Cyclodextrin Sulfates: Characterization as Polydisperse and Amorphous Mixtures

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Alpha- and beta-cyclodextrins and their hydroxypropyl derivatives were converted by the reaction with chlorosulfonic acid in pyridine to the corresponding sulfates. Cyclodextrin sulfates were shown by fast-atom bombardment mass spectrometry (negative ion mode, triethanolamine matrix) to be mixtures with nearly symmetrical distributions of degree of substitution by sulfate groups and by powder X-ray diffraction to be amorphous. Thus, in these aspects, cyclodextrin sulfates are similar to the potent drug solubilizers hydroxypropylcyclodextrins.

KEY WORDS: cyclodextrin sulfates; hydroxypropylcyclodextrin sulfates; amorphous cyclodextrin derivatives; mass spectrometry.

INTRODUCTION

Cyclodextrin sulfates (Fig. 1) were found to have anti-inflammatory and antilipemic activities (1–4) and, more recently, to inhibit angiogenesis when used in combination with hydrocortisone or with fumagillin (5,6). Cyclodextrin sulfates have also been found to be antiviral and to inhibit the replication of HIV by either prevention of viral absorption or budding (7). One of our aims was to improve on the characterization of cyclodextrin sulfates so that they may be more useful in pharmacy and pharmacology. Using elemental analysis, the sulfur content was 6% when sulfuric acid was used in the preparation and 16.5% when chlorosulfonic acid was used (8). The latter composition is close to that expected for two sulfate groups per glucose residue. Consequently, the names that have been given to such derivatives (i.e., β-cyclodextrin tetradecasulfate) give the impression that only one degree of substitution is present. The second aim was to test the suitability of the reaction used for preparation of cyclodextrin sulfates for the conversion of hydroxyalkylcyclodextrins to their sulfates.

MATERIALS AND METHODS

Preparation of Cyclodextrin Sulfates. The procedure is illustrated with a preparation of β-cyclodextrin sulfate (preparation 2 in Table I). The procedure is principally that of Hamuro and Akiyama (1) and thus the product is equivalent to that described as β-cyclodextrin tetradecasulfate.

To prepare β-cyclodextrin sulfate the anhydrous pyridine (200 ml) was cooled to −10°C, and while stirring chlorosulfonic acid (50 ml) was added dropwise so that the temperature was kept below 10°C. Afterward, the flask was heated in an oil bath to 70°C, and anhydrous β-cyclodextrin (30 g, prepared from β-cyclodextrin donated by Chinoin, Budapest Hungary, which contained 14% water) was slowly added and the mixture kept at 70°C for another hour. Thereafter, the reaction was, while cooling with ice, terminated by the addition of water (400 ml), and the solution was concentrated by evaporation *in vacuo*. To the concentrate, acetone was added, and the precipitate was washed with ether, dried, and again dissolved in water. The aqueous solution was neutralized with sodium carbonate, dialyzed (48 hr, on shaker), concentrated *in vacuo*, and again precipitated by acetone. The precipitate, after washing and drying, was dissolved in water, clarified and sterilized by filtration through a membrane filter (0.45 μm), and freeze-dried to give 33.5 g of β-cyclodextrin sulfate.

The other sulfates were prepared similarly, but omitting the precipitation with acetone, i.e., the reaction mixture, after decomposition with water, was neutralized, dialyzed, and the solution freeze-dried. To make preparations 3 and 4, reagents in 60 and 20% excess to those given above were used.

Mass Spectrometry. The instrumentation and conditions used were previously established as optimal for the analysis of heparin fractions (9). Spectra were obtained using a VG ZAB-HF high-resolution mass spectrometer equipped with a standard VG analytical fast-atom bombardment (FAB) ion source operating in the negative ion mode. Xenon gas was used to form the fast-atom beam; typical conditions were beam energies of 8 keV and a neutral beam current equivalent to 2 mA. The spectra were obtained by signal adding up to eight scans using the multichannel analysis (MCA) scanning software of the VG 11 250J data system. The samples of cyclodextrin sulfates were dissolved in distilled, deionized water to form a concentration of 10–20 μg/μl. To obtain the spectra, 1 μl of triethanolamine and 2 μl of the solution of cyclodextrin sulfate were placed on a standard VG stainless-steel probe tip.

X-Ray Powder Diffraction. A Siemens D-500 automated powder diffractometer with graphite monochromator was used. Cu radiation (λ = 1.54 Å) was generated from a source operated at 50 kV at 40 mA. Two-theta calibration was performed using NBS mica standard. The samples were kept together in a tightly closed vessel for 100 hr at room temperature to equilibrate their water content and then, just before the measurements, were lightly ground in a mortar. The sample size actually used in measurement was close to 100 mg.

Elemental Analyses. Samples of compounds were dried overnight *in vacuo*, those of 1–3 at 100°C, and that of 4 at room temperature. Analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tennessee; the size of samples actually used in the analyses varied between 5 and 10 mg.

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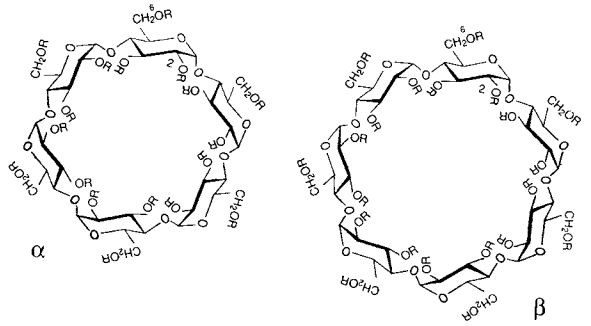

Fig. 1. Structures of α- and β-cyclodextrin sulfates; R is either H or SO_3Na. Number of sulfate groups per molecule may be denoted by a number (degree of substitution; DS) or by a prefix (e.g., DS14 is β-cyclodextrin tetradecasulfate). In several reactions of cyclodextrins (8) the hydroxyls 2 and 6 are more reactive than hydroxyl 3.

RESULTS

The method for the preparation of sulfates by reaction with chlorosulfonic acid in pyridine was applied to α- and β-cyclodextrin and their hydroxypropyl derivatives. The products thus obtained were denoted **1**, **2**, **3**, and **4**, respectively, and all were white, water-soluble powders of the elemental compositions summarized in Table I. On the basis of composition the substitution in preparations **1** and **2** was not far from two sulfate groups per glucose residue; in preparations **3** and **4**, which were made using a larger excess of sulfatation reagents, substitution was higher.

Mass spectra of sulfated carbohydrates are difficult to obtain and no useful results were obtained on cyclodextrin sulfates using ^{252}Cf plasma desorption spectrometry and fast-atom bombardment with glycerol matrix (unpublished). However, fast-atom bombardment in the negative ion mode using triethanolamine as a liquid matrix gave well-defined mass spectra; those of α- and β-cyclodextrin sulfate (i.e., preparations **1** and **2**) are presented in Fig. 2. The contaminating sodium sulfate produced a series of intense cluster ions of the type $(Na_2SO_4)_nNaSO_4^-$ (for example, n = 3–13, 545–1964 amu, in Fig. 2B); nevertheless, the molecular ions of the cyclodextrin sulfates were well defined and clearly separated. The major peaks correspond to monoanions formed by loss of one sodium ion from a neutral cyclodextrin sulfate. Each of the major ions had a less intense ion which differed by 22 amu, corresponding to monoanion of an acidic monoprotonated cyclodextrin sulfate. The distribution of degree of substitution by sulfate groups was about symmetrical in both cases. For example, degrees of substitution from 8 to 14 were discernible for α-cyclodextrin sulfate; the most prominent molecular ion was that belonging to degree of substitution 12. In the case of β-cyclodextrin sulfate degrees of substitution from 10 to 17 were observed; the most prominent molecular ion was that belonging to degree of substitution 15. Preparations **3** and **4** were made from mixtures of hydroxypropyl derivatives of α- and β-cyclodextrins, and consequently the spectra were too complex for detailed analysis. Powder X-ray diffraction data were measured on preparations **1–4** and are reproduced in Fig. 3. All the samples have an amorphous pattern, rather similar to that of hydroxypropylcyclodextrin mixtures. Preparation **4** was made from crystalline 2-*O*-2-(*S*)-hydroxypropyl-β-cyclodextrin and is nearly fully substituted; thus, introduction of sulfate groups into cyclodextrins strongly deters crystallization. It is of interest to compare the results of mass spectra and diffraction data. In the mass spectra of preparations **1** and **2**, the peaks of sodium sulfate and its aggregates were prominent, whereas in the X-ray diffractions the maxima of crystalline sodium sulfate (thenardite, Joint Committee on Powder Diffraction Standards, file number 37-1465) were not detectable. Fast-atom bombardment mass spectrometry can detect even traces of low molecular weight polar impurities, while signals seen in X-ray diffraction data are more representative of composition.

Table I. Sodium Salts of Sulfates of Cyclodextrins and Hydroxypropylcyclodextrins

Preparation No.	Name		Elemental composition				
			C	H	S	Na	O
1	α-Cyclodextrin sulfate	Found	19.39	2.57	18.19		
		Calc. for 12 sulfates	19.68	2.20	17.51		
2	β-Cyclodextrin sulfate	Found	16.74	2.60	18.40	13.05	44.43
		Calc. for 14 sulfates	19.68	2.20	17.51	12.55	48.06
		16 sulfates	18.23	1.98	18.54	13.30	47.98
3	Hydroxypropyl-α-cyclodextrin sulfate[a]	Found	21.24	2.57	16.83	13.44	
		Calc. for 16 sulfates	21.80	2.70	17.44	12.50	
4	Hydroxypropyl-β-cyclodextrin sulfate[b]	Found	14.45	2.62	17.92		
		Calc. for 21 sulfates & 25 waters	14.27	2.80	17.78		

[a] Prepared from hydroxypropyl-α-cyclodextrin mixture which, according to ^{252}Cf mass spectrum, had an average degree of substitution of 5.8. Found: C, 47.87; H, 7.37; ash, <0.4. Calc.: C, 48.97; H, 7.30.

[b] Prepared from hydroxypropyl-β-cyclodextrin with a degree of substitution close to 1.

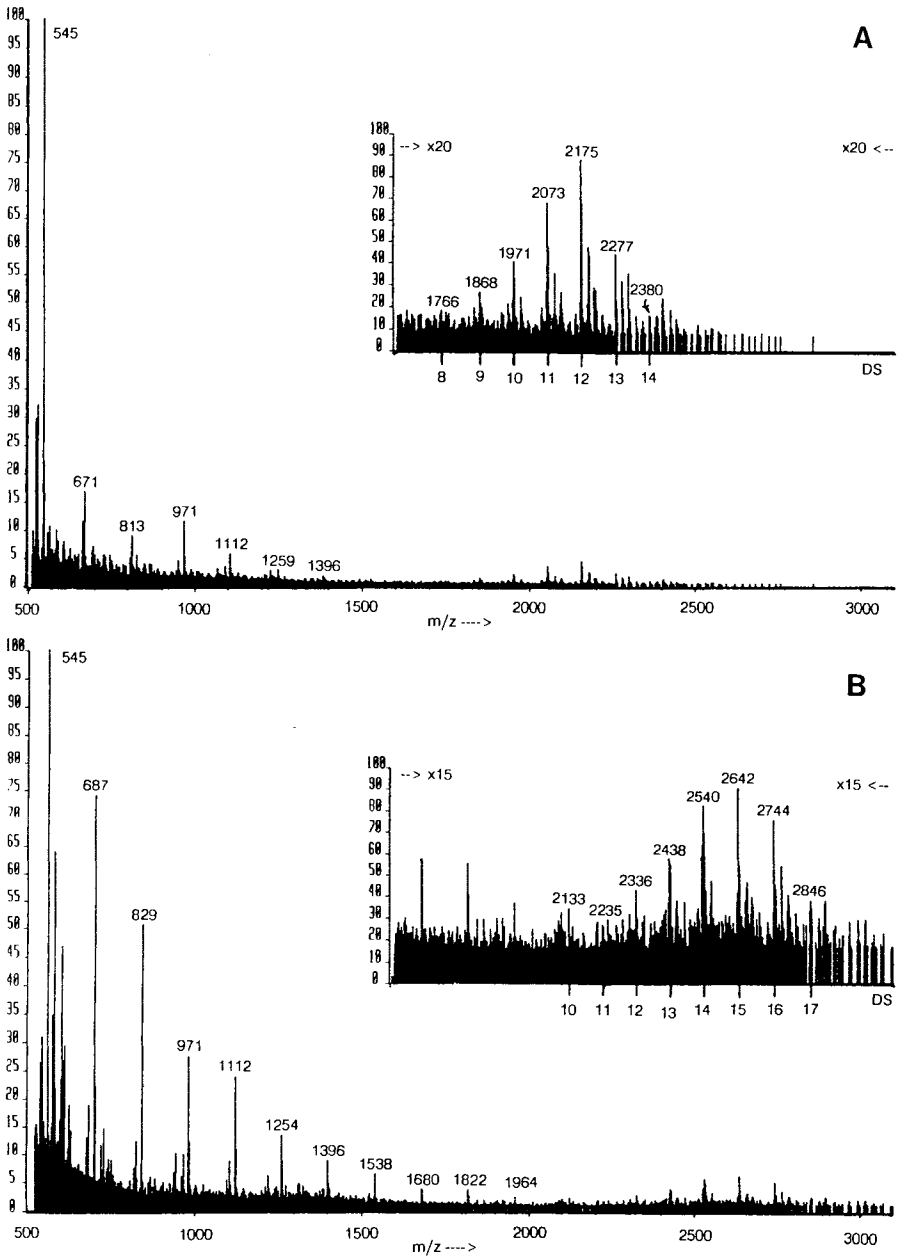

Fig. 2. Mass spectra of (A) α- and (B) β-cyclodextrin sulfates, preparations 1 and 2 in Table I. The series of major peaks with the period 142 amu belongs to clusters of sodium sulfate, a minor contaminant of the preparation. The molecular ion area is to the right of this series and is magnified in the inset; DS denotes the degree of substitution, i.e., the number of sulfate groups per cyclodextrin molecule.

DISCUSSION

Cyclodextrin derivatives are more potent drug solubilizers than cyclodextrins themselves (10). Several types of cyclodextrin derivatives have been used. Hydroxypropylcyclodextrins are mixtures of a great number of isomeric and homologous compounds; both these and their complexes with drugs are amorphous (11,12). Other useful derivatives are so-called branded cyclodextrins, which really are glycosyl and maltosyl derivatives of cyclodextrins. Some of these are chemically individual and crystalline compounds but of very high water solubility, as are their complexes with drugs (13–15). Dimethylcyclodextrins have also been used; these derivatives are crystalline but are highly water soluble and are mixtures of cyclodextrins methylated in all O-2 and O-6 positions and of several under- and overmethylated species (16–20).

Cyclodextrin sulfates prepared by nonregioselective methods are mixtures of many compounds with a symmetrical distribution of degree of substitution; further, these mixtures are amorphous. Thus, cyclodextrin sulfates are alike in their polydisperse character and amorphous phase to hydroxypropylcyclodextrins (11,21). The reaction which was originally developed for the conversion of cyclodextrins

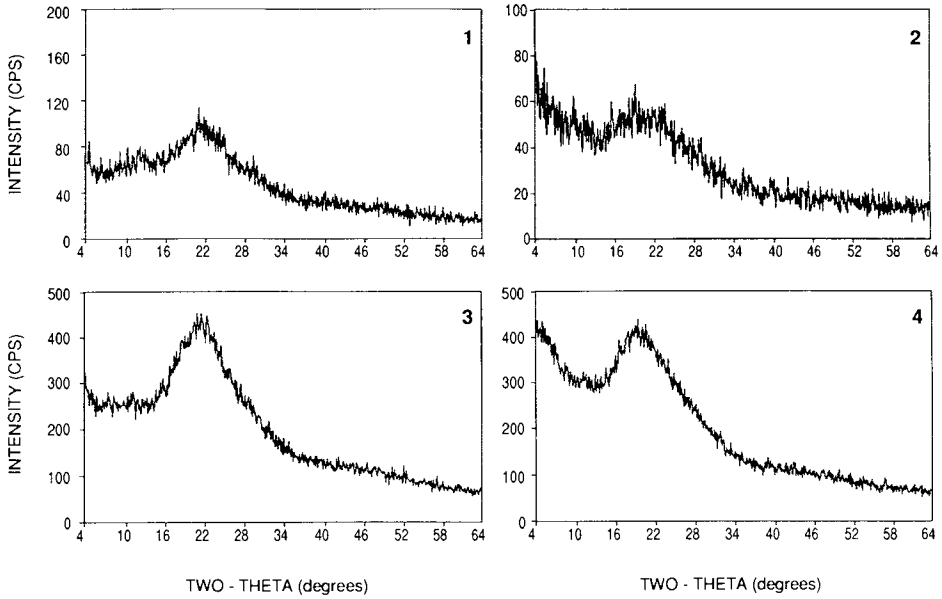

Fig. 3. X-ray powder diffraction patterns of samples of preparations 1–4.

to their sulfates (1) has been found to be applicable to hydroxypropylcyclodextrins as well.

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