0059

08:45 h

STUDIES ON THE INTERACTION OF THROMBIN AND PLATELET PROTEINS BY CROSSED IMMUNOELECTROPHORESIS USING IMMOBILIZED THROMBIN IN THE INTERMEDIATE GEL. I. Hagen, F. Brosstad, R. Korsmo and N.O. Solum. Research Institute for Internal Medicine, and Hematological Research Laboratory, University of Oslo, Oslo, Norway.

The first step in the thrombin-platelet interaction is the binding of thrombin to the platelet surface. In order to identify platelet proteins that interact with thrombin Triton extracts of platelets were examined by crossed immunoelectrophoresis (CIE) against poly-specific anti-platelet antibodies under conditions where the proteins had to pass a gel of thrombin coupled to Sepharose 4B. Proteins which bind to thrombin will stick to the immobilized enzyme and their corresponding immunoprecipitates will be absent or reduced in area compared to a control without thrombin. Four immunoprecipitates representing platelet factor 4, glycocalicin-related protein, factor XIII and a hitherto unidentified protein were absent or reduced in area, whereas the arc representing GP IIb and GP IIIa and the other immunoprecipitates were unaffected. The interaction between glycocalicin-related protein and thrombin seemed to be dependent on both the macromolecular binding site and the active serine site since this protein passed unaffected when either or these sites were bloched by NBS, heparin, TLCK or PMSF. Treatment of the Triton extract with immobilized thrombin followed by CIE indicated some proteolysis of factor XIII, whereas no effect was observed on the immunoprecipitates representing glycocalicin-related protein and platelet factor 4. It is concluded that crossed immunoelectrophoresis with immobilized thrombin in the intermediate gel provides a means of studying the interaction between thrombin and individual platelet proteins directely without prior purification

0060

09:00 h

STUDIES ON GLYCOCALICIN AND GLYCOPROTEIN GP Ib. N.O. Solum, T. Sletbakk, I. Hagen and G. Gogstad. Research Institute for Internal Medicine, University of Oslo, Rikshospitalet, Oslo, Norway.

Crossed immunoelectrophoresis (CIE) of extracts of human platelets in 1% Triton X-100 using antiserum to purified glycocalicin shows an immunoprecipitate consisting of two peaks. Previous experiments have shown the small fast-moving peak to represent free glycocalicin whereas the slow-moving one corresponds to a larger amphiphilic protein, probably the integral membrane protein GP Ib. Glycocalicin is probably derived from the latter secondary to an activation of a calsium-dependent protease during platelet lysis. Further studies on these problems are presented. A gradual reduction of the concentration of Triton X-100 in the extraction buffer (tris-glycine, pH 8.7, 135 mOsM) gradually reduced the area of the slow-moving peak and increased that of the fast-moving one untill all was present as free glycocalicin. Reduction of the concentration of Triton in an extract already prepared with 1% Triton X-100 by adsorption to Bio-Beads SM-2 had no such effect. The presence of the protease inhibitor leupeptin during extraction at a low concentration of Triton (0.2%) reduced the peak corresponding to free glycocalicin. GP Ib was purified from Triton extracts by precipitation with con A, affinity chromatography of the supernatant on WGA-Sepharose and elution with NAGA, and gel filtration of the eluate on Ultrogel AcA 22. Triton X-100, EDTA and sodium azide were present at all steps. The characteristic change in mobility of GP Ib on SDS PAGE comparing unreduced to reduced samples deduced from studies on whole platelet proteins, was confirmed with the purified material, as was the correspondence between the GP Ib band on SDS and the slow-moving component of the CIE.

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09:15 h

PLATELET AGGREGATION BY MEMBRANE GLYCOPROTEIN I. K.Watanabe, M.Yamamoto, Y.Ando, H.Iri, K.Furihata, Y.Yoshii M.Handa,M.Imai,K.Sugiura, Y.Ikeda and K.Toyama. Department of Laboratory Medicine and Department of Hematology, Keio University, Tokyo, Japan.

It has been recently shown that platelet membrane components,particularly glycoproteins,have a lectin activity, thus mediating an aggregation of platelets. To obtain further evidences for a crucial role of glycoproteins in an aggregation mechanism,we have investigated the possibility that membrane glycoprotein can directly induce an aggregation of platelets. The membrane glycoproteins (GP I, GP II and GP III) were isolated from 3-4 mg of human platelet membranes using preparative electrophoresis on 5 % polyacrylamide gels with 0.1% SDS. Platelet aggregation by isolated GP I, GP II or GP III was examined under phase-contrast microscopy after the incubation of these peptides with platelet rich plasma at 37°C for 15 min.. Among glycoproteins tested, only GP I(20 µg/ml <) exerted an apparent platelet aggregation. No such aggregation was induced by either GP II or GP III even at concentration of 80 µg/ml. GP I isolated separately using the wheat germ agglutinin affinity column also produced a platelet aggregation. Aggregation curve recorded with an aggregometer showed a long lag phase (10 min. <) followed by an irreversible aggregation.The GP I-induced platelet aggregation occured in a dose dependent manner. This aggregation was completely inhibited by the addition of aggregating inhibitors such as indomethacin (25 µM), PGE_1 (1 µM), EDTA (0.5 mM) and TMB-8 (1mg/ml). A significant amount of serotonin (27%) and β-thromboglobulin (14.6%) was released from platelets by GP I (100 µg/ml). Treatment of GP I with either trypsin (50 µg/ml) or chymotrypsin (40 µg/ml) reduced the aggregating activity of this glycopeptides. The platelet aggregation by GP I was inhibited in the presense of 30 mM N-acetylneuraminic acid, arginin or L-lysine, but N-acetylated amino sugars and neutral sugars were without effect. This GP I-induced platelet aggregation may be an important findings in elucidating platelet aggregation mechanism.

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09:45 h

ISOLATION AND PURIFICATION OF COLLAGEN AND α1 RECEPTOR FROM PLATELET MEMBRANE. T.M. Chiang and A.H. Kang, VA Medical Center and Departments of Medicine and Biochemistry, University of Tennessee Center for the Health Sciences, Memphis, Tennessee 38104

We have previously demonstrated that chick skin type I collagen and the α1(I) chain mediate platelet aggregation. Aggregation is associated with specific binding of these substances by platelet membranes. We now describe the isolation and purification of the receptor. Platelet membranes were prepared as described previously and isolated membranes were solubilized in 0.5% Triton. The receptor was then purified by a combination of gel filtration, affinity chromatography on α1(I)-sepharose or type I collagen-sepharose and preparative polyacrylamide gel electrophoresis. The receptor activity was assayed either directly by a binding assay using (^{14}C)-glycine-labeled α1(I) or indirectly by an adhesion inhibition assay on Sepharose 2B with (^{14}C)-serotinin-labeled platelets.

The results show that the α1(I) receptor can be purified to a single band on SDS-gel electrophoresis with a recovery of 2.5%. Its activity is destroyed by preincubation with trypsin or pronase indicating it is a protein. The apparent molecular weight as estimated by gel filtration and SDS-gel electrophoresis is 95,000 daltons. The binding of (^{14}C)-labeled α1(I) is specifically displaced by unlabeled α1(I), and the bound radioactivity can be removed by treatment with purified bacterial collagenase. The binding of (^{14}C)-labeled α1(I) by the purified α1(I) receptor can also be inhibited by the receptor isolated from collagen-sepharose affinity chromatography. These data suggest that the α1(I) binding site is identical to the collagen binding site.