FABAD J. Pharm. Sci., 29, 27-38, 2004

SCIENTIFIC REVIEW

INVITED REVIEW

Large Ring Cyclodextrins - Recent Progress

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Born in 1948 in Tokyo, Japan, Haruhisa Ueda received his bachelor's degree in pharmaceutical sciences from Tokyo College of Pharmacy in 1972, and his M. S(c) from Hoshi University in 1974 and his Ph. D. from Hoshi University in 1982. From 1983 to 1984, he was a postdoctoral fellow in Pharmaceutical Sciences at the University of Florida, U. S. A. Since 1975, he has been working as a research associate, assistant professor and associate professor in Faculty of Pharmaceutical Sciences, Hoshi University, Tokyo, Japan. Since 2001, he has been a professor and chair in Department of Physical Chemistry, Hoshi University. His field of research include: Pharmaceutical use of cyclodextrins in various drug formulations, and the production, purification and characterization of large-ring cyclodextrins and their applications.

Large Ring Cyclodextrins - Recent Progress

Summary

Cyclodextrin (CD) is a common name for cyclic a-1,4-glucans. Not only the well known α-, β- and γ-CDs, which are composed of six, seven and eight D-glucopyranose units, but also large-ring CDs (LR-CDs), with more than nine D-glucopyranose units, exist in the group. LR-CDs were found by French et al. in the 1960s; however, because of difficulties in the purification and isolation of LR-CDs, there were only two papers, written by French et al., before 1985. After the development of a preparation method for the mixture of LR-CDs in 1986, studies on LR-CDs started again. In the last decade, knowledge about the enzymatic production, physicochemical properties, structural features and inclusion complex formation abilities of LR-CDs has increased substantially. In this mini review, recent findings on the production, physicochemical properties, inclusion complex formation ability and applications of LR-CDs are discussed and shown.

Key Words : *Cyclodextrin, large-ring cyclodextrin, production, physicochemical property, inclusion complex formation ability.*

Received *: 27.07.2004*

INTRODUCTION

Cyclodextrin (CD) is a common name for cyclic α-1,4-glucans. In particular, α-CD (cyclomaltohexaose), β-CD (cyclomaltoheptaose) and γ-CD (cyclomaltooctaose) have been well studied since crystalline compounds (namely CD) were found in starch degradation products treated with *Bacillus amylobacter* at the end of the 19th century, and α-CD and β-CD were subsequently isolated in the beginning of the 20th century[1]). CDs can include a variety of hydrophobic and hydrophilic guest molecules in

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their annular cavity, and while inside the cavity the CDs can modify their chemical stability and water solubility. As a result, they and their derivatives have applications in many fields, for example pharmaceutical, food and cosmetic industries. Basic studies and the applications of α-, β- and γ-CD (regular CDs), and their derivatives, have been shown in several invaluable reviews[1-4]).

Conversely, small- and large-ring CDs (LR-CDs), which are composed of less than five and more than nine D-glucopyranose units, respectively, were not generally studied until the end of the 20th century, with the exception of two papers on LR-CDs with a degree of polymerization (DP) of 9 to 13, by French et al[5,6]). More studies were reported in the 1990s. From a small-ring CD, a CD with a DP of 5 (cyclomaltopentaose) was chemically synthesized by Nakagawa et al. in 1994[7]). Unfortunately, the physicochemical properties and inclusion complex formation ability of cyclomaltopentaose have not been reported yet. On the other hand, several papers on LR-CDs, including enzymes for preparation, physicochemical properties, molecular structures and inclusion complex formation abilities, have been published, and LR-CDs have attracted a good deal of attention since Kobayashi et al. developed a preparation method for the mixture of LR-CDs, and isolated a CD with a DP of 9 in 1986[8]). Some reviews have already become available on the nomenclature, enzymatic preparation, molecular structures and inclusion formation abilities of LR-CDs[9-13]). In this mini review, recent advances (mainly after 2002) in LR-CDs are discussed and shown.

I. PRODUCTION OF LARGE-RING CDs.

1. Enzymatic Preparation

1) Cyclodextrin glucanotransferases (CGTases, EC 2.4.1.19)

It is well known that cyclodextrin glucanotransferases (CGTases) can convert starches into a mixture of CDs by an intramolecular transglycosylation reaction (cyclization reaction), and regular CDs are industrially produced by the use of CGTases from starches. CGTases are extracellular enzymes originating from bacteria such as the *Bacillus* species, and catalyze not only the cyclization reaction, but also a transglycosidic linearization reaction (coupling reaction), intermolecular transglycosylation reactions (disproportionation reactions) and hydrolysis reactions[14-15]). It was clear that CGTases could produce LR-CDs, because LR-CDs with a DP from 9 to 21 were found in a commercially available CD mixture produced by CGTase from potato starch[8, 16-22]), and CGTase from the alkalophilic Bacillus sp. strain A2-5a, *B. macerans* and *B. stearothermophilus* produced LR-CDs from synthetic amyloses[23-25]); however, an elongation of reaction times caused decreased amounts of LR-CDs because of their conversion into regular CDs by repeated coupling and cyclization reactions. This result shows that the balance between coupling reaction activity and cyclization reaction activity is an important factor in the production of LR-CDs. If the coupling reaction activity in CGTase was selectively depressed, LR-CDs would be produced more effectively. Recently, two papers supported the possibility of the above mentioned hypothesis. Yoon and Robyt reported the effect of molar ratios of D-glucose on the transglycosylation reactions of *B. macerans* CGTase[26]). α-CD was incubated with CGTase and different amounts of D-glucose for 24 h at 37°C, and the products were analyzed by a thin layer chromatography. It was shown that an increase in the ratio of D-glucose/a-CD caused decreases in all CDs, in contrast to an increase of maltodextrins (Fig. 1). The D-glucoses that are added, and maltodextrins produced during the CGTase reaction, act as acceptors during the coupling reaction. This result showed that the amount of acceptors present during the CGTase reaction played a pivotal role in the production of CDs, especially LR-CDs. In another paper, Qi et al. reported the effect of reaction temperature on the transglycosylation reactions of B. macerans CGTase for the production of LR-CDs[27]). It is shown in Figure 2 that chromatograms of the CDs obtained from synthetic amylose as substrate were catalyzed by the B. macerans CGTase at 40 and 60°C. Almost all LR-CDs were converted into regular CDs at 40°C after a reacti-

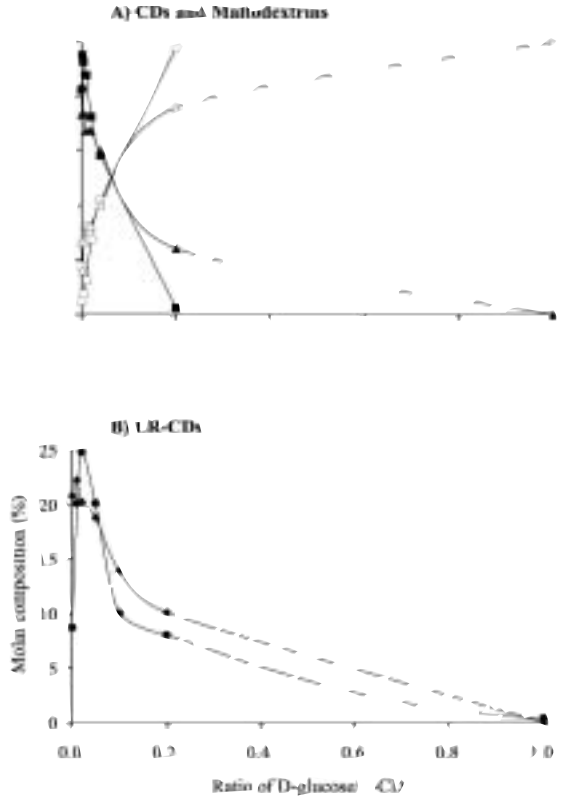

Fig. 1 Percent molar composition of the CDs, maltodextrins (A) and LR-CDs (B) with various molar ratios of D-glucose to 50 and 100 mM α-CD after 24 h at 37°C (Data modified from reference No. 26).
Each symbol indicates CDs in 50 mM α-CD ( ), CDs in 100 mM α-CD ( ), maltodextrins in 50 mM α-CD ( ), maltodextrins in 100 mM α-CD ( ), LR-CDs in 50 mM α-CD ( ), LR-CDs in 100 mM α-CD ( )

on time of 20 h, and only some LR-CDs remained after a reaction time of 2 h, as reported in a previous study[23]). On the other hand, a number of LR-CDs could be detected even after a reaction time of 20 h. The maximum yield of LR-CDs after 2 h of incubation at 60°C reached about 50% of the total glucan level. And they remained approximately constant during longer incubation times, although the maximum yield of LR-CDs at 40°C was 35% of the total glucan level at the same incubation time. The observed effect of temperature on the production of LR-CDs using B. macerans CGTase was caused by differing optimum temperatures for the cyclization reaction activity and the coupling reaction activity, as shown in Figure 3. These results suggest that inhibition of the coupling reaction by the removal of acceptors, and the control of incubation temperatures throughout CD production, might enable the selection and control of the type and amount of products.

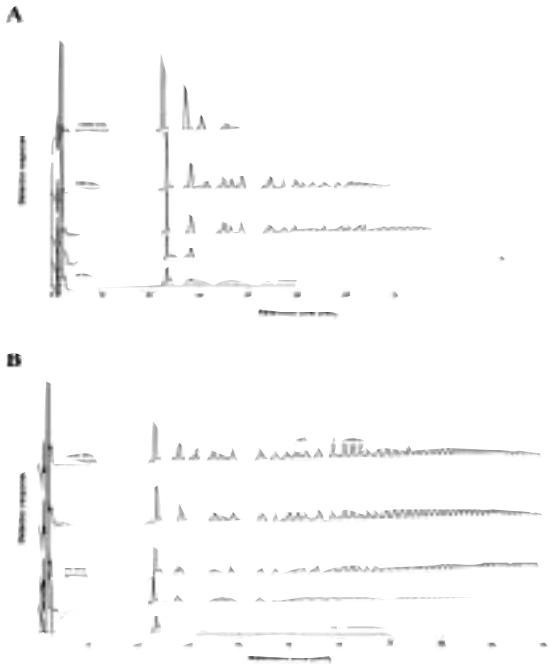

Fig. 2 High performance anion exchange chromatographic analysis of CDs produced after 10, 30, 60, 120 and 1200 min by CGTase from *B. macerans* at (A) 40°C and (B) 60°C (Reprinted from reference No. 27 by permission of Elsevier Ltd).
Numbers above peaks indicate the DPs.

2) Other enzymes

It has already been reported that several other 4-α-glucanotransferases (EC 2.4.1.25), such as D-enzyme

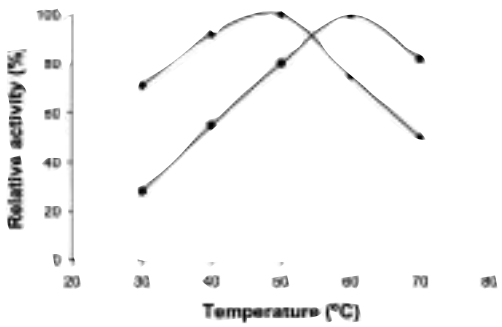

Fig. 3 Comparison of the coupling reaction activity ( ) and the cyclization reaction activity ( ) of the CGTase from *B. macerans* at different temperatures (Reprinted from reference No. 27 with permission from Elsevier Ltd.).

and amylomaltase, could also produce LR-CDs[28,29]). Recently, Yanase *et al.* found that the glycogen debranching enzyme (GDE, EC 2.4.1.25/EC 3.2.1.33) was also able to produce LR-CDs[30]). This enzyme has 4-α-glucanotransferase activity and amylo-1,6-glucosidase activity in the single polypeptide chain[31,32]), and had some interesting effects on LR-CD production, as follows: (1) The GDE from Saccharomyces cerevisiae was incubated with synthetic amyloses of differing average molecular mass and some starches. Figure 4 indicates that the GDE can produce LR-CDs within a DP range of 11 to around 40, from not only synthetic amyloses but also starches such as amylopectin and debranched starch (short-chain amylose). (2) The yield of LR-CDs using the GDE and soluble starch attained about 60% after 6 h of incubation time without any other enzymes; however, it was 40% in the case of D-enzyme combined with isoamylase and pullulanase. (3) Although the coupling reaction activity by CGTase, D-enzyme and amylomaltase was enhanced in the presence of D-glucose, it had no effect on the production of LR-CDs using the GDE at the 0.01 and 0.1% D-glucose concentrations, because the GDE could not use D-glucose and maltose as acceptors of the coupling reaction. These results suggested that the GDE was a useful enzyme for LR-CD production, because commercially available starches could be used as a substrate, and inactivation of GDE was not required to simplify the production process.

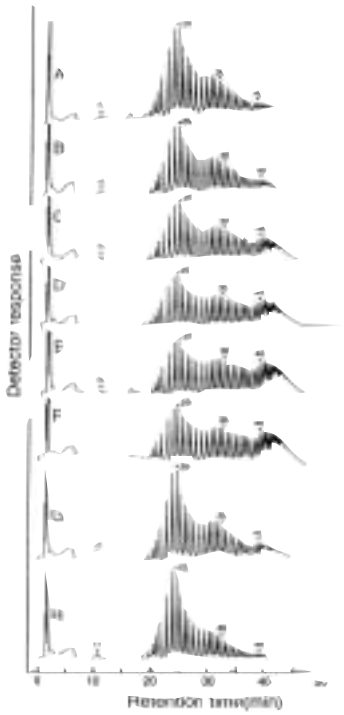

Fig. 4 High performance anion exchange chromatographic analysis of CDs produced by GDE from various substrates after 24 h at 40°C (Reprinted from reference No. 30 with permission from American Society for Microbiology).
The substrates were synthetic amyloses with average molecular masses of 5 kDa (A), 10 kDa (B), 30 kDa (C), 70 kDa (D), 110 kDa (E) and 320 kDa (F); amylopectin (G); and debranching starch (short-chain amylose) (H).

2. Chemical Synthesis

The chemical synthesis of LR-CD was not developed until quite recently, although chemical syntheses of a- and g-CD were reported by Takahashi and Ogawa in 1987[33,34]). In 2002, the first chemical synthesis of LR-CD was reported. Wakao et al. synthesized a-CD and LR-CD with a DP of 9 (CD_9), by the molecular clamp method using the phthaloyl bridge[35]). In the case of CD9, maltotriose was selected as the starting material and the chemical synthesis consisted of 26 steps. As mentioned above, several enzymes with LR-CD production ability were found, and production methods of LR-CDs also developed, in the latest decade; however, LR-CD production using enzymes provides a mixture of many CDs with different DPs. If a specific LR-CD is needed, we have to purify it using chromatography and precipitation, etc. Unfortunately, an effective purification method for LR-CD has not yet been developed, which prevents the advance of LR-CD chemistry. Although regular CDs are industrially produced by enzymatic reaction due to cost and productivity concerns, chemical synthesis advancements may break the deadlock in LR-CD production in the future.

II. X-RAY CRYSTAL STRUCTURE AND PHYSICOCHEMICAL PROPERTIES OF LARGE-RING CDs.

The crystal structures of four kinds of LR-CDs with only water molecules, which are composed of 9

(CD9)[36]), 10 (CD10)[18, 37-40]), 14 (CD_{14})[37,38,41]) and 26 (CD_{26})[42,43]) D-glucopyranose units, have already been described using X-ray crystallographic analysis. The structure of regular CDs, a truncated cone with a round cavity, is well known. In CD_{10} and CD_{14}, the macrocyclic rings were deformed elliptical shapes, and the cavity shape was a narrow groove. Furthermore, the arrangement between two adjacent glucopyranose units showed *anti* type conformation (band flip) at two sites in their macrocyclic rings, although the normal arrangement between two adjacent glucopyranose units is *syn* type conformation, so that the primary and secondary hydroxyl groups exist on the same side of the macrocyclic ring. CD_{26} had a structure where two antiparallel V-amylose helices were bound through band flips. CD_9 had an intermediate structure between that of regular CDs, and CD_{10} and CD_{14}, such as a distorted elliptical macrocyclic ring without a band flip. To understand the above-mentioned structural features of LR-CDs in depth, some reviews should be consulted[9-12]). On the other hand, although there have been molecular structure studies of LR-CDs from molecular dynamics simulations and small-angle X-ray scattering analysis[44,45]), X-ray crystal structure analyses of other LR-CDs have not been reported due to the difficulties in making single crystals. It was generally found that high crystallinity of β-CD was caused by the rigid macrocyclic ring, derived from the continuous intramolecular hydrogen bonds between adjacent D-glucopyranose units and its high crystal lattice energy[3]). The low crystallinity of most LR-CDs might be caused by the difficulties in forming intramolecular and intermolecular hydrogen bonds, due to the high flexibility of their macrocyclic rings. In addition, it has been reported that the nucleation rates of sugars are generally very low, and this has to precede crystal growth, so sugar solutions often form supersaturated solutions of syrup-like liquid[46,47]). The molecular behavior in water of LR-CDs with high aqueous solubility may resemble that of sugars.

Table 1 lists some physicochemical properties of regular CDs and LR-CDs with DPs ranging from 9 to 21. The aqueous solubilities of CD_9, CD_{10} and CD_{14} were lower than those of α- and γ-CD; however, the other LR-CDs had high aqueous solubilities over 100 g/100 mL. This may be a consequence of high structural flexibility, on the basis of the formation of int-

Table 1. Nomenclature and some physicochemical properties of CDs

Number of glucopyranose units	Systematic name	Generic name	Abbreviation	CAS No.	Molecular weight	Aqueous solubility[a)] (g/100 mL)	Surface tension[a)] (mN/m)	Specific rotation $[\alpha]^{25}_D$	Half-life of macrocyclicopening[b)] (h)
6	cyclomaltohexaose	α-cyclodextrin	α-CD	10016-20-3	972[c)]	14.5[d)]	72[h)]	+147.8[h)]	33[h)]
7	cyclomaltoheptaose	β-cyclodextrin	β-CD	7585-39-9	1135[c)]	1.85[d)]	73[h)]	+161.1[h)]	29[h)]
8	cyclomaltooctaose	γ-cyclodextrin	γ-CD	17465-86-0	1297[c)]	23.2[d)]	73[h)]	+175.9[h)]	15[h)]
9	cyclomaltononaose	δ-cyclodextrin	CD_9	85220-53-7	1459[e)]	8.19[e)]	72[h)]	+187.5[e)]	4.2[h)]
10	cyclomaltodecaose	ε-cyclodextrin	CD_{10}	156510-98-4	1621[f)]	2.82[h)]	72[h)]	+204.9[h)]	3.2[h)]
11	cyclomaltoundecaose	ζ-cyclodextrin	CD_{11}	156510-97-3	1783[f)]	>150[h)]	72[h)]	+200.8[h)]	3.4[h)]
12	cyclomaltododecaose	η-cyclodextrin	CD_{12}	156510-96-2	1946[f)]	>150[h)]	72[h)]	+197.3[h)]	3.7[h)]
13	cyclomaltotridecaose	θ-cyclodextrin	CD_{13}	156510-95-1	2107[f)]	>150[h)]	72[h)]	+198.1[h)]	3.7[h)]
14	cyclomaltotetradecaose	ι-cyclodextrin	CD_{14}	156510-94-0	2270[g)]	2.30[h)]	73[h)]	+199.7[h)]	3.6[h)]
15	cyclomaltopentadecaose	κ-cyclodextrin	CD_{15}	156510-99-5	2432[g)]	>120[h)]	73[h)]	+203.9[h)]	2.9[h)]
16	cyclomaltohexadecaose	λ-cyclodextrin	CD_{16}	200501-75-3	2594[g)]	>120[h)]	73[h)]	+204.2[h)]	2.5[h)]
17	cyclomaltoheptadecaose	μ-cyclodextrin	CD_{17}	200501-91-3	2756[g)]	>120[h)]	72[h)]	+201.0[h)]	2.5[h)]
18	cyclomaltooctadecaose	ν-cyclodextrin	CD_{18}	219720-42-0	2919[i)]	>100[i)]	73[i)]	+204.0[i)]	3.0[i)]
19	cyclomaltononadecaose	ζ-cyclodextrin	CD_{19}	219720-44-2	3081[i)]	>100[i)]	73[i)]	+201.0[i)]	3.4[i)]
20	cyclomaltoeicosaose	σ-cyclodextrin	CD_{20}	219720-45-3	3243[i)]	>100[i)]	73[i)]	+199.7[i)]	3.4[i)]
21	cyclomaltoheneicosaose	π-cyclodextrin	CD_{21}	219720-46-4	3405[i)]	>100[i)]	73[i)]	+205.3[i)]	3.2[i)]

a) Observed at 25°C; b) In 1 mol/L at 50°C

c) Ref. No. 2, d) Ref. No. 48, e) Ref. No. 16, f) Ref. No. 49, g) Ref. No. 50, h) Ref. No. 51, i) Ref. No. 52

ramolecular and intermolecular hydrogen bonds, similar to crystallinity. No significant difference was observed in the acid-hydrolysis rate among LR-CDs with DPs of 10 to 21. This suggested that the increases in decomposition points (parts of glucosidic linkage) in the macrocyclic rings accompanied by an increasing number of D-glucopyranose units were not the major reason for the macrocyclic ring opening reaction catalyzed by protons. On the other hand, the half-lives of macrocyclic ring openings paralleled ^{13}C NMR chemical shifts of C1 and C4 in D-glucopyranose units, as shown in Figure 5. This relationship may show that steric strains in macrocyclic rings contribute to the rate of macrocyclic ring opening in acidic conditions.

III. INCLUSION COMPLEX FORMATION ABILITY OF LARGE-RING CDs.

As summarized in Table 2, there have been several papers regarding the inclusion complex formation

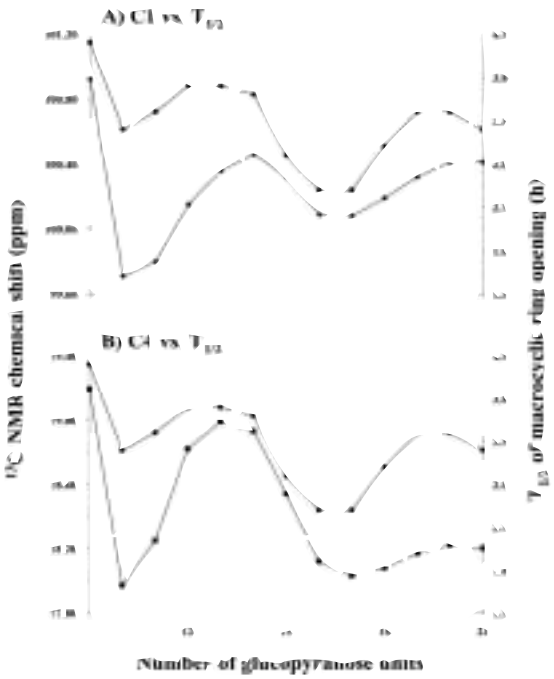

Fig. 5 Relationship between half-life of macrocyclic ring opening and of ^{13}C NMR chemical shifts of C1 (A) and C4 (B) in LR-CDs with DP of 9 to 21 (Data modified from reference No. 52).
Each symbol indicates half- life of macrocyclic ring opening ( ), chemical shift of C1 ( ), chemical shift of C4 ( ).

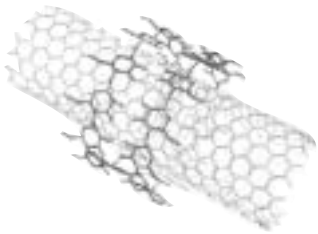

Fig. 6 Model of SWNT included by two CD_{12} with the head-to head arrangement by molecular dynamic simulation (Reprinted from reference No. 63 with permission from The Royal Society of Chemistry)

between pure LR-CD or a mixture of LR-CDs and guest compounds. For details of these, some reviews should be mentioned[10-13]). Recently, Dodziuk et al. reported that not only fullerenes, but also single-wall carbon nanotube (SWNT), were dissolved into water using LR-CD[63]). It was shown that CD_{12} with a DP of 12 formed inclusion complexes with SWNT, which were cut into shorter pieces by co-grinding with β- or γ-CD to thread, from ^{1}H and ^{13}C NMR data and simulations on the basis of molecular mechanics and molecular dynamics (Fig. 6); however, X-ray crystallographic studies on the inclusion complex of LR-CD have not been reported until quite recently. Since 2002, two crystallographic studies on the inclusion complex of LR-CD were reported, and inclusion complex formation ability was clearly proven. The first report was the X-ray crystal structure analysis between CD_9 and cycloundecanone by Harata *et al.* in 2002[64]). The structure of the inclusion complex was described as four cycloundecanone molecules entrapped in a barrel-like structure, where two CD_9 molecules form a head-to-head dimer using many hydrogen bonds between secondary hydroxyl groups, as shown in Figure 7. It has already been reported that CD_9 in the crystalline state with only water molecules had an elliptically distorted macrocyclic ring, and that the cavity was narrower than that expected from its ring size[36]); however, it was demonstrated that the inclusion of cycloundecanones caused induced-fit structural change to make the macrocyclic ring of CD_9 symmetrical. This result suggested that CD_9 had the possibility of forming inclusion complexes with guest molecules to fit its cavity, created by the induced-fit structural change when CD_9 would try to include relatively

Fig. 7 Structure of the asymmetric unit of the crystalline inclusion complex of CD_9 with cycloundecanone (Reprinted from reference No. 64 with permission from The Royal Society of Chemistry).
The cycloundecanone molecules are shaded. Thin lines indicate the intermolecular hydrogen bonds between CD_9 molecules.

large guest molecules. Another study reported on the X-ray crystal structures of inclusion complexes between CD_{26} and iodine (Nimz *et al.*, 2003[65]). Two single crystals of inclusion complexes of $CD_{26}$with triiodide were prepared using a mixture of CD_{26}, NH_4I_3 or $Ba(I_3)_2$, and polyethylene glycol (PEG400) by the hanging drop vapor diffusion method. The brown prismatic crystals obtained had the following chemical formula: $2CD_{0.5}{\cdot}6NH_4I_3{\cdot}2NH_4I{\cdot}91.5H_2O$ and $(CD_{26})_{0.5}{\cdot}Ba_{0.5}I_3{\cdot}23.75H_2O{\cdot}(PEG400)_{0.5}{\cdot}$ glycerol, respectively. The X-ray crystallographic data showed that triiodide was included in the V-amylose helices of CD_{26}. Furthermore, raman and UV/VIS spectrum data also supported the existence of triiodide in CD_{26}, but not in other species such as iodine atoms, iodine molecules, pentaiodide, etc.

IV. APPLICATIONS OF LR-CDs.

In 2003, the first chemically modified LR-CD (CD_9) was synthesized to enhance chemiluminescence efficiency by forming a CD-bound luciferin analogue[66]). Cypridina luciferin, in aqueous solutions, is able to emit blue light in the presence of Cypridina luciferase and triplet oxygen with high efficacy, but no light is observed without luciferase[67,68]). Cypridina luciferin analogues, such as 2-methyl-6-(4-methoxyphenyl)-imidazo[1,2-α]pyrazin-3(7H)-one (MCLA), which generate chemiluminescence following reactions with triplet oxygen without enzymes in aqueous solutions, have already been developed for use as tools in analyses of superoxide anions and lipid hydroperoxides[69]); however, their chemiluminescence efficiency was lower than that of *Cypridina* bioluminescence. Teranishi *et al.* have already prepared several regular CDs modified by MCLA to overcome this problem, and tested the enhancement effect of CDs as chromophor[70,71]). The results showed the order of overall chemiluminescence efficiency (ΦCL) on the basis of luminol in 0.03 M phosphate buffer (pH 8.0) as follows: γ-CD-MCLA (Φ_{CL}: 0.021) > CD_9-MCLA (Φ_{CL}: 0.0058) > β-CD-MCLA (Φ_{CL}: 0.0036) > α-CD-MCLA (Φ_{CL}: 0.00062) > MCLA alone (Φ_{CL}: 0.00048). This suggested that the entrance and cavity of CD_9 was so large that CD_9 could not include enough of the singlet-excited amide moiety produced during chemiluminescence reactions in comparison with that of γ-CD-MCLA, although CD_9 was also able to interact with MCLA appended.

A mixture of LR-CDs has also become of interest in recent years, since the effect of an LR-CD mixture as an artificial chaperone for protein refolding was reported by Machida *et al.*[72]). At present, a protein refolding kit containing a mixture of LR-CDs as one of the active components is on the market[73]). Furthermore, it was also reported in 2003 that the LR-CDs mixture provided an efficient method for refolding denatured antibody to correct active structures[74]). In the pharmaceutical field, interactions between drugs and mixtures of LR-CDs have been studied. Tomono *et al.* evaluated the interactions between mixtures of LR-CDs with DPs of 20 to 50 (average molecular weight, 7720) and drugs such as prednisolone, cholesterol, digoxin, digitoxin and nitroglycerin, using the solubility method[75]). Although nitroglycerin did not interact with the mixture of LR-CDs, the solubilities of prednisolone, cholesterol, digoxin and digitoxin were enhanced by the presence

Table 2. Studies of inclusion complex formation between pure LR-CD or mixture of LR-CDs and compounds

CD	Indicator or Method	Compound
(Pure LR-CD)		
CD_9[16]	Enhancement of solubility (UV/VIS absorption)	Anthracene Amphotericin B Ajmalicine Ajmaline Carbamazepine Digitoxin Spironolactone 9,10-Dibromoanthracene Perylene-3,4,9,10 tetracarboxylic dianhydrate
	Solubility method	Spironolactone
CD_9[53]	Enhancement of solubility (Spectrophotometry)	Fullerene C_{70}
CD_9[54]	Enhancement of solubility (Spectrophotometry)	Reserpine [2,2]-Paracyclophane Perylene Triphenilene 1,8-Naphthalic anhydride Naphthalene-1,4,5,8-tetracarboxylic dianhydride Digitoxin Gitoxin Digoxin Methyldigoxin Lanatoside C G-Strophanthin Proscillaridin A
	Solubility method and NMR	Digitoxin
CD_9[55]	Simple precipitation	1,5-Cyclooctadiene Cyclononanone Cyclodecanone Cycloundecanone Cyclododecanone Cyclotridecanone Cyclopentadecanone
	Powder X-ray diffraction DSC	Cycloundecanone Cyclododecanone
CD_9-CD_{13}[56-58]	Capillary electrophoresis	Benzoate 2-Methyl benzoate 3-Methyl benzoate 4-Methyl benzoate 2,4-Dimethyl benzoate 2,5-Dimethyl benzoate 3,5-Dimethyl bezoate 3,5-Dimethoxy benzoate Salicylate 3-Phenyl propionate 4-tert-Butyl benzoate Ibuprofen anion 1-Adamantane carboxylate
CD_{14} - CD_{17}[59,60]	Capillary electrophoresis	Salicylate 4-*tert*-Butyl benzoate Ibuprofen anion
CD_{21} - CD_{32}[61]	Isothermal titration calorimetry	Iodine
(Mixture of LR-CDs)		
CA(S)* and CA (L)**[15,62]	Spectrofluorometry	8-Anilino-1-naphthalene sulfonic acid
CA (S)* and CA (L)**[15,62]	Simple precipitation	1-Octanol 1-Butanol Oleic acid

* CA (S) : Mixture of LR-CDs with DP around 20 to 55, mainly DP of 25 to 50.
** CA (L) : Mixture of LR-CDs with average DP of ca. 150, except CDs with DP less than 50.

of the mixture of LR-CDs, and the phase solubility diagrams showed complex formations between them and the mixture of LR-CDs. In particular, the mixture of LR-CDs showed the highest solubilization effect for cholesterol among β-, γ-CD and the mixture of LR-CDs.

V. CONCLUSIONS

Our knowledge of LR-CDs has significantly progressed in the last decade. The description of the cyclization reaction mechanism by CGTase, and the discovery of some new enzymes for LR-CD production such as D-enzyme, amylomaltase and GDE have provided an efficient production method for LR-CDs. The physicochemical properties and the structural features of LR-CDs showed unexpected and attractive results, such as the low solubilities observed in some LR-CDs and "band flip", the unique conformation between glucopyranose units. Furthermore, inclusion complex formation ability of LR-CDs, a subject of great interest, was evidently confirmed. At the present time, we already have a product containing a mixture of LR-CDs. On the other hand, the purification method for each LR-CD has remained almost the same since Kobayashi et al. and our group purified and isolated each LR-CD with DPs of 9 to 21. This will prevent basic and application studies of each single LR-CD. Probably, pharmaceutical applications of mixtures of LR-CDs might exceed those of each single LR-CD, because it would be possible to supply a mixture of LR-CDs. Development of more effective purification methods, as well as effective and selective production methods for LR-CDs will be required to advance basic studies and applications of single LR-CDs.

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