SUMMARY AND CONCLUSIONS

The role of lysine residues in lysozyme molecule was examined. The problem was approached by the chemical modification method. Since lysozyme shows two pH optima, 6.2 and 9.2 for maximum activity, lysine residues were modified with FDNB at neutral pH 7.2 and at alkaline pH 9.1. Derivatives in which increasing number of lysine residues were blocked were prepared. The effect of modification on the enzyme activity and physico-chemical properties of lysozyme was investigated in detail.

Studies carried out at pH 7.2 provided a good deal of information regarding the role of lysine residues. Three derivatives designated as P_1, P_2 and P_3 were prepared around neutral pH; they contained 1, 3 and 6 DNP groups per mole of the enzyme and retained 100%, 55% and 40% of the original enzyme activity respectively. All the derivatives were found to have enzymatic properties similar to the native lysozyme. The activity-pH profile in the acid and alkaline region remains unchanged; activity-buffer concentration profile also remains unaltered at pH 6.8 and 9.2. This suggests that the electrostatic forces involved in the formation of the enzyme substrate complex were not affected due to the blocking of the lysine residues. Specific (NAG) and non-specific (Poly Tyr-Glu) inhibitors inhibit the activity of the modified enzyme to the same extent as they affect that of native enzyme. The specific inhibitor NAG does not mask the lysine participating in the

modification reaction. These results indicate that binding of the inhibitor is not affected and therefore the lysine residues do not belong to the active site. It was therefore concluded that lysine residues are not involved in the catalytic activity of the enzyme.

The enzyme activity of the derivatives could also be reversed after the derivatives were subjected to physical (heat) or chemical (urea and guanidine) denaturing agents. In these experiments it was expected that if small conformational changes had occurred due to the introduction of the bulky DNP-groups these would be amplified due to the effect of the denaturing agents. The fact that the enzyme activity could be reversed indicated that the overall conformation of the derivatives was not altered to any appreciable extent. This was also confirmed by UV spectra, ultracentrifuge and gel filtration studies, which showed no difference in the shape and size of the DNP-derivatives as compared to the native lysozyme. The data of these experiments excludes the possibility of involvement of the lysine residues in the maintenance of the enzyme conformation.

No aggregation was found in the derivatives prepared at pH 7.2. Although these derivatives were soluble around neutral pH they precipitated slightly and slowly in alkaline pH range. Derivatives prepared at pH 9.1 were not only more or less completely inactive, but they were relatively insoluble in aqueous solutions and formed large aggregates. Because of the relative insolubility and aggregation further studies on the

activity and physicochemical properties were not possible. The data suggest the role of lysines in the solubility of the enzyme in aqueous solution . The function of lysine side chains in the solubility of the protein molecule is discussed.

From the studies discussed above it appears that lysine residues of the lysozyme molecule perhaps energetically favour the interaction with solvent molecules over interaction with other side chains and in that way they perform the function of solubilizing the enzyme molecule; this is indicated by the observation that blocking of these residues affects the solubility of the enzyme in aqueous solution.