Investigation of Enantiomer Separation Using Chiral Crown Ethers as Chiral Selectors

Wonjae Lee[†]

Abstract

A number of chiral selectors have been developed and applied for enantiomer separation of a variety of chiral compounds. Among these chiral selectors are chiral crown ethers, a class of synthetic host polyether molecules that bind protonated chiral primary amines with high selectivity and affinity. In this paper, two important chiral crown ethers as chiral selectors of bis-(1,1'-binaphthyl)-22-crown-6 and (18-crown-6)-2,3,11,12-tetracarboxylic acid (18-C-6-TA) are focused. They have been widely used to resolve the enantiomers of chiral compounds containing a primary amino moiety using chiral stationary phases (CSPs) or chiral selectors by high-performance liquid chromatography (HPLC), capillary electrophoresis (CE) and so on in chirotechnology. Also, it was described that the commercially available covalent type HPLC CSPs derived from (+)- and (−)-18-C-6-TA have been developed and successfully applied for the resolution of various primary amino compounds including amino acids.

Keywords: Chiral Crown Ether, Chiral Selector, Chiral Stationary Phase, 18-Crown-6-2,3,11,12-tetracarboxylic Acid, Capillary Electrophoresis, High-performance Liquid Chromatography.

1. Introduction

The Nobel Prize in Chemistry 1987 was awarded to Donald J. Cram, Jean-Marie Lehn and Charles J. Pedersen "for their development and use of molecules with structure-specific interactions of high selectivity"[1,2]. They were recognized as pioneers of host-guest chemistry in biomolecular study using crown ethers. As synthetic macrocyclic polyethers, crown ethers have cavity in a specific size, which were synthesized by Pedersen for the first time in 1967[3]. Ether oxygens of crown ether surround inner wall of its cavity, which play a role as coordination atoms of electron donor. As a result, metal ions or ammonia are bound inside the cavity, which enables forming a complex. Afterward, Cram of the pioneering leader in host-guest chemistry prepared various chiral crown ethers with chiral 1,1′-binaphthyl unit as chiral selectors used in the liquid-liquid extraction and in HPLC for the first time in the late 1970s[2]. He mainly applied the chiral crown ethers for chiral separation of α-amino acids. Chiral crown ethers have been developed in the way that chiral barrier is added to inside the crown ether as a proper chiral unit[2,4]. For example, chiral aromatic cyclic compounds such as binaphthyl or biphenanthryl units, helicence derivatives, and chiral natural compounds such as tartaric acid or carbohydrate play a role as chiral barrier in crown ether, which leads to preparation of an effective chiral crown ether. Besides, chiral aza crown ether, chiral pyridino crown ether and phenolic chiral crown ether have been introduced and reported[4]. Since biomolecular study in host-guest chemistry using crown ether is a very important research field, chiral crown ethers with various structures as chiral selectors have been developed and employed. This paper focused on two chiral crown ethers; the one is bis-(1,1′-binaphthyl)-22-crown-6 derived from chiral binaphthyl moiety and the other is (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid (18-C-6-TA) derived from L-tartaric acid, both of which have been used the most widely until recently for enantiomer separation as chiral selectors (Fig. 1). Therefore, the purpose of this study is to describe chirotechnology that is related to various studies on development and application for enantiomer separation performed in our group where the two aforementioned chiral crown ethers of chiral selectors have been used in HPLC and CE.

College of Pharmacy, Chosun University, Gwangju, 501-759, Korea

[†]Corresponding author : wlee@chosun.ac.kr
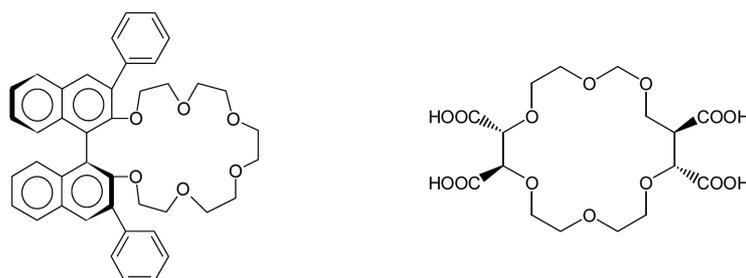
Fig. 1. The structures of two chiral crown ethers as chiral selectors of bis-(1,1'-binaphthyl)-22-crown-6 for Crownpak CR(+) (left) and (+)-18-C-6-TA derived from L-tartaric acid (right), respectively.

2. Enantiomer Separation Using 1,1'–Binaphthyl Based Chiral Crown Ether

As explained above, a primitive chiral crown ether type HPLC CSP derived from chiral binaphthyl moiety was first introduced by Donald J. Cram in 1979[2]. Thereafter, a CSP using bis-(1,1'-binaphthyl)-22-crown-6 compound as a chiral selector was developed in 1987 by Shinbo (Fig. 1, left)[5]. This HPLC CSP has been employed very effectively for enantiomer separation of chiral compounds with the primary amino group including amino acids and it was commercialized in the name of Crownpak CR by Daicel company (www.chiral-tech.com)[5,6]. The chiral crown ether coated type Crownpak CR was prepared by taking advantage of lipophilic interaction between the chiral crown ether and the octadecyl group of silica gel. The chiral selector used for Crownpak CR has a chiral cavity of chiral crown ether in molecule due to chiral binaphthyl group. Therefore, chiral separation is caused by host-guest stereoselective complexation between the chiral cavity of crown ether and the racemic protonated primary amino group[2,5]. However, because Crownpak CR HPLC CSP is prepared by coating the chiral crown ether of chiral selector on ODS solid support, use of a mobile phase containing more than 15% methanol in water is not permitted for column safety. If more than 15% methanol in water or other solvents rather than methanol is used as a mobile phase, there is a problem that adsorbed chiral selector may be washed out, which causes damage to column. In addition, if any analyte has high hydrophobicity, elution itself may not take place or it may take a tremendously long time for analysis under the above limited mobile phase conditions[6,7].

In order to develop improved Crownpak CR CSP,

Shinbo prepared a CSP that 6,6'-dioctyl-3,3'-diphenyl-1,1'-naphthyl)-20-crown-6 (two octyl groups were added to the previous chiral selector) was coated on octadecyl silica gel[8]. As the two added octyl groups were attached to chiral crown ether moiety, it was expected to improve lipophilic interaction between the octadecyl group of ODS silica gel and the chiral crown ether with two octyl groups. And it was successful in enantiomer separation of several analytes like α-amino acids, 1-phenylethylamine and 3-aminocaprolactam. However, when more than 40% methanol in water is used as a mobile phase in HPLC, the chiral crown ether may still come off from coating, which causes damage to column. On the other hand, as a way to overcome the fundamental drawback of the crown ether coated type Crownpak CR, new CSPs were developed by covalently bonding of the previous chiral selector of (3,3'-diphenyl-1,1'-naphthyl)-20-crown-6 to silica gel[9,10]. Owing to covalent bonding of chiral selector to silica gel, it was possible to use any kind of mobile phases in HPLC column phase without any solvent limitation. It was reported that the newly developed CSPs were very useful for enantiomer separation of various chiral primary amino analytes including amino acids.

## 3. Enantiomer Separation Using 18-C-6-TA of Chiral Crown Ether in CE

Kuhn group reported a chiral crown ether of (+)-18-C-6-TA as chiral selector to conduct enantiomer separation of amino acids in CE analysis in 1992 (Fig. 1)[11,12]. Originally, the chiral crown ether (+)-18-C-6-TA was synthesized through various steps, starting from L-(+)-tartaric acid for the first time in 1980 by Lehn who was awarded with the Nobel Prize in Chemistry for

development of crown ether[13]. Also, our group applied (+)-18-C-6-TA and/or (−)-18-C-6-TA to perform enantiomer separation of various aromatic amino acids and their esters in CE[14-16]. The report was the first to conduct enantiomer separation using (+)- and (−)-18-C-6-TA in order to ensure the exactly opposite elution order of these analytes in CE, respectively[14]. In addition, when β-blockers are enantiomerically separated in CE, additional use of 18-C-6-TA as a dual chiral additive, rather than use of only DMβCD as chiral selector, leads to better results of chiral separation[16]. The better simultaneous enantiomer separation in CE was shown when 11 β-blockers were applied along with DMβCD and (+)-18-C-6-TA as dual chiral selectors.
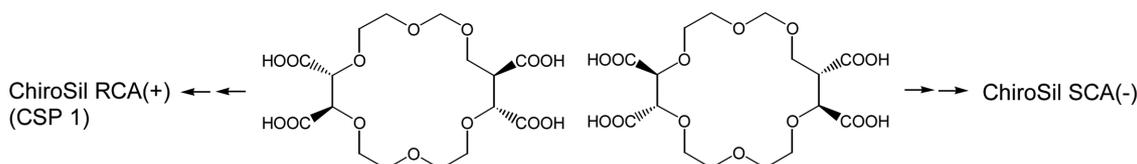
4. Enantiomer Separation Using 18-C-6-TA of Chiral Crown Ether in High-performance Liquid Chromatography

Crownpak CR was the unique coated type HPLC CSP with chiral crown ether of 1,1′-binaphthyl based chiral selector on ODS solid support, although it has been widely used[5,6]. In order to prepare CSP that was superior to the coated type Crownpak CR with an intrinsic drawback, we intended to develop and commercialize a new crown ether type CSP with a covalently bonding between chiral selector and silica gel[17,18]. To this end, we paid attention to chiral crown ether of (+)-18-C-6-TA as a chiral selector employed by Kuhn group in CE. So, we used (+)-18-C-6-TA with acetyl chloride to convert it to (+)-18-C-6-TA dianhydride,

which was reacted with aminopropyl silica gel in the presence of triethylamine. As a result, the chiral selector of (+)-18-C-6-TA was covalently bonded to prepare a new chiral stationary phase (CSP 1) (Fig. 2, left)[17-24]. In the meantime, Machida group also used (+)-18-C-6-TA to react with aminopropyl silica gel using 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) as a coupling agent to prepare CSP 2 (Fig. 2, right)[25,26]. Interestingly, at the same time the two research groups independently developed each new HPLC CSP, starting from the same crown ether of (+)-18-C-6-TA as a chiral selector. However, the different preparation process for (+)-18-C-6-TA derived CSP resulted in its structural difference, as shown in Fig. 2. When the same analytes of amino acids and chiral amines were resolved, CSP 1 developed by our research group provided more excellent results of enantiomer separation than CSP 2 developed by Machida group. Especially, in case of asparagine, aspartic acid, isoleucine, threonine, valine, and baclofen, CSP 1 showed that the enantiomer separation was successful, whereas CSP 2 showed that enantiomer separation did not take place at all[23,25]. The different chiral discrimination ability between CSP 1 and CSP 2 is due to the structural difference, even if the same chiral selector of (+)-18-C-6-TA was used. CSP 1 was used for enantiomer separation of not only α-amino acids but also α-amino acid esters. We reported that α-amino acid esters showed worse results of enantiomer separation in general than α-amino acids. Because amino acid with the primary amino group was resolved, proline without the primary amino group was
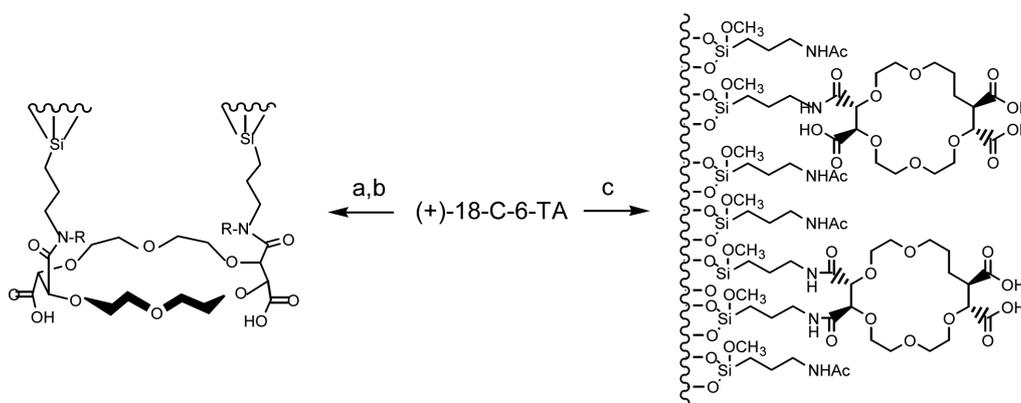
Fig. 2. The structures of CSP 1 (R=H) and CSP 1a (R=CH₃) (left) developed by our group, and CSP 2 (right) by Machida group. These covalent type CSPs are derived from (+)-18-C-6-TA of the same chiral selector by different process. (a) acetyl chloride (b) aminopropyl silica gel, Et₃N (c) EEDQ, aminopropyl silica gel.

Fig. 3. The commercially available covalently bonded ChiroSil RCA(+) (CSP 1) and ChiroSil SCA(-) derived from (+)- and (−)-18-C-6-TA, respectively[24].

not resolved. Furthermore, successful results of enantiomer separation could be found in various kinds of chiral primary amines, amino alcohols, fluoroquinolones including gemifloxacin, tocainide derivatives, β-amino acids, and aryl α-amino ketones[10,17-24].

Therefore, the covalently bonded crown ether type CSP 1 has the strong point in terms of column stability as it can be used in any kind of HPLC mobile phases without limitation. This covalent type CSP 1 has strong advantages in chiral separation, compared to the coated type Crownpak CR. If compound has high hydrophobicity, Crownpak CR has the drawback that elution itself may not take place or it may take a very long time for elution. In order to shorten the elution time, the use of mobile phase is limited for Crownpak CR[7,22]. For an example, gemifloxacin (Factive®), developed as the fourth generation antibiotic and approved by the FDA, showed good results of enantiomer separation in Crownpak CR. However, as it has the high hydrophobicity, it takes about one hour for elution in the flow rate 1.5 mL/min[7,21]. On the other hand, it showed much more excellent results of enantiomer separation on the covalent type CSP 1 along with a fast elution. Also, when preparative separation for hydrophobic analytes is performed using Crownpak CR, the coated chiral crown ether on the silica gel could be washed off, which is a significant weak point. Currently, the chiral crown ether covalent type HPLC CSP is commercially available as ChiroSil RCA(+) derived from (+)-18-C-6-TA and ChiroSil SCA(−) derived from (−)-18-C-6-TA (RS Tech Corp., Daejeon, Korea)[24]. As the two chiral selectors are an enantiomer to each other, ChiroSil RCA (+) and ChiroSil SCA (−) can be used respectively to change the elution order as desired. In general, the (D)-enantiomers of the investigated amino acids are strongly retained on ChiroSil RCA(+), while the (L)-enantiomers are strongly retained on ChiroSil SCA(-) [24]. This is a strong advantage for an actual analysis process for

both CSPs where chiral purity is measured. Also, in terms of the elution order, it might be said that ChiroSil RCA(+) is equivalent to Crownpak CR(-), whereas ChiroSil SCA(−) is equivalent to Crownpak CR(+) [23]. Therefore, the elution orders described by Machida group that (+)-18-C-6-TA derived CSP is similar to Crownpak CR(+) is not correct[23,25].

Much effort has been made to develop improved (+)-18-C-6-TA derived CSPs to enhance chiral recognition efficiency of CSP 1[10]. Thanks to such effort, CSP 1a was developed as N-H of two amide tethers was replaced by N-CH₃ in aminopropyl silica gel of CSP 1[27]. In enantiomer separation of amino acids, CSP 1 showed slightly better results than CSP 1a, whereas CSP 1a was slightly superior to CSP 1 in enantiomer separation of amino acid esters. As examples, the investigated analytes including phenylalanine and leucine as methyl esters were well resolved in CSP 1a even though these two analytes were not resolved in CSP 1. In addition, CSP 1a was found to be better than CSP 1 in enantiomer separation of some primary chiral amines. These results showed that different connecting structures for CSPs might influence their ability to resolve the analytes depending on their structures related to the chiral recognition mechanism[18,27]. Furthermore, we prepared a new CSP by dynamic coating of chiral selector of (+)-18-C-6-TA on ODS silica gel solid support just as the case with Crownpak CR[28]. To this end, N-dodecyl diamide of (+)-18-C-6-TA was prepared and it was adsorbed in ODS silica gel to make the (+)-18-C-6-TA coated type CSP. Enantiomer separation on this coated type CSP did not lead to better results generally than that on the covalent type CSP 1[28]. For example, the former coated type CSP failed to resolve several amino acids including aspartic acid. On the contrary, amino acid esters showed slightly better results of enantiomer separation on this coated type CSP than on the covalent type CSP 1.

5. Application of Enantiomer Separation Using 18-C-6-TA of Chiral Crown Ether

The chiral crown ether of 18-C-6-TA as chiral selector was not only used only in HPLC but also used in capillary electrochromatography and capillary liquid chromatography to be applied to enantiomer analysis[29,30]. The same covalently bonded stationary phase derived from (−)-18-C-6-TA for capillary chromatography was used for enantiomer separation of aromatic amino acids in aqueous methanol/Bis-Tris or Tris-citric buffer solution. In general, enantiomer separation in capillary electrochromatography was found to be superior to that in capillary liquid chromatography. When enantiomer separation is performed on CSP 1 where (+)-18-C-6-TA is used as chiral selector, it is mainly applied to resolve analytes with the primary amino group, even though there is an exceptional report for beta-blockers of amino alcohols with the secondary amino group[10]. Therefore, the application of enantiomer separation of chiral carboxylic acids using the chiral crown ether type CSP 1 is not proper. However, we have attempted to extend the application of crown ether derived CSP 1 to chiral 2-aryloxypropionic acids used as herbicides[31]. The corresponding N-hydrazide derivatives were prepared for complexation between the N-hydrazide and the crown ether moiety. Consequently, several 2-aryloxypropionic acids as N-hydrazide derivatives were well resolved on CSP 1. Also, enantiomer separation of thyroxine was conducted as an applied research on the chiral crown ether type CSP[32-34]. Thyroxine is a bio-material with amino acid structure and L-thyroxine (L-T4; levothyroxine) of the naturally occurring thyroid hormone has been used for the treatment of thyroid dysfunctions. But there have been a very few reports on the direct enantiomer separation using CSPs[32]. ChiroSil RCA (+) and SCA (−) columns were used to newly develop and apply enantiomer separation of thyroxine in order to measure enantiomeric purity of not only currently available L-T4 chemicals but also levothyroxine sodium tablets prepared by various pharmaceutical companies that are sold as pharmaceutical domestically and internationally[32,33]. In addition, simultaneous chiral HPLC analysis method was developed and verified for quantitative analysis of L-T4 and D-T4 that exist in human plasma[34].

6. Conclusion

Chiral crown ethers, a class of synthetic macrocyclic polyethers have been widely used in the resolution of various chiral compounds containing a primary amino group. We focused on the two compounds of 1,1′-binaphthyl based chiral crown ether and 18-C-6-TA chiral crown ether that have been essentially applied as chiral selectors. In a variety of research areas like liquid chromatography, capillary electrophoresis and NMR spectroscopy and so on, chiral discrimination studies using these chiral crown ethers have been generally performed and applied for chiral compounds containing a primary amino moiety[10,35,36]. Also, covalently bonded CSPs derived from (+)- and (−)-18-C-6-TA have been developed in our groups and utilized for the resolution for various types of analytes[24]. Since each enantiomer of chiral drugs may have different activities in terms of pharmacological, toxic and pharmacokinetic behaviors, it is essential for pharmaceutical company to conduct study on enantiomer separation in the process of development of chiral drugs[37,38] It is expected that this study on the development and application of enantiomer separation using chiral crown ethers would make a great contribution to development of chiral drugs along with advanced chirotechnology[37].

Acknowledgment

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