Matrix assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometric identification of human erythrocyte membrane associated proteins separated by one dimensional gel electrophoresis

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MALDI-TOF- Peptide Mass Fingerprinting (MALDI-TOF-PMF) is fast, accurate, and an ideal method to identify gel separated proteins in biological samples. This technique, which is being developed at the Department of Physics, University of Colombo, could be applied to compare erythrocyte membrane proteins in leukemia patients in Sri Lanka, and has the potential as a tool in clinical investigations. A feasibility study has been carried out to create a comprehensive protein profile of the associated proteins of erythrocyte membranes from blood samples of healthy individuals in Sri Lanka. Erythrocyte membranes were isolated following washing, osmotic lysis and centrifugation. Membranes were then re-suspended in a dissolving solution in order to release membrane-associated proteins and the samples were electrophoresised under denaturing conditions. Silver staining was carried out in order to visualize proteins. A total of 60 - 70 bands were detected in healthy individuals. Selected protein bands were excised, trypsinized and then subjected to MALDI-TOF analysis. The identification of proteins was performed by Peptide mapping using sequence databases. The mass of peptides derived from an in-gel proteolytic digestion were measured and subsequently searched against the computer based protein databases available in Internet.

The molecular weight marker Glyceraldehyde-3-phosphate dehydrogenase (36 KDa) identified with 29% sequence coverage and 12 matched peptides with 0.05 Da mass tolerance, used as a control. Further analyses of two bands positioned at the top of the gel enabled the identification of α spectrin (281 KDa) with 15% sequence coverage and 27 matched peptides with mass tolerance of 0.10 Da and β spectrin (247 KDa) with 18% sequence coverage and 31 matched peptides with mass tolerance of 0.06 Da.

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