CHAPTER I

INTRODUCTION

Cyclodextrin glycosyltransferase (CGTase; 1,4-α-D-glucan 4-α-D-(1,4-α-D-glucano)-transferase : E.C.2.4.1.19) degrades starch by catalyzing cyclization , coupling, and disproportionation reactions as illustrated below (Bender, 1986)

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

$$\text{glc(m)} + \text{glc(n)} \underset{}{\overset{\text{disproportionation}}{\rightleftharpoons}} \text{glc(m+x)+glc(n-x)}$$

glc(m) and glc(n) = 1,4-α-D-glucopyranosyl chains

m and n = D-glucopyranosyl residues

x = 6 α-cyclodextrin

x = 7 β-cyclodextrin

x = 8 γ-cyclodextrin

The cyclization reaction produces three main types of cyclodextrins (CDs) known as α-,β-and γ-forms with 6,7 and 8 glucose residues respectively, linked by α-1,4 linkages (Fig.1.1). Three dimensional structures of CDs reveal that CDs possess hydrophobic cavities surrounded by hydrophilic belts of the sterically positioned hydroxyl groups of glucose (Fig.1.2). The property leads to the ability to form complex with hydrophobic substances. Complex formation is, in effect, molecular encapsulation, and this results in many advantages for the trapped molecules (Schmid, 1989), for examples

- protection against oxidative degradation or destruction by UV light,
- improvement of the solubility of hydrophobic substances in aqueous solutions,
- stabilization of volatile compounds,
- alteration of the chemical reactivity,
- modification of liquid substances to powders, and
- improvement of bad smell and taste.

Clearly, complexation with CDs is beneficial for the practical use of a large number of sophisticated compounds, and opens up many applications in the pharmaceutical, food, cosmetic and chemical industries and in agriculture. CDs' uses in foods and drugs are supported by the finding that their toxicity is low. The Ministry of Health and Public Welfare in Japan have permitted the use of β-cyclodextrin and its mixtures in food as the stabilizer of flavors and pigments, anti-oxidants and emulsifiers (Horikoshi

and Akiba, 1982).

Although the potential of cyclodextrins is well documented by more than 3,000 publications and patents, the market for cyclodextrins is still limited as shown in Table 1.1 (Schmid, 1989). Table 1.1 also indicates an expected seven-fold increase in the demand for cyclodextrins in the year 1995 A.D. The chemical and industrial grade of cyclodextrins currently cost $ 400-2000 kg^{-1} and $ 8-80 kg^{-1}, respectively (Pongsawasdi , 1994). This projection is based partly on an assumption of lower production cost, and partly on the trends towards greater acceptability of cyclodextrins.

Low cost processes for the production of cyclodextrins can be developed by using gene cloning technique. Gene cloning and overexpression of CGTase genes provide a straight forward way of satisfying the anticipated expansion of the cyclodextrin market. The growing economic importance of CGTase is also reflected in the magnitude of the supporting research effort. In the past, many CGTase genes have been cloned from bacterial sources,for example in Table 1.2 It is expected that the large-scale production of CGTase, rendered possible by gene over-expression, will significantly influence the world demand for cyclodextrins.

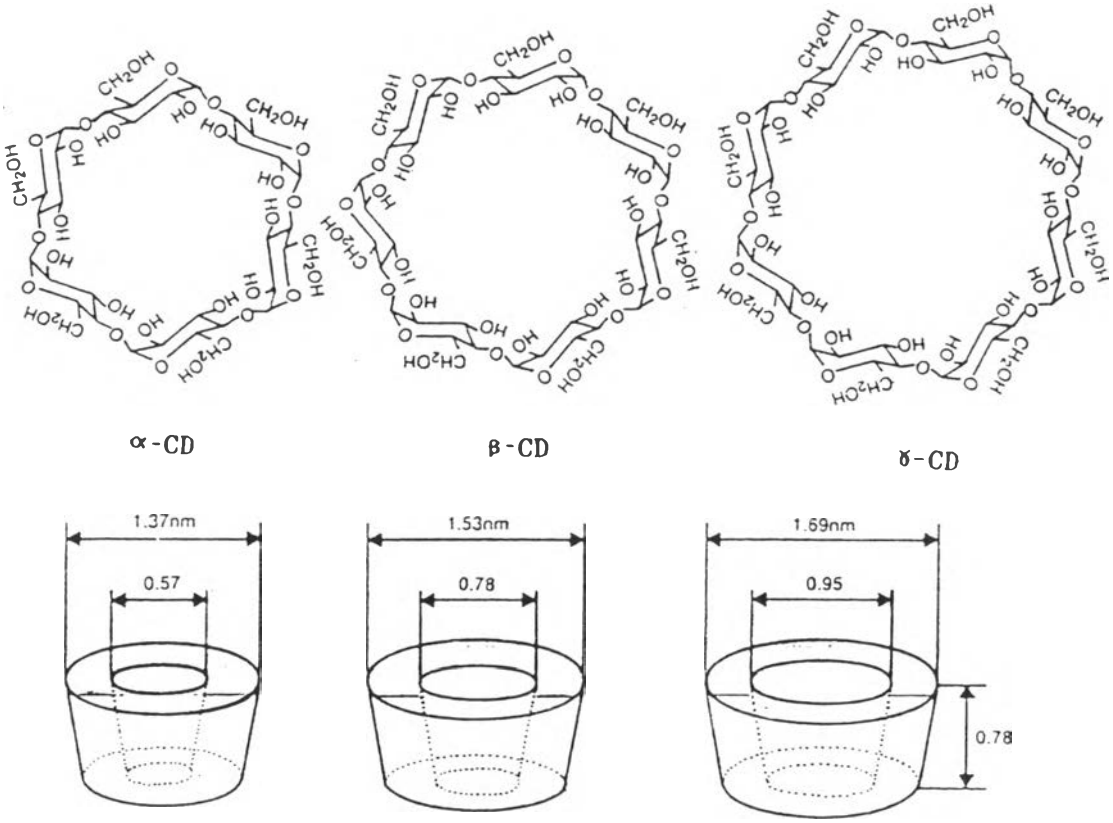

Figure 1.1 Structure of α-, β- and γ-cyclodextrins (Szejtli, 1988)

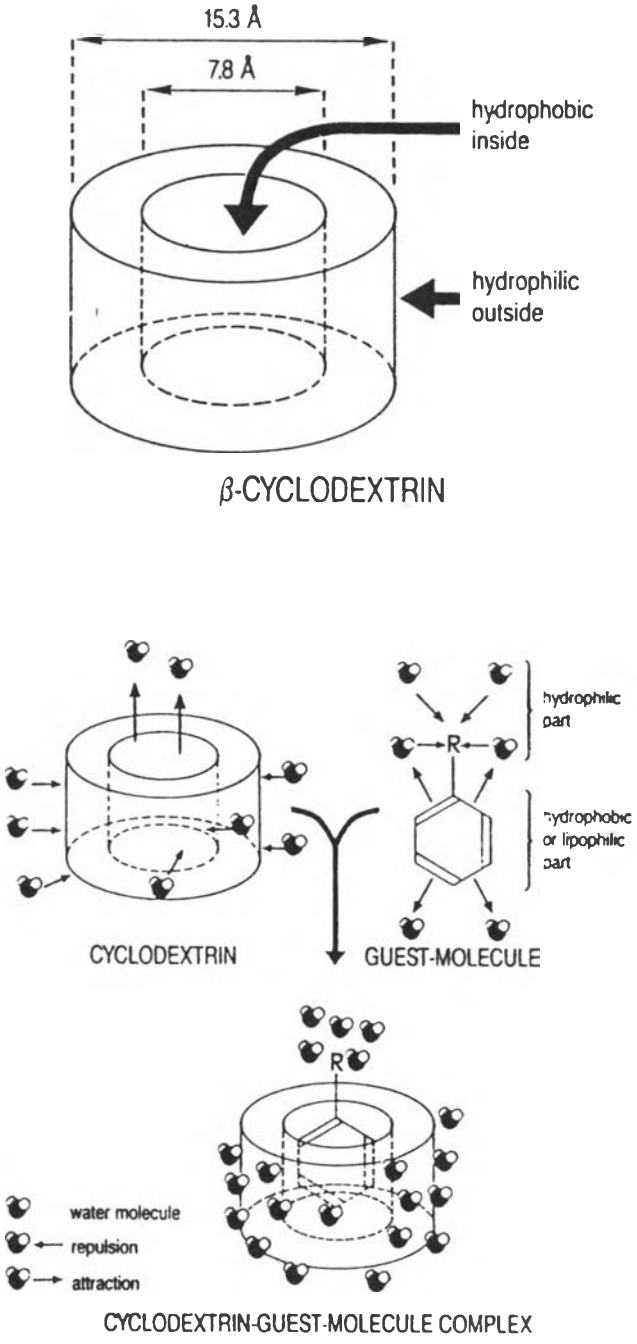

Figure 1.2 Illustration of inclusion of cyclodextrin and guest-molecule (Janssen, 1992)

Table 1.1 World market for cyclodextrin *

Application	Market (ton year^{-1})	
	1989	1995
Pharmaceutics	50	2,000
Food	700	2,500
Cosmetics	50	500
Agriculture	10	500
Chemical industry (biotransformations, separation, catalysis)	30	300
Other purposes (e.g. diagnostics)	10	200

* Source : Consortium fuer electrochemische Industrie GmbH.

Table 1.2 Bacteria which produce CGTases

Organisms	CGTase type	Gene cloned	References
Klebsiella oxytoca M5a1	α	+	Binder *et al.*,1986
Bacillus macerans	α	+	Takano *et al.*,1986
Alkalophilic *Bacillus* 1011	β	+	Kimura *et al.*,1987
Alkalophilic *Bacillus* 38-2	β	+	Kaneko *et al.*,1988
Bacillus circulans strain no.8	β	+	Nitschke *et al.*,1990
Bacillus ohbensis nov.c-1400	β,γ	+	Sin *et al.*,1991
Bacillus subtilis No. 313	γ	+	Kato *et al.*,1986

Bacillus sp. A11 was selected as one of the best strain producing extracellular CGTase (Pongsawasdi and Yagisawa, 1987). In 1993, Surasak partially digested chromosomal DNA from *Bacillus* sp. A11 with *Sau*3AI and inserted to the *Bam*HI site of plasmid pBR322. The CD-producing clone was selected. Its insert was subcloned into *Bam*HI site of pUC18 resulting in a recombinant plasmid pSV3 (renamed pCSBC5) with a 5.2 kb insert. This fragment was transferred to pSE411 vector giving rise to pSV5 (renamed pCSBC8). Both pCSBC5 and pCSBC8 when transformed into *E. coli* JM101 were tested positive for CGTase (Fig.1.3).

However, CGTase activity detected was not high and certain amount was found not to secret, being accumulated in the periplasmic space. CGTase was actually secretory protein in *Bacillus* sp. A11. This was due to the lack of secretion system in *E. coli*. On the other hand, *Bacillus subtilis* is well known to secrete a large number of proteins efficiently into the culture medium (Sin *et al.*, 1993). It is therefore possible to produce CGTase proteins extracellularly using the expression and secretion system of *B. subtilis*. The aim of this thesis is to construct an expression system using *B. subtilis* as a host for production.

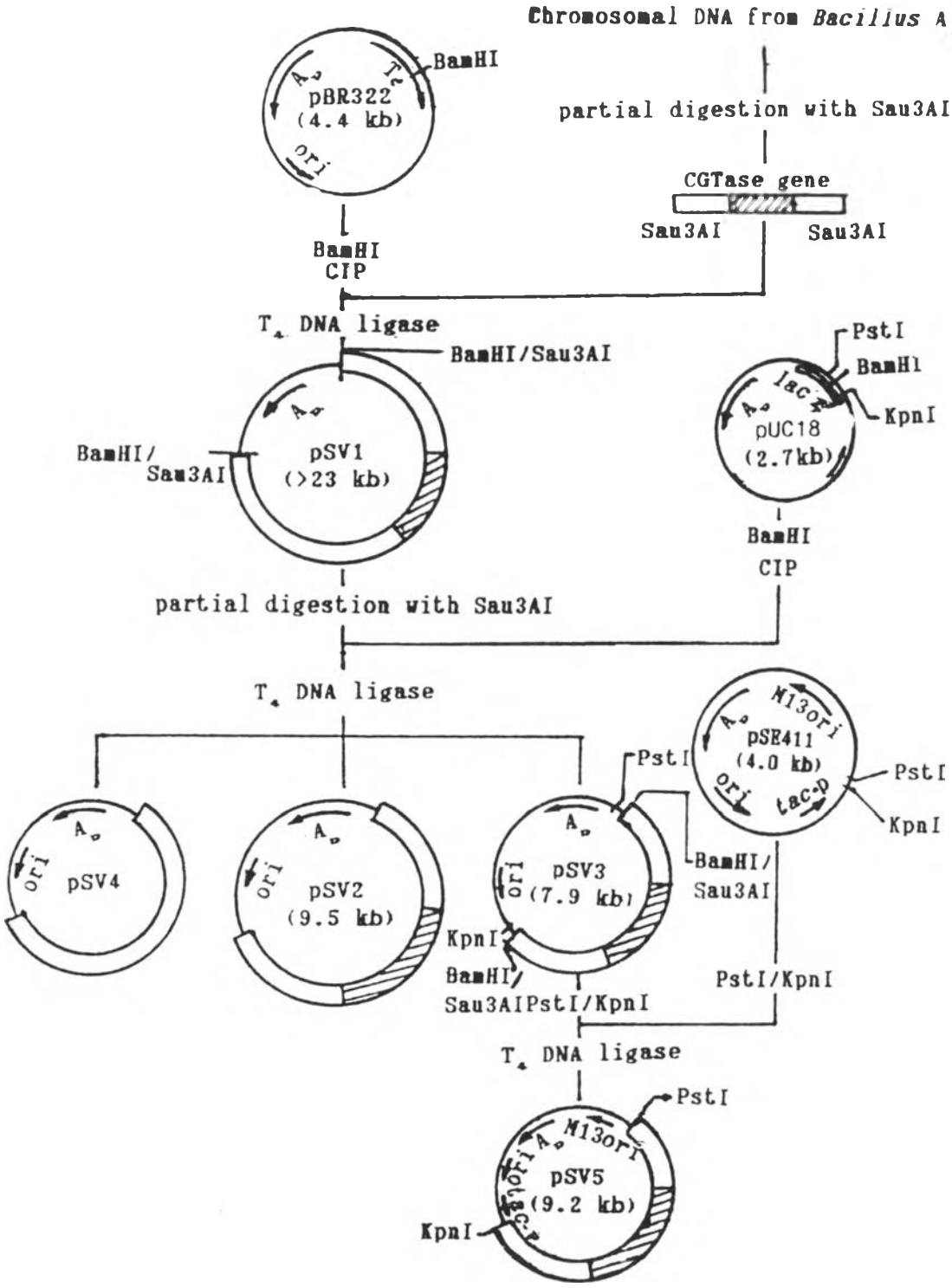

Figure 1.3 Illustration of the recombinant plasmids construction pSV1, pSV2, pSV3, pSV4 and pSV5 (สรศักดิ์, 1993)