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Proteases secreted by the infective larvae of *Toxocara canis* and partial purification of a 50 kDa protease

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Toxocara canis is a nematode parasite of dogs, a causative agent for human toxocariasis. It has become one of the major zoono-parasitic infections in Sri Lanka. Infective second-stage larvae of *T. canis* secrete proteolytic enzymes which are suggested to be instrumental in their tissue-migration process. Therefore, this study aims at identification of proteolytic enzymes which are involved in the invasion and processing of proteins of these parasites and thereby targeting a specific enzyme for the control of the infection.

Proteolytic activity of these larvae during culture in vitro was determined by gelatin - zymography, pH optimum and substrate and inhibitor specificity. A partial purification of a 50 kDa protease was done using DEAE-anion exchange chromatography which was characterized for its optimum pH, temperature and inhibitor susceptibility.

Excretory-secretory products of infective larvae showed proteolytic activity as seven bands in gelatin zymography with their molecular weights lie between 175 kDa to 20 kDa. Serine, cysteine and metalloprotease activity were identified optimally at pH 5.5 to 6.5. Metalloprotease found to be predominated. Proteolytic activity was optimum against albumin over gelatin and casein. 50 kDa protease was partially purified by using DEAE-anion exchange chromatography and its activity was optimum at pH 8.5 and 70 °C. This protease activity was inhibited by metalloprotease inhibitor EDTA.

Proteases secreted by *T. canis* infective larvae exhibit diversity in classes of proteases, based on the differential migration in polyacrylamide gels containing gelatin. This result clearly demonstrates the heterogeneity of larval proteases that might be involved in important functions during the larval migration. Partially purified 50 kDa protease might be involved in a specific function and inhibition of this enzyme activity may arrest the activity of the infective larvae. Therefore, this enzyme could be a target candidate in the control of toxocariasis by inhibition with chemical or immunological methods.

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