Inclusion excluded: Chiroptical sensing of the external surface of sulfated cyclodextrins

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ABSTRACT

It is shown that the heparin antagonist *bis*-aminoquinoline derivative surfen interacts with sulfated cyclodextrins in a unique fashion. Analysis of the UV spectroscopic data revealed exceptionally strong association ($K_a \sim 10^7\ M^{-1}$) of several surfen molecules to the external surface of the cyclodextrin hosts. H-bonded to the sulfate groups in 1:1 stoichiometry, the drug molecules form a chiral layer around the macrocycles. Due to the steric proximity, dipole–dipole coupling occurs between the adjacent amino-quinoline rings that accounts for the large UV hypochromism and the induced exciton couplet in the circular dichroism spectra.

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1. Introduction

Sulfated cyclodextrins (CDXs) are highly hydrophilic, effective chiral selectors for the separation of enantiomers of chemically diverse cationic and neutral drugs [1]. Attached to the hydroxyl sites of the macrocycle, sulfate moieties can form hydrogen and ionic bonds with guest compounds. Despite of the combination of polar and hydrophobic cavity interactions, inclusion complexes of sulfated CDXs are featured with low-stability ($K_a \sim 10^1$–$10^3\ M^{-1}$) and low capacity [2–5].

In contrast to these data, the present contribution demonstrates the unique, very high-affinity association of several surfen molecules on the outer surface of sulfated α-, β- and γ-CDX (the average degree of substitution (DS) is 11, 12, and 14, respectively). Surfen is a *bis*-aminoquinoline derivative (Scheme 1) that can bind to proteins [6,7] as well as anionic, linear-chain polysaccharides (*e.g.*, heparin, chondroitin sulfate, dermatan sulfate) modulating a number of functional glycosaminoglycan–protein interactions [8–10].

2. Materials and methods

2.1. Materials

Surfen (cat# 362,330, purity ≥ 98.00%) was obtained from Calbiochem. α-Cyclodextrin sulfate sodium salt (DS ~ 11, Mw 2095), β-cyclodextrin sulfate sodium salt (DS ~ 12, Mw 2360), γ-cyclodextrin sulfate sodium salt (DS ~ 14, Mw 2730) and natural cyclodextrins (α, β, γ) were supplied by Cyclolab Ltd. (Budapest, Hungary).

2.2. Preparation of surfen and CDX solutions

Stock solution of surfen was prepared freshly before each measurement in spectroscopy grade DMSO. The volume of DMSO added into the buffer samples during the spectroscopic titrations never exceeded 2% (v/v). Cyclodextrin samples were made in 50 mM, pH 7.4 Tris–HCl buffer containing 100 mM sodium chloride.

2.3. Circular dichroism (CD) and UV absorption spectroscopic measurements

All spectroscopic experiments were conducted in 50 mM, pH 7.4 Tris–HCl buffer containing 100 mM sodium chloride. CD and UV absorption spectra were recorded on a JASCO J-715
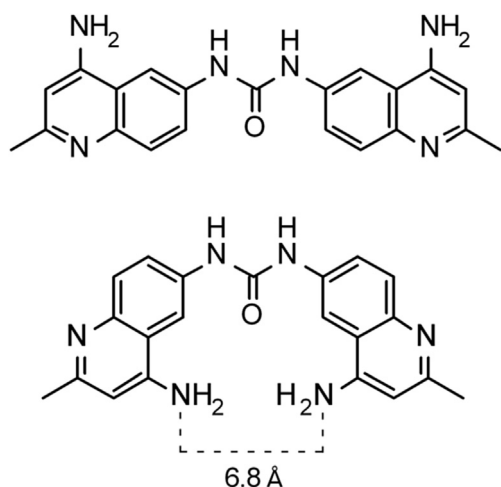
Scheme 1. Linear and crescent-shaped planar structures of surfen. The crescent-shaped form represents the structure found in the cocrystal complex of anthrax lethal factor (PDB id. 1PWP). Dotted line shows the nitrogen–nitrogen interatomic distance.

spectropolarimeter at 25 ± 0.2 °C. Temperature control was provided by a Peltier thermostat equipped with magnetic stirring. CD/UV titration experiments were performed in a rectangular quartz cell of 1 cm optical path length (Hellma, USA). Each spectrum represents the average of two scans obtained by collecting data at a scan speed of 200 nm/min. Absorption spectra were obtained by conversion of the high voltage (HT) values of the photomultiplier tube of the CD equipment into absorbance units. CD and UV curves of surfen–CDX and CDX–surfen mixtures were corrected by blank buffer or buffer solutions of CDXs, respectively. JASCO CD spectropolarimeters record CD data as ellipticity ('Θ') in units of millidegrees (mdeg).

2.4. Calculation of the CDX binding parameters of surfen

The surfen–CDX binding interaction can be quantified by the association constant (K_a):

$$S + CDX = SCDX; \quad K_a = \frac{[SCDX]}{[S][CDX]} \quad (1)$$

It is evident that

$$[S] = c_s - [SCDX] \quad (2)$$

and

$$[CDX] = c_{cdx} - [SCDX] \quad (3)$$

where c_s and c_{cdx} mean the total molar concentrations of surfen and CDX, respectively.

Since the formation of surfen–CDX complexes is responsible for the UV hypochromism (see Fig. 2), it can be written that

$$\varepsilon_0 - \varepsilon = k \, [SCDX] \quad (4)$$

where ε_0 is the molar absorption coefficient ($M^{-1} cm^{-1}$) of the main UV peak of surfen measured in CDX-free buffer solution, ε is the molar absorption coefficient of the same peak measured after addition of increasing amounts of CDXs into the sample solution and k is a constant. Using Equations (1)–(4), we obtain

$$(\varepsilon_0 - \varepsilon) = \frac{k}{2} \left(c_s + c_{cdx} + K_a^{-1} - \sqrt{\left(c_s + c_{cdx} + K_a^{-1} \right)^2 - 4 c_s c_{cdx}} \right) \quad (5)$$

where K_a is the surfen–CDX apparent averaged association constant.

In order to calculate the value of K_a, non-linear regression analysis was applied using NLREG® (statistical analysis program, version 3.4). Number of surfen molecules bound per a CDX host (n) was estimated by adjusting the molar concentration of surfen (c_s) during the curve fitting procedure. For instance, the best curve fit on the UV titration data obtained with sαCDX was achieved by entering 2.85 μM of surfen concentration instead of the experimental value of 32 μM. The ratio of 32/2.85 gives the average number of surfen molecules bound per a macrocycle (see Fig. 3).

3. Results and discussion

Gradual addition of the optically inactive drug into the buffer solution of sulfated CDXs induced an intense, oppositely signed CD band pair (Fig. 1). This CD couplet consists of a longer-wavelength positive and a shorter-wavelength negative peak with a zero cross-over point around 255–257 nm, close to the λ_{max} of the main UV absorption band of surfen. These spectroscopic features are characteristic to strong excitonic interactions between the $\pi-\pi^*$ transitions of the aminoquinoline rings of surfen molecules. The induced ellipticity − drug concentration plots exhibit a steeper ascending and a second, rather flat phase (Fig. S1 in Supplementary data). The CD extrema reached a saturation above 10:1 surfen:CDX molar ratio.

In comparison with the UV profile of free surfen molecules, the blue shifted (~8 nm) absorption peak displayed a significant hypochromism in the presence of sulfated CDXs (Fig. 1). The hypochromism is more prominent and can be better studied by a reverse titration adding sulfated CDXs to a constant amount of surfen. Each CDX produced a large, at least 50% reduction in the absorbance value (Fig. 2). In contrast to the CD titration data, the absorption changes were more uniform and thus could be used to estimate the apparent averaged host-guest affinity constants. Non-linear regression analysis of the molar absorption coefficients measured at increasing sulfated CDX/surfen ratios indicated exceptionally strong surfen–CDX association (Fig. 3). This tight binding interaction ($K_a \sim 10^7 M^{-1}$) enables the chiroptical detection of sulfated CDXs at a concentration as low as 10^{-8} M (Fig. S2 in Supplementary data).

In the case of sγCDX, the substantial deviation of the data set in the plateau phase of the titration prevented the calculation of the stability constant. However, the overall similarity of the UV titration profiles suggests a K_a value of the same order of magnitude for sγCDX, too (Fig. 3).

It was found that the best non-linear fit for the UV data occurs at 11:1 surfen–sαCDX and 12:1 surfen-sβCDX stoichiometry that correlates well with the average DS values of these host compounds. This suggests that each sulfate group binds a single surfen molecule on the outer surface of CDXs. In analogy with the stacked base pairs of DNA, the proximity of the sulfate substituents may favor $\pi-\pi$ stacking of the surfen molecules bound to them producing a large hypochromism in the UV spectrum. Due to the asymmetric spatial disposition of the sulfate groups, the intermolecularly stacked aminoquinoline rings form a supramolecular, chiral layer around the macrocycle resulting in strong exciton CD splitting of intermolecular origin. Application of the exciton coupling theory [11,12] predicts that the observed positive-negative CD feature (Fig. 1) correlates with the right-handed (P-helical) orientation of long axes of adjacent aminoquinoline moieties
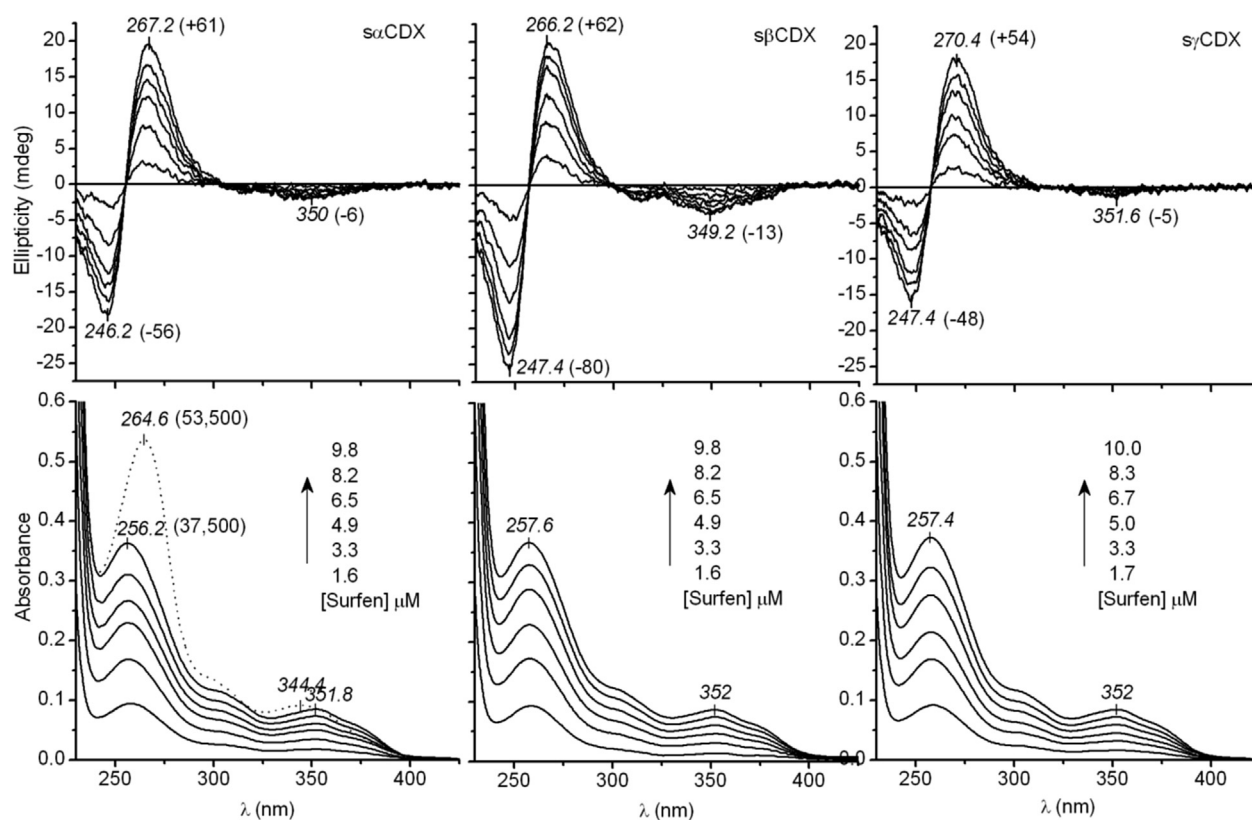
Fig. 1. Induced CD and UV absorption spectra of surfen added into 3 μM solution of sulfated α-, β-, and γ-CDX (50 mM Tris–HCl buffer pH 7.4, 100 mM NaCl, 25 °C). Molar absorption (ε) and dichroic absorption coefficients (Δε) calculated by using the total molar concentration of surfen in the sample solutions are shown in parentheses. Dotted line (left panel): UV spectrum of 10 μM surfen recorded in CDX-free buffer solution.
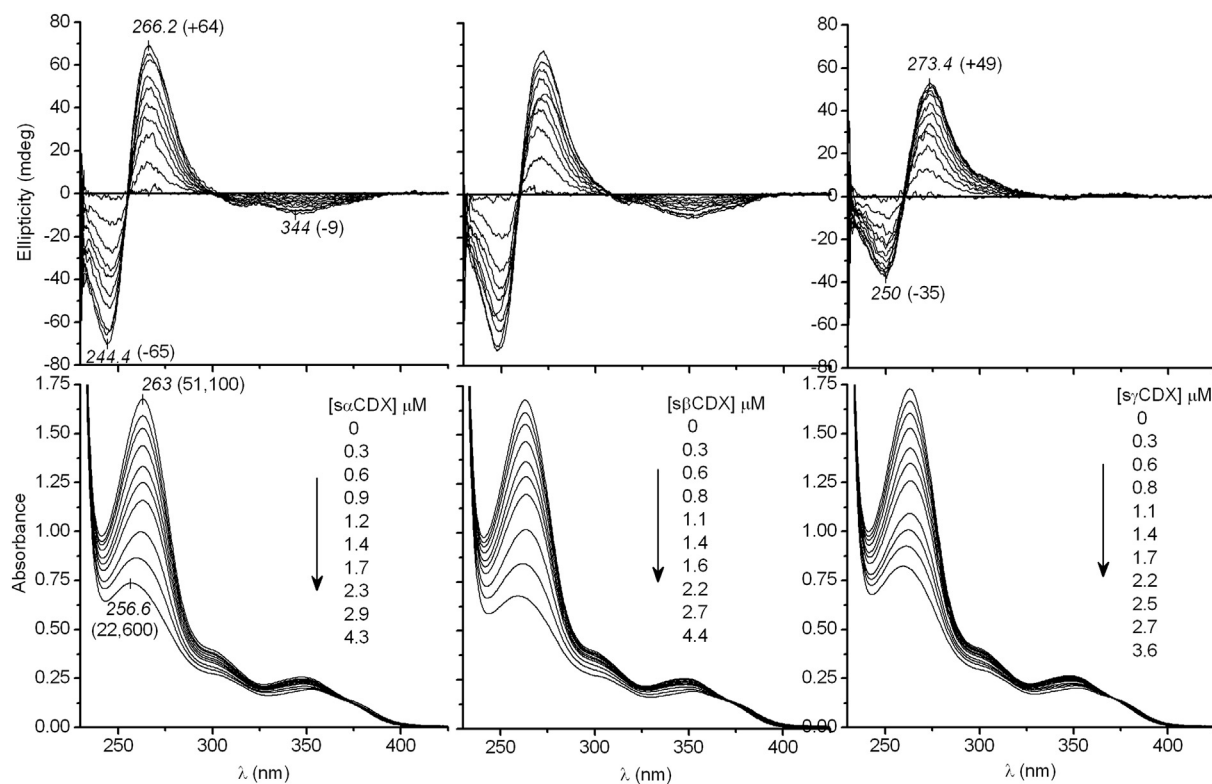

Fig. 2. Induced CD and UV absorption spectra of 32 μM surfen titrated with sulfated α-, β-, and γ-CDX (50 mM Tris–HCl buffer pH 7.4, 100 mM NaCl, 25 °C). Molar absorption (ε) and dichroic absorption coefficients (Δε) calculated by using the total molar concentration of surfen in the sample solutions are shown in parentheses.
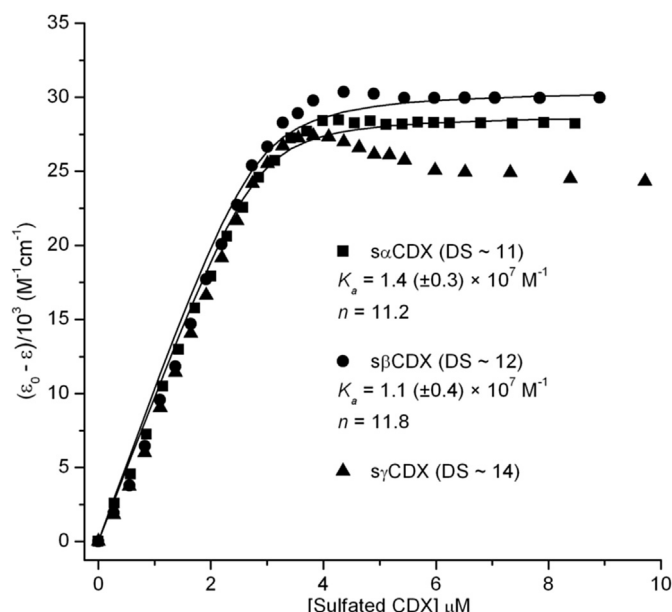
Fig. 3. UV absorption changes plotted against sulfated CDX concentration of the sample solution containing 32 μM surfen. ε_0: molar absorption coefficient of the main UV peak of surfen measured in CDX-free buffer solution. ε: molar absorption coefficient of surfen measured with added CDX. Solid lines are the results of non-linear curve fitting analysis. Estimated association constants (K_a) and the number of surfen binding sites (n) per a CDX molecule are shown.

(linear dichroism measurements showed that in quinoline the electronic transition moment of the main UV band centered around 221 nm is oriented along the long axis of the molecule [13]).

Surfen can attach to the sulfate groups through ionic and H-bonding. The protonated basic ring nitrogens (pK_a 9.17 [14]) may be involved in electrostatic interactions while the −NH$_2$ substituents act as H-donors. Noticeably, the replacement of the −NH$_2$ groups with −H, −OH or −OMe substituents completely abolishes the heparan sulfate binding of surfen [15,16]. In addition, electrostatic complexes of basic organic compounds formed with sulfated glycosaminoglycans are highly sensitive to the ionic strength of the solution and dissociate at physiological salt concentration [17,18]. In contrast to this, surfen does bind to sulfated CDXs in the presence of 0.1 M sodium chloride and an increase of the Na$^+$ concentration up to 1.25 M caused only a small fluctuation of the ε_{max} value but did not cancel the UV hypochromism, the spectroscopic sign of CDX association of the drug molecules (Fig. S3 in Supplementary Data). Interestingly, crystallographic studies showed that surfen can adopt a crescent-shaped conformation [6] where the amino groups face toward the same side occupying a favorable steric position to establish intermolecular H-bonds with the same sulfate substituent (Scheme 1). Concomitantly, the charged edge of the molecule points toward the bulk aqueous phase. This possible CDX binding mode of surfen is consistent with the 1:1 drug:sulfate stoichiometry (Fig. 3).

By contrast to the sulfated CDXs, native CDXs were unable to induce any ellipticity signal in the CD spectrum of surfen (data not shown). The main UV peak, however, exhibited a moderate (~10%) hypochromism (Fig. S4 in Supplementary data). Analysis of the absorption changes indicated a much weaker association ($K_a \sim 10^5$ M^{-1}) of 4–7 surfen molecules per a CDX host (Fig. S4 in Supplementary data). These results imply that presumably via the hydroxyl groups, surfen can bind to the external surface of natural CDXs, too. The small hypochromism, however, suggests a substantially larger intermolecular spacing between the guest

molecules, the accommodation of which occurs in a non-chiral fashion.

In conclusion, the results described herein show that the hydrophilic external surface of sulfated CDXs may serve as an asymmetric template enabling a very strong, chiral supramolecular association of guest molecules. These data help to better understand the molecular recognition properties of sulfated cyclodextrins with a potential to extend further their applications.

Conflict of interest

None.

Acknowledgments

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.03.123.

Transparency document

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