A-07 Purification and characterization of an acid proteinase from filarial parasite *Setaria digitata*

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Parasite proteinases play a significant role in host tissue invasion, evasion of host immune response, larval development and nutrient uptake. Therefore inhibition of parasite proteinases will be a potential target in the control of filariasis. We have started to characterize filarial parasite proteinases with the long term objective of inhibitor screening. Isolation of acid and neutral proteinases of the filarial parasite *Setaria digitata* were presented last year. In this report, the purification procedure and enzymatic properties of the major acid proteinase of *S. digitata* are presented.

A crude extract of the parasite. *S. digitata* was prepared and it was centrifuged. The supernatant was dialyzed and applied onto a column of DEAE cellulose-52. The protein was eluted with a linear gradient of 0-1M NaCl. Fractions of the major peak with proteolytic activity were combined and applied into a column of Sephacryl S-200. Fractions with proteolytic activity were combined and dialyzed against 0.04M acetate buffer, 0.2M NaCl at pH 4.0 and applied into pepstatin-Sepharose column. The protein was eluted with 0.05M Tris-HCl buffer, pH 8.0, containing 1M NaCl. The fractions with proteolytic activity were pooled and applied onto a Mono Q(HR 5/5) column and the proteins were eluted with a linear gradient of 0-1.0M NaCl in 0.02M Tris-HCl buffer at pH 7.8 in FPLC system.

The acid proteinase of *S. digitata* was purified by elution through successive chromatographies until a single band was seen on SDS-PAGE. The molecular mass of the purified proteinase was estimated to be 40kD by SDS-PAGE. The enzyme preferentially hydrolysed denatured bovine haemoglobin at pH 2.0-3.0. Further significant activity was not observed above pH 7.0. The proteolytic activity of the enzyme was completely inhibited by 0.1mM pepstatin at pH 3.0. Furthermore, the enzyme was stable at the neutral pH where pepsin loses its activity. These results suggest that the major proteinase of *S. digitata* belongs to the family of non pepsin type aspartic proteinase.