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ISOLATION, PURIFICATION AND CHARACTERISATION OF CLINICALLY IMPORTANT PROTEINASES OF FILARIAL PARASITE *SETERIA DIGITATA*

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Filariasis is a public health problem in the Sri Lanka and world. Available chemotheraputic agents are not effective to control the disease in endemic areas. Specific inhibition of parasite key enzymes by chemotheraputic agent is considered as a more effective method of controlling filarial infection. Parasite proteinases are believed to play a significant role in host tissue invasion, evasion of host immune responses, larval development and nutrient uptake. Therefore inhibition of parasite proteinase will be a potential target in the control of filariasis. However few studies have been reported on characterisation of proteinases of filarial parasite. We have started to characterise filarial proteinases with long term objective of development of their inhibitor as a chemotheraputic agent to control filariasis.

Filarial parasites *Seteria digitata* were collected from slaughtered cattle and washed with PBS and stored at –30 °C. Crude extract of whole parasite was prepared and acid proteinase activity was identified by analysing in polyacrylamide gel electrophoresis followed by activity staining. Three acid proteinase activity zones were detected and suggesting the presence of three acid proteinases in crude extract. Assay procedure was developed to detect acid and neutral proteinase activities by using denatured haemoglobin and casein as the substrates. Acid proteinases in crude extract were partially purified by chromatographies on DEAE cellulose 52, Sephacryl S-200 and pepstatin Sepharose.

Parasites were dissected into three parts, body wall, digestive system(oesophagus, intestine) and reproductive system. Crude extract of each part was prepared separately. Acid proteinase activities were 2.04 , 0.75, 2.50 and 0.47 U/mg in crude extract of whole parasite, reproductive system, digestive system and body wall, respectively. Reproductive tissues were further dissected to ovarian tubes and uterus and higher acid proteinase activity was observed in extract of ovarian tubes. Relatively low activity of neutral proteinase was detected in all parts of the parasite, except intestine.

Acid proteinases of crude extracts of dissected tissues were purified by successive chormatographies as described above. Two DEAE bound acid proteinases were identifed in crude extract of intestinal tissues and body wall. They were eluted at 0.4 and 0.6M NaCl . Three acid proteinases(two DEAE bound and one DEAE unbound) were identified in extract of reproductive system and bound proteinases were eluted at 0.4 and 0.8M NaCl. These results suggest the specific localisation of acid proteinases and their specific roles in different tissues.

Optimum pH and temperature of DEAE unbound acid proteinase of reproductive system were at 3.0 and 45 ^{0}C and molecular weight was 42,000 as analysed in SDS-PAGE. Complete inhibition of acid proteinase activity was observed with 1 mM pepstatin suggest that it belongs to family of aspartic proteinase. Further purification of acid proteinases and clarification of their physiological role are in progress to identify a better target proteinase for inhibition.