CHAPTER V

CONCLUSION

1. The optimal conditions for PheDH production of *Bacillus badius* strain BC1 was 24 hours of cultivation in 1 % peptone medium, pH 6.5 containing 0.8 % L-phenylalanine at 37°C and those of bacterial strain BC2 was 24 hours of cultivation in 1 % peptone medium, pH 5.0 containing 0.8 % L-phenylalanine at 37°C. PheDH from *B. badius* BC1 was chosen for further studies in purification, characterization and kinetic mechanism because it was higher in total activity and specific activity than those of strain BC2 in the experiments on optimization .

2. PheDH from *B. badius* BC1 was purified to homogeneity by 40-50 % saturated ammonium sulfate precipitation, DEAE-Toyopearl, first Butyl-Toyopearl and second Butyl-Toyopearl column chromatography with 20% yield and 160.7 purification folds.

3. The enzyme had a relative molecular weight of about 358,000 with 8 identical subunits (approximately 44,500) as analyzed by gel filtration and SDS-polyacrylamide gel electrophoresis.

4. The enzyme showed high substrate specificity in the oxidative deamination on L-phenylalanine while that of the reductive amination was on phenylpyruvate.

5. The enzyme required NAD^+ as a natural coenzyme for oxidative deamination. $NADP^+$ was inert while the NAD^+ analog, 3-acetylpyridine-NAD^+, showed 1.65 times higher activity than NAD^+.

6. The optimum pH of the enzyme for the oxidative deamination and reductive amination were 10.7 and 8.3 , respectively.

7. The optimum temperature of the enzyme for the oxidative deamination and reductive amination were 50°C and 45°C , respectively.

8. The enzyme was stable over a pH range from 6.0 to 11.0.

9. No loss of the enzyme activity was observed upon incubation at 40°C for 2 hours and 80% and 50% of the activity was retained after incubation at the same temperature for 20 and 30 hours, respectively.

10. Sulfhydryl group was important to enzyme activity since the enzyme activity was completely lost in the presence of 1 mM final concentration of $AgNO_3$, $HgCl_2$. Fe^{2+} and Fe^{3+} (1 mM final concentration) strongly inhibited the enzyme activity and Mn^{2+} (10 mM final concentration) showed notable inhibitory effect on the enzyme activity.

11. D- and L- amino acids with non-polar side chain and those with acidic side chain and *o*-fluoro-DL-phenylalanine significantly inhibited the oxidative deamination of L-phenylalanine, while α-keto acids showed no significant inhibition on the reductive amination of phenylpyruvate.

12. The enzyme was chemically modified with a series of group-specific reagents to identify essential amino acid residues. Incubation of the enzyme with 10 mM of *N*-bromosuccinimide (NBS), chloramine T (CT), diethylpyrocarbonate (DEPC) and 2,4,6-trinitrobenzenesulfonic acid (TNBS) which were specific for tryptophan, methionine, histidine and lysine, respectively, led to complete loss of enzyme activity. In addition 10 mM of phenylglyoxal (PG) which was specific for arginine reduced the enzyme activity to about 10 %, while *N*-acetylimidazole (NAI), dithiothreitol (DTT) and phenylmethylsulfonyl fluoride (PMSF), the modifiers of tyrosine, cysteine and serine, respectively, did not affect the enzyme activity. Therefore, arginine, methionine, histidine, tryptophan and lysine are all likely involved in PheDH activity as the essential residues for enzyme biological function.

13. The apparent K_m values for L-phenylalanine, NAD^+, NADH, phenylpyruvate and ammonium were 0.59, 0.28, 0.07, 0.33 and 200 mM, respectively.

14. Initial velocity and product inhibition studies showed that the oxidative deamination proceeded through a sequential ordered binary-ternary mechanism, in which the sequence of substrate binding to the enzyme was NAD^+ and L-phenylalanine and then the sequence of product release was ammonia, phenylpyruvate and NADH, respectively.