

HYALURONIDASES IN *Loxosceles intermedia* (BROWN SPIDER) VENOM ARE ENDO- β -N-ACETYL-D-HEXOSAMINIDASES HYDROLASES

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In studying *Loxosceles* spp. venom, we detected degradation of purified hyaluronic acid (HA) and hydrolysis of purified chondroitin sulphate (CS) while neither dermatan sulphate, heparin or heparan sulphate were affected. In addition we showed that a hydrolase enzyme was involved in the HA cleavage and found that venom hyaluronidase is an endo- β -N-acetyl-d-hexosaminidase that generates terminal N-acetylglucosamine residues upon cleavage of HA. Zymogram analysis of *L. intermedia* venom showed both HA and CS lytic activities at 41 and 43 kDa. Thus, these results suggest that the same molecules are involved in cleaving HA and CS residues. *L. intermedia* electrostimulated venom and venom gland extract demonstrated very similar HA lytic activity, suggesting that hyaluronidases are self-components of venom instead of oral egesta contamination. These hydrolase enzymes showed no apparent activities upon HA at low or high pH, with optimal activity at 6.0–8.0 pH. Finally, we confirmed the cleaving action of the venom hyaluronidases on HA in the extracellular matrix of the dermis of rabbit by fluorescence reaction to HA. Thus, hyaluronidases type hydrolases endo- β -N-acetyl-d-hexosaminidase are implicated as self-components of *Loxosceles* spp. spider venom and can be involved in venom effects as spreading factors.