Novel Channel Structure of Bile Acid-Guest Inclusion Complex Formed between Ursodeoxycholic Acid and Phenanthrene

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The crystal structure of inclusion complex between ursodeoxycholic acid (UDCA), and phenanthrene has been determined. UDCA molecules formed hydrogen bond network to provide the channel structure along b axis, and phenanthrene molecules were accommodated in the cavity with a stoichiometry of 1 : 1 molar ratio. The channel structure observed in the UDCA-phenanthrene complex was significantly different from that of inclusion complex previously reported for deoxycholic acid (DCA) and cholic acid (CA). Because of the mesh-like hydrogen bond network, channel framework of UDCA could have less flexibility than that of DCA and CA. The difference of molecular state of phenanthrene was clearly observed in solid-state fluorescence measurement.

Key words ursodeoxycholic acid; inclusion complex; channel structure; phenanthrene

During a few past decades, several attempts were made to clarify the molecular complexation between bile acids and guest compounds. Deoxycholic acid, 3α,12α-dihydroxy-5β-cholanic acid (DCA), have long been known as host compounds for formation of inclusion complex with a variety of guest compounds.[1,2] Recently, Miyata and co-workers found that cholic acid, 3α,7α,12α-trihydroxy-5β-cholanic acid (CA), also included several kinds of chemical compound.[3] Numerous guest compounds were reported to form complexes with CA; including fatty acids,[4] aliphatic esters[5] and aromatic compounds.[6] The characteristic structure of DCA and CA inclusion complex was proposed as tunnel-like spaces called "channels".[7,8] Further studies were also advanced in terms of the fine-tuning of nanocavity,[9] molecular and/or chiral recognition.[10,11]

Although ursodeoxycholic acid, 3α,7β-dihydroxy-5β-cholanic acid (UDCA), has a similar chemical structure to DCA or CA, neither inclusion complex nor solvate of UDCA has been demonstrated so far. Recently, we reported that UDCA formed complex when being ground with guest compounds. The specific channel structure was suggested since only phenanthrene and anthrone could be accommodated in the channel.[12] However, the molecular arrangement of inclusion complex is not yet established. In the present study, we attempted to clarify molecular packing of the inclusion complex between UDCA and phenanthrene. The molecular state of phenanthrene was also studied by solid-state fluorescence spectroscopy.

Experimental

Sample Preparation An equimolar mixture of UDCA (17.19 g) and phenanthrene (7.81 g) was suspended in 50 ml tetrahydrofuran and dissolved on heating at 45 °C. This solution was stored for 30 d at 25 °C. Precipitated crystal was dried at the room temperature, and a rectangular transparent crystal, mp 135 °C, was obtained for the X-ray crystal analysis measurement.

Solid-state Fluorescence Spectroscopy Solid-state fluorescence emission spectra were obtained by FP-6500 spectrofluorometer (JASCO, Tokyo, Japan) equipped with attachment for solid sample measurement. Excitation wavelength of phenanthrene was 315 nm.

Single Crystal X-Ray Analysis All measurements were made on a RIGAKU Raxis IIc CCD plate area detector with graphite monochromated Mo$K\alpha$ radiation. The structure was solved by direct methods[13] and expanded using Fourier techniques.[14] The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included but not refined. The final cycle of full-matrix least-square refinement was based on 2499 observed reflections ($I>3.00\sigma(I)$) and converged with unweighted and weighted agreement factors of 0.088 and 0.104, respectively. All calculations were performed using the teXsan[15] crystallographic software package of Molecular Structure Corporation.

Results and Discussion

The molecular structure of UDCA-phenanthrene was determined by using X-ray diffraction analysis. The crystal data and experimental conditions were summarized in Table 1. Figure 1 shows the molecular arrangement of UDCA and phenanthrene inclusion complex with labeling scheme. In order to describe the arrangement of CA in complexes, Mi-

Table 1. Crystal and Experimental Data

Empirical formula: $C_{38}H_{50}O_4$
Formula weight=570.81
Crystal color, habit: clear, prism
Crystal dimensions: 0.50×0.35×0.25 mm
Crystal system: orthorhombic
a=27.84(1) Å
b=6.928(3) Å
c=16.662(8) Å
V=3213.6(24) Å^3
Space group: $P2_12_12_1$ (#19) Z=4
D_{calc}: 1.180 g/cm^3
μ(Mo$K\alpha$): 0.74 cm^{-1}
Radiation: Mo$K\alpha$ (λ=0.71069 Å)
Monochromator: graphite
Temperature: −173 °C
No. of observations: 2499 ($I>3.00\sigma(I)$)
No. of variables: 380
Residuals: R=0.088 R_w=0.104
Goodness of fit indicator: 1.89
Maximum peak in final diff. map: 1.16 e Å^{-3}
Minimum peak in final diff. map: −0.40 e Å^{-3}
Measurement: Rigaku RAXIS IIc
Program system: teXsan
Structure solution: Direct methods (SIR92)

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yata called the chemical structure for the steroidal A ring and the side chain containing COOH as "head" and "tail" moieties, respectively.[3] In addition, inside and outside of bending molecules were called as belly and back sides, respectively. According to the X-ray data, it is proposed that phenanthrene molecule is located on the belly side of UDCA in the inclusion complex. The O(2)-H was on the back side of UDCA molecule, hence the belly side was considered to provide hydrophobic environment for accommodating phenanthrene molecules. On the contrary, all OH groups are located on the belly side in cases of DCA (3α,12α-OH) and CA (3α,7α,12α-OH).

Figure 2 shows the molecular packing aspect in the complex. UDCA molecules formed hydrogen bond network to provide the channel structure along b axis, and phenanthrene molecules stacked in the cavity with a stoichiometry of 1 : 1 molar ratio. The molecular arrangement of UDCA in the complex was quite different from that of UDCA with no guest compound as reported by Higuchi *et al.*[16]

Solid-state fluorescence spectroscopy was performed to estimate molecular state of phenanthrene in the single crystal of inclusion complex. Figure 3 illustrates the fluorescence spectra of phenanthrene with excitation at 315 nm. Equimolar physical mixture of phenanthrene and UDCA showed same emission spectrum to that of phenanthrene crystal, whereas the spectrum was significantly changed after grinding. In the case of inclusion complex, phenanthrene complex showed almost the same emission spectrum as that of the ground mixture with UDCA. Oguchi *et al.* reported that phenanthrene showed the characteristic emission peaks at 372 and 390 nm when phenanthrene crystals were ground with UDCA, and they mentioned that the phenanthrene molecules might be accommodated in the channels of the UDCA-phenanthrene complex.[12] Compared to the emission spectra of ground mixture and single crystal of inclusion complex, the molecular state of phenanthrene in the ground mixture with UDCA would be an identical state to that in the single crystal.

The channel structure of UDCA was compared with those of DCA and CA. Figure 4 demonstrates the channel space of present UDCA complex, in comparison with those of DCA and CA with acetophenone, as a typical framework. In the case of the DCA inclusion complex, the DCA molecules were arranged in one dimensional array by hydrogen-bond formation between the OH and COOH groups and provide hydrophobic spaces for accommodating guest compounds (shown as ellipse in Fig. 4). These spaces were adjustable depending on the size of the guest molecules, so that wide variety of compounds were accommodated. Such amphiphilic

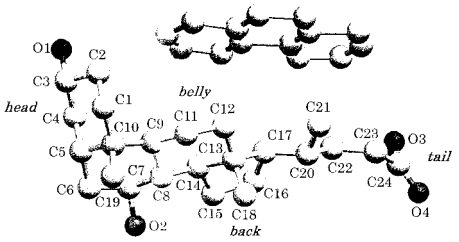

Fig. 1. Perspective View of Molecular Complex Consists of UDCA and Phenanthrene with the Numbering Scheme

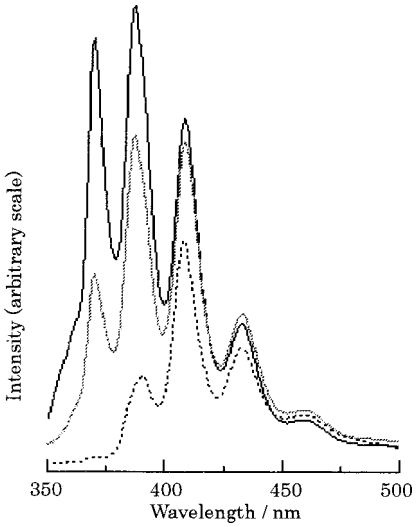

Fig. 3. Solid-State Fluorescence Emission Spectra of Phenanthrene in (a) Equimolar Physical Mixture with UDCA (···), (b) Ground Mixture with UDCA Ground for 1 h (—), (c) Single Crystal of Inclusion Complex (—)

The excitation wavelength was 315 nm.

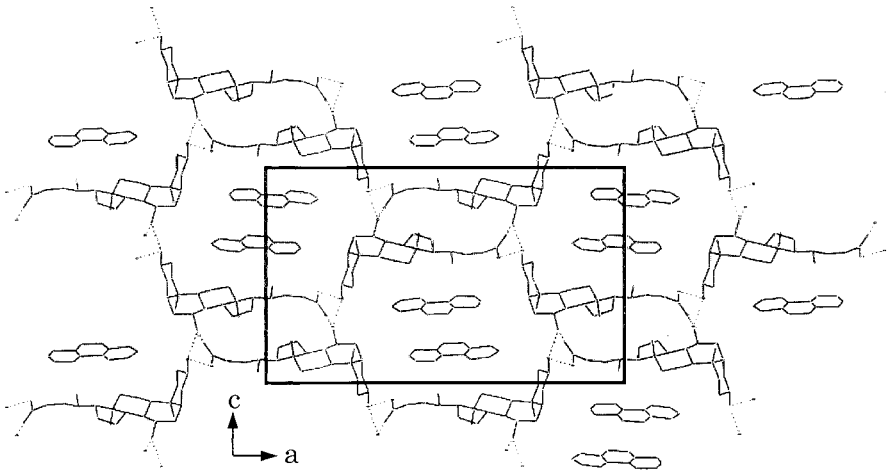

Fig. 2. Molecular Packing in the Complex Consists of UDCA and Phenanthrene Viewed along *b* Axis

The dashed lines indicated hydrogen bonding.

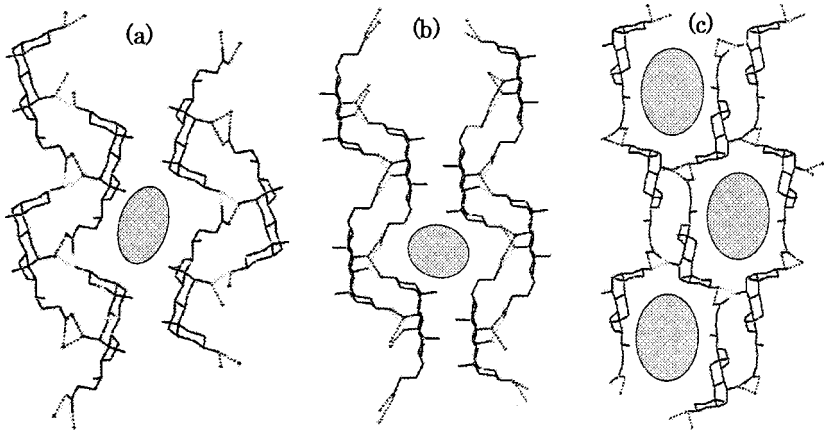

Fig. 4. Schematic Drawings of the Typical Channel Structures of Deoxycholic Acid (DCA), Cholic Acid (CA) and Ursodeoxycholic Acid (UDCA)
Guest molecules were omitted for the sake of clarity. (a) DCA–acetophenone 5 : 2 complex,[2] (b) CA–acetophenone 1 : 1 complex,[3] (c) UDCA–phenanthrene 1 : 1 complex.

layered structure was also observed in the CA complex crystal. On the other hand, the channel structure observed in the UDCA-phenanthrene complex was different from that of DCA or CA by the following two points: (i) each of the back sides of UDCA molecules combined with the others to form hydrogen bond, (ii) mesh-like channel structure was established with more complicated hydrogen bond network rather than layer structure, resulting in more specific complexation with the guest compounds. In the former point, the β-O(2)H of UDCA could affect to form hydrogen bond combined with reverse direction. Because of the latter view point, channel framework of UDCA having less flexibility than that of DCA or CA, the property of this framework would cause the higher guest selectivity of UDCA in the complex formation.

In conclusion, channel framework of UDCA-phenanthrene complex was different from that of inclusion complexes previously reported for DCA or CA. This indicated one of the reasons for specific complexation of UDCA with organic compounds. Anyhow, the new member could be added to inclusion compound family of bile acids.

References and Notes

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