Aspartic Proteinase in *Dugesia tigrina* (Girard) Planaria

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A proteolytic activity was identified in *Dugesia tigrina* planaria using the chromogenic substrate Phe–Ala–Ala–Phe (4-NO_2)–Phe–Val–Leu–O_4MP. The activity of the enzyme increased four times during the regeneration and presented a maximum at 120 hr being higher in tail than head regenerating segments. The protease that displays this activity was purified from worms by a single step on pepstatin-agarose followed by gel-filtration high performance liquid chromatography. The purification resulted in a 34-fold increase in specific activity and the final yield was 10%. The active *D. tigrina* hydrolase appears to be a dimeric protein composed of identical subunits with 34 kDa associated by disulphide bridges similar to vertebrate cathepsin D. By SDS-PAGE several bands were detected but upon gel filtration HPLC one proteolytically active component, termed Asp-68, was detected and isolated. The maximal activity was observed in a range between pH 3.5–5.0 and the enzyme became inactivated at a pH value above 7.2. The purified enzyme was not inhibited by inhibitors from serine (aprotinin, TPCK, PMSF and TLCK), metallo (EDTA) and cysteine proteinase (E-64) classes. In contrast, inhibitors such as pepstatin, EPNP, and 4-β-PMA efficiently inhibited the activity of the 68-kDa protease.