LIST OF FIGURES

Figure 1.1 Diagrammatic representation of the chain structures of proteins in the cystatin superfamily

Figure 1.2 Schematic illustration of evolutionary relationships

Figure 1.3 3D structure of a typical type II cystatin and its secondary structure elements

Figure 1.4 Scheme of the proposed model for the interaction of chicken cystatin with papain

Figure 4.1 Gel filtration chromatography

Figure 4.2 Ion-exchange chromatography

Figure 4.3 Gel electrophoresis of goat kidney cystatin during various stages of purification

Figure 4.4 SDS Polyacrylamide gel electrophoresis of the purified isoforms of goat kidney cystatin

Figure 4.5 SDS PAGE of the purified goat cystatin in the presence of 8M urea

Figure 4.6 Molecular weight determination of goat cystatin by SDS PAGE

Figure 4.7 Molecular weight estimation of purified goat cystatin using Sephadex G-75 gel filtration chromatography

Figure 4.8 Determination of Stoke's radius of the purified cystatin by Laurent and Killander plot

Figure 4.9 Effect of proteolytic enzymes on the purified cystatin isoforms

Figure 4.10 Effect of temperature on thiol proteinase inhibitor
Figure 4.11 Effect of pH on purified cystatin isoforms. 74
Figure 4.12 Thermal denaturation of cystatin isoforms. 76
Figure 4.13 Direct binding ELISA. 77
Figure 4.14 Ouchterlony immunodiffusion. 78
Figure 4.15 Immunodiffusion of goat cystatin with sheep LMW kininogen. 79
Figure 4.16 Inhibition ELISA of isoforms I and II. 80
Figure 4.17 N-terminal amino acid sequence of goat cystatin 82
Figure 4.18 UV absorption spectra of native isoforms I and II 85
Figure 4.19 Ultraviolet absorption spectra of papain complex with I and II isoforms 86
Figure 4.20 Ultraviolet Absorption spectra of isoform I and II under denaturing conditions. 87
Figure 4.21 Intrinsic fluorescence of native isoform I and II 88
Figure 4.22 Intrinsic fluorescence of isoform I and II complex with papain 90
Figure 4.23 Fluorescence spectra of isoform I on incubation with different concentrations of urea and GdnHCl 91
Figure 4.24 Fluorescence spectra of isoform II after treatment with different concentrations of urea and GdnHCl 92
Figure 4.25 Fluorescence spectra of isoform I and II after denaturation in reducing condition 93
Figure 4.26 Far-UV circular dichroism spectra of native purified isoforms I and II. 95
Figure 4.27 Far-UV circular dichroism spectra of papain complex with isoforms I and II 97
Figure 4.28 Far UV circular dichroism spectra of urea and GdnHCl treated isoforms I and II 98
Figure 4.29 Near-UV circular dichroism spectra of native isoforms 99
Figure 4.30 Circular dichroism spectra of the complex of 4.4μM purified isoforms I and II with papain. 100
Figure 4.31 Ki determination with papain 102
Figure 4.32 Ki value determination with ficin 104
Figure 4.33 Ki determination with bromelain 105
Figure 4.34 Determination of association rate constants for papain. 107
Figure 4.35 Association rate constant determination for ficin 109
Figure 4.36 Association rate constant determination for bromelain 110
Figure 4.37 Determination of rate constants for dissociation with papain 111
Figure 4.38 Determination of rate constants for dissociation with ficin 112
Figure 4.39 Determination of rate constants for dissociation with bromelain 113
Figure 4.40 Effect of riboflavin on the activity of cystatin 115
Figure 4.41 PAGE of cystatin damage by riboflavin 116
Figure 4.42 PAGE of cystatin and riboflavin in the presence of free radical scavengers 119
Figure 4.43 Fluorescence spectra of cystatin after treatment with 100μM riboflavin 121
Figure 4.44 Fluorescence spectra of cystatin alone (—) and riboflavin treated cystatin (---) and cystatin after removal of riboflavin (——). 122
Figure 4.45 UV absorption spectra of riboflavin. 123