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Effect of photo-dimerization of coumarins on their interaction with polymeric β-cyclodextrin

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Abstract−Coumarin and 7-hydroxycoumarin (7HC) were photo-dimerized in an aqueous solution of polymeric β-cyclodextrin (PβCD) to investigate the effect of the photo-dimerization on the viscosity of PβCD solution. PβCD was prepared by cross-linking β-cyclodextrin (βCD) using epichlorohydrin. The content of βCD residues in PβCD, determined by a colorimetric method using phenolphthalein as an indicator, was 28.8 wt%. The solubility of coumarin increased with increasing concentration of PβCD, because the benzene ring of coumarin was included in the hydrophobic cavity of βCD residue. PβCD also increased the solubility of 7HC, but the solubilizing effect on 7HC was much less than that on coumarin, possibly because the hydroxy group on the benzene ring was likely to suppress the inclusion in the cavity of βCD. And, the photo-dimerization degree of 7HC in an aqueous solution of PβCD was about half than that of coumarin, possibly because the vinyl ether of pyrone group of 7HC could be concealed in the cavity of βCD. The photo-dimerization of coumarin in an aqueous solution of PβCD could significantly increase the viscosity of the solution. One coumarin dimer would hydrophobically interact with two βCD residues so it can act as a cross-linker for PβCD.

Key words: Coumarin, Polymeric β-Cyclodextrin, Photo-dimerization, Viscosity

INTRODUCTION

Photo-responsive vehicles, which release their contents in response to light irradiation, have been developed for decades for the on-demand release of drugs. One of the extensively studied vehicles is a coumarin-based photo-responsive one due to its unique photo-reactive property. Coumarin (1,2-benzopyrone) and its derivatives could become dimers through the formation of cyclobutane bridge and the dimers can be cleaved to monomers upon the irradiation of UV light at 254 nm [1,2]. Based on the photo-dimerization and the photo-de-dimerization property, a few kinds of photo-responsive carries were developed [3-5]. An amphiphilic block copolymer having coumarin in its hydrophobic block was self-assembled into micelles in an aqueous phase, and the cores of the micelles were cross-linked by the photo-dimerization of coumarin residues [6]. The promoted release of a hydrophobic compound was observed from the micelles under the irradiation of UV light at 254 nm. The photo de-dimerization was reported to be responsible for a promoted release. A photo-responsive nanogel was prepared using a thermo-sensitive block copolymer having coumarin in its thermo-sensitive block [7]. Micelles were formed in an aqueous solution due to the hydrophobic interaction of the thermo-sensitive blocks when the temperature of the solution was raised up to a temperature higher than the lower critical temperature (LCST). The micelle became nanogels when coumarins in the micelle core were photo-dimerized and then the micelle solution was cooled down to a temperature lower than LCST. The nanogel was sensitive to the UV light irradiation in terms of the size change and the release of their contents. Based on the same principle, a photo-responsive polymer vesicle was prepared using another thermo-sensitive block copolymer containing coumarin [8].

Recently, coumarin and its derivatives were reported to form inclusion complexes with cyclodextrins (CDs), and they became dimers even in the presence of CDs [9]. In this study, the effects of photo-dimerizations of coumarin and 7-hydroxycoumarin on their interaction with polymeric β-cyclodextrin (PβCD) were investigated by measuring the viscosities of aqueous mixture solutions of couamrin/PβCD and 7-hydroxycoumarin/PβCD. The viscosities of the mixture solutions, before and after the irradiation of λ at 365 nm, were measured on a viscometer with varying temperatures. First, PβCD was prepared by reacting βCD and epichlorohydrin in a strong alkalic condition. And then, each of coumarin and 7-hydroxycoumarin was dissolved in the aqueous solution of PβCD. The mixture solutions were photo-treated under the irradiation of UV light at 365 nm in order to dimerize coumarin and 7-hydroxycoumarin. Our idea is that the dimers formed by the photo-treatment will act as a cross linker for PβCD because one dimer could interact with two βCD residues. Therefore, the dimerization could have a significant effect on the viscosities of the mixture solutions (Fig. 1).

MATERIALS AND METHODS

1. Materials

β-Cyclodextrin (βCD, M.W. 1135) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), epichlorohydrin (EPI, M.W. 92.53) and 7-hydroxycoumarin (7HC, M.W. 162.14) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA), coumarin (M.W. 146.14) was obtained from TCI (Tokyo, Japan), water was

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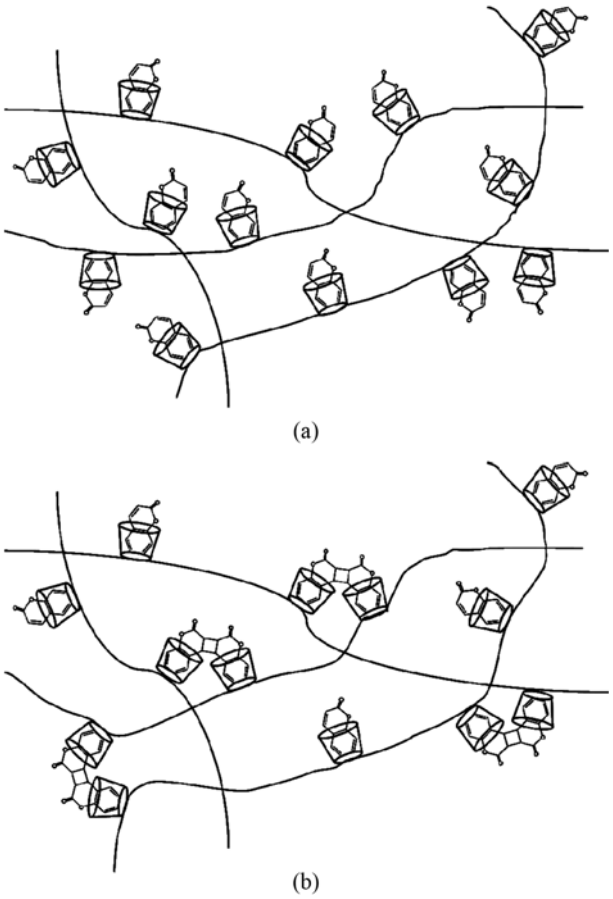

Fig. 1. A possible interaction between coumarin and PβCDs. Benzene ring of coumarin can be included in the cavity of βCD residue in PβCD (a). Upon the irradiation of λ at 365 nm, the vinyl ethers of pyrone react with each other to form a cyclobutane bridge, leading to cross-linking of PβCDs (b).

doubly distilled in a Milli-Q water purification system (Millipore Corp.) until the resistivity was 18 MΩ/cm. All other reagents were of analytical grade.

2. Preparation of PβCD

βCD polymer was prepared by the method reported in a previous work [10]. 5 g of βCD was put into 8 ml of NaOH aqueous solution (30%, w/w) contained in a 50 ml-beaker, and then it was gently stirred overnight at room temperature. EPI (a chemical linker for βCDs) was added to βCD solution in a dropwise manner so that the molar ratio of βCD to EPI was 1 : 10. The polymerization was done after 3 hr and 10 min around 30 ℃, and it was stopped upon the addition of 30 ml of acetone. After acetone was removed, 6 N HCl was added to the reaction mixture until the value of pH reached to 12, and then it was stood overnight in an oven kept at 50 ℃. After the reaction mixture was cooled to room temperature, it was neutralized using 6N HCl. For purification, the reaction mixture was dialyzed using a dialysis bag (MWCO 1,000, Spectra/Por, Cole-Parmer, USA) against 1,000 ml of distilled water for 72 hr with six-time exchanges of water. White precipitate was obtained by mixing the dialyzed solution with 1,000 ml of acetone, and they were filtered through a filter paper (24.0 cm cycles, Whatman, England). The filtrate was dried in a vacuum oven at 50 ℃.

3. ^{13}C NMR Spectroscopy of PβCD

PβCD was dissolved in D_2O and the ^{13}C NMR spectrum was taken on a Bruker Avance 400 spectrometer (Karlsruhe, Germany, located in the Central Laboratory of Kangwon National University.).

4. Determination of βCD Contents in PβCD

The content of βCD residues in PβCD was determined using a method described elsewhere [11]. 2 ml of phenolphthalein solution (0.373 mM, pH 11.0), 2 ml of sodium carbonate solution (0.028 M, pH 11.0) and various amounts of βCD solution (0.25 ml-4 ml, 10 mg/ml, pH 11.0) were mixed together and the volume of the mixture solution was made up to 25 ml using distilled water (pre-adjusted to pH 11.0). The calibration curve was set up by measuring the absorbance of the mixture solution at 550 nm on a UV spectrophotometer (6505 UV/Vis. Spectrophotometer, JENWAY, U.K.). To determine the contents of βCD residues in PβCD, 4 ml of PβCD solution (10 mg/ml, pH 11.0) was employed in the same procedure as used for the set-up of the calibration curve.

5. Interactions of Coumarin and 7-Hydroxycoumarin with β-Cyclodextrin Residue of PβCD

To investigate the interaction of coumarin and 7HC with βCD residue of PβCD, their solubilities in aqueous solutions of PβCD were determined. PβCD was dissolved in distilled water so that the concentrations were 1.5 wt%, 3 wt%, 6 wt% and 12 wt% (corresponding to 0.0038 M, 0.0076 M, 0.0152 M and 0.0305 M of βCD residue). And then, excess amounts of coumarin and 7HC were put into PβCD solutions contained in 15 ml-conical tubes, respectively. The conical tubes were capped and the contents were agitated overnight on a roller mixer (205RM, Hwashin Technology Co., Korea). Undissolved coumarin and 7HC were removed by filtration using a syringe filter (0.45 μm, Acrodisc). Coumarin and 7HC in the filtrates were quantified by measuring the absorbance at 280 nm and 324 nm, respectively, on a UV/Vis spectrophotometer (6505 UV/Vis. Spectrophotometer, JENWAY, U.K.).

6. FTIR Spectroscopy

The FT-IR spectra of PβCD, coumarin-included PβCD, and 7HC-included PβCD were taken in KBr pellet on a Fourier transformed infrared spectrophotometer (FT-IR, FT-3000-Excalibur, Varian Inc., CA, USA).

7. Dimerization Degree of Coumarin and 7-Hydroxycoumarin in PβCD Solution under Cyclic UV Irradiations

PβCD was dissolved in distilled water so that the concentration was 20 wt%. Each of coumarin and 7HC was dissolved in the PβCD solution so that the concentrations were 1.43 wt% and 0.81 wt%, respectively. The cyclic dimerizations and de-dimerizations of coumarin and 7HC in PβCD solutions were investigated by cyclic UV irradiations, the UV light at 365 nm (400 W) for 40 min and 254 nm (6 W) for 20 min. The degree of dimerization was determined as follows [4]:

$$\text{Dimerization (\%)} = (1 - A_t/A_0) \times 100\%$$

Where, A_0 was the absorbance before irradiating upon the UV light, and A_t was the absorbance after irradiating upon the UV light for a certain period. The absorbance was measured at 280 nm for coumarin and at 324 nm for 7HC.

8. Effects of Dimerizations of Coumarin and 7-Hydroxycoumarin on Viscosity of PβCD Solution

PβCD was dissolved in distilled water so that the concentration

was 20 wt%. Each of coumarin and 7HC was dissolved in the PβCD solution so that the concentration was 1.43 wt% and 0.81 wt%, respectively. PβCD/coumarin solution and PβCD/7HC solution were subjected to the irradiation of λ at 365 nm (400 W) for 3 hr for the dimerizations of coumarin and 7HC. The viscosities of solutions, before and after the irradiation, were measured on a viscometer (LV, Brookfield, USA) with varying temperatures.

RESULTS AND DISCUSSION

1. ^{13}C NMR Spectroscopy of PβCD

Fig. 2 shows the ^{13}C NMR spectrum of PβCD. The carbons of βCD residue were found at 101.0 ppm, 81.2 ppm, 73.5 ppm, 72.5 ppm, 69.3 ppm, and 60.7 ppm. Carbons on the primary face were found at 60.7 ppm, and substituted carbons on the primary face were observed at 69.3 ppm. Carbons on the secondary face were found at 73.5 ppm and 72.5 ppm, and substituted carbons on the secondary face were scarcely observed. Therefore, it is believed that the primary face of βCD is the major site for the substitution reaction. It was reported that the reactivity of the primary face hydroxyl groups was much higher than that of the secondary face hydroxyl groups. On the other hand, Carbons of EPI residues were found at 70.8 ppm and 63.0 ppm. The ^{13}C NMR signals obtained in present work accorded well with the signals reported in a previous report [12].

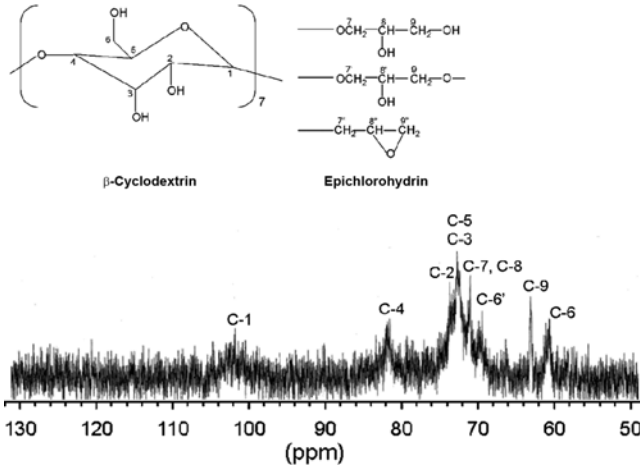

Fig. 2. ^{13}C NMR spectrum of PβCD.

2. Determination of βCD Contents in PβCD

Fig. 3 shows the calibration curve for the determination of βCD contents in PβCD. The equation was expressed as $Y=-0.8059\,X-1.2254$ ($R^2=0.9997$), where Y was the logarithmic value of absorbance at 550 nm and X was the logarithmic value of βCD concentration in mg/ml. The absorbance of the solution was inversely proportional to the concentration of βCD. Phenolphthalein forms inclusion complexes with βCD in an aqueous solution so the color intensity of the solution will decrease with increasing amount of βCD due to the sequestering effect [13,14]. Using the calibration curve and the absorbance of PβCD solution, the content of βCD residues in PβCD was calculated to be about 28.8 wt%. The content of βCD in the reaction mixture of βCD and EPI was 55.1 wt%. The difference indicates that the reactivity of βCD was less than EPI.

3. Interactions of Coumarin and 7-Hydroxycoumarin with β-Cyclodextrin Residue of PβCD

Fig. 4 shows the solubilities of coumarin and 7HC in PβCD solutions. The solubility of coumarin was proportional to the concentration of PβCD. This would be ascribed to the hydrophobic interaction of the aromatic rings of coumarin with the cavities of βCD residues of PβCD. Owing to the hydrophobic cavities, CDs can solubilize various kinds of hydrophobic compounds of comparable size to form inclusion complexes [15,16]. The equation for the solubility of coumarin in PβCD solutions is expressed as $Y=0.0201\,X+0.1443$ ($R^2=0.9988$), where Y is the concentration of coumarin in

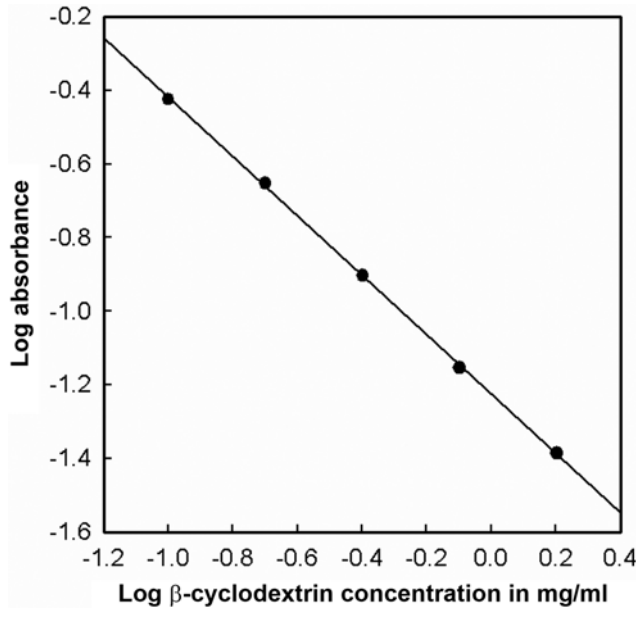

Fig. 3. Calibration curve for determination of βCD contents in PβCD.

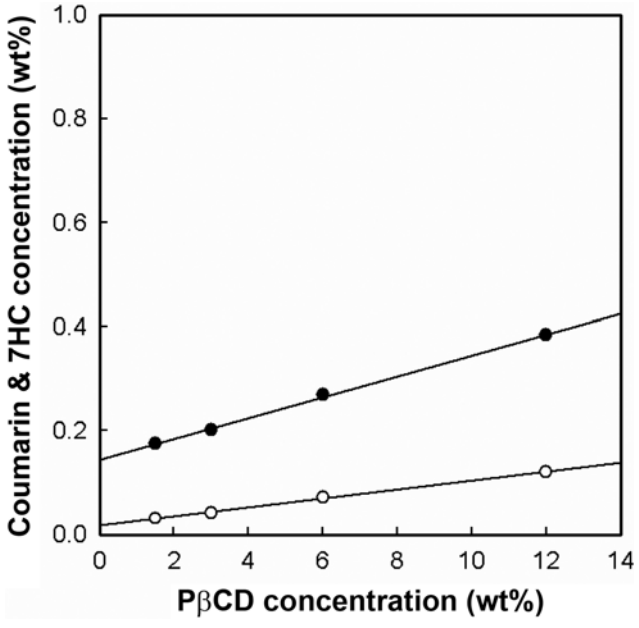

Fig. 4. Solubility of coumarin (●) and 7HC (○) in PβCD solution.

% (w/w) and X is the concentration of PβCD in % (w/w). Based on the content of βCD residues in PβCD was 28.8 wt%, the equation could be converted to Y=0.5417X+0.0010, where Y is the concentration of coumarin in M and X is the concentration of βCD residues of PβCD in M. The equation is comparable to the equation for the solubility of coumarin in (2-Hydroxypropyl)-β-cyclodextrin (HPβCD) solution (Y=0.5737X+0.0127) [9]. It means that the solubility of coumarin depends mainly on the number of βCD, whether it exists in an aqueous solution as monomeric form or polymeric form. The solubility of 7HC also increased with the increasing concentration of PβCD. The equation for the solubility in PβCD solutions is expressed as Y=0.0086 X+0.0181 (R^2=0.9978), where Y is the concentration of 7HC in % (w/w) and X is the concentration of PβCD in % (w/w). By taking advantage of the fact that the content of βCD residues in PβCD was 28.8 wt%, the equation could be converted to Y=0.2081 X+0.0001, where Y is the concentration of 7HC in M and X is the concentration of βCD residues of PβCD in M. The equation is comparable to the equation for the solubility of 7HC in HPβCD solution (Y=0.2175X+0.0014) [9].

4. FTIR Spectroscopy

Fig. 5 shows the FT-IR spectra of PβCD, coumarin-included PβCD, and 7HC-included PβCD. In the spectrum of PβCD, the peak at 756 cm^{-1} was the signal of C-H, the strong peak at 1,027 cm^{-1} was the signal of C-C, the peak at 1,078 cm^{-1} on the shoulder of the strong peak was the signal of C-O, the peak at 1,363 cm^{-1} was the signal of CH_2, the peak at 2,923 cm^{-1} was the signal of C-H, and the broad peak centered at 3,371 cm^{-1} was the signal of OH. In the spectrum of coumarin-included PβCD, the C=O signal and the C-C signal of coumarin were observed at 1,710 cm^{-1} and 1,607 cm^{-1}, respectively, together with the characteristic peaks of PβCD. Thus, coumarin was believed to be included in the cavity of βCD residues of PβCD. In the spectrum of 7HC-included PβCD, the C=O signal and the C-C signal of 7HC were observed at 1,706 cm^{-1} and 1,613 cm^{-1}, respectively, together with the characteristic peaks of PβCD, indicating that 7HC was successfully included in the cavity of βCD residues of PβCD.

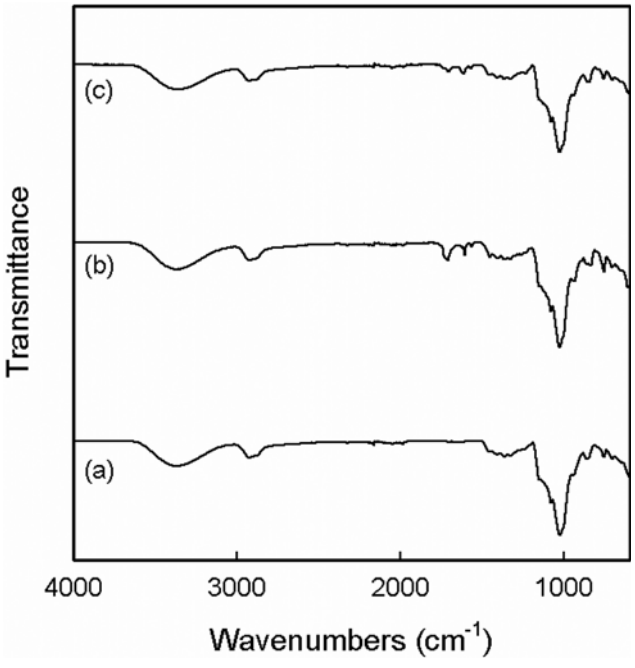

Fig. 5. FT-IR spectra of PβCD (a), coumarin-included PβCD (b), and 7HC-included PβCD (c).

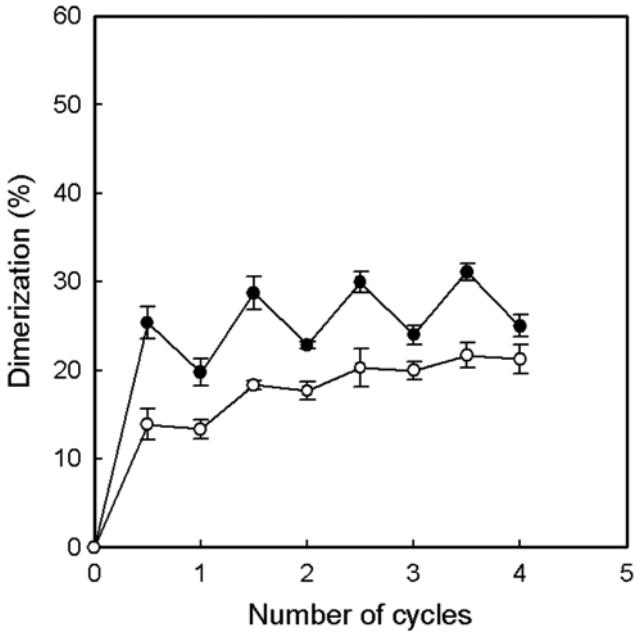

Fig. 6. Cyclic dimerization and de-dimerization of coumarin (●) and 7HC (○) in PβCD solution (n=3).

5. Dimerization Degree of Coumarin and 7-Hydroxycoumarin in PβCD Solution under Cyclic UV Irradiations

Fig. 6 shows the cyclic dimerization and de-dimerization of coumarin and 7HC in PβCD solution. The dimerization degree of coumarin increased to 25.5% during the first 40 min-irradiation of λ at 365 nm. The dimerization degree decreased to 20% by a subsequent 20 min-irradiation of λ at 254 nm. Coumarins become dimers through the formation of cyclobutane bridges under the irradiation of λ at 365 nm, and the dimers can be cleaved under the irradiation of λ at 254 nm [1,2]. Subsequent cyclic irradiation led to a periodic increase and decrease in the dimerization degree. In a previous work, there was no significant effect of HPβCD on the photo-dimerization of coumarin [9]. The benzene ring of coumarin was likely to be included in the hydrophobic cavity of HPβCD, and the double bond of the vinyl ester of coumarin could be exposed to the outside of the cavity [9]. This might account for why the cyclic dimerization and de-dimerization of coumarin could take place even in the presence of PβCD.

On the other hand, the dimerization degree of 7HC increased to 13.5% by the first 40 min-irradiation of λ at 365 nm. The dimerization degree of 7HC was much lower than that of coumarin obtained under the same irradiation condition. In a previous work, the photo-dimerization of 7HC was suppressed by HPβCD [9]. The hydrophobic part (the opposing part to -OH, -O-, and C=O) of 7HC would fit well with the hydrophobic cavity of βCD residue of PβCD due to their comparable size, so a stable complex would be formed owing to a strong hydrophobic interaction. Under this condition, the double bond of the vinyl ester of 7HC could be concealed by the cavity of βCD residue. This could explain why the photo-dimer-

ization degree of 7HC in PβCD solution was lower than that of coumarin in the same solution. The dimerization degree of 7HC decreased from 13.5% to 12.5% by the first 20 min-irradiation of λ at 254 nm. Subsequent cyclic irradiation gave a rise to a periodic increase and decrease in the dimerization degree, but the photoreaction sensitivity was much less than that of coumarin. In the case of 7HC, the dimerization could be occurring due to the photo-reaction not only between double bonds but also between 7-hydroxy group and double bond [17]. Because the chemical bonding formed by the photo reaction between 7-hydroxy group and double bond was hardly photo-cleaved, the photo-cleavage under the irradiation of λ at 254 nm would not be as much as that of coumarin.

6. Effects of Dimerizations of Coumarin and 7-Hydroxycoumarin on Viscosity of PβCD Solution

Fig. 7 shows the viscosities of PβCD solution, PβCD solution containing 7HC, and PβCD solution containing coumarin at temperature of 25 °C, 30 °C, 37 °C, and 42 °C. The viscosities were around 20 cP, and the value slightly decreased while increasing temperature. There was no significant difference in the viscosities among these three kinds of solutions. It means that neither 7HC nor coumarin had any significant effect on the viscosity of PβCD solution. Coumarin and 7HC interacted with βCD residue in PβCD and their solubilities were proportional to the concentration of PβCD (Fig. 4). The benzene ring of coumarin was likely to be included in the hydrophobic cavity of βCD residue in PβCD, and the hydrophilic moiety (pyrone group) would face toward bulk aqueous phase. Due to the hydrophilicity, the pyrone group would hardly interact with another cavity of βCD residue, so it could scarcely act as a cross-linker for PβCD. Similarly, the hydrophobic part (the opposing part to -OH, -O-, and C=O) of 7HC could fit well with the hydrophobic cavity of βCD residue in PβCD. However, the hydrophilic moiety of 7HC would face outward of the cavity and hardly interact with another cavity, failing in acting as a cross-linker. This may explain why neither coumarin nor 7HC had any significant effect on the viscosity of PβCD solution.

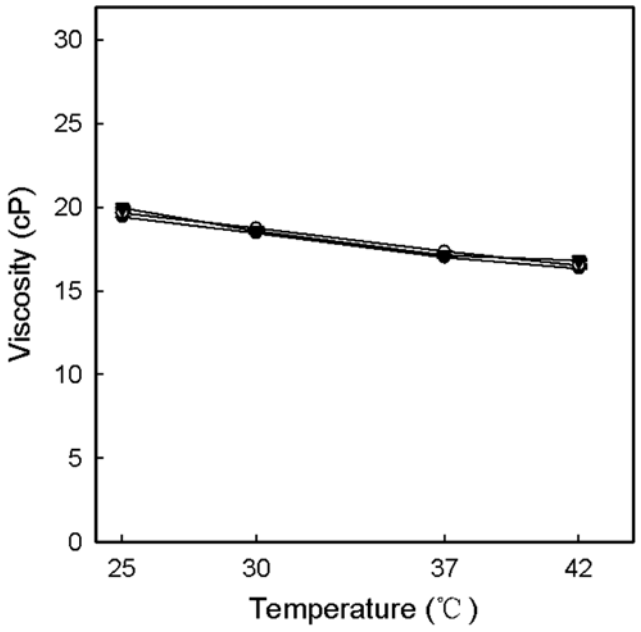

Fig. 7. Viscosities of PβCD solution (●), PβCD solution containing coumarin (▼), and PβCD solution containing 7HC (○) at temperature of 25 °C, 30 °C, 37 °C, and 42 °C (n=3).

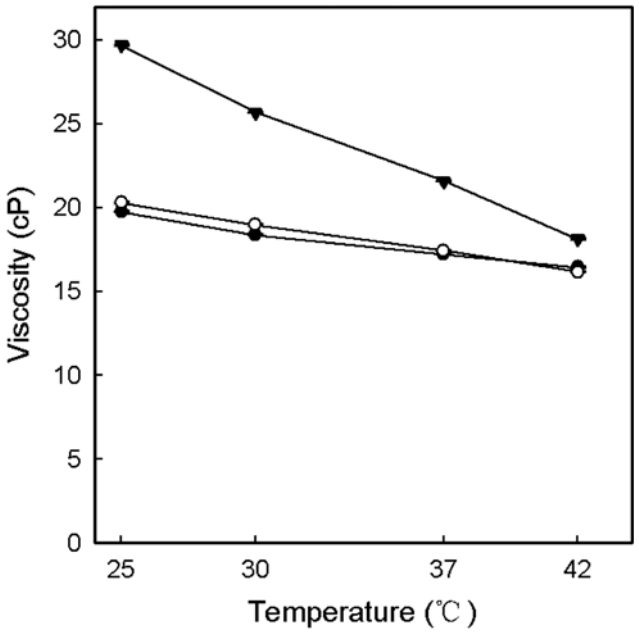

Fig. 8. Viscosities of PβCD solution (●), PβCD solution containing coumarin (▼), and PβCD solution containing 7HC (○) at temperature of 25 °C, 30 °C, 37 °C, and 42 °C (n=3), after the solutions were subjected to the irradiation of λ at 365 nm (400 W).

Fig. 8 shows the viscosities of PβCD solution, PβCD solution containing coumarin, and PβCD solution containing 7HC at temperature of 25 °C, 30 °C, 37 °C, and 42 °C, after the solutions were subjected to the irradiation of UV light at 365 nm (400 W). No significant change in the viscosity of PβCD solution was observed. In fact, the only functional group in PβCD was hydroxyl group, which was not photo-reactive. However, the viscosity of PβCD with coumarin markedly increased upon the irradiation. Coumarin could interact with βCD residue of PβCD (Fig. 4) and it could photo-dimerize in the presence of PβCD (Fig. 6). In this circumstance, the irradiation of UV light at 365 nm would cross-link PβCD since a dimer produced by the irradiation can interact with two βCD residues. A possible interaction between the dimer and PβCD is illustrated in Fig. 1. On the other hand, the viscosity of PβCD solution containing 7HC was also little affected by the irradiation of UV light at 365 nm. 7HC could also interact with βCD residue of PβCD (Fig. 4) and it could also photo-dimerize in the presence of PβCD (Fig. 6). However, as described previously, both the interaction of 7HC with βCD residue and the dimerization degree of 7HC in the presence of PβCD were much less than those of coumarin. As a result, the cross-linking degree of PβCD, obtained by the photo-dimerization of 7HC, would be insufficient to increase the viscosity of PβCD solution.

CONCLUSIONS

The solubility of coumarin in an aqueous solution was enhanced

by PβCD, possibly due to the inclusion complexation of the benzene ring with βCD residue. The solubility of 7HC was also booted-up by PβCD, but the solubility enhancement of 7HC was not as much as that of coumarin. The hydroxyl group on the benzene ring seemed to suppress the complexation of 7HC with βCD residue of PβCD. Coumarin and 7HC could be photodimerized even in an aqueous solution of PβCD, but the photodimerization degree of coumarin was almost double than that of 7HC. Due to the hydroxyl group, the vinyl ether of pyrone group of 7HC was likely to be sequestered by the cavity of βCD residue. Since one coumarin dimer would hydrophobically interact with two βCD residues and it could act as a cross-linker for PβCD, the photo-dimerization of coumarin in an aqueous solution of PβCD could significantly increase the viscosity of the solution. However, the viscosity of PβCD solution containing 7HC was also little affected by the photodimerization, possibly due to the shortage of the amount of dimer, and the lack of the hydrophobic interaction between the dimers and the βCD residues. PβCD solution containing coumarin could be used as photo-responsive vehicle for a drug delivery system.

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REFERENCES

1. S. R. Trenor, A. R. Shultz, B. J. Love and T. E. Long, *Chem. Rev.*, **104**, 3059 (2004).
2. C. A-Lorenzo, L. Bromberg and A. Concheiro, *Photochem. Photobiol.*, **85**, 848 (2009).
3. N. K. Mal, M. Fujiwara and Y. Tanaka, *Nature*, **421**, 350 (2003).
4. Q. Jin, X. Liu, G. Liu and J. Ji, *Polymer*, **51**, 1311 (2010).
5. J. Babin, M. Lepage and Y. Zhao, *Macromolecules*, **41**, 1246 (2008).
6. J. Jiang, B. Qi, M. Lepage and Y. Zhao, *Macromolecules*, **40**, 790 (2007).
7. J. He, X. Tong and Y. Zhao, *Macromolecules*, **42**, 4845 (2009).
8. X. Jiang, R. Wang, Y. Ren and J. Yin, *Langmuir*, **25**(17), 9629 (2009).
9. J. Dai and J.-C. Kim, *Effects of hydroxypropyl cyclodextrins on photo-reactions of coumarins*, *J. Disper. Sci. Technol.*, In press.
10. E. Renard, A. Dertani, G. Volet and B. Sebille, *Eur. Polym. J.*, **33**, 49 (1997).
11. C. Basappa, P. Rao, D. N. Rao and S. Divakar, *Int. J. Food. Sci. Technol.*, **33**, 517 (1998).
12. X. Yang and J.-C. Kim, *Int. J. Pharamaceut.*, **388**, 58 (2010).
13. S. Divakar and M. M. Maheswaran, *J. Sci. Ind. Res.*, **53**, 924 (1994).
14. W. Li, J. X. Zhou, L. T. Zhang, H. Corke and L. S. Yang, *Anal. Lett.*, **29**, 1201 (1996).
15. J. Szejtli, *Chem. Rev.*, **98**, 1743 (1998).
16. E. M. Martin Del Valle, *Process. Biochem.*, **39**, 1033 (2004).
17. Y. Chen and K.-H. Chen, *J. Polym. Sci. Pol. Chem.*, **35**, 613 (1997).