Modified Surface Metal Oxide for Enrichment of Phosphopeptides

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INTRODUCTION

The application of TiO2 and ZrO2 and other metal oxides is well established in the purification and enrichment of phosphopeptides. However, the nonspecific binding of non-phosphopeptides is still a hurdle in the enrichment of low concentrations of phosphopeptide molecules. Metal oxides bind to the phospho group as Lewis acid base pairs. In the instance of some pure metal oxides, the Lewis acid base pair formation is so strong that it is difficult to isolate the desired molecules. Besides phospho group containing molecules, other negatively charged groups (such as carboxyl, sulfate) also bind to metal oxides. The modification of the Lewis acid property of these metal oxides may help in more specific enrichment of phosphopeptides

MATERIAL & METHOD

Materials

Molecular Biology Grade Water from Biowittaker (Walkersville, MD, USA) was used for the matrix and sample preparation solutions. albumin and standard peptides were obtained from Sigma. The matrix material, á-cyano-4-hydroxycinnamic acid (4-HCCA) was obtained from Fluka (Buch, Switzerland). Bovine trypsin was immobilized on Poros beads by using Glygen (Columbia, MD, USA) Technology.

A mixture of synthetic non-, mono-, di- and tri- phospho peptides is used for this study. Different chemically and structurally modified TiO2 and ZrO2 metal oxides are used in a NuTip column format for the binding of peptides at low pH in 10-100mM formic acid and eluted with ammonium hydroxide solution. The purified peptides are analyzed by MALDI mass spectrometry. The mass spectrometric analysis (AP-MALDI) is prepared on a gold-plated target plate by mixing aliquots of 1.0 ml of the purified peptide mixture with 1.0 ml of a matrix solution and dried at room temperature. Matrix composition was 1.25 mg/mL of 4-HCCA dissolved into a solution of 50% ACN and 0.1% TFA.

Mass Spectrometry

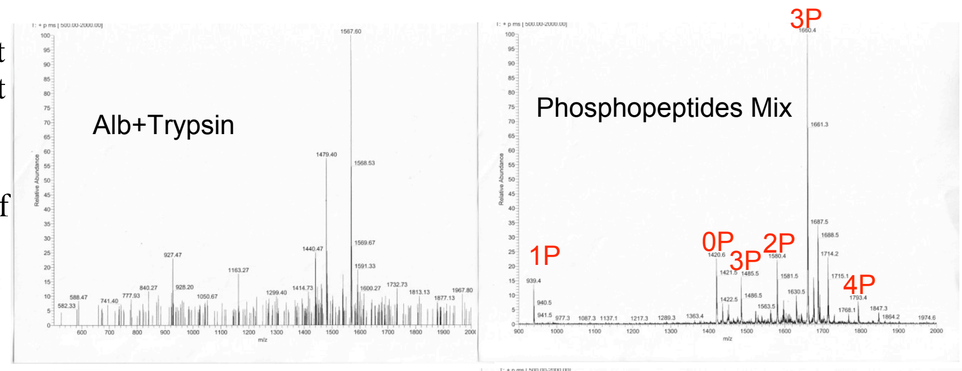
Experiments were carried out on a Thermo Finnigan (San Jose, CA, USA) LCQ Deca XP ion trap mass spectrometer integrated with an AP/MALDI ion source (MassTech Inc., Columbia, MD, USA). The AP/MALDI source is described in detail in ref. [8-10], but our specific arrangement is outlined here. A Thermo Laser Science Inc. (Franklin, MA, USA) Model 337 Si nitrogen UV laser was used. Its wavelength was =337 nm, laser pulse duration was about 4 ns, and the laser beam was focused to approximately 500 mm size spot. The maximum laser energy on the target was measured to be about 140 J/pulse, using a Molectron Detector (Portland, OR, USA) Model EM-400 laser energy meter. During operation, the laser energy was attenuated to the level of about 60 J/pulse. Sample and laser spots were observed on a TV monitor at a viewing angle of 450 with a total magnification of about 100x using a CCD camera.

Operation

Experiments were carried out in a repetitive laser shot mode (frequency 10 Hz). The laser trigger times were not synchronized with the LCQ operation. Operating conditions for all full MS and MS/MS experiments were as follows: automatic gain control (AGC) was off, ion injection time was 220 ms, the temperature of the LCQ input capillary was held at 280 C. Typical high voltage applied to the target plate was 2000 V. Spectra were averaged over a 30 s to 3 min collection time. The LCQ was operated in high mass range mode (up to 4000 Da), and was calibrated using the AP/MALDI source and a standard protein. Mass spectra were obtained by averaging 50–200 individual laser shots.

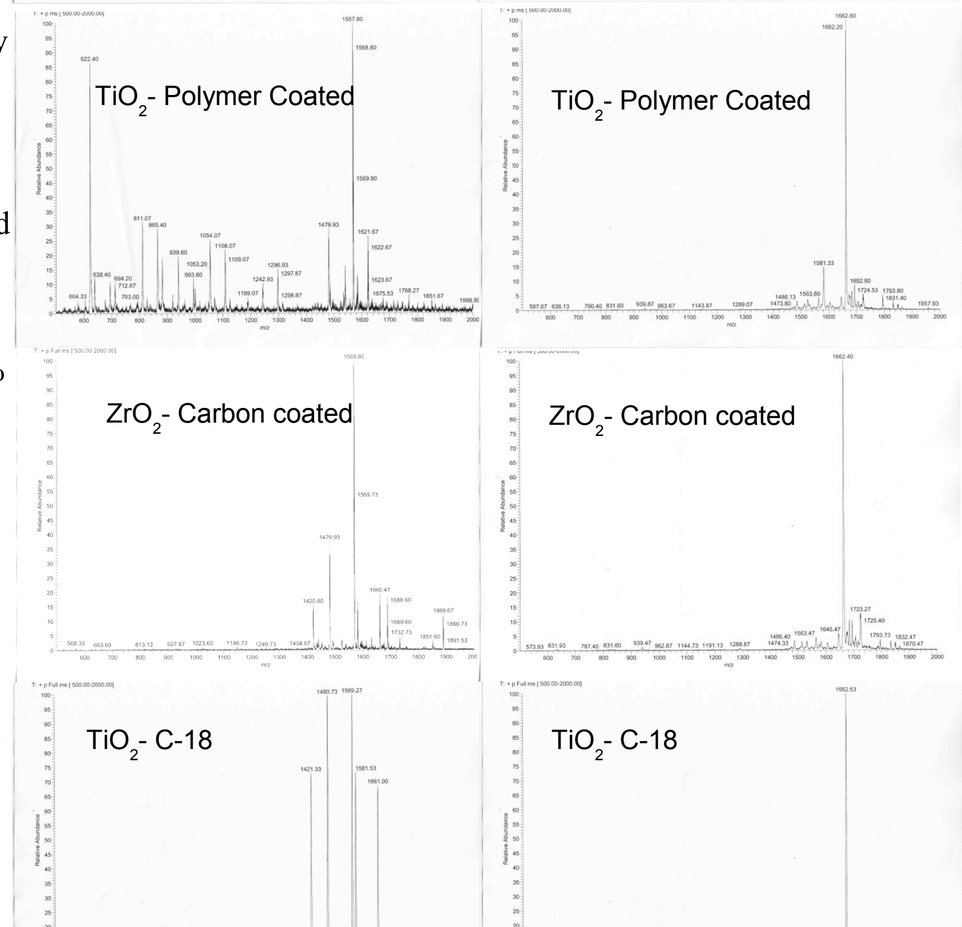
RESULTS

In this poster, we describe a new method (patent pending) that demonstrates the use of a different modified surface of the metal oxide for purification and concentration of phospho and non-phospho peptides. Furthermore, the edges of perforation (depending upon the material that the Grid is made of) may have an affinity for the sample molecules.



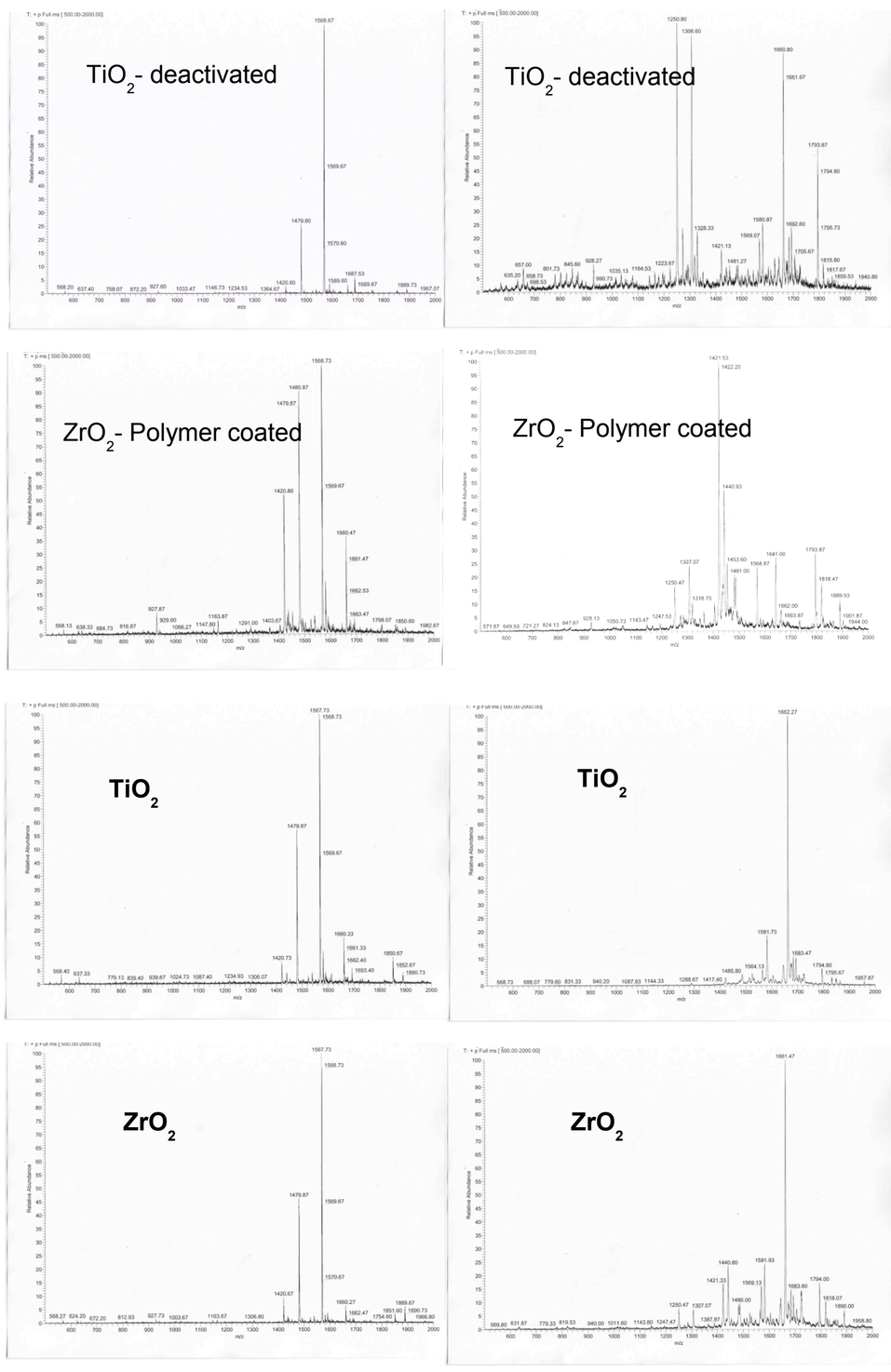
Modification of the metal oxides is performed by chemically attaching different hydrophobic groups (such as C18, polymer and carbon), which reduces the density of free Lewis acid groups on the surface of metal oxides. This modification reduces the formation of Lewis acid base pairs with non-phosphopeptides. The phospho and non phospho peptides are bound at low pH and after washing, the non phospho peptides can be eluted by using standard 50-70% organic solution containing 0.01% TFA or 30-50mM HCOOH. The phospho peptides can be eluted with 100mM ammonium hydroxide.

For the identification of peptides in the range of 10 fmole by AP-MALDI, some precautions are required such as : pre-mix the peptide and matrix in 1:1 ratio and let it dry at room temp. Donot air dry or heat. A slow crystallization gives better spectra. Furthermore, when working in fmole range, the matrix concentration should be 1/10 of the regular concentration of matrix.



Phospho Peptides

NH_2-GGGGGLGSLGK-COOH MW-938.9
WWGS GPSG SGGS GGGK MW-1420.5
NH_2-GSRAAKTLGSLGK-COOH MW-1485.4
WWGS GPSG SGGS GGGK MW-1580.4
WWGS GPSP SGGS GGGK MW-1659.5
NH_2-RSRVVKLGSSSLGK-COOH MW-1793.7



All Samples are Eluted with 50% AcCN with 20mM HCCOH

All samples are Eluted with 100mM NH_4OH, after AcCN elution

DISCUSSION

The coating of metal oxides does not cover the entire surface of the metal oxides. A small percentage of the surface of metal oxides remains uncoated and binds the phosphopeptides. The non phospho peptides are eluted with 50-70% organic solvent, and the phospho peptides are eluted by further increasing the pH. The advantage of this method is that due to a low density of metal oxides, the di-, tri-, tetra- or more phosho groups bind as mono phospho groups and this results in an easier elution as compared to the non modified metal oxide. In the case of non modified metal oxides, more centers are available to bind multi phospho group peptides.
This method allows purification of different peptides in one step. By using different coatings on metal oxides, it will be easy to purify the specific mixture of peptides. Furthermore, this method allows the study of phosphorylation in the proteins.

*Easy to use

*Suited for small sample volumes (micro/nano liters)

*Simultaneous preparation and analysis of a large number of samples

*Simultaneous use of many different types of separation/chromatographic materials

*Versatile, Economical, Fast, Minimal sample loss.

Glygen likes to thank Prof. Karl Mechtler, Viena, Austria for providing phospho-peptides