CHAPTER V

CONCLUSION

1. Besides the catalytic A/B domains in CGTase, other domains, i.e. domain D, also contribute to the specificity and ratios of cyclodextrin production.
2. The mutation site I plays an important role in β-cyclodextrin production.
3. More space as well as other interaction in substrate -7 (mutation site II) are important for the γ-cyclodextrin production.
4. We propose the sliding model of CGTase during its binding to the substrate; the glycosyl chain is sliding through the subsite +2 before continuing to the subsite-7.

is deleted, the interaction between the sugar residue and the amino acid side chains at this site may be decreased. The glycosyl chain can thus reach subsite -8 and is cyclized to be γ-cyclodextrin. In pNan7 which contains mutations in subsites -3 (site I) and -7 (site II), the effect of mutant site II is shadowed by that of mutant site I since there is no production of γ-cyclodextrin. The mutation site I (subsite-3) may change the geometry of active site making it suitable to accommodate glycosyl chain with seven sugar residues. Consequently, the glycosyl chain cannot reach the subsite -8. This may be the reason why there is no γ-cyclodextrin production with the mutation site I and mutation sites I+II. Therefore, the glycosyl chain should slide through the subsite-3 before continuing to the subsite-7. The result then supports our sliding model of CGTase during binding of the substrate.

We might then conclude that the mutation site I plays an important role in β-cyclodextrin production and the mutation site II is important for the γ-cyclodextrin production. The results of the mutation site III indicate that domain D also participates in cyclodextrin synthesis.

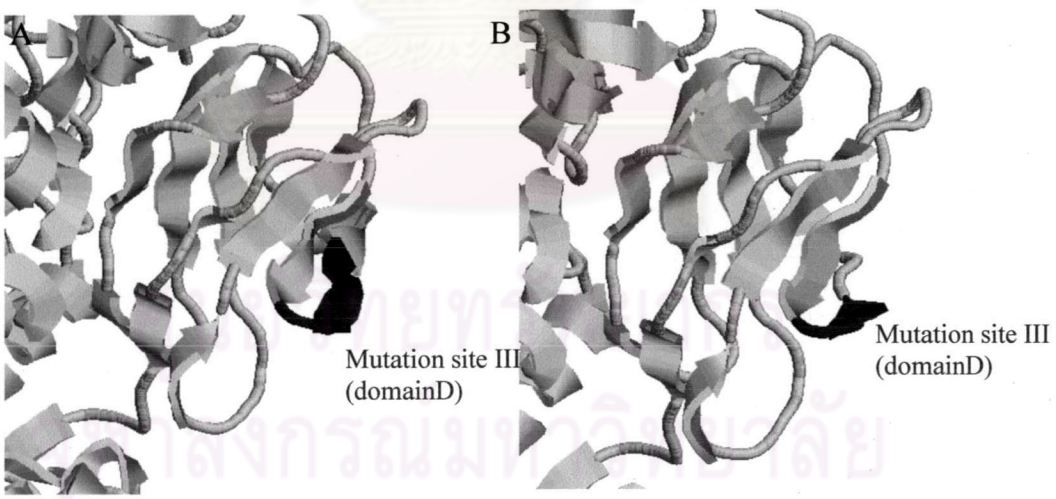

Figure.34 Comparison of the three dimensionanl structures of CGTase at domain D between wild type CGTase(A) and mutant CGTase(B).

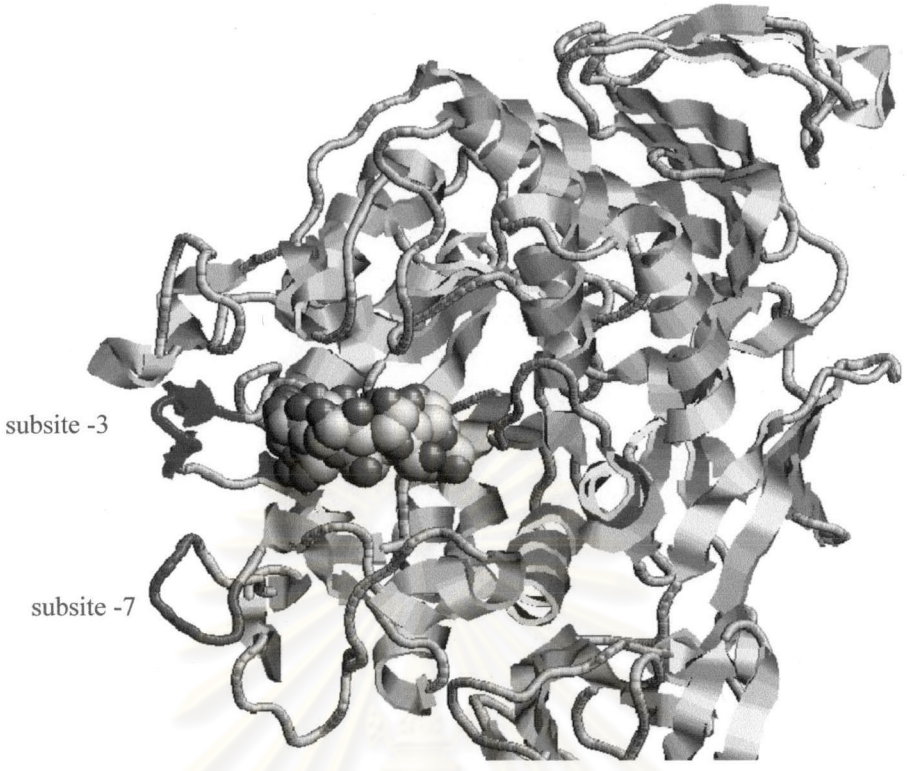

Figure.35 The three dimensionanl structures of CGTase from *B. circulans* 251(PDB I.D. 1D3C) in complex with γ-cyclodextrin

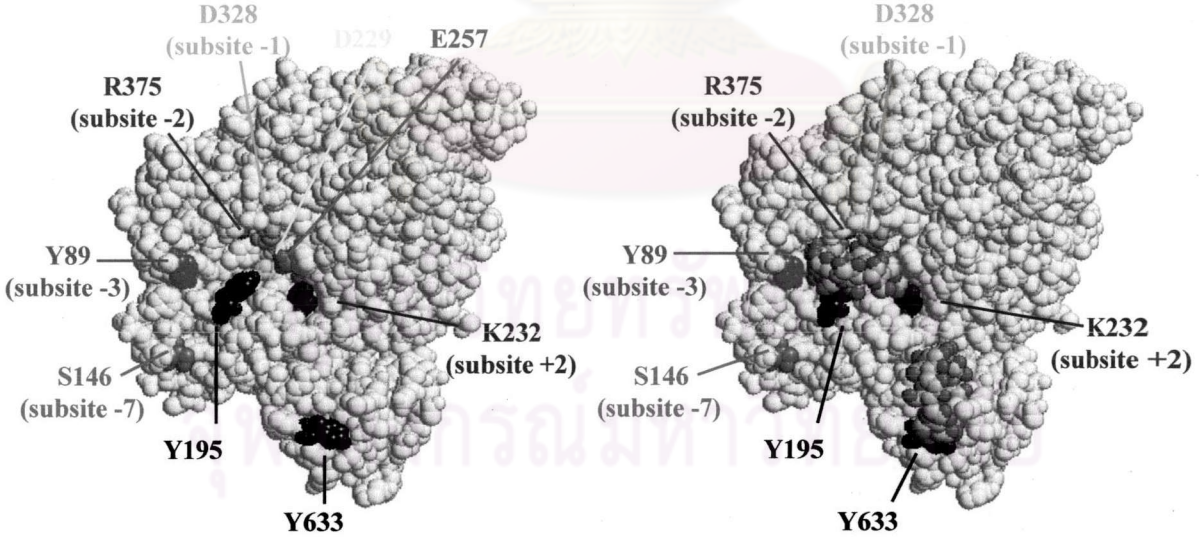

Figure. 36 The active site of CGTase from *B. circulans* 251 (PDB I.D. 1D3C) in complex with γ-cyclodextrin. The amino acids involved in the binding of substrate are colored. D229 and E257 are catalytic residues. Y195 is located at the center of active site and Y633 is the residue that involved in the binding of substrate in domain E.