CHAPTER I

INTRODUCTION

Cyclodextrins

Cyclodextrins (Cycloamyloses, cyclomalto-oligoses, Schardinger dextrins, CDs) are cyclic, non-reducing oligosaccharides composed of glucose units linked by α - 1, 4 glycosidic bonds. The main CDs synthesized naturally by the enzyme cyclodextrin glycosyltransferase (CGTase) are built up from six to eight glucopyranose units, respectively known as α-cyclodextrin (cyclomaltohexaose, cG_6), β-cyclodextrin (cyclomaltoheptaose, cG_7), and γ-cyclodextrin (cyclomalto-octaose, cG_8), as shown in Figure 1 (French and Rundle, 1942 ; Freudenberg and Cramer, 1948). CDs with less than six glucopyranose units cannot be formed for steric reasons and CDs containing more than nine glucopyranose units have been shown to be branched (Sundararajan and Rau, 1970). Recently, δ-CD (cyclonanoamylose, composed of nine α-1,4-linked D-glucose units) and η-CD (cyclododecaamylose, composed of twelve α-1,4-linked D-glucose units) have been reported. They were produced from cyclodextrin powder by the action of β-amylase and pullulanase. Even if these large ringed cyclodextrins are inapplicable for various industrial uses, they may have some unique characters in comparison with the conventional cyclodextrins. Elucidation of their structures and physicochemical properties may provide much information on a basic knowledge and development of oligosaccharides (Tomohiro *et al*, 1994).

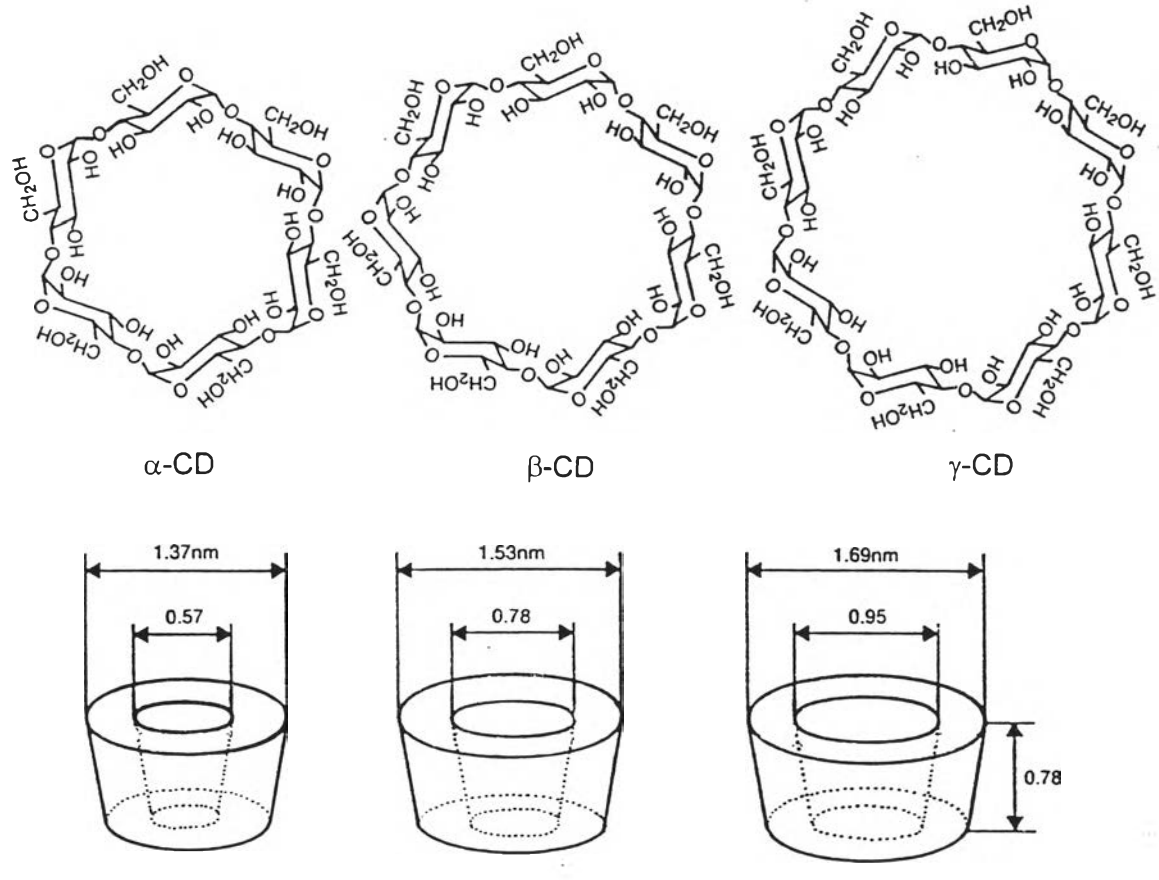


Figure 1 Structure and molecular dimension of cyclodextrins (CDs). (Szejtli, 1990)

Some physical properties of natural cyclodextrins are summarized in Table 1 (Szejtli,1988). The cyclodextrins are water-soluble. This fundamental characteristic derives from the location of all free hydroxyl groups of each successive glucose unit on rims of these doughnut - shaped molecules - the C_6 primary hydroxyls on the narrower side and the C_2 and C_3 secondary hydroxyls occupying the wider side. These two hydrophilic planes thus confer hydrophilicity upon the molecule, while the inside cavity of CDs is hydrophobic because it is lined with C-H groups and glycosidic oxygen bridges (Saenger, 1979, 1982), see Figure 2.

This unique spatial structure enhances its chemical stability comparing to its building units. The chemical properties markedly differ from those of non-cyclic carbohydrates are as follows :

A) The ring structure has neither a reducing nor non-reducing end group.

B) They are not decomposed by hot alkali.

C) They are rather resistant to hydrolysis by most organic acids and many common α-amylases, and completely resistant to yeast fermentation and β-amylases.

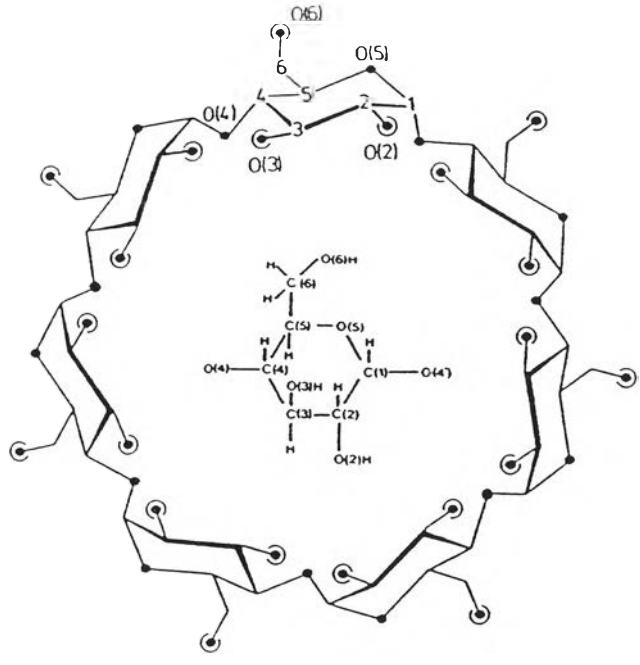
D) They demonstrate the enhanced thermal stability with a decomposition temperature approaching 300 °C.

The most important characteristic of cyclodextrins is their ability to form three-dimensional inclusion complexes with a wide variety of suitable size "Guest" molecules, including these molecules wholly or partially within the central cavity of the "Host" cyclodextrin (Bender, 1986). The inclusion complex is held together by non-covalent bonding forces such as hydrophobic interaction, Van der Waal forces, London dispersion forces, and hydrogen bonding. The binding of "Guest" molecules

Table 1 Characteristics of cyclodextrins. (Saenger, 1982 ; Szejtli, 1988)

	α-CD	β-CD	γ-CD
Number of glucose unit	6	7	8
Molecular weight	972	1,135	1,297
Cavity dimensions			
Cavity diameter (A°)	4.7 - 5.3	6.0 - 6.5	7.5 - 8.3
Cavity depth (A°)	7.9 ± 0.1	7.9 ± 0.1	7.9 ± 0.1
Cavity volume $(A°)^3$	174	262	472
Solubility in water (g/100 ml at 25°C)	14.40	1.85	23.20
Crystal form (from water)	hexagonal plates	monoclinic paralelograms	quadratic prisms

(a)



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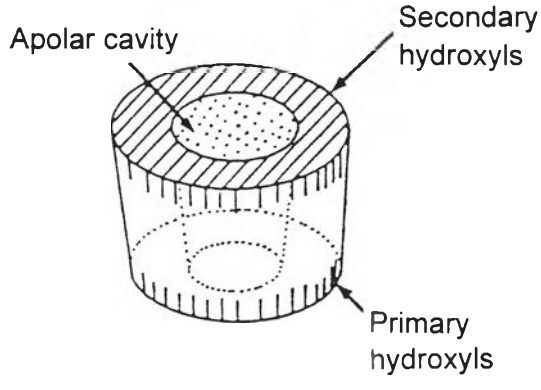

Figure 2 Structure of β - cyclodextrin. (Bender, 1986 ; Szejtli, 1990)

(a) Chemical structure ; O = oxygen atoms, • = hydroxyl groups

(b) Functional structure scheme

within the "Host" cyclodextrin is not fixed or permanent, but rather is governed by a dynamic equilibrium and thereby affording an ease of assembly and disassembly. Binding strength depends on how well the Host-Guest molecules fit together geometrically and on specific local interactions between their surface atoms.

Complexes can be formed either in solution or in the crystalline state and while water is typically the solvent of choice, inclusion complexation can be accomplished in co-solvent systems and with some non-aqueous solvents (Amaizo, 1993). Inclusion in cyclodextrins can markedly improve the chemical and physical properties of guest molecules as they are "temporarily docked or caged" within the host cavity, giving rise to the following beneficial modifications of guest molecules (see Figure 3).

A) Solubility enhancement.

B) Stabilization of labile guests against the degradative effects of oxidation, visible or UV light, and heat.

C) Control of volatility and sublimation.

D) Physical isolation of incompatible compounds.

E) Chromatographic separations.

The potential guest list for molecular encapsulation in cyclodextrins as in Figure 3, is quite varied and includes such compounds as straight or branched chain aliphatics, aldehydes, ketones, alcohols, organic acids, fatty acids, aromatics, gases, and such polar guests as the halogen and oxy-acids and amines. Additionally, some ionic binding might occur externally. Consequently, considerable interest has been generate recently to commercially exploit cyclodextrins in various industrial sectors. The applications of cyclodextrins as emulsifiers, antioxidants, and stabilizing agents

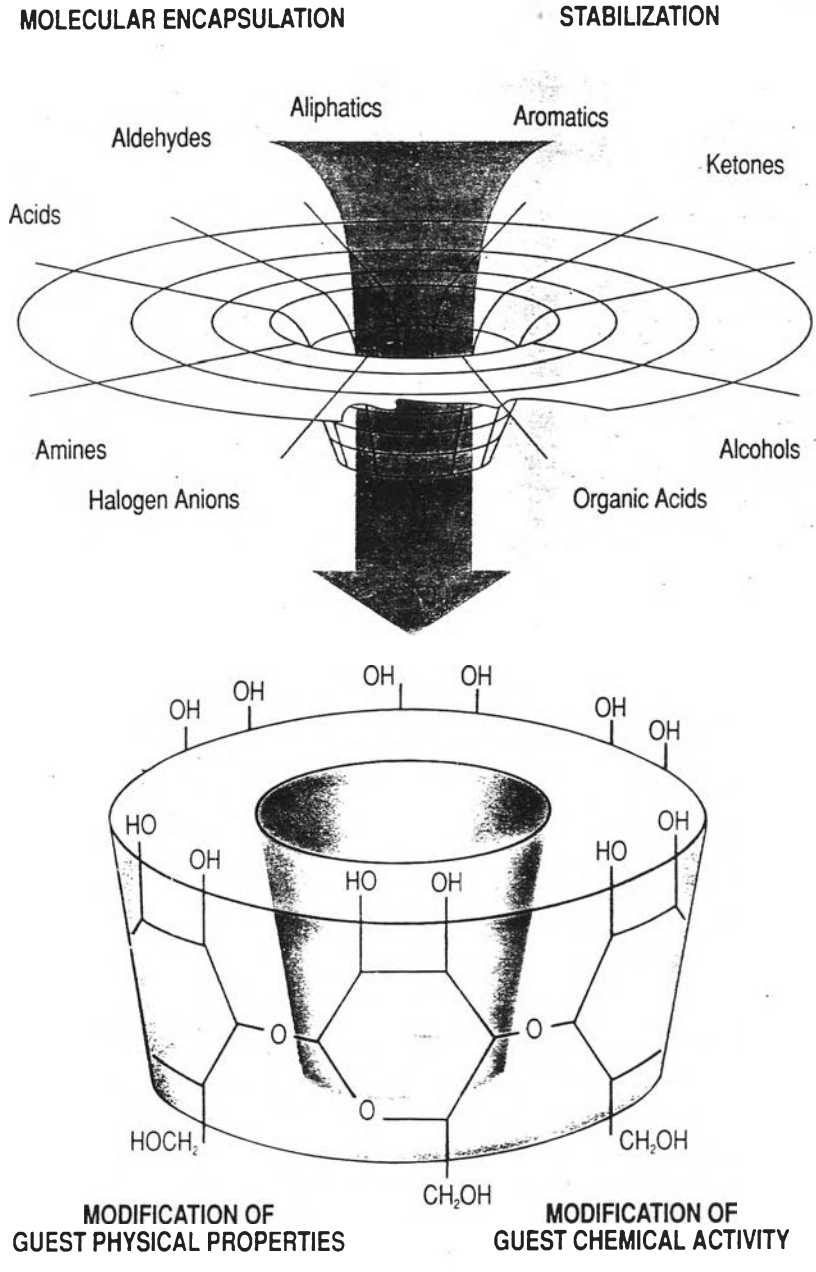

Figure 3 Beneficial modification of guest molecules by cyclodextrins.

(Amaizo, 1993)

have been rapidly increased in the food, cosmetic, pharmaceutical, agrochemical, and plastic industries (Nagatomo, 1985), see Table 2.

Cyclodextrins are not only important examples of simple natural compounds capable of complex formation, they can act as covalent or noncovalent catalysts and therefore, are useful models for enzymes (Breslow, 1984). Cyclodextrins form stereospecific complexes and can be used for the separation of enantiomers (Van den Berg and Benshop, 1970 ; Mikolajczek and Drabowicz, 1978) .

As cyclodextrins have been widely used in various industries especially in food, pharmaceutical, and cosmetics since early 1970s, many countries have approved their uses (in different levels). For example, Japan, Germany, France, Netherland, Denmark, Spain, Italy, Belgium, Hungary, USA, Taiwan have approved the use of cyclodextrins in food (Amaizo, 1991). The world market for cyclodextrins are growing, Table 3 indicates a ten-fold increase in demand during 1989-1995.

As the field of application of cyclodextrins expands, cyclodextrins with specific properties, and capable of meeting more specific needs than the parent cyclodextrins, have been developed by bonding various substances onto the parent cyclodextrins through chemical syntheses or enzymatic reactions. They are called second generation or modified cyclodextrins. Currently available cyclodextrins are listed in Table 4.

Modification of cyclodextrins through substitution of the hydroxyl groups by other functional groups may drastically alter the properties of cyclodextrins such as solubility and inclusion ability. Likewise the inclusion complexes of the methylated cyclodextrins are highly water soluble and more stable (Casu and Reggiani, 1979). Solubility in water of partial methylated β-CD of Wacker Company (Wacker, 1994) is 170 g/100 ml (25°C) which is 90 times higher than its parent, β-CD, and is 6 times

Table 2 Industrial applications of cyclodextrins. (Horikoshi, 1982 ; Bender, 1986 ; Szejtli, 1988 ; Cyclodextrin News, 1991).

Use	Guest compounds / end products
Foods	
1) Emulsification	Eggless mayonnaise, seasoning oil, whipping cream, *etc.*
2) Increase of foaming power	Egg white (freeze-dry), hotcake-mix, cake-mix, *etc.*
3) Stabilization of flavours and seasoning	Chewing gum flavour, biscuit flavour, seasoning powder, instant noodles, seasoning paste,*etc.*
4) Taste masking	meat paste
5) Reduction of hygroscopicity	Powder flavour products
6) Elimination of unpleasant tastes	Juice, milk casein, ginseng, propylene glycol
7) Elimination of cholesterol	Egg yolk, milk, butter
8) Reduction of odour	Mutton, fish, soybean
Cosmetics and toiletries	
1) Color masking and control	Fluorescein, bath agents
2) Stabilization of fragrance	Menthol
3) Stabilization	Chalcone, perfume, dihydrochalcone (toothpaste)
4) Preventing inflammation of skin	Skin lotion, sun block cream
5) Deodorant	Mouth wash, refrigerator, wash room
6) Reduction of irritation	Shampoo, cream, skin powder
7) Enhancement of attained concentration	Skin moisturizing lotion
8) Defoaming effect	Laundry
Pharmaceuticals	
1) Increase of solubility	Prostaglandin, phenobarbital, chloramphenical
2) Taste masking	Prostaglandin
3) Powdering (non-volatile)	Nitroglycerin, clofibrate
4) Stabilization (UV, thermal)	Pyrethrins, pyrethroids, isoprenoid
5) Decrease of irritation	Cu-alcanomine complex, tiamulin
6) Enhancement bioavailability	Barbiturate, flufenamic acid, digoxin
7) Reduction of systemic toxicity	DDVP, DIMEB

(continued)

Use	Guest compounds / end products
Agriculture	
1) Stabilization of volatility	Tobacco aroma
2) Stabilization of nutrient	Animal-feed
3) Improvement of palatability	Bone-powder, microbial cell-mass
Pesticides	
1) Stabilization (UV, thermal)	Pyrethrin, pyrethroid, isoprenoid
2) Powdering (non-volatile)	DDVP and other phosphorus pesticides
Chemical technology	
Catalyzation for reaction	Hydrolysis, substitution, Diels-Alder reaction, stereospecific reaction, *etc.*
Plastics	
Stabilization	Colors, flavours
Others	Adhesives

Table 3 World market of cyclodextrins.

Application	Market (ton per year)	
	1989	1995
Pharmaceutic	50	2,000
Food	700	2,500
Cosmetics	50	500
Agriculture	10	100
Chemical industry (biotransformations, separation, catalysis)	30	300
Other purposes (e.g. diagnostics)	10	200

Source : Consortium fur electrochemische Indutrie GmbH (Schmid, 1989)

Table 4 Classification of cyclodextrins. (Ensuiko, 1993)

Parent CD	Modified CD		
	Substituted CD	Branched CD	CD Polymers
α-, β-, γ-CD	methylated CD	homogeneous branched CD	- Cross-linked CDs
	- dimethylated	-glucosyl, maltosyl	- Matrix coupled CDs
	- trimethylated	Heterogeneous branched CD	
	ethylated CD	-galactosyl, mannosyl,	
	- diethylated	maltosyl	
	- triethylated		
	hydroxyalkylated CD		
	- 2-hydroxyethylated		
	- 2-hydroxypropylated		
	- 3-hydroxypropylated		

CD : cyclodextrin

better than the best solubilized parent CD, γ-CD. Hydroxypropyl-β-CD is also highly soluble and has been used in pharmaceutical (Lin *et al.*, 1990) and cosmetic industries (Matsuda et al., 1994). In addition, branched cyclodextrin that has excellent solubility in water and organic solvents has now been developed. New branched cyclodextrin (Maltosyl CD) (Figure 4) is produced from cyclodextrin and maltose by the action of an enzyme (pullulanase) which combines both compounds efficiently (Ensuiko, 1994). Maltosyl CD that has the branch of maltose has greatly improved solubility in water (170 g/100 ml, 25 °C) due to the high hydrophilicity of the maltose, and still keeps the inclusion activity comparable to ordinary cyclodextrins. Accordingly, it is a cyclodextrin product already used in various industries fields (Yamamoto, *et al.*,1990 ; Ueda, Saijo and Nagai, 1990).

Cyclodextrin producing enzymes

Cyclodextrin glycosyltransferase (1, 4 - α - D - glucan : 1, 4 - α - D-glucopyranosyl transferase, EC 2.4.1.19, CGTase) is known to catalyze the degradation of starch to form cyclodextrins. This enzyme catalyzes three possible mechanisms (cyclization, coupling and disproportionation reactions) (Kitahata and Okada, 1975) as shown in the following equations :

$$G_n \xrightarrow{\text{cyclization}} G_{(n-x)} + cG_x$$

$$G_n \xleftarrow{\text{coupling}} G_{(n-x)} + cG_x$$

$$G_m + G_n \xrightarrow{\text{Disproportionation}} G_{(m-x)} + G_{(n-x)}$$

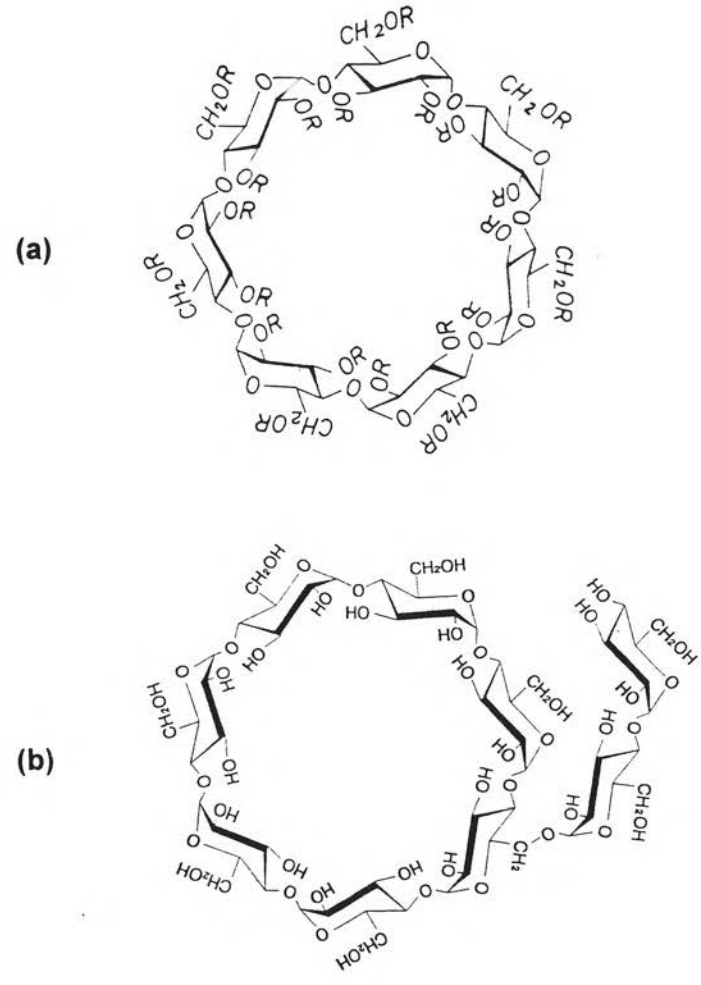

Figure 4 Modified cyclodextrins.

Figure 4 Modified cyclodextrins. (Wacker, 1994 ; Ensuiko, 1994 and Cyclodextrin News, 1987)

(a) Substituted CD : Methylated CD (R = methyl group),

Hydroxypropyl CD (R = 2 or 3-hydroxypropyl group)

(b) Branched CD : Maltosyl-β-CD

(c) CD-Polymer : Cross-linked CDs (C.1,C.2) , Matrix coupled CDs (C.3)

Where G_n and G_m are 1, 4 -α- D-glucopyranosyl chains with " n " and " m " are D - glucopyranosyl residues ; x is a part of the 1, 4 -α- D- glucopyranosyl chain, and CG_x is a symbol for CDs. These mechanisms are summarized in Table 5.

The cyclization reaction is thought to be a special type of disproportionation, the non-reducing end of one chain itself serving as acceptor, whereas the helical conformation of substrate is thought to be a prerequisite for cyclization. It should be mentioned that the acceptor binding sites of enzyme are not absolutely specific for glucose or malto-oligosaccharides (Bender,1986). The cyclization reaction is efficient for long chain substrates containing 16-80 glucopyranosyl residues. If chain length is greater than 100 units, disproportionation reaction dominates. This reaction occurs mainly at the beginning of the enzymatic reaction if long chain starch is used. Higher concentration of malto-oligosaccharides or glucose favours the reversed coupling reaction resulting in linear end products with negligible amount of CDs (Kitahara, Okada and Fukai, 1978). The action of CGTase is different from that of other starch-degrading enzymes in that the products are cyclic and non-reducing.

CGTase is produced by various microorganisms, mainly the *Bacillus* species, as listed in Table 6 (a-b). The CGTase can be divided into three types ; α -, β - and γ - according to the major type of CD formed (Horikoshi, 1988). The enzymes from different sources show different characteristics, such as working pH and temperature and molecular weight. Each CGTase enzyme yields different ratio of cyclodextrin products for example, the CGTase of *B.macerans* produces α -, β -and γ -CD in relative amount of 2.7: 1.0: 1.0 (Depinto and Campbell, 1968), while the CGTase of Alkalophilic *Bacillus* no. 38-2 produces CDs in relative ratio of 1.0: 11: 1.5 (Matzuzawa *et al*, 1975) and the CGTase of *Bacillus fermus / lentus* 290-3 was known to produce γ-CGTase in the initial phase of the enzyme production

Table 5 Summarization of CGTase mechanism. (Okada and Kitahara, 1975)

Reaction	Action
Cyclization	starch ⟶ cyclodextrins
Coupling	cyclodextrin + glucose ⟶ oligosaccharide terminated at the reducing end by the added glucose
Disproportionation	$(\text{oligosaccharide})_m$ + $(\text{oligosaccharide})_n$ ⟶ various oligosaccharides

(Englbrecht *et al*, 1990).

With regard to the isolation of the microorganism producing CGTase, species that synthesize mainly β-CD are once desirable. Since, β-CD is known to be more suitable for practical use because the inclusion complexes are easily prepared and stable due to the size of the apolar cavity being optimum for a large number of molecules such as drugs and preservatives (Horikoshi and Akiba, 1982; Horikoshi, 1979). In addition, β-CD is easily separated from the reaction mixture because of its low solubility in water. However, acquiring a γ-CD producing strain is becoming an attractive point at present. Since γ-CD is more suitable especially in pharmaceutical industry due to its higher solubility and bigger inner cavity compared with α- or β-CD. Moreover, the production of γ-CD is still a problem because very few CGTases produce γ-CD preferentially have been reported (Englbrecht *et al*, 1990) and their enzymatic properties were not suitable for large scale production. Biochemical and genetic characterizations of γ-CGTase have been performed and accumulated in order to compare with those of α- and β-CGTases. Basic knowledge obtained would facilitate construction of a genetically overproduced γ-CGTase strain or an appropriate protein engineered γ-CGTase in the near future.

Production of cyclodextrins

Various groups of researchers have tried to produce cyclodextrins. However, the production cost of cyclodextrins is still relatively high, which restricts their extensive use. For practical use, the CDs must be available at a reasonable price. Production of low cost CDs depends on both an easily available CGTase of well-known characteristics and economic methods for manufacturing the cyclic compounds.

Table 6-a Properties of cyclodextrin glycosyltransferase.

Organism	Predominant product [a]	Optimum pH (activity)	Optimum temp. (°C)	Molecular weight (dalton)	pI	Reference
Klebsiella pneumoniae M 5 al	α-CD	6.0-7.2	ND	68,000	4.8	Bender ,1982
Alkalophilic *Bacillus* 38-2 [b]	β-CD	1) 4.6 2) 7.0 3) 9.5	45-50	88,000	5.3	Nakamura and Horikoshi, 1976
Alkalophilic *Bacillus* 17-1	β-CD	6.0	ND	74,000	ND	Yamamoto *et al.*, 1972
Bacillus macerans IFO 3490	α-CD	5.0-5.7	55	65,000	4.6	Kitahata *et al.*, 1974
Bacillus megaterium	β-CD	5.0-5.7	55	ND	6.07	Kitahata and Okada, 1974
Bacillus stearothermophilus	α-CD	6.0	ND	68,000	4.5	Kitahata and Okada, 1982
Bacillus macerans IAM 1243	α-CD	5.5-7.5	60	145,000	ND	Kobayashi *et al.*, 1977
Bacillus macerans ATCC 8514	α-CD	6.2	ND	139,300	ND	Depinto and Campbell, 1986
Micrococcus sp.	β-CD	5.8	55-65	88,000	4.2	Yagi *et al.*, 1980
B. fermus/lentus 290-3	γ-CD	6-8	50	75,000	4.1	Englbrecht *et al.*, 1990

[a] Main CD produced in the initial phase of transfer reactions

[b] Three CGTases are produced having their optimum pH for activity in the acidic, neutral, and alkaline pH range

ND, no data

Table 6- b CGTase-producing bacteria. (Bender, 1986)

Organism	Cultivation mode	mg CGTase/litre culture filtrate[a]	References
Bacillus macerans	Batchwise	360-480	Miskolci-Török et al., 1980
Bacillus megaterium	Batchwise	260	Kitahata, Tsuyama and Okada, 1974
Bacillus stearothermophilus	Batchwise	ND	Kitahata and Okada, 1982a, 1982b
Bacillus circulans	Batchwise	100	Kitahata and Okada, 1982b
Bacillus ohbensis	Batchwise	24	Yagi and Iguchi, 1974
Alkalophilic *Bacillus* 38-2	Batchwise	430	Horikoshi, Ando and Yoshida, 1982 ; Nakamura and Horikoshi, 1976
Micrococcus sp.	Batchwise	199	Yagi, Kouno and Juni, 1980
Klebsiella pneumoniae M 5 al	Continuous	120	Bender, 1977a, 1977b , 1982

[a] CGTase-protein was calculated from the enzyme activities.

ND, no data

Various approaches have been made to increase the production yield of cyclodextrins.

First, the study of microorganisms producing CGTase as well as cultivation conditions yielding high amount of CGTase, and characterization of the CGTases were carried out. Table 6 summarizes the bacterial species, cultivation mode, and amount of CGTase produced (Bender, 1986). These bacteria are all inducible wild-type organisms producing CGTase when growing with starch or related compounds as the source of carbon. The CGTase from *Bacillus macerans* strains have been widely used for the production of cyclodextrins. A need for a thermostable CGTase which gives a high cyclodextrin yield has been recognized. CGTase from an alkalophilic *Bacillus* strain No. 38-2 (ATCC 21783) was observed to provide the required properties (Horikoshi and Akiba, 1982). Furthermore, overexpression of CGTase genes following cloning could significantly increases the enzyme yield. It provides a straight forward way of satisfying the anticipated expansion of the cyclodextrin market (Schmid, 1989).

Second, several methods for manufacturing the cyclic compounds from starch substrate and the enzyme cyclodextrin glycosyltransferase (CGTase) were carried out. For starch substrate, potato starch or maize starch has been generally used. In a conventional process, starch is usually liquefied by heating or treatment with hydrolyzing enzyme (pretreatment step), then the CGTase is added to synthesize cyclodextrins (production step). Partial hydrolysis improves the solubility and lowers the viscosity of the starch solution. Pretreatment of raw starch to obtain the liquefied/dissolved starch by enzymatic means using bacterial α-amylase (Vakaliu, *et al*, 1977) or thermostable CGTase (Horikoshi, Ando and Yoshida, 1982) was reported for industrial scale preparation of β-CDs. Another method of

pretreatment, the physical means of heating at 80-120°C for a certain time period (Flaschel, Landert, Renken, 1982 ; Horikoshi *et al*, 1981), was also reported for industrial scale preparation of α-CDs. In the production step : the conversion to cyclodextrins can be performed with or without complexing agent. The enhancement in cyclodextrin production by addition of organic solvents as complexing agents (e.g. cyclohexane, bromobenzene, trichloroethylene, toluene, long-chain aliphatic alcohols) and studies on the cause of the increase in yield have been reported. By adding such compounds capable of forming insoluble inclusion complexes with CDs, the equilibrium of the transfer reaction can be shifted drastically towards cyclization (French, Levine, Pazur and Norberg, 1949). For example, the Chinoin Pharmaceutical Industries patented a process for β-CD production, using the CGTase from *B.macerans* and toluene as complexing agent (Bender, 1986). One hundred and fifteen kilograms of pure β - CD were obtained from 238 kg of maize starch (48.3% yield). In addition, long-chain (> C_8) primary aliphatic alcohols are known to form nearly insoluble inclusion complexes with cyclodextrins, especially with α-CD. 1-Decanol or n-decyl alcohol (FDA -approved, permitted by the Health Council of Europe up to 5 ppm) was selected as the complexing agent (Flashel, Landert, Renken, 1982). Using the CGTase of *K.pneumoniae* and n-decyl alcohol, 41 % of soluble potato starch was obtained as crude cyclic product. Moreover, the addition of bromobenzene (solubility of the γ-CD-bromobenzene complex 0.1 g/l at 20°C) in suitable conversion conditions will render crude γ-CD at 14-17 % of the potato or maize starch (Bender, 1983). Therefore, selective cyclodextrin conversion and the yield were affected by complexing agent and conditions used. Otherwise, cyclodextrin production can be performed without the use of organic solvents and further purified by chromatographic methods, and by

crystallization. For instance, production of the cyclic compound by the CGTase of alkalophilic *Bacillus* 38-2 from potato starch yielded 19 % of pure β-CD crystals which are commercially available by Nihon Shokuhin Kako Co. Furthermore, Nihon Shokuhin Kako Co. also patented a process for the production of α-CD by using the CGTase of *B. macerans*, 27 % of substrate is converted into α-CD (Bender, 1986).

Moreover, the technique of enzyme immobilization has been rapidly developed, and investigations on the preparation of immobilized CGTase (ICGTase) have been reported (Nakamura and Horikoshi, 1977 ; Hashimoto, Hara, and Kuwahara, 1986, Steignardt and Kleine, 1993 ; Rutchtorn, 1993 ; Kuttiarcheewa, 1994). Using ICGTase in cyclodextrin production has the advantage of recovery of enzyme, higher yield of cyclodextrins and continuous operation. However, the investment cost is still higher than using the free enzyme. Report on using ICGTase in industrial production of cyclodextrins has not yet been available.

By scaling-up the production processes, the prices of cyclodextrins dropped drastically during the last two decades (e.g. 1975: $ 1500 /kg β-CD ; 1985: $ 7-10 /kg β-CD ; 1995 $ 5-10 /kg β-CD). At present, about 60 tons per year of β-CD are produced by the Japanese company : Nihon Shokuhin Kako Co. and Hungarian company : Chinoin Pharmaceutical Industries. It is also planned to produce α-CD and γ-CD of relatively low cost on an industrial scale (1986 prices : approx. $ 85 /kg α-CD ; approx. $ 850 /kg γ - CD) (Bender, 1986).

Objectives of the thesis

Thailand has long been known as an agricultural based country and rice starch is one of our top agricultural products. To turn raw rice starch (≅ $ 1 /kg)

into cyclodextrin, a value-added product (Chemical grade : $ 400-2000 /kg ; Industrial grade : $ 8-80 /kg) (Pongsawasdi, 1994) is obviously an advantage. Moreover, it would be of interest and economized to replace commercial soluble starch with local starch to lower the cost of cyclodextrin production.

This research work continues from the cyclodextrin group's work of Biochemistry Department, Faculty of Science, Chulalongkorn University. We had screened more than 70 strains of microorganisms from South-East Asian soil, and selected *Bacillus* sp. A 11 which was found to be one of the best strains, producing high amount of CGTase (Pongsawasdi and Yagisawa, 1987; Techaiyakul, 1991). We found that the CGTase produced was mainly β-CGTase (Techaiyakul, 1991). Our group had also studied the optimization of CGTase production in 5 liter-fermenter (Rutchtorn, 1993) and we had tried to produce cyclodextrins by using immobilized CGTase in both batchwise and continuous production (Rutchtorn, 1993 ; Kuttiarcheewa, 1994). The present study aims at production of cyclodextrins by using free CGTase. The focus will be on the development of efficient cyclodextrin production from rice starch. The objectives of the present thesis cover the following schemes :

1. Study the pretreatment of starch in order to obtain the suitable material for cyclodextrin production.
2. Optimize the ratio of CGTase to starch substrate.
3. Observe the use of complexing agents on cyclodextrin production.
4. Study the procedure of product separation.