INTEGRATED MULTI-TIP PHOTOCATALYTIC NANOREACTOR ELECTROSPRAY CHIP FOR PHOSPHOPEPTIDE ENRICHMENT AND OXIDATION

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ABSTRACT

Many biological processes are controlled by reversible phosphorylation of proteins. Identification of protein phosphorylation sites is crucial for thorough understanding of cell signaling pathways. This study aims at developing tools for phosphoproteomic research. Applicability of an integrated multi-tip photocatalytic micropillar nanoreactor electrospray ionization (µPESI) chip for phosphopeptide analysis has been preliminary studied. Easy and fast switching between individual tips allows rapid mass spectrometry (MS) analysis of large sample series. The titanium dioxide (TiO₂) coating of the chip allows rapid and simple enrichment of phosphopeptides as well as enables photocatalyzed oxidation reactions.

KEYWORDS: Electrospray Ionization, Mass Spectrometry, TiO₂ Photocatalysis, Phosphopeptide Enrichment

INTRODUCTION

Analysis of phosphoproteins or phosphopeptides is a major challenge in proteomics. Protein phosphorylation is one of the most important post-translational modifications, regulating most cellular processes. Identification of phosphorylation sites of proteins is essential for understanding complex biological signal transduction cascades controlled by reversible phosphorylation. The MS analysis of phosphopeptides from complex peptide mixtures is hampered by ion suppression caused by more easily ionizable non-phosphorylated peptides. Therefore various techniques have been developed for enrichment of phosphopeptides from complex peptide samples prior to MS analysis, TiO₂ based techniques being especially promising [1]. In addition, TiO₂ is capable of catalyzing both oxidative and reductive reactions when exposed to ultraviolet (UV) light. A TiO₂ coated µPESI chip with a single tip combined with MS has been recently used for photocatalytic oxidation of drug molecules, mimicking phase I metabolic reactions [2]. The main aim in this study is to use a rotating multi-tip µPESI platform with TiO₂ coating for rapid phosphopeptide enrichment and oxidation studies.

EXPERIMENTAL

The fabrication of the rotating silicon platform with 60 identical electrospray (ESI) emitter tips at 6 degrees separation from each other (Fig. 1) has been described in detail elsewhere [3]. First, the sharp ESI tips were etched through the wafer using deep reactive ion etching (DRIE), after which the second DRIE step defined the microchannels with embedded micropillars. The entire platform was coated with photocatalytically active TiO₂ (100 nm thick) by atomic layer deposition.

The multi-tip µPESI platform was placed on a computer controlled rotating table (Thorlabs CR1-Z7/M, Thorlabs Sweden AB) in front of a triple quadrupole mass spectrometer (API 300 or API 365 triple quadrupole, Perkin Elmer Sciex, Toronto, Canada). The ESI voltage of 4.5 kV was applied through a platinum electrode placed on top of the µPESI platform.

Figure 1. a) One quarter of the multi-tip µPESI platform with 60 independent chips at the periphery of the silicon wafer without the 100 nm thick TiO₂-nanolayer coating. b) A micrograph of a µPESI tip.
RESULTS AND DISCUSSION

Switching between tips was easy and fast using a rotating, computer controlled table. This allows rapid analysis of large sample series. Integration of a photocatalytic nanoreactor and a μPESI source on the same microchip enables rapid online analysis of reaction products with MS.

The photocatalytic activity of the TiO$_2$ coating was confirmed by photocatalyzed reactions of verapamil according to a recently published procedure [2]. Briefly, 1 µl of aqueous solution of verapamil (10 µM) was deposited onto the nanoreactor spot. The reactor spot was kept wet by applying aliquots of water during UV exposure (5 minutes) with an external UV lamp (TEK-Lite, Union Bridge, MD, USA, $\lambda_{max}$ 365 nm, 100 mW/cm$^2$). After exposure, the aqueous droplet was evaporated and high voltage (4.5 kV) was turned on. The reaction products were eluted by applying 1 µl of 0.1 % HCOOH, 95 % MeOH to the reactor spot. Five photocatalytic dealkylation reaction products of verapamil were observed in the MS spectrum measured (Fig. 2).

![Figure 2. a) A mass spectrum of verapamil and its photocatalytic reaction products. b) The structure of verapamil and the photocatalytically produced reaction products. Lines and arrows show the cleavage sites.](image-url)

The μPESI conditions for phosphopeptide enrichment were optimized using non-phosphorylated angiotensin II (5 µM) and singly phosphorylated insulin receptor 1142-1153 (10 µM) as test compounds. 1 µl of acidic solution (0.1 % TFA) containing both peptides was deposited on the nanoreactor spot and evaporated. The phosphopeptide, insulin receptor 1142-1153, was adsorbed onto TiO$_2$, while the non-phosphorylated angiotensin II was not adsorbed. The high voltage was switched on and angiotensin II was eluted by applying 1 µl of 95 % MeOH, while the phosphopeptide remained adsorbed onto TiO$_2$ and was not observed in the MS spectrum (Fig 3a). The phosphopeptide was eluted by applying first 1 µl of 1 % NH$_4$OH and then 1 µl of 0.1 % NH$_4$OH, 95 % MeOH several times on the reactor spot (Fig 3b). However, residues of angiotensin II could still be observed in the MS spectrum, indicating that further optimization of elution conditions might be needed.
Figure 3. Phosphopeptide enrichment by TiO$_2$ nanolayer of the multi-tip µPESI platform. 1 µl of a solution containing non-phosphorylated peptide (5 µM) and singly phosphorylated peptide (10 µM) in 0.1 % TFA was deposited on the reactor spot of the microchip and evaporated. a) The non-phosphorylated peptide, angiotensin II, was eluted by applying 1 µl of 95 % MeOH several times on the reactor spot. The phosphopeptide was not observed in MS spectrum. b) The singly phosphorylated peptide, insulin receptor 1142-1153, was eluted after addition of 1 % NH$_4$OH by applying 1 µl of 0.1 % NH$_4$OH, 95 % MeOH several times on the reactor spot. Residues of angiotensin II were still detectable in the MS spectrum.

CONCLUSION
The initial results demonstrate that the rotating TiO$_2$ coated multi-tip µPESI platform enables rapid and simple separation of a phosphorylated and a non-phosphorylated peptide and also allows photocatalytic oxidation reactions. Our future work includes examination of photocatalytical oxidation of phosphopeptides and developing a µPESI method towards a tool for identification of protein phosphorylation sites.

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