

## 键合磷酸锆开管毛细管柱制备及富集磷酸肽方法研究

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### Study of Preparation of Open Tubular Capillary Column (OTCC) Coated with Zirconium Phosphonate to Isolate Phosphopeptides

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**Abstract:** A new approach that combines the open tubular capillary column coated with zirconium phosphonate (ZrP-OTCC) and matrix-assisted laser dissociated ionization time of flight mass spectrometry (MALDI TOF MS) for selective enrichment and identification of phosphopeptides from complex samples were described. A standard phosphoprotein  $\alpha$ -casein was chosen as the model and sample, and MALDI TOF MS was used to demonstrate the performance of the ZrP-OTCC. The optimal conditions of the ZrP-OTCC, such as interior diameter, length of the silica-fused capillary and flow rate et al, were discussed for preparation and application of the ZrP-OTCC. In addition, urea, SDS, and sodium chloride which often decrease the MS sensitivity were added into the tryptic digest of  $\alpha$ -casein to test the compatible capacity of the ZrP-OTCC, respectively. By using the synthetical phosphopeptide (FLpTEYVATR) as a model sample, the loading capacity of the ZrP-OTCC (150 cm $\times$ 50  $\mu$ m) was 32.52 pmol averagely and the recovery is 78.1% by iTRAQ reagent labeling and MS. Repeated experiments of enrichment for phosphopeptides from an  $\alpha$ -casein digest for 20 times indicate that the approach has a good reproducibility. The selectivity and sensitivity of the capturing method were also evaluated by the tryptic digest mixture of  $\alpha$ -casein and bovine serum albumin at molar ratio of 1:1, 1:10 and 1:100, respectively, and the results show that the ZrP-OTCC can efficiently trap the phosphopeptides even when the concentration is as low as  $10^{-8}$  mol $\cdot$ L $^{-1}$ . Most importantly, the approach was successfully used to realize automated online enrichment for phosphopeptides from the tryptic digest of  $10^{-6}$  mol $\cdot$ L $^{-1}$  proteins in bovine milk as a complex real world sample, making this method be promise for use in future studies of phosphoproteomes on large scale.

**Key words:** open tubular capillary columns; zirconium phosphonate; phosphopeptides; mass spectrometry

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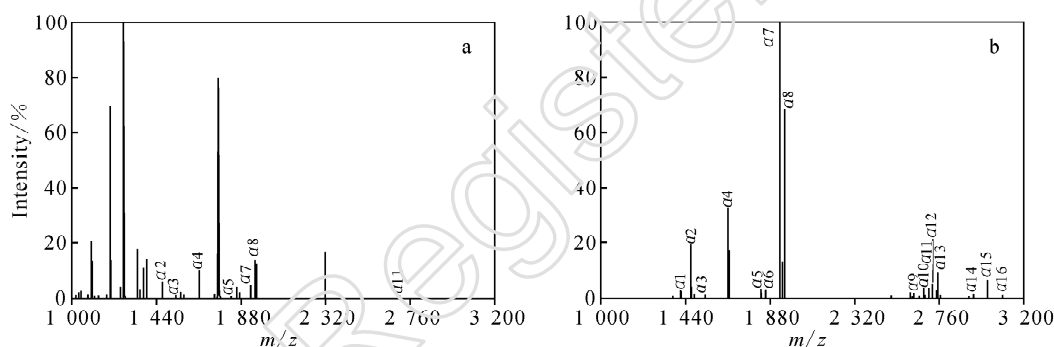
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蛋白质磷酸化是生物体内最重要的翻译后修饰之一, 与细胞的许多生命活动, 如信号转导、细胞通讯、分化、增殖、代谢等密切相关。由于磷酸化蛋白在生物体内含量很低, 且在质谱分析时易

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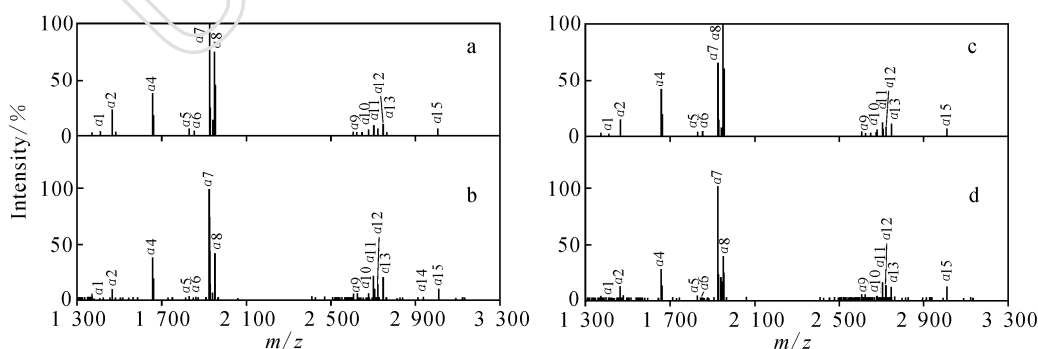
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受到非磷酸肽的干扰,因而磷酸肽和磷酸化蛋白质的富集成为磷酸化肽和磷酸化蛋白质分析的一个关键步骤。目前,虽然有很多富集磷酸肽的方法和技术,但这些方法在应用时仍然存在不少问题,因而需要发展新的富集方法,以满足大规模磷酸化蛋白质组分析的需要。因此,我们开展了一种磷酸肽富集和质谱分析的新方法,并将其应用于复杂样品的分析中。本方法为一种利用键合磷酸锆开管毛细管柱的制备及用于快速富集磷酸肽的新方法。通过酸碱活化熔融石英毛细管内表面,使其带有自由的硅羟基,然后对硅羟基进行氨基修饰、磷酸基团修饰并与锆离子生成磷酸盐(示于图1),最终合成具有特异性吸附磷酸肽分子的开管毛细管柱。进一步采用正交实验对磷酸肽的富集条件进行优化,确定最佳柱径、柱长及进样流速分别为 $50\ \mu\text{m}$ 、 $150\ \text{cm}$ 和 $0.3\ \mu\text{L}\cdot\text{min}^{-1}$ 。另外,如图2所示,还考察了显著影响MALDI源质谱灵敏度的常用试剂尿素、SDS和氯化钠对富集效果的影响。并用合成的磷酸肽段(FLpTEYVATR)与iTRAQ试剂相结合,对开管柱的富集效率、负载量和回收率等进行考察,确定负载量 $32.52\ \text{pmol}$ ,回收率 $78.1\%$ ( $150\ \text{cm}\times 50\ \mu\text{m}$ )。20次重复富集和质谱分析结果表明,该功能化的开管毛细管柱性能稳定。利用 $\alpha$ 酪蛋白与BSA混合酶切溶液,考察了功能化毛细管开管柱的选择性以及MALDI TOF MS检测灵敏度的影响,结果表明此方法对磷酸肽有较高的选择性,最低富集浓度可达到 $10^{-8}\ \text{mol}\cdot\text{L}^{-1}$ 。在优化的实验条件下,利用牛 $\alpha$ 、 $\beta$ 酪蛋白对功能化的开管毛细管柱的富集性能进行考察,实验结果显示 $\alpha$ 酪蛋白的理论磷酸化位点覆盖率达到 $90.9\%$ , $\beta$ 酪蛋白覆盖率为 $100\%$ 。最后,通过将所建富集和质谱鉴定方法用于牛奶样本中磷酸化蛋白质的富集和分析,结果表明,此功能化的开管毛细管柱可以用于复杂样本中磷酸化肽的富集,并提高其质谱鉴定效率。



注: a. 直接质谱分析,  $2\ \text{pmol}$ ; b. 经键合磷酸锆开管毛细管柱富集后质谱分析; 富集条件为:  $2\ \mu\text{mol}\cdot\text{L}^{-1}$   $\alpha$ 酪蛋白酶切肽段以  $0.2\ \mu\text{L}\cdot\text{min}^{-1}$  进样  $100\ \text{min}$ , 洗脱液为  $10\ \mu\text{L}\cdot 0.1\ \text{mol}\cdot\text{L}^{-1}$  氨水溶液, 开管柱内径  $50\ \mu\text{m}$ , 柱长  $150\ \text{cm}$ 。

图1  $2\ \mu\text{mol}\cdot\text{L}^{-1}$   $\alpha$ 酪蛋白酶切肽段的富集前后的MALDI-TOF MS质谱图



注: a. 对照实验, 不含尿素、SDS或氯化钠; b.  $1\ \text{mol}\cdot\text{L}^{-1}$  尿素存在下键合磷酸锆开管毛细管柱富集结果; c.  $1\%$  SDS存在下键合磷酸锆开管毛细管柱富集结果; d.  $1\ \text{mol}\cdot\text{L}^{-1}$  NaCl存在下键合磷酸锆开管毛细管柱富集结果; 富集条件为:  $2\ \mu\text{mol}\cdot\text{L}^{-1}$   $\alpha$ 酪蛋白酶切肽段以  $0.2\ \mu\text{L}\cdot\text{min}^{-1}$  进样  $100\ \text{min}$ , 洗脱液  $0.1\ \text{mol}\cdot\text{L}^{-1}$  氨水溶液  $10\ \mu\text{L}$ , 开管柱内径  $75\ \mu\text{m}$ , 柱长  $50\ \text{cm}$ 。

图2  $2\ \mu\text{mol}\cdot\text{L}^{-1}$   $\alpha$ 酪蛋白酶切肽段的MALDI-TOF MS质谱图