CHAPTER V

SUMMARY AND CONCLUSIONS
Ribonucleases are ubiquitous in mammalian tissues, body fluids, secretory organs as well as animal secretions. They play important roles in metabolic processes such as RNA processing and maturation, cytoplasmic RNA degradation and RNA degradation during viral infection. RNases present in animal secretions and body fluids form a distinct family (pancreatic RNases) of structurally and enzymatically related proteins, for which RNase A is a representative member. Several other proteins with known biological properties are also found to be homologous to RNase A, example for which are angiogenin, onconase, eosinophil derived neurotoxin and eosinophil cationic protein etc. The cytosolic compartment of many mammalian tissues also contains RNase activity which resembles pancreatic RNase A in enzymatic characteristics. Their cytosolic location is debatable since all the pancreatic RNases have properties of secretory proteins, targeted to secretory organelles. Nevertheless, they are present in the cytoplasm in significant amounts (in terms of their activity) and it is important to understand their physiological role and how they are regulated.

An acidic protein, known as protein RNase inhibitor (PRI), present in the cytosol of many mammalian tissues is known to strongly bind and inhibit RNase A and other pancreatic RNases in vitro. The postulated physiological role of PRI includes regulation of cytosolic RNase activity. To explore and understand this possibility further, it was aimed to study structure-function relationship between RNase and PRI.

BS RNase, present in large quantities in bovine seminal plasma is a unique member of the RNase super family. It is the only pancreatic type RNase which exists in dimeric form. Dimeric BS RNase has two identical monomeric units held together by two inter-subunit disulfide bonds and non-covalent interactions. Monomeric form of BS RNase is homologous to RNase A in its sequence, structure, enzymatic properties as well as active site residues: the active site of both RNase A and BS RNase monomer include His 12 near the N-terminal and His 119 towards the C-terminal end. Recently, two quaternary structural forms (MxM and M=M forms) have been shown to be present in the native dimeric BS RNase and are in
equilibrium with each other. The MxM form has two composite active sites formed as a result of the exchange of N-terminal polypeptides between the two subunits (i.e. the two active site amino acids His 12 and His 119 originate from different monomer subunits), while in M=M, such an exchange is absent during active site formation. Treatment of the enzyme with dithiothreitol (DTT) reduces the inter-subunit disulfides of BS RNase selectively and causes partial monomerization of BS RNase. This is because the two forms differ in their monomerization capabilities: on reduction the M=M form monomerizes but the MxM form remains as a non-covalent dimer (NCD). Methods for preparation of dimeric BS RNase - native and reduced forms, the active monomeric forms as well as the homogeneous MxM and M=M forms are available and BS RNase in this way offers various forms of the enzyme which are catalytically active.

PRI proteins present in many mammalian tissues are closely related in properties. Placental RNase inhibitor is a representative member of the group and is available in abundant quantities commercially.

Interaction of RNase A and different quaternary structural forms of BS RNase (including the native form) with placental RNase inhibitor was therefore investigated as a model study to examine, in the context of the regulation of RNase activity, if and how the structure of the enzyme plays any significant role in interaction with the inhibitor.
MAJOR OBSERVATIONS

The main conclusions from this study are:

1. RNase A as well as the monomeric BS RNase are sensitive to PRI and are inhibited completely and to a comparable extent while native BS RNase is virtually insensitive to PRI.

2. MxM form of BS RNase remained insensitive to PRI, both in the absence and in the presence of DTT as expected (as it remains in a dimeric structure even in the presence of DTT). Further, M=M form of BS RNase was found to be insensitive to PRI in the absence of DTT, while in the presence, it readily acquired PRI sensitivity on account of monomerization.

3. Exposure of native BS RNase to DTT prior to the assay rendered the enzyme partially sensitive to PRI, on account of partial monomerization.

The above results taken together imply that (a) Dimeric structure is the basis for PRI insensitivity of BS RNase, and (b) Monomerization is the means by which BS RNase acquires PRI sensitivity.

4. Reduction of the MxM and M=M forms of BS RNase by DTT transforms them into NCD and M respectively. As MxM and M=M forms interconvert and are in equilibrium, their reduced enzyme forms NCD and M also interconvert, in fact at much faster rates.

5. DTT monomerizes the enzyme partially; presence of PRI during DTT treatment monomerizes the enzyme completely, implying that PRI promotes monomerization of BS RNase (conversion of even NCD to M).

6. Other experiments suggested a probable effect of the substrate on the enzyme structure - stabilization of dimer by substrate.
7. PRI sensitivity studies as well as physical characterization of BS RNase after exposure to DTT in the presence of different amounts and kinds of the substrate confirmed the effect of the substrate on the enzyme structure in a concentration dependent manner.

8. Thus, substrate and PRI seem to influence the inter conversion of NCD and M, under reducing conditions and draw the equilibrium in opposite directions. Substrate draws the equilibrium towards the PRI insensitive NCD, while PRI draws it towards the PRI sensitive monomer.

**IMPLICATIONS OF THE STUDY**

The presence of an equilibrium between the monomeric (M) and dimeric (NCD) forms of the enzyme under reducing conditions and the effects of substrate and PRI to draw the equilibrium towards the dimeric and monomeric forms respectively, suggest a regulatory relationship for enzyme activity. Reducing conditions and low RNA concentrations seem to favour monomerization resulting in the inhibition of the enzyme activity, while at high RNA concentrations dimerization is favoured resulting in resistance to inhibition by PRI and increase in RNA degradation (as the monomer is sensitive and NCD is insensitive to PRI). Thus the ratio between the two enzyme forms and the relative contents of substrate and PRI appear to be the determinants of BS RNase activity. One of the special biological properties of BS RNase is the selective cytotoxic effect against tumour cells. The dimeric structure, presence of composite active sites (see the review on BS RNase in "Introduction") and the catalytic activity were found to be necessary for the special biological properties of BS RNase. Since the cytosolic environment is generally reducing and as MxM and M=M transform into NCD and M, under these conditions, the present study offers (i) information for a probable modulation of NCD and M forms in the cytosolic compartment and (ii) at least suggests conditions under which the entire enzyme can be held as NCD, that ultimately helps in increasing the cytosolic RNase activity. BS RNase is an extracellular enzyme, whose
physiological function is not known. In view of this, to examine if these results can be made applicable for the regulation of the RNase activity of the cytosolic compartment, attempts are underway (i) to study if other enzymes like RNase A, a representative of pancreatic type RNases, also form a similar monomer-dimer equilibrium system, and (ii) to look for PRI sensitive and insensitive enzyme forms and a probable structure-function relationship between them.

Thus the present studies, (1) provide structural basis for the differential inhibition of BS RNase activity by PRI. (2) suggest possible existence of PRI-sensitive and -insensitive RNases in tissues, which may be structurally related, and may be important for PRI mediated regulation of RNase activity in cells. (3) give a rationale for the cytotoxic action of BS RNase on tumour cells, based on the resistance of the dimeric enzyme to PRI.

PUBLICATIONS BASED ON THE WORK
