Thesis Title: Chemical Modification of *Bacillus* Penicillin Acylase

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ABSTRACT

Extracellular penicillin acylase from *Bacillus subtilis* pBA 401 was studied using chemical modification to modify the enzyme molecules. Many reagents were chosen based on their specific reaction on certain amino acids such as lysine, histidine, methionine and those with carboxyl groups. The results showed that only methylacetimidate and o-methylisourea which reacted with ϵ-amino group of lysine modified the enzymes still retained their enzymatic activity. Results with kinetic studies showed that methylacetimidate modified enzyme had the Km similar to that of the native one. O-methylisourea on the other hand, had 2 times

higher value of Km than that of the native penicillin acylase. The result implied that only o-methylisourea modified the enzyme molecule in such a way that there were some changes of the substrate binding site. Vmax of both modified enzymes were similar and about 2 times less than that of the native one.

It was possible that any changes occured for both modified enzymes regarding their catalytic sites were the same. The kinetic data of both modified enzymes with 6-APA showed that the substance acted as a competitive inhibitor instead of a non-competitive inhibitor as done with the native penicillin acylase. Higher thermostability seen with the methylacetimidate modified enzyme might be useful for industrial uses. Further studies need to be continued in order to understand the relationship between components of the enzyme molecule and the increase in enzyme stability.