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论文

质谱法分析蛇毒蛋白翻译后修饰

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摘要:

采用SDS-PAGE分离大连黑眉蝮蛇(*Gloydius Shedaoensis*)蛇毒蛋白组分, Pro-Q Emerald 488糖蛋白和Pro-Q Diamond磷酸化蛋白荧光染料用于糖蛋白和磷酸化蛋白泳带染色, 采用高效液相色谱电喷雾电离串联质谱(HPLC-nESI-MS/MS)法鉴定蛋白. SDS-PAGE胶上的8条糖蛋白带被分别鉴定为*L*-氨基酸氧化酶、金属蛋白酶、谷氨酰环化酶、C-端缺失*L*-氨基酸氧化酶、纤溶酶原激活物、磷脂酶A_2(PLA_2)和神经生长因子; 5条磷酸化蛋白带被分别鉴定为Stejaggregin-A、PLA_2、Crisp、金属蛋白酶 P-Ⅲ和Acutolysin e precursor, 与其它蛇毒来源蛋白具有一定的同源性. 为进一步验证方法的可靠性, 采用离子交换和凝胶过滤层析技术纯化得到了PLA_2, Pro-Q Diamond染色结果显示PLA_2被磷酸化. 研究所得结果为进一步研究蛋白质翻译后修饰对蛇毒蛋白的生物活性、结构与功能提供了依据.

关键词: 蛇毒 糖基化 磷酸化 高效液相色谱串联质谱法

Investigation of Post-translational Modifications of Proteins in the Venom of Chinese *Gloydius Shedaoensis* Snake by Mass Spectrometry

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Abstract:

SDS-PAGE was employed to the separation of the venomic complex of Chinese *Gloydius Shedaoensis* snake localized at Lüshun. The glycosylated and phosphorylated protein components were visualized by the Pro-Q Emerald 488 glycoprotein staining and Pro-Q Diamond phosphoprotein fluorescent dyes. Protein identification of the selected protein bands were performed the HPLC-nESI-MS/MS proteomic approach. Eight glycoprotein bands in the gel were identified as the homology proteins of *L*-amino acid oxidase, metalloproteinase, glutaminyl cyclase, salmobin, plasminogen activator, halystase, phospholipase A_2(PLA_2), nerve growth factor and truncated/degraded *L*-amino acid oxidase products at the N-terminus, which posses homology peptides originated from other kinds of snake venoms. The five phosphoprotein bands visualized by Pro-Q Diamond dye were identified as stejaggregin-A, PLA_2, Crisp, metalloproteinase P-Ⅲ and acutolysin e precursor homology proteins. To validate this approach, a novel PLA_2 was purified from this venom to homogeneity by the ion-exchange and gel filtration chromatography. The results from Pro-Q Diamond staining and mass spectrometry identification indicate that the purified PLA_2 sharing certain homology peptides of PLA_2s from other snake venoms. Our experimental results provide new insights for further making a research on the relationship between the post-translation modifications of snake venom proteins and their biological functions and structures.

Keywords: Snake venom Glycosylation Phosphorylation HPLC-nESI-MS mass spectrometry

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