

Structural diversity of hyaluronidases from animal venoms

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Hyaluronidases (HYAL) are a diverse group of enzymes present across all domains of life. These enzymes primarily degrade hyaluronan and other glycosaminoglycans found in the extracellular matrix (ECM). This degradation can lead to a loss of integrity in the ECM of soft connective tissues, which is crucial in various biological processes including fertilization, cell differentiation, inflammation, and the growth and metastasis of tumor cells. The ability of HYAL to break down ECM components has been harnessed to enhance the absorption and dispersion of injected drugs. Beyond this application, their potential in medical and biotechnology fields is significant, with HYAL being utilized in antitumor therapies, immunotherapy, and cosmetic procedures. In this study, we performed a structural analysis of HYALs from the venoms of several species: *Apis mellifera* (bee - Am-Hyal), *Lasiadora subcanens* (spider - Ls-Hyal), *Bothrops jararacussu* (snake - Bj-Hyal), and *Scorpaena plumieri* (fish - Sp-Hyal), using bioinformatics tools. Initially, the presence of HYAL in these venoms was evidenced through SDS-PAGE zymography assays, which demonstrated catalytic activity on hyaluronan. All venoms exhibited marked activity, indicated by colorless bands on an otherwise blue gel. The migration patterns were as follows: approximately 45 kDa for Am-Hyal and Ls-Hyal, and approximately 60 kDa for Sp-Hyal. In contrast, Bj-Hyal displayed two colorless bands at approximately 15 and 20 kDa. Amino acid sequence alignment of HYALs was performed using Align (UniProt). Sequences for Am-Hyal, Bj-Hyal, and Sp-Hyal were obtained from UniProt, while Ls-Hyal was obtained through transcriptomic and proteomic analysis. For Sp-Hyal, the sequence of the fish *Pterois volitans* was used as a template, since the sequence fragment from *S. plumieri* venom showed 100% similarity to the HYAL from this fish. Sequence alignment revealed approximately 26–38% of identity among these HYALs and found several key features: a probable region with high consensus, predictions of protein domains (EGF-like I and II; Multicopper oxidase-Prosite), catalytic/specificity amino acid residues, disulfide bonds, a glycosyl hydrolase family 56 signature (Pfam), and putative N-glycosylation sites (NetNGlyc) in asparagine residues. Specifically, there are three N-glycosylation sites in Am-Hyal (5, 83, and 231), two in Ls-Hyal (62 and 140), nine in Bj-Hyal (25, 47, 76, 138, 193, 214, 304, 311, and 329) and four in Sp-Hyal (50, 141, 336, and 422). Molecular modeling (Chimera-X) revealed distinct three-dimensional structures. The in silico data revealed both structural similarities and differences among HYALs from various animal venoms, enhancing our understanding of their biological properties. This information paving the way for future research that may explore the biotechnological potential of these enzymes.

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