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Physicochemical Properties of Large-Ring Cyclodextrins (CD_{18}~CD_{21})

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Abstract

Cyclomaltooctadecaose (CD_{18}), cyclomaltononadecaose (CD_{19}), cyclomaltoeicosaose (CD_{20}) and cyclomaltoheneicosaose (CD_{21}) are cyclic oligosaccharides composed of 18, 19, 20 and 21 D-glucose units, respectively. This report describes the physicochemical properties of CD_{18}, CD_{19}, CD_{20} and CD_{21} in terms of aqueous solubility, surface tension, optical rotation and acid-catalyzed hydrolysis.

Introduction

Cyclodextrin (CD) is a cyclic oligosaccharide produced by cyclodextrin glucanotransferase (CGTase), and α-, β-, γ-CD and their derivatives have been well studied. With regard to large-ring CDs (LR-CDs) composed of more than 9 D-glucose units, we have reported a method for preparation and purification from a commercially available CD powder [1–4]. We characterized the physicochemical properties and inclusion complex formation abilities of CD_9 composed of 9 D-glucose units with several guest molecules [1, 5]. Furthermore, inclusion complex formation constants of CD_{10}, CD_{11}, CD_{12}, CD_{13}, CD_{14}, CD_{15}, CD_{16} and CD_{17}, composed of 10, 11, 12, 13, 14, 15, 16 and 17 D-glucose units, respectively, were determined by capillary zone electrophoresis [6, 7]. However, it is difficult to investigate the physicochemical properties and complexation abilities of LR-CDs with more than 10 D-glucose units in detail, because of the low yields of these CDs prepared using commercially available CD powder as the starting material. Recently, it has been reported that LR-CDs were preferentially produced in the initial stage of CGTase cyclization reaction and were subsequently converted into smaller CDs [8]. We have already prepared and purified CD_{10}, CD_{11}, CD_{12}, CD_{13}, CD_{14}, CD_{15}, CD_{16} and CD_{17}, from LR-CD mixture produced by the initial action of CGTase and elucidated their physicochemical properties [9]. In this study, CD_{18}, CD_{19}, CD_{20} and CD_{21}, composed of 18, 19, 20 and 21 D-glucose units, respectively, were purified from the LR-CD mixture. Their physicochemical properties, i.e., aqueous solubility, surface activity, optical rotation, and acid-catalyzed hydrolysis rate, were elucidated in comparison with those of conventional α-, β-, γ-CD and other LR-CDs (CD_9~CD_{17}).

Materials and methods

Materials

The production of LR-CD mixture by the initial action of CGTase was carried out as described previously [8]. α-CD was a gift from Nihon Shokuhin Kako Co., Ltd. (Tokyo, Japan). Other chemicals were obtained from commercial sources and were used without further purification. Milli-Q Water (Milford, MA, USA) was used in all experiments.

Purification of CD_{18}, CD_{19}, CD_{20} and CD_{21}

The purification procedure was almost the same as that reported previously [9]. However, purification with HPLC consisted of several steps using an ODS column and an NH2 column to save time and obtain a good yield. Figure 1 shows an outline of the purification of CD_{18}, CD_{19}, CD_{20} and CD_{21} by HPLC and their yields.

Identification of CD_{18}, CD_{19} and CD_{20}

Identification of the CD_{18}, CD_{19} and CD_{20} fractions obtained was carried out with HPLC and mass spectrometry. The chromatographic behaviors of the CD_{18}, CD_{19} and CD_{20} fractions obtained were compared with those of standard CDs on an ODS column and an NH2 column. The LR-CDs used as standards were the same samples that were isolated and purified previously by Endo *et al.* [3]. The elution times of the purified products on the two different columns agreed with those of the standard CDs. The purities of the CD_{18}, CD_{19} and CD_{20} fractions were >98% as determined by HPLC. The molecular weights of the CD_{18}, CD_{19} and CD_{20} fractions determined by FAB-MS agreed with the calculated values.

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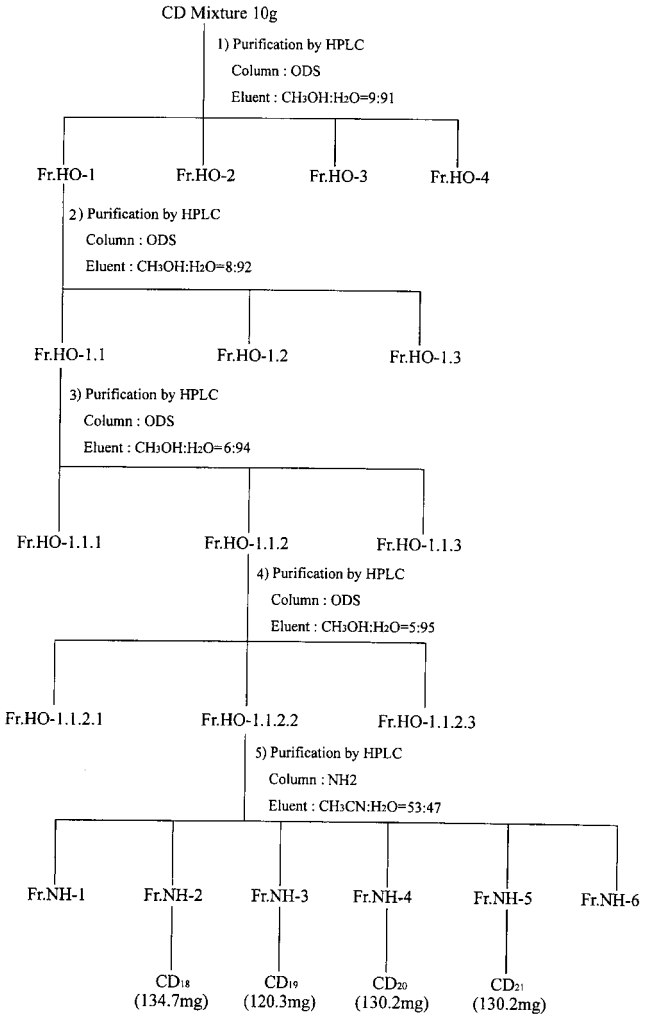

Figure 1. Purification of CD_{18}, CD_{19}, CD_{20} and CD_{21}.

Identification of CD_{21}

HPLC with an ODS column and an NH2 column demonstrated that the purity of the obtained CD_{21} fraction was >98%. Its molecular weight, as determined by FAB-MS, was m/z 3427.3 ($[M + Na]^+$). This value agreed with the calculated molecular weight of CD_{21}. The ^{13}C-NMR spectrum of the obtained CD_{21} had six clear single peaks.

Physicochemical properties of CDs

The solubilities of CDs were determined using the following methods. Water was carefully added to a glass vessel containing 100mg of CDs. The quantity of water varied progressively from 0.01 to 0.1 mL. The samples were vigorously shaken for periods of 1min at 10min intervals at 25 °C. The cycle was continued until the CDs dissolved completely. The total volume of water added was measured, and the saturated solubility was calculated. Surface tension measurements were taken on a Wilhelmy surface tensiometer. The glass vessels used were treated with 20% sulfuric acid before each measurement. Optical rotation measurements were taken on a polarimeter. The polarimeter was calibrated with sucrose solution before measurement. In the acid-catalyzed

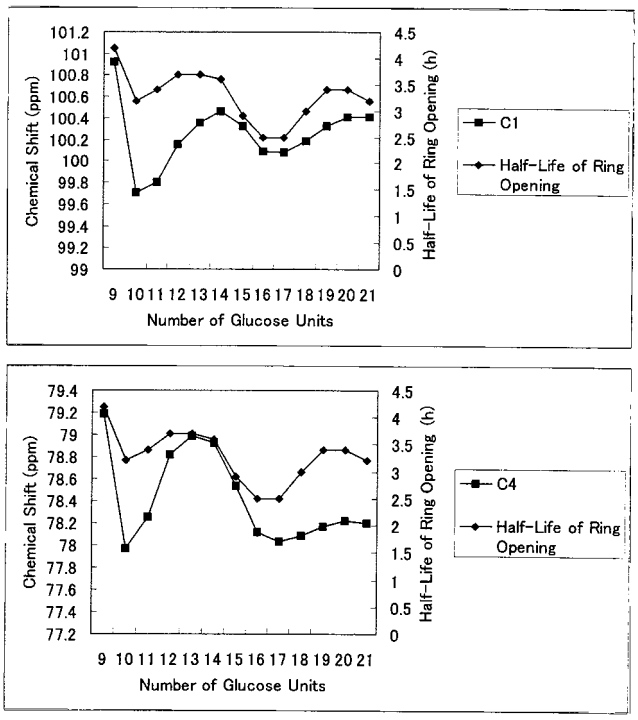

Figure 2. Relationship between half-life of ring opening and ^{13}C chemical shifts of large-ring CDs with number of glucose units.

hydrolysis, samples of 100 mg of CDs were dissolved in 5 mL of 1 M HCl, and the reaction solution was heated in a heating bath at 50 °C. Samples of the reaction solution were taken at appropriate intervals and neutralized by addition of 1 M NaOH. The samples were quantified by HPLC. The internal standard used was α-CD.

Results and discussion

Physicochemical properties of CD_{18}, CD_{19}, CD_{20} and CD_{21}

Table 1 lists some of the physicochemical properties of α-, β-, γ-CD and LR-CDs. The aqueous solubilities of CD_{18}, CD_{19}, CD_{20} and CD_{21} were greater than those of α-, β-, γ-CD, CD_9, CD_{10} and CD_{14}. CD_{18}, CD_{19}, CD_{20} and CD_{21} showed no surface activity. Based on this result, we assumed that the surface activity of LR-CDs composed of more than 22 D-glucose units does not change with increasing number of D-glucose units. The optical rotation increased in the order: α-CD < β-CD < γ-CD < CD_9 < $CD_{12} \approx CD_{13} \approx CD_{14} \approx CD_{20} \approx CD_{11} \approx CD_{17} \approx CD_{19} < CD_{15} \approx CD_{18} \approx CD_{16} \approx CD_{10} \approx CD_{21}$. There were no marked differences in specific rotation among LR-CDs (from $CD_{10} \sim CD_{21}$). The acid-catalyzed hydrolysis rates of CD_{18}, CD_{19}, CD_{20} and CD_{21} were faster than those of α-, β-, γ-CD and CD_9. There were no marked differences in the acid-catalyzed hydrolysis rate among LR-CDs (from $CD_{10} \sim CD_{21}$). The above results showed that the increases in simple disorder and decomposition points (α-1, 4 linked parts) of the intact rings with increasing number of D-glucose units did not have a marked effect influence on the breakdown of LR-CDs. The

Table 1. Physicochemical properties of CDs

	Glucose unit	Molecular weight	Aqueous[a] solubility (g/100 mL)	Surface[a] tension (mN/m)	Specific rotation $[\alpha]_D^{25}$	Half-life of[b] ring opening (h)
α-CD	6	973	14.5[c]	72	+147.8[d]	33[d]
β-CD	7	1135	1.85[c]	73	+161.1[d]	29[d]
γ-CD	8	1297	23.2[c]	73	+175.9[d]	15[d]
CD_9	9	1459	8.19[c]	73	+187.5[d]	42[d]
CD_{10}[d]	10	1621	2.82	72	+204.9	3.2[d]
CD_{11}[d]	11	1783	>150	72	+200.8	3.4
CD_{12}[d]	12	1946	>150	72	+197.3	3.7
CD_{13}[d]	13	2107	>150	72	+198.1	3.7
CD_{14}[d]	14	2270	2.30	73	+199.7	3.6
CD_{15}[d]	15	2432	>120	73	+203.9	2.9
CD_{16}[d]	16	2594	>120	73	+204.2	2.5
CD_{17}[d]	17	2756	>120	72	+201.0	2.5
CD_{18}	18	2919	>100	73	+204.0	3.0
CD_{19}	19	3081	>100	73	+201.0	3.4
CD_{20}	20	3243	>100	73	+199.7	3.4
CD_{21}	21	3405	>100	73	+205.3	3.2

[a] Observed at 25 °C.
[b] In 1 mol/L HCl at 50 °C.
[c] I. Miyazawa, H. Ueda, H. Nagase, T. Endo, S. Kobayashi, and T. Nagal: *Eur. J. Pharm. Sci.* **3**, 153 (1995).
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hydrolysis rates of CD_{18}, CD_{19}, CD_{20} and CD_{21} were almost the same as those of CD_{10} and CD_{11}. Saenger *et al*. proposed that the excessive steric strain of the CD ring was relieved by band flip structures [10]. The small differences in LR-CD hydrolysis rates may be explained by taking the band flips and ^{13}C-NMR chemical shifts into account. As shown in Figure 2, the half-lives of ring opening paralleled ^{13}C chemical shifts of C1 and C4 with number of D-glucose units [11]. We assumed that the signals of C1 and C4 were shifted upfield with the strength of steric strain, and the steric strain of the CD ring of CD_{18}, CD_{19}, CD_{20} and CD_{21} was gradually relieved in comparison with CD_{15}, CD_{16} and CD_{17}. The hydrolysis rates of CD_{18}, CD_{19}, CD_{20} and CD_{21} were slower than those of CD_{15}, CD_{16} and CD_{17} despite the increase in number of decomposition sites with increasing number of D-glucose units. However, this hypothesis should be confirmed by further experiments using LR-CDs composed of more than 22 D-glucose units.

Acknowledgments

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