Title: Isolation of angiogenin and lactoferrin from buffalo milk
&
inhibition of ribonuclease A and angiogenin by flavonoids

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Abstract

The central thesis of DNA to RNA to protein is largely dependent on the action of RNA polymerases and RNA depolymerases as there exists a crucial and delicate balance between the formation and degradation of RNA. Ribonucleases can be cytotoxic at higher levels by entering into the cytosol and retaining their ribonucleolytic activity thus degrading the cellular RNA. Within the ribonuclease superfamily of proteins, ribonuclease A (RNase A) is the most abundant and most studied protein. It has stimulated a lot of research by virtue of the fact that several pancreatic RNase A homologues, including angiogenin, eosinophil-derived neurotoxin and bovine seminal RNase A utilize their enzymatic activities to elicit potent physiological effects.

A fluorescence based ribonucleolytic kinetic method was developed using ethidium bromide as a fluorescence probe to monitor the degradation process of RNA in the presence of ribonucleases. To understand the structure-activity relationship for the inhibition of RNase A in terms of the positional preferences for the –OH groups of the polyphenols, we have carried out agarose gel electrophoresis followed by a precipitation assay. Enzyme kinetics was also performed to determine the inhibition constant values for the polyphenols. The secondary structural changes due to binding of the polyphenols with RNase A were monitored via circular dichroism (CD) and Fourier transform infrared (FTIR) studies. Fluorescence quenching studies were performed to calculate the number of binding sites and the binding constant values of various polyphenols with RNase A. The inhibitory property of copper complexes of rutin and quercetin were also investigated. In addition ^{1}H NMR studies of RNase A confirmed the noncompetitive binding mode of the ligands. The presence of hydroxyl groups in the cinnamoyl and benzoyl moieties of the flavonoids are required for the inhibitory activity against RNase A.

Angiogenin and lactoferrin (LF) have been isolated from buffalo milk via cation exchange chromatography. The purity of the isolated angiogenin was verified by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and a zymogram assay. The isolated angiogenin exhibits the ribonucleolytic activity and is inhibited by the ribonuclease inhibitor (RI). The ribonucleolytic activity of angiogenin is lost upon treatment with diethyl pyrocarbonate (DEPC). The angiogenesis potency of the isolated angiogenin was verified by the chorioallantoic membrane (CAM) assay. The polyphenols have been found to inhibit the ribonucleolytic and angiogenic activity of angiogenin. Docking studies and fluorescence resonance energy transfer (FRET) calculations show that the polyphenols bind at a distance of 20-30Å from Trp 89 of human angiogenin. LF shows the superoxide scavenging activity and antiangiogenic activity. Interestingly RI is unable to inhibit the ribonucleolytic activity of LF. The DEPC modified form of LF is unable to cleave RNA indicating that His residues are present at the active site. The DNA binding property of LF was determined by the agarose gel based assay and was found to be an entropy driven process. The ribonucleolytic activity of LF was not affected upon binding with DNA. DNA binds at the N terminal site of LF as estimated by the Adaptive Poisson-Boltzmann Solver (APBS).

Keywords: Ribonuclease A; Buffalo angiogenin; Buffalo lactoferrin; Ribonuclease Inhibitor; Inhibition; Polyphenols.