New cysteine protease inhibitors in physiological secretory fluids and their medical significance

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Abstract: New cysteine protease inhibitors in human tears and milk were found and their medical significance was studied. As the protective components against bacterial infection in eyes, we detected four kinds of biologically active proteins in normal human tears including three kinds of cysteine protease inhibitors. Using our reverse zymography of normal tears, the three kinds of cysteine protease inhibitors were found to be 78, 20 and 15 kDa and were determined to be lactoferrin, VEG protein and cystatin S, respectively. The C-terminus area 17 mer peptide, Y_{679}-K_{695} of lactoferrin molecule showed strong homology with a common active domain of cystatin family and the synthesized peptide itself showed considerable inhibition of cysteine proteases. Not only disease-specific changes of these inhibitor contents, but disease-specific new inhibitors were also found in patient tears in special autoimmune diseases. The characteristic 35 kDa inhibitor band which was detected specifically in the cases of Behcet's disease tears, an autoimmune disease, was determined to be a lachrymal acidic proline-rich protein family based on the N-terminus sequence analysis. The 65 kDa inhibitor of tears in Harada's autoimmune-disease was determined to be a human Ig heavy chain V-III region. Also lactoferrin content in Harada's disease was very low compared with that of normal tears. Also we found two cathepsin inhibitors, lactoferrin and ?-casein, in milk of human and bovine using reverse zymography. They may also play a role in bacterio-cidal and viro-cidal functions in milk. The L_{133}-Q_{151} in human ?-casein molecule is the active inhibitory domain. It is most important to know from biological aspects that the concentration of these inhibitors in natural milk can inhibit cysteine proteases of bacteria. Surprisingly, the 50 times diluted milk inhibited papain completely, because lactoferrin and casein contents in milk are very high. We want to emphasize that these inhibitors in milk play a sufficient role in the protection of bacteria.

Index Keywords: casein; cathepsin; cystatin; cysteine proteinase inhibitor; lactoferrin; papain; peptide; transferrin; amino acid sequence; animal; breast milk; cattle; chemistry; conference paper; dose response; human; kinetics; liver; metabolism; milk; molecular genetics; polyacrylamide gel electrophoresis; protein tertiary structure; rat; sequence homology; structure activity relation; Amino Acid Sequence; Animals; Caseins; Cathepsins; Cattle; Cystatins; Cysteine Proteinase Inhibitors; Dose-Response Relationship, Drug; Electrophoresis, Polyacrylamide Gel; Humans; Kinetics; Lactoferrin; Liver; Milk; Milk, Human; Molecular Sequence Data; Papain; Peptides; Protein Structure, Tertiary; Rats; Sequence Homology, Amino Acid; Structure-Activity Relationship; Transferrin; Animalia; Bacteria (microorganisms); Bos taurus; Bovinae

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