Preparation of Human Plasma Sample

- 20 mL of blood were drawn from a male adult.
- Each blood sample was collected into tubes containing EDTA, centrifuged at 1000 rpm for 45 minutes at 4°C.
- The plasma was carefully removed, aliquoted and frozen at -40°C.
- The protein concentration was determined by biuret assay.

2D Liquid Chromatography with Mass Spectrometry for Multidimensional Proteome Profiling

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ABSTRACT

The discovery stage of proteome profiling typically involves the comparison of different states of a cell or tissue. One approach utilizes fractionation of the proteome followed by mass spectrometry (MS). A two-dimensional, liquid chromatographic fractionation system, the ProteomeLab PF 2D, followed by a third dimension with MALDI-TOF MS has been used for this approach. The first dimension separation is chromatofocusing where proteins are separated by pI and collected in fractions based on pH fractions. Upstream of 2-D pH fractions are then used for second dimension reversed phase chromatography. The third dimension is MALDI-TOF MS analysis of intact proteins. The combination of the ProteomeLab PF 2D with the Biomek 3000 Laboratory Automation Workstation was used. The Biomek 3000 Laboratory Automation Workstation prepared and spotted the fractions from the second dimension run along with the appropriate matrix on a 180-well format MALDI target. The third dimension measures the mass/charge ratio of the proteins. Human plasma was analyzed with the multidimensional system developed and the Biomek 3000 Laboratory Automation Workstation facilitated the complete analysis of a functional proteome by overcoming the potential bottleneck resulting from the large number of samples collected from the first two dimensions.

Objectives

- Use the Biomek 3000 Laboratory Automation Workstation as the interface between the ProteomeLab PF 2D and MS by spotting the MALDI plate with 2nd dimension fractions (Figure 3).
- To illustrate "proof of concept" by performing automatic two-dimensional separation of human plasma with the ProteomeLab PF 2D followed by analysis of intact proteins by MALDI-TOF MS as the 3rd dimension.
- To combine the benefits of an advanced two-dimensional liquid chromatographic technique for complex protein mixtures with the ultra-high performance of MALDI-TOF MS analysis for proteomics (Figure 2).

Biomek 3000 Laboratory Automation Workstation

- Schematic Diagram of MALDI Plate Spotting
- Table 1: A description of the methods for the ProteomeLab PF 2D System
- Table 2: General overview of MALDI spotting parameters on the Biomek 3000
- Table 3: Representative MALDI plate spotting method
- Figure 3: The 1st and 2nd dimensions are performed by the ProteomeLab PF 2D, which is interfaced to the 3rd dimension MALDI-TOF MS by the Biomek 3000 Laboratory Automation Workstation.

2nd Dimension Results: Reversed Phase

- Figure 4: A representative image from the Biomek Software showing the Instrument Setup for the MALDI plate spotting method.
- Figure 5: Schematic representation of the MALDI spotting protocol.
- Figure 6: Shown are mass spectra of intact proteins for fraction 52 (A) and fraction 61 (B) of the pH fraction 5.33-5.63, and fraction 50 (C) of the pH fraction 6.21-6.48 from the 2nd dimension run. These fractions correspond to the peaks with single charge m/z 28273, m/z 82289, respectively, which have the same retention time in the 2nd dimension and a pI that corresponds to the 1st dimension pH data.

3rd Dimension Results: MALDI-TOF MS of Intact Proteins

- Figure 7: Schematic representation of the MALDI-TOF MS method.
- Table 1: A description of the methods for the ProteomeLab PF 2D System
- Table 2: General overview of MALDI spotting parameters on the Biomek 3000
- Table 3: Representative MALDI plate spotting method
- Figure 8: A representative image from the Biomek Software showing the Instrument Setup for the MALDI plate spotting method.

SUMMARY

- Successful creation of a method run for MALDI plate spotting on the Biomek 3000 Laboratory Automation Workstation.
- The ProteomeLab PF 2D effectively fractionates proteomes for subsequent MS analysis. Since the fractions are liquid, no sample solubilization is required prior to MS.
- MALDI-TOF MS analysis of 2nd-dimension fractions and gives information on exact masses of intact proteins.
- The combination of the ProteomeLab PF 2D for separation of complex protein mixtures interfaced with MALDI-TOF MS represents an advanced tool for proteomics. This combination is adaptable to automation with minimum user participation to remove the potential bottleneck resulting from the large number of samples collected from the first two dimensions.