Improved identification of membrane proteins by MALDI-MS/MS using matrix pre-deposited MALDI chips

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Introduction

Due to their low abundance and limited number of tryptic cleavage sites, integral membrane proteins are difficult to analyze by mass spectrometry. Our approach is based on a novel technology for peptide sample preparation that uses plates with pre-deposited matrix spots of submicron size CHCA crystals prepared by vacuum sublimation onto an ultrathin surface (Mass Spec Turbo Chips, QIAGEN). This design allows analyte concentration in the top layers of the matrix thin layer providing highest MS sensitivity. To evaluate the performance of this system in membrane proteomics two model proteins (ATP synthase subunit β from spirochetes and bacteriorhodopsin from Halobacteriun salinarum) containing several transmembrane domains were analyzed and spectra quality was compared to standard MALDI preparations. In a second step the chips were tested with a membrane protein preparation sample from the bacterium Rhodococcus sp. RHA1, a soil actinomycete. The proteomic workflow comprised the isolation of the membrane fraction and proteins were separated using the recently developed technology based on ion-exchange chromatography and SDS-PAGE (see Methods). The gel bands were analyzed by MALDI-TOF/TOF resulting in the identification of cytosolic, membrane-associated, and integral membrane proteins. Our results clearly show that more integral membrane proteins were identified using the Mass Spec Turbo Chips, which prove their advantage for membrane proteomics.

Methods

ABC/SDS-PAGE

For details see Schläusener, 2005. Briefly, cells were disrupted by French pressure treatment and membranes washed with 2.5 M NaBr to remove membrane-associated proteins. The resulting membrane fraction was solubilized in buffer containing 2% (v/v) ASB-14 and applied onto an anion-exchange column (Poros20 HQ material, Applied Biosystems, Darmstadt, Germany). Fractions were precipitated by TCA and SDS-PAGE was performed according to Laemmli.

MALDI-MS/MS

After visualization with Coomassie blue, protein bands were excised from the SDS-polyacrylamide gel and prepared for overnight tryptic digestion according to standard protocols. To elute the peptides, 10 µl of 50% (v/v) acetonitrile, 0.5% (v/v) TFA was added to the samples and sonicated for 5 min. Aliquots of 1 µl were applied either onto the steel target plate and immediately mixed with an equal volume of 70% (v/v) acetonitrile or onto the MALDI-TOF/TOF chips. This was particlarly the case for model membrane proteins. Spectra were acquired on a 4800 MALDI-TOF/TOF instrument (Applied Biosystems) and analyzed using the Data Explorer software. Proteins were identified by searching extracted peak lists against the NCBInr DB (Actinobacteria) using the Mascot search engine.

Separation of membrane protein extracts

Mascot scores: Stainless steel vs. Turbo Chip

A direct comparison of the preparation methods shows that Mass Spec Turbo Chips typically deliver higher Mascot scores (and hence a more reliable identification) than stainless steel plates.

MALDI-MS spectra of model membrane proteins

A direct comparison of the preparation methods shows that Mass Spec Turbo Chips deliver higher signal-to-noise ratios than stainless steel plates.

In addition, the total number of peptides observed was higher in the case of samples processed using Mass Spec Turbo Chips. This was particularly the case for highmolecular peptides, typically bound in membrane protein preparations, which could be analyzed with higher sensitivity.

Summary

Sample preparation on chips possessing sublimated CHCA matrix spots (Mass Spec Turbo Chips) resulted in the observation of a larger number of peptides and higher S/N peak ratios of spectra from model membrane protein digests, compared to classical dried-droplet sample preparation.

The recently developed 2-dimensional AIEC/SDS-PAGE technique provided an excellent tool for efficient separation of bacterial membrane proteomes.

In an exemplary membrane proteomics study of a Rhodococcus strain 2/3 of the proteins identified were annotated by sequence-based prediction of their cellular localization as integral or associated-membrane proteins.

Sample preparations on sublimated CHCA matrix delivered higher protein identification rates based on total Mascot scoring or MS/MS-based protein identification.

A comparison of the total Mascot score demonstrated on average a higher confidence of the identified proteins resulting from higher sequence coverage, however either no peptide mass or fragment spectrum could be matched to protein transmembrane domains.

References


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