Thermodynamics of Binding of Neutral Molecules to Sulfobutyl Ether β-Cyclodextrins (SBE-β-CDs): The Effect of Total Degree of Substitution

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Purpose. To understand the role of degree of substitution on binding of molecules to β-Cyclodextrins (β-CDs) with varying degrees of sulfobutyl ether (SBE) substitution.

Methods. Using UV spectroscopy, complexation constants of molecules to SBE-β-CDs were estimated as a function of temperature, allowing for calculation of thermodynamic parameters, including the enthalpy and entropy of binding.

Results. Binding constants of various molecules to SBE-β-CDs did not show a uniform trend to total degree of SBE substitution. However, a distinct pattern was observed with the enthalpy and entropy of complexation. The results showed the complexation of substrates to SBE-β-CDs to be more entropy-favored as the number of SBE groups increased. This favorable entropy of interaction was compensated by a less favorable enthalpy of interaction.

Conclusions. Enthalpy and entropy of complexation provided additional insight into the role that the alkylsulfonate groups may play in the complexation of molecules with SBE-β-CDs.

KEY WORDS: cyclodextrin; complexation; thermodynamics; sulfobutyl ether β-cyclodextrin (SBE-β-CD).

INTRODUCTION

Various researchers have attempted to modify β-cyclodextrin (β-CD) to improve its physicochemical and toxicological limitations (1,2). One family of β-CD derivatives which have shown potential improved solubility and complexation ability without the significant toxicity associated with the parent or certain other alkylated cyclodextrins are the sulfobutyl ether cyclodextrins (SBE-β-CDs) (3,4).

One reason for the improved complexation ability of most alkyl-substituted cyclodextrins is believed to be due to cavity extension phenomena (5). This increase in cavity dimension (increased height) seems to improve complexation by providing additional surface for interaction. However, the improvement in complexation may become limited by induced steric hindrance upon addition of fragments near and around the cavity entrance. This was observed in the cases of methylated-β-CD (6,7), hydroxypropyl-β-CD (HP-β-CD) (8) and acetylated β-CD (9). For example, the 2,6-dimethyl-β-CD showed improved binding and solubility over β-CD, but when all 21 hydroxyl moieties were methylated, the binding capacity decreased. This reduction in complexation was attributed to possible narrowing of the cavity opening at high degrees of substitution. Also, the solubility of HP-β-CD was shown to increase exponentially with an increase in the number of substituents, while the binding capacity decreased (8). Similar trends in binding ability were observed for acetylated cyclodextrins (9).

The goal of the present study was to determine the effect of the degree of SBE side chain substitutions on the complexation of some pharmaceutically relevant molecules to SBE-β-CDs. Such studies may provide information in predicting complexation parameters, and/or help in developing other modified cyclodextrins with superior complexation properties. Most studies to date, with some exceptions [10,11 (and references therein)], have only looked at the binding constant (K). However, since K is a derived parameter related to the free energy (ΔG°) of complexation, which has contributions from enthalpic (ΔH°) and entropic (ΔS°) terms, the estimation of these thermodynamic parameters may provide additional insight into the mechanism/s for altered binding.

MATERIALS AND METHODS

Chemicals

All chemicals were of analytical or reagent grade and were used without further purification unless otherwise noted. The preparation and characterization of the SBE-β-CDs has been described elsewhere (12–15). Mixtures of SBE-β-CDs with varying Total Degrees of Substitution (TDS) were utilized in this study (16). Cyclodextrin water contents were determined so that solution concentrations could be prepared accurately. More accurately, $(SBE)_{1M}$-β-CD (TDS = 1.1; MW = 1309.8, water = 5.2% w/w), $(SBE)_{7M}$-β-CD (TDS = 6.8; MW = 2207.6, water = 9.85% w/w), and $(SBE)_{12M}$-β-CD (TDS = 11.5; MW = 2953.6, water = 10.5% w/w) were utilized. Hydrocortisone, prednisolone, 6α-methylprednisoline, testosterone, progesterone, warfarin sodium, and papaverine hydrochloride were obtained from Sigma™ Chemical Company (St. Louis, Missouri). Prazosin hydrochloride and naproxen were gifts form Pfizer and Syntex laboratories, respectively. 6-O-benzyl guanine was obtained from the National Cancer Institute. Water was deionized and charcoal filtered prior to glass distillation with a Corning Mega-Pure System MP-1 (Corning, New York). Solvents were obtained from conventional sources.

Instrumentation

Karl Fischer water analysis was performed on a Brinkmann™ 652 KF-Coulometer. UV analysis was performed using a Perkin Elmer™ double beam UV/Vis Lambda 6 instrument equipped with a data manager software and water-jacketed multicell attachment. A circulating water bath was used to provide constant temperature during experiments. Temperatures were accurately monitored by a temperature probe and measured to ±0.2°C.

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Methods

The cyclodextrin complexation constants were determined using UV analysis as described by Connors (17), where changes in the absorbance of the substrate were monitored upon formation of inclusion complexes. The experiments were carried out at fixed wavelengths chosen appropriately for each molecule to give the largest possible absorption change. The range of cyclodextrin concentrations studied was large enough to produce much of the complexation isotherm. The spectral change data were linearized and complexation constants were determined using the x-reciprocal method (17).

Due to limited water solubility of some of the tested compounds, the drug solutions were made from a 1.0 mg/ml stock solution of drug in methanol. Solutions of appropriate drug concentration containing less than 1% (v/v) methanol were prepared to provide absorbance values between 0.6–0.9 absorbance units. These solutions were filtered through 0.22 μm laminated PTFE filters and degassed by means of sonication under vacuum prior to experiments. Cyclodextrin solutions were filtered through 0.45 μm nylon filters. Matched quartz cuvets of 1.5 ml capacity and 1.0 cm path length were used.

The process involved equilibration of 800 μl of drug solution in the cuvets at the appropriate temperature for 20 minutes. The absorbance value of the drug was obtained prior to addition of cyclodextrin and following the addition of each increment of CD solution. Cyclodextrin solution was added 1 μl at a time up to a maximum volume of 10 μml, using a Hamilton™ syringe. The solutions were inverted 30 times prior to measurement to allow for thorough mixing and left in water jacketed sample holders to come to equilibrium temperature for eight minutes before measurements were taken. Cyclodextrin solutions were also added to reference cells to account for dilution effects. Table I shows some properties of the molecules studied along with the conditions for UV analysis. Solution pH values were chosen so that only the neutral forms of the molecules were present (see Table I).

Thermodynamic Measurements

Enthalpy and entropy of binding were calculated from the K values at various temperatures between 9–45 °C. Van't Hoff plots were generated to determine the enthalpy and entropy of complexation of the molecules to cyclodextrins.

RESULTS AND DISCUSSION

Effect of Total Degree of Substitution on Binding Constant (K)

A representative example of the x-reciprocal plots for binding constant determination is depicted in Fig. 1. Two samples were run at each temperature and the final K values were determined as the average of the two. The linear x-reciprocal plots for all compounds studied suggested a 1:1 interaction between the cyclodextrin and the molecules, similar to what was observed with the phase solubility studies (18). It is worthwhile to mention that the binding constant values obtained from UV analysis differed somewhat from those obtained by the phase solubility method. This discrepancy between the two methods may be attributed to the experimental approaches of each method.

The binding constants derived in this paper, and most others, are concentration and not activity equilibrium constants. Thus, the binding constants reported are apparent binding constants, and therefore can be system dependent, i.e., they are affected by concentration, ionic strength, etc. (17,19). Thus, phase solubility or other studies performed at relatively high concentrations may generate intermolecular interactions resulting in apparent stability constants different than those obtained in dilute solutions (10^{-4} M), such as those reported here. Also, in determining the thermodynamic parameters, the UV analysis method proved to be superior in performing temperature studies compared to the phase solubility method, because of more accurate temperature control. Specifically, the phase solubility technique requires equilibration of samples at certain fixed temperatures, but subsequent sample handling cannot be easily performed at constant temperature. In other words, the sampling technique usually introduces dramatic temperature differences that result in inaccurate binding constants at temperatures other than those around room temperature.

The effect of the total degree of substitution on the ability of SBE-β-CD mixtures to complex with various molecules is depicted in Figs. 2a and 2b. Unfortunately, due to limited availability of SBE derivatives, some complexation constants could not be determined. The binding potential of all molecules varied with the degree of substitution, but not all molecules behaved similarly. Molecules such as hydrocortisone and prednisolone exhibited a decrease in K, as the numbers of substituents were increased. Warfarin acid binding increased linearly with an increase in the substitution number, while testosterone showed an apparent maximum. The varying patterns of complexation with an increase in the degree of substituents suggests the possibility of specific interactions or mixed modes of binding between different components or functional groups in the structure of the substrate and specific cyclodextrin. Even within relatively common structures such as the steroids (having only differed in the D ring substituents) various patterns of binding were observed. Thus, suggesting the possibility of specific interactions, which has been observed with some molecules interacting with cyclodextrins and cyclophanes (20–22).

The observed differences in the binding of molecules to different SBE-β-CDs may be attributed to changes in the mode of complexation due to derivatization of the cyclodextrin torus (6,7). For example, upon increasing the number of SBE substituents, the cyclodextrin torus may be distorted in such a way as to result in decreased hydrophobic interactions between some guest molecule and the interior cavity of the cyclodextrin, thereby decreasing the driving force for inclusion complexation. In addition, steric interference may also play a role in the observed decrease in binding capacity of modified cyclodextrins. For example, the cavity opening may be capped by the substituents alone, or by the formation of an aqueous solvation shell near the entrance of the cavity associated with charged substituents (23,24). The presence of bulky, highly charged, and hydrated sulfonate groups near the entrance of the cavity may inhibit the approach of a hydrophobic molecule, thus reducing complex formation.

On the other hand, derivatization of the cyclodextrin torus may provide additional sites of interaction for stabilization of some complexes. For example, the alkyl chains may provide additional hydrophobic binding sites, or the sulfonate moieties may induce specific interactions with the guest molecule.

Table I. Molecular Properties and Conditions at Which the Thermodynamic Parameters Were Performed

Substrate	MW	pKa	λ(nm)	pH	*I* (M)[d]	Conc. (μg/ml)[e]
Prednisolone	360.4	NA	246	6.5[a]	0.15	20
6α-Methylprednisolone	374.5	NA	246	6.5[a]	0.15	20
Hydrocortisone	362.5	NA	246	6.5[a]	0.15	15
Progesterone	314.5	NA	248	6.5[a]	0.15	7
Testosterone	288.4	NA	246	6.5[a]	0.15	15
6-O-Benzylguanine	240.8	3.5 & 9.0	281	6.5[a]	0.15	20
Naproxen	230.3	4.8	213	2.0[b]	0.15	2.5
Warfarin	330.3	4.8	282	2.0[b]	0.15	20
Prazosin	419.9	6.5	250	12.0[c]	0.15	7
Papaverine	375.9	8.1	237	12.0[c]	0.15	6

[a] 50 mM phosphate buffer.
[b] 10 mM hydrochloride solution.
[c] 10 mM sodium hydroxide solution.
[d] Solution ionic strength adjusted using NaCl.
[e] Drug solution concentration at which the experiments were performed.

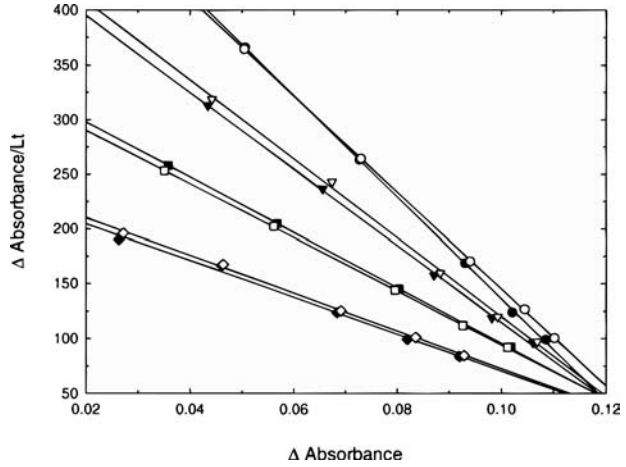

Fig. 1. A representative example of an X-reciprocal plot of prednisolone/$(SBE)_{1M}$-β-CD complexation at pH 2.0 and various temperatures of 17° (○, ●); 25° (▼, ▽); 32° (□, ■); and 45°C (♦, ◇).

Therefore, the stabilizing or destabilizing effects of the sulfoalkyl ether groups may depend on the substrate structure.

Effect of TDS on Enthalpy and Entropy of Complexation

The complexation constant and free energy of interaction are dependent on the enthalpy and entropy of interaction. Therefore, the evaluation of the enthalpy and entropy of interaction may provide additional insight into the role of the degree of substitution on complexation.

The effect of the degree of substitution on the enthalpy and entropy of complexation is shown in Table II, and graphically in Fig. 3. These studies were performed on mixtures of cyclodextrins possessing a TDS as large as twelve. $(SBE)_{1M}$-β-CD represented derivatized cyclodextrin most similar to the parent β-CD structures. $(SBE)_{12M}$-β-CD, with an average TDS of twelve, represented a derivatized cyclodextrin least like β-CD, with $(SBE)_{7M}$-β-CD somewhere between the two extremes. The points on the graph represent the thermodynamic complexation parameters of different molecules to each cyclodextrin derivative tested, and the encircled areas emphasize the regional occurrence of these parameters. All molecular inclusion compounds displayed enthalpically-driven processes with varying favorable (positive values) or unfavorable (negative values) entropies. The enthalpy of complexation became systematically more unfavorable (less negative) while the entropy of complexation became more favorable, and even positive, with increasing total degrees of substitution (Table II). This gradual change in entropy and enthalpy of complexation is also shown by the encircled areas in Fig. 3, where molecules complexing to $(SBE)_{1M}$-β-CD displayed the most unfavorable entropic parameters (bottom left corner), molecules complexing to $(SBE)_{12M}$-β-CD displayed the most favorable entropic parameters (top right corner), with $(SBE)_{7M}$-β-CD occupying the region in between the two.

Traditionally, hydrophobic interactions between two apolar molecules at room temperature have been known as an entropy-driven processes, where the entropy of interaction is large and positive while the enthalpy of the process is often small (25–30). However, cyclodextrin inclusion complexations

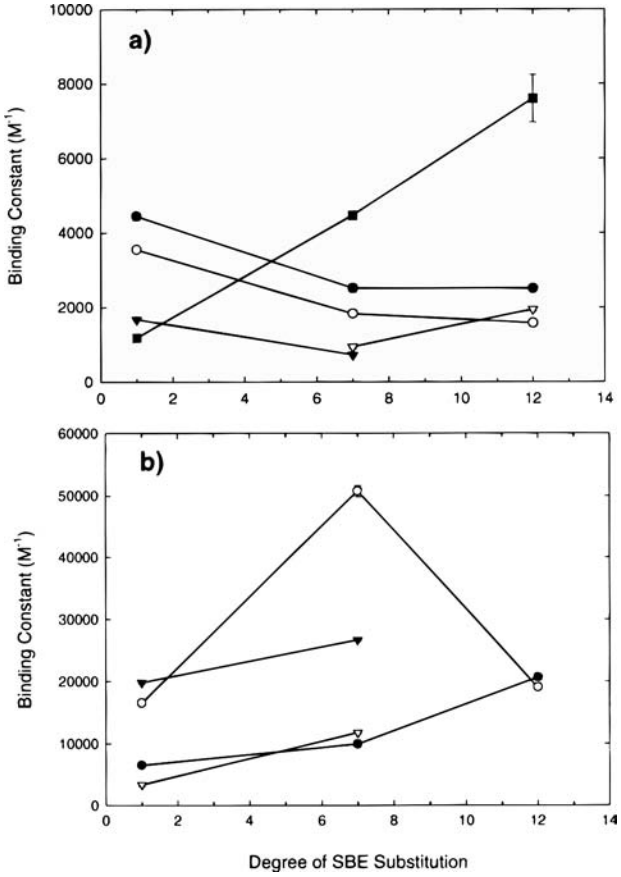

Fig. 2. Effect of increasing degree of sulfobutyl ether (SBE) substitution on complexation of molecules with SBE-β-CD. a) Hydrocortisone (●); Prednisolone (○); Methylprednisolone (▼); 6-O-Benzylguanine (▽); Warfarin (■). b) Naproxen (●); Testosterone (○); Progesterone (▼); Prazosin (▽). All data are the average of two determinations with the error bars representing the upper and lower ends (some error bars are small enough to be within the symbols).

at room temperature are usually enthalpy-driven processes with minor favorable or unfavorable entropies of interaction (10,11) due to features specific to the cyclodextrin cavity. With one exception, the systematic increase in the entropy of interaction with higher SBE substitution patterns seen in Fig. 3 may be explained in terms of additional hydrophobic interactions. As stated earlier, chemical modification of β-cyclodextrin with sulfobutyl ether substituents may provide additional areas of interaction between the cyclodextrin and a substrate molecule. For example, the alkyl chains of the sulfobutyl ether moieties may interact with hydrophobic portions of the molecule outside the cyclodextrin cavity. Derivatization of cyclodextrins may also distort the cavity, limiting the complexation of a guest with the cavity interior, inducing interaction with the alkyl chains around the torus. This is expected to be similar to those hydrophobic interactions where entropy is the dominant term. Thus the interaction of molecules with modified cyclodextrins may be a combination of an enthalpy-driven process, and entropy driven process associated with the alkyl chains of SBE. The more positive and favorable entropic term of higher substituted SBE-β-CD compared to the lower substituted cyclodextrins (Fig. 3) is consistent with this hypothesis.

Table II. Comparison of Binding Constant, Free Energy, as Well as Enthalpy and Entropy Change of Complexation of Various Substrates to SBE-β-CDs with Varying Total Degrees of Substitution (TDS)

Substrate	SBE-β-CD TDS	$K_{1:1}$ 25°C	ΔG° (KJ/mole)	ΔH° (KJ/mole)	TΔS° (KJ/mole)
Hydrocortisone	1	4440 (96)	−20.8 (0.05)	−27.1 (2.30)	−6.29 (0.55)
"	7	2516 (106)	−19.4 (0.82)	−18.0 (0.68)	1.41 (0.05)
"	12	2506 (50)	−19.4 (0.05)	−11.6 (0.79)	7.78 (0.53)
Prednisolone	1	3554 (51)	−20.3 (0.04)	−28.1 (0.87)	−7.81 (0.27)
"	7	1821 (58)	−18.6 (0.59)	−15.4 (0.43)	3.16 (0.09)
"	12	1578 (85)	−18.2 (0.14)	−13.0 (1.58)	5.22 (0.64)
Methylprednisolone	1	1674 (9)	−18.4 (0.01)	−49.2 (3.65)	−30.9 (2.29)
"	7	726 (32)	−16.3 (0.11)	−15.6 (1.41)	0.71 (0.07)
"	12	—	—	—	—
6-O-Benzylguannine	1	—	—	—	—
"	7	994 (48)	−17.1 (0.83)	−16.4 (1.47)	0.68 (0.06)
"	12	1944 (46)	−18.8 (0.06)	−14.2 (1.22)	4.58 (0.39)
Testosterone	1	16506 (261)	−24.1 (0.04)	−39.8 (2.52)	15.7 (1.03)
"	7	50728 (918)	−26.8 (0.48)	−34.8 (3.64)	−7.75 (0.81)
"	12	18965 (115)	−24.4 (0.02)	−13.9 (1.30)	10.49 (0.98)
Progesterone	1	19811 (211)	−24.5 (0.03)	−38.1 (2.63)	−13.6 (0.95)
"	7	26644 (34)	−25.3 (0.03)	−30.5 (2.53)	−5.33 (0.44)
"	12	—	—	—	—
Naproxen	1	6496 (6)	−21.8 (0.00)	−29.6 (2.70)	−7.81 (0.71)
"	7	9913 (336)	−22.8 (0.77)	−24.9 (1.59)	−2.12 (0.13)
"	12	20603 (438)	−24.6 (0.05)	−20.8 (1.03)	3.83 (0.19)
Warfarin	1	1184 (28)	−17.5 (0.06)	−27.9 (1.40)	−10.4 (0.58)
"	7	4463 (32)	−20.8 (0.15)	−21.6 (1.95)	−0.79 (0.07)
"	12	7604 (635)	−22.1 (0.21)	−20.9 (1.83)	1.26 (0.11)
Prazosin	1	3367 (276)	−20.1 (0.21)	−31.4 (2.30)	−11.3 (0.96)
"	7	11733 (138)	−23.2 (0.27)	−27.5 (1.47)	−4.40 (0.23)
"	12	—	—	—	—
Papaverine	1	—	—	—	—
"	7	725 (68)	−16.3 (1.53)	−36.2 (2.87)	−20.2 (1.63)
"	12	—	—	—	—

Note: Results are expressed as mean ± average deviation of two experiments.

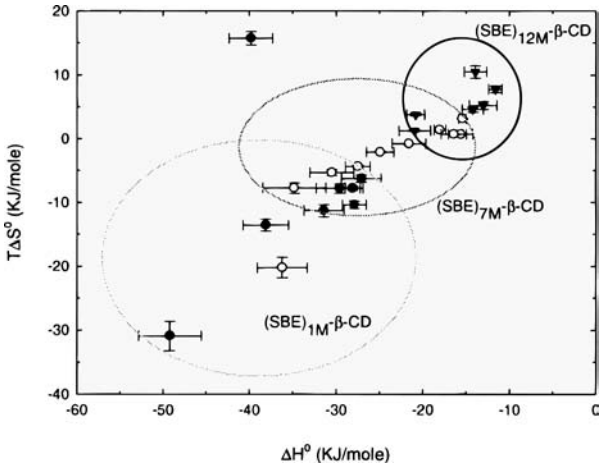

Fig. 3. A plot of TΔS° versus ΔH° showing the changes in complexation thermodynamics of all molecules with each cyclodextrin with an increase in the number of sulfobutyl ether side chains; $(SBE)_{1M}$-β-CD (●); $(SBE)_{7M}$-β-CD (○); $(SBE)_{12M}$-β-CD (▼). All data are the average of two determinations with the error bars representing the upper and lower ends.

The interaction of a specific substrate with larger derivatives of SBE-β-CD does not automatically increase the binding since the enthalpies and entropies of interaction counterbalance each other's contribution towards the complexation constant. Therefore, the modification in binding constant with increasing SBE derivatization is dependent on the magnitude of the enthalpy and entropy change. This will be the subject of further discussion in a subsequent paper.

CONCLUSIONS

The number of sulfobutyl substituents may change the complexation capability of cyclodextrins. The alkyl chains of SBE may provide additional hydrophobic regions for stabilization of the complex, potentially counterbalancing the negative effects of steric interference. SBE alkyl chains may also provide an extension of the cyclodextrin cavity with which the guest molecules may interact.

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