A-27: Isolation and characterization of proteinases of filarial parasite *Setaria digitata*

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Filariasis is a public health problem. Available chemotherapeutic agents are not effective to control filariasis in endemic areas. Specific inhibition of parasite key enzymes by chemotherapeutic agents is considered as a more effective method. Parasite proteinases play a significant role in host tissue invasion, evasion of host immune response, larval development and nutrient uptake. Therefore inhibition of parasite proteinase will be a potential target in the control of filariasis. However few parasite proteinases have been characterized so far. We have started to characterize filarial parasite proteinases with the long term objective of inhibitor screening. This paper presents the isolation and characterization of proteinases of filarial parasite *Setaria digitata*

Parasites *S.digitata* were collected from infected cattle in Kandy slaughter house and washed 3 times with phosphate buffer saline and stored at -30°C. Crude extract was prepared by homogenizing parasites in phosphate buffer at pH 7.5. Proteolytic activities at acidic and neutral pH were analysed by activity staining of electrophoretically fractionated crude extract on

polyacrylamide gel with haemoglobin and casein as the substrate. Assay procedures were developed to determine proteolytic activities at the acidic and neutral pH by using denatured haemoglobin and casein & 7-amino-4-methylcoumarin (MCA) derivatives as the substrates respectively. Proteinases identified in crude extract were separated by using DEAE-Cellulose and Q-Sepharose chromatographies. Properties of partially purified proteinases were investigated.

Several proteolytic active zones were observed at acidic and neutral pHs in activity staining of electrophoretically fractionated crude extract on polyacrylamide gel. Significant proteolytic activities of crude extract were determined at acidic pH and neutral pH toward haemoglobin and casein, respectively with developed assay procedures. Further significant hydrolysis of synthetic substrate Ala-MCA and Arg-MCA were observed with crude extract at pH 8.0. These results suggest the presence of acid & neutral endoproteinases and aminopeptidases in *S.digitata*.

Acid proteinase activity was observed with fractions of 2 major peaks and one minor peak eluted at 0.25, 0.70 and 0.50M NaCl respectively, in DEAE-cellulose 52 chromatography, suggesting presence of 3 types of acid proteinases. Complete inhibition of proteolytic activities of these fractions at the acidic pH were observed with 0.1mM pepstatin indicating acid proteinases of *S.digitata* belong to family of aspartic proteinase. Further maximum proteolytic activities of these 3 peaks were observed at pH 3.0-4.0. Purification, characterization and structural determination of these acid proteinases will clarify whether *S.digitata* contain different types of acidic proteinases or isoenzymes.

Neutral proteinase activity was detected in fractions collected during sample injection and washing of DEAE-Cellulose 52 chromatography. Fractions with proteolytic activity at neutral pH were pooled and further purified by Q-sepharose chromatography. Endopeptidase activity (casein hydrolysis) was observed with fractions of peak eluted at 0.25M NaCl. However amino peptidase activity (hydrolysis of Ala-MCA & Arg-MCA) was observed with fractions of peak eluted at 0.15M NaCl. These results confirm the presence of 2 different types of neutral proteinases in *S.digitata*. Further 99% and 40% inhibition of amino peptidase activity was observed with 10 mM 1,10 phenathroline and 10μM bestatin suggesting *S.digitata* aminopeptidase belongs to a family of metallo proteinases.

Three different types of proteinases: acid(aspartic) neutral & aminopeptidase (metallo) were identified in crude extract of *S.digitata*. These proteinases were separated by chromatographies on DEAE-Cellulose 52 and Q-sepharose.