Abstract
ABSTRACT

Cystatins are the endogenous thiol proteinase inhibitors found ubiquitously in the physiological system. Inhibitors showing evolutionary, structurally and functionally significant correlation to cystatins have been grouped into cystatin superfamily. Cystatin superfamily has been divided into three families stefin, cystatin and kininogen on the basis of the number of amino acids, disulphide bonds and carbohydrate content. Regulation of the activities of CP by their inhibitors is important to maintain a balance between the proteinase and antiproteinase. Cystatins are competitive, non-covalent and tight binding inhibitors. They have reportedly been purified from a number of mammalian sources like muscle, spleen, heart and liver. However, lacuna existed in the isolation and purification of cystatin from most important mammalian organ that is kidney.

In the present study purification of cystatin from goat kidney has been achieved by simple three step procedure which includes ammonium sulphate fractionation, gel filtration on Sephadex G-75 and ion-exchange chromatography on DEAE-cellulose. The preparation was found to be homogenous on the basis of molecular weight however when the pooled fractions from gel chromatography were subjected to DEAE-cellulose column two homogenous inhibitor isoforms were obtained with a yield of about 60.8% and 40% and fold purification of 266.5 and 229, respectively. The isoforms I and II have Mr of 67KDa consisting of single polypeptide chain as found by gel filtration and SDS PAGE. The stokes radius of the isoforms was also identical, 35.3Å. They are devoid of disulphide bonds and contain carbohydrate content as 1.8% and 2.9% for isoforms I and II, respectively. Both the inhibitors exhibited remarkable stability in the temperature range of 35°C - 95°C and wide range of pH (3-10) and showed antibacterial activity against Staphylococcus aureus, Bacillus subtilis, Streptococcus hemolyticus, Escherichia coli and Pseudomonas fluorescens.
The 15 residue N-terminal sequence analysis showed low homology to chicken cystatin and human cystatin S and no homology to cystatin A, B, D, E and F. Comparatively higher sequence homology to type III cystatins (33.3%) was observed. The conserved glycine (which is present at position 9 in many cystatins) was found at position 15 in the purified cystatins. Analogous cleavage pattern by nonspecific proteases was observed. Chymotrypsin cleaved the cystatin into two domains although trypsin did not cleave the inhibitors. The antiserum against the isoform II showed cross reactivity among the two isoforms and complete identity with LMW kininogen.

The purified cystatin exhibited major fluorescence spectral changes in the presence of papain. The CD studies revealed precisely the secondary structural composition of isoforms I and II. Isoform I was composed of 29.6% α-helix, 9% β-structure, 27.9 β-turn and 33.5% random structure. Similarly, a predominant helical structure was obtained in isoform II with 25.5% α-helix, 17% β-structure, 22.4% β-turn and 35.2% random structure.

The inhibition kinetics studies revealed that the cystatin isoforms are competitive tight binding inhibitors. The major finding being the inhibition of bromelain by these isoforms, although the affinity was low. The Ki values obtained were 2.1x10^{-10}M and 3x10^{-10}M with papain, 9x10^{-10}M and 5x10^{-10}M with ficin and 2.2x10^{-9}M and 1.7x10^{-9} with bromelain for isoforms I and II, respectively. Papain exhibited highest affinity compared to ficin and bromelain. Highest association rate of 1.5x10^{6}M^{-1}s^{-1} and 8.6x10^{4}M^{-1}s^{-1} was obtained for papain for isoform I and II respectively. Dissociation rate constants of 6.14x10^{2}s^{-1} and 5.75x10^{4}s^{-1} with papain, 5.37x10^{4}s^{-1} and 4.6x10^{4}s^{-1} with bromelain and 1.63x10^{3}s^{-1} and 2.3x10^{3}s^{-1} with ficin for isoform I and II was obtained, respectively.
Cystatin underwent damage and degradation by the ROS produced by riboflavin mainly due to singlet oxygen and hydroxyl radical. The damage was further examined by fluorescence spectra. The results showed rapid and major spectroscopic changes in the cystatin conformation on exposure to ROS.

The novel properties of cystatin like high molecular weight, absence of disulphide bonds, presence of carbohydrate moieties, antibacterial activity, high stability to wide temperature and pH range, unique N-terminal 15 amino acid residue sequence, different cleavage pattern by chymotrypsin and trypsin, composition of secondary structures, the kinetics of inhibition which describe these isoforms to be competitive and tight binding inhibitors and susceptibility to ROS suggest that it cannot be placed in any of the known families of cystatin. The characteristics are intermediate between type I, II and III cystatins. Hence, the name cystatin K is proposed to place it in a new class of cystatins.