(Format No. 3)

SUMMARY OF DOCTORAL THESIS

Name: Maizom Binti Hassan

Title: **Enzymatic Studies on the Degradation of 4-*N*-Trimethylamino-1-butanol by *Pseudomonas* sp. 13CM**
（Pseudomonas sp. 13CM による4-トリメチルアミノ-1-ブタノールの分解に関する酵素的研究）

The microbial degradation and metabolism of quaternary ammonium compounds such as choline, L-, and D-carnitine have been well investigated. Furthermore, the structure and function of quaternary ammonium compound degrading enzymes have been studied. The author assumed that the quaternary ammonium compound degrading enzymes consisted of two binding sites; a negatively charged or 'anionic' site which recognized the quaternary ammonium moiety of substrate, and catalytic site which recognized the alcohol or aldehyde moiety of substrate. To elucidate the structure and function of quaternary ammonium compound degrading enzymes, the author took up 4-*N*-trimethylamino-1-butanol (TMA-Butanol), which shows a considerable structural resemblance to choline as a theme.

In chapter 1, the isolation and identification of TMA-Butanol degrading microorganism were described. Six strains with high NAD^+-dependent TMA-Butanol dehydrogenase activity in the cell-free extract were selected from 450 strains grown on TMA-Butanol. Among these strains, a strain, which showed the highest enzyme activity was selected. The isolated microorganism was identified as the *Pseudomonas* species by the morphological and biochemical analysis and the 16S rDNA sequence of the bacterium. TMA-Butanol degradation in *Pseudomonas* sp. 13CM is postulated to proceed as follows: TMA-Butanol → 4-Trimethylaminobutyraldehyde (TMABaldehyde) → γ-butyrobetaine → L-carnitine.

In chapter 2, the purification and characterization of TMA-Butanol dehydrogenase from *Pseudomonas* sp. 13CM were described. TMA-Butanol dehydrogenase was purified 526-fold to apparent homogeneity by 5 chromatographic steps. The molecular mass of the enzyme was 45 kDa and appeared to be a monomer. The isoeletric point was found to be 4.8. The N-terminal twelve amino acid residues were identified as followed; MIDNLSPLSRQS. The optimum temperature is 50ºC, and the optimum pH for the oxidation and the reduction reaction are 9.5 and 6.0, respectively. TMA-Butanol dehydrogenase was specific for NAD^+ and oxidized TMA-Butanol, trimethylaminopentanol, trimethylaminohexanol, dimethylaminobutanol, dimethylaminopentanol, dimethylaminohexanol, and 4-ethylamino-1-butanol. The K_m

values for TMA-Butanol and NAD^+ were 0.54 mM and 0.22 mM, respectively. The enzyme catalyzes the following reaction: TMA-Butanol + NAD^+ ⇄TMABaldehyde + NADH + H^+. The K_i values for triethylamine, diethylamine, and ethylamine increased with decreasing number of ethyl groups. The same phenomenon also was observed in trimethylamine, dimethylamine, and methylamine.

The purification and characterization of NAD^+-dependent TMABaldehyde dehydrogenase from *Pseudomonas* sp. 13CM were described in chapter 3. The enzyme was purified to apparent homogeneity by hydrophobic and affinity chromatography. The molecular mass of the enzyme was 150 kDa. And, the enzyme consisted of three identical subunits. The N-terminal 13 amino acid residues were identified as PQLRDAAYWRAWS. The pI of the enzyme was 5.5 and optimum temperature and pH were 40ºC and pH 10.0, respectively. The Km values for TMABaldehyde, DMABaldehyde, and NAD^+ were 7.4, 51, and 125 μM respectively. The enzyme catalyzed the following reaction: TMABaldehyde + NAD^+ → γ-Butyrobetaine + NADH + H^+. The K_i values for triethylamine, ethyltrimethylammonium iodide, and dimethylethylamine decreased in increasing number of ethyl groups. The same phenomenon also was observed in acetaldehyde, 3-methylbutyraldehyde, and trimethylbutyraldehyde.

In conclusion, the results of the kinetic studies on substrates and substrate analogs of both enzymes have shown that positively charged triethylammonium or trimethylammonium groups of substrate have effect to the catalytic activity of quaternary ammonium compound degrading enzymes and are a major determinant in defining the specificity for its substrate.

In chapter 4, the expression cloning of TMABaldehyde dehydrogenase gene and the characterization of recombinant enzyme were described. The electrophoretic pattern on native-PAGE and the kinetic properties of recombinant enzyme (TMABaldehyde dehydrogenase II) were different from those of the enzyme purified from *Pseudomonas* sp. 13CM (TMABaldehyde dehydrogenase I). Furthermore, the nucleotide sequencing of 7.2 kbp fragment contained TMABaldehyde dehydrogenase II gene was determined. The 7.2 kbp fragment contained four ORFs. The deduced amino acid sequence of the ORF1 exhibited 92% identical to putative betainealdehyde dehydrogenase from *P. putida* KT2440. It was suggested that TMABaldehyde dehydrogenase I was an induced enzyme and TMABaldehyde dehydrogenase II was a constitutive enzyme in *Pseudomonas* sp. 13CM.