

Enantioselective Recognition of Aliphatic Amino Acids by β -Cyclodextrin Derivatives Bearing Aromatic Organoselenium Moieties on the Primary or Secondary Side

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Spectrophotometric titrations have been performed in order to determine the stability constants of inclusion complexation of some aliphatic amino acids with four structurally related organoselenium-modified β -cyclodextrins: mono(6-phenylseleno-6-deoxy)- β -cyclodextrin (**1a**), mono[6-(*p*-methoxyphenylseleno)-6-deoxy]- β -cyclodextrin (**1b**), mono(2-phenylseleno-2-deoxy)- β -cyclodextrin (**2a**), and mono[2-(*p*-methoxyphenylseleno)-2-deoxy]- β -cyclodextrin (**2b**). Conformation analysis by circular dichroism and 2D NMR spectroscopic studies revealed that the aryl-substituted β -cyclodextrins gave self-inclusion intramolecular complexes in aqueous solution, while the extent of penetration depended both on the positions and on the structures of substituents. Quantitative investigation on the binding ability of the hosts with amino acids showed that they were able to recognize the size and

the shape of guests, affording supramolecular complexes with quite small stability constants ranging from 24 to 355 M^{-1} . The molecular recognition abilities are discussed from the viewpoints of induced-fitting mechanisms, geometric complementary, and cooperative binding processes. Furthermore, these β -cyclodextrin derivatives displayed considerable enantioselectivity towards L/D-amino acid isomers, giving the highest L-enantioselectivity (up to 8.4) for inclusion complexation between leucine and **2a**. The binding modes of L/D-leucine with **1b** were elucidated from NOESY studies and the chiral recognition phenomena were interpreted accordingly.

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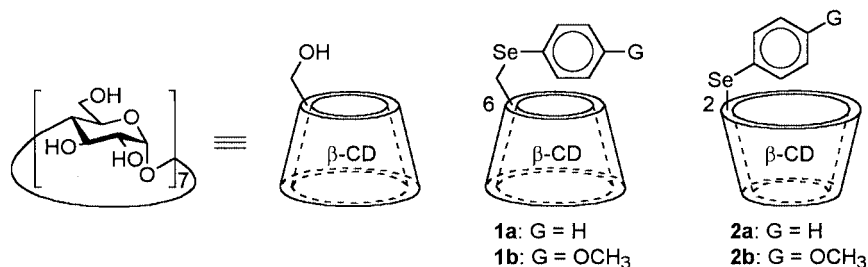
Introduction

Numerous efforts over the past three decades have been devoted to molecular recognition studies of biological molecules by artificial receptors, and among them protein recognition is currently a significant research topic.^[1,2] In the protein recognition procedure, receptors usually react with amino acid residues, the active sites on the protein surface, to give inclusion complexes or supramolecular species. The technique may therefore provide essential information relating to protein recognition through investigation of the non-covalent interactions between receptors and small biological molecules such as amino acids and peptide segments. As semi-natural artificial receptors made up of cyclic oligosaccharides, cyclodextrins and their derivatives have been successfully used in the enantioselective recognition of L/D-amino acids^[3–7] and also sequence recognition of oligopeptides,^[8–12] thanks to their fascinating intrinsically chiral inner cavity. These studies strongly suggest that cyclodextrins may form inclusion complexes or association complexes with small biological molecules in polar solvents. Although cyclodextrins have even been extensively used as

chiral stationary phases for the enantiomeric separation of amino acids in analytical chemistry,^[13,14] the molecular recognition mechanism for this system is still debated.

In principle, cyclodextrin derivatives bearing both positively charged and negatively charged substituents can produce excellent chiral discrimination of amino acid enantiomers through triple recognition (one hydrophobic and two electrostatic sites).^[15] On the basis of coordination interaction, metallacyclodextrins have also been applied to the selective binding of amino acids.^[16–18] In this system, the amino and carboxylic acid groups of the amino acid take part in coordination with metal center (copper or nickel, for example), which fixes the amino acid's orientation relative to the cyclodextrin's cavity and affords enantioselectivity. This nature of this procedure is also based on triple recognition. Although positively charged cyclodextrins can provide only an electrostatic site for the carboxylic acid in an amino acid, this kind of host has also been confirmed to be capable of recognizing amino acid isomers.^[6,19–21] It is our experience, however, that the introduction of a simple aromatic moiety onto the cyclodextrin rim can also influence its original chiral binding ability.^[22–25] For instance, mono[6-(*o*-tolylseleno)-6-deoxy]- β -cyclodextrin affords the highest enantioselectivity (27) for L-alanine over the antipodal D-alanine.^[23] Our previous studies have revealed that the selectivity depends not only on the size and the shape of the α -amino acid, but also on the structures and proper-

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ties of the substituent(s) on the modified cyclodextrins. Obviously, a substituent may interfere with the interaction between amino acid guests and cyclodextrin hosts in a certain manner. To the best of our knowledge, however, the influence on amino acid recognition of a substituent's position in a cyclodextrin derivative has seldom studied been before.^[26] In this context we wish to report here our investigation into the molecular recognition of aliphatic amino acids by several structurally related organoselenium-modified β -cyclodextrins. Our particular interests are in examination of how minor structural differences in the hosts can affect molecular chiral recognition ability. From the hosts' microstructure changes to the resulting complex conformation, it may be possible to infer the molecular selective binding ability and the chiral recognition mechanism.

Results and Discussion

Conformation Analysis

An achiral chromophore should show induced circular dichroism (ICD) signals around its corresponding transition bands when it is embedded in or entering into a cyclodextrin's cavity.^[27] This phenomenon has become a powerful tool for the elucidation of the inclusion modes of cyclodextrin complexes and the conformation of modified cyclodextrins. We therefore measured the ICD spectra of compounds **1a**, **1b**, **2a**, and **2b** in order to examine their initial conformations in aqueous solution, which are essential to understanding of their molecular recognition ability.

Figure 1 gives the ICD spectra of hosts **1a**, **2a**, **1b**, and **2b**. It can be seen that hosts **1a** and **2a** have ICD spectra of closely similar shape, but with different intensities. Compound **1a** and compound **2a** each exhibit a positive Cotton effect peak for the 1L_b band around 280 nm and a negative Cotton effect peak for the 1L_a band around 230 nm, respectively. Similarly, the ICD spectrum of β -cyclodextrin derivative **1b** has a substantial resemblance in shape to that of its counterpart **2b**. An empirical rule for the inclusion complexation of cyclodextrins with chromophoric molecules, known as Kajtár's sector rule,^[28] states that the sign of the induced circular dichroism Cotton effect depends on the orientation of the transition dipole moment in the cyclodextrin's cavity. From the observed ICD signals, it can therefore be concluded that the aromatic substituents on **1a**, **1b**, **2a** and **2b** were each self-included into the cavity according to Kajtár's sector rule. Typical energy-minimized representations of **1a** and **2a** are shown in Figure 2. This result is

common and consistent with the conformations of most other aromatic substituted cyclodextrins in aqueous environment, which should be attributable to the intramolecular hydrophobic interaction.

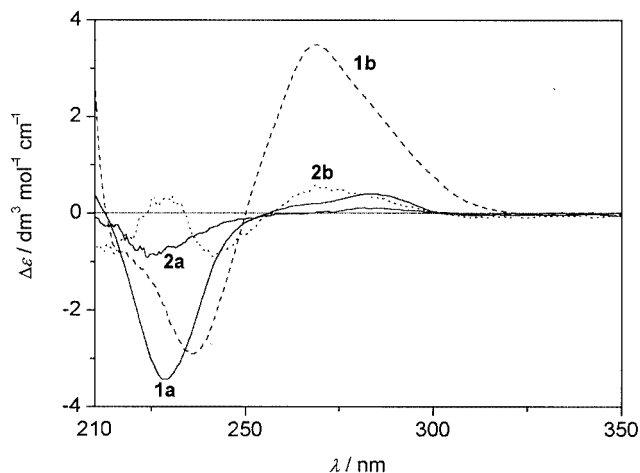


Figure 1. Circular dichroism spectra of monoarylseleno- β -cyclodextrins **1a**, **2a**, **1b**, and **2b** (0.1 M) in aqueous buffer solution (pH, 7.2) at 25 °C

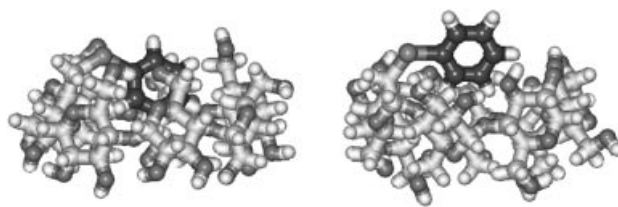


Figure 2. Structures of **1a** and **2a** estimated from ICD spectra; the energy was minimized by use of MM2 force field techniques

It should be noted from Figure 1, however, that the intensities of the ICD signals of **1a** and **1b** are markedly stronger than those of **2a** and **2b**, although they afford very similar ICD spectral shapes. Around the 1L_b band, for instance, the ICD intensity of **1a** is about 3.5 times that of **2a**, while the corresponding value for **1b/2b** couple is about 6. A result from theoretical calculation suggests that the magnitude of ICD is greater when a guest molecule exists in the narrower-rim outside than in the wider-rim outside.^[29,30] If so, the phenomena observed above are normal and closely dependent on the substituent's position. Additionally, from the calculation result (as illustrated in Figure 2) we may note that the extent of self-inclusion in **2a** is less than that in **1a**, due to the short linkage between the substituent and

cyclodextrin rim, which also accounts for the difference in the intensities of the ICD signals. It is also interesting to note that the ICD intensities of **1b** and **2b** are stronger than those of **1a** and **2a**, respectively. If the electron densities of

aromatic moieties are taken into account, it is reasonable to conclude that the self-inclusion stabilities of **1b** and **2b** are higher than those of their counterparts.^[31,32]

To obtain further evidence for the initial geometries of the self-inclusion modes of cyclodextrin derivatives with similar substituent groups, ¹H NOESY experiments were performed on **1a** and **1b**, on a Varian INVOA 300 spectrometer. As shown in Figure 3 (a), the NOESY spectrum of **1a** displays clear NOE cross-peaks between H5 and H3 of the cyclodextrin and the *meta* and/or *para* protons of the benzene ring (peaks B), as well as between H5 and the *ortho* protons (peaks A), which clearly indicate that the benzene ring in **1a** is self-included in the cavity from the primary side of the cyclodextrin. However, the NOESY spectrum of **1b** exhibits different NOE cross-peaks, there being only one correlation peak in the peaks B', implying interaction between H3 of the cyclodextrin and the *meta* protons of the benzene ring (the NOE cross-peaks between the methoxy protons and the *ortho* protons in the benzene ring overlap each other perfectly), and two correlation peaks in the peaks A' between H5 and H3 and the *ortho* protons of the benzene ring. These facts suggest that the anisyl substituent in **1b** is deeply self-included in the hydrophobic cavity from the primary hydroxy side, and unambiguously delineate the differences in conformational features between compounds **1a** and **1b**. The obtained results not only further support the ICD investigations into the conformation of modified cyclodextrins, but may also serve to establish correlations between the initial conformations of modified cyclodextrins and their molecular recognition abilities.

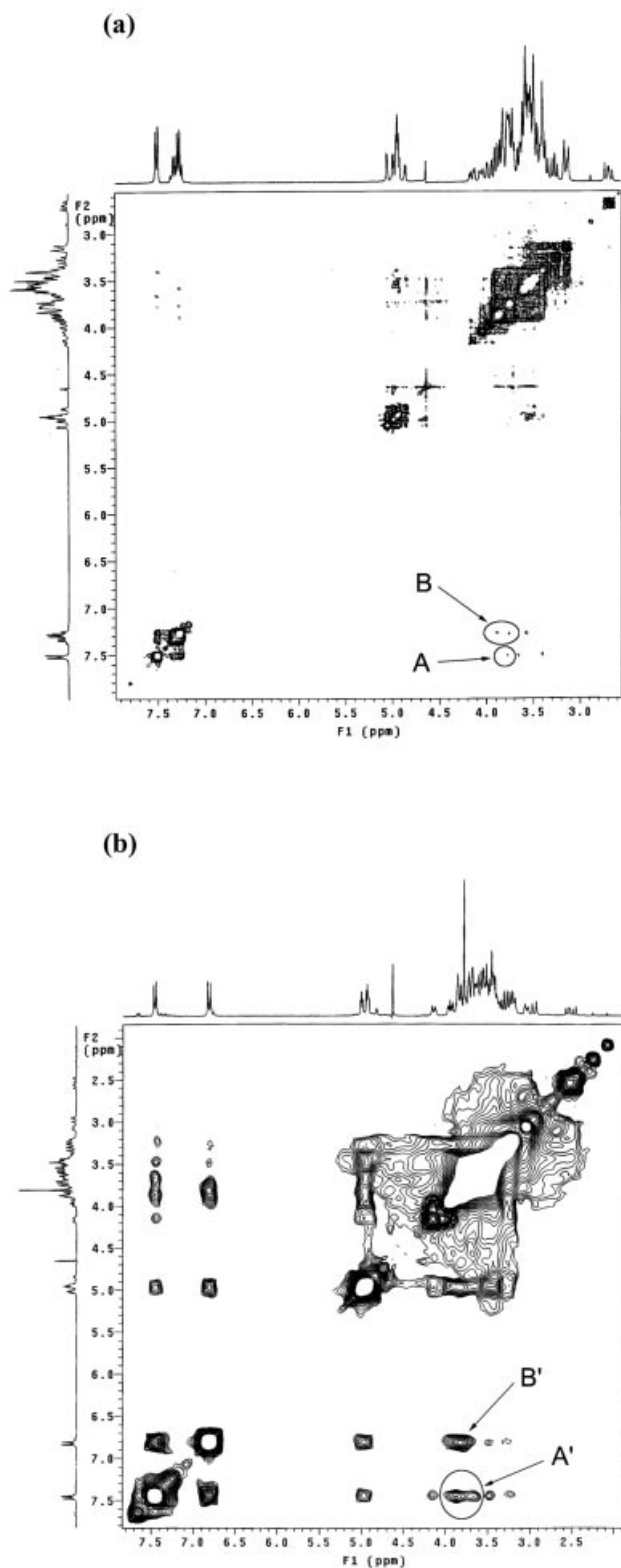


Figure 3. ¹H NOESY spectra (300 MHz) of (a) **1a** and (b) **1b** in D₂O at 25 °C with a mixing time of 800 ms

Complex Stability Constant

In order to investigate the molecular recognition behavior of cyclodextrin hosts **1** and **2** with amino acid guests quantitatively, spectrophotometric titrations were performed to measure the complexation stability constants of the supramolecular species formed. In each titration, the concentration of the host was kept constant, while the concen-

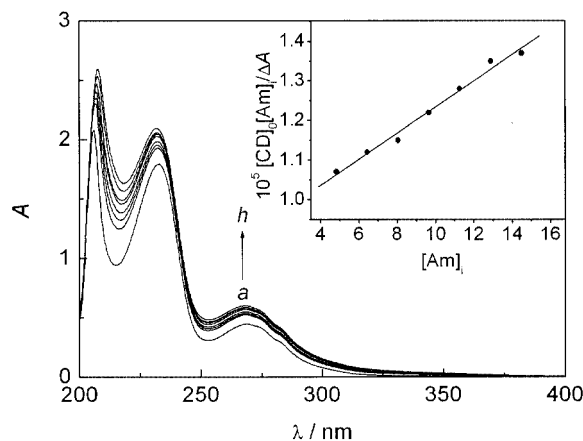


Figure 4. UV/Vis spectral changes of phosphate buffer solution of **2a** (1.46×10^{-4} M) in the presence of D-alanine, added as a guest. The concentration of D-alanine ranges from 0 to 14.49 mM; $\lambda_{\max} = 269$ nm; the inset shows the corresponding Benesi–Hildebrand plot

tration of the guest was varied. The spectral changes depend critically on the formation of new species, the host-guest complex. Representative spectral changes are shown in Figure 4 for complexation between **2a** and D-alanine. Similar spectral changes were also observed for other host-guest interactions.

If conventional 1:1 host/guest stoichiometry is assumed, complexation between amino acids (= Am) and cyclodextrin hosts (= CD) can be expressed as presented in Equation (1).



The UV spectral changes (ΔA) upon addition of amino acid guest are proportional to the concentrations of complex formed, and the difference in the molar extinction coefficients of the free host and the complexed one may be taken as the proportionality coefficient (i.e., $\Delta A = \Delta \varepsilon \cdot [\text{CD} \cdot \text{Am}]$). Thus, the complex stability constant (K_S) should be defined as noted in Equation (2), where $[\text{CD}]_0$ and $[\text{Am}]_i$ denote the initial concentrations of cyclodextrin host and amino acid guest, respectively.

$$K_S = \frac{[\text{CD} \cdot \text{Am}]}{[\text{CD}][\text{Am}]} = \frac{[\text{CD} \cdot \text{Am}]}{([\text{CD}]_0 - [\text{CD} \cdot \text{Am}])([\text{Am}]_i - [\text{CD} \cdot \text{Am}])} = \frac{\Delta A / \Delta \varepsilon}{([\text{CD}]_0 - \Delta A / \Delta \varepsilon)([\text{Am}]_i - \Delta A / \Delta \varepsilon)} \quad (2)$$

After some manipulation, Equation (2) is converted into Equation (3).

$$\frac{[\text{CD}]_0[\text{Am}]_i}{\Delta A} = \frac{1}{K_S \cdot \Delta \varepsilon} + \frac{[\text{Am}]_i}{\Delta \varepsilon} \quad (3)$$

Equation (3) is also known as the Benesi–Hildebrand equation,^[33] with the restriction that the initial concentration of one component is far higher than that of the other component. In the current study, the value of $[\text{Am}]_i/[\text{CD}]_0$ is larger than 30.

The inset of Figure 4 depicts the Benesi–Hildebrand plot for complexation between cyclodextrin derivative **2a** and D-alanine. The good correlation between the experimentally determined and the calculated result strongly indicates that the complexation process is actually 1:1. The binding constants (K_S) can easily be calculated from the slope ($1/\Delta \varepsilon$) and intercept ($1/K_S \cdot \Delta \varepsilon$) of linear regression. The binding constants obtained for the 1:1 complexation between the cyclodextrin derivatives **1** and **2** and the amino acids are compiled in Table 1. When repeated measurements were

made, the K_S values were reproducible within an error of $\pm 7\%$.

The small but substantial spectral changes in the hosts' absorption bands observed upon addition of amino acid guests clearly indicated that the substituents' positions were disturbed by inclusion complexation with the guests. That is, the aromatic substituent originally perched on the edge of or inside the β -cyclodextrin cavity should be either far away from or embedded more deeply in the cavity, suggesting the formation of inclusion complexes. In general, several non-covalent weak forces, including van der Waals, hydrophobic, electrostatic, and hydrogen-bonding interactions, cooperatively contribute to the formation of the cyclodextrin complexes. Since water is an excellent medium for the maximization of many interactions, due to its extremely low polarizability,^[34] it should favor the molecular binding between a cyclodextrin host and an amino acid guest in this solvent system. We may note from Table 1, however, that the stability constants for inclusion complexation between the four β -cyclodextrin derivatives and the amino acids are quite small: **1a**, for example, affords the highest stability constant (355 M^{-1}) with D-alanine, while **2a** shows the lowest stability constant (24 M^{-1}) with D-leucine. These small complex stability constants should be attributed to the essentially hydrophilic character of amino acids, which cannot produce effective hydrophobic interaction with cyclodextrin's hydrophobic cavity.

Extensive studies on molecular recognition of acyclic and cyclic aliphatic alcohols with native cyclodextrins^[35,36] and modified cyclodextrins^[31,37] have revealed that the complex stability constants ($\log K_S$) increase practically linearly with increasing carbon number in the guests. Hydrophobic and van der Waals interactions are believed to be the major driving forces in the binding process. In the current case, however, the binding constants seem not to be strictly correlated with the guest amino acids' chain lengths. For instance, the complex stability constant for **2a**/D-alanine is 246 M^{-1} , while the corresponding value for **2a**/D-leucine is only 24 M^{-1} , about 1/10 of the preceding one. Structurally, leucine possesses an additional isopropyl residue as compared with alanine, but the additional hydrophobic moiety does not provide effective interaction with cyclodextrin cavity, indicating that hydrophobic interaction is not the dominant force in these inclusion complexation processes. The hydrophilic groups in acyclic aliphatic guests (such as amido, hydroxy, and carboxylic acid) are commonly believed to remain outside the cavity upon binding to cyclodextrin hosts.^[38] However, this complexation mode is not applicable to the current amino acid recognition, as the amido/carboxylic acid of amino acids must take part in the complex formation.

In the current solvent system (pH = 7.20), amino acids would be transformed into zwitterions. There should therefore be hydrophilic or hydrogen-bonding interactions between the amphoteric ions and the hydroxy groups situated at the rim of the cyclodextrin molecule,^[39] which also contribute to the complex stability. In this context, hydrophobic and hydrophilic/H-bonding interactions can occur

Table 1. Complex stability constant (K_S) and Gibbs free energy change ($-\Delta G^\circ$) for the supramolecular system formed by organoselenium modified β -cyclodextrins (**1a**, **1b**, **2a**, and **2b**) with some aliphatic amino acids in aqueous buffer solution (pH, 7.2, 0.1 M) at 25 °C

| Host | Guest | K_S/M^{-1} | $\log K_S$ | $-\Delta G^\circ/kJ\cdot mol^{-1}$ | $\Delta\Delta G^\circ/kJ\cdot mol^{-1}$ [a] | Ref. | |
|-----------|-----------|--------------|------------|------------------------------------|---|-----------|-----------|
| 1a | L-Ala | 91 | 1.96 | 11.2 | -3.4 | [23] | |
| | D-Ala | 355 | 2.55 | 14.6 | | [23] | |
| | L-Ser | 100 | 2.00 | 11.4 | 1.6 | [23] | |
| | D-Ser | 51 | 1.71 | 9.8 | | [23] | |
| | L-Leu | 288 | 2.46 | 14.0 | -0.1 | [23] | |
| | D-Leu | 295 | 2.47 | 14.1 | | [23] | |
| 2a | L-Ala | 117 | 2.07 | 11.8 | -1.8 | this work | |
| | D-Ala | 246 | 2.39 | 13.6 | | this work | |
| | L-Ser | 273 | 2.44 | 13.9 | 3.1 | this work | |
| | D-Ser | 78 | 1.89 | 10.8 | | this work | |
| | L-Val | 59 | 1.77 | 10.1 | 1.2 | this work | |
| | D-Val | 36 | 1.56 | 8.9 | | this work | |
| | L-Leu | 201 | 2.30 | 13.1 | 5.2 | this work | |
| | D-Leu | 24 | 1.38 | 7.9 | | this work | |
| | 1b | L-Ala | 237 | 2.37 | 13.6 | 1.6 | this work |
| | | D-Ala | 126 | 2.10 | 12.0 | | this work |
| L-Ser | | 118 | 2.07 | 11.8 | 2.3 | this work | |
| D-Ser | | 46 | 1.66 | 9.5 | | this work | |
| L-Val | | 61 | 1.78 | 10.2 | 0.7 | this work | |
| D-Val | | 46 | 1.66 | 9.5 | | this work | |
| L-Leu | | 329 | 2.52 | 14.4 | 4.0 | this work | |
| D-Leu | | 66 | 1.82 | 10.4 | | this work | |
| 2b | L-Ala | 115 | 2.06 | 11.8 | 2.0 | this work | |
| | D-Ala | 53 | 1.72 | 9.8 | | this work | |
| | L-Ser | 267 | 2.47 | 13.9 | 3.9 | this work | |
| | D-Ser | 58 | 1.76 | 10.0 | | this work | |
| | L-Val | 70 | 1.85 | 10.5 | -1.0 | this work | |
| | D-Val | 102 | 2.01 | 11.5 | | this work | |
| | L-Leu | 59 | 1.77 | 10.1 | -0.4 | this work | |
| | D-Leu | 68 | 1.84 | 10.5 | | this work | |

[a] $\Delta\Delta G^\circ$ signifies the difference in the free energy changes for the complexation behavior with L/D-amino acids, i.e. $\Delta\Delta G^\circ = \Delta G^\circ(\text{D isomer}) - \Delta G^\circ(\text{L isomer})$.

simultaneously and stabilize the host-guest complexes. However, these weak interactions are not always cooperative, but may instead act independently, due to the hydrogen-bonding interaction requiring appropriate spatial orientation. Because the aliphatic side chains in the amino acids used are quite small in relation to the bulky cavity of β -cyclodextrin, it is not surprising that hydrophobic interaction did not dominate the complex formation.

It is generally believed that secondary hydroxy side-substituted cyclodextrins are superior to the corresponding primary hydroxy side-substituted cyclodextrins.^[26] We are also interested in substituent effects of these β -cyclodextrin derivatives on amino acid recognition. From Table 1, however, it can be seen that we were unable to observe significant differences between secondary hydroxy side-substituted hosts (**2a**, **2b**) and primary hydroxy side-substituted hosts (**1a**, **1b**) on molecular binding. Although the hydrophilic terminals of the amino acids prefer to interact with the secondary hydroxy side, which bears far more hydroxy groups, the introduction of aromatic substituents onto the secondary side would not enhance this kind of interaction. In contrast, the presence of the hydrophobic substituent on the secondary side could potentially prevent the guest amino acid from accessing the secondary peripheral hydroxy groups. Consequently, hosts **2a** and **2b** in some cases show

binding abilities even lower than those of their primary analogues **1a** and **1b**. On the other hand, it has been reported previously that the extent of self-inclusion in primary side-substituted cyclodextrins is greater than in secondary side-substituted ones. Compounds **1a** and **1b** can thus provide more effective van der Waals and hydrophobic interactions with small-sized guests (such as alanine), and show marginal molecular binding enhancement.

Chiral Recognition

The data listed in Table 1 also show that the cyclodextrin hosts bearing one arylseleno moiety as a probe can recognize not only the sizes and shapes of amino acids but also the L and D enantiomers, affording poor to moderate chiral recognition ability. The L enantioselectivities calculated from the K_S values are up to 8.4 and 5.0 for the complexation of leucine with hosts **2a** and **1b**, respectively, while the D enantioselectivities are up to 3.9 and 2.1 for complexation between alanine and hosts **1a** and **2a**, respectively. Cyclodextrin's chiral discrimination ability should originate from its intrinsic chiral cavity. After modification, however, the self-included substituent may distort the initial regular cavity^[40] and then influence its chirality, so the cyclodextrin hosts show reversed chiral recognition results in some cases. Another point that should be emphasized is that the cyclo-

dextrin hosts used do not possess the characters required by the classical “triple recognition” mechanism at all, but they still show considerable chiral recognition ability. It was recently found that the water molecules located within the hydrophobic region of a cyclodextrin cavity play a crucial role in the chiral discrimination of D/L-amino acid derivatives in the crystalline state.^[41] If this rule is also applicable to solution systems, the role of water molecules in the chiral recognition of amino acids by cyclodextrin hosts must be taken into account.

In order to elucidate the structural features responsible for the strong chiral recognition of L/D-Leu upon complexation with mono[6-(*p*-methoxyphenylseleno)-6-deoxy]- β -cyclodextrin (**1b**), ¹H NOESY experiments were performed on a Varian INVOA 300 spectrometer. As shown in Figure 5, the NOESY spectrum of an equimolar mixture of host **1b** and guest L-Leu (1.0 mM each) in D₂O solution displays complicated NOE cross-peaks, originating not only from intermolecular interaction between the cyclodextrin host and the amino acid molecule, but also from intramolecular interactions of **1b** and/or L-Leu, respectively. Although the NOE correlation peaks between H3 and H5 of the cyclodextrin and the methyl protons (peaks A) and H ^{α} or H ^{β} (peaks B) in L-Leu were not clear enough to estimate their relative intensity, we were able to declare unambiguously that the corresponding NOE correlations exist. Analogous phenomena occur between H3 and H5 of cyclodextrin and the *meta* (peaks C) and *ortho* protons (peaks D) of the benzene ring. However, the clear correlations between the aromatic protons of the anisyl group in compound **1b** and the side chain protons in L-Leu (peaks E, F, and G in Figure 5, a) reasonably allow us to deduce the detailed orientation of the side chain of L-Leu relative to the aromatic ring. The strong and similar correlations between the aromatic protons and H ^{α} or H ^{β} (peaks E) imply that these protons (H ^{α} or H ^{β}) should be located above the aromatic ring, while the diminishing intermolecular NOE intensities from peak G (between the methyl protons in L-Leu and the *meta* protons of **1b**) to peak F (between the methyl protons and the *ortho* protons) indicate that the methyl group must be situated at the end of the methoxy group of the anisyl substituent. In view of the possible cation- π interaction^[42] between RNH₃⁺ in zwitterionic leucine and the aromatic ring in **1b**, together with the NOESY spectrum, we were able to deduce reasonably the binding mode between L-Leu and mono[6-(*p*-methoxyphenylseleno)-6-deoxy]- β -cyclodextrin (**1b**, Figure 5, b). In this inclusion mode, the whole L-leucine molecule is embedded in the cyclodextrin's cavity, while the substituent stays in the cavity simultaneously.

It is interesting to compare the difference in binding modes between D-Leu and L-Leu upon complexation with **1b**, since the *K*_S value of the former is only up to 20% that of the latter. This comparative study may reveal not only the origin of the moderate L-enantioselectivities achieved by **1b**, but also a detailed mechanism of chiral discrimination by modified cyclodextrins. In the NOESY spectrum of the **1b** complex of D-Leu (Figure 6, a), NOE cross-peaks between the H5 and methyl protons (peaks A') and H ^{α} and/or H ^{β} (peaks B') in D-Leu indicated that the side chain of the amino acid is shallowly included in the cavity from the primary side of the cyclodextrin. However, no NOE cross-peaks between the cyclodextrin's H5 and/or H3 and the aromatic protons were observed in Figure 6 (a). In fact, peaks C' represent the NOE between H ^{α} in D-Leu and the *o*-phenyl protons of **1b**, and peaks D' signify an intramolecular NOE of the substituent group in **1b** between H_o and methoxy protons. These results imply that the *p*-anisylseleno group is not self-included in the cavity of cyclodextrin at all. Moreover, the intermolecular NOE between the *o*-phenyl protons in **1b** and the methyl protons (peaks F') and H ^{α} and/or H ^{β} (peaks E') of D-Leu further confirm that the side

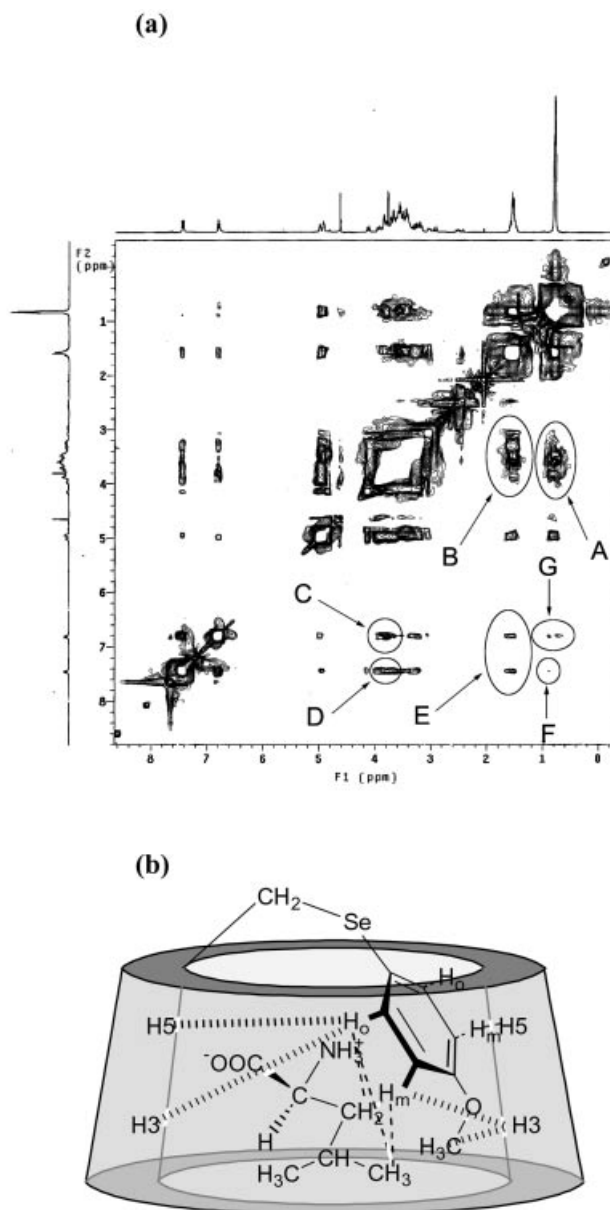


Figure 5. (a) ¹H NOESY spectrum (300 MHz) of the **1b** complex of L-Leu, and (b) plausible complex structure of L-Leu with **1b**; thin and thick dashes indicate intermolecular and intramolecular NOEs, respectively

or H ^{β} (peaks B') in D-Leu indicated that the side chain of the amino acid is shallowly included in the cavity from the primary side of the cyclodextrin. However, no NOE cross-peaks between the cyclodextrin's H5 and/or H3 and the aromatic protons were observed in Figure 6 (a). In fact, peaks C' represent the NOE between H ^{α} in D-Leu and the *o*-phenyl protons of **1b**, and peaks D' signify an intramolecular NOE of the substituent group in **1b** between H_o and methoxy protons. These results imply that the *p*-anisylseleno group is not self-included in the cavity of cyclodextrin at all. Moreover, the intermolecular NOE between the *o*-phenyl protons in **1b** and the methyl protons (peaks F') and H ^{α} and/or H ^{β} (peaks E') of D-Leu further confirm that the side

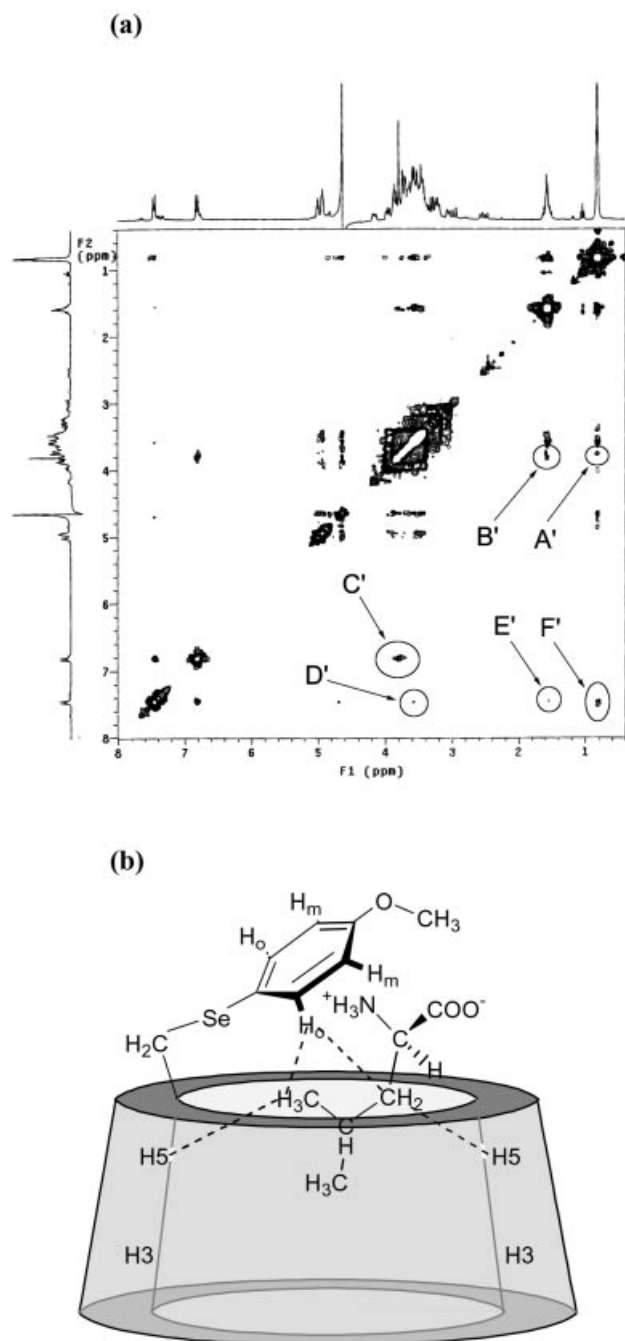


Figure 6. (a) ^1H NOESY spectrum (300 MHz) of the **1b** complex of D-Leu, and (b) plausible complex structure of D-Leu with **1b**; dashes indicate intermolecular NOEs

chain of D-Leu is located over the primary side of cyclodextrin, as shown in Figure 6 (b). As the side chain of D-Leu does not fully enter the cavity, it is out of question that the magnitude of hydrophobic interaction not be attributable to L-Leu. Thus, the K_S value and the 2D NMR result are in excellent agreement with each other. Indeed, the induced-fitting process (for L-Leu) gives strong interaction between the modified cyclodextrins and the guest molecules, giving rise to large free energy changes, while the competitive inclusion (for D-Leu) lacks the fixed conformation of the re-

sulting complexes, thus affording small free energy changes. In this context, the establishing of a correlation between the conformations of complexes and their molecular binding abilities is used to understand the molecular recognition mechanism between biological receptors and substrates.

Conclusion

In summary, we have investigated the inclusion complexation behavior between monoarylseleno-substituted β -cyclodextrins and aliphatic amino acids in aqueous systems. The hydrophobic aromatic substituents are generally self-included in the cavity, to give intramolecular inclusion complexes that compete with interaction with amino acids. Accordingly, hosts of this kind show relatively weak binding affinities to hydrophilic amino acids. The introduction of simple aromatic substituents on the secondary side of the cyclodextrin would not enhance its original binding ability, as there is a lack of the additional effective interaction between substituents and guests. Thus, the introduction of functional groups, such as amido, carboxylic acid, or even crown ether species, would be essential to improve the binding affinity to amino acids through hydrophilic, hydrogen-bonding, and electrostatic interactions. The inclusion mode was elucidated by 2D NMR studies, suggesting that the complex stability depends primarily on whether a guest enters the cavity, which also accounts for the chiral discrimination result.

Experimental Section

All guest amino acids (Tianjin Chemical Reagents Co.) were commercially available and were used directly. Mono(6-phenylseleno-6-deoxy)- β -cyclodextrin (**1a**),^[37] mono[6-(*p*-methoxyphenylseleno)-6-deoxy]- β -cyclodextrin (**1b**),^[31] mono(2-phenylseleno-2-deoxy)- β -cyclodextrin (**2a**),^[43] and mono[2-(*p*-methoxyphenylseleno)-2-deoxy]- β -cyclodextrin (**2b**)^[43] were prepared as reported previously.

The synthetic procedure used for **2a** and its characterization are described as follows: sodium borohydride (0.038 g, 1.0 mmol) was added in batches under nitrogen at room temperature to a yellow solution of diphenyldiselenide (0.156 g, 0.5 mmol) in dry ethanol (25 mL). After the solution had turned colorless, a solution of mono[2-*O*-(*p*-toluenesulfonyl)]- β -cyclodextrin (1.29 g, 1.0 mmol) in dry DMF (25 mL) was added dropwise, and the solution was heated at 85 °C for 8 h with stirring. The resulting solution was evaporated under reduced pressure to give a light yellow powder, which was dissolved in the minimum possible amount of water, and the solution was then poured into acetone (200 mL). The precipitate formed was filtered to give a pale yellow powder. The crude product was purified on a column of Sephadex G-25 with water as eluent to give 0.27 g of **2a** (yield 21%). FAB-MS (NaI): m/z = 1275 [$M + H^+ - 8H_2O$]. IR (KBr): $\tilde{\nu}$ = 3366, 2909, 1636, 1399, 1335, 1302, 1145, 1072, 1021, 926, 847, 812 cm^{-1} . UV/Vis (H_2O): λ_{max} (ϵ , $\text{dm}^3\text{mol}^{-1}\text{cm}^{-1}$) = 222.0 (7600), 262.0 nm (850). ^1H NMR ($[D_6]DMSO$, TMS): δ = 3.0–4.0 (m, 42 H), 4.1–4.7 (m), 4.7–4.9 (m, 7 H), 5.0–5.8 (m), 7.2–7.7 (m, 5 H) ppm. ^{13}C NMR ($[D_6]DMSO$, TMS): δ = 129.19, 128.00, 126.77, 125.29, 101.55, 96.94, 81.29, 80.97, 72.84, 72.00, 71.13, 68.32, 59.68, 48.50 ppm.

C₄₈H₇₄O₃₄Se·8H₂O (1418): C 40.65, H 6.40; found C 40.14, H 6.41.

Compound **2b** was prepared from mono[2-*O*-(*p*-toluenesulfonyl)]-β-cyclodextrin and bis(*p*-methoxyphenyl)diselenide by procedures similar to those used in the synthesis of **2a** (yield 30%). IR (KBr): $\tilde{\nu}$ = 3372, 2900, 1628, 1588, 1489, 1392, 1346, 1282, 1240, 1147, 1072, 1021, 950, 813 cm⁻¹. UV/Vis (H₂O): λ_{max} (ϵ , dm³·mol⁻¹·cm⁻¹) = 230.6 (12600), 267.0 nm (3550). ¹H NMR ([D₆]DMSO, TMS): δ = 3.0–4.0 (m, 42 H), 4.1–4.6 (m), 4.8 (m, 7 H), 5.9 (m), 6.8 (d, 2 H), 7.4 (d, 2 H) ppm. ¹³C NMR ([D₆]DMSO, TMS): δ = 162.00, 132.08, 128.17, 117.74, 104.71, 100.20, 83.39, 82.00, 76.19, 75.94, 74.55, 71.71, 62.70, 58.16, 52.09 ppm. C₄₉H₇₆O₃₅Se·5H₂O (1394): C 42.21, H 6.22; found C 41.88, H 5.89.

UV/Vis spectra and CD spectra were measured in a conventional quartz cell (light path length 1 cm) on a Shimadzu UV 2401PC spectrophotometer and a JASCO J-715S spectropolarimeter at 25 °C, respectively. 2D NMR spectra were recorded in D₂O at 25 °C on a Varian INVOA 300 spectrometer. Spectrophotometric titrations were performed to determine the binding constants between the host (cyclodextrin) and the guest (amino acid). Disodium hydrogen phosphate and sodium dihydrogen phosphate were dissolved in distilled, deionized water to make 0.1 M phosphate buffer solution of pH 7.2 for UV and circular dichroism (CD) spectral measurements.

The molecular modeling was performed with the CAChe 3.2 program (CAChe 3.2, Oxford Molecular 1999). The starting geometry for β-cyclodextrin was taken from the crystalline data in the literature^[44] and its energy was minimized by use of MM2 force-field techniques.

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