Summary & Conclusion

SUMMARY

Plant latex proteases serve a protective function in several plants and often are found to exhibit remarkable stability towards high temperatures and a wide range of pH. Proteases such as papain, bromelain and ficin have been in extensive use in several industrial and medical applications. Since the latex of *Synadenium grantii,* which is a widespread, cosmopolitan species, was found to contain a highly active protease, this latex was taken for further investigation on the nature of its proteolytic activity. The present thesis has dealt with the isolation, purification and characterization of serine proteases from the *Synadenium grantii* latex.

Preliminary studies performed on the crude latex extract revealed proteolytic activity occurring at temperatures in the range of 25-65 °C. Therefore, attempts were made to isolate and purify the proteases from the *S.grantii* latex, as discussed in the first chapter of this thesis.

Among the different methods adopted, clarification of the latex by water-dilution followed by ammonium sulphate precipitation yielded the best results. Persistent lipid contaminants were removed from this fraction by treatment with diethyl ether after which the fraction was subjected to gel-filtration chromatography on the Sephacryl S-200 matrix. Active fractions thus obtained by gel-filtration were pooled and further subjected to anion-exchange chromatography using a Resource-Q column.

Among the resolved peaks, caseinolytic activity was detected in two peaks that eluted at a salt concentration of 0.17 and 0.2 M. The rechromatography of each of these fractions confirmed that they are distinct proteins.

The physico-chemical studies form the second chapter of this thesis. The molecular mass of both the proteases was found to be 76.7 kDa. In the purified enzymes, no carbohydrate was detected by the periodic acid-Schiff staining method. The proteases were inhibited by PMSF and DEPC, thus implying that they were most likely to be serine proteases, wherein histidine residues also played an important role in catalysis. They were unaffected by a wide range of other inhibitors, such as iodoacetamide, o-phenanthroline, pepstatin, TLCK and TPCK. Thus they are unlikely to be either thiol or metalloproteases. The other naturally occurring proteolytic inhibitors such as soyabean trypsin inhibitor, ovomucoid inhibitors, aprotinin and leupeptin also had no effect on the latex proteases.

Unlike papain, the *S.grantii* proteases were not inhibited by Fe (III),Cu (II) or Zn ions at a concentration of 1 mM.

The proteases were maximally active at 60 °C and at a pH of 7.0. Stability over a pH range of 5-10 and retention of 75% activity following incubation of the proteases at 65 °C are among their desirable characteristics. There was very little difference between the K_m and the V_{max} of the proteases with respect to azocasein. However, in the case of the synthetic peptide substrate, $(Glu: Ala:Tyr)_n$ the value of Km for protease 'a' was nearly twice that of protease 'b'.

The caseinolytic activity of the proteases was comparable with that of other well-characterized serine proteases like chymotrypsin. The hydrolysis of azocasein was linear upto 60 min of the reaction while the breakdown of $(Glu: Ala: Tyr)_n$ was linear for only 30 min of the reaction. The proteases also cleaved various other protein substrates in their native state, which included bovine serum albumin, ovalbumin and ribonuclease A. At enzyme: substrate ratios of 1: 500 or less, the *S.grantii* proteases were found to inactivate RNAse A completely. Denatured human haemoglobin was also hydrolyzed by the proteases. In addition, the proteases also exhibited moderate collagenolytic activity.

As evidenced by HPLC profiles, the cleavage patterns of proteins (studied using RCM-RNAse A) clearly differed from those produced by trypsin or chymotrypsin- a fact already shown by the inability of these proteases to degrade BAEE or BTEE. The proteases were also inactive against succinyl-trialanyl-nitroanilide and the synthetic peptide substrates such as $(Glu: Tyr)_n$,Poly-Lys and Poly-Arg. Among the synthetic peptides the proteases were active only against $(Glu: Ala: Tyr)_n$. Based on these observations it can be inferred that the proteases possibly cleave proteins at the N-terminus of alanine residues in proteins.

The final chapter of the thesis deals with the potential biotechnological applications of these proteases. Since the *S. grantii* proteases underwent very little loss of activity at 0.5% (w/v) SDS (final concentration, used in reaction mixtures for genomic DNA isolation), the possibility of using them as substitutes for proteinase k was explored. Studies performed on the isolation of genomic DNA from *E.coli* and *Saccharomyces cerevisae* indicated that the performance of the *S.grantii* proteases in this regard compared favourably with proteinase k. Hence, these proteases could be utilized as cost-effective substitutes for proteinase k.

Studies performed on the hydrolysis of meats indicate that the activity of the proteases was comparable with that of papain that is widely used in meat processing. Moreover, the

proteases also are capable of degrading fish wastes, which forms an important area of protein recovery. The *S.grantii* proteases were also compatible with papain and hence could be used in combination with it for enhanced proteolysis.

The performance of the *S.grantii* proteases in detergency was also tested. Although the proteases are unaffected by a number of additives used in detergency, there was no significant improvement of the wash performance, when they were used. The *S.grantii* proteases also cannot be used without encapsulation in the presence of hydrogen peroxide, which is a constituent of bleaches.

However the *S. grantii* proteases were unaffected by pharmaceutical diluents and binders/disintegrants. Wetting agents such as ethyl alcohol and isopropanol retarded their performance especially at concentrations of 60% or more. Other proteases such as trypsin, chymotrypsin and papain were without effect on the activity of the *S.grantii* proteases.

Proteases find a wide range of application in research, industry and medicine. In this regard, the *S.grantii* proteases could also be recognized as potential candidates for use either singly or in combination with other proteases. As evidenced by the information gathered by us in the present study, these proteases exhibit several desirable characteristics that could enable their utilization in various industries.