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Synthesis of Cyclic Glycerol Ether Cyclodextrin Derivatives and Investigation of their Binding Properties with Drugs

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Abstract. Epichlorohydrin was reacted with cyclodextrins to form the non-cyclic and cyclic glycerol ethers of β- and γ-cyclodextrin (abbreviated as glyc-CD). Cyclic substitution extends the cyclodextrin cavity in a way that is as rigid and non-polar as the cavity of the parent cyclodextrin. Derivatives with extended cavities should better accommodate large or odd shaped molecules. The binding of drugs to the new cyclodextrin derivatives was investigated, through degradation rate studies and solubilization studies, and compared to that of β-cyclodextrin, γ-cyclodextrin and hydroxypropyl-β-cyclodextrin. The inclusion binding of small molecules such as acetazolamide, ethoxyzolamide and chlorambucil, in the glyc-CDs was either increased or decreased compared to the other cyclodextrins. However, larger molecules, such as indomethacin and hydrocortisone, always bound better to the glyc-CDs with up to 180% increase in the stability constant. The degradation rate within the cyclodextrin cavity was not affected by the above derivation.

Key words: cyclodextrin derivatives, cyclodextrin glycerol ethers, synthesis, complexation, solubilization.

1. Introduction

A variety of cyclodextrin derivatives have been described [1]. Derivatives which have been introduced for pharmaceutical applications are usually present as mixtures of compounds differing in the number of substituents per molecule and their isomers. These mixtures generally lead to more highly soluble complexes than are obtained with a single parent compound [2]. The derivatives of present interest are formed by introducing side groups (R) on the hydroxyl groups which line the "rim" of the cyclodextrin "cone" as shown in Figure 1. The introduction of the side groups can affect the ability to form inclusion complexes in two ways. The side group can extend the cavity and help form complexes. Alternatively, the substituent can enter into the cyclodextrin cavity of the same or other cyclodextrin molecule and reduce its ability to encapsulate hydrophobic compounds. It has been shown that, in the case of hydroxypropyl β-cyclodextrin (HP-β-CD), both effects are present and the

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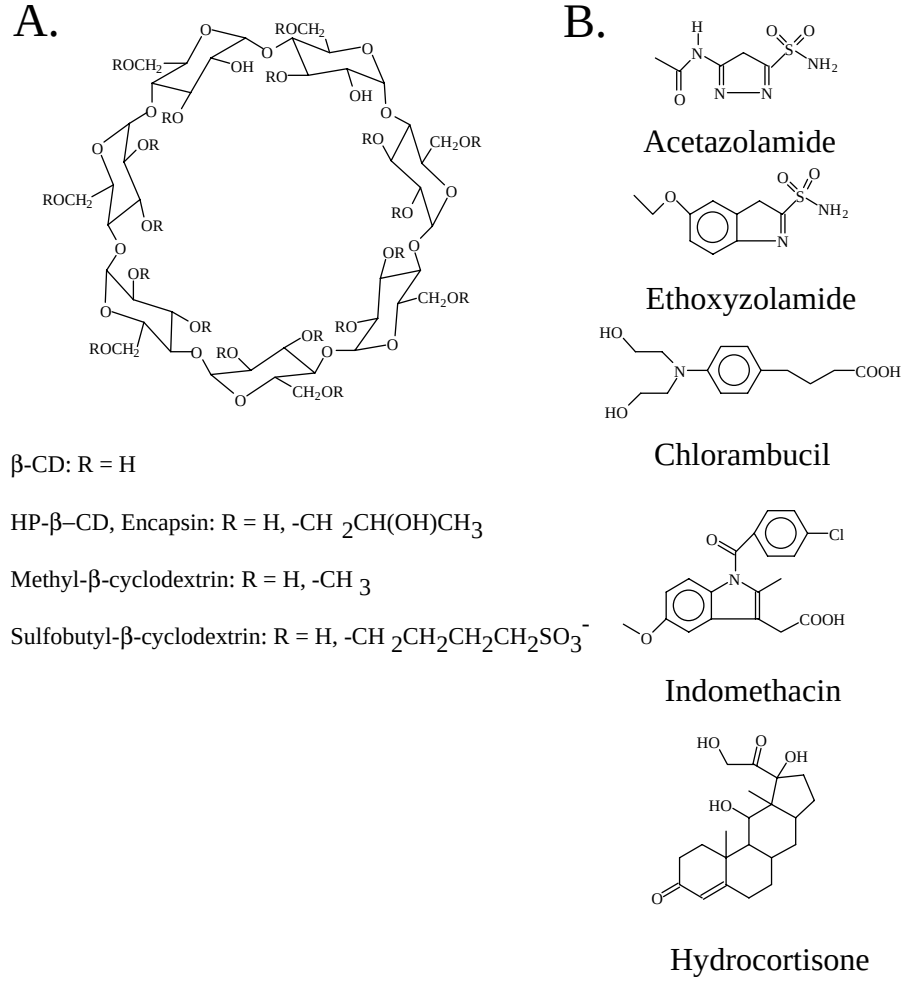

Figure 1. A. The structure of β-cyclodextrin and some of its commercially available derivatives. B. The drugs used in the present study.

stability constant (K_c) of complexation is either greater or smaller than that of the parent β-cyclodextrin depending on the degree of substitution and the position of the substituents [3,4].

In the present work, a new class of cyclodextrin derivatives, glyc-CD, was investigated. The glyc-CDs were made by forming –$CH_2CH(CH_2OH)$– bridges between the 2 and 3 hydroxyl oxygens on the glucosyl units (Figure 2). This extends the cavity in a relatively rigid manner, the side group cannot enter the cavity, and the extension keeps the interior of the new cavity non-polar. Entering guest compounds are accommodated in a cavity which is larger and of different shape from that of the parent cyclodextrin. Therefore, the K_c value for large and odd shape molecules

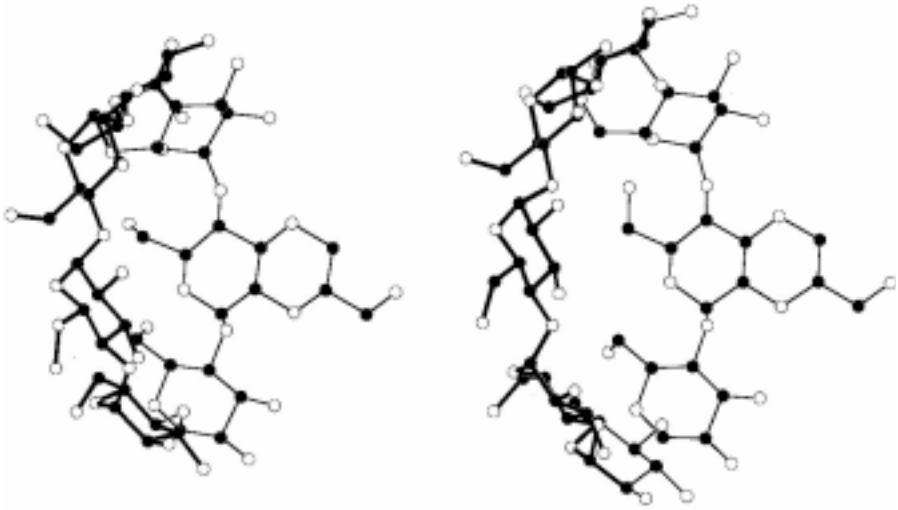

Figure 2. The structure of mono-glycerol ether cyclodextrin derivatives showing how the bridge formed by the cyclic ether will extend the cavity. Our products contained a mixture of compounds possessing one or more cyclic glycerol ethers and some non-cyclic glycerol ethers.

should increase. Nevertheless, not all bridges formed in the reaction are closed and some simple monoethers of glycerol are formed as well.

Unstable compounds in the cyclodextrin cavity are often protected from hydrolysis and other degradation [5]. The rate of degradation for the compound included in the cyclodextrin cavity (k_c) is much smaller than the degradation rate (k_o) for the free compound in solution. The k_c value can vary for the different cyclodextrin derivatives and, in some cases, is much smaller than in the natural analogue and sometimes not significantly different from zero [6]. Glyc-CD offered the possibility to study how k_c is affected by the shape and size of the cavity.

The K_c and k_c/k_o ratios were obtained from degradation rate studies of two drugs, i.e. chlorambucil and indomethacin. The relative K_c of the different cyclodextrins was also estimated from the solubilization of drugs at a single cyclodextrin concentration. Glyc-γ-CD and glyc-β-CD were compared to the chemical analogue HP-β-CD and their parent cyclodextrins.

2. Experimental

2.1. MATERIALS

Hydroxypropyl-β-cyclodextrin (DS = 0.6) and γ-cyclodextrin were kindly donated by Wacker-Chemie (Germany); Encapsin® (i.e. hydroxypropyl-β-cyclodextrin with DS = 0.42) was purchased from American Maize-Products (Indiana, USA); β-cyclodextrin was obtained from Nihon Shokuhin Kako Co. Ltd. (Tokyo, Japan). The drug compounds were obtained from the following suppliers: acetazolamide

from Icelandic Pharmaceuticals (Reykjavik), indomethacin and 6-ethoxyzolamide from Sigma Chemical Company, and hydrocortisone from Norsk Medisinaldepot (Oslo, Norway). Chlorambucil was supplied by courtesy of the Wellcome Foundation Ltd. (UK). All other chemicals were commercially available chemicals of reagent or analytical grade.

2.2. SYNTHESIS

Previously, we have synthesized monosubstituted glyc-β-CD through intramolecular cyclization of 2-O-(2′,3′-epoxypropyl)-β-CD performed in anhydrous trifluoroacetic acid [7]. In the present work, mixtures of the glyc-CDs were obtained through condensation of the parent cyclodextrins with epichlorohydrin in mildly alkaline media.

Glyc-β-CD: A suspension of β-cyclodextrin (15 g), calcium hydroxide (3.48 g) and sodium borohydride (0.15 g) in water (800 mL) was stirred and heated until reflux occurred; then epichlorohydrin (6.6 mL) was added drop-wise. The addition took 30 minutes and, thereafter, the reflux was continued for another 75 minutes. The reflux condenser was then replaced by a descending condenser and part of the water (about 200 mL) distilled off at atmospheric pressure; no epichlorohydrin could be detected in the distillate. The suspension was then cooled to room temperature, filtered and the basic solution neutralized by hydrochloric acid. The solution was then dialyzed (Spectra/Por Molecular Porous Membrane Tubing, MWCO : 3500) exhaustively against water and evaporated in vacuo to dryness, leaving 15.59 g of white amorphous material. This material still contained small amounts of calcium chloride which made it hygroscopic and adhered firmly to the glass of the evaporation flask. Calcium salts had to be removed by the addition of an excess of sodium carbonate solution and another dialysis. The final product was just marginally hygroscopic and could be easily scraped from the flask and powdered. The same procedure as above was applied to γ-cyclodextrin to form glyc-γ-CD.

The product was analyzed after permethylation and conversion to the alditol acetates with the following results: (a) average degree of substitution – 4.6 hydroxyls of gamma-cyclodextrin substituted, (b) 97% of the substitution occurred on the secondary hydroxyls, (c) 55% of all possible ring closures (that is cross-linking of 2-O and 3-O hydroxyls of glucopyranosyl residues by bridges formed from epichlorohydrin) occurred.*

The average molecular weights (1202 for glyc-β-CD and 1473 for glyc-γ-CD) were determined by FABMS.

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2.3. CHROMATOGRAPHY CONDITIONS

The HPLC system consisted of a ConstaMetric 3000 (Milton Roy) solvent delivery system with a SP8450 (Spectra-Physics) variable wavelength detector, using a 150 mm, 4.6 mm i.d., 5 μm bead, C18 reverse-phase column and 1.5 mL/min flow rate. The mobile phases, detection wavelengths and retention times for the different drugs were as follows: for chlorambucil, acetonitrile, acetic acid and water (55 : 1 : 44), 257 nm, 3.6 min.; for indomethacin, acetonitrile, tetrahydrofuran, acetic acid and water (55 : 5 : 0.4 : 39.6), 256 nm, 3.4 min.; for acetazolamide, acetonitrile, acetic acid and water (10 : 2 : 88) containing 0.015% 1-octanesulfonate, 263 nm, 4.0 min; for ethoxyzolamide, acetonitrile and water (35 : 65) containing 0.1% hexane sulfonate, 254 nm; for hydrocortisone, acetonitrile, tetrahydrofuran and water (30 : 1 : 69), 254 nm, 2.6 min.

2.4. KINETIC STUDIES AND DATA FITTING

Stock solutions of indomethacin were made in methanol; chlorambucil stock solutions were made in methanol : buffer (20 : 80). In the experiments, 10 μL of the stock solution was added to 1.5 mL of the cyclodextrin solution, and the mixture kept on a AS-4000 (Merck-Hitachi) autosampler temperature controlled sample rack. Changes in drug concentration with time were monitored by HPLC. In indomethacin degradation studies, a 10 mM Na_2CO_3/HCl (pH 10 at 40 °C) buffer was used; a 10 mM NaH_2PO_4/NaOH (pH 7.5 at 30 °C) buffer was used for the chlorambucil studies. The degradation constant (k_{obs} or k_o) for drug compounds was obtained from linear regression of the logarithm of the HPLC peak intensity plotted against time. The overall degradation constant (k_{obs}) depends on the degradation constant for the drug in solution (k_o), the degradation rate constant for the drug within the complex (k_c), the stability constant of the drug-cyclodextrin complex (K_c) and the total cyclodextrin concentration $[CD]_t$ as described by equation 1 [5].

$$k_{obs} = \frac{k_o + K_c[CD]_t}{1 + K_c[CD]_t}. \tag{1}$$

The data were fitted using non-liner fitting of the Kaleidagraph program (Synergy Software, USA) which uses the Levenberg-Marquardt algorithm for fitting of a user-defined equation. All the data were fitted to a 1 : 1 complex model, according to the formula above.

The values of k_c and K_c were obtained from the best fit, but k_o was determined from solutions containing no cyclodextrin.

2.5. SOLUBILITY STUDIES

Cyclodextrin solutions (1%) were made in H_2O or in 10 mM NaH_2PO_4/NaOH (pH 6.0) in the case of ethoxyzolamide. After dissolution of the cyclodextrin, the pH was adjusted to the set value (pH 6 to 8 in case of water) with strong HCl or NaOH solutions.

Three mL of the solutions were added to vials containing approximately 10 mg of the drug; three vials were measured at each cyclodextrin concentration. The vials were then autoclaved for 20 minutes, cooled to room temperature, equilibrated for up to 5 days, and the suspensions filtered through a 0.45 μm cellulose acetate membrane filter (Schleicher & Schuell, Germany). As a precaution, the first mL of the filtrate was discarded. Nevertheless, another experiment established that the concentration of drug cyclodextrin solutions did not change by filtration indicating insignificant adsorption of drug to the filter. The concentration of drug in the solution (filtrate) was measured by HPLC.

3. Results and Discussion

At conditions where basicity was minimal, but high enough to allow the reaction to proceed, epichlorohydrin formed the non-cyclic and cyclic glycerol ethers of cyclodextrins. Characteristically, at these conditions, more than half of the ethers formed were cyclic; that is, the secondary hydroxyls of anhydroglucose residues became connected by $–CH_2CH(CH_2OH)–$ bridges, forming cyclodextrins with extended cavities as shown in Figure 2. Drugs containing aromatic or polycyclic cores of various sizes were selected for this study in order to investigate how this change in shape of the cyclodextrin cavity influenced their complexation properties and rates of drug degradation.

The K_c values and the degradation rate (k_c/k_o) ratios were obtained from nonlinear fitting of the data for degradation in various cyclodextrin concentrations as shown in Figure 3. Complexation of chlorambucil was larger with β-CD, which was followed by the cyclodextrin glycerol ethers, which were more powerful complexation agents than the corresponding HP-β-CD and γ-CD (Table 1). Indomethacin has a larger aromatic core than chlorambucil and K_c with glyc-β-CD was 26 or 180% larger than with the corresponding β-CD and HP-β-CD. The k_c/k_o ratios were similar for all the chlorambucil cyclodextrin inclusion complexes, except for glyc-γ-CD where it was significantly smaller. Indomethacin was better protected in the glyc-γ-CD complex than in the γ-CD complex, but less protection was observed for glyc-β-CD than for the two other β-cyclodextrins tested.

Acetazolamide, ethoxyzolamide and hydrocortisone form 1 : 1 complexes with cyclodextrins at low cyclodextrin concentrations [8, 9]. Thus, the complexation

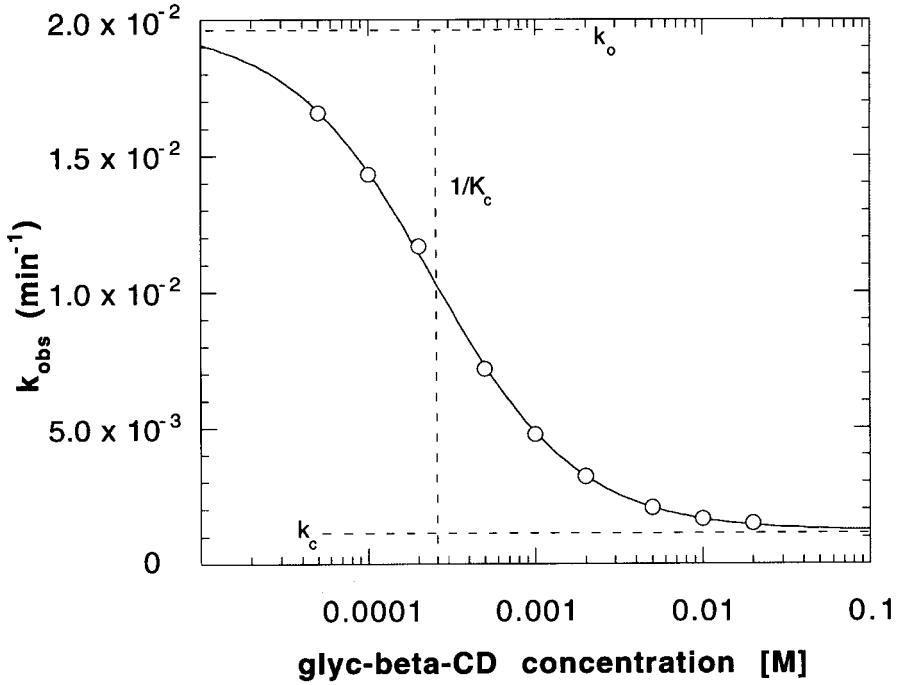

Figure 3. Example of non-linear fitting of the degradation data for chlorambucil. The broken lines are shown to indicate k_o, k_c and $1/K_c$.

Table I. K_c and the k_c/k_o ratio as determined in the degradation rate study

Cyclodextrin	$K_c \times 10^{-3}$ M^{-1}	k_c/k_o
Indomethacin (40 °C; pH 10), $k_0 = (8.53 \pm 0.36) \times 10^{-3}$ min^{-1}		
β-CD	0.31 ± 0.03	0.13
γ-CD	0.27 ± 0.04	0.65
HP-β-CD	0.69 ± 0.06	0.09
glyc-β-CD	0.87 ± 0.09	0.22
glyc-γ-CD	0.22 ± 0.06	0.43
Chlorambucil (30 °C; pH 7.5) $k_0 = (1.95 \pm 0.03) \times 10^{-3}$ min^{-1}		
β-CD	5.34 ± 0.11	0.071
γ-CD	0.35 ± 0.01	0.056
HP-β-CD	3.84 ± 0.09	0.059
glyc-β-CD	4.10 ± 0.08	0.061
glyc-γ-CD	0.38 ± 0.01	0.029

Table II. Drug solubility in 1% cyclodextrin solutions

Solution	Conc. of CD ($M \times 10^{-3}$)	Solubility of drug (mg/mL)	Solubility of drug (M)	Calculated $K_c[D]_o$
Acetazolamide				
Water		0.066	2.97×10^{-4}	
glyc-β-CD	8.32	0.106	4.77×10^{-4}	0.0221
glyc-γ-CD	6.79	0.076	3.55×10^{-4}	0.0086
Encapsin	7.65	0.100	4.50×10^{-4}	0.0204
β-CD	8.80	0.098	4.41×10^{-4}	0.0130
γ-CD	7.71	0.076	3.35×10^{-4}	0.0050
Ethoxyzolamid (pH 6.0)				
Buffer		1.10	0.43×10^{-5}	
glyc-β-CD	8.32	6.96	2.69×10^{-5}	0.0027
glyc-γ-CD	6.79	1.88	0.73×10^{-5}	0.0004
Encapsin	7.65	7.67	2.97×10^{-5}	0.0033
β-CD	8.80	6.10	2.36×10^{-5}	0.0022
γ-CD	7.71	1.96	0.76×10^{-5}	0.0004
Hydrocortisone				
Water		0.049	1.34×10^{-4}	
glyc-β-CD	8.32	0.281	7.74×10^{-4}	0.083
glyc-γ-CD	6.79	0.296	8.17×10^{-4}	0.112
Encapsin	7.65	0.211	5.81×10^{-4}	0.062
β-CD	8.80	0.290	8.00×10^{-4}	0.082
γ-CD	7.71	0.239	6.59×10^{-4}	0.073

efficacy ($K_c[D]_o$) can be calculated from the solubilization of the drug at a single cyclodextrin concentration. The efficacy was calculated according to the equation:

$$K_c = \frac{[CD \cdot D]}{[CD][D]_o} \Rightarrow K_c[D_o] = \frac{[D]_t - [D]_o}{[CD]_t - ([D]_t - [D_o)}$$

$$= \frac{([D]_t - [D]_o)/[CD]_t}{1 - ([D]_t - [D]_o)/[CD]_t} \tag{2}$$

The intrinsic solubility of each drug ($[D]_o$) was determined and also the total solubility of the drug in cyclodextrin solution ($[D]_t$). The total cyclodextrin concentration in the solution was 1% and the molar concentrations ($[CD]_t$.) could be calculated from the respective average molecular weights of the derivatives.

The complexation with glyc-γ-CD increased relative to γ-CD with acetazolamide and hydrocortisone. The complexation with glyc-β-CD also improved rela-

tive to β-CD and hydroxypropyl-β-cyclodextrin (Encapsin®) in the case of acetazolamide and hydrocortisone.

4. Conclusion

The general observation was that complexation with glyc-CD was improved relative to other cyclodextrins, especially with glyc-γ-CD, where the extended shape of the cavity would lead to better complexation. In the case of glyc-β-CD, the complexation increased with larger molecules like indomethacin and hydrocortisone. Changing the shape of the cavity had little influence on k_c which suggests that other factors, like polarity, are responsible for the reduction in k_c seen with some other cyclodextrin derivatives.

Acknowledgement

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