EXPERIMENTS were designed and performed to analyze the cytoskeleton assembly and the interaction of glycoprotein (GP)IIb, IIIa and cytoskeletal proteins during platelet activation. 125I-stimulated platelets were solubilized with Triton X-100 solution and centrifuged. The non-soluble fraction were analyzed by two dimensional electrophoresis and the soluble fraction were fractionated with 5-25% sucrose gradient centrifugation and analyzed by SDS PAGE. In Triton X-100 insoluble fraction, high molecular weight protein fraction (MW > 10^3) was present after stimulation which were consisted of actin binding protein (ABP), myosin heavy chain (MHC), actin and GPlb and IIa. And some of the ABP and MHC formed dimer. ABP and actin in this fraction were increased with 1 mM CaCl_2 treatment but the reduction of ABP was inhibited by leupeptin. In Triton X-100 soluble fraction after stimulation, some of the ABP, MHC, F235 protein, actin and small amount of GPlb, IIa were sedimented in the same high density fraction but most proteins were sedimented as a monomer form or GPIIIb-IIIa complex form. The GPIIb, IIa incorporation in high molecular weight protein fraction or high density fraction was absent in Ca_2+ chelating condition or the presence of competitive fibrinogen binding inhibitor which blocked the platelet aggregation. It is concluded that cytoskeletal proteins and GPIIb, IIa are assembled each other and formed high molecular weight protein fraction or dimer formation during activation. In stimulated platelets these assembled cytoskeletal proteins containing GPIIb, IIa were also found in Triton X-100 soluble fraction as a precursor of high molecular weight fraction in Triton X-100 insoluble fraction. The binding of fibrinogen to GPIIb-IIIa complex induce the linkage of GPIIb, IIIa to assembled cytoskeletal proteins.

CONTACT ACTIVATED PLATELETS BIND VON WILLEBRAND FACTOR TO GPIIb-IIIa. D.M. Estry (1), J.C. Mattson (2) and J. Cheidiss (3). Medical Technology Program, Michigan State University, East Lansing, MI, U.S.A. (1), Department of Clinical Pathology, William Beaumont Hospital, Royal Oak, MI, U.S.A. (2) and Michael Reese Medical Center, Chicago, IL, U.S.A. (3).

Using a rabbit polyclonal anti-von Willebrand factor (vWF) antibody, normal human adherent platelets extensively bind vWF in a diffuse pattern as detected by immunogold electron microscopy. This pattern differed significantly from the sonial pattern observed for direct fibrinogen-gold labelling in contact activated platelets. In order to determine if contact activated platelets bind vWF to GPIIb-IIIa or GPIb, the extent and pattern of bound vWF in platelets from patients with Glanzmann’s thrombasthenia (GT) and Bernard Soulier Syndrome (BSS) was determined. Virtually no bound vWF was detected by immuno-gold labeling in GT platelets previously characterized as being deficient in GPIIb-IIIa. On the other hand, BSS platelets, lacking GPIb, demonstrated extensive labeling of vWF in a pattern identical to that seen in normal platelets. This data is consistent with vWF binding to GP Ib-IIIa in contact induced adhesion and spreading.