#### 研究论文

反相高效液相色谱/基质辅助激光解吸电离飞行时间质谱分离鉴定螺蛳血管紧张素转换酶抑制肽

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摘要 运用反相高效液相色谱(RP-HPLC)对酶解螺蛳腹足肌得到的血管紧张素转换酶(ACE)抑制肽进行两步分离提纯,第一步主要得到8个组分;选取其中活性最高的组分进一步分离,得到2个组分,其中活性较高组分的ACE半抑制浓度为43.5 µmol/L,基本为单一肽组分。对提纯的组分分别使用高效液相色谱/电喷雾离子质谱法(HPLC/ESI-MS)和基质辅助激光解吸电离飞行时间质谱法(MALDI-TOF MS)进行分析,同时结合氨基酸组成分析结果,最终得到的肽链一级结构为Lys-Glu-Ile-Trp(KEIW),符合已知的高活性ACE抑制肽的结构规律。经过对两种方法分析过程的比较,认为ESI-MS可以得到多方面的信息,但无法确定肽的序列;MALDI-TOF MS可以得到精确的二级质谱图(m/z精确至0.0001),从而可以得到确定的肽的序列。

关键词 <u>螺蛳</u> <u>血管紧张素转换酶抑制肽</u> <u>反相高效液相</u> <u>电喷雾离子质谱</u> <u>基质辅助激光解吸电离飞行时间</u> 质谱

分类号

# Purification and Identification of a Novel ACE Inhibitory Peptide Derived from the Mud Snail Bellamya purificata by RP-HPLC/MALDI-TOF

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#### **Abstract**

Bellamya purificata is one of mud snails in fresh water found in China. The purification and identification of an angiotensin I -converting enzyme (ACE) inhibitory peptide extracted from Bellamya purificata hydrolysate are described. The peptide was purified twice with semi-preparative reversed-phase high performance liquid chromatography (RP-HPLC) to obtain an active fraction with an inhibitory concentration 50% ( IC50) of 43.5 µmol/L. The primary structure of the purified peptide was identified by the high performance liquid chromatography/electrospray ionization mass spectrometry (HPLC/ESI-MS) and the martix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) combining with the amino acid composition analysis. Finally, it was identified as a tetrapeptide and sequenced as Lys-Glu-Ile-Trp (KEIW), which has the common characters of ACE inhibitory peptide extracted from selfish muscle. The structure identification results from the two methods were also compared in this study. The results from ESI-MS included a lot of information, such as the total ion current chromatogram and ultraviolet scan spectrum. However, the exact structure could only be from the MALDI-TOF MS analysis, in which the exact MS/MS spectrum could be obtained. Furthermore, the m/z measurement precision of MALDI-TOF MS was 0.0001 and much better than that of 0.1 of ESI-MS.

**Key words** Bellamya purificata Angiotensin I -converting enzyme (ACE) inhibitory peptide reverse-phase high performance liquid chromatography (RP-HPLC) electrospray ionization mass spectrometry (ESI-MS) martix-assisted laser desorption/ ionization time-of-flight mass spectrometry (MALDI-TOF-TOF MS)

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