PURIFICATION AND MOLECULAR CHARACTERIZATION OF ACID PROTEINASES FROM FILARIAL PARASITE *Setaria digitata*

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ABSTRACT

Acid proteinases are important as new targets for chemotherapy in the control of parasitic diseases. Proteinases of cattle filarial parasite *Setaria digitata* were characterized for this purpose.

Crude extract of the female cattle filarial parasites separated on PAGE showed three different acid proteinase activities. When the body parts of the parasite were separated into uterus and oviducts, esophagus and intestine and body wall, three acid proteinases appeared differently. The uterus and oviducts representing the reproductive system, showed the highest levels of activity for all three enzymes. But in the oesophages and intestine, which represent the digestive system, there were only two enzyme activities and these two were at a lower level than those of the reproductive system. In the case of body wall there were only two enzyme activities, similar to the digestive system but at a much lower level of activities.

In developing methods for purification of the acid proteinases, crude extract of the whole parasite was used. The steps included DEAE cellulose chromatography, S-200 gel filtration, pepstatin sepharose affinity chromatography followed by mono-Q chromatography. DEAE cellulose chromatography resolves three distinct enzyme activities. Each of these was then separated by S-200 gel filtration, pepstatin sepharose affinity chromatography and mono-Q chromatography. In DEAE cellulose chromatography the enzyme activity detected in a fraction of single peak eluted during sample injection was referred to as DE-

unbound (DEUB) proteinase, the second peak eluted at 0.4M NaCl was referred to as DE-bound major (DEBMJ) proteinase, it showed the highest activity and the third peak eluted at 0.8M NaCl was referred to as DE-bound minor (DEBMN) proteinase.

All these proteinase activities separated had a molecular weight of 42kDa as determined by SDS-PAGE. Since all these proteinase activities couldn't be resolved further using SDS-PAGE under reducing conditions. It is suggested that three acid proteinases are made from a single polypeptide.

The optimum pH for enzyme activity was observed to be pH 2.0, 1.5 and 2.5 for DEUB, DEBMJ and DEBMN proteinases, respectively. Optimum temperature was observed at 45 °C for all three proteinases. The DE-unbound proteinase was stable over a wider pH range than the DE-bound types. Even though DE-bound proteinases were stable in neutral and alkaline pH, they had a very little stability in acidic pH. They were not inhibited with soybean trypsin inhibitor, phenol methane sulphonyl fluoride (PMSF) and EDTA, but totally inhibited by pepstatin confirming that they belong to the family of aspartic proteinases.

Proteinases isolated from different tissues showed some similar as well as different characteristics. The results confirm the presence of three types of proteinases localized in different tissues of the parasite. The N-terminal amino acid sequencing is required to clarify whether they are isozymes of the same enzyme or different enzymes. Identification of cleavage specificity of the acid proteinases against susceptible biological peptides is necessary to clarify the physiological role

of these proteinases. These investigations will clarify the physiological role of three acid proteinases and the possibility of identification as a therapeutic target point to control the filarial infections in the future.