CHAPTER V
CONCLUSIONS

The results of this study may be concluded as follows:

1. Evaluation of solvent effect on the conformational modification of lysozyme to MG state was done after exposure of lysozyme to various environments.

1.1 Aqueous-ethanolic environment

At 80% v/v of ethanol in the aqueous-ethanolic solution, [L(80)(0)], the CD spectra of lysozyme showed a loss in the tertiary structure and retaining the native-liked secondary structure. In addition, the fluorometric results of the modified lysozyme obtained by this condition signified MG state. This MG conformation could be reversed to its native structure when it was diluted at twenty times of the original volume.

1.2 Acidic environment

Acidic environment alone could not induce lysozyme structural changes into the MG state even at high HCl acid concentrations.

1.3 Combined environment

At 35% v/v or 40 % v/v of ethanol in the aqueous-ethanolic solution in 20 mM of HCl acid [L(35)(20) and L(40)(20), respectively], lysozyme showed characteristics of MG state. The tertiary structure was disturbed, while the secondary structure showed high contents of α-helix. In addition, ANS binding results of these conditions signified MG state. Furthermore, dilution of modification of these environments with water at twenty times of the original volume resulted in reversibility of lysozyme to its native structure.

Lysozyme formulas L(80)(0) and L(35)(20) were in the MG state and were selected for the subsequent experiments.

2. The penetration study of modified lysozyme was done using modified Franz-diffusion cell technique.

2.1 The evaluation for the integrity of the in vitro model skin (pig's ear skin) was done using propranolol HCl as a marker. The permeation of propranolol HCl from the ethanolic or combined environments was reduced when compared to that from aqueous solution. This finding implied changes in the skin structure without leakage of the skin. Cryo-SEM revealed that ethanolic environment affected the skin by dehydration and lipid extraction while combined environment induced swelling of the skin structure and loss of the skin structure.

2.2 The amount of lysozyme permeated through the skin into the receiver compartment was not successfully detected due to interferences from endogenous substance. Therefore, the amount of lysozyme remaining in the donor side was detected by CD instead. The lysozyme from L(80)(0) and L(35)(20) disappeared from the donor side faster than that from L(0)(0) and L(35)(0). The reason for this finding still needed further investigation. However, penetration of lysozyme in MG state with increasing hydrophobic character was one of the possibilities.