

Specific Complexation of Ursodeoxycholic Acid with Guest Compounds Induced by Co-grinding. II.[#] Effect of Grinding Temperature on the Mechanochemical Complexation

Toshio Oguchi, Kazuo Kazama, Toshiro Fukami, Etsuo Yonemochi,[†] and Keiji Yamamoto*

Graduate School of Pharmaceutical Sciences, Chiba University, 1-33 Yayoicho, Inage-ku, Chiba 263-8522

†School of Pharmaceutical Sciences, Toho University, 2-2-1 Miyama, Funabashi, Chiba 274-8510

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Ursodeoxycholic acid (UDCA) formed an inclusion complex with phenanthrene or with anthrone when being ground at an ambient temperature, while grinding at lower temperatures did not provide the complex but rather a mixture of amorphous UDCA and the guest compound crystals. Since heat treatment of the sample ground at low temperature did not provide the complex, it was concluded that the interplay of mechanochemical and thermal factors is responsible for the complexation of UDCA with the guest compound.

In pharmaceutical manufacture, grinding is a fundamental and important process to pulverize solid materials. In some cases, pharmaceutical solids are converted to the amorphous state through grinding process,[1,2] and such amorphization can be utilized for enhancing the solubility of poorly soluble drugs.[3] When being co-ground with microcrystalline cellulose, the drug effectively became amorphous by forming a molecular dispersion in the hydrogen bond network of microcrystalline cellulose.[4] It was reported that an inclusion complex was obtained in the amorphous state by using cyclodextrin as an additive.[5] Tanaka and Toda reviewed the solvent-free organic synthesis based on host–guest chemistry and explained that the enantioselective complexation could be achieved only by simply mixing or grinding for various kinds of host-guest combinations.[6]

Cholic acids are known as possible host compounds for formation of inclusion complexes with a variety of compounds like cyclodextrin.[7] In the crystal structure of the inclusion complex, adjacent host molecules form channels in a cooperative fashion, and accommodate guest molecules in the channels.[8,9] In our recent work, the co-grinding process was introduced to the deoxycholic acid system, and was found to induce the inclusion complex formation similarly to cyclodextrin systems.[10,11]

With regard to ursodeoxycholic acid (UDCA, Fig. 1), there has been no report for the formation of the inclusion complex. In the previous study we attempted the coprecipitation method from the alcoholic solution of UDCA with a variety of guest compounds, but no complexation was found. When the co-grinding process was applied for UDCA systems, however, phenanthrene and anthrone exceptionally formed the inclusion complex with UDCA among a variety of guest candidates.[12]

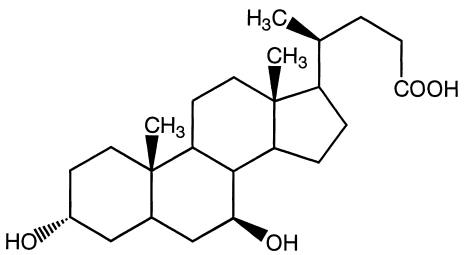

Fig. 1. Ursodeoxycholic acid (UDCA).

This phenomenon attracts our attention because this may give useful clues to clarify the mechanism of mechanochemical complexation which we have been discussing for other bile acid systems. In the present study, we focused on the effect of temperature during the grinding process on the complexation between UDCA and phenanthrene/anthrone in order to investigate more details of the specific inclusion formation.

Experimental

Materials. UDCA was received as a gift from Mitsubishi Tokyo Pharm. Co., Ltd. (Tokyo, Japan). Phenanthrene and anthrone were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). All the chemicals were of analytical reagent grade and were used without further purification.

Preparation of Ground Mixture. A physical mixture of UDCA and a guest compound was prepared using a vortex mixer for 5 min. For the preparation of ground mixture, two grams of an equimolar physical mixture of UDCA and a guest compound was loaded into a 50 mL mill chamber, and was ground using a Vibrating Freeze Mill, CMT TI-500ET (Fukushima, Japan). The mill chamber consists of a mill pot, made of SUS F5 stainless steel, and a cylindrical rod, made of SUS C stainless steel.

During the grinding process, the sample chamber was set in a cold box. It contained cold nitrogen gas or was filled with dry ice, ice or water (20 °C). The temperature in the cold box was measured by a copper-constantan thermocouple connected to a data

Part I: T. Oguchi, K. Kazama, E. Yonemochi, S. Chutimaworapan, W.-S. Choi, S. Limmatvapirat, and K. Yamamoto, *Phys. Chem. Chem. Phys.*, **2**, 2815-2820 (2000).

logger Keyence NR-1000 (Osaka, Japan). When cold nitrogen gas was introduced to the stationary cold box, the temperature was equilibrated at ca. −70 °C after 10 min. In the case of using dry ice and ice, the temperature of the cold box was observed to be −55 and 0.0 °C, respectively.

To avoid increasing the temperature of the sample, the grinding process was performed intermittently, i.e., in sequential repetitions of 5 min grinding followed by 5 min intermission. This grinding program was assumed to be proper because the same results were obtained when the intermission time was prolonged to 10 min.

Powder X-ray Diffraction. A Rigaku Miniflex diffractometer (Tokyo, Japan) was employed for powder X-ray diffraction. The measurement conditions were as follows: target Cu, filter Ni, voltage 30 kV, current 15 mA, scanning speed 4 ° min^{-1}.

Determination of Heat of Dissolution. The heat of dissolution was determined on an isothermal heat-conduction twin-type multipurpose calorimeter (model MPC-11, Tokyo Riko Co., Ltd., Tokyo, Japan). Measurement conditions were as follows: solvent, ethanol (95%)–water (5%) 20 mL; temperature, 25 °C; temperature precision, $\pm 1 \times 10^{-5}$ °C; sample weight, 0.200 g; and rotational speed of stirrer, 50 rpm. Calibration was carried out at known quantities of heat for each determination.

Results and Discussion

The co-grinding process was applied for the UDCA and phenanthrene/anthrone system. Figure 2 shows the changes in the powder X-ray diffraction (PXRD) pattern of the equimolar physical mixture of UDCA and phenanthrene during the grinding process. The diffraction pattern was clearly changed after the grinding for 15 min, we demonstrated in the previous paper[9] that this resulted from the inclusion complex formation between UDCA and phenanthrene through the co-grinding process.

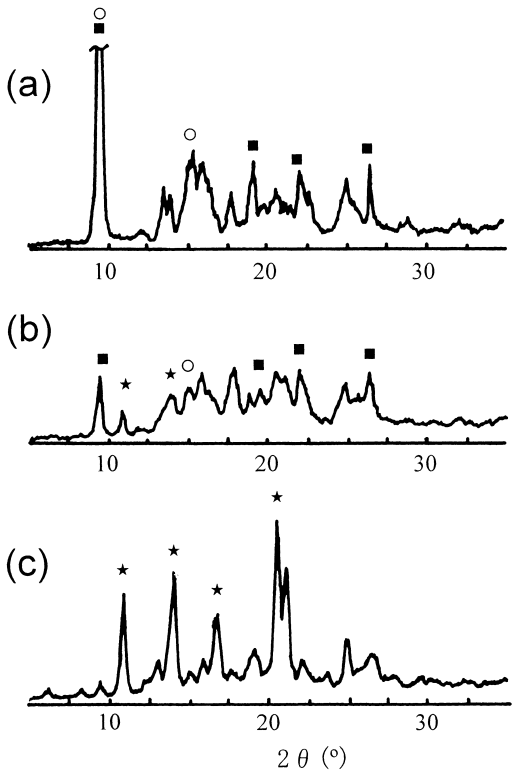

Fig. 2. Changes in the powder X-ray diffraction (PXRD) pattern of the equimolar mixture of ursodeoxycholic acid (UDCA) and phenanthrene by grinding at ambient temperature, grinding time: (a) 0, (b) 5, (c) 15 min. The diffraction peaks due to UDCA, phenanthrene and the complex crystals are indicated by ○, ■, and ★, respectively.

Organic compounds, especially with low molecular weight, can not necessarily be converted to the amorphous state even by use of a high-energy mill.[13,14] During the grinding process by a high-energy mill, the mechanical force can be exerted to the molecular level for organic compound crystals and is assumed to be large enough to cause plastic deformation of the ground specimen. Since the deformed or dissembled state is thermodynamically unstable for molecules, spontaneous reconstitution of the crystal structure would tend to take place immediately as a relaxation process. In cases of sublimable compounds, the process may also occur via gas phase, in which the vapor pressure of the solid should be enhanced due to the disordering of the crystal structure. Anyhow, this process is promoted by molecular mobility and, therefore, the temperature should affect the reconstitution process to a great extent.

Heat is evolved in grinding process. During the grinding process of inorganic materials, there seems to be the "hot-spots", localized high-temperature domains, in the milled mass. In the case of organic compounds, plastic deformation can easily be caused by mechanical force because of weakness of lattice energy. Therefore, we speculate that an organic specimen is unlikely to have a high strain region or a hot-spot in the grinding process. In addition, most organic compounds are chemically stable against the grinding process, although thermal decomposition of organic compounds resulting in the cleavage of the covalent bonds usually takes place around 200–250 °C. This may also support our speculation about the absence of hot-spots.

Immediately after grinding for 15 min at ambient temperature, we measured the temperature of the inside of the mill chamber by an infrared thermometer. The temperature rose to ca. 45 °C. In order to dissipate the heat evolved and to investigate the effects of temperature, we performed the grinding process at low temperatures.

The equimolar physical mixture of UDCA and phenanthrene was ground with cooling by cold nitrogen gas, the PXRD pattern of the ground mixture obtained is shown in Fig. 3a. As mentioned in the experimental section, the surrounding temperature of the mill chamber was about −70 °C. No diffraction peaks due to the complex appeared in the diffraction pattern, but diffraction peaks due to phenanthrene crystals were observed on a broad diffraction. This indicated that the low-temperature grinding did not induce the complexation, but just amorphized UDCA in the binary mixture of UDCA and phenanthrene. Figures 3b, c and d show the PXRD patterns of the ground mixtures obtained by using other cooling media. When cooled with dry ice or with ice, the ground mixtures obtained showed no diffraction peaks due to the complexation. On the other hand, grinding at 20 °C was found to induce the formation of the inclusion complex between UDCA and phenanthrene. These results indicate that mechanochemical complexation between UDCA and phenanthrene took place

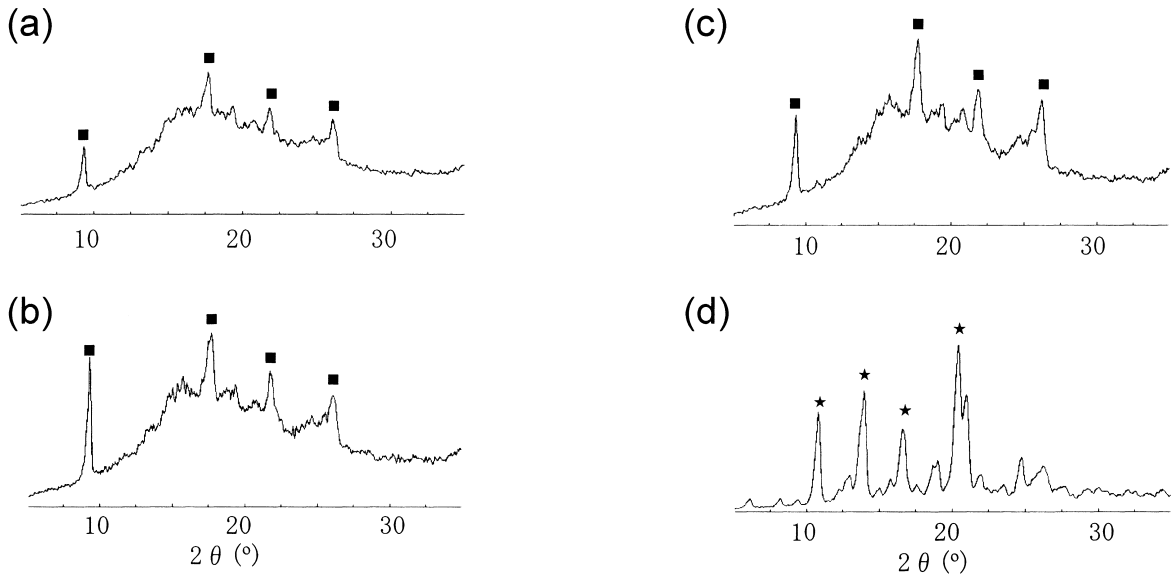

Fig. 3. PXRD patterns of the equimolar ground mixtures of UDCA with phenanthrene system prepared with various cooling conditions. During the grinding process for 60 min (total time), the sample chamber was cooled with; (a) liquid nitrogen (−70 °C), (b) dry ice (−55 °C), (c) ice (0 °C), (d) water (20 °C). The diffraction peaks due to phenanthrene and the complex crystals are indicated by ■ and ★, respectively.

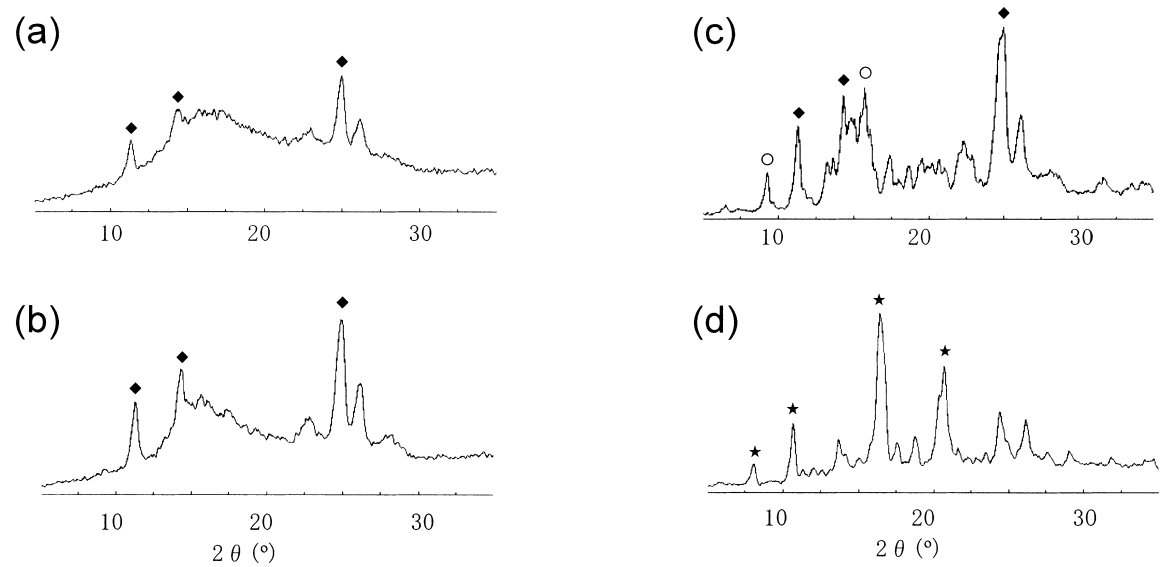

Fig. 4. PXRD patterns of the equimolar ground mixtures of UDCA with anthrone system prepared with various cooling conditions. During the grinding process for 60 min (total time), the sample chamber was cooled with; (a) liquid nitrogen (−70 °C), (b) ice (0 °C), (c) water (20 °C), (d) ambient temperature. The diffraction peaks due to UDCA, anthrone and the complex crystals are indicated by ○, ◆, and ★, respectively.

above a certain temperature that was assumed to be in the range of about 0 to 20 °C.

Figure 4 shows the PXRD patterns of equimolar ground mixtures of UDCA with anthrone. When the grinding process was performed at ambient temperature, the PXRD result indicated the formation of the inclusion complex, as shown in Fig. 4d. Similarly to the results in the UDCA–phenanthrene system, Figures 4a and b indicated that the complex was not formed during the grinding process at lower temperatures. Interestingly, when the temperature was controlled at 20 °C using water, the ground sample showed diffraction peaks due to UDCA crystals as well as peaks due to anthrone crystals (Fig. 4c). This ground mixture could be regarded as a mixture of UDCA crystals and anthrone crystals, although diffraction peak intensities were different from those of the physical mixture before grinding.

To investigate why the physical mixture of UDCA and anthrone remained unchanged after grinding at 20 °C, we applied the grinding process to the other ground mixtures obtained under different conditions. Figures 5a and b demonstrates the

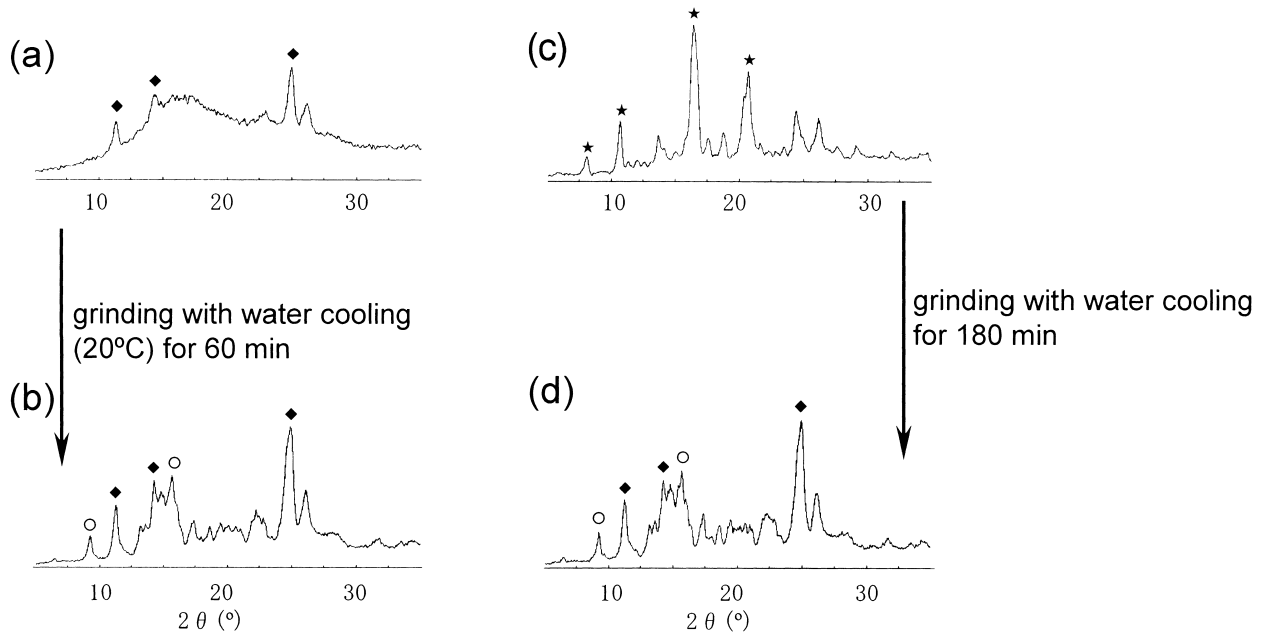

Fig. 5. Changes in the PXRD patterns of equimolar ground mixtures of the UDCA with anthrone during the process of grinding with cooling; (a) ground mixture prepared with cooling by liquid nitrogen and (b) the same mixture after grinding at 20 °C for 60 min, (c) ground mixture prepared at ambient temperature and (d) the same mixture after grinding at 20 °C for 180 min. The diffraction peaks due to UDCA, anthrone and the complex crystals are indicated by ○, ◆, and ★, respectively.

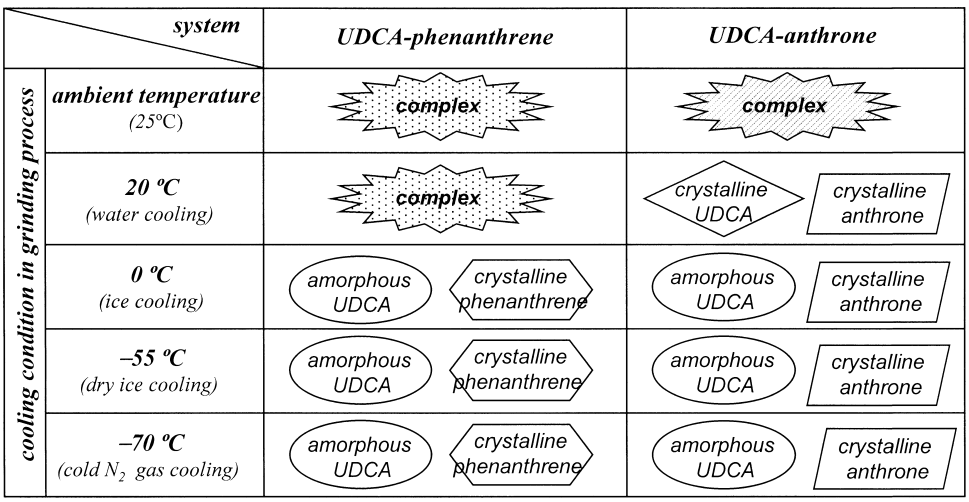

Fig. 6. Scheme for the effect of the temperature on the mechanical complexation of UDCA with phenanthrene/anthrone.

changes in the PXRD pattern through grinding at 20 °C for the ground mixtures, obtained at lower temperatures, which consisted of amorphous UDCA and crystalline anthrone (see Fig. 4a). After the grinding for 60 min, the PXRD pattern was changed to a pattern similar to Fig. 4c. With regard to the inclusion complex obtained by the grinding at ambient temperature, the grinding process at 20 °C for 180 min also provided changes in the PXRD pattern toward the mixture of the two crystals (Fig. 5c and d). Consequently, the final state of the ground specimen was not dependent on the state before grinding, but was determined by the temperature during the grinding process.

Figure 6 summarizes the effects of the grinding temperature on the mechanochemical complexation of UDCA with phenanthrene/anthrone. In both the systems, complexation was completed when the grinding process was carried out above a certain temperature. Grinding at a lower temperature provided a mixture of amorphous UDCA and guest crystals. The threshold temperature seemed to depend on the guest compound. Namely, for the UDCA–phenanthrene system the threshold temperature was in the range between 0 and 20 °C, while for the UDCA–anthrone system the temperature was above 20 °C. Interestingly, an intermediate state was found in the UDCA–anthrone system when the grinding was carried out at 20 °C.

For both the UDCA–phenanthrene and the UDCA–anthrone

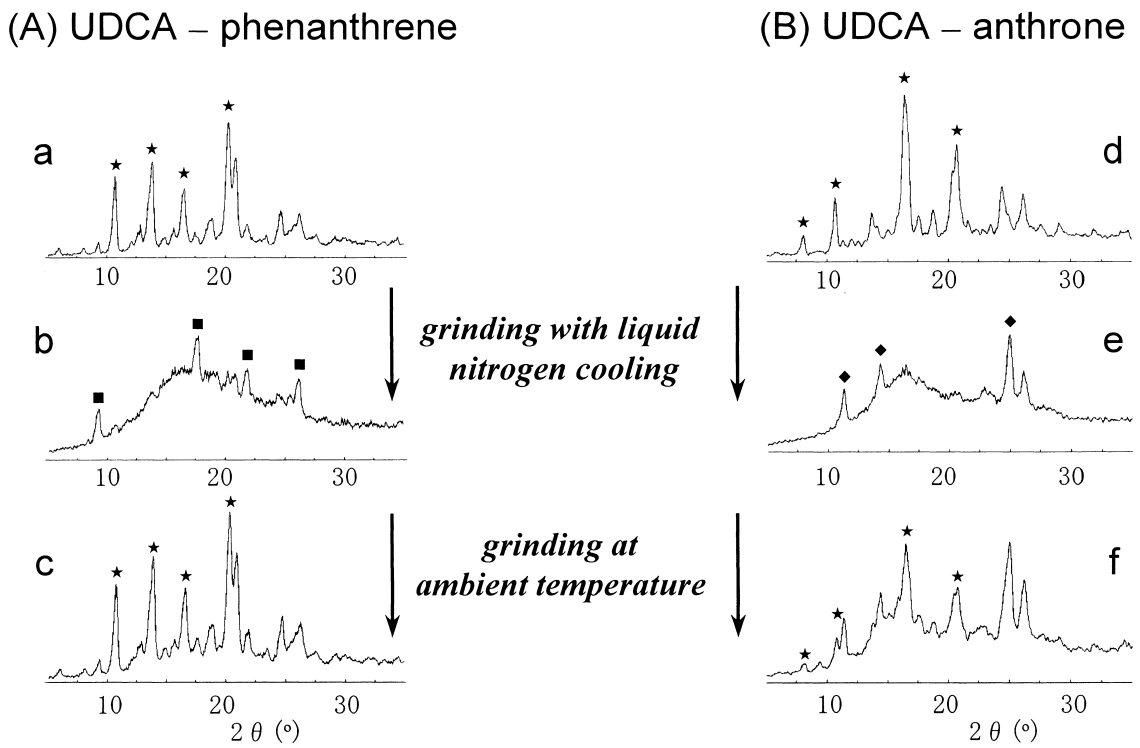

Fig. 7. Reversible mechanochemical complexation controlled by the temperature during the grinding process. (A) UDCA–phenanthrene system, (B) UDCA–anthrone system. The diffraction peaks due to UDCA, phenanthrene, anthrone and the complex crystals are indicated by ○, ■, ◆, and ★, respectively.

systems, the grinding processes were successively performed by alternating the temperature. Figures 7a and 7d represent the PXRD patterns of the inclusion compounds prepared by conventional grinding of each equimolar physical mixture. When these inclusion compound crystals were ground with cooling by cold nitrogen gas (Figures 7b and 7e), the PXRD patterns changed to the same patterns as shown in Figs. 3a and 4a, indicating that the complexes transformed to the mixture of amorphous UDCA and guest compound crystals. Further, when the grinding process at ambient temperature was sequentially applied, the PXRD patterns indicated transformation back to the initial inclusion compound (Figs. 7c and 7f). These results indicated that the mechanochemical transformation in UDCA and phenanthrene/anthrone systems proceeded reversibly and the grinding temperature did determine the state of the systems.

As described above, the grinding process was assumed to consist of two steps: i.e., breaking-up process of the ordered structure, and building-up process of the stable crystal structure of inclusion complex. We assumed that the mechanical force is related to the first step, and that the second step can be markedly affected by the temperature conditions.

To investigate the involvement of heat on the complexation, only heating was applied for the mixtures of amorphous UDCA and guest crystals that were obtained by low-temperature grinding with cold nitrogen gas cooling. Figure 8 illustrates the change in the PXRD patterns due to the heating process. For both UDCA–phenanthrene and UDCA–anthrone systems, the diffraction peaks characteristic of the inclusion complex crystals were not observed after heating at 75 °C for 40min, but the PXRD patterns indicated that the sample corresponded to the mixture of UDCA and guest crystals. Interestingly, the heating process just induced the crystallization of UDCA in the sample. These results suggest that for the complex formation of UDCA the mechanochemical and heating effects must act co-operatively.

To access the physicochemical stability of the inclusion complex, the heat of dissolution for each specimen was determined for the UDCA-phenanthrene system. A mixed solvent of 95% ethanol and 5% water was used as a dissolution medium and the measurement was carried out at 25 °C. The results are listed in Table 1, where the heats of dissolution for specimens (c)–(g) are expressed as the value per a sample containing both of one mole UDCA and one mole phenanthrene. The heats of dissolution for the physical mixture (28.6 kJ/mol) appear to demonstrate the additivity of the contributions from UDCA (8.8 kJ/mol) and from phenanthrene (19.4 kJ/mol), suggesting no significant interaction between UDCA and phenanthrene in the ethanolic medium.

When the physical mixture (*specimen c*) was ground with liquid nitrogen cooling, the conversion into a meta-stable state of lower crystallinity resulted in a decrease in the heat of dissolution (*specimen d*, 19.6 kJ/mol). Heating the *specimen d* induced the crystallization of amorphous UDCA; the heat of dissolution increased to 28.5 kJ/mol (*specimen e*), which was almost the same as that of the initial physical mixture (*specimen c*). On the other hand, the inclusion compound (the *specimen g*, derived from grinding the *specimen c* at ambient temperature followed by the heating at 70 °C) showed the heat of dissolution of 31.6 kJ/mol. The result of the heat of dissolution

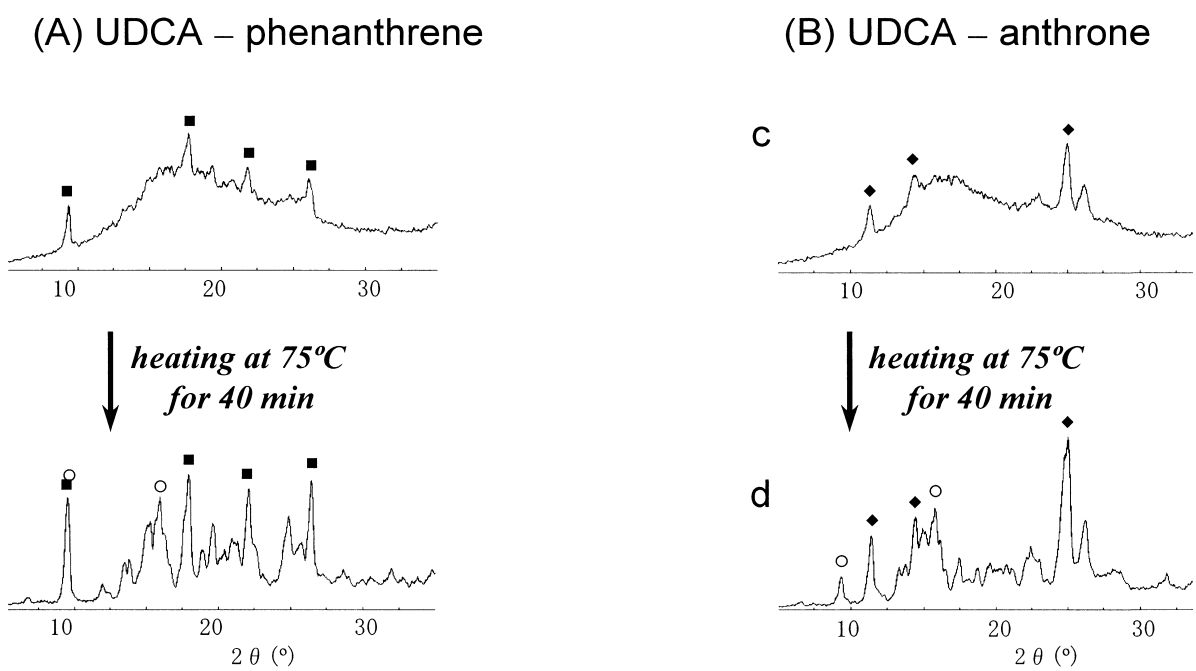

Fig. 8. Changes in the PXRD patterns by heating the ground mixture prepared with liquid nitrogen cooling. (A) UDCA–phenanthrene system, (B) UDCA–anthrone system. The diffraction peaks due to UDCA, phenanthrene and anthrone crystals are indicated by ○, ■, and ◆, respectively.

Table 1. Heat of Dissolution in the UDCA–Phenanthrene (1:1) System at 25 °C Solvent: Ethanol–Water (95:5 w/w)

Specimen	Heat of dissolution kJ mol^{-1}
(a) UDCA	8.8
(b) Phenanthrene	19.4
(c) Equimolar physical mixture of UDCA and phenanthrene	28.6
(d) Ground specimen (c) with liquid nitrogen cooling	19.6
(e) Heated specimen (d) at 70 °C	28.5
(f) Ground specimen (c) at ambient temperature	28.6
(g) Heated specimen (f) at 70 °C	31.6

indicated that the inclusion complex was enthalpically more stable than the physical mixture (or *specimen e*). Considering that complexation was not completed by the heating of the *specimen d* (shown in Fig. 7), we may say that the complexation of UDCA and phenanthrene was not controlled only by a thermodynamic factor.

Figure 9 summarizes the interconversion in the UDCA–phenanthrene/anthrone system. From above results we can assume three different states of the systems: i.e., the mixture of crystalline UDCA and crystalline guest compound (*mixture A*), the mixture of amorphous UDCA and crystalline guest compound (*mixture B*), and the crystalline complex. When the physical mixture (*mixture A*) was ground, either the crystalline complex or *mixture B* was produced depending on the temperature. Heating the *mixture B* did not provide the complex, but induced crystallization of amorphous UDCA to produce *mixture A*. The final states of the ground mixtures were independent of the initial states, i.e., interconversion between the complex and *mixture B* was possible by controlling grinding temperature.

Conclusions

In this paper, the temperature effects on the mechanochemical complexation were investigated. It was found that the complexation depended on the temperature in the UDCA–phenanthrene/anthrone systems, i.e., the complexation was completed by the conventional grinding at ambient temperature, while grinding at lower temperatures (cooling with ice, dry ice or cold nitrogen gas) provided a mixture of amorphous UDCA and crystalline phenanthrene/anthrone. Since the coprecipitation from the alcoholic solution, which is the conventional method for inclusion complex preparation of cholic acids, did not give the complex, there must be some other factors as well as thermodynamic stability for determining the state of the system during the grinding process. The interconversion between the two different states proceeded reversibly. These results suggest that the physicochemical change in the grinding field proceeds in an equilibrium fashion. Especially, it is noteworthy that the mechanochemical and heating effect should function co-operatively for the complex formation of UDCA with

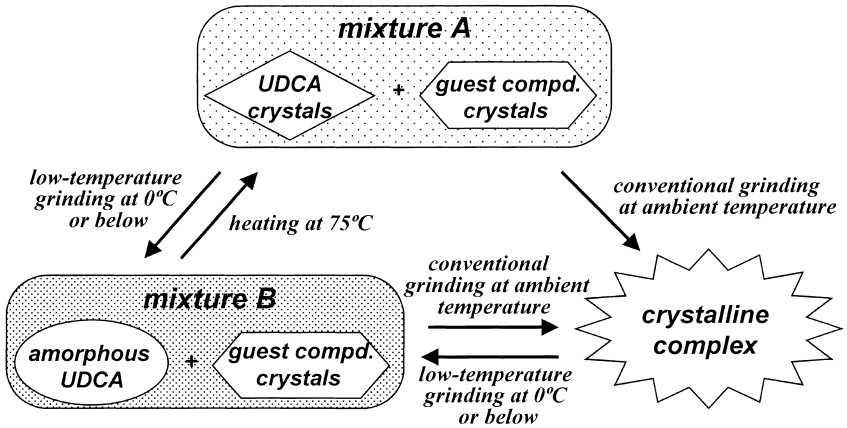

Fig. 9. Mechanochemical conversion in UDCA–phenanthrene/anthrone systems.

phenanthrene/anthrone. Such a phenomenum has not been reported before for organic binary systems and is quite interesting.

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