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Formation of unexpected substitution patterns in sulfonylbutylation of cyclomaltoheptaose promoted by host–guest interaction

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Abstract

The distribution of substituents in sulfonylbutylethers of cyclomaltoheptaose (β-cyclodextrin) formed in aqueous medium has been determined by gas chromatography after hydrolysis and formation of the permethylated sulfonylfluoride derivatives. In contrast to other etherification reactions of β-cyclodextrin, preferred substitution in position 3 of the glucose units has been detected. From ^{1}H NMR and microcalorimetric experiments, the formation of host–guest complexes by β-cyclodextrin and the reagent 1,4-butane sultone in water became evident. This spatial preorganization presumably favors the reaction with the O-3. In contrast, in methyl sulfoxide preferred 2-*O*-alkylation was obtained, indicating that host–guest interaction does not influence regioselectivity in this solvent.

Keywords: Sulfonylbutyl cyclomaltoheptaoses; Substitution pattern; Host–guest interaction; Regioselectivity

1. Introduction

Cyclodextrins (CDs) and their derivatives find several applications in chromatography [1–3] and in capillary electrophoresis [4], especially for enantiomer separation, and as excipient for pharmaceutical formulations [5]. Valued properties of these components are their chirality, their inclusion properties and their high functionality that allows the formation of a wide range of randomly or regioselectively substituted derivatives [6]. Sufficient water solubility of β-cyclodextrins (β-CDs) that is essential for the application as a drug carrier is achieved by methylation or hydroxypropylation [5]. Sulfonylbutylation with 1,4-butane sultone in aqueous sodium hydroxide also gives appropriate water soluble products (Captisol) due to the ionic character of the sulfonic acid residues [7]. For pharmaceutical applications these products must be thoroughly characterized. Luna et al. determined the distribution of sulfonylbutyl groups in modified cyclodextrin molecules by capillary electrophoresis [8]. Monokis[*O*-sulfonylbutyl]-CDs (2-, 3- and 6-regioisomers) were isolated and characterized by NMR spectroscopy [9]. We developed a method for the analysis of the regioselectivity of the sulfonylbutylation reaction in the glucose unit via gas

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chromatographic analysis of the corresponding sulfonylfluoride derivatives [10]. We now report on the application of this method on sulfonylbutylethers of cyclodextrins (SBE-β-CD) with different degrees of substitution (DS) and, for comparative purposes, on a sulfonylbutyl amylose (SBE-amylose).

2. Results and discussion

Three SBE-β-CDs (**1**, DS 0.17, 0.73, and 1.23/glucose unit, termed SBE1-, SBE7- and SBE12-β-CD) and one SBE-amylose (DS 0.19/glucose unit), prepared by addition of 1,4-butane sultone to amylose in aqueous sodium hydroxide, were investigated after hydrolysis and formation of the permethylated sulfonylfluoride derivatives by GLC as described [10]. Table 1 gives the relative distribution in the mono- and disubstituted fractions for all samples. All cyclodextrin derivatives show preferred sulfonylbutylation at position 3. While at low DS (SBE1-β-CD) only 4.3% of the substituents are located in position 6 of the glucose unit, substitution at this primary position strongly increases with increasing DS at the cost of 2-*O*-substitution. In the disubstituted fraction, combinations corresponding to the composition of the monosubstituted glucose moieties dominate (2,3- for SBE7-β-CD, 3,6- for SBE12-β-CD).

In contrast, 2-*O*-alkylation is strongly preferred for SBE-amylose with nearly equal amounts of the 3-*O*- and 6-*O*-substituted regioisomers, and consequently similar ratios of 2,3- and 2,6-di-*O*-SBE-substitution as it is well known for other starch ethers [11].

These results were unexpected since cyclodextrins can be *O*-alkylated in positions 2 and 6 with high selectivity without protecting groups [12]. This is explained by the higher acidity of the 2-OH proximate to the anomeric carbon, assisted deprotonation at 2-OH due to participation of the α-glucosidic bond, the better accessibility of primary hydroxy groups compared with secondary ones, and the overall structure of the cyclic molecule, where 3-OH groups are involved in cooperative hydrogen bonds [13]. At low base concentration, etherification with alkyl halides or oxiranes occurs preferably in position 2 [11,12,14]. The same is true for starch or amylose [11,15].

NMR spectroscopy.—Since the 1,4-butane sultone exhibits very poor water solubility, it was investigated whether it is included in the more hydrophobic cavity of the β-CD. ^{1}H NMR spectra in D_2O were recorded from both the β-CD and its equimolar mixture with the sultone. Since the sultone was slowly hydrolyzed in water, the mixture was freshly prepared. In the presence of the sultone, H-3 of the CD is clearly upshifted indicating interaction and spatial proximity of this position and the reagent (Fig. 1). In addition, a smaller but also significant upfield shift is observed for H-5 of the CD, which is also located in the cavity. These effects correspond with those observed for the inclusion complex of octylamine and α-CD, as reported by Rekharsky et al. [16]. The same experiment was performed with Me_2SO-d_6 as the solvent, and proton shifts were no longer apparent.

Microcalorimetry.—In order to confirm these first indications of a host–guest interaction, microcalorimetric measurements were

Table 1
Distribution of sulfonylbutylether groups in the monosubstituted and disubstituted fraction of SBE-β-CDs and in SBE-amylose

Position	Relative ratios of the monosubstituted fraction (%)				Position	Relative ratios of the disubstituted fraction (%)			
	SBE1 DS 0.17	SBE7 0.73	SBE12 1.23	Amylose 0.19		SBE1 DS 0.17	SBE7 0.73	SBE12 1.23	Amylose 0.19
2	46.2	36.0	16.1	72.4	2.3	(100) [a]	67.5	21.1	51.0
3	49.8	49.7	49.1	15.5	2.6		14.5	18.7	37.8
6	4.3	14.3	34.8	12.1	3.6		18.0	60.2	11.2

[a] Only traces of 2,3-SFB-Glc detected.

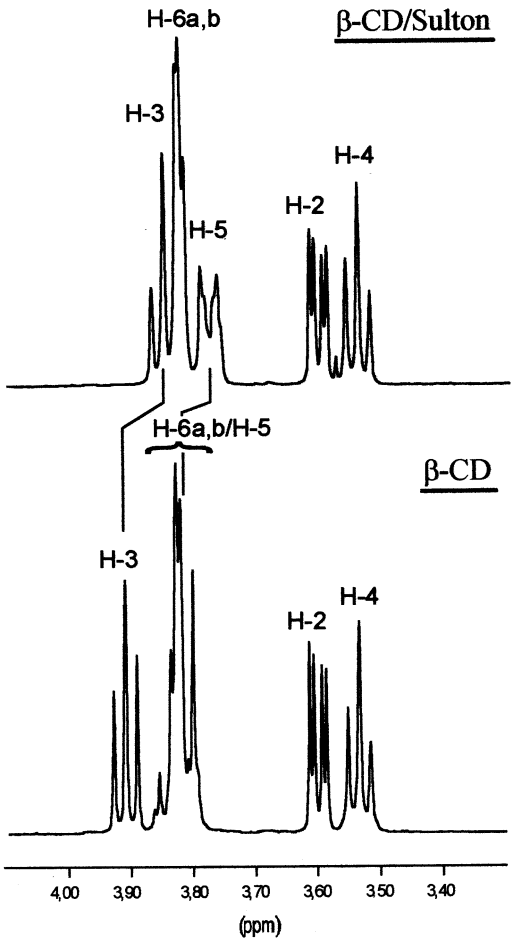

Fig. 1. ^{1}H NMR spectrum of β-CD and 1:1 β-CD + 1,4-butane sultone in D_2O. H-3 and H-5 are shifted upfield by host–guest interaction. No shifts were observed in Me_2SO-d_6.

carried out at 25 °C. A solution of β-CD in water (0.2 mL per step, 0.011 mol/L) was added stepwise at a rate of 20 mL/h to a solution of 1,4-butane sultone in water (2 mL, 0.032 mol/L). The next titration step was always started after reaching chemical and thermal equilibrium. The heat flow was recorded as a function of time and converted into enthalpies by integration of the appropriate reaction peaks. Dilution effects were corrected by subtracting the results of blank experiments with pure water as reactant performed under the same experimental conditions (Fig. 2). The experimental results were then fitted to a first-order reaction model with the enthalpy of reaction and the equilibrium constant as adjustable parameters [17,18]. The fit quality was always satisfactory, but due to the poor water solubility and slow hydrolysis of the sultone to the 4-hydroxybutane sulfonic acid during the experiment, the calculated equilibrium constants scattered strongly and were not reliable. However, the enthalpy of reaction could be determined with sufficient precision and it is evident that a remarkable interaction of β-CD and 1,4-butane sultone takes place. The observed enthalpy of reaction of -6.0 ± 1 kJ/mol is comparable with the formation of other CD-inclusion complexes [16,19], suggesting the existence of specific host–guest interactions.

On the basis of these results, the substitution patterns of SBE-CDs are interpreted as follows. Due to its poor water solubility and its appropriate size and shape, 1,4-butane sultone is included in the less polar cavity of β-CD. From this preorganized supramolecular structure, reaction with the 3-OH is sterically

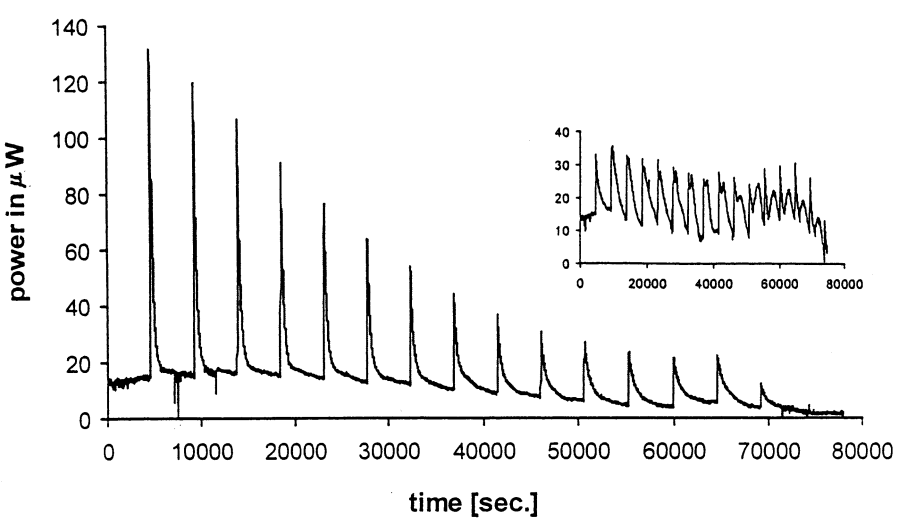

Fig. 2. Microcalorimetric titration of 1,4-butane sultone in water with β-CD. Insert: blank experiment without β-CD.

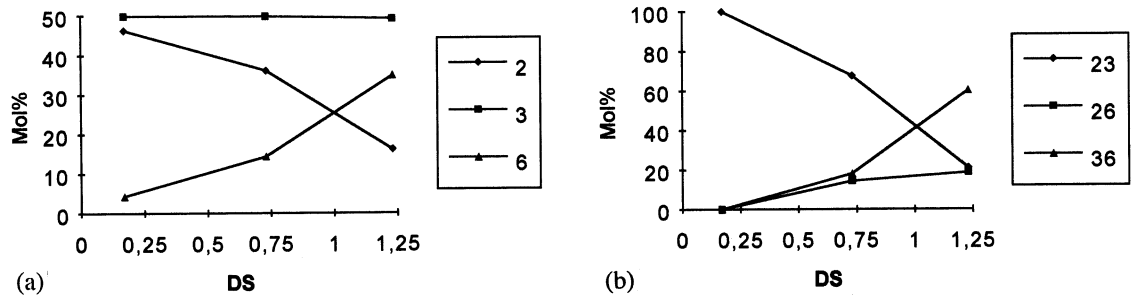

Fig. 3. Relative ratios of the mono- (a) and disubstituted (b) sulfonylbutyl regioisomers with respect to the DS of β-CD.

favoured. With increasing ratio of the sultone and sodium hydroxide, the probability of the competing reactions of deprotonated 2- and 6-OH with the 1,4-butane sultone is enhanced. Low base concentration favours the more acidic 2-OH (SBE1-β-CD). At higher base concentration differences in acidity are leveled off and the primary 6-OH is favoured over position 2 [9], resulting in preferred 3-, 6- and 3,6-di-*O*-sulfonylbutylation (SBE12-β-CD). This is illustrated by the course of the relative ratios of the regioisomers with the DS of the β-CD derivative in Fig. 3.

In contrast, reaction of 6-*O*-protected β-CD with 1,4-butane sultone and sodium hydroxide in the aprotic solvent methyl sulfoxide showed nearly exclusive 2-*O*-substitution. The sultone is well dissolved in the methyl sulfoxide and regioselectivity is no longer influenced by host–guest interaction. These results are in agreement with the ^{1}H NMR experiments.

Sulfonylbutylation of β-CD with 1,4 butane sultone in water yields CD derivatives with an unusual regioselectivity. Fujita et al. reported on the regioselective sulfonation of β-CD with β-naphthalenesulfonyl chloride at the C-3-OH in aqueous acetonitrile [20]. The authors also assume that the reaction proceeds through formation of an inclusion complex. Further examples of regioselective substitution of CD, promoted by host–guest interaction, are the 2-*O*-tosylation with 3-nitrophenyltosylate [21] and 6-*O*-tosylation in water in the presence of Cu^{2+} [22].

3. Conclusions

Sulfonylbutylation of β-CDs with 1,4-butane sultone in aqueous sodium hydroxide solution appears to follow two competing mechanisms. We consider that, due to its poor water solubility, the sultone forms an inclusion complex with the β-CD and, therefore, preferably reacts with the sterically favoured C-3-OH. With increasing concentration of the sultone, the probability of the reaction with the known regioselectivity in positions 2 and 6 increases. The relative reactivity of the primary 6-OH is promoted at higher base concentrations. In contrast, the open chain α-(1→4)-glucan amylose is not able to form such a preorganized complex with the sultone, and therefore shows only poor reactivity at position 3 as has been found for other etherification reactions. The same 'normal' regioselectivity is observed, when sulfonylbutylation of β-CD is performed in methyl sulfoxide.

4. Experimental

General.—The SBE-β-CDs (sodium form) were obtained from Cydex, Kansas, USA, and from Pfizer, Sandwich, UK (Captisol®). These samples were prepared as described by Rajewski [7] according to the following general procedure: β-CD was dissolved in NaOH–water at an elevated temperature under stirring. Initial base concentration was about 50% of the total amount added during the course of the reaction to keep the pH in the range 10–12. Total molar equivalents of NaOH were 4 (SBE1), 16 (SBE7) and 32 (SBE12) per β-CD. To the alkaline solution, 1.7 (SBE1), 8 (SBE7), and 25 (SBE12) equiv/β-CD of the 1,4-butane sultone were added, respectively. Reaction mixtures were neutralized with HCl, subjected to ultrafiltration and freeze dried. The DS was calculated from the sulfur content of the

purified sodium salt. SBE1- (DS 0.17), SBE7- (DS 0.73), and SBE12-β-CD (DS 1.23/glucose unit).

For all reactions in DMF (permethylation) and CH_2Cl_2 (sulfonylchlorination) the sulfonates were transformed to their triethylammonium salts by ion exchange as described [10]. All reagents were of highest purity available and purchased from Fluka, Aldrich or from E. Merck.

SBE-amylose.—SBE-amylose was prepared in our laboratory. Amylose (446.6 mg, 2.8 mmol) was dissolved in water (25 mL) by heating under pressure for 5 min to 150 °C. Sodium hydroxide (167.4 mg, 4 mmol) and 1,4-butane sultone (420 μL, 4.1 mmol) were added and the mixture was stirred at room temperature for 16 h. To destroy the unreacted sultone, another portion of NaOH (330 mg, 8.3 mmol) was added and the reaction mixture was heated to 100 °C for 2.5 h. The solution was neutralised with HCl and dialysed for 4 days. After freeze drying 350.6 mg (1.8 mmol) sulfonylbutylated amylose (sodium salt) was obtained. C, 38.26; S, 2.87, C/S 13.33 corresponding to a DS of 0.19/glucose unit. (Yield 65%).

Analysis of the sulfonylbutyl pattern.—SBE-β-CDs and SBE-amylose were hydrolysed and transformed to the permethylated sulfonylfluoride derivatives (SFB-Glc) as described [10].

GLC.—GLC separations were carried out on a Carlo Erba GC 6000 Vega Series 2 instrument equipped with an on-column injector, a flame ionization detector (FID), a 25 m capillary column CPSil 8CB (Chrompack) connected with a retention gap (2 m), and an E. Merck Hitachi D-2500 integrator. Hydrogen was used as carrier gas (80 kPa). Complete separation of all monosubstituted SFB-Glc derivatives was obtained with a 60 m DB5, i.d. 0.25 mm, helium as carrier gas at 2.0 kg/cm^2, splitless injection (40 s) and the following temperature program: 100 °C (1 min), with 7 °C/min to 240 (10 min isotherm), with 10 °C/min to 290 °C (hold). Data from the FID were collected with a Shimadzu Techlab C-R6A Chromatopac integrator.

GLC–MS.—EI (70 eV) mass spectra were recorded on a VG Analytical VG/70-250S instrument. For CI, ammonia was used as a reactant gas.

NMR spectroscopy.—^{1}H NMR spectra were measured with a 400 MHz Bruker WM 400 instrument; the solvents D_2O and Me_2SO-d_6 (internal standard: acetone, δ 2.18) were purchased from E. Merck.

Microcalorimetry.—Measurements were performed by means of a twin heat conduction microcalorimeter of the Calvet-type (DAK 1A-1, Russia). A home-made titration insertion cell equipped with a calibration heater and a stirrer was used for the experiment. Typical signal noise and drift were $<$ 0.5 μW (point to point) and <1 μW/h, respectively. Reagents were injected by means of a Hamilton syringe (2.5 cm^3) mounted on a step-motor driven precision syringe pump (SP250i, WPI, USA). A thin stainless steel tube (i.d. 0.2 mm) connected to the syringe reached directly into the calorimetric vessel. The vessel can be filled with 2 up to 6 cm^3 of solution. More details of the titration calorimeter and the experimental procedure are described in Ref. [23].

Dialysis.—For dialysis, a cellulose membrane from Spectra Por with a MWCO of 3500 was used. It was dialysed for 2 days against tap water and for another 2 days against dest. water (batchwise).

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