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多肽的磷酸化修饰及多肽与蛋白质、核酸
相互作用研究

Investigation on The Phosphorylation of Peptides and The
Interactions between Peptides and
Proteins/Deoxyribonucleic Acids

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**Investigation on The Phosphorylation of Peptides and The
Interactions between Peptides and
Proteins/Deoxyribonucleic Acids**

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中文摘要

磷酸化修饰是一种重要且常见的蛋白质翻译后修饰方式,蛋白质的可逆磷酸化与去磷酸化过程是真核细胞生命活动中最普遍的调控手段,广泛参与到细胞周期、分化和发育、代谢和神经活动、肌肉收缩和转录调节等生命过程中。

研究蛋白质、多肽的磷酸化方法和磷酸化识别对了解生命过程具有重要科学意义。经典的磷酸化多肽的合成方法,均需要对多肽中的氨基或羧基进行保护。因此,本文的主要工作在于寻找一种水相中非保护的多肽磷酸化方法,为磷酸化多肽的相关研究提供有效的、丰富的样品来源;并且利用电喷雾质谱技术研究磷酸化多肽与蛋白质及活性多肽与核酸的相互作用规律,试图探寻一种通过多肽与生物分子的相互作用,来筛选并利用多级质谱技术同步鉴定多肽结构的研究新思路。

具体来讲,本论文的研究工作主要包括以下两个方面:

一、利用磷酸一酰胺盐作为小分子磷给体,探索一种新型的水相中非保护的多肽 *O*-磷酸化方法。

运用高效液相色谱-电喷雾离子阱质谱监测磷酸一酰胺与多肽在室温、水相的反应,考察其反应可行性及普适性,并确定磷酸化修饰位点。研究结果表明:1) 磷酸一酰胺钠盐在水相中可以实现对多肽的磷酸化修饰;2) 排除该反应条件下肽链上氨基、羧基的磷酸化可能性,最终确定其磷酸化位点为侧链羟基;3) 考察 pH 值和反应投料比对反应转化率的影响,得出磷酸一酰胺与多肽的最佳反应条件是: pH 值为 8.0 的硼酸缓冲体系,磷酸一酰胺与多肽投料摩尔比为 200:1;4) 对比最优条件下各条多肽的磷酸化转化率,发现对序列相近、长度一致的多肽序列,转化率比较接近,约为 20%左右。

该方法一步进行,绿色、便捷,对肽链中的三种侧链羟基(丝氨酸、苏氨酸及酪氨酸)的磷酸化修饰具有普适性。因此,磷酸一酰胺盐是一种有效的多肽侧链羟基的小分子磷给体,将成为一种理想的用于蛋白激酶生物功能、作用机制研究的小分子模型。

二、利用电喷雾离子阱多级质谱、高分辨质谱技术研究多肽与蛋白质及寡聚核酸的相互作用。

本论文充分利用离子阱质谱的多级质谱解析能力,研究模型磷酸化多肽

Ac-pYEEI 和 pYEEI 与 SH2 domain 的相互作用，确定磷酸化多肽与 SH2 domain 相互作用研究的质谱条件，为今后相关研究提供实验基础。通过对复合物进行多级质谱解析，以期同步给出配体小分子化合物的结构信息，拟发展一种快速、便捷的活性小分子质谱筛选新技术。

首先，经非变性电喷雾质谱研究发现，Ac-pYEEI 和 pYEEI 与 SH2 domain 蛋白都具有较强的相互作用，但是由于肽链 N-端的乙酰化修饰，使得 Ac-pYEEI 与 SH2 domain 有更强的相互作用。利用薛定谔软件对该研究结果进行进一步的理论验证，计算结果显示 Ac-pYEEI 与 SH2 domain 确有更强的结合能。上述研究结果说明，多肽 N-端乙酰化修饰会对多肽与底物蛋白的相互作用产生影响。理论计算结果也可说明，电喷雾质谱上体现的 Ac-pYEEI 和 pYEEI 与 SH2 domain 蛋白结合作用强弱的差异，来源于真实的溶液状态中的作用差异，而非质谱气化过程导致的结果。

此外，利用离子阱质谱在多级方面的实验优势，对 Ac-pYEEI 与 SH2 domain 的非共价复合物进行多级质谱解析，顺利得到配体小分子磷酸化多肽 Ac-pYEEI 的结构信息。实验结果表明该电喷雾多级质谱方法可用于筛选与 SH2 domain 蛋白有较强弱相互作用的配体小分子，并可同步获得配体小分子结构信息，该思路有望发展成一种快速、便捷的活性小分子质谱筛选新技术。

丝组二肽是本课题组最早发现的具有切割核酸和蛋白功能的活性二肽。本论文试图利用电喷雾高分辨质谱技术来评价丝组二肽与寡聚核酸的切割活性，探寻其对寡聚核酸的序列选择性。通过结合聚丙烯酰胺凝胶电泳等手段，对丝组二肽与七条不同序列的寡聚核酸的作用产物进行分析，发现丝组二肽对寡聚核酸的切割可能具有一定的序列选择性。但是，具体何种序列的寡聚核酸有利于丝组二肽的切割，还需更为深入的研究。该研究结果也说明高分辨质谱技术也是一种评价丝组二肽与寡聚核酸切割活性的可行手段，可以实现多种研究方法的有效互补。

关键词：磷酸一酰胺；SH2 domain 蛋白；磷酸化多肽；液相色谱；电喷雾离子阱多级质谱

Abstract

Protein phosphorylation is one of the most common and important post translational modifications in cells, and the reversible phosphorylation of proteins regulates nearly every aspect of cell life, such as cell cycle, differentiation and development, metabolism, nerve activity, muscle constriction and transcriptional regulation.

Knowing the methods of phosphorylation and its identification is of significance in understanding the process of cell life. The traditional synthesis methods of phosphopeptides require the protecting of the amino or carboxyl groups. And in this thesis, we focus on developing a novel method of phosphorylation in water without protection and a strategy of detecting the interaction of peptides and proteins/oligo-DNA by electrospray mass spectrometry, containing electrospray mass spectrometry and high resolution spectrometry.

The major contents of this graduate thesis can be divided into two parts.

The first part of this thesis is utilizing the small molecular compound phosphoramidate as phosphor donor to design a novel phosphoryl method for peptide in water phase.

The reaction between peptides and phosphoramidate in water phase was monitored by High Performance Liquid Chromatography Electrospray Ion trap mass spectrometry and the phosphorylation site was characterized by on-line tandem mass spectrometry. The results suggested that: 1) The peptides can be phosphorylated by phosphoramidate in water phase. 2) The possible phosphorylation site is on the side chain hydroxyl group instead of the amino group or carboxy group. 3) The pH value and the molar ratio have significant affect on the reaction. The optimistic reaction condition is at pH 8.0 and a 200:1 molar ratio of phosphoramidate to peptides 4) The transform ratio of peptides with similar sequence are almost the same, nealy 20%. Finally, the Cytochrome C was used as model for trypsin cleavage to generate an oligopeptide library. The oligopeptide library and its phosphorylation products treated

with phosphoramidate were identified by Matrix-Assisted Laser Desorption Ionization mass spectrometry in preliminary. The experiment results indicated that the method is not suitable for the phosphorylation of a peptide library.

In the second part we used electrospray mass spectrometry to study the interaction of phosphopeptides and the protein / the interaction between Seryl-Histidine and Deoxyribonucleic acids.

In the native condition, the interaction difference between SH2 domain protein with pYEEI peptide and Ac-pYEEI peptide was studied by electrospray ion trap mass spectrometry. Ac-pYEEI has stronger interaction with SH2 domain protein than pYEEI. The Schrödinger software was applied as auxiliary evidence to the interaction occurred in the electrospray ionization mass spectrometry and helped to evaluate the affection of the peptide *N*-terminal acetyl group in the interaction. The results indicated that the *N*-terminal acetyl protection contribute to the binding energy and strengthen the interaction.

Futhermore, the tandem mass spectrometry function of the ion trap mass spectrometry is developed to meet the requirement of understanding the structure of the small molecules which might have strongly binding to the protein.

Seryl-Histidine is a functional dipeptide found by our group, which has the cleavage function to proteins and Deoxyribonucleic acids. In this paper, the cleavage function of Seryl-Histidine to oligoDeoxyribonucleic acids was investigated by mass spectrometry for understanding of the cleavage site or selectivity. The reactions between Seryl-Histidine and seven kinds of Deoxyribonucleic acids were arranged separately, and the resulting products were detected by high resolution Electrospray mass spectrometry and polyacrylamide gel electrophoresis bothly. The results indicated that Seryl-Histidine might have cleavage selectivity to parts of the sequence of the oligoDeoxyribonucleic acids used, to some extend.

Key words: phosphoramidate, SH2 domain, phosphopeptides, High Performance Liquid Chromatography, electrospray tandem mass spectrometry.

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