REGULATION OF PLATELET CYTOSKELETAL ASSEMBLIES AND FUNCTION

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It has been recognized for quite some time that contractile or cytoskeletal proteins play a major role in platelet stimulus-response coupling. Ultrastructure studies (1-3) demonstrated that while unactivated platelets contain few microfilament structures, upon activation, parallel arrays of microfilament bundles form within the pseudopods of shaped changed platelets. These microfilament bundles were shown to have a uniform polarity so pseudopods could be retracted and were continuous with a central network of microfilaments also arising during platelet activation. This latter network, referred to as the contractile gel, has been proposed to centralize secretory granules augmenting their fusion with the surface connected canalicul system and forcefully expelling the granule contents. Concurrent with these extensive cytoskeletal changes within the cytoplasm, the platelet surface membrane also undergoes changes upon activation which are essential to its role in hemostasis.

The platelet surface expresses at least three de novo sites upon activation. These sites are: 1) a fibrinogen/fibrin binding site mediating platelet aggregation which is composed of the glycoprotein IIb-IIIa complex (4-7); this site may also bind other adhesive proteins such as von Willebrand factor and fibronectin (8-10), although a separate adhesion site, 2) composed of glycoprotein Ib is implicated in platelet adherence and agglutination by ristocetin-von Willebrand factor (8,11), and 3) a platelet prothrombin-converting active (PPCA) site composed of co-factor Va and factor Xa bound to acidic phospholipids (12,13) and possibly a specific Va receptor (14).

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These sites are latent on the unactivated platelet and expressed as a consequence of activation. The fibrinogen binding site as well as the PPCA site have been suggested to be regulated by cytoskeletal assemblies. The observation with both sites is that they are retained with the cytoskeletal core precipitate after detergent extraction of the platelet \( (14,15) \). This extraction procedure lysis the platelet and removes soluble proteins as well as the bulk of the membrane phospholipids. In our hands, the cytoskeletal core retains between 10 and 15% of the total platelet phospholipids \( (16) \) with a substantial acidic phospholipid component. These chloroform:methanol extracted phospholipids fully support factor Xa and Va prothrombinase activity. In view of the phospholipid retention and the uncertainty regarding a specific Va receptor, the significance of the PPCA association with the cytoskeletal core will be difficult to assess.

Our efforts have been focused over the last three years on the association of the glycoprotein IIb-IIIa complex with the platelet cytoskeleton. We had several questions we wanted to address. First was to establish if the glycoprotein IIb-IIIa complex was specifically associated with the pseudopodal or the contractile gel microfilament assembly or both. Second, we wished to determine if modifying cytoskeletal assembly would alter the expression of the fibrinogen binding site. Third, exactly which cytoskeletal elements were involved in linking the glycoprotein IIb-IIIa complex to the cytoskeletal core and how might this linkage be regulated? Lastly, did cytoskeletal disassembly reverse expression of the fibrinogen binding site and could the site be re-expressed upon re-activation?

With respect to the first question, we had already developed the necessary tools to provide an answer. Utilizing an assay for DNAase I inhibition by monomeric but not filamentous actin (F-actin), we had shown that the unactivated platelet contains a large pool of about 70% monomeric or G-actin with less than 10% of the actin recoverable in a cytoskeletal core \( (17) \). Although there have been higher estimates of the amount of preformed cytoskeleton in the unactivated platelet \( (18,19) \), these lower values have been generally accepted over the last few years \( (20) \). Upon activation by thrombin or any of the physiologic platelet activators, this actin pool polymerizes to give rise to F-actin-tropomyosin \( (21) \), microfilaments crosslinked by alpha-actinin \( (22) \), actin-binding protein \( (23) \), and myosin \( (24,25) \) into the cytoskeletal core structure recovered by detergent extraction and centrifugation.