CHAPTER V

CONCLUSION

1. Alanine dehydrogenase from *Aeromonas hydrophila* was purified approximately 150 folds with a 28% yield and specific activity of 18 units/ mg protein.

2. Alanine dehydrogenase activity was lost after the modifications of methionine, histidine, arginine, and lysine residues by chloramine T, diethylpyrocarbonate, phenylglyoxal, and 2,4,6-trinitrobenzenesulfonic acid, respectively, while the modification of tyrosine by *N*-acetylimidazole showed slight effect on the enzyme activity. In contrast, dithiothreitol, which specifically modified at cysteine residue, did not inhibit the enzyme activity.

3. The loss of alanine dehydrogenase activities after the modifications of histidine, arginine and lysine residues were reduced in the presence of pyruvate and/or NADH. In contrast, substrate protection did not work for methionine modification.

4. The protection by substrate against modification at arginine and histidine residues showed the highest efficiency when the ternary complex E·NADH·pyruvate was formed.

5. Inactivation kinetic of histidine and arginine with DEPC and PG, respectively, result in a simple bimolecular reaction with preudo-first order kinetics. The second order rate constant was 1.0 mM^{-1} min^{-1} and 1.2 mM^{-1} min^{-1} for modification of histidine and arginine, respectively.

6. Inactivation constant (k_{inact}) of the modified enzyme at histidine residues was 1.0 while that of arginine residues was 1.1. Thus, the inactivations result from the reaction of 1 mole DEPC or PG: 1 mole enzyme subunit.

7. Histidine-95 was proposed to be an essential residue in the active site.