Formation of a Heptakis-(2,6-di-*O*-methyl)- β -cyclodextrin-*p*-nitrophenol Inclusion Compound by Sealed-Heating

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The complexes of heptakis-(2,6-di-*O*-methyl)- β -cyclodextrin (DM β CD) and *p*-nitrophenol (*p*-NP), which were prepared by sealed-heating and a slow evaporation method, were comparatively evaluated. From the results of differential scanning calorimetry, powder X-ray diffraction, and infrared spectroscopy, the sealed-heated sample was considered as a new complex which was different from the slow evaporation complex. Also, the data from a sublimation experiment indicated that the inhibition of sublimation of *p*-NP was observed only in the sealed-heated sample. These results suggested that *p*-NP molecules were included within the cavity of DM β CD molecules, whereas in case of the slow evaporation complex, *p*-NP molecules were located in the intermolecular space among the DM β CD molecules.

Key words heptakis-(2,6-di-*O*-methyl)- β -cyclodextrin; *p*-nitrophenol; sealed-heating; inclusion compound; thermal analysis

In the previous paper, we reported that an inclusion compound was obtained by heating the physical mixture of cyclodextrin (CD) and a drug in a sealed container.^{2–5)} When the physical mixture of heptakis-(2,6-di-*O*-methyl)- β -cyclodextrin (DM β CD) and benzoic acid (BA) was heated in a glass ampoule, an inclusion compound was formed, and the combining molar ratio of BA to DM β CD increased with an increase in the heating temperature. The mechanism was described as follows: the guest molecules were sublimated then adsorbed to the surface of DM β CD particles, followed by inclusion within the cavity of the DM β CD crystals. The non-sublimable guest molecules could not be included within the cavity of CDs as the sublimation of the guest molecules was important in the first step.³⁾ *p*-Nitrophenol (*p*-NP) was included within the cavity of the DM β CD in the aqueous solution,⁶⁾ whereas in the solid complex prepared by the slow evaporation method, the *p*-NP molecule was not included within the CD cavity, but was located in the intermolecular space among DM β CD molecules.^{7,8)} In this report, we discussed the formation mechanism of an inclusion compound of *p*-NP and DM β CD made by a sealed-heating method, as well as the physicochemical properties of the sealed-heated sample.

Experimental

Materials DM β CD was purchased from Toshin Chemical Co. and used without further purification. *p*-NP was of special reagent grade purchased from Wako Pure Chemical Industries, Ltd.

Preparation of Sealed-Heated Sample An equimolar physical mixture of DM β CD and *p*-NP was prepared by blending them in a vial using a vortex mixer. The mixture (300 mg) was sealed in a 2 ml glass ampoule. Then, the ampoule was heated in an oven at a heating rate of 8 °C/min from 40 °C to 100 °C, and was kept at 100 °C for a definite time. The temperature around the glass ampoule in the oven was monitored by thermocouple.

Preparation of Slow Evaporation Complex 2.857×10^{-3} mol of DM β CD was dissolved in 40 ml of distilled water and 2.857×10^{-3} mol of *p*-NP was added, then stirred. The solution was kept at room temperature and was allowed to evaporate slowly.

Powder X-Ray Diffraction Measurement Powder X-ray diffraction patterns were measured using a Rigakudenki 2027 diffractometer. Condition: target Cu, filter Ni, voltage 30 kV, current 5 mA, count range

2000 cps, scanning speed 4°/min.

Infrared Absorption Spectroscopy Measurements were carried out by the Nujol method using a Nicolet FT-IR spectrophotometer (SZDX).

Thermal Analysis DSC measurements were carried out on a Du Pont TA9900 thermal analysis system under a stream of nitrogen gas by using about 2.0 mg of sample at a heating rate of 10 °C/min.

Heating Experiment in an Aluminum Cell An equimolar physical mixture of DM β CD and *p*-NP was filled in a 0.8 ml aluminum cell, and the cell was well closed with aluminum foil as previously described.⁹⁾ The well-closed cell was heated at a heating rate of 8 °C/min, and the heated sample was obtained when the sample was heated to a desired temperature.

Sublimation Experiment Sublimation of *p*-NP from various preparations (30 mg) by heat treatment at 100 °C and reduced pressure was determined. The remaining sample was dissolved in a 0.1N HCl solution, and the quantity of remaining *p*-NP was assayed by the UV absorption method.

Results and Discussion

An equimolar physical mixture of DM β CD and *p*-NP was sealed-heated at 100 °C for a definite time. Figure 1 shows the powder X-ray diffraction patterns of the equimolar physical mixture of DM β CD and *p*-NP. The

Fig. 1. Powder X-Ray Diffraction Patterns of Sealed-Heated Mixture of *p*-NP and DM β CD (Mixing Molar Ratio 1:1) at 100 °C

Heating time; a) 0 h, b) 6 h, c) 12 h, d) 24 h.

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Fig. 2. Powder X-Ray Diffraction Patterns of *p*-NP-DM β CD Systems

a) Physical mixture (mixing molar ratio 1:1), b) sealed-heated sample (heated at 100 °C for 24 h), c) complex prepared by slow evaporation of aqueous solution, d) *p*-NP.

diffraction pattern of the physical mixture (curve a) was simply a superposition of those of two components, *i.e.* the diffraction peaks at $2\theta = 9.7^\circ$ and $2\theta = 8.1^\circ$ were due to DM β CD crystals, and the peak at $2\theta = 18.6^\circ$ was due to *p*-NP crystal. Curves b, c and d show the powder X-ray diffraction patterns of the physical mixture of DM β CD and *p*-NP after sealed heating at 100 °C for 6, 12 and 24 h respectively. After 6 h of heating, the diffraction peaks observed in curve a were still the same. However, the diffraction peaks due to DM β CD crystals and *p*-NP crystals disappeared, and new diffraction peaks appeared at $2\theta = 11.0^\circ$, 19.3° and 20.1° when the mixture was heated for 12 h. The intensities of these new diffraction peaks were increased up to 24 h and no change was found after heating for more than 24 h. As no changes were observed in the X-ray diffraction patterns when only DM β CD crystal was sealed-heated for 24 h at 100 °C, the changes observed in the sealed-heated process were presumed to be related to the molecular interaction between DM β CD and *p*-NP molecules.

Figure 2 shows the powder X-ray diffraction patterns of various *p*-NP-DM β CD systems. The X-ray diffraction peak positions of the slow evaporation complex (curve c) were identical to the theoretical 2θ values calculated from the unit-cell constants ($a = 14.779$ Å, $b = 18.965$ Å, $c = 28.741$ Å) reported by Harata.^{7,8)} Thus, it was confirmed that *p*-NP molecules were located in the intermolecular space among DM β CD molecules. The powder X-ray diffraction pattern of the sealed-heated sample (curve b), however, was different from those of the slow evaporation complex and the physical mixture. These results indicated that the sealed-heated sample formed a

Fig. 3. Infrared Spectra of *p*-NP-DM β CD Systems

a) Physical mixture (mixing molar ratio 1:1), b) sealed-heated sample (heated at 100 °C for 24 h), c) complex prepared by slow evaporation of aqueous solution.

new complex which was distinctly different from the slow evaporation complex.

To confirm the results from X-ray diffractometry, IR spectroscopy was employed. Figure 3 shows the IR spectra of various *p*-NP-DM β CD systems. Curve a shows the IR spectrum of the physical mixture. A stretching vibration band of the NO₂ group was observed at 1335 cm⁻¹. However, the stretching vibration band of the NO₂ group was shifted to a higher wave number (1335–1339 cm⁻¹) in the spectrum of the sealed-heated sample (curve b). The shift of stretching vibration band was considered to be related to the formation of a complex of *p*-NP and DM β CD by sealed-heating. The sealed-heating process caused the disappearance of intermolecular hydrogen bonding formed in crystals between the nitro group and the hydroxyl group of *p*-NP molecules.^{10,11)} Curve c shows the spectrum of the slow evaporation complex showing the peak of the NO₂ stretching vibration band at 1340 cm⁻¹. In the slow evaporation complex, *p*-NP molecules are not included within the DM β CD cavity, but are located in the intermolecular crevice which had a highly hydrophobic property.⁷⁾ A small peak at 1318 cm⁻¹ was observed in the IR spectrum of the slow evaporation complex, while in the IR spectrum of the sealed-heated sample, only a small peak was observed at 1318 cm⁻¹. This peak might indicate the difference in molecular state of *p*-NP between the sealed-heated sample and the slow evaporation complex.

Thermal properties were determined by DSC using a sealed pan which was considered to be similar in condition to the inside of the glass ampoule. Figure 4 shows the DSC curves of various *p*-NP-DM β CD systems. *p*-NP (curve a) showed an endothermic peak due to the fusion at 113 °C, whereas no peak was detected in the case of DM β CD. Two endothermic peaks were observed at 135 °C and 195 °C in the physical mixture (curve c) which

indicated that some changes took place in the *p*-NP crystals before they reached 113 °C in the DSC measurement. One endothermic peak was observed at 195 °C in the sealed-heated sample (curve d), and this curve was different from the DSC curve of the slow evaporation complex. The slow evaporation complex showed endothermic peaks at 145 °C and 195 °C (curve e).

To clarify about the endothermic peaks which were found in the DSC thermogram of the physical mixture, a heating experiment in an aluminum cell was employed. The results from this experiment were investigated by X-ray diffractometry and IR spectroscopy, and are shown in Figs. 5 and 6, respectively. The physical mixture and the sample heated to 80 °C showed the same X-ray

diffraction pattern (Fig. 5, curves a and b) and IR spectra (Fig. 6, curves a and b). These results indicated that no complex formation occurred. The sample heated to 190 °C, which was the temperature between the two endothermic peaks, showed some changes both in the powder X-ray diffraction pattern (Fig. 5, curve d) and in the IR spectrum (Fig. 6, curve d). They were exactly identical to those of the sealed-heated sample. In the previous report,⁴⁾ two endothermic peaks were also observed in the DSC curve of the physical mixture of DM β CD and BA, and the endothermic peak at a lower temperature originated from the formation of an inclusion compound. Thus, the endothermic peak at 135 °C in this study was considered to correspond to the formation of an inclusion compound as well. The sample which was heated up to 220 °C (Fig. 5, curve e) showed the same powder X-ray diffraction pattern as intact DM β CD crystal (Fig. 5, curve f). The IR spectrum (Fig. 6, curve e) demonstrated that the NO₂ stretching peak intensity was decreased as compared with the sample heated at a lower temperature (Fig. 6, curve d). All of these results indicated that the endothermic peak at 195 °C was considered to represent the elimination of *p*-NP from the inclusion compound. From the DSC curve of the sealed-heated sample (Fig. 4, curve d), an endothermic peak at 135 °C was not observed, while the endothermic peak at 195 °C was clearly observed. This indicated that the sealed-heated sample was a complex in which *p*-NP molecules had already been included within the cavities of the DM β CD molecules. The peak at 195 °C simply explained the elimination of *p*-NP from the inclusion compound.

The endothermic peak which was found in the DSC thermogram of the slow evaporation complex (Fig. 4, curve e) was also investigated by a heating experiment in an aluminum cell (data not shown). The powder X-ray diffraction pattern of the slow evaporation complex heated to 180 °C was identical to that of the sealed-heated sample. The powder X-ray diffraction pattern of the slow

Fig. 4. DSC Curves of *p*-NP-DM β CD Systems

a) *p*-NP, b) DM β CD, c) physical mixture (mixing molar ratio 1:1), d) sealed-heated sample (heated at 100 °C for 24 h), e) complex prepared by slow evaporation of aqueous solution.

Fig. 5. Powder X-Ray Diffraction Patterns and DSC Curve of the Mixture of *p*-NP and DM β CD

a) Physical mixture (mixing molar ratio 1:1), b) heated to 80 °C in aluminum sealed cell, c) sealed-heated sample (heated at 100 °C for 24 h), d) heated to 190 °C in aluminum sealed cell, e) heated to 220 °C in aluminum sealed cell, f) DM β CD.

Fig. 6. Infrared Spectra of the Mixture of *p*-NP and DM β CD

a) Physical mixture (mixing molar ratio 1:1), b) heated to 80°C in aluminum sealed cell, c) sealed-heated sample (heated at 100°C for 24 h), d) heated to 190°C in aluminum sealed cell, e) heated to 220°C in aluminum sealed cell.

evaporation complex heated to 220°C was identical to that of DM β CD crystals alone. So, it may be concluded that the endothermic peak at 145°C was considered to be related to the formation of an inclusion compound, and the endothermic peak at 195°C resulted from the elimination of *p*-NP from the inclusion compound.

Recently, Kawashima *et al.* reported an inclusion compound formation of a new kind between Naphthalene and DM β CD by a sealed-heating method,¹²⁾ and they also discussed a difference in the drug elimination between inclusion compounds. The sublimation behavior of *p*-NP from the physical mixture, the sealed-heated sample, and the slow evaporation complex under reduced pressure at 100°C was investigated. Figure 7 shows the changes in *p*-NP contents under reduced pressure at 100°C. The physical mixture and the slow evaporation complex showed faster sublimation as compared with the sealed-heated sample. After 10 h of sublimation treatment, the physical mixture and the slow evaporation complex showed a lower combining molar ratio of 0.23 and 0.30, respectively, while the sealed-heated sample showed a high combining molar ratio of 0.79. As all samples had similar high crystallinity, the differences in sublimation behavior are likely related to the molecular state of *p*-NP in each preparation. From the slow evaporation complex in which the *p*-NP molecules were located in the intermolecular space of DM β CD and the physical mixture in which *p*-NP

Fig. 7. Sublimation of *p*-NP from Various Preparations by Heat Treatment at 100°C and Reduced Pressure

○, Sealed-heated sample (heated at 100°C for 24 h); □, physical mixture (mixing molar ratio 1:1); △, complex prepared by slow evaporation of aqueous solution.

molecules existed separately from DM β CD, sublimation took place more easily than in the sealed-heated sample. Therefore, the sealed-heated sample was considered to be one in which the *p*-NP molecules are located in the cavity of DM β CD molecules.

From all of the results mentioned above, the sealed-heated sample was estimated to be a different complex from the slow evaporation complex, and the sealed-heated sample might be an inclusion compound in which the *p*-NP molecules are located in the cavity of DM β CD molecules.

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References

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- 2) Nakai Y., Yamamoto K., Terada K., Watanabe D., *Chem. Pharm. Bull.*, **35**, 4609–4615 (1987).
- 3) Nakai Y., Yamamoto K., Terada K., Oguchi T., Saitou H., Watanabe D., *Chem. Pharm. Bull.*, **37**, 1055–1058 (1989).
- 4) Nakai Y., Yamamoto K., Oguchi T., Yonemochi E., Hanawa T., *Chem. Pharm. Bull.*, **38**, 1345–1348 (1990).
- 5) Nakai Y., Yamamoto K., Oguchi T., Yonemochi E., Hanawa T., *Chem. Pharm. Bull.*, **39**, 1532–1535 (1991).
- 6) Nakai Y., Yamamoto K., Terada K., Oguchi T., Nakamura G., *Yakugaku Zasshi*, **107**, 802–807 (1987).
- 7) Harata K., *Bull. Chem. Soc. Jpn.*, **61**, 1939–1944 (1988).
- 8) Harata K., *Chem. Lett.*, 1641–1644 (1984).
- 9) Hanawa T., Nakai Y., Yonemochi E., Oguchi T., Yamamoto K., *Netsu Sokutei*, **22**, 11–15 (1995).
- 10) Coppens P., Schmidt G. M. J., *Acta Crystallogr.*, **18**, 62–67 (1965).
- 11) Coppens P., Schmidt G. M. J., *Acta Crystallogr.*, **18**, 654–663 (1965).
- 12) Kawashima H., Yonemochi E., Oguchi T., Yamamoto K., *J. Chem. Soc., Faraday Trans.*, **90**, 3117–3119 (1994).