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Physicochemical Properties and Complex Formation Abilities of Large-Ring Cyclodextrins

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Abstract

Large-ring cyclodextrins (LR-CD) are cyclic α-1,4-glucans composed of nine to more than several hundred glucopyranose units. The first definitive evidence for the existence of LR-CD with a degree of polymerization between 9 and 13 was reported in 1965. That LR-CD study did not reveal anything that attracted attention. LR-CD with a degree of polymerization between 9 and 31 were isolated and characterized during the past decade, and so began to attract considerable attention. This mini-review summarizes the findings of LR-CD with regard to the potential for host-guest interactions and corresponding applications.

Introduction

Cyclodextrin (CD) is a common name for cyclic oligosaccharides composed of a number of α 1,4-linked glucopyranoses, in which numbers 6, 7, and 8 are well known as α-, β-, and γ-CD, respectively. Owing to their annular cavity of 5–8 Å, they are able to form an inclusion complex with a variety of guest molecules. They and their derivatives have been thoroughly studied and used in many fields. Several excellent reviews are available on basic studies and their applications [1–3].

On the other hand, it is very difficult to find reports on LR-CD with a degree of polymerization of greater than nine units prior to 1985. In 1965, French *et al.*, reported the first definitive evidence for the existence of LR-CDs with a degree of polymerization from 9 to 13 [4]. However, this early LR-CD study did not reveal anything that attracted attention, and moreover it was forgotten because of the difficulties in their purification and the preparation of reasonable yields. In 1986, Kobayashi *et al.* developed a preparation method for LR-CD mixtures and succeeded in isolating δ-CD (the degree of polymerization was 9) [5]. The crystal structure of δ-CD was characterized by Fujiwara *et al.* in 1990 [6]. Our group isolated and characterized LR-CD from commercially available CD-mixtures as a food additive in Japan and confirmed the existence of LR-CDs with a degree of polymerisation from 9 to 21 [7–13]. Takaha *et al.* isolated and characterized LR-CD with a degree of polymerization up to 31, and also reported their new synthesis in significant amounts using various glucanotransferase enzymes [14–19]. In addition, Machida *et al.* reported that LR-CD mixtures with a degree of polymerization from 22 to 45, and greater than 50 exhibited an efficient artificial chaperone for protein refolding [20]. In the autumn of 2001, the new product, a protein refolding kit using LR-CDs came onto the Japanese market. Based on the above situation, LR-CD studies began to attract considerable attention. This mini-review summarizes the findings of LR-CD, focusing on the potential of LR-CD for host-guest interactions and corresponding applications. Throughout this mini-review, the genetic names will be used for α-, β-, γ- and δ-CD, whereas the semisystematic names, which includes the degree of polymerization in the macrocycle, will be used for LR-CDs with a degree of polymerization greater than 10 (abbreviated, CDn, where n designates the degree of polymerization). Larsen expounded on the nomenclature of LR-CDs in his recent review [21].

Production and purification of LR-CDs

Regular CDs (α-, β-, and γ-CD) are currently synthesized industrially using various CGTases. However, LR-CDs preparations are not established industrially or were extensively studied using many kinds of CGTases and/or other enzymes on a laboratory scale. From previous findings, the degree of polymerisation and yield of LR-CD obtained on a laboratory scale depended on the type of enzyme chosen and was strongly influenced by the ingredients and reaction conditions (especially, reaction time) used. Zimmermann *et al.* expounded on the detail of the enzymatic synthesis of LR-CDs in their recent review [22].

The isolation and purification procedures of LR-CDs were reported by our group and Takaha *et al.* [7–14]. These procedures included common initial purification steps and several chromatographic methods. At present, the isolation of relative large amounts of LR-CDs requires tedious pretreatment and a number of chromatographic separations.

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Table 1. Physicochemical properties of CD

	Number of glucopyranose units	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	14.5	72	+147.8	33
β-CD	7	1.85	73	+161.1	29
γ-CD	8	23.2	73	+175.9	15
δ-CD	9	8.19	73	+187.5	4.2
CD_{10}	10	2.82	72	+204.9	3.2
CD_{11}	11	>150	72	+200.8	3.4
CD_{12}	12	>150	72	+197.3	3.7
CD_{13}	13	>150	72	+198.1	3.7
CD_{14}	14	2.30	73	+199.7	3.6
CD_{15}	15	>120	73	+203.9	2.9
CD_{16}	16	>120	73	+204.2	2.5
CD_{17}	17	>120	72	+201.0	2.5
CD_{18}	18	>100	73	+204.0	3.0
CD_{19}	19	>100	73	+201.0	3.4
CD_{20}	20	>100	73	+199.7	3.4
CD_{21}	21	>100	73	+205.3	3.2

[a] Observed at 25 °C.
[b] In 1 mol/L HCl at 50 °C.

This is a problem that cannot avoid a great deal of labor and a relatively high cost.

Physicochemical properties and structures of LR-CDs

Table 1 lists some of the physicochemical properties of regular CDs and LR-CDs. The aqueous solubilities of LR-CDs except δ-CD, CD_{10}, CD_{14} are greater than those of regular CDs. Both regular CDs and LR-CDs show no surface activity. The optical rotation increases in the order: α-CD $<$ β-CD $<$ γ-CD $<$ δ-CD $<$ $CD_{12} \cong CD_{13} \cong CD_{14} \cong CD_{20} \cong CD_{11} \cong CD_{17} \cong CD_{19} < CD_{15} \cong CD_{18} \cong CD_{16} \cong CD_{10} \cong CD_{21}$. There are no marked differences in the specific rotation among LR-CDs ($CD_{10} \sim CD_{21}$). The acid-catalyzed hydrolysis rates of LR-CDs ($CD_{10} \sim CD_{21}$) are faster than those of regular CDs and δ-CD. There are no marked differences in the acid-catalyzed hydrolysis rates among LR-CDs ($CD_{10} \sim CD_{21}$). The structure of four LR-CDs (δ-CD, CD_{10}, CD_{14}, and CD_{26}) were reported [6, 9, 23–26]. The detailed structural features of those solid state structures was reviewed by Saenger et al. [27]. The structure of δ-CD exhibits a distorted elliptic boat-like shape, but it retains a similar structure to regular CDs. CD_{10} and CD_{14} also exhibit a more elliptical macrocyclic ring folded in a saddle-like shape. The structure of CD_{26} has channel-like cavities composed of two short V-amylose helices in anti-parallel orientation, and its structure is very different from the regular CDs. Other LR-CDs structures have not been reported, because their single crystals could not be prepared. However, several LR-CDs have been deduced from molecular dynamics simulations and small angle X-ray scattering analysis [28–30].

Table 2. Precipitation of CD by the formation of insoluble inclusion complexes with macrocyclic compounds (25 °C) [32]

	α-CD	β-CD	γ-CD	δ-CD
1,5-Cyclooctadiene	38%	72%	53%	–
Cyclononanone	63%	75%	55%	–
Cyclodecanone	33%	96%	66%	–
Cycloundecanone	–	84%	87%	35%
Cyclododecanone	–	33%	36%	42%
Cyclotridecanone	–	73%	89%	56%
Cyclopentadecanone	–	4.4%	22%	53%

–: Not detected.

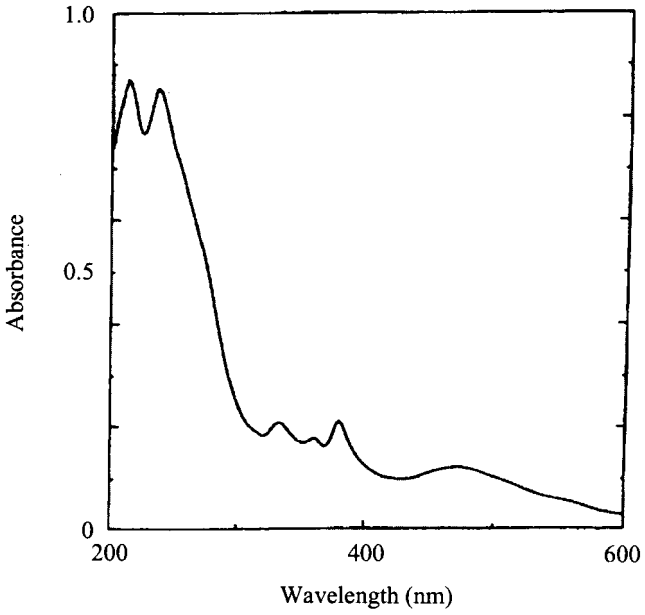

Figure 1. UV-VIS spectrum of aqueous solution of C_{70}/δ-CD complex (diluted 12.5-fold) [33].

Table 3. Inclusion complex formation constants of the 1:1 complexes between cyclodextrins and various anions measured by capillary electrophoresis at 25 °C [36]

Compound	Inclusion complex formation constant (M^{-1})							
	α-CD	β-CD	γ-CD	δ-CD	CD_{10}	CD_{11}	CD_{12}	CD_{13}
Benzoate	16	23	3	3	3	5	4	5
2-Methyl benzoate	13	13	7	6	6	5	6	7
3-Methyl benzoate	26	40	6	3	5	6	7	8
4-Methyl benzoate	36	66	8	2	4	6	6	7
2,4-Dimethyl benzoate	45	42	8	3	4	5	7	6
2,5-Dimethyl benzoate	41	27	6	4	5	5	6	6
3,5-Dimethyl benzoate	39	9	7	2	5	7	8	8
3,5-Dimethoxy benzoate	47	63	10	8	9	10	9	12
Salicylate	11	65	13	9	8	8	9	10
3-Phenyl propionate	35	79	7	2	3	5	4	6
4-*tert*-buthyl benzoate	51	382	74	47	3	9	15	25
Ibuprofen anion	56	>2500[a]	67	27	2	12	29	39
1-Adamantane carboxylate	114	501	42	8	–[b]	4	4	8

[a] Too high to be accurately determined.
[b] Could not be determined.

Inclusion complex formation

The effect of complex formation with δ-CD on the solubility of drugs which are poorly soluble or insoluble in water was reported [7, 31], but δ-CD did not show any significant solubilization effect on these drugs in comparison with regular CDs. The relation between the complex forming ability of δ-CD and guest molecule structure was elucidated in detail using eight kinds of macrocyclic compounds with 8 to 16 carbon atoms in the ring as a model of large guest molecules. Table 2 shows that α-CD and β-CD formed rather stable complexes with small guest molecules, while γ- and δ-CD were more efficient in binding larger guest molecules [32]. These results suggested that LR-CDs may be good host molecules for relative large guest compounds. The interaction between δ-CD and Buckminster fullerene (C_{70}) has been elucidated and an effective solubilization of this molecule into water has been observed (Figure 1) [33]. The effect of δ-CD on the solubilization of C_{60} into water has also been elucidated, its effect was superior to that of γ-CD [34]. The solubilization of fullerene (C_{60}, C_{70}) into water using LR-CD ($CD_{10} \sim CD_{17}$) has been studied by the measurement of UV-VIS spectrum. For some of these LR-CDs, the UV-VIS spectra of the C_{60}, C_{70}/LR-CDs systems were in agreement with those of C60, C_{70} in hexane solution, respectively, although the intensities of C_{60}, C_{70}/LR-CDs were much weaker than those of and C_{60}, C_{70}/δ-CD [35]. As shown in Table 3, the inclusion complex formation constants between LR-CD (δ-CD $\sim$ CD_{17}) and various anions has been measured by capillary electrophoresis [36–38]. The findings showed that LR-CDs have a certain extent of inclusion ability. The complex formation of LR-CDs ($CD_{21} \sim CD_{32}$) with iodine in water has been studied by isothermal titration calorimetry [15]. The complex formation between an LR-CD mixture with a degree of polymerization from 22 to more than 100 and iodide and other chemical compounds has been reported [39]. LR-CD mixtures with a degree of polymerisation from 22 to 45 and over 50, respectively, exhibited an efficient artificial chaperone for protein refolding [20]. The findings showed that chemically denatured citrate synthase was folded with in 2 h, and over 50% of its activity was recovered with in 30 min. As a consequence, a protein refolding kit using an LR-CD mixture came onto the Japanese market. This product was the first practical application of LR-CDs. To investigate further applications of LR-CDs, it is necessary to prepare large amounts of each isolated pure LR-CD efficiently.

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