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Comparative evaluation of the chiral recognition potential of single-isomer sulfated beta-cyclodextrin synthesis intermediates in non-aqueous capillary electrophoresis

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ABSTRACT

The enantioselectivity of neutral single-isomer synthetic precursors of sulfated-β-cyclodextrins was studied. Four neutral single-isomer cyclodextrins substituted on the secondary side with acetyl and/or methyl functional groups, heptakis(2-*O*-methyl-3,6-dihydroxy)-β-cyclodextrin (HM-β-CD), heptakis(2,3-di-*O*-acetyl-6-hydroxy)-β-cyclodextrin (HDA-β-CD), heptakis(2,3-di-*O*-methyl-6-hydroxy)-β-cyclodextrin (HDM-β-CD), heptakis(2-*O*-methyl-3-*O*-acetyl-6-hydroxy)-β-cyclodextrin (HMA-β-CD), and their sulfated analogs the negatively charged heptakis(2,3-di-*O*-methyl-6-sulfato)-β-cyclodextrin (HDMS-β-CD) and heptakis(2,3-di-*O*-acetyl-6-sulfato)-β-cyclodextrin (HDAS-β-CD) were investigated by non-aqueous capillary electrophoresis in the view of enantiodiscrimination for various drugs and related pharmaceutical compounds. The focus of the present work was on the chiral selectivity studies of the neutral derivatives, which are the synthesis intermediates of the sulfated products. The chiral recognition experiments proved that among the neutral compounds the HMA-β-CD shows remarkable enantioselectivity towards chiral guests in non-aqueous capillary electrophoresis, while HM-β-CD, HDA-β-CD and HDM-β-CD failed to resolve any of the 25 studied racemates under the applied experimental conditions. In order to get deeper insight into the molecular interactions between the studied single-isomer cyclodextrin and chiral fluoroquinolones (ofloxacin, gatifloxacin and lomefloxacin) and β-blockers (propranolol), ^{1}H and ROESY NMR experiments were performed. The 2-*O*-methylation in combination with the 3-*O*-acetylation of the host was evidenced to exclusively carry the essential spatial arrangement for chiral recognition.

1. Introduction

The understanding of chiral recognition and chiral selection is not only essential in biology, but also instructive in stereoselective synthesis and chiral separation. Separating chiral compounds is one of the most popular applications of capillary electrophoresis (CE), commonly achieved by adding chiral selectors, most frequently cyclodextrins (CDs) to the background electrolyte. These popular selectors are composed of (1,4)-linked α-D-glucopyranose units and contain a rather lipophilic cavity and a hydrophilic outer surface. Synthetic modification of the native, 6-, 7- and 8-membered products provides a large variety of selectors decorated with various substituents to achieve the desired enantiorecognition properties [1]. The complexity of the interactions between the selector and the analyte makes it particularly difficult to predict the success of the enantioseparation. Until now, the consensus is that no general scheme of the enantiomeric discrimination of CDs has resulted from studies i.e., how to design cyclodextrin modifications in order to improve effectiveness for resolution of enantiomers.

Several single-isomer α-, β- and γ- cyclodextrin derivatives, all completely sulfated in the C6-positions, several of them additionally substituted on their larger secondary rims with moderately hydrophobic (acetyl) and/or hydrophobic (methyl) functional groups, have already been examined for enantiomeric discrimination of drugs by capillary electrophoresis [2–12]. Sulfation at

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primary side provides an anionic site where chiral guest cationic part can reside. This electrostatic-supported interaction may play role in separation. The use of single-isomer structures in these studies has been stressed as an absolutely mandatory condition for two reasons: (a) to eliminate the drawback of several isomers in commercially available sulfated CDs resulting in ill-defined complex mixtures and (b) to provide a comprehensive theoretical framework on the enantiomer migration in electrophoresis and identify the variables in order to predict successful separations [3,8,10]. The sulfated-derivatives of di-*O*-methyated-, di-*O*-acetylated- and the 2-*O*-methylated-3-*O*-acetylated-α-CD hosts have different separation selectivity toward the tested analytes and could be used as complementary chiral resolving agents having specific applications in enantioseparation [3,7,10]. In order to facilitate the choice of the adequate chiral selector, various screening methods and theories have been established [13,14]. The most widespread used CHARM model is only applicable for charged chiral selectors, but no such model is available for neutral derivatives. The main focus of our study was on the neutral single-isomer cyclodextrin derivatives, which are the intermediates of the final sulfated products.

To date, no extensive study has been reported on the comparison of chiral recognition abilities of structurally-related single-isomer neutral CDs. The research groups of Holzgrabe and Chankvetadze conducted several studies evaluating the application of heptakis(2,3-di-*O*-acetyl-6-hydroxy)-β-cyclodextrin (HDA-β-CD) and heptakis(2,3-di-*O*-methyl-6-hydroxy)-β-cyclodextrin (HDM-β-CD) as a chiral selector in aqueous systems [15–25].

In the current study, our aim was to reveal whether the neutral synthetic precursors of the frequently applied single-isomer sulfated CDs already bear chiral selectivity prior to the final modification or it is associated with the sulfate group essentially. Due to the low solubility of these neutral CD derivatives in water, the chiral resolving ability was investigated by non-aqueous capillary electrophoresis (NACE).

2. Materials and methods

2.1. Materials

All CD derivatives, four neutral hosts being heptakis(2-*O*-methyl-3,6-dihydroxy)-β-cyclodextrin (HM-β-CD), HDM-β-CD, HDA-β-CD and heptakis(2-*O*-methyl-3-*O*-acetyl-6-hydroxy)-β-cyclodextrin (HMA-β-CD) and two sulfated analogs, heptakis(2,3-di-*O*-methyl-6-sulfato)-β-cyclodextrin (HDMS-β-CD) and heptakis(2,3-di-*O*-acetyl-6-sulfato)-β-cyclodextrin (HDAS-β-CD) were products of Cyclolab (Budapest, Hungary), synthesized by either Vígh and coworkers or by Cyclolab according to the well-described procedures in the literature [26–29].

Methanol, acetonitrile, *N*,*N*-dimethylformamide (DMF), H_3PO_4, dichloroacetic acid, trimethylamine, sodium acetate, NaCl, citric acid, Tris and NaOH used for the preparation of buffer solutions were of analytical grade and purchased from commercial suppliers. All reagents were used without further purification. Purified water was used throughout the capillary electrophoretic study.

D_2O (99.9% D atom) and methanol (99.8% D atom) used in NMR studies were products of Merck KGaA (Darmstadt, Germany).

Altogether 25 racemic pharmaceutical compounds purchased from Sigma-Aldrich (Budapest, Hungary), as test molecules with diverse chemical structures were selected for the study: mandelic acid, 2-chloromandelic acid, 4-chloromandelic acid, 4-methoxymandelic acid, ephedrine, hexobarbital, vildagliptin, atropine, lomefloxaxin, ofloxacin, gatifloxacin, atenolol, carvedilol, propranolol, metoprolol, pindolol, prasugrel, terbutaline, amlodipine, citalopram, venlafaxine, cetirizine, naproxen, and ibuprofen. Levofloxacin and *S*-(−)-propranolol applied for EMO studies were also purchased from Sigma-Aldrich. Alogliptin was a generous gift from a local pharmaceutical company. Chemical structures of 16 compounds, shortlisted after the preliminary experiments are depicted in Fig. 1.

2.2. Capillary electrophoresis

All non-aqueous capillary electrophoretic measurements were carried out on an Agilent 7100 instrument (Agilent Technologies, Waldbronn, Germany), equipped with a photodiode array detector (DAD) and the Chemstation software for data handling. Measurements were performed in untreated fused silica capillaries (33.5 cm total and 25.0 cm effective length and 25 μm i.d.) purchased from Agilent. Six different buffer compositions were evaluated as potential background electrolytes (BGEs) based on previous NACE experiments. Vígh et al. elaborated the systems containing (i) 0.02 M H_3PO_4, 0.01 M NaOH in methanol [30], (ii) 0.05 M dichloroacetic acid, 0.025 M triethylamine in methanol [10], (iii) 0.025 M dichloroacetic acid, 0.05 M triethylamine in methanol [10]. In these papers, separation characteristics of HDA-β-CD and HDM-β-CD as chiral selectors are emphasized. Altria and Bryant reported a BGE of (iv) 0.002 M sodium acetate in methanol/acetonitrile 50-50% suitable for the chiral analysis of acidic compounds with acetylated-β-CD [31], Valkó et al. described (v) 0.01 M NaCl in DMF for the separation of dansyl-amino acids by β-CD [32], and Wang and Khaledi used (vi) 0.1 M citric acid, 0.05 M Tris in DMF to separate racemic guests using native and derivatized cyclodextrins (methylated, hydroxypropylated and sulfated CDs) [33]. All solutions were filtered through 0.45 μm pore size PTFE filters prior to use. Due to the low boiling point of methanol and the limited hydrolytic stability of all acetylated cyclodextrins, the BGEs were prepared on a daily basis to avoid undesirable changes in their compositions. The electrophoretic conditions were identical for each buffer: 20 °C temperature, 200 nm detection wavelength (supported with 214 nm, if necessary), 360 nm reference wavelength, +30 kV applied voltage, 50 mbar * 4 s hydrodynamic injection. At the beginning of each work day, the capillary was rinsed with 0.1 M NaOH for 5 min and methanol for 10 min. Between runs, the preconditioning comprised of washing with 0.1 M NaOH for 90 s, followed by 90 s of methanol and 300 s of the appropriate BGE.

The racemic samples were dissolved in methanol at 1 mg/mL concentration and applied without further dilution.

In the case of enantioseparation, resolution values were calculated according to the general formula:

$$\mathbf{R_S} = 2\,(\mathbf{t}_1 - \mathbf{t}_2)\,/\,(\mathbf{w}_1 + \mathbf{w}_2) \quad (1)$$

where w_1 and w_2 stand for the extrapolated peak widths at the baseline, t_1 and t_2 for the migration times of the enantiomers.

2.3. Nuclear magnetic resonance spectroscopy

^{1}H NMR spectra were recorded at 298 K on a 600 MHz Varian DDR NMR spectrometer equipped with a 5 mm inverse-detection gradient (IDPFG) probe. Stock solutions 1 mg/mL in CD_3OD containing 0.02 M H_3PO_4, 0.01 M NaOD and 0.01–0.015 M of a selected CD (HDM-β-CD, HDA-β-CD or HMA-β-CD), were prepared from the ofloxacin, gatifloxacin, lomefloxacin, and propranolol racemic samples. In the case of ofloxacin, the enantiospecific assignment of all ^{1}H resonances (as a result of diastereomeric complex formation) were completed by spiking the sample with levofloxacin. Chemical shifts were referenced to the residual solvent resonance of CHD_2OD at 3.35 ppm.

In the case of 2D ROESY NMR experiments for ofloxacin-HMA-β-CD, gatifloxacin-HMA-β-CD and propranolol-HMA-β-CD systems, the parameters were as follows: the molar ratio of the racemic