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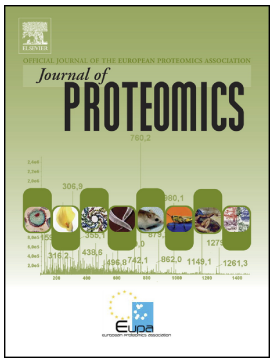

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Isolation and characterization of Conohyal-P1, a hyaluronidase from the injected venom of *Conus purpurascens*

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Running title: Conohyal-P1, a novel hyaluronidase

Abstract

Hyaluronidases are ubiquitous enzymes commonly found in venom and their main function is to degrade hyaluran, which is the major glycosaminoglycan of the extracellular matrix in animal tissues. Here we describe the purification and characterization of a 60 kDa hyaluronidase found in the injected venom from *Conus purpurascens*, Conohyal-P1. Using a combined strategy based on transcriptomic and proteomic analysis, we determined the Conohyal-P1 sequence. Conohyal-P1 has conserved consensus catalytic and positioning domain residues characteristic of hyaluronidases and a C-terminus EGF-like domain. Additionally, the enzyme is expressed as a mixture of glycosylated isoforms at five asparagine sites. The activity of the native Conohyal-P1 was assess MS-based methods and confirmed by classical turbidimetric methods. The MS-based assay is particularly sensitive and provides the first detailed analysis of a venom hyaluronidase activity monitored with this method. The discovery of new hyaluronidases and the development of techniques to evaluate their performance can advance