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Interaction between Cycloamylose and Various Drugs

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Abstract

Cycloamylose (CA), has a cyclic structure like cyclodextrin (CD), but has a very large number of molecules, and its physical properties are still unclear. The CA used in this study was supplied by Ezaki Glico Co., Ltd, and was a mixture (mean molecular weight 7720). Predonisolone, cholesterol, digoxin, digitoxin and nitroglycerin were chosen as guest molecules. We evaluated the interaction between CA and the guest molecules using the solubility method described by Higuchi and Connors. The concentration of each dissolved guest molecule was determined by HPLC. This solubility method was performed at a temperature of 5 °C. The phase solubility diagrams of drugs with CA showed type A or type B profiles. Cholesterol, digoxin, digitoxin and predonisolone formed a complex with CA, but nitroglycerin did not.

Introduction

Cycloamylose (CA) [1] is α-1,4 glucan, with the same ring structure as cyclodextrin, and the degree of polymerization is considered to be higher than 17. CA with such a large ring structure may have different properties from α-, β- and γ-CD. In this study, CA at degrees of polymerization 20–55 (mean molecular weight, 7720) was examined [2]. We investigated CA using phase solubility diagrams [3] with cholesterol, predonisolone, digitoxin, digoxin and nitroglycerin, and examined the stability of CA in acidic solution.

Materials and methods

Materials

Cycloamylose (CA) [1, 2] (mean molecular weight, 7720) was provided by Ezaki Glico Inc. Co., Ltd. (Osaka, Japan). α-, β- and γ-CD were provided by Nippon Shokuhin Kako Co., Ltd. (Tokyo, Japan). Nitroglycerin was provided by Nippon Kayaku Co., Ltd. (Tokyo, Japan). Digitoxin and digoxin were the Japanese pharmacopoeia standard. Prednisolone was biochemical grade from Wako Pure Chemical Industries Ltd. (Osaka, Japan). All other chemicals and solvents were of special grade from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Distilled water was used in all experiments.

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Stability study

10 mg each of α-, β- and γ-CD and CA was put into a 1-ml measuring flask, and measured up to 1 mL with 1 mol/L hydrochloric acid. The solutions were moved into screw cap test tubes, and stored in a water-bath at 50 °C after insertion of a stirring stick and capping. Samples were periodically collected from the solutions, and the remaining α-, β- and γ-CD and CA were quantified by HPLC. HPLC measurement conditions were as follows: Column, Capcell Pak NH_2 (Shiseido Co. Ltd., Tokyo, Japan) 5 μm, 4.6 i.d. $\times$ 50 mm; Mobile phase, 47% CH_3CN; Flow rate, 0.7 mL/min; Monitor, RI (ERC-7515B type, EAC Inc., Tokyo Japan).

Solubility Study

Prednisolone: 10 mg prednisolone (2.77×10^{-6} mol) was added to 1 mL of 0–4.5 $\times 10^{-3}$ mol/L CA, subjected to sonication (1000 mW) for 5 min, and shaken in a water-bath at 5 °C for 1 day. This sample solution was filtered using a disc filter (0.45 μm), and measured by HPLC. HPLC measurement conditions were as follows: Column, Inertsil ODS 5 μm, 4.6 i.d. $\times$ l50 mm; Mobile phase, Methanol : tetrahydrofuran : distilled water = 62 : 250 : 688; Flow rate: 1.0 mL/min; Detection wavelength, UV 245 nm.

Cholesterol: 2 mg cholesterol (2.77×10^{-6} mol) powder and CA powder was kneaded with several drops of distilled water in an agate mortar for 15 min, and then the mixture was dissolved in 1 ml of water giving a final CA concentration of 0–0.5 $\times 10^{-3}$ mol/L. This sample solution was shaken at 5 °C for 1 day, and centrifuged at 3000 rpm for 15 min. The supernatant was filtered using a disc filter (0.45 μm), and measured by HPLC. HPLC measurement condi-

tions were as follows: Column, Inertsil ODS 5 μm, 4.6 i.d. $\times$ 150 mm; Mobile phase, Methanol : distilled water = 98 : 2; Flow rate: 1.0 mL/min; Detection wavelength, UV 210 nm.

Digitoxin: 3 mg digitoxin (3.92×10^{-6} mol) was added to 1 mL of 0–7.8 $\times 10^{-2}$ mol/L CA, subjected to sonication (1000 mW) for 5 min, and shaken in a water-bath at 5 °C for 3 days. This sample solution was filtered using a disc filter (0.45 μm), and measured by HPLC. HPLC measurement conditions were followed; Column, Inertsil ODS 5 μm, 4.6 i.d. $\times$ 150 mm; Mobile phase, Methanol : distilled water = 3 : 1; Flow rate: 1.2 mL/min; Detection wavelength, UV 220 nm.

Digoxin: 3 mg digoxin (3.8×10^{-6} mol) was added to 1 mL of 0–5.3 $\times 10^{-3}$ mol/L CA, subjected to sonication (1000 mW) for 5 min, and shaken in a water-bath at 5 °C for 3 days. This sample solution was filtered using a disc filter (0.45 μm), and measured by HPLC. HPLC measurement conditions were as follows: Column, Inertsil ODS 5 μm, 4.6 i.d. $\times$ 150 mm; Mobile phase, Methanol : dil. Acetic acid (3 : 2500) = 4 : 3; Flow rate: 1.2 mL/min; Detection wavelength, UV 245 nm.

Nitroglycerin: 13 mg nitroglycerin (5.7×10^{-5} mol) was added to 1 mL of 0–2.1 $\times 10^{-2}$ mol/L CA, subjected to sonication (1000 mW) for 5 min, and shaken in water-bath at 5 °C for 3 days. This sample solution was filtered using a disc filter (0.45 μm), and measured by HPLC. HPLC measurement conditions were as follows: Column, Inertsil ODS 5 μm, 4.6 i.d. $\times$ 150 mm; Mobile phase, Methanol : distilled water = 6 : 4; Flow rate: 1.0 mL/min; Detection wavelength, UV 210 nm.

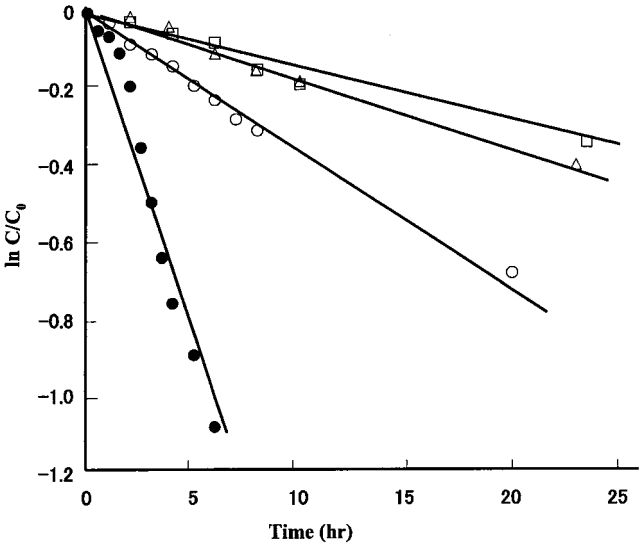

Figure 1. Decomposition of CA and CDs at 60 °C. ●: CA; □: α-CD; △: β-CD; ○: γ-CD.

Table 1. Degradation rate of CA and Cds in 1 mol/L HCl at 50 °C

	Rate k (h^{-1}	Half life (h)	R^2
α-CD	0.015	46.20	0.970
β-CD	0.017	46.53	0.985
γ-CD	0.035	19.74	0.992
CD	0.170	4.07	0.945

Results and discussion

Stability study

As shown in Figure 1, the decomposition of CA in 1 mol/L hydrochloric acid was analyzed as the first-order reaction. The decomposition of CA was more rapid than that of α-, β-, and γ-CD, which agreed with the study of Motohama et al. showing that the decomposition of macrocyclic CD was rapid [4]. As shown in Table I, the decomposition rate of CA was faster than that of α-, β-, and γ-CD. This decomposition rate was slower than that of isolated large-ring cyclodextrin, which is composed of 17 D-glucose units. We must not jump to conclusions without further experiments, but the reason might be that CA is a mixture of large-ring cyclodextrin, which is composed of more than 17 D-glucose units.

Solubility study

Prednisolone: As shown in Figure 2a, the phase solubility diagram of prednisolone an CA was of the A_L type. Previously, the solubility of prednisolone at 37 °C had been determined to be 6.3-fold higher in aqueous solutions containing α-CD (1.18%), 8.3 times higher in solution containing β-CD (1.06%), and 4.3 times higher solution containing γ-CD (8.4%) compared to solution containing no cyclodextrin [5]. This corresponds closely to our results. The solubility of prednisolone became 1.6-fold higher by adding 3% CA. The stability constant determined from the initial slope was 314 M^{-1} in α-CD, 12,500 M^{-1} in β-CD, 404 M^{-1} in γ-CD, and 433 M^{-1} in CA.

Cholesterol: As shown in Figure 2b, the phase solubility diagram of cholesterol and CA was of the B_S type. The solubility of cholesterol with CA was higher than that obtained with β-, and γ-CD. The stability constant determined from the initial slope was 1570 M^{-1} in β-CD, 558 M^{-1} in γ-CD, and 53,200 M^{-1} in CA

Digitoxin: As shown in Figure 2c, the phase solubility diagram of digitoxin and CA was the A_L type. The stability constant determined from the initial slope was 33,000 M^{-1} in β-CD (B_S type), 63,000 M^{-1} in γ-CD (B_S type), and 813.95 M^{-1} in CA (A_L type). Since Ueda et al. [6] reported that δ-CD showed an A_L type phase solubility diagram, this CA may have similar properties to those of δ-CD.

Digoxin: As shown in Figure 2d, the phase solubility diagram of digoxin and CA was the A_L type. The phase solubility diagram of digoxin and CD was the A_L type in α-CD, A_L type in β-CD and B_S type in γ-CD. The stability constant was 1110 M^{-1} in α-CD, 12,631 M^{-1} in β-CD, 1254 M^{-1} in γ-CD and 354 M^{-1} in CA.

Nitroglycerin: The solubility of nitroglycerin was not changed, indicating that there was no interaction between nitroglycerin and CA (Figure 2e). We reported that the stability constant was 154 M^{-1} in β-CD, 26.25 M^{-1} in β-CD polymer [7].

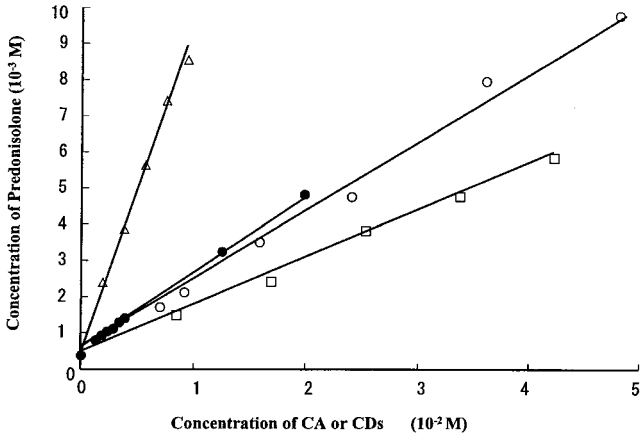

Figure 2a. Phase solubility diagrams of predonisolone-CA or predonisolone-CD system in water at 5 °C. ●: CA; □: α-CD; △: β-CD; ○: γ-CD.

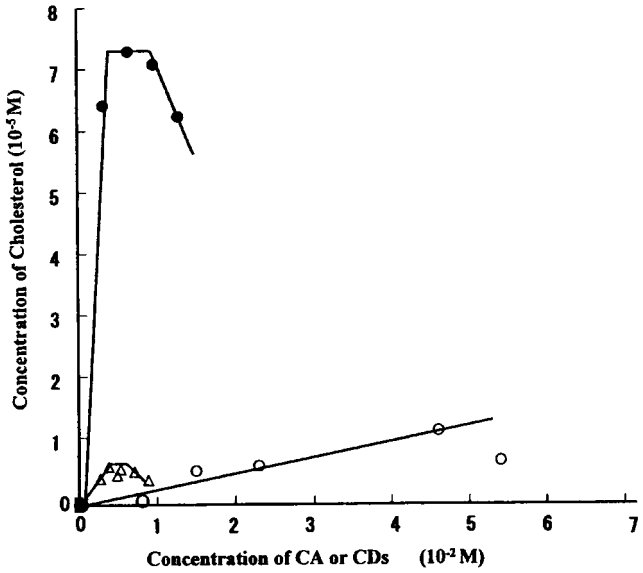

Figure 2b. Phase solubility diagrams of cholesterol-CA or cholesterol-CD system in water at 5 °C. ●: CA; △: β-CD; ○: γ-CD.

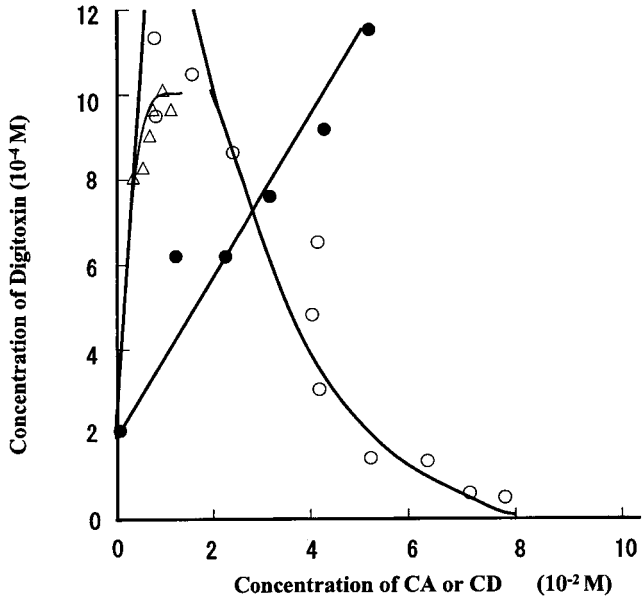

Figure 2c. Phase solubility diagrams of digitoxin-CA or digitoxin-CD system in water at 5 °C. ●: CA; △: β-CD; ○: γ-CD.

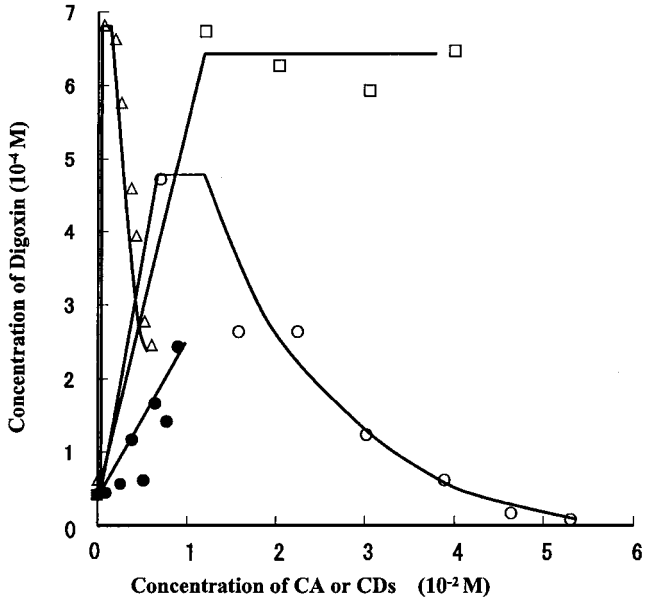

Figure 2d. Phase solubility diagrams of digoxin-CA or digoxin-CD system in water at 5 °C. ●: CA; □: α-CD; △: β-CD; ○: γ-CD.

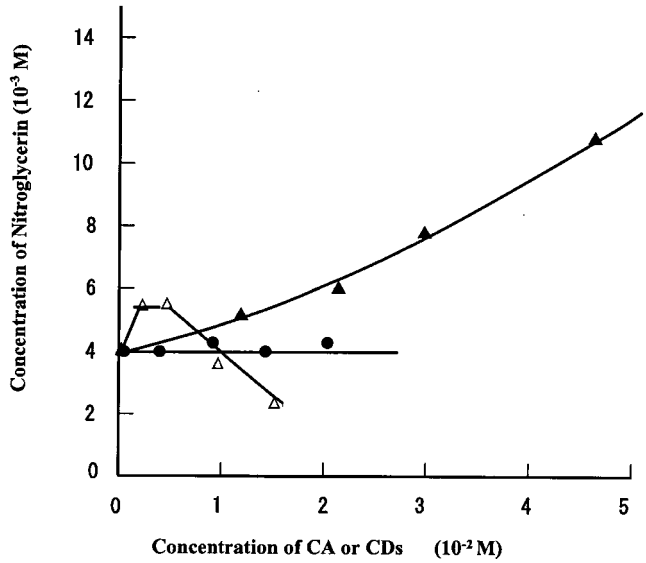

Figure 2e. Phase solubility diagrams of nitroglycerin-CA or nitroglycerin-CD system in water at 5 °C. ●: CA; △: β-CD; ▲: β-CD polymer.

Conclusions

Like other cyclomalto-oligosaccharides, CA was decomposed by acid. The decomposition rate of CA was faster than that of α-, β-, and γ-CD. However, the decomposition rate of CA was slower than that of isolated large-ring CD. Analysis of the phase solubility diagrams revealed that CA formed complexes with prednisolone, cholesterol, digitoxin and digoxin. The phase solubility diagrams of CA were the A or B types, like those of various CDs, and the diagram types were determined by guest molecules. CA as well as CDs may basically have inclusion ability with guest molecules. The state of inclusion appears to differ between CA and CD.

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