Interpretation of Enantioresolution in Nordeoxycholic Acid Channels Based on the Four-Location Model

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ABSTRACT Nordeoxycholic acid (NDCA) forms three kinds of host frameworks, M1, M2, and M3, with channels where aliphatic alcohols (1–7) are accommodated. $^{13}$C-NMR studies clarified that racemic alcohols 1 or 2–6 are enclosed in the M1- or M2-type channel with lower than 15% enantiomeric excess, respectively, while 3-methyl-2-pentanol (7) is done in the M3-type with 47% ee. These inclusion phenomena can be explained due to the Difference Fourier maps of electron densities of their enantiomers in the channels. In addition, analysis of the manner of packing indicates that four locations in the channels should be fixed for the enantioresolution of the alcohols. These results support the four-location model, which has been proposed by Mesecar et al.20 with respect to enantioresolution on protein surfaces. Chirality 15:53–59, 2003.

KEY WORDS: chiral recognition; inclusion compounds; packing manner; difference Fourier map; three-point attachment model

Many crystalline inclusion compounds have been used so far for enantioresolutions of racemic compounds. It is known that some of the compounds selectively encapsulate either of the enantiomers into the host frameworks. For example, Toda et al. reported that diol host compounds include 100% enantiopure guests. We have been studying the enantioresolution by using inclusion compounds of bile acids and their derivatives. In most of these cases, the resulting values of enantioexcesses were quite low and its mechanism is still unclear.

The “three-point attachment model” has been accepted for stereospecific inclusion of guest compounds and for design of enantioselective complexation. As illustrated in Figure 1a, this model is based on fixation of three locations (A, B, and C) of a guest component on the surface of a host component (A’, B’, C’). However, Mesecar et al. represented that three recognition sites (A’, B’, C’) are not always enough to distinguish either of the isomers. The reason is that the guest molecules can theoretically approach the recognition sites from both sides, as shown in Figure 1b. They concluded that a four-location model is needed to explain the enantioselective ability of some proteins.

We consider that this model is applicable for channel-type inclusion compounds. Figure 1b illustrates that a channel has three binding or recognition sites (A’, B’, and C’) on the inner surface. In this case, the (R)-isomer can approach the recognition sites from one side of the channel and the (S)-isomer from the other side. Only when the direction of the approach or the fourth position of D is fixed can either of the isomers be distinguished in the channel. This means that the four-location model is necessary for enantioresolution in the channel. This article concerns a

Fig. 1. Three-point attachment model for enantioresolution on the surface (a) and four-location model for enantioresolution in a channel (b).
detailed mechanism of the enantioresolution in the channel of nordeoxycholic acid (3α, 12α-dihydroxy-5β-23-norcho-23-norlan-23-oic acid, NDCA; Scheme 1) from the viewpoint of a four-location model.

MATERIALS AND METHODS

Preparation of Inclusion Crystals

NDCA was prepared by degradation of the sidechain of deoxycholic acid.21 Seven kinds of racemic alcohols were employed: 2-butanol (1), 3-methyl-2-butanol (2), 2-pentanol (3), 3,3-dimethyl-2-butanol (4), 3-methyl-2-pentanol (5), 2-hexanol (6), and 4-methyl-2-pentanol (7), which were commercially available and used without further purification. The inclusion compounds of NDCA were prepared by recrystallization from the corresponding alcohols. The resulting crystals were dried on filter paper.

Method of Enantioresolution

The volatile guest components were separated from the inclusion crystals by a microdistillation method and converted to their camphorsulfonated derivatives. The enantiopurities of the alcohols were established by 13C-NMR spectroscopy.22

Measurements

Infrared spectra were recorded on a JASCO IR-Report-100 spectrometer. 1H-NMR and 13C-NMR spectra were measured with a JEOL 270MHz spectrometer. TG-DTA were performed on a Rigaku TAS 100 system to give molar ratios of the host to the guest. X-ray powder diffractions were recorded using a Rigaku RINT-2000 with Cu-Kα radiation at room temperature. X-ray diffraction data were collected on a Rigaku RAXIS-RAPID diffractometer equipped with a 2D area detector, using Cu Kα radiation monochromatized by graphite. All calculations were performed by using the TEXSAN crystallographic software package (Molecular Structure Corp., The Woodlands, TX).

Molecular Graphics

Sectional views of host channels were depicted by using Chime 2.6 (SP3) plug-in. The corresponding molecular assemblies were constructed on the basis of the data obtained by the X-ray crystallographic method.

RESULTS AND DISCUSSION

Inclusion, Enantioresolution, and Polymorphism

We obtained inclusion crystals of NDCA with seven kinds of secondary alcohols (1–7), which have hydroxyl groups at their second positions. Their enantioresolutions measured by 13C-NMR spectroscopy are summarized in Table 1.

Table 1. Enantioselectivity of the aliphatic alcohols, CH(OH)(CH3)R, by inclusion method using NDCA

<table>
<thead>
<tr>
<th>Guest</th>
<th>R</th>
<th>e.e.%</th>
<th>Predominant configuration</th>
<th>Polymorphism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Et</td>
<td>8</td>
<td>R</td>
<td>M1</td>
<td></td>
</tr>
<tr>
<td>i-Pr</td>
<td>11</td>
<td>S</td>
<td>M2</td>
<td></td>
</tr>
<tr>
<td>s-Pr</td>
<td>6</td>
<td>S</td>
<td>M2</td>
<td></td>
</tr>
<tr>
<td>t-Bu</td>
<td>15</td>
<td>R</td>
<td>M2</td>
<td></td>
</tr>
<tr>
<td>sec-Bu</td>
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<td></td>
<td>d</td>
<td>M2</td>
</tr>
<tr>
<td>n-Bu</td>
<td>3</td>
<td>R</td>
<td>M2</td>
<td></td>
</tr>
<tr>
<td>t-Bu</td>
<td>47</td>
<td>S</td>
<td>M3</td>
<td></td>
</tr>
</tbody>
</table>

Notes:
- Determined by 13C-NMR.
- Determined by 13C-NMR.
- Determined by XRD.
- Not determined.

Scheme 1.

Fig. 2. Crystal structure of the inclusion compound of NDCA with 3-methyl-2-butanol (2). The M2-type host framework involving a schematic channel (a), a columnar arrangement (b), and the accompanying helical hydrogen bonding network (c).
Table 1. The alcohols with fewer than five carbon atoms (1–6) exhibit quite low enantio excess (ee) values and various priority for the enantiomers. However, in the case of 7 the ee value is quite higher than the others and its (S)-isomer is clearly dominant in the crystals rather than its (R)-isomer.

NDCA forms guest-dependent polymorphic crystals with different channels, which were classified by means of XRD patterns. As shown in Table 1, the crystal structures are classified into three kinds; M1, M2, and M3 (Sada et al., to be submitted). Only 1 is included in the M1-type structure, while 2–6 and 7 in the M2- and M3-type, respectively.

The enantioselectivities mentioned above suggest that M1- and M2-type crystals consist of frameworks with loose packing modes, while M3-type crystal have a tight packing manner. Such a steric relation among the host and guest components may be evaluated from detailed electron densities. Therefore, we analyzed the densities in the channels of two inclusion compounds of NDCA with 2 (monoclinic, P2₁2₁2₁, a = 22.061(1) Å, b = 10.116(5) Å, c = 12.6721(7) Å, β = 94.460(0) Å, V = 2819.5(2) Å³) and 7 (orthorhombic, P2₁2₁2₁, a = 12.385(5) Å, b = 23.215(4) Å, c = 10.224(3) Å, V = 2939.0(1) Å³).

Observation of Electron Densities in the M2-Type Channel

Figure 2 illustrates the crystal structure of the inclusion compound of NDCA with 2. The host molecules form an M2-type framework (Fig. 2a) with a helical arrangement and hydrogen bonding network (Fig. 2b). The bonds involve not only hydroxyl groups on the 3rd and 12th positions of the steroidal skeleton but also hydroxyl groups of the guest alcohols (Fig. 2c).

During the structural analysis, we observed separated peaks of the electron density around chiral carbon (C2) of the alcoholic guest. Figure 3 shows the Difference Fourier maps calculated without the carbon (C1) of the guest. The maps are composed of two sets, I and II, which are observed from two different directions. Each set consists of five maps taken at every 0.5 Å depth.

Set I consists of the sectional views taken horizontally to the plane formed by three atoms; (C3), (C2), and (O), and
Fig. 4. Sectional views of the M2-type channel (a) and its magnified front views (b) and side views (c) to display packing manners of the (R)-isomer (above) and (S)-isomer (below) of 2. The front views (b) correspond to magnified squares depicted with dashed lines in the channel view (a). The front views (b) are rotated around the dashed lines to yield the side views (c). For example, in the case of the (R)-isomer the hydroxyl (O), isopropyl (C3) groups and hydrogen (H) linked to the chiral carbon (C2) are visualized in the upper front view, except for the methyl group (C1), which is visualized in the upper side view.

Fig. 5. A scheme for simulating a four-location model in a channel. a: The guest components with four sites (A, B, and C) are fixed on the basis of the corresponding recognition sites of a host (A', B', and C') in the channel, but there are two possible locations for the fourth site (D) of the guest. b: Such a guest in the channel is schematically depicted like a triangular plane formed by A, B, and C with two possible sites; (D(R)) and (D(S)). c: In the case of the alcohol 2, the sites of hydroxyl (O), chiral carbon (C2), isopropyl (C3), and methyl (C1) are symbolized as A, B, C, and D, respectively. The fourth site, methyl (C1), has two possible sites: D(R) and D(S). d: Actually, molecule 2 is analyzed like the fourth map of set II in Figure 3.
shows that two peaks are observed above and under the plane. One peak, which is observed about 1.0 Å above the plane, corresponds to the carbon (C1) of the (R)-isomer. The other peak, which is observed about 1.0 Å under the plane, corresponds to the carbon (C1) of (S)-isomer. These Difference Fourier maps directly indicate that both enantiomers of the alcohol are simultaneously accommodated in the same channel.

Set II is based on planes taken perpendicularly to the bond axis between the chiral carbon (C2) and the neighboring oxygen (O). It is confirmed from these maps that the two peaks of set I mentioned above are directly linked to the chiral carbon (C2).

**Packing Manner of the Guest Molecule in the M2-Type Channel**

As shown in Figure 1, a chiral carbon and four substituents at the chiral carbon should be fixed in the channel for the enantioresolution. In order to confirm this, we explored the detailed packing manner among the host and guest molecules.

Figure 4a shows a sectional view of the M2-type channel. Figure 4b,c shows its magnified front views and side views, respectively. We see how to accommodate the chiral carbon (C2) and four different substituents: hydroxyl (O), methyl (C1), isopropyl (C3) groups, and hydrogen (H), of the (R)- and (S)-isomers of 2. First, (O) is fixed in the channel by the hydrogen bonds. Second, (C3), the largest group among them, is enclosed within the largest cavity, because the other cavities do not have enough size. Third, (C2) is fixed according to the electron density in Figure 3. In this way, three locations are fixed in the channels.

One more location should be fixed in the channel for achievement of the enantioresolution. That is, the position of methyl group (C1) is crucial. In Figure 4c there are two possible positions where (C1) can be located. One is the cavity for (C1) of the (R)-isomer, and the other is the cavity for that of the (S)-isomer. When (C1) occupies one cavity, the final (H) at (C2) should occupy the remaining one. Since these two cavities have almost similar sizes, the (C1) in both cavities appears to be equally packed. Therefore, it is possible to mutually replace the methyl group and the hydrogen in this channel. This is the reason why enantioresolutions are scarcely achieved in the M2-type channel.

**Four-Location Model for Chiral Recognition in the Channel**

Figure 5 represents a state where both enantiomers are enclosed in the channel. In this figure the three locations of (O), (C2), and (C3) of the alcohol 2 are symbolized as A, B, and C, respectively. Even if three sites of a guest (A, B, and C) are fixed by the recognition sites of a host (A', B', and C'), the enantiomers cannot be distinguished because of the existence of two possible directions in the channel (Fig. 5a). The two triangle pyramids in the channel correspond to the pyramids overwritten on the alcoholic guest (Fig. 5b,c). Its fourth sites, D(S) and D(R), locate on the upper and lower sides of the fixed triangular plane formed by A, B, and C. Such a schematic representation is based on the Fourier map in the M2-type channel (the fourth map of set II in Figs. 3, 5d). This coexisted state of the racemic guest reveals the imperfection of the three-point attachment model. Instead, the four-location model is required for chiral recognition in the channel.

**Fixation of the Fourth Location in the M3-Type Channel**

Next, we consider the enantioresolution of 7, which displays the most effective resolution among the seven alcohols employed and is enclosed in the M3 channel. Figure 6 illustrates the crystal structure and the hydrogen bonding network of the inclusion compound of NDCA with 7. It can be seen from comparison of Figures 2 and 6 that the assembly of host molecules is changed to a herringbone framework (Fig. 6a) from parallel (Fig. 2a), and that the helical arrangement and hydrogen bonding network of M3 (Fig. 6b,c) is the same as those of M2 (Fig. 2b,c).

Figure 7 shows a sectional view of the M3-type channel and a simulation of the packing manner of the enantiomers. As in the case of 2, the chiral carbon (C2) of 7 has four
substituents: hydroxyl (O), methyl (C1), isobutyl (C3) groups, and hydrogen (H). First, the position of (O) is fixed by the hydrogen bond. Second, the front view indicates that the largest substituent, (C3), is enclosed in the largest cavity. Third, judging from the electron-density maps, (C2) is fixed in the channel. In this way three locations in the channel are fixed like those in the M2-type channel.

As for the fourth location occupied by (C1), however, there is a significant difference. It can be seen from the side view (Fig. 7c) that the methyl group (C1) of (S)-isomer is tightly surrounded by the wall composed of the host molecules. On the other hand, the one of the (R)-isomer is extremely close to the wall, leading to repulsion by a van der Waals interaction. Such a packing manner can explain the fact that the (S)-isomer of 7 is predominantly enclosed in the M3-type channel, rather than the (R)-isomer, indicating that the four-location model is applicable to the enantioresolution of 7.

CONCLUSION

We demonstrate that the enantioresolutions in the NDCA channel are interpreted based on the four-location model instead of the three-point attachment model. This study suggests that the four-location model may be generally applied for the enantioresolutions in the inclusion compounds.

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