CHAPTER I

INTRODUCTION

Cyclodextrins

Cyclodextrins (celluloseine, cycloamylose, cyclomaltose, schardinger dextrin: CD) are cyclic-oligosaccharides of glucoses linked by α-1,4-glucosidic bonds. They are named α-, β-, and γ- CDs, according to the number of six, seven, or eight glucose molecules, respectively, as shown in Figure 1 (Schardinger, 1903, 1904; French *et al.* , 1942, 1949; Pulley and French,1961).

Some physical properties of cyclodextrins are summarized in Table 1 (Szejtli, 1988). 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 molecules. The C6 primary hydroxyls on the narrower side and the C2 and C3 secondary hydroxyls accupying the wider side. These oreintation make cyclodextrin molecules hydrophilic on the outside. The hydrophobic inside cavity of cyclodextrins is lined with C-H groups and glycosidic oxygen bridges (Figure 2) (Saenger, 1979, 1982; Bender, 1986). γ- CD, having bigger cavity and more flexible characteristic is more soluble than α- and β- CDs, respectively. The C2 and C3 hydroxyl groups of the nearby glucose unit in the molecule of β- CD can form seven hydrogen bonds, called the secondary belt, that is why β- CD is the most stable but the lowest soluble

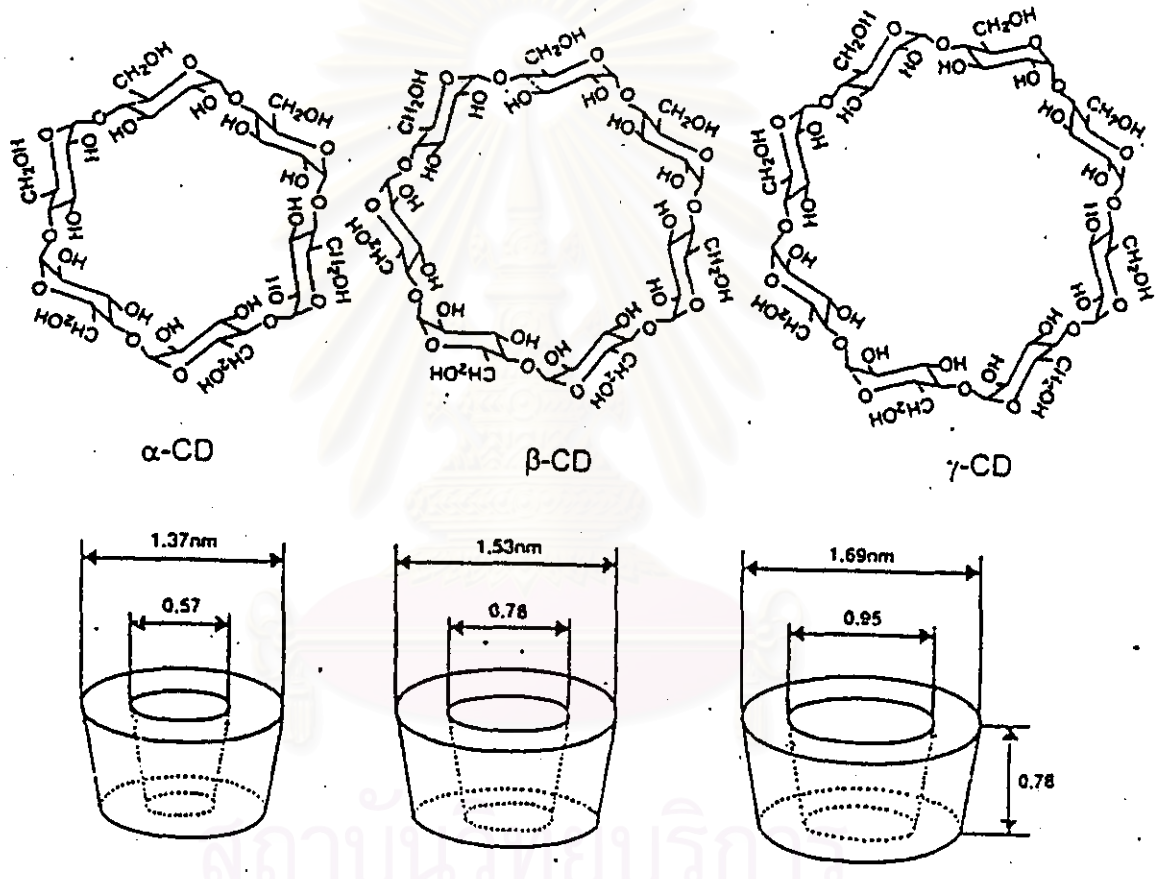


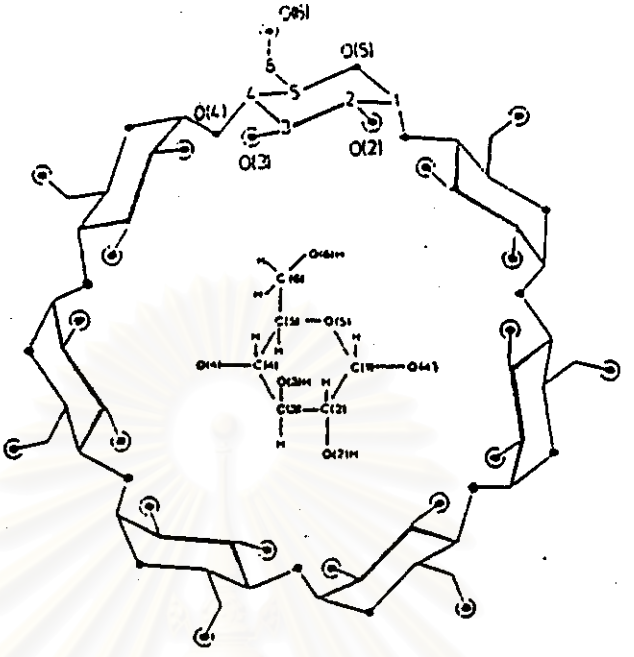
Figure 1 Structure and molecular dimension of cyclodextrins (CDs)

(Szejtli, 1990)

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	1135	1297
Solubility in water g/100 ml at ambient temperature	14.5	1.85	23.2
Cavity diameter A^0	4.7-5.3	6.0-6.5	7.5-8.3
Height of torus A^0	7.9+0.1	7.9+0.1	7.9+0.1
Volume of cavity (approx.) $(A^0)^3$	174	262	472
Cavity vol.(approx.);			
per mol CD (ml)	104	157	256
per g CD (ml)	0.10	0.14	0.20
Crystal form (from water)	hexagonal plates	monoclinic paralellogram	quadratic prisms
Crystallographic parameters			
C_1-O_4-C_4 angle (^{0})	119.0	117.7	112.6
ϕ/(^{0})	116/-169	169/-172	165/-169
O_4...O_4, distance A^0	4.23	4.39	4.48
O_2...O_3, distance A^0	3.00	2.86	2.81
Crystal water (wt %)	10.2	13.2-14.5	8.13-17.7
Diffusion constant 40(^{0}C)	3,443	3,224	3,000
Hydrolysis by *A. oryzae* α-amylase	negligible	slow	rapid
Partial molar volumes in solubility	611.4	703.8	801.2

(a)



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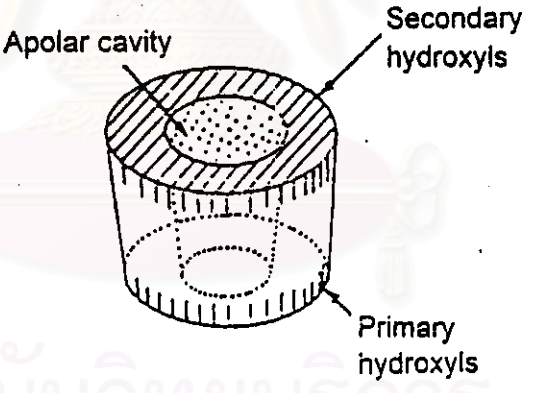

Figure 2 Structure of β- cyclodextrin (Bender, 1986; Szejtli, 1990)

(A) Chemical structure; 0 = oxygen atoms, ● = hydroxyl groups

(B) Functional structure model

form. α- CD has a glucose unit in a distorted position and can form only four hydrogen bonds. (Szejtli, 1988).

The cavity of cyclodextrins are filled with water molecules. It can also form three-dimensional inclusion complex in the solid state or in solution, which consists of guest molecules (small hydrophobic molecules) held in the cavity of the host (cyclodextrin) (Saenger, 1980; 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 (Komiyama and Bender, 1984). The guests which can be encapsulated in cyclodextrins, include such compounds as straight or branched chain hydrocarbons, gasses, and some relatively polar compounds, as shown in Figure 3 (Amaizo, 1993) and Figure4 (Janssen, 1992).

β- CD is earlier known to be more suitable for practical use because the inclusion complexes are easily prepared and more stable due to the size of the apolar cavity being optimum for a large varity of guest molecules (Horikoshi and Akiba, 1992; Horikoshi, 1979). In addition, it can be easily separated from the reaction mixture because of its low solubility in water. At present, γ- CD is becoming an attractive molecule especially in pharmaceutical industry due to its higher solubility and bigger inner cavity. However, the production of γ- CD is still a problem because very few CGTases preferentially produced γ- CD have been reported (Englbrecht *et al.*, 1990).

Several cyclodextrin derivatives have been developed through chemical or enzymatic means in order to obtained CDs with specific desirable properties. Examples are those with solubility better than parent

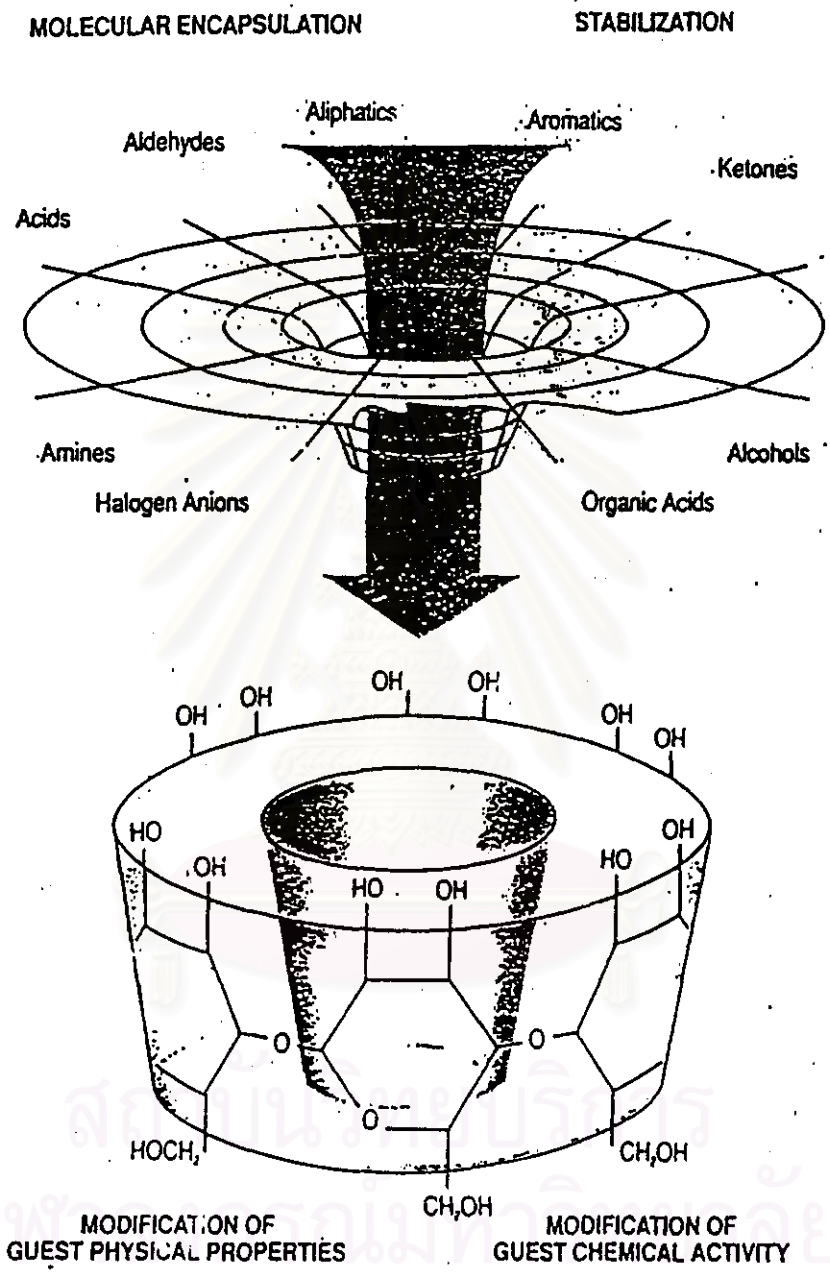

Figure 3 Inclusion complex formation between CDs and variable guest leading to modification of guest physical and chemical properties (Amaizo, 1993)

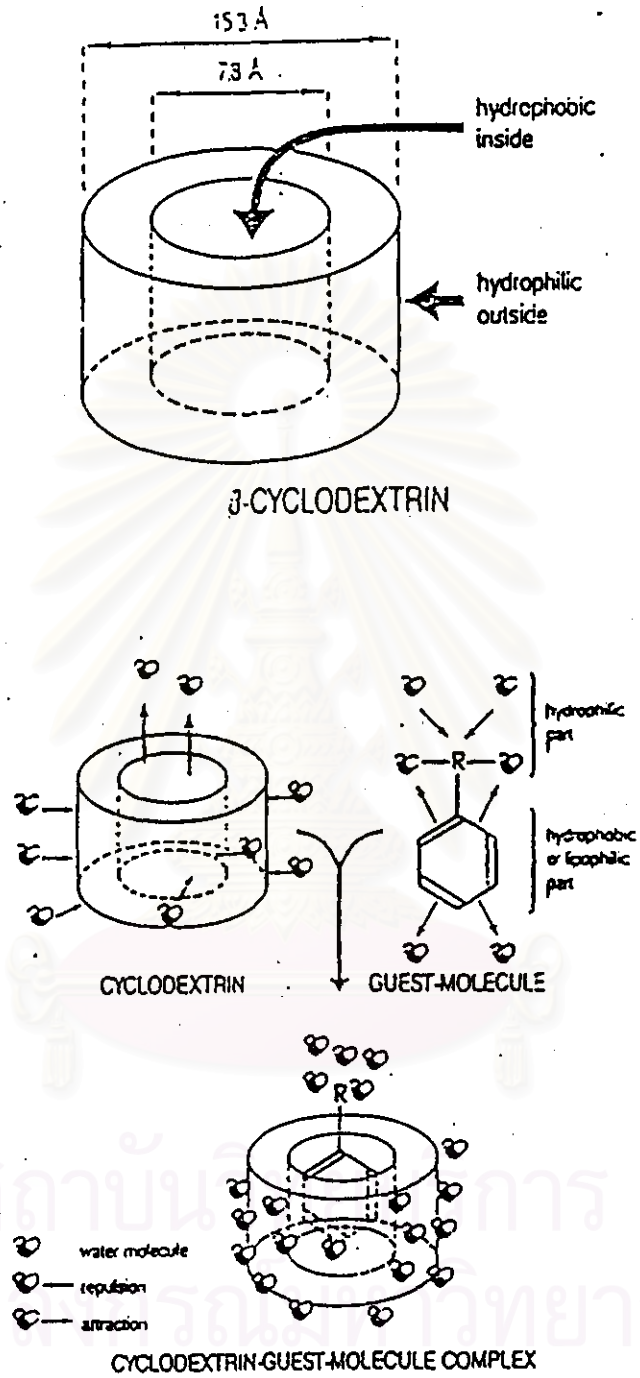

Figure 4 Guest orientation in CD-guest complex (Janssen, 1992)

compounds e.g. methylated, hydroxypropylated, and maltosyl-cyclodextrins (substitution of the hydroxyl groups by methyl, hydroxypropyl, and oligosaccharides, respectively). CD-polymers (linked cyclodextrins) are used often as stationary phase in various liquid chromatography system. (Casu and Roggiani, 1979; Ensuiko, 1994; Yamamoto et al., 1990). These modified CDs, in addition to their native or parental CDs (the α-, β-, and γ-CD), can be chosen according to their properties to be used as the suitable host molecules.

Complex formation of cyclodextrins and guest molecules leads to the change in the physical or chemical properties of guest molecules. Protection against oxidative degradation or destruction by UV light, improvement of the solubility of hydrophobic substances in aqueous solution, stabilization of volatile compounds, alternation of the chemical reactivity, modification of liquid substances to powders, or reduction of undesirable smell or taste in products e.g. foodstuffs are among those known useful properties (Schmid, 1989).The applications of cyclodextrins as emulsifiers, antioxidants, and stabizing agents have rapidly increased in food, cosmetics, pharmaceutical, agrochemical, and plastic industries (Table 2 and 3) (Nagamoto,1985). Since early 1970s, many countries, for example, Japan, Germany, France, Netherland, Denmark, Spain, Italy, Belgium, Hungary, USA, and Taiwan, have approved the use of cyclodextrins (at different levels) in several fields of industries. (Amaizo, 1991) .

Table 2 Industrial applications of cyclodextrins
(Horikoshi, 1982; Bender, 1986; Szejtli and Pagington, 1991)

Use	Guest compound/ end product
Food	
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 power, 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 agent
2) Stabilization of fragrance	Menthol
3) Stabilization	Chalcone, dihydrochalcone (toothpaste), perfume
4) Preventing inflammation of skin	Skin lotion, sun block cream
5) Deodorant	Mouth wash, refrigerator,
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

Table2 (continued)

Use	Guest compound/ end product
2) Taste masking	Prostaglandin
3) Powdering (non-volatile)	Nitroglycerin, clofibrate
4) Stabilization (UV, thermal)	Pyrethrins, pyrethroids, isoprenoid
5) Decrease irritation	Cu-alcanomine complex, tiamulin
6) Enhancement bioavailability	Barbiturate, flufenamic acid, digoxin
7) Reduction of systemic toxicity	2-amino,4-methyl-phosphynobutyric acid
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)	Pyrethrins, pyretenoids, isoprenoid
2) Powdering (non-volatile)	2-amino,4-methyl-phosphyno-butyric acid and other organic phosphorus pesticides
Chemical technology	
Catalyzation for reaction	Products of hydrolysis, substitution, Diels-Alder reaction, stereospecific reaction, etc.
Plastic	
Stabilization	Colors, flavours
Others	Adhesives

Table 3 The uses of cyclodextrins in Japan during 1984-1987 (tons)
(Hashimoto, 1988)

Year	Manufac turing process	Foods	Pharma ceuti cals	Cosmetics	General indus tries	Others	Total
1984	3	16	33	2	15	7	76
1985	10	35	18	2	41	3	109
1986	20	20	31	11	50	8	145
1987	15	17	18	10	35	4	99
Total	48	88	105	25	141	22	429

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,

$$Gn \underset{\text{coupling}}{\overset{\text{cyclization}}{\rightleftharpoons}} G(n-x)+cGx$$

$$Gm+Gn \xrightarrow{\text{disproportionation}} G(m-x)+G(n-x)$$

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

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 site of the enzyme is not absolutely specific for glucose or maltooligosaccharides (Bender, 1986). The cyclization reaction is efficient for long chain substrate containing 16-80 glucopyranosyl residues. If the chainlenght is greater than 100 units, disproportionation reaction

Table 4 Summarization of CGTase mechanisms
(Okada and Kitahara, 1975)

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

Table 5 Relationship between lenght of substrate and mechanism of CGTase (Szejtli, 1988)

Substrate chainlenght (residues)	Effect on mechanism of CGTase
1(D-glucose)	- no catalysis
2-4	- inhibit initial reaction of cyclization - substrate for coupling reaction
5-14	- good substrate for coupling reaction - poor substrate for disproportionation reaction
16-80	- good substrate for cyclization reaction
> 100	- good substrate for disproportionation reaction

dominates. The relationship between chain-length of substrate and reaction of CGTase is summarized in Table 5. Higher concentration of maltooligosaccharides or glucose favours the reversed coupling reaction resulting in linear end products with negligible amount of cyclodextrins (Kitahata, 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.

Model of mechanism for the cyclization has been postulated, that CGTase binds eight to ten (or even more) glucose units of a starch molecule. The active site of CGTase thus consisted of eight to ten (or more) subsites. The reaction is an exoattack on glucose chains from the non- reducing ends. The resulting maltohexaose intermediate is bound to an aspartyl group of enzyme by ester bond. The non-reducing end of the maltohexaose subsequently binds to subsite two and new α-1,4 glycosidic bond is formed between glucose residues one and six of maltohexaose, as shown in Figure 5 (Bender, 1988).

CGTase is produced by various organisms, for example *Klebsiella pneumoniae* (Bender, 1977), *Brevibacterium sp.* (Mori *et al.*, 1994), and mainly the *Bacillus sp.* (Bender, 1986; Komitani *et al.*, 1993), as listed in Table 6 and Table 7. 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 showed different characteristics, such as working pH, temperature, and molecular weight. Each CGTase yields different ratio of CD products for example, the CGTase of *B. macerans* produced α-, β-, and γ-CD in relative amount of 2.7: 1.0: 1.0 (Depinto and Campbell, 1986), while the CGTases of Alkalophilic *Bacillus sp* no. 38-2

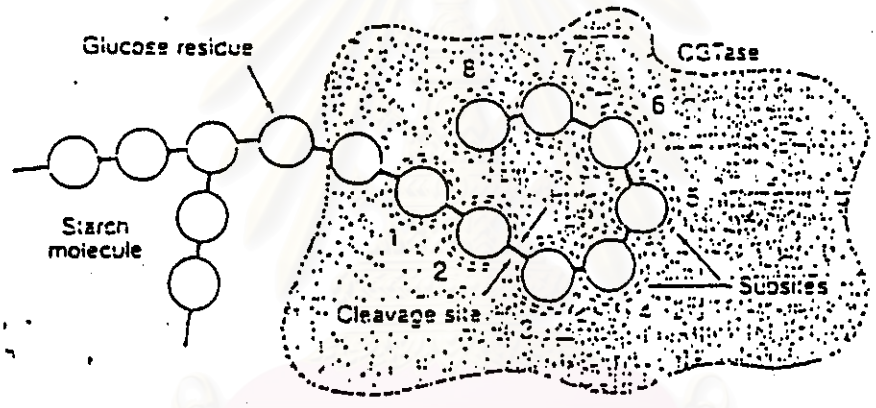

Figure 5 Model of CGTase mechanism from *Bacillus oxytoca* M5a1 (Bender, 1988)

Table 6 Properties of cyclodextrin glycosyltransferases

Organism	Predominant product [a]	Optimum pH (activity)	Optimum temperature (^{0}C)	Molecular weight (dalton)	pI	Reference
Klebsiella pneumoniae M 5aI	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	5,000	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 Cambell, 1986
Micrococcus sp.	β-CD	5.8	55-65	88,000	4.2	Yagi *et al.*, 1980

Table 6 (continued)

Organism	Predominant product [a]	Optimum pH (activity)	Optimum temperature (^{0}C)	Molecular weight (dalton)	pI	Reference
Bacillus fermus/lentus 290-3	γ-CD	6.0-8.0	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 ranges
ND = no data

Table 7 CGTase-producing bacteria (Bender, 1986)

Organism	Cultivation mode	mg CGTase/litre culture filtrate[a]	Reference
Bacillus macerans	Batchwise	360-480	Miskolci-Torok *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 5aI	Continuous	120	Bender, 1977a, 1977b, 1982

(a) = CGTase-protein was calculated from the enzyme activities.
ND = no data

and *B. circulans* produced cyclodextrins in relative ratio of 1.0: 11.0: 1.5 (Matzuzawa *et. al*, 1975) and 1.0: 10.0: 1.0 (Pongsawasdi and Yagisawa, 1987), respectively. The CGTase of *Bacillus fermus*I *lentus* 290-3 was known to produce γ- CGTase in the initial phase of the enzyme production (Englbrecht *et. al*, 1990).

Studies on amino acid sequences of CGTases were deduced from determination of nucleotide sequences and amino acid sequencer (only about 20-30 residues from N-terminal end) (Makela *et al.*, 1988; Hamamoto *et al.*, 1987). Studies on amino acid compositions were determined directly from amino acid analyzer (Schmid *et al.*, 1988; Takano *et al.*, 1986; Kimura *et al.*, 1987; Binder *et al.*,1986). CGTase genes from various microorganisms consisted of 2,100-2,800 bases encoding the CGTases of 680-690 amino acids. These enzymes are homologous with 60-80% identical amino acid residues and molecular weights around 66,000-80,000 daltons.

The amino acid compositions of Bacillus CGTases are presented in Table 8. Cysteine cannot be detected in CGTase produced from *B. circulans* and *B. macerans* while a few can be detected in CGTases from other microorganisms. Asx (aspartic and asparagine) was the most in (100-120 residues) CGTases (Bovetto *et al.*, 1992).

N-terminal sequences of 20 amino acids of 8 CGTases from Bacillus strains and *Klebsiella pneuminiae* are shown in Table 9. A close homology exists among the sequences of all CGTases from Bacillus strains, whereas little coherence between them and CGTase from *K pneumoniae* exists.

Table 8 Amino acid compositions of CGTases from *Bacillus* strains

Amino acid	residues per mole of purified enzyme			
	***B. circulans* E192**	***B. macerans***	***B. alkalophilus***	***B. circulans* strain8**
Asx	119	101	110	107
Gsx	37	40	48	33
Ser	45	47	39	53
Gly	67	79	64	59
His	12	9	12	12
Thr	78	67	58	75
Arg	8	21	23	18
Ala	74	57	57	59
Pro	25	24	29	23
Tyr	28	34	34	32
Val	45	49	48	49
Met	9	12	14	11
Cys	0	0	2	2
Ile	37	36	43	33
Leu	40	40	36	37
Phe	40	32	32	37
Trp	19	13	13	14
Lys	33	26	23	30
total	716	687	685	684
Reference	Bovetto *et al.*, 1992	Takano *et al.*, 1986	Kaneko *et al.*, 1988	Nitschke *et al.*, 1990

Table 9 Comparison of 20 amino acid sequences at the N-terminal of CGTases from various bacterial strains

Bacterial strains	1				5					10					15					20	Reference
Bacillus sp. 38-2	A	P	D	T	S	V	S	N	K	Q	N	F	S	T	D	V	I	Y	Q	I	Kaneko *et al.*,1988
Bacillus sp. 1011	A	P	D	T	S	V	S	N	K	Q	N	F	S	T	D	V	I	Y	Q	I	Kimura *et al.*, 1989
Bacillus sp. 17-2	A	P	D	T	S	V	S	N	K	Q	N	F	S	T	D	V	I	Y	Q	I	Kaneko *et al.*, 1989
Bacillus macerans	S	P	D	T	S	V	S	N	K	Q	N	F	S	T	D	V	I	Y	Q	I	Takeno *et al.*, 1986
Bacillus circulans	D	P	D	T	A	V	T	N	K	Q	S	F	S	T	D	V	I	Y	Q	V	Bovetto *et al.*, 1992
Bacillus licheniformis	D	A	D	T	A	V	T	N	K	Q	N	F	S	T	D	V	I	Y	Q	V	Hill *et al.*, 1990
Bacillus ohbensis	D	V	T	N	K	V	N	Y	T	R	D	V	I	Y	Q	I	V	T	D	R	Sin *et al.*, 1991
Bacillus stearothermopholus	A	G	N	L	N	K	V	N	F	T	S	D	V	V	Y	Q	I	V	V	D	Fujiwara *et al.*, 1992
Klebsiella pneumoniae	A	E	P	E	E	T	Y	L	D	F	R	K	E	T	I	Y	F	L	F	L	Binder *et al.*, 1986

The three-dimensional structure of CGTase from X-ray crystallographic technique showed that CGTase consisted of five domains, labeled A to E. It is similar to amylase at A, B, and C domains, but amylase lacks the additional D and E domains that are unique for CGTase. Although CGTase and α-amylase showed a low overall degree of similarity in amino acid sequence, the N-terminal domain of CGTase contains three distinct regions also present in α-amylase. The E domain of CGTase from *B. circulans* strain 251 was found to bind two maltoses at putative raw starch binding site and a third maltose was observed to bind at the C domain (Lawson *et al.*, 1994). These regions have been proposed to form the active center of the amylase enzyme. The same regions were also found in isoamylase and pullulanase but not in glucoamylase or β-amylase. There are about 95 amino acids at C-terminal ends of CGTase which shows similarity to C-terminal end of the glucoamylase of *Aspergillus niger* (Schmid, 1989).

Although, potential application of cyclodextrins in industry is well known, but the market for cyclodextrins is still limited due to high cost and the availability of α- and γ- cyclodextrins. Various studies have been emphasized on improvement of cyclodextrin production. Development of cultivation for cyclodextrin over-production was carried out under optimized culture condition and with complex nutrient media. 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 this required properties (Horikoshi and Akiba, 1982). The immobilized CGTase can be utilized in several conversion cycles to steadily increase the volume of production, hence

reduce the production cost (Nakamura and Horikoshi, 1976; Kato and Horikoshi, 1984; Yang and Su, 1989). Protein engineering, site-directed mutagenesis, and gene cloning were also used to increase the yield of enzyme product. These techniques were based partly on an assumption of lower production costs, but partly also on the trends towards greater acceptability of cyclodextrins (Schmid, 1989). Studies on molecular cloning and mapping of CGTase gene were summarized in Table 10 and Figure 6.

Gene cloning and over-expression of CGTase gene, not only provides satisfying cyclodextrin production, but also provides more enzyme for studies on structure and mechanisms including determination of its nucleotide sequence. For examples, the CGTase gene from *K. pneumoniae* and Alkaliphilic *Bacillus* sp. 1-1 was each placed under tac promotor and was cloned into mutant *E. coli*. The expression of the gene was higher than wild type, and when the gene was cloned into *B. subtilis*, the activity was even higher than in *E. coli* (Bender, 1986; Schmid, 1989). The CGTase gene from *B. circulans* ATCC 21783 was inserted into pUB110 and was cloned into *B. subtilis*. The expression of the gene was very low. The gene was then placed under the control of the α-amylase promotor, which resulted in 100 fold increase in activity (Paloheimo *et al.*, 1988). The CGTase from Alkalophilic *Bacillus sp.* strain 1011 was cloned into *E. coli* and the activity obtained was very low. The gene was then placed under three promotors; lac promotor, trp promotor, and P_L promotor. It was found that, trp promotor was higher expressed than lac and P_L promotors (Kimura *et al.*, 1990). The CGTase from Alkalophilic *Bacillus sp.* no.38-2 was cloned into *E. coli* and *B. subtilis*; and it was

Table 10 Molecular cloning of CGTase genes

Bacterial strains	CGTase type	Host	Plasmid	DNA insert sizes (kb)	ORF* (kb)	Reference
Bacillus ohbensis	β	*B. subtilis*	pUP110Ce-CGTase pUP110Ce-CGT degQ	4.8	2.1	Sin *et al.*, 1992
Alkalophilic *Bacillus sp. No.17-1*	β	*E. coli*	pUP1	5.5	2.9	Kaneko *et al., 1989*
Bacillus circulans var. alkalophilus ATCC21783	β	*B. subtilis*	pAKL153 pAKL156 pAKL159	5.3	3.0	Paloheimo *et al., 1992*
Bacillus subtilis No.313	γ	*E. coli*	pMT2	2.8	-	Kato *et al.*, 1989

ORF*= Open reading frame

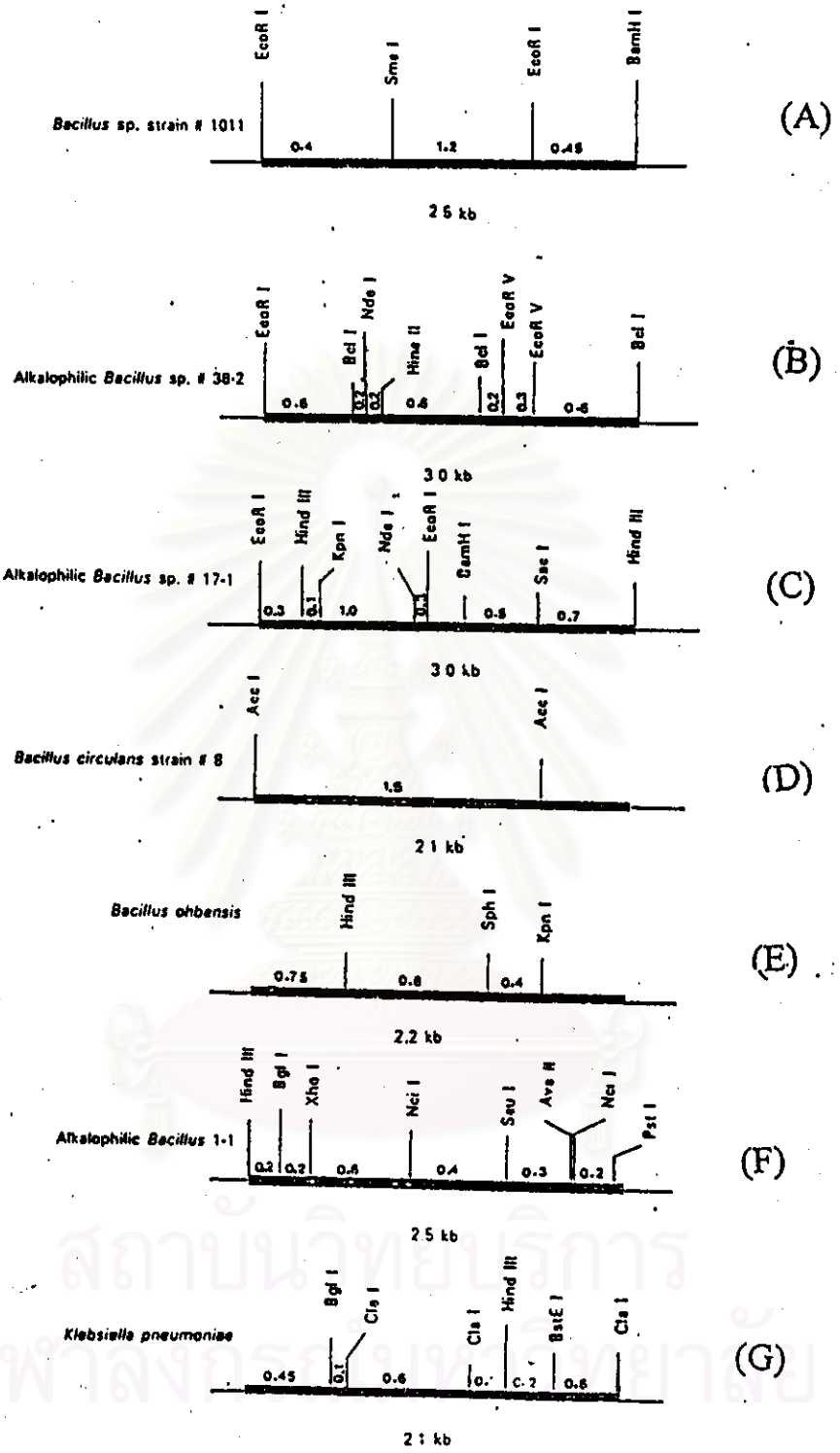

Figure 6 Restriction map of CGTase genes from some CGTase producing bacteria (A) Kimura *et al.*,1989 (B) Sin *et al.*, 1991 (C) Kancko *et al.*, 1988 (D) Schmid *et al.*, 1988 (E) Kaneko *et al.*, 1989 (F) Binder *et al.*, 1986 (G) Nitschke *et al.*, 1990

found that *E. coli* mutant gave higher activity than *B. subtilis* mutant (Georgia *et al.*, 1991). When the CGTase gene of *B. macerans* was inserted into *B. subtilis* and *B. bravis*; the latter system gave higher activity (Toshiya *et al.*, 1991).

Our cyclodextrin research group in the Department of Biochemistry have been working on β-CGTase of *Bacillus sp.* A11, a strain isolated from South-East Asian soil (Pongsawasdi and Yagisawa, 1987). The enzyme was purified and characterized (Techaiyakul, 1991; Rojtinnakorn, 1994). Specific antibody against CGTase was prepared (Rojtinnakorn, 1994) and was used in enzyme purification through immunoaffinity column chromatography (Kim, 1996). Siripornadulsil(1992), Vittayakitsirikul (1995), and Boonchai (1996) reported on molecular cloning techniques, gene expression, mapping and partial nucleotide sequence determination, but there were problems on identifying CGTase gene. Specific oligonucleotide probes for CGTase gene, will be helpful since they can be used to confirm the gene. Hybridization techniques, based on the specific interaction between the DNA template and oligonucleotide probes (labelled with non-radioactive or radioactive), such as southern blot hybridization, dot blot hybridization, and colony hybridazation, have proved to be highly specific and efficient means for identification and selection of target gene.

With the main focus of the work on the synthesis of oligonucleotide probes for CGTase gene from *Bacillus* sp.A11, the N-terminal amino acid of the purified enzyme will be determined. The nucleotide sequence will be deduced from the amino acid sequence and the oligonucleotide will be synthesized and used as a probe to detect and clone DNA fragments,

containing the CGTase gene by colony hybridization technique. The DNA fragment detected will be characterized concerning its size and sequence.