Intercalation and Polymerization in Chenodeoxycholic Acid Channels with Retention of a Crystalline State

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(Received February 13, 1999)

ABSTRACT: Chenodeoxycholic acid (CDCA) was found to form hexagonal crystals including various vinyl and diene monomers. The crystals consist of helical assemblies of CDCA. The inner channels are one-dimensional spaces with a diameter of 0.7 to 0.8 nm. The monomers are incorporated into the channels through a guest exchange method (called intercalation), starting from the original compounds with *n*-octyl acetate. γ -Ray irradiation from a ^{60}Co source yielded polymers with relatively narrow molecular weight distributions. The microstructures are similar to those obtained from usual radical polymerization. The crystals maintained their shape during the intercalation and polymerization. The propagating radicals were detected by ESR spectroscopy. The spectra were dependent on the temperature. These results indicate that the polymerization proceeds in the relatively large channels with a living-like radical mechanism. This research contributes to the establishment of the concept; molecular information and their expression through nano-organized systems of sequential and chiral carbon-chain oligomers.

KEY WORDS Inclusion Polymerization / Chenodeoxycholic Acid / Helical Assemblies / Molecular Channels / Alkyl Methacrylate / Propagating Radicals / ESR Spectroscopy /

The molecular information at a nanometer level may originate from sequential and chiral carbon-chains, which are chemically modified for making the best use of non-covalent bonds.¹ As shown in Figure 1, the modified carbon-chains involve proteins with amide bonds, steroids with polycycles, nucleic acids with sugar moieties, and so on. Their information can be expressed with the corresponding nano-organized systems through spontaneous non-covalent processes; molecular architecture, host-guest complexation, reactions of the included guest components, and so on.

Our research project concerns with one of the nano-organized systems by using the steroids.^{2,3} In particular, we notice naturally occurring bile acids and their derivatives, since they form various molecular assemblies including guest components through multiple hydrogen bonds. The systematic study of the assemblies would lead us to a hypothesis about a relation between the molecular structures and the assemblies. This relation can be compared as that between the sequences of twenty kinds of amino acids and the folded structures of proteins.

A perspective of the project is represented in Figure 2. This involves all aspects of the steroidal inclusion compounds. Over twenty years ago we started from inclusion polymerization by using deoxycholic acid (DCA, Figure 3(b)).⁴ DCA forms parallel bilayered assemblies.⁵ The wavy layers leave channels with a rectangular cross section of 0.4 to 0.7 nm (Figure 3(e)). The first important result was the accomplishment of asym-

Figure 1. Information carriers at a nanometer level. A sequential and chiral carbon-chain (a) is chemically modified to the corresponding compound, such as protein (b), steroid (c), or nucleic acid (d).

metric and stereoregular polymerization in the channels. The second is based on the usage of a pair of DCA and apocholic acid. Such a research established the concept; the space effect on polymerization processes at a nanometer level.^{6,7}

During the study, we discovered channel-type inclusion compounds of cholic acid (CA, Figure 3(a)).^{8,9} Since CA forms antiparallel bilayers with larger channels than those of DCA, CA can include various organic substances (Figure 3(d)).² Moreover, this finding directed us to an extension of a scope of the steroidal host-guest complexes.^{10,11} Namely, over one thousand derivatives of bile acids have latent abilities to form inclusion compounds. This extension gave the idea that the inclusion phenomena are general for middle-molecular-weight

Figure 2. A perspective of our research project by using steroidal inclusion compounds.

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Figure 3. Molecular and crystal structures of three bile acids. Cholic acid (CA) (a), deoxycholic acid (DCA) (b), chenodeoxycholic acid (CDCA) (c) form antiparallel bilayers (d), parallel bilayers (e), and helical assemblies (f), respectively. The molecular channels are left between the bilayers in the cases of CA and DCA or in the helices in the case of CDCA.

molecules with multiple hydrogen bonding groups.¹

We continue to search the inclusion compounds by using more than fifty derivatives of bile acids. Until now this study has clarified that the host molecules basically form layered or helical assemblies, some of which leave channels where polymerization can take place. Recently, it has been found that chenodeoxycholic acid (CDCA, Figure 3(c)) form inclusion compounds with various organic substances. This paper concerns with the inclusion behaviors and the polymerization in the channels of helical assemblies of CDCA (Figure 3(f)).

EXPERIMENTAL

Materials

CDCA was commercially available from Tokyo Chemical Industry Co., Ltd. and used without further purification. Vinyl, diene monomers and solvents of reagent grade quality were purchased commercially and used without further purification.

Preparation of Inclusion Crystals

CDCA (100 mg) was located in a screw vial (5mL) and dissolved in *n*-octyl acetate (usually 2-3 mL) with heating. The resulting gel was allowed to stand at room temperature until crystals began to form. Columnar crystals gradually grew up

from the gel. The resulting crystals were collected and air-dried on a sheet of filter paper for several minutes.

The guest exchange was carried out in the following way. The crystals (100mg) were soaked into liquid guest components (usually 0.5 mL) and allowed to stand at room temperature for several days. The crystals were collected and air-dried on a sheet of filter paper for several minutes. The resulting crystals were characterized by IR, NMR spectroscopy and X-ray diffractions. The host-guest ratios were determined by NMR spectroscopy and thermogravimetry (TG).

Polymerization

500mg of the crystals including a monomer were placed in a glass tube of 10 mm diameter. The tube was sealed under reduced pressure and exposed to γ -irradiation from a ^{60}Co source at 77 K. The total dose was 1.0 Mrad. Post-polymerization was carried out by allowing to stand at 313 K for some days. The contents were poured into an excess amount of methanol to separate the polymers from the host. The methanol-insoluble fraction was filtered, dried under vacuum and weighed.

Measurements

IR spectra were taken on a JASCO IR-810 grating spectrometer using KBr disk. ^1H NMR spectra were recorded on a

Figure 4. Photographs of crystals of the inclusion compounds of CDCA with *n*-octyl acetate (a), with methyl methacrylate (b), with poly(methyl methacrylate), and the separated polymers (d). Schematic representation of the processes of intercalation (e), polymerization (f) and separation (g).

JEOL 270 MHz FT-NMR spectrometer. TG-DSC was measured by a Rigaku Thermoflex TG 8120 instrument. X-ray powder patterns were taken on a Rigaku RINT-2000 diffractometer with Cu-K α radiation (0.6 KW sealed tube) at room temperature. ESR spectra were recorded on a Bruker ESP 300 spectrometer after γ -ray irradiation at 77 K by using quartz tubes of 4 mm diameter.

X-ray diffraction data of single crystals were collected on a Rigaku RAXIS-IV diffractometer with graphite-monochromatized Mo-K α radiation. Crystal data; (a) CDCA+*n*-octyl acetate, C₂₄H₄₀O₄ • C₁₀H₂₀O₂ (213 K), *M*=392.6 (excluding *n*-octyl acetate), hexagonal, space group *P*6₃, *a*=22.195(5), *b*=22.195(5), *c*=10.27(1) Å, *V*=4382(1) Å³, *Z*=6, *D*_c=1.28 g cm⁻³. (b) CDCA+styrene, C₂₄H₄₀O₄ • C₈H₈ (207 K), *M*=392.6 (excluding styrene), hexagonal, space group *P*6₃, *a*=22.31(1), *b*=22.31(1), *c*=10.252(1) Å, *V*=4418(2) Å³, *Z*=6, *D*_c=1.12 g cm⁻³. Reflections of 1666 for (a), 1961 for (b) were unique. Observed reflections with $|F_0| > 3\sigma(|F_0|)$ of 1309 for (a), 1495 for (b) were used for further calculations after Lorentz and polarization corrections. The structures were solved by direct method SIR 92 and refined against the *F*_o data by full-matrix least-squares methods. The host molecules were fixed but the guest molecules were disordered. All non-hydrogen atoms of the host were refined anisotropically, and hydrogen atoms attached to the carbon atoms were placed in the calculated positions, while O-H were obtained from difference Fourier synthesis. Total parameters of 113 for (a) and 113 for (b) were refined to final residuals; *R*=0.177 and *R*_w=0.217 for (a), *R*=0.185 and *R*_w=0.240 for (b). All calculations were performed using the TEXSAN crystallographic software package of the Molecular Structure Corporation.

RESULTS AND DISCUSSION

Inclusion Compounds of CDCA with Organic Substances

It is known that CDCA has a crystal structure with helical columns accommodating a limited range of guest components, such as ethyl acetate¹² and chloroform.¹³ The guest molecules are disordered in the channels with diameter of 0.7 to 0.8 nm. However, the conventional recrystallization of CDCA from organic liquid substances yields amorphous powders, guest-free or very small crystals. Accordingly, there are only a few reports^{14,15} about the inclusion ability of CDCA in spite of many papers about those of DCA and CA.

Recently, we have obtained relatively large crystals (0.5 to 2.0 mm length) of CDCA by chance. When a gel of CDCA with *n*-octyl acetate was allowed for a long time, columnar crystals of CDCA grew up in the gel. Such a crystal is shown in Figure 4(a) photographically. ¹H NMR and IR spectroscopy indicate that the crystal includes *n*-octyl acetate as the guest. Another crystals were suitable for X-ray diffraction analysis. The crystal belongs to a hexagonal system, space group *P*6₃. Its structure was isomorphous to that with ethyl acetate, as indicated by about the same crystal lattice. Figure 3(f) shows a schematic view of the crystal structure. The host molecules are connected through intermolecular hydrogen bonding networks. Therefore, the channels have hydrophobic interiors and hydrophilic exteriors.

The acquisition of the large crystals enabled us to prove the inclusion of various organic substances by means of the intercalation method.^{16,17} Thus, the original crystals with *n*-octyl acetate were soaked into the other insoluble guest components for some days. Judging from their IR and ¹H NMR spectra,

Figure 5. X-ray powder patterns of the inclusion compounds of CDCA with *n*-octyl acetate (a), with methyl methacrylate (b), with poly(methyl methacrylate).

the original guest *n*-octyl acetate was displaced with the desired guest component. The notable feature is that the guest exchange proceeded without destroying the crystals. In the case of styrene, the crystals after the guest exchange were analyzed by X-ray diffraction method. The unit cell of the crystal was almost the same before and after the intercalation, as described in the experimental section.

In this way it was firstly established that CDCA forms inclusion compounds with various organic substances. This finding opened a new way for inclusion polymerization by using CDCA as a host. This polymerization would give us valuable information about the space effect at a nanometer level as compared with that by using DCA and CA.

Monomer Inclusion Compounds through the Intercalation

Next, the intercalation method was applied to various vinyl and diene monomers. For example, *n*-octyl acetate were exchanged by methyl methacrylate as follows. The original crystals were immersed in methyl methacrylate in bulk for several days at room temperature. IR and ¹H NMR spectra of the crystals indicate that *n*-octyl acetate was substituted by methyl methacrylate. The molar ratio of CDCA to the monomer was about 1:1 from TG measurements. A photograph and X-ray powder pattern of such a crystal are shown in Figure 4(b) and Figure 5(b), respectively. Both are very similar to those of the original sample. X-ray crystallographic study clarified that the crystal structure is isomorphous to that with ethyl acetate, as indicated by almost the same crystal lattice. These results established that the intercalation takes place with retention of the crystalline state. Similarly, the other monomers were confirmed to be incorporated into the channels.

Table I. Inclusion polymerization in channels of CDCA ^a

Monomer	Yield / % ^b	<i>M</i> _n / x10 ⁴	<i>M</i> _w / <i>M</i> _n ^c
2,3-Dimethyl-1,3-butadiene	55	4.5	2.6
Methyl methacrylate	50	11.0	2.8
Ethyl methacrylate	80	8.0	1.2
Isopropyl methacrylate	0	—	—
<i>n</i> -Butyl methacrylate	45	7.1	1.3
Isobutyl methacrylate	35	6.9	1.3
<i>tert</i> -Butyl methacrylate	0	—	—
Styrene	80	13	3.2

^a Polymerized at 313 K for one day after γ -irradiation with a dose of 1.0 Mrad at 77 K. ^b Methanol-insoluble fractions were weighed. ^c *M*_w/*M*_n is based on the calibration of standard polystyrene.

Figure 6. ESR spectra derived from the inclusion compounds of CDCA with methyl methacrylate. The measurements were carried out at 183 K (a) and 273 K (b) after γ -ray irradiation at 77 K with a dose of 1.0 Mrad.

Polymerization in Crystalline Channels

In order to avoid the polymerization outside of the channels, the inclusion crystals with the monomers were dried on filter paper for several minutes. After the irradiation at low temperature, postpolymerization was carried out at an elevated temperature. The striking feature is that the crystals maintained the shape in appearance. For example, one of the crystal with methyl methacrylate is shown in Figure 4(c). The X-ray powder patterns were similar before and after the polymerization, meaning retention of the helical assemblies. These results indicate that the polymerization proceeded in the channels. The melting points did not change as compared with those before the polymerization.

The crystals were poured into a large amount of methanol to separate the resulting polymers. The results are summarized in Table I. Several *n*-alkyl methacrylates polymerized to yield rubbery polymers, although the monomers with isopropyl or *tert*-butyl group did not polymerize. This is in contrast to the fact that these monomers did not polymerize in the channels of DCA. Their stereoregularities are similar to those obtained by usual radical polymerization in solution. Relatively narrow molecular weight distributions of the polymers suggest that the polymerization was initiated simultaneously by elevating temperature after the irradiation and the propagation reaction proceeded at a similar speed in the channels.

The resulting polymer obtained from 2,3-dimethyl-1,3-butadiene was rubbery, indicating that its stereoregularity was not high. ^1H NMR spectra confirmed that the microstructure of the polymer consists of a mixture of 1,4-*trans* and 1,4-*cis* units and only a few percents of 1,2 units.

ESR Measurements of the Propagating Radicals

After γ -ray irradiation, the samples were subjected to ESR measurements. The spectra were assigned to the propagating radicals of vinylic type.¹⁸ Since the spectra did not disappear at room temperature, the radicals were thermally stable. For example, Figure 6 shows the ESR spectra in the case of methyl methacrylate. Moreover, the spectra changed with increasing temperatures. ESR simulation study suggests that the movement of the radicals increase with increasing temperatures. These results indicate that the polymerization proceeded in constrained media by a free-radical mechanism.¹⁹ Other monomers also gave temperature-dependent ESR spectra. A comparative study of these radicals in the channels of CDCA, DCA and CA would give us numerous information about the nanospace effect.

CONCLUSION

This study demonstrates that helical assemblies of CDCA serve as reaction spaces suitable for inclusion polymerization. Since other bile acids and their derivatives provide the channel-type assemblies, it is expected that such channels also may function as the polymerization spaces when monomers suitable for the hosts are selected. Further comparative study by using CA, DCA and CDCA will make clear a relation between the channels and the monomers from a viewpoint of molecular recognition in size, shape, polarity and chirality. This research contributes to the establishment of the concept; molecular information and their expression through nano-organized systems of sequential and chiral carbon-chain oligomers.

Acknowledgments. The authors wish to thank the Radiation Laboratory, the Institute of Scientific and Industrial Research, Osaka University, for allowing them to use the ^{60}Co facilities. They are also gratefully indebted to Mr. H. Miyamoto of the Analytical Center, Faculty of Engineering, Osaka University, for his ESR measurements. This work was supported by a Grant-in-Aid for Scientific Research on Priority Areas, "New Polymers and Their Nano-Organized Systems" from the Ministry of Education, Science, Sports and Culture, Japan.

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