

论文
 磺基异硫氰酸苯酯化学辅助方法对新蛋白质进行从头测序

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摘要:

利用基质辅助激光解吸电离-串联飞行时间(MALDI-TOF-TOF)质谱结合磺基异硫氰酸苯酯化学辅助的方法对一种从拟青霉(*Paecilomyces bainier*)分离纯化到的新人参皂苷Rb1水解酶的部分肽段进行了从头测序. 共获得了这个新蛋白质8条肽段的序列, 一些磺化后信噪比非常低的肽段也获得了比较完整的序列. 同时通过从头测序分析确定了一对甲硫氨酸非氧化和氧化肽段的序列. 结果表明, 磺化后的肽段离子化效率大大增强, 在PSD(源后裂解)过程中只有肽键断裂产生的C端的碎片离子系列(γ 离子系列)出现在质谱图中, 图谱背景清晰, 信噪比高, 单纯的 γ 离子系列使得图谱解析变得非常容易. 将这8条序列在NCBI(美国国立生物技术信息中心)数据库中进行BLAST(蛋白质序列比对工具)检索印证这种 β -葡萄糖苷酶是一个新蛋白质, 发现的两条相对保守的序列为进一步研究奠定了基础.

关键词: 磺基异硫氰酸苯酯 基质辅助激光解吸电离-串联飞行时间质谱 新蛋白质 从头测序

De Novo Sequence of Novel Protein Based on Chemically-assisted Method Using 4-Sulfophenyl Isothiocyanate Derivatization by Mass Spectrometry

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Abstract:

Eight tryptic peptides of a novel ginsenoside Rb1 hydrolase(β -glucosidase) purified from *Paecilomyces bainier* were identified with chemically assisted de novo sequencing followed 4-sulfophenyl isothiocyanate(SPITC) derivatization by MALDI-TOF-TOF mass spectrometry. Some relatively complete sequences were obtained from sulfonated peptides with very low signal/noise ratio. A pair of peptides were identified with non-oxidation and oxidation at methionine, respectively. The results indicated that the sulfonated peptide by SPITC significantly enhanced MALDI-TOF-PSD fragmentation signals and produced a spectrum containing only γ^+ ions. This method facilitated de novo sequencing and spectrum interpretation. By comparing the eight identified peptide sequences with the NCBI database, this purified β -glucosidase proves to be a novel protein that has not yet been reported. Two relatively conserved peptides are remarkable contribution to further research.

Keywords: SPITC MALDI-TOF-TOF MS Novel protein De novo sequence

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