CHAPTER 5

CONCLUSION

Over expression was achieved in 37°C, 40°C, 42°C and 45°C for the pETSK construct in BL21 (DE3). Inclusion bodies were identified for the constructs pETSK and pRSETB-SKH. About 60% of the streptokinase protein was recovered from the pETSK construct by Hydrophobic Interaction Chromatography and 57% of streptokinase protein was recovered from pRSETB-SKH construct by Immobilised Metal Affinity Chromatography. The purified products were further confirmed by western blot and bioassay.

The lipid modified streptokinase (LM-SK clone) was over expressed in LBON medium at 30°C and hence 30°C was found to be an ideal temperature for the protein expression. No leaky expression was observed in BL21 (DE3) E.coli strain. Lipid modified streptokinase at 30°C showed more activity than at any other temperatures. Expression of lipid modified streptokinase at 48.5 kDa in the membrane fraction, confirmed the membrane targeting of the protein and the mobility difference of about 1.5 kDa between the modified and native streptokinase in Tricine SDS-PAGE confirmed the lipid modification. Comparison of the radioactivity of lipid modified streptokinase with the control, the incorporation of radioactive [9, 10 3H] palmitate in the expressed protein has confirmed the addition of lipid moiety to streptokinase protein. This result indicates that the lipid modified SK is having more activity than native streptokinase.

The streptokinase gene was cloned in pRSETB vector with the replacement, partial deletion and complete deletion of ribosome binding site.
The expression of the SD-REP, SD-parDEL and SD-comDEL clones were optimized for overexpression in LB, GYE and LBON. In spite of nil expression of SD-comDEL under any conditions, its activity was confirmed by caseinolytic assay. There was mild expression of SD-parDEL at 37°C. No expression of SD-REP, SD-parDEL and SD-comDEL were observed in BL21 (DE3) pLysS. The recombinant protein was confirmed by Western blot and in vitro bioassay.

5.1 FUTURE WORK

- Further characterization of lipid modified streptokinase has to be carried out.

- In vivo biological activity of all the expressed streptokinases has to be analysed in animal models.

- Ribosome toe print assay has to be conducted to understand the mechanism of initiation process SD-dependent and SD-independent pathways.