, *3*, 188−191.
(15) Zhong, N.; Byun, H. S.; Bittman, R. *Tetrahedron Lett.* **1998**, *39*, 2919−2920.
(16) Chan, T. R.; Hilgraf, R.; Sharpless, K. B.; Fokin, V. V. *Org. Lett.* **2004**, *6*, 2853−2855.
(17) Mitsunobu, O.; Yamada, Y. *Bull. Chem. Soc. Jpn.* **1967**, *40*, 2380−2382.
(18) Chatijgakis, A. K.; Donze, C.; Coleman, A. W. *Anal. Chem.* **1992**, *64*, 1632−1634.
(19) Krieger, E.; Darden, T.; Nabuurs, S.; Finkelstein, A.; Vriend, G. *Proteins* **2004**, *57*, 678−683.
(20) Duan, Y.; Wu, C.; Chowdhury, S.; Lee, M. C.; Xiong, G.; Zhang, W.; Yang, R.; Cieplak, P.; Luo, R.; Lee, T. *J. Comput. Chem.* **2003**, *24*, 1999−2012.
(21) Jakalian, A.; Jack, D. B.; Bayly, C. I. *J. Comput. Chem.* **2002**, *23*, 1623−1641.
(22) Dang, X. J.; Nie, M. Y.; Tong, J.; Li, H. L. *J. Electroanal. Chem.* **1997**, *437*, 53−59.
(23) Osa, T.; Matsue, T.; Fujihira, T. *Heterocycles* **1977**, *6*, 1833−1839.
(24) Nakazawa, H.; Andrews, P. A.; Callery, P. S.; Bachur, N. R. *Biochem. Pharmacol.* **1985**, *34*, 481−490.
(25) Powis, G. *Pharmac. Ther.* **1987**, *35*, 57−162.
(26) Mukhopadhyay, M.; Banerjee, D.; Mukherjee, S. *J. Phys. Chem. A* **2006**, *110*, 12743−12751.
(27) Vasquez, T. E. Jr.; Bergset, J. M.; Fierman, M. B.; Neslon, A.; Roth, J.; Khan, S. I.; O'Leary, D. J. *J. Am. Chem. Soc.* **2002**, *124*, 2931−2938.