

Direct On-Membrane MS/MS Analysis using the Chemical Printer (CHIP-1000) and AXIMA-QIT

Microscale on-membrane PMF analysis can be conducted on proteins transferred to a PVDF membrane utilizing the microdispensing function of a chemical printer (refer to AXIMA Applications 14, 17). Moreover, using the AXIMA-QIT ion trap MS in conjunction with the chemical printer enables MS analysis with high mass accuracy ($<0.1\text{Da}$). In this application, we succeeded in conducting MS/MS analysis of proteins immobilized on PVDF transfer membranes, something which has been difficult up to now due to the irregularities of the membrane surface and electrical charge. Direct on-membrane MS/MS analysis using the chemical printer together with the AXIMA-QIT, in addition to the established methodology for on-membrane PMF analysis, is expected to be extremely effective in identifying unknown proteins.

Protein spots from *E. coli* separated by 2-dimensional electrophoresis were transferred to a PVDF membrane for use as the sample (Fig. 1). A chemical printer was then used to perform enzymatic digestion (trypsin digestion) on selected protein spots. Following enzymatic digestion, matrix solution was microdispensed on each print position, and on-membrane MS analysis was then conducted using the AXIMA-QIT. The obtained MS spectra are shown in Fig. 2.

Next, the AXIMA-QIT was used to conduct on-membrane MS/MS analysis of precursor ions identified from characteristic signals of protein spots verified through PMF analysis. We were able to identify the respective protein spots by conducting an MS/MS ion search based on the obtained MS/MS spectra.

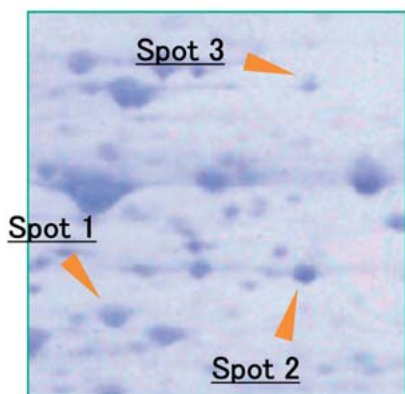


Fig. 1 shows a portion of the transfer membrane stained with Direct Blue 71 after transferring *E. coli* extract separated by 2-dimensional electrophoresis to the PVDF membrane. The MS spectra obtained from microscale on-membrane PMF analysis using the chemical printer are shown in Fig. 2. Combining the chemical printer with the AXIMA-QIT ion trap mass spectrometer enables on-membrane MS analysis with high mass accuracy ($<0.1\text{Da}$).

Fig. 1: PVDF Membrane with Transferred 2-Dimensional Electrophoresis-Separated *E. coli* Extract

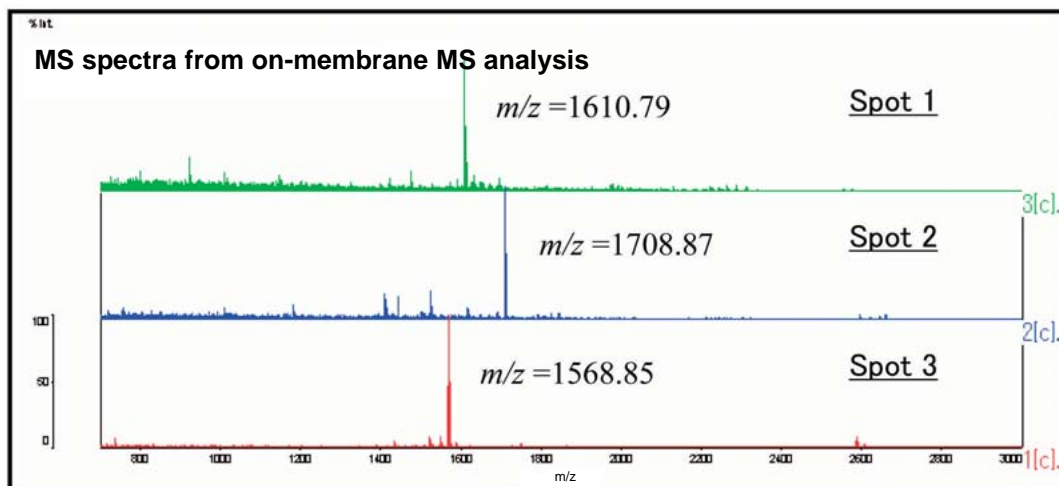


Fig. 2: MS Spectral Data from Direct On-Membrane Analysis of Protein Spots

Fig. 3 shows the results obtained from on-membrane MS/MS analysis of the precursor ions identified from characteristic signals (Spot 1: $m/z = 1610.79$, Spot 2: $m/z = 1708.87$, Spot 3: $m/z = 1568.85$) confirmed by PMF analysis. The results of MS/MS ion search using these MS/MS spectra provided identification of these protein spots with convincing scores (Fig. 4).

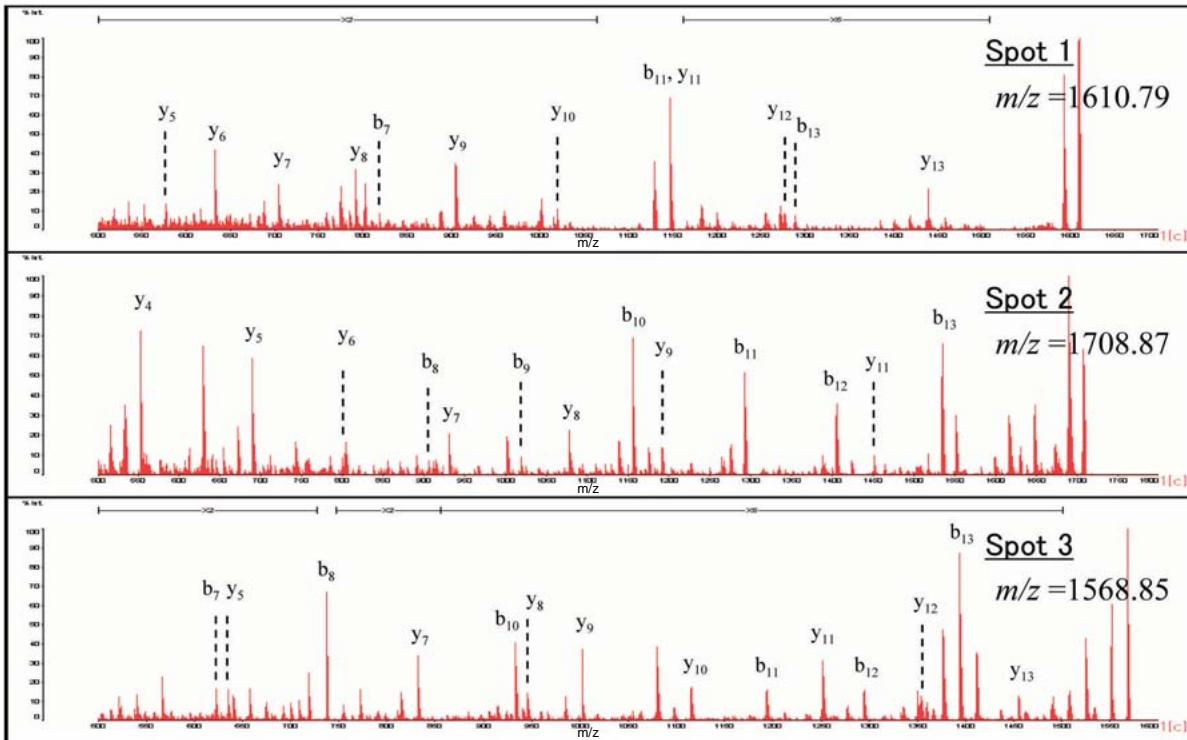


Fig. 3: Spectral Data from Direct On-Membrane MS/MS Analysis of Protein Spots

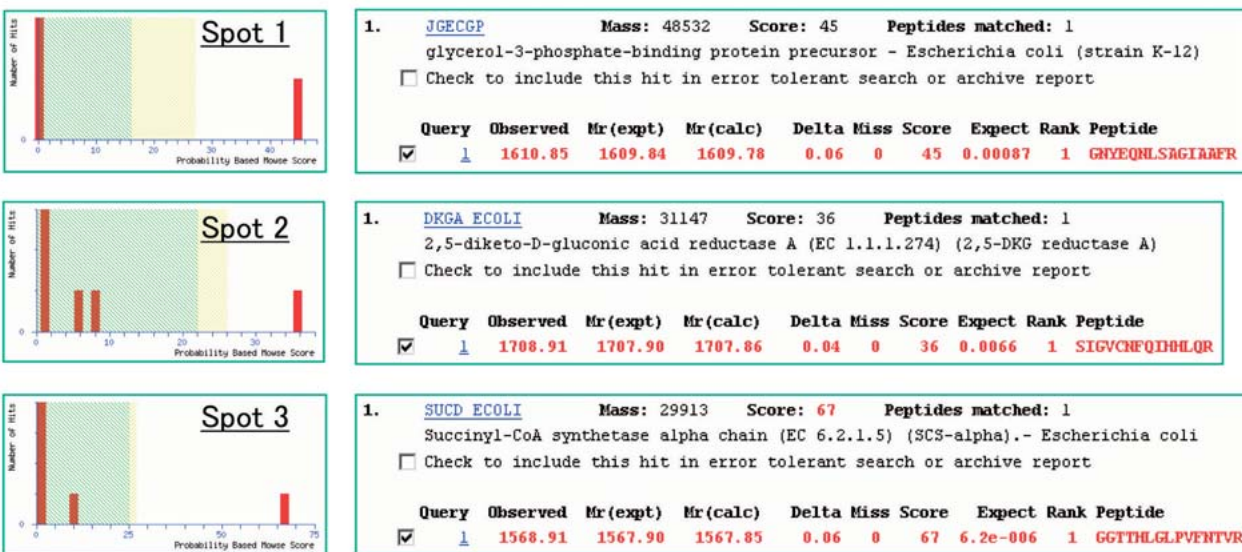


Fig. 4: MS/MS Ion Search Results for Direct On-Membrane Analysis of Protein Spots

Literature cited: T. Nakanishi *et al.*, *J. Proteome Res.*, 2005, 4, 743-747



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