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Abstract of thesis:

Dimers of Ribonuclease A (RNase A) and others members of the RNase superfamily are promising targets for therapeutic applications as some of them exhibit antitumor activity *in vitro* and *in vivo*. With the knowledge that several DNA binding proteins exist in the dimeric form and that RNase A shows DNA melting properties, dimers of the protein were prepared by dityrosine (DT) cross-linking, considered to be one of the major reasons behind natural protein dimerization. DT cross linking is associated with various pathological disorders and used as a biomarker of oxidative stress, aging and disease. Formation of dimers and oligomers also occurs due to non-enzymatic glycation of proteins. Prolonged non-enzymatic glycation of proteins results in the formation of advanced glycation end products (AGEs) that cause several diseases. In the present study we have prepared and characterized the DT cross-linked RNase A dimer and glycated RNase A (^{g}RNase A). We have compared the ribonucleolytic activity, ribonuclease inhibitor (RI) binding ability and DNA melting properties of the dimer and ^{g}RNase A with the monomer.

The dimer was prepared by a photoirradiation method followed by isolation using size exclusion chromatography. ^{g}RNase A was obtained by incubating RNase A with ribose, glucose and fructose at 37 °C and pH 7.4 over a time period of 30 days. The dimer and ^{g}RNase A were characterized via gel electrophoresis and various spectroscopic techniques. The ribonucleolytic activity of the dimer and ^{g}RNase A was monitored by employing agarose gel based assays, precipitation assays and kinetic studies. An agarose gel based assay was also conducted to monitor the DNA binding ability of the dimer and ^{g}RNase A. The RI binding ability of the dimer and ^{g}RNase A was checked by an RI binding assay and confirmed by native gel electrophoresis analyses.

The synthesized dimer showed reduced ribonucleolytic activity in comparison to the monomer, both in absence and presence of RI, competitive and noncompetitive inhibitors. Isothermal titration calorimetric studies indicated that the lower activity of the dimer is most likely due to the partial blocking of the active site of the dimer. One molecule of the dimer was found to be bound with two molecules of RI. The possible structure of the dimer and the Tyr residues involved in DT bond formation were determined by employing docking studies and accessible surface area calculations. Tyr92 of Chain 1 and Tyr76 of Chain 2 of the dimer are most likely involved in intermolecular DT bond formation. Each monomer unit of the dimer binds with a greater number of DNA nucleotides compared to the monomer. The DNA binding affinity of the dimer is found to be higher than that of the monomer. The melting profile for the ct-DNA–dimer indicated that the melting temperature (T_m) for the complex is lower compared to the ct-DNA–monomer complex. The ribonucleolytic activity of the dimer is also inhibited upon DNA binding. The glycation studies indicated that with an increase in incubation time high molecular weight AGEs are formed with a decrease in ribonucleolytic activity. Ribose exhibited the highest potency as a glycating agent along with the greatest reduction in the ribonucleolytic activity of the enzyme. Interestingly, ^{g}RNase A was unable to bind with the RI and DNA. ^{g}RNase A was also found to be ineffective in DNA melting unlike native RNase A.

Keywords: Dityrosine cross-linking; Ribonuclease A dimer; Ribonucleolytic activity; Ribonuclease inhibitor; Protein−DNA binding; DNA melting; Glycation