

PLATELET MEMBRANE ACTIN AND MYOSIN. H. Horák, P.G. Barton and C.M. Gibbs, Department of Biochemistry, University of Alberta, Edmonton, Canada.

Horse platelet membranes isolated by the glycerol lysis technique and subjected to SDS-PAGE showed large amounts of actin and variable amounts of myosin relative to other membrane proteins and glycoproteins. [^{14}C]-2-dinitrothioadenosine diphosphate, when briefly incubated with whole cells, rapidly labeled the membrane actin component. Retention of myosin by the membranes during their isolation was optimized by lysing the cells and resuspending the membranes in Tris-HCl, pH 7.35, with 0.13 M KCl, 0.01 M NaCl, 2 mM MgCl_2 and 0.01 mM CaCl_2 . Subsequently, significant amounts of actin and myosin could be eluted from the membranes with 10^{-3} M ADP but not with CDP, GDP or UDP. Actin was also eluted effectively from membranes prepared in Tris-NaCl, pH 7.35, by washing with 0.1 mM EDTA (in presence or absence of ADP). Despite repeated washings with either elution system, more than 50% of the actin remained associated with the membranes. When membrane vesicles with right side out (RO) and inside out (IO) orientation, separated by chromatography on Con A-Sepharose, were similarly washed identical results were obtained. Two dimensional electrophoresis of the membrane protein of IO vesicles separated two major actin components, one of which was differentially removed by prior treatment of the vesicles with 10^{-3} M ADP. It is concluded that (1) platelet myosin and two forms of platelet actin are associated with the cell membrane and that myosin and one form of actin can be displaced by ADP or EDTA while the second form of actin is more firmly attached, and (2) some actin is present on both membrane surfaces.

IDENTIFICATION OF A THROMBIN PROTEOLYTIC RECEPTOR ON HUMAN PLATELETS, by David R. Phillips and Patricia Poh Agin, Department of Biochemistry, St. Jude Children's Research Hospital, Memphis, Tennessee

Thrombin is one of the most potent physiological agents causing platelet stimulation. It would appear that proteolysis is intimately linked to stimulation because trypsin, but not thrombin inactivated with PMSF, also stimulates platelets. Our approach to identifying the proteolytic substrate was to radioactively label the membrane surface proteins and determine which of these were hydrolyzed by thrombin. A glycoprotein labeling method (neuraminidase/galactose oxidase/ ^3H)- NaBH_4) was employed. Twelve membrane glycoproteins were labeled, including most of those labeled by lactoperoxidase-catalyzed iodination. Secretion and aggregation experiments showed that platelets labeled by this procedure are equally responsive to thrombin, collagen, and ADP as unlabeled platelets.

Of the glycoproteins labeled by this procedure, only glycoprotein V ($\text{Mr} = 89,000$) was decreased as a result of thrombin action. Although low thrombin concentrations (0.2 U/ml) were sufficient to obtain significant hydrolysis, the complete loss of glycoprotein V occurred at the ratio of 1 U thrombin per 10^9 platelets; no further changes were observed when the thrombin concentration was increased to 10 U/ 10^9 platelets. A soluble glycopeptide hydrolytic product ($\text{Mr} = 70,000$) was released into solution. The kinetics of glycoprotein V hydrolysis were comparable to those of secretion and aggregation. Glycoprotein V hydrolysis was not observed when platelets were aggregated by collagen, ADP, or the Ca^{++} ionophore A-23187. It is proposed that glycoprotein V is a proteolytic receptor of thrombin.