Full Papers

溶胶凝胶微流控酶反应器用于蛋白质肽谱分析的研究

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摘要 报道了以硅溶胶凝胶基质为流动支持载体的聚二甲基硅氧烷(PDMS)微流控酶反应器的加工及其与MALDI TOF MS 和ESI MS的联用技术。利用浇注和氧等离子体表面处理技术加工而成的PDMS微流控芯片,其微通道的表面会呈现出一层SiOH基团,这些基团进一步与溶胶基质的羟基发生缩合反应,使包埋有胰蛋白酶的凝胶基质牢固地结合于微通道壁上,从而有效防止了微通道壁上凝胶基质的脱落。通过固定在微流控酶反应器上的不锈钢电极和可取代的石英喷头可实现蛋白质在线酶解,酶解时间约为2秒。利用CE、MALDI TOF 和ESI MS方法对该酶反应器进行研究,结果表明该微酶反应器具有稳定、快速、高效酶解蛋白质的能力,可为蛋白质分析鉴定提供高通量的技术平台。

关键词 微流控酶反应器,硅溶胶凝胶,MALDI TOF MS, ESI MS,高通量蛋白质分析鉴定 分类号

Sol-gel-derived Poly(dimethylsiloxane) Enzymatic Reactor for Microfluidic Peptide Mapping

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Abstract The silica-based poly(dimethylsiloxane) (PDMS) microfluidic enzymatic reactor was reported along with its analytical features in coupling with MALDI TOF and ESI MS. Microfluidic chip was fabricated using PDMS casting and O₂-plasma techniques, and used for the preparation of enzymatic reactor. Plasma oxidation for PDMS enabled the channel wall of microfluidics to present a layer of silanol (SiOH) groups. These SiOH groups as anchors onto the microchannel wall were linked covalently with the hydroxy groups of trypsin-encapsulated sol matrix. As a result, the leakage of sol-gel matrix from the microchannel was effectively prevented. On-line protein analysis was performed with the microfluidic enzymatic reactor by attachment of stainless steel tubing electrode and replaceable tip. The success of trypsin encapsulation was investigated by capillary electrophoresis (CE) detection, and MALDI TOF and ESI MS analysis. The lab-made device provided excellent extent of digestion even at the fast flow rate of 7.0 mL/min with very short residence time of ca. 2 s. In addition, the encapsulated trypsin exhibits increased stability even after continuous use. These features are the most requisite for high-throughput protein identification.

Key words microfluidic enzymatic reactor silica sol-gel matrix-assisted laser desorption ionization time-of-flight mass spectrometry electrospray ionization mass spectroscopy high-throughput protein identification

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