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Chiral recognition and racemic resolution of drug enantiomers by electrophoresis based on host–guest complexation

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Abstract

The use of cyclodextrins (CDs) and their derivatives as enantioselective modifiers for chiral discrimination in various capillary electrophoresis (CE) modes has been growing in popularity. The current studies have successfully applied chiral CE mediated with cyclodextrins for the chiral recognition and racemic resolution of several water-soluble melatonergic drug enantiomers such as BMS-191435, BMS-191602 and BMS-193587. Cyclodextrins including β-cyclodextrin (β-CD) and its derivative heptakis(2,6-di-O-methyl)-β-cyclodextrin (DM-β-CD) were chosen as chiral selectors incorporated in the buffer system. Comparative studies of these two CDs on the chiral recognition of drug enantiomers indicated specific inclusion of the guest molecules in the CD cavity. Optimum chiral capillary electrophoresis conditions such as concentration of chiral selectors and proper choice of pH were studied. Complexation binding constants for the interaction between chiral selectors and drug enantiomers were determined, indicating higher affinity for *S* enantiomers. Molecular interactions with DM-β-CD were also investigated using a computer modelling approach. Two distinct molecular interactions relating to the inclusion complexes were simulated. Energy minimized structures indicated the interaction differentiation of 1 kcal/mol in favor of *S* enantiomer (1 cal=4.184 J), which is consistent with chiral CE results. NMR spectroscopy was also utilized to determine the sites of molecular interaction between enantiomers and chiral selectors. These studies provide a structural basis for the understanding and application of CD mediated CE chiral separations in pharmaceutical research.

Keywords: Enantiomer separation; Complexation; Buffer composition; Drugs; Cyclodextrins

1. Introduction

The resolution of a racemic mixture into its enantiomers is becoming an increasingly relevant issue during recent years in drug discovery and pharmaceutical development [1]. Chirality, or optical activity, is a significant characteristic of many synthetic and biologically active compounds. Drug substances are often administered as racemates even if the two enantiomers exhibit different pharmacological activity profiles. In fact, many of the widely prescribed drugs contain at least one chiral center and approximately 75–90% are marketed as racemates [2]. During recent years, these issues have become a major concern for regulatory agencies addressing the development of optically pure enantiomer drugs [3]. This has led to an increased understanding of the biological action of drugs with respect to their stereochemistry. The development of enantioselective analytical methodologies has accelerated the already extensive investigation of the pharmacologic and toxicologic properties of individual drug enantiomers. Among the various research activities associated with these areas, the

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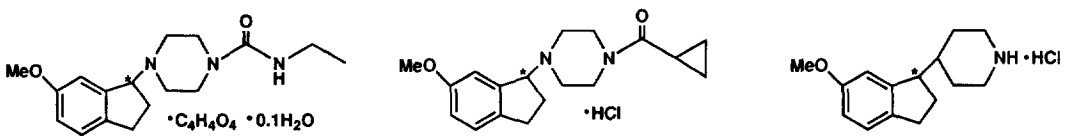
rapid and efficient separation of stereoisomers continues to be a challenging task.

Chiral recognition and racemic resolution are generally difficult due to the similar physico-chemical properties which often challenge existing analytical methods. Among many analytical techniques such as polarimetry, circular dichroism, nuclear magnetic resonance (NMR) spectroscopy and crystallization, chromatography-based separation methodologies are perhaps the predominant choice for direct enantiomeric resolution. In particular, capillary gas chromatography (GC), modern high-performance liquid chromatography (HPLC) and supercritical fluid chromatography (SFC) have been the most widely used methods in this area over the past decade [4–8]. The success of chiral chromatography has been predominantly driven by the advances in HPLC techniques and the wide availability of commercial chiral columns. However, the design of appropriate chiral stationary phases has been considered an expensive and complex process. Column selection generally requires extensive screening and usually may only be applied to a limited number of racemates. The alternative use of chiral selectors as an additive in the HPLC mobile phase has not proved to be popular and is limited by the concerns of consuming large amounts of chiral reagent, especially costly chiral selectors.

Recently, capillary electrophoresis (CE) and electrokinetic capillary chromatography (EKCC) have generated tremendous interest in chiral separation [9–19]. Generally, these methods can be classified into two broad categories, techniques that use a chiral additive in the mobile phase and techniques that involve a chiral stationary phase immobilized either on the capillary wall or on an appropriate support such as gel matrix. Chiral separation based on electromigration methods possesses various advantages such as the inheritance of the high resolving power of CE techniques to the beneficial of maximum resolution of enantiomeric mixtures, rapid screening of various chiral selectors in conjunction with an appropriate buffer system for the most satisfactory resolution, flexibility of column selection and buffer additives, and considerably reduced consumption of chiral reagents in chiral CE format as compared to HPLC. Among various chiral reagents, cyclodextrins (CDs) and their derivatives are perhaps the most commonly used selectors in chiral CE [2,15,16] although other media such as proteins (e.g. serum albumin and α_1-acid glycoprotein) and macrocyclic antibiotics (e.g. rifamycins) have also been reported [17–19]. The host–guest complexation between CDs and enantiomeric analytes provides a unique mechanism of molecular interaction to achieve chiral separations. In addition, the lack of a chromophore and readily available modified forms are also advantages of CDs. The use of cyclodextrins and their derivatives as enantioselective modifiers for chiral discrimination in various CE modes has been growing in popularity, particularly in pharmaceutical applications.

In our recent efforts in drug discovery research, a class of central nervous system (CNS) related substances such as water-soluble melatonergic agents have been identified as potential drug candidates. Most of these compounds possess one or two chiral centers and have dramatically different activity between their enantiomers. Some representative structures of these compounds are displayed in Fig. 1. Racemic separation of these enantiomers and the understanding of molecular interaction with a specific selector appears to be significant for further development of these drug candidates. In current studies, we have utilized the host–guest interaction approaches with a selection of CDs and derivatives for the resolution of racemic mixtures. The fundamental molecular interactions between enantio-



BMS-191435-02 BMS-191602-02 BMS-195387-01

Fig. 1. Structures of selected water-soluble melatonergic drug compounds.

meric compounds and chiral selectors are studied through computer-modelling approaches to calculate host–guest complexation. NMR studies aimed at determining interaction sites are also described.

2. Experimental

2.1. Apparatus

2.1.1. Chiral capillary electrophoresis

Chiral capillary electrophoresis was performed using untreated fused-silica capillaries (Polymicro Technology, Phoenix, AZ, USA) with 50–75 μm I.D. and 360 μm O.D. and effective separation lengths of 40.5–50.6 cm. The detection window was made by burning the polyimide coating and placing it about 7 cm from the outlet. A Beckman P/ACE model 2100 CE system (Beckman Instruments, Fullerton, CA, USA) equipped with a UV absorbance detector was used for all separations. The temperature-controlled capillary chamber was maintained at 25°C by employing a liquid coolant. Pressure injection mode (0.5 p.s.i. low pressure; 1 p.s.i.=6894.76 Pa) was employed throughout all experiments. The components were monitored with the UV detector at a wavelength of 214 nm. Data were collected and analyzed with Beckman System Gold software.

2.1.2. Nuclear magnetic resonance spectroscopy

NMR data were collected on a Varian Unity 500 spectrometer using a standard presaturation pulse sequence. Residual water was suppressed with low-power continuous-wave radio-frequency irradiation. All data were collected at 25°C. Proton 90° pulses were calibrated to within 0.2 μs accuracy. Data were processed using VNMR, a software package supplied with the spectrometer. Chemical shifts were referenced to the internal residual water resonance line.

2.2. Materials and reagents

β-Cyclodextrin (β-CD) and heptakis(2,6-di-O-methyl)-β-cyclodextrin (DM-β-CD) were purchased from Sigma (St. Louis, MO, USA). The following compounds were obtained in house: BMS-191435, BMS-191602, BMS-191435(*R*), BMS-191435(*S*) and BMS-195387. HPLC-grade water and methanol, sodium phosphate, sodium hydroxide and phosphoric acid were obtained from Fisher Scientific (Fair Lawn, NJ, USA).

The basic buffer for chiral CE experiments consisted of 50 m*M* sodium phosphate adjusted to an appropriate pH value, depending on the specific experimental requirement, with varied concentrations of CD additives. All samples were dissolved in HPLC-grade water at a concentration of 0.2 mg/ml and were subsequently diluted by a running buffer as needed for injection. For NMR experiments, a 50 m*M* (pH=2.58) phosphate buffer was made with [$^2H_2$] water. Samples selected for NMR studies were dissolved in this buffer to a concentration of 5 m*M*. CDs were prepared in the same phosphate buffer at a concentration of 30 m*M*. Both sample and CD solutions were combined for complexation measurement.

2.3. Molecular modelling

Molecular-modelling calculations were carried out using Macromodel to study the complexation of DM-β-CD with enantiomers of BMS-191435. Molecular complexes were initially constructed by docking chiral BMS-191435 into the cavity of DM-β-CD using the X-ray crystallographic structure of DM-β-CD. To find the optimal structure, several docked structures were obtained and followed by energy minimization using molecular mechanics. Calculations were performed using the AMBER force field as implemented in the MacroModel program. The effect of solvent was treated by the continuum solvation model for water developed by Still et al. [20]. The basic amine of BMS-191435 was assumed to be protonated.

3. Results and discussion

The selectivities of appropriate chiral selectors in combination with the favorable kinetics of capillary separations are key factors for successful separations. Among various available chiral media, CDs and corresponding derivatives are by far the most powerful chiral mobile-phase selectors for a wide range of compounds. CDs are chiral cyclic oligosaccharides,

consisting of D-(+)-glucopyranoside unit arranged in a truncated cone shape. Their unique features to form host–guest complexes with various compounds and pharmaceutical drugs and the chiral recognition mechanism have been extensively studied [21–23]. In the present studies, β-CD and its derivative DM-β-CD were utilized as an additive in the separation buffer for host–guest complexation with several chiral drug compounds. Applications of this approach for the separation of racemic mixtures of CNS related substances such as water soluble melatonergic agents, BMS-191602, BMS-191435 and BMS-195387 were studied. As shown in Fig. 2A, single peaks were observed for the three components in the absence of CDs. Upon addition of DM-β-CD to the buffer solution, separation of racemic mixtures into their enantiomers for each of the studied compounds was achieved. Fig. 2B illustrates the separation of a mixture consisting of the three melatonergic agents. With the presence of 28 m*M* DM-β-CD in the buffer, BMS-191602 and BMS-191435 were well resolved into their individual enantiomers. BMS-195387 was only partially resolved under these experimental conditions. The change in the migration order upon introduction of the CD in the electrolyte may be largely related to the structural tendency of the inclusion complex although other parameters such as the charge status, molecular mass and hydrophobic or hydrophillic property may also contribute. The overall inclusion binding for BMS-195387 appears to be the most favored for both *R* and *S* enantiomers as they are retained longer by neutral CD molecules with partial resolution. The association constants determined for BMS-191602 and BMS-191435 in this study perhaps can be also linked to this observation. These phenomena indicate that the inclusion interaction between CD and guest molecules is often more favorable towards one enantiomer rather than another, depending on the specific structural orientation. It is known that CDs in the presence of water are mediated by their exterior hydrophillicity, where the inner hydrophobic cavity becomes the primary site of interaction with organic molecules. Derivatization of the secondary hydroxyl groups can indeed affect the inclusion phenomena as observed in this study. The use of β-CD for the resolution of these racemic mixtures is somewhat different from its derivative. The differences in molecular size with the CD cavity determines the inclusion of various guest molecules. Possible hydrogen bond interactions near the mouth region of the CD cone may also have some contribution to the chiral recognition phenomena.

During a kinetically favorable chiral CE separation process, there are many parameters which can affect a specific chiral recognition. Even slightly different stability constants of the dynamic inclusion complexation for each enantiomer can result in appreciably different mobilities in an electric field. A proper concentration of CDs, choice of pH to ensure a charge on the molecules, control of electroosmosis, and various additives can be useful in 'fine-tuning' the component, presumably through various competitive interactions. As discussed in the above studies, specific molecular interactions are involved between CDs and the studied molecules. Optimization of separation was conducted in terms of the concentration of selected chiral selectors and buffer pH. Various separation patterns were obtained for both BMS-191435 enantiomers and BMS-191602 enantiomers as a function of either DM-β-CD or β-CD concentration. As the CD concentration increases to a certain value, the resolution increases as well. Beyond this concentration, however, the resolution either remains constant or decreases slightly, depending on the structure of an individual compound itself. It appears that there is an optimal chiral selector concentration for obtaining the maximum resolution of racemic mixtures. Fig. 3 shows the correlation between peak resolution and the concentration of CDs. The maximum concentration was found to be in the range 15–28 mM for both DM-β-CD and β-CD.

We described that the degree of enantiomeric separation depends on the concentration of chiral selector. The experimental results appear to be consistent with a simple model proposed by Wren et al. [24,25]. The basic concept is that the enantiomers and the chiral selector are in rapid equilibrium with the formed complex which has a different electrophoretic mobility from that of the enantiomer alone during an electrophoretic run. The apparent electrophoretic mobility (μ_{ap}) of the enantiomers is, therefore, related to both the concentration ([C]) of the chiral selector and the association constant (K) between the enantiomers and the selector as described in the following equation:

$$\mu_{ap} = (\mu_f + \mu_c K[C])/(1 + K[C]) \quad (1)$$

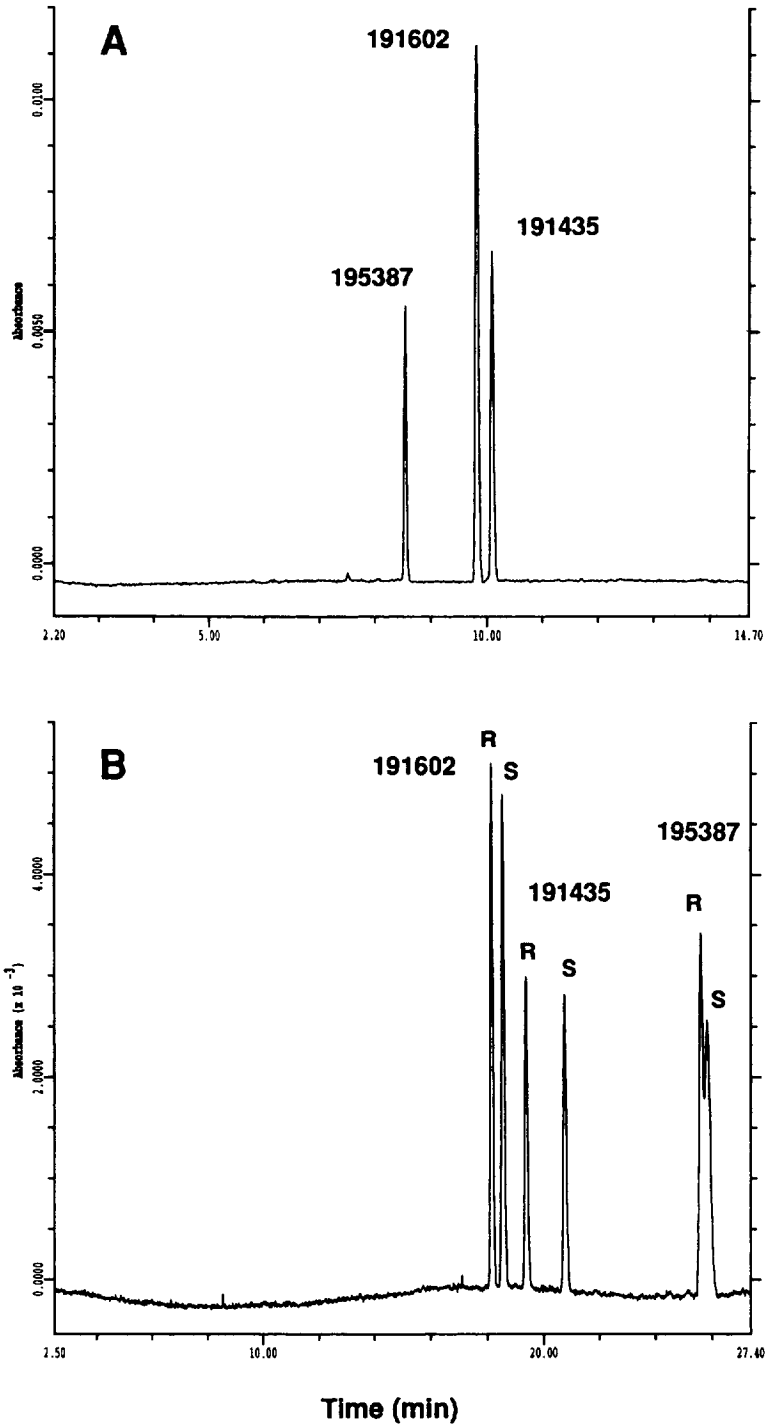


Fig. 2. Separations of racemic mixture using (A) CE, and (B) chiral-CE mediated with DM-β-CD. Peak identification is indicated in electropherograms. Capillary column: 57.3 cm (50.6 cm effective separation length 75 μm I.D.×360 μm O.D.). Background electrolyte: (A), 50 m*M* phosphate buffer with pH=2.58; (B), 50 m*M* phosphate buffer (pH=2.58) with 28 m*M* DM-*β*-CD. Applied voltage: 20 kV.

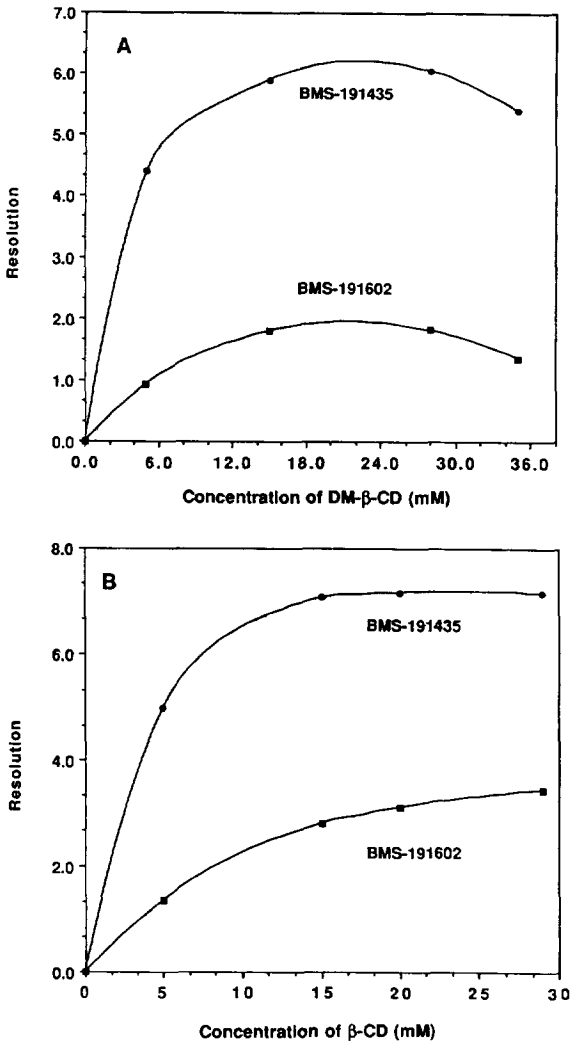


Fig. 3. Resolution plots of chiral separations of melatonergic drug enantiomers in the presence of (A) DM-β-CD, and (B) β-CD. Conditions are the same as in Fig. 2.

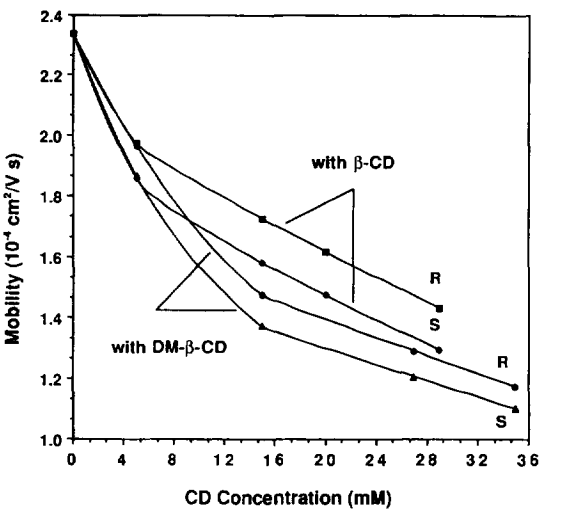


Fig. 4. Binding curves of complexation between BMS-191435 enantiomers and cyclodextrins. Binding constants were determined through curve fitting using Systat software. Conditions are the same as in Fig. 2.

A representative plot of the measured apparent electrophoretic mobility of BMS-191435 as a function of both DM-β-CD and β-CD concentrations in the separation buffer is displayed in Fig. 4. The measured mobility of free enantiomers (μ_f) in the absence of the chiral selector was found to be 2.34×10^{-4} cm^2/V s. The binding constants determined through a non-linear curve fitting procedure are 60.8 M^{-1} and 95.1 M^{-1} for BMS-191435(R) and BMS-191435(S) enantiomers, respectively, in the presence of β-CD. These values are 67.4 M^{-1} and 85.5 M^{-1} in the case of the DM-β-CD selector. Furthermore, the thermodynamic association constant is also related to the free energy change by the standard relationship $\Delta G = -RT\ln K$. In our studies, this energy differentiation was determined to be around 0.3 kcal/mol in favor of the BMS-191435(S) enantiomer (1 cal=4.184 J). The electrophoretic mobility of the formed intermediate complex (μ_c) was found to be around 1.0×10^{-4} cm^2/V s. The large binding constant indicates a higher affinity for the complexation, and, thus, a lower electrophoretic mobility since the CDs used in this study are neutral molecules which tend to drag the charged species and cause a slow migration towards the detection window.

In addition to the CD concentration, the buffer pH has direct effects on the electrophoretic mobility of enantiomers and the formed complex with the chiral selector. Optimal pH assays were thus performed with a buffer consisting of 28 mM DM-β-CD as previously demonstrated for both BMS-191435 and BMS-191602 enantiomers. Similar results were also obtained for β-CD with a concentration of 15 mM in buffer. The plots correlating resolution and electrolyte pH are displayed in Fig. 5. With both CDs, an

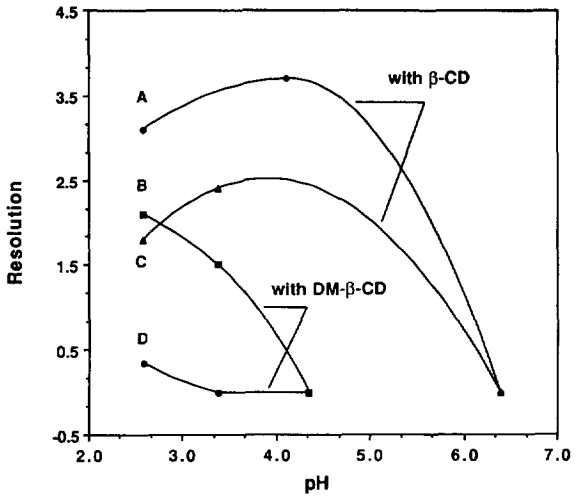


Fig. 5. Resolution plots of chiral separations of melatonergic drug enantiomers in the presence of 15 m*M* of β-CD, (A) and (C), and 28 m*M* of DM-β-CD, (B) and (D). Plots (A) and (B): BMS-191435; plots (C) and (D): BMS-191602. Other conditions are the same as in Fig. 2.

optimal resolution pH appears to exist. Some of the melatonergic drug enantiomers contain secondary aliphatic amines which may not be fully ionized in the pH range studied. The formation of the hydrogen bond between this functional group and the CD moiety appears to be crucial to the enantiomeric separation. It was evidenced that higher pH results in the partial or total loss of resolution. The optimal pH determined in this study was 2.58 for DM-β-CD and 4.10 for β-CD. Other parameters such as organic modifiers, surfactant additives and capillary treatment may also have a significant impact on the resolution, but are not the subject of current studies.

The fundamental molecular interactions between enantiomeric compounds and chiral selectors can also be assessed through computer-modelling approaches and NMR spectroscopy for determining interaction sites and configure host–guest complexation. As previously discussed, both DM-β-CD and β-CD facilitate the chiral recognition and racemic resolution of various melatonergic drug enantiomers. It was determined that chiral discrimination varies to a great extent although most of the compounds investigated possess similar structure and functional groups. This raises fundamental concerns about how an individual chiral enantiomer interacts with a particular chiral selector. To address this question, other analytical methods are needed to gain insights into chiral discrimination during complexation.

Computer modelling of known structures coupled with energy minimization calculations is a powerful technique for evaluating and understanding chiral interactions. CDs are particularly amenable to this approach because of their well-defined and relatively static structure. There are a number of requirements for chiral recognition by CDs. For example, an inclusion complex must be formed with a relatively tight fit between the complexed guest and the CDs. In addition, the chiral center or a substituent of the chiral center is assumed to be proximate and to interact with the mouth of the CD cavity. The unidirectional 2- and 3-hydroxyl groups located at the cavity mouth are considered to be particularly important in chiral recognition. Using a water-soluble chiral melatonergic agent BMS-191435 as a model, molecular-modelling studies were carried out to gain insights into the chiral discrimination in the complexation of DM-β-CD with the two enantiomers, BMS-191435(*R*) and BMS-191435(*S*). To facilitate the calculation, the basic amine group of both enantiomers was assumed to be protonated, which is consistent with experimental conditions where an acidic buffer (pH=2.58) was used. Computer modelling indicated two distinct molecular interactions, as demonstrated in Fig. 6. The hydrophobic head of each enantiomer was complexed to the relatively immobile CD core. The hydrophillic tail protrudes from the CD mouth and forms hydrogen bonds to the mouth's external surface where hydroxyl groups are located. Simple energy minimization indicated a differentiation in the complexation of DM-β-CD with enantiomeric BMS-191435(*R*) and BMS-191435(*S*). The predicted interaction differentiation is 1 kcal/mol in favor of BMS-191435(*S*), indicating tighter binding between the BMS-191435(*S*) enantiomer and DM-β-CD. This is reasonably consistent with the chiral CE separation and the determined binding constant as well as the thermodynamic free energy change, as previously discussed. The chiral recognition and discrimination by host–guest complexation achieved in the electrophoretic experiments appears to be partially related to the binding energy. The difference in this type of binding behavior together with changes of other

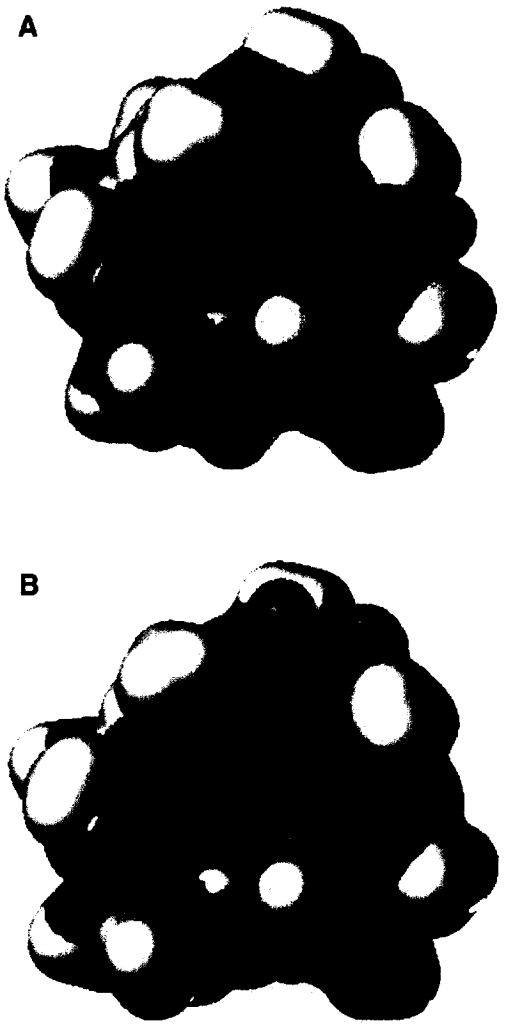


Fig. 6. Computer modelling of complexation of (A) BMS-191435(*R*) (green) and (B) BMS-191435(*S*) (blue) enantiomers with DM-β-CD (red and white). The configurations, with the aromatic group of BMS-191435 inside the cavity of DM-β-CD, represent energy-minimized inclusion complexes using MacroModel and AMBER force field. The interaction differentiation is 1 kcal/mol in favour of BMS-191435(*S*) enantiomer.

parameters could thus result in a separation of racemic mixtures.

The molecular details of these interactions were also studied by NMR spectroscopy. A comparison of the 1D NMR spectra of BMS-191435(*R*) in the absence and presence of DM-β-CD shows a significant change in the resonance lines assigned to the 5, 5′ protons of BMS-191435(*R*). A similar change in the position of the 5, 5′ resonances are seen for the BMS-191435(*S*) enantiomer. No other ^{1}H resonance lines of these compounds changed in the 1D spectra and many resonances were obscured by the presence of excess DM-β-CD. This change in chemical shift is a direct result of a change in the magnetic environment which is likely to be the result of a molecular interaction with DM-β-CD. Although these data do not show direct evidence of differential structural interactions between the two enantiomers and the DM-β-CD, the interacting sites identified seem to be reasonably consistent with the common assumption that the most active sites are generally close to the chiral center.

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