A-39 Purification and characterization of a minor 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. In this paper purification procedure and enzymatic properties of minor acid proteinase of *S. digitata* are presented.

Crude extract of parasite *S. digitata* was prepared and it was centrifuged. The supernatant was dialyzed and applied into a column of DEAE cellulose-52 and washed with 0.02M phosphate buffer, pH 7.5. The protein was eluted with a linear gradient of 0-1M NaCl. Fractions, collected during sample injection and washing with proteolytic activity were combined and dialyzed against 0.04M sodium acetate buffer pH 4.0. It was applied into a column of pepstatin-Sepharose. 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 into 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 a FPLC system.

The acid proteinase of *S. digitata* was purified by elution through successive chromatographies to a single band at the SDS-PAGE. Elution positions of the minor enzyme from DEAE-cellulose 52, Mono Q chromatography were different from those of major one. The molecular mass of the purified proteinase was estimated to be 42 kd by SDS-PAGE. The enzyme preferentially hydrolyzed denatured bovine haemoglobin at pH 2.5-3.0 and at 37°C. Further significant activity was not observed above pH 7.0.

The proteolytic activity of enzyme was completely inhibited by 0.1mM pepstatin at pH 3.0 like major enzyme. Further, enzyme was stable at the neutral pH where pepsin loses its activity. These results suggesting that minor acid proteinase of *S. digitata* belongs to the family of aspartic proteinase as the major protein.

Investigation of substrate specificity, structure and physiological function of this enzyme and comparison with a major one will provide necessary information for the future screening of proteinase inhibitors for therapeutic approach.

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