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FREE COMMUNICATIONS VII

Platelets: Membranes.

TWO-DIMENSIONAL ELECTROPHORETIC "MAPPING" OF HUMAN PLATELET MEMBRANE PROTEINS. W.L.Nichols and K.G. Mann. Hematology Research, Mayo Clinic, Rochester, MN 55901.

Two-dimensional electrophoresis of solubilized human platelet membrane proteins utilizing polyacrylamide gel isoelectric focusing followed by sodium dodecyl sulfate (SDS) slab gel electrophoresis with discontinuous buffer systems yields a high resolution "fingerprint" of platelet membrane proteins. Membranes were prepared from citrate-phosphate-dextrose (CPD) platelet concentrates by differential centrifugation, repeated washes and lysis by means of sonication or glycerol hypotonic lysis. Membrane vesicles were isolated on step gradients of 27%(w/v) or 30% (w/w) sucrose. Following further washing of the vesicles, membrane pellets were extracted by heating in SDS and β -mercaptoethanol. After pelleting the insoluble material, the resulting supernatant was found to contain most of the total protein content of the membrane, and to yield SDS gel patterns similar to platelet membranes totally solubilized with larger amounts of SDS. Extracts were dialyzed against 9M urea, 2% Triton X-100 at 37° for 12 hrs., then electrofocused in large-pore gels containing 9M urea, 1% Triton X-100 and 1.6% to 2.0% ampholyte (pH 3-10). Electrofocused samples were equilibrated with 1% SDS in half-strength stacking gel buffer (D.M. Neville, J.Biol. Chem. 246:6328, 1971) and electrophoresed in the second dimension into slab gels.

Coomassie Blue R-250 staining revealed more than 40 components, the majority of which electro-focused between pH 4.5 - 7. The major glycoproteins, as assessed by periodic acid-Schiff staining, electrofocus at acidic pH. The greater resolution obtained by this analytical technique will be useful in the further characterization of the membrane proteins of normal and abnormal platelets.

THE CHARACTERIZATION OF PIG PLATELET MEMBRANE PROTEINS: STUDIES ON MEMBRANE-ASSOCIATED ACTIN AND THE SURFACE-ASSOCIATED PHOSPHODIESTERASE ACTIVITY. D.G. Taylor. Department of Biochemistry, University of Birmingham, Birmingham B15 2TT, UK.

Analysis of the polypeptides of a pig platelet surface membrane fraction by SDS-polyacrylamide gel electrophoresis revealed the presence of approx. 12 components (molecular weight range 12000-200000). A component of 43-45,000 daltons was particularly prominent and this has been identified as actin, and shown to be similar in many respects to the cytoplasmic protein. The membrane-associated actin has been isolated as a pure band by preparative SDS-polyacrylamide gel electrophoresis and analysis of its amino acid composition, although very similar to that of actin purified from whole platelets, showed a much lower content of 3-methylhistidine (approx. 0.05 residues/mole compared with 0.62 residues/mole). The surface membrane-associated phosphodiesterase (towards bis-(p-nitrophenyl) phosphate) has also been partially characterised. This enzyme activity can be completely solubilised from the membrane using 0.5% Triton X-100, and can then be isolated as a single peak on Sepharose 4B. The separated enzyme fraction showed considerable purification over the original membrane as illustrated by SDS-polyacrylamide gel electrophoresis with the prominent component being the 95,000 dalton polypeptide. Our studies have also shown that this phosphodiesterase is inhibited competitively by ADP and ATP.