Full automation of sample preparation on MALDI targets using StageTips

Christopher Lößner[1], Heinrich Gausepohl[2]
[1] German Cancer Research Center, INF 580, 69120 Heidelberg, Germany
[2]INTAVIS Bioanalytical Instruments AG, Nattermannallee 1, 50829 Köln, Germany

Introduction

In-gel digestion and subsequent preparation on MALDI targets are crucial steps of large-scale protein identification projects and must be automated to achieve reasonable throughput and reliability. Usually the extracted solution volume after digestion is too large for direct loading of an MS system and concentration and/or desalting is required. This can conveniently be done by solid phase extraction using ZipTips (™ Millipore), which are reliable but quite expensive. Manual use of ZipTips is tedious but they can also be handled automatically. Here we tried to optimize extraction of peptides out of the gel supernatant using very economic self-made StageTips (1). The problem of StageTips is their very high back pressure which makes manual use of them very tedious and so far prevented their application in automated instruments.

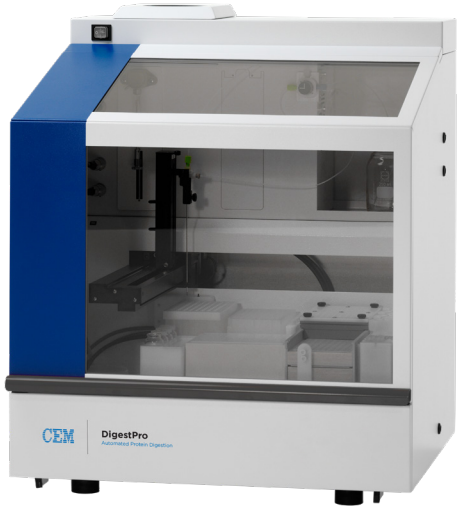

Fig. 1 DigestPro instrument for digestion and MALDI sample preparation with StageTips

Methods

Rabbit muscle Phosphorylase b was run on SDS-PAGE. After staining of the gels with colloidal Coomassie two spots per lane were excised. The digestion protocol including washing, reduction, alkylation, trypsin digestion and elution of peptides was performed according to the manufacturers standard procedure. C-18 affinity purification was compared to direct spotting of an aliquot of acidified supernatant (2 μl of 20 μl supernatant). Digests were spotted onto a Bruker AnchorChip (800 μm). All steps were carried out on an INTAVIS DigestPro MS. Mass spectra were acquired on a Bruker Ultraflex I and database searches were performed using Mascot.

Fig. 2: StageTip on steel needle over target

StageTips were prepared manually as published (1) and stored in the tip holder of the instrument. From here they are picked up by a stainless steel needle which seals just above the C-18 bed of the StageTip, leaving only a few μl of air trapped. The original ZipTip protocol was modified to allow for sample enrichment via the back of the tip as the back pressure is too high for aspiration through the C-18 bed. Washing and elution was also performed from the rear at flow rates of 10-400nl/min.

Principle of Operation

When handled manually, ZipTips are loaded by repeated aspiration/dispense steps of up to 10μl of peptide solution with a standard air displacement pipette (Fig. 3a). In the automated fashion (Fig. 3b) virtually unlimited volumes of solutions can be aspirated through the tip into the stainless steel needle of the robot and then dispensed again. Due to the very low air volume between sealing of the stainless steel needle and the C-18 bed (Fig. 3d), the dispense speed is fully controlled by the instrument. A single pass of the peptide solution at low speed is sufficient for complete extraction. After peptide binding the C-18 bed is washed and the tip is deposited back in the storage rack. For elution onto the target a small volume of 60% acetonitrile or matrix solution is aspirated into the needle, the tip is picked up from the storage rack again and the peptides are eluted slowly from the back of the packing (red arrow).
For the StageTips the sample must be aspirated into the stainless steel needle first and dispensed very slowly through the bed (Fig. 3c, green arrow) due to the high back pressure of the C-18 packing in teflon mesh. Splashing during elution is avoided by very slow dispensing at only 10nl/min.

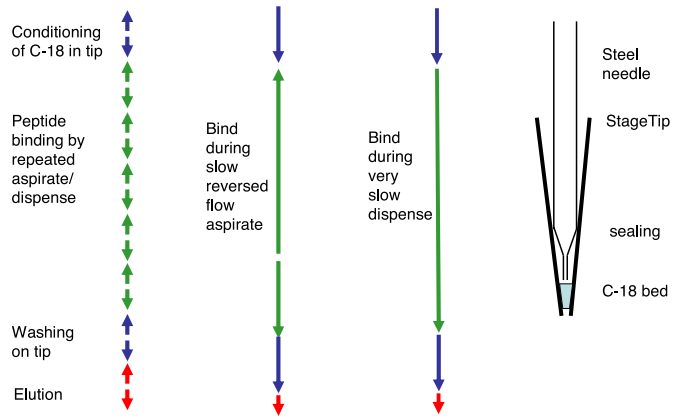

Fig. 3: (a) manual ZipTip (b) automated ZipTip (c) automated StageTip (d) steel needle in tip

Results

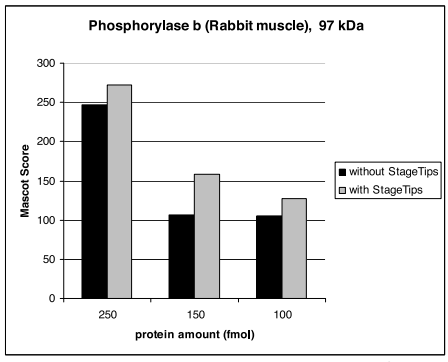

Fig. 4: Protein identification results with and without use of StageTips

With a special needle sealing just above the extraction bed and a low air volume entrapped in the tip it is possible to automate sample preparation on economic self-made StageTips. Peptides are desalted and enriched in a small volume which can be eluted directly onto MALDI targets.

Reference

[1] J. Rappsilber et al, Nature Protocols 2007, Vol. 2, No. 8.