Reciprocal Transmembranous Receptor–Cytoskeleton Interactions in Concanavalin A–activated Platelets

M. ELYSE WHEELER, JONATHAN M. GERRARD, and ROGER C. CARROLL
Department of Pathology, Harvard Medical School, Brigham and Women’s Hospital, Boston, Massachusetts 02115; Department of Pediatrics, University of Manitoba, Winnipeg, Canada; and Department of Medical Biology, University of Tennessee Memorial Research Center, Knoxville, Tennessee 37920

ABSTRACT Concanavalin A (Con A) has been used to activate platelets, inducing a specific interaction between the glycoprotein IIb-IIIa complex and the cytoskeleton of the activated platelet. In agreement with this, we have shown that Con A activates human platelets, initiating phosphorylation, secretion, and cytoskeletal formation. Con A and cytochalasin B were used to demonstrate a reciprocal interaction of the glycoprotein complex with the platelet cytoskeleton. Additionally, we have shown that a similar reciprocity is provided by the multivalent fibrin–fibrinogen platelet interaction found in the thrombin-induced clot. Con A differs from other activators in precipitating an apparent cytoskeletal core despite a complete inhibition of platelet activation by prostaglandin E1. We suggest, from this result, that Con A may be cross-linking a membrane-associated cytoskeletal complex present in the unactivated platelet.

Concanavalin A (Con A), a multivalent lectin, has been used to demonstrate transmembranous receptor–cytoskeleton interactions in fibroblasts (1), lymphocytes (2), and adenocarcinoma cell lines (3). In addition, Con A binds to platelet surface glycoproteins, especially each component of the glycoprotein IIb-IIIa complex (4). The tetrameric Con A molecule, as well as other multivalent lectins, also activates platelets by an as yet unknown mechanism (5). It has been proposed that Con A activation leads to cytosolic calcium elevation with subsequent protein phosphorylation and cytoskeletal core formation in the same manner as thrombin or arachidonate (6). Painter et al. (7) have reported that Con A induces a specific transmembranous interaction between the glycoprotein IIb-IIIa receptor complex and the cytoskeleton of the activated platelet. This interaction is more extensive than that seen with typical platelet activators in that 85–90% of the surface-labeled glycoprotein IIb-IIIa complex is retained in the Con A-induced cytoskeletal core, whereas thrombin-aggregated platelets retain only ~26% of this complex (8).

The terms contractile gel and pseudopodal cytoskeletons have been ultrastructurally defined as the dense microfilament network responsible for platelet secretory granule centralization and the parallel microfilament bundles observed in platelet pseudopodia (9). These cytoskeletal structures resist nonionic detergent extraction and can be collected as cytoskeletal cores which, remarkably, retain the ultrastructural form observed in the intact platelet (8).

Our previous studies have shown that the contractile gel and pseudopodia can be separately assembled with unique cytoskeletal core components (9). In the presence of cytochalasin B (CB), the activated platelets form a contractile gel and secrete granules normally but have limited pseudopodal development, as observed by transmission electron microscopy. Although CB did not affect the extent of actin polymerization or actin-binding protein phosphorylation, we observe an almost complete loss of actin-binding protein and about half of the actin from the detergent extract. There was also a comparable loss of alpha-actinin and tropomyosin, which were not quantitated in this study. Under these conditions, the cytoskeletal core consisted of actin–tropomyosin, alpha-actinin, and an unreduced quantity of myosin.

Our conclusion from these studies was that CB was preventing the formation of long actin filaments which, when cross-linked by actin-binding protein, made up the pseudopodal cytoskeleton. The question of whether or not actin-binding protein was interacting with the nonsedimenting filamentous actin was not resolved in this study.

More recently, we have shown that the glycoprotein IIb-IIIa complex, which forms the fibrinogen binding site ex-
pressed upon platelet activation, is specifically linked to the CB-sensitive pseudopodal cytoskeleton (10). This conclusion is also based on the observation that retention of the glycoprotein IIb-IIIa complex in the detergent-insoluble core is not observed when platelets are activated in the presence of CB. Again, we attribute this to an inhibition by CB of long actin filament formation, since fibrinogen binding and platelet aggregation dependent on the glycoprotein IIb-IIIa complex were not blocked by CB. This left open the possibility that linkages between glycoprotein IIb-IIIa complexes and the non-sedimenting actin-binding protein–actin filaments still occurred but that such complexes were insufficiently cross-linked to precipitate after detergent extraction. Although the exact nature of this linkage and its importance to the expression of the fibrinogen binding site is unknown, we did determine in this study that receptor occupancy alone was insufficient to retain the complex in the isolated cytoskeletal core. Rather, a multivalent interaction with these surface receptors is required to stabilize their interaction with the cytoskeleton.

This entire cytoskeletal assembly process of actin polymerization and interaction with phosphorylated myosin and actin-binding protein can be completely reversed by agents that elevate platelet cytosolic cyclic AMP levels (11, 12). This oppositional control is most likely due to the lowering of cytosolic calcium levels elevated during activation (13). Moreover, these studies showed that the cytoskeletal assembly-disassembly cycles correlated completely with the reversal of platelet shape change, aggregation, and expression of the fibrinogen binding site.

In this study, we have used Con A and CB to demonstrate a reciprocal interaction of the glycoprotein IIb-IIIa complex with platelet pseudopodal elements. Moreover, using prostaglandin E1 (PGE1) and the reciprocal Con A–cytoskeleton interaction, we demonstrate a membrane-associated cytoskeletal complex that may be pre-organized in the unactivated platelet. Evidence is also presented that the physiological correlate to the multivalent binding of Con A may be the fibrin–fibrinogen–platelet interaction found in the forming thrombin-induced clot.

MATERIALS AND METHODS

Lyophilized human fibrinogen was purchased from Helena Laboratories, Beaumont, TX. [3H]-Hydroxytryptamine (serotonin) and carrier-free Na251 were obtained from Amersham, Radiochemica Center, Amersham, England. 32p-Orthophosphate was obtained from New England Nuclear, Boston, MA. [3H]-5-Hydroxytryptamine (serotonin) and carrier-free Na251 were obtained from Amersham, Radiochemica Center, Amersham, England. 32p-Orthophosphate was obtained from New England Nuclear, Boston, MA. 32p-Orthophosphate was obtained from New England Nuclear, Boston, MA. [3H]-5-Hydroxytryptamine (serotonin) and carrier-free Na251 were obtained from Amersham, Radiochemica Center, Amersham, England. 32p-Orthophosphate was obtained from New England Nuclear, Boston, MA. [3H]-5-Hydroxytryptamine (serotonin) and carrier-free Na251 were obtained from Amersham, Radiochemica Center, Amersham, England. 32p-Orthophosphate was obtained from New England Nuclear, Boston, MA. [3H]-5-Hydroxytryptamine (serotonin) and carrier-free Na251 were obtained from Amersham, Radiochemica Center, Amersham, England. 32p-Orthophosphate was obtained from New England Nuclear, Boston, MA. [3H]-5-Hydroxytryptamine (serotonin) and carrier-free Na251 were obtained from Amersham, Radiochemica Center, Amersham, England. 32p-Orthophosphate was obtained from New England Nuclear, Boston, MA. [3H]-5-Hydroxytryptamine (serotonin) and carrier-free Na251 were obtained from Amersham, Radiochemica Center, Amersham, England. 32p-Orthophosphate was obtained from New England Nuclear, Boston, MA. [3H]-5-Hydroxytryptamine (serotonin) and carrier-free Na251 were obtained from Amersham, Radiochemica Center, Amersham, England. 32p-Orthophosphate was obtained from New England Nuclear, Boston, MA. [3H]-5-Hydroxytryptamine (serotonin) and carrier-free Na251 were obtained from Amersham, Radiochemica Center, Amersham, England. 32p-Orthophosphate was obtained from New England Nuclear, Boston, MA. [3H]-5-Hydroxytryptamine (serotonin) and carrier-free Na251 were obtained from Amersham, Radiochemica Center, Amersham, England.

RESULTS

Platelet Aggregation, Secretion, and Cytoskeletal Formation

Con A induces the formation of a complete cytoskeleton (Fig. 1, lane 3), similar to the thrombin-induced cytoskeleton (lane 2). The cytoskeletal core content of filamentous actin is slightly reduced for activation with Con A with respect to thrombin as assessed by DNase I inhibition assay (Table 1). At 1 U/ml thrombin, maximal aggregation occurs between 2 and 3 min, while activation by Con A follows a slower time course with maximal aggregation between 7 and 8 min (Fig. 2).

When the cytosolic levels of cyclic AMP are elevated by pretreatment with PGE1 or dibutyryl cyclic AMP sufficient to
Polyacrylamide gradient–SDS gels of concanavalin A–activated platelets. Aliquots of 125I–surface labeled–platelets were activated and cytoskeletal cores prepared as described in Materials and Methods. Equivalent aliquots of cytoskeletal core were subjected to electrophoresis on 6–17.5% polyacrylamide gradient–SDS slab gels, and the gels were stained with Coomassie Blue. The cytoskeletal cores are from: (1) control, unactivated platelets; (2) platelets activated with thrombin (1 U/ml); (3) platelets activated with Con A (100 μg/ml); (4) platelets pretreated with PGE1 (1 μg/ml), then activated with Con A; (5) platelets pretreated with CB (5 μg/ml), then activated with Con A; (6) platelets pretreated with CB (5 μg/ml), then activated with thrombin (1 U/ml). The cytoskeletal components indicated are: actin-binding protein (ABP), myosin heavy chain (MHC), alpha-actinin (α-A), actin, and tropomyosin (TM). Also indicated are the fibrin (f) and concanavalin A (Con A) subunits.

Table 1. Cytoskeletal Core Composition

| Treatment          | Actin* | ABP*  | Myosin* | GP IIa* |
|--------------------|--------|-------|---------|---------|
| Controls           | <10%   | <5%   | <5%     | <3%     |
| Thrombin           | 60–85  | 60–80 | 60–80   | 25 ± 1  |
| CB + thrombin      | 40–50  | 6–14  | 62–70   | <3%     |
| Con A              | 60–80  | 60–80 | 60–80   | 79 ± 4  |
| CB + Con A         | 42–45  | 27–37 | 29–35   | 84 ± 5  |
| Con A + PGE1       | 40–45  | 28–42 | 3–9     | 84 ± 5  |

* Determined by laser densitometry of Coomassie Blue–stained bands (ABP, 260-kD actin-binding protein; myosin 200-kD heavy chain) resolved on SDS slab gels (n = 3).

** Determined by DNase I inhibition assay (n = 3).

† Determined by laser densitometry of 125I–labeled autoradiograms (± SEM, n = 5).

§ Controls include unactivated platelets with and without pretreatment with CB or PGE1, platelet lysates to which Con A or fibrinogen have been added before pelleting, and platelets pretreated with PGE1, before addition of fibrinogen and thrombin.

Block cytoskeletal formation by thrombin, the pseudopodal cytoskeletal elements (actin cross-linked by actin-binding protein and alpha-actinin) still form a precipitable matrix in the presence of Con A (Fig. 1, lane 4) with only slight reduction in the core content of actin. This actin in the core is most likely that filamentous component present in the resting platelet (typically in the 30–40% range) since no more than a 5–10% increase in filamentous actin occurs, as measured by

DNase inhibition assays of whole platelet lysates. This precipitation of an apparent pseudopodal cytoskeleton occurs even though platelet activation is also largely suppressed in terms of aggregation (Fig. 2), secretion (Fig. 3), and actin–myosin interaction in the contractile gel (Fig. 1, lane 4).

Pretreatment with CB enhances the Con A–induced aggregation and secretory responses, as has been observed with other activators (9). CB pretreatment, which inhibits the formation of the long actin filaments necessary for pseudo-
podal formation, does not reduce the retention of pseudopodal elements (actin-binding protein and alpha-actinin) from the Con A–stimulated cytoskeletal core (Fig. 1, lane 3). This is in contrast to the thrombin-induced cytoskeleton in which pretreatment with CB completely releases the actin-binding protein and a significant quantity of the alpha-actinin from the cytoskeletal core (Fig. 1, lane 6). No additional precipitate, over control level, is observed (data not shown) if the Con A is added after lysis of the unactivated platelets. This is in agreement with the results of Painter et al. (7).

**Electron Microscopy of Con A–activated Platelets**

Electron micrographs of unactivated controls, with or without PGE₂ pretreatment, show discoidal platelets containing numerous randomly distributed granules (Fig. 4A). In agreement with the above results, at 5 min after stimulation with Con A, small aggregates of platelets exhibiting a loss of discoid shape, granule centralization, degranulation, and pseudopodal formation are evident (Fig. 4, B and C). As has been previously noted (5), the surface-connected canalicular system appears dilated and is more prominent than is typically observed with other activation conditions. Pretreatment with CB followed by Con A activation produces a similar picture of activated platelets but with somewhat fewer pseudopodal projections (not shown).

Platelets pretreated with PGE₂ and then exposed to Con A show some degree of cell–cell adherence by 10 min but do not show any significant loss of discoid shape, granule centralization, or degranulation (Fig. 4, D, E, and F).

**Phosphorylation of Platelet Proteins**

Activation of platelets by thrombin has been shown to induced rapid two- to threefold increases in phosphorylation of actin-binding protein (16) and myosin light chain (17), as well as of a cytosolic 47,000-D protein (18) designated 40P (5–10-fold) with peak ³²P-labeling between 30 and 60 s. Activation with Con A produces similar increases in phosphorylation of myosin light chain and 40P (Fig. 5A) (however, the phosphorylation of actin-binding protein is less than two-fold). The time course, like the aggregation and secretion response, is slower with peak ³²P-labeling occurring between 5 and 6 min. Pretreatment with CB followed by Con A activation does not alter the ³²P-labeling pattern (data not shown). Pretreatment with PGE₂ completely blocks the ³²P-labeling of the myosin light chain (Fig. 5B). The actin-binding protein, along with 24- and 22-kD peptides (19), is phosphorylated by PGE₂, independent of activation as previously reported for PGI₂ or dibutyl cyclic AMP (11).

**Cytoskeletal Core Content and Retention of Glycoprotein IIb-IIIa**

Quantitation of the cytoskeletal cores of the activated platelets by DNase I inhibition assay for actin content and laser densitometry of the Coomassie Blue–stained gels for myosin and actin-binding protein content is summarized in Table I. The effect of pretreatment with PGE₂ on Con A activation is a slight reduction in cytoskeletal core actin content with a significant decrease in myosin. Pretreatment with CB produces the same slight decrease in actin content with either Con A or thrombin activation. The release of actin-binding protein from the core, under the influence of CB, is far greater with thrombin activation than with Con A.

Retention of the ¹²⁵I-labeled glycoprotein IIb-IIIa complex in the cytoskeletal cores was assessed by autoradiography and laser densitometry. The retention, expressed as a percentage of the whole platelet, was calculated using the glycoprotein IIIa band as a marker for the complex, since it is more heavily labeled and easier to quantitate. In all platelet samples activated with Con A, irrespective of pretreatment, the retention was in excess of 80%. Thrombin-activated platelet cores contained 25% of the total glycoprotein IIb-IIIa complex, and pretreatment with CB reduced the core content to control levels. Similar data was previously reported for arachidonate-activated platelets, with or without pretreatment with CB (10).

**Cytoskeletal Formation and Glycoprotein IIb-IIIa Retention in the Presence of Fibrinogen**

To investigate whether the multivalent surface binding provided by Con A might be responsible for retention of the pseudopodal elements in the presence of CB, platelets were activated with thrombin in the presence of 0.1 mg/ml exogenous fibrinogen (0.5 kD for fibrinogen–receptor binding). In addition to activating the platelets, thrombin will convert the soluble fibrinogen into the insoluble fibrin network. The fibrin network can then provide multiple sites for glycoprotein IIb-IIIa interaction. The cytoskeletal composition of ¹²⁵I-labeled platelets activated with and without pretreatment with CB and in the presence or absence of exogenous fibrinogen are shown in Fig. 2A. The autoradiogram of the retained surface-labeled protein is shown in Fig. 2B. The cytoskeletal core from platelets activated with Con A (100 μg/ml) is shown in lane 1. In addition to retaining 79 ± 4% of the glycoprotein IIb-IIIa complex, other glycoproteins, including one ~140,000 D (possibly glycoprotein Ib), can be detected in these cores. Thrombin activation in the absence (lane 2) and presence of exogenous fibrinogen (lane 3) yield the same core compositions. However, the retention of the glycoprotein IIb-IIIa complex in the core is enhanced by the presence of exogenous fibrinogen (Fig. 2B, lanes 2 and 3). Quantitation by laser densitometry indicated that without exogenous fibrinogen, 25 ± 1% of the surface-labeled glycoprotein IIa is retained in the cytoskeletal core, whereas in the presence of exogenous fibrinogen, 36 ± 3% of the glycoprotein IIIa is retained. CB-pretreated platelets activated without exogenous fibrinogen do not contain a significant proportion of actin-binding protein (lane 4), and the core content of glycoprotein IIIa is reduced to control levels (<3%). In the presence of fibrinogen, the cytoskeletal cores from CB-pretreated platelets activated by thrombin contain a significant portion of actin-binding protein (lane 5), and the retention of the glycoprotein IIIa in the core is increased 10-fold (31 ± 3%).

As with Con A, the addition of a similar amount of fibrinogen to the lysates of unactivated platelets does not increase the levels of any retained core component, including the glycoprotein IIb-IIIa complex (Table 1). This control demonstrates the need for intact membrane surface–cytoskeletal system for these interactions to take place. In addition, platelets, exposed to thrombin and fibrinogen after pretreatment with PGE₂ to block activation, do not show increased retention of cytoskeletal core components. This latter finding demonstrates the need for platelet activation expressing the fibrinogen binding site in order for the transmembrane cytoskeletal interaction with the fibrin network to occur.
Figure 4. Electron micrographs of Con A activated with platelets. Platelets were either unactivated controls (A); activated with 100 μg/ml Con A for 5 min (B); activated with 100 μg/ml Con A for 10 min (C); or pretreated for 10 min with 1 μg/ml PGE1, then activated with Con A for 10 min (D, E, and F). Bar, 1 μm.
FIGURE 6 Polyacrylamide gradient-SDS gels of platelet cytoskeletons and retention of surface-labeled membrane proteins. Equivalent aliquots of platelets were subjected to electrophoresis on 6–17.5% polyacrylamide gradient-SDS slab gels, and the gels were stained for protein with Coomassie Blue (A). Cytoskeleton was from: (1) platelets activated with 100 μg/ml Con A; (2) after activation with 1 U/ml thrombin; (3) activation with 1 U/ml thrombin in the presence of 0.1 mg/ml fibrinogen; (4) pretreated with CB, activated with 1 U/ml thrombin; (5) pretreated with CB, activated with 1 U/ml thrombin in the presence of 0.1 mg/ml fibrinogen. B is the autoradiogram of the protein-stained gel in A showing retention of the 125I-surface labeled proteins, IIb, glycoprotein IIb; IIIa, glycoprotein IIIa.

DISCUSSION

The activation of platelets by Con A has been suggested to be an analogue of the normal receptor-mediated activation pathway (6). In agreement with this, we have shown that Con A activates human platelets, initiating phosphorylation, secretion, and the formation of a cytoskeletal structure that contains all the structural elements found in the thrombin or arachidonate-activated platelet.

Platelet stimulus–response coupling is most likely mediated by increased phospholipid metabolism (20). The subsequent phospholipase C–mediated formation of diacylglycerol leads to activation of protein kinase C (21, 22). Protein kinase C phosphorylation of actin-binding protein (23) may regulate its cross-linking of filamentous actin into a pseudopodal cytoskeleton (16, 24). At the same time, formation of thromboxane A2 from the arachidonic acid metabolic pool, lysophosphatidic acid, and/or inositol 1, 4, 5-trisphosphate mobilizes calcium from the intracellular pool (25–28). Cytosolic calcium–mediated phosphorylation of the myosin light chain stimulates the actin–myosin interaction (29) and the contractile activity responsible for granule centralization (9).

This entire process of actin polymerization and interaction with actin-binding protein and myosin is both blocked and even reversed by cyclic AMP inhibition of the elevation of cytosolic calcium triggered by thrombin or arachidonic acid (10–12). Unlike these activators, Con A precipitates pseudopodal (actin-tropomyosin, alpha-actinin, and actin-binding protein) core components along with most of the glycoprotein IIb-IIIa complex in the presence of PGE2 or dibutyryl cyclic AMP. Under the influence of PGE2 or dibutyryl CAMP, Con A cannot stimulate phosphorylation of the 20-kD light chain, and the actin–myosin interaction is blocked.

To explain these results, we propose the following model. In the unactivated platelet, it is now generally accepted that ~60–70% of the platelet actin is in a monomeric actin pool (9, 10, 30), perhaps stabilized by profilin (31). The organization and subcellular distribution of the remaining 30–40% actin is not known but has been proposed to be an unorganized or amorphous network (30, 32), possible associated with the platelet membrane (33, 34). This actin may be in short filaments, the ends of which could serve as nucleation sites, and are blocked by capping proteins (35). In our model, the monomeric actin pool is induced to polymerize by receptor occupancy uncapping of these nucleation sites on the membrane or by formation of de novo membrane or cytoplasmic nucleation sites. Regulation of this latter process may be accomplished by elevation of cytoplasmic calcium levels causing actin nucleation by gelsolin (36, 37) or, perhaps, by phosphorylation of cytoskeletal proteins such as actin-binding
protein (16, 38). That long actin filaments can form in the presence of gelsolin and micromolar calcium is, perhaps, due to the stabilizing influence of tropomyosin (39), also a component of the platelet cytoskeleton (40).

The cytoskeletal proteins incorporated into the Con A-stimulated cytoskeleton are the same as in the cores recovered from platelets activated with thrombin or arachidonate. However, we suggest that activation may be initiated by an altered mechanism. The binding of Con A to the surface glycoproteins may collect or cap the receptors, resulting in linkage and clustering of polymerized actin (41). This membrane-associated actin could bind alpha-actinin and actin-binding protein on the cytosolic face of the plasma membrane (34). This rearrangement of membrane proteins may also displace calcium from the plasma membrane and initiate phospholipid metabolism resulting in diacylglycerol-mediated protein kinase C activation and cytosolic calcium-mediated myosin kinase activation. This, coupled with actin polymerization triggered by one of the above mechanisms, leads to assembly of the complete cytoskeletal system.

An apparent cytoskeletal core was precipitated by Con A despite cyclic AMP elevation and inhibition of platelet activation in terms of shape change, phosphorylation of 40P and myosin light chain, contractile gel core formation, granule centralization, and secretion. Our interpretation of this result is that we are precipitating membrane-associated complexes of actin–tropomyosin, alpha-actinin, and actin-binding protein already present in the unactivated platelet. A less likely alternative is that these complexes form in the presence of Con A despite cyclic AMP opposition of cytosolic calcium elevation and protein phosphorylation. Although this latter possibility could be analogous to the receptor occupancy–triggered cytoskeletal assembly model mentioned above, we did not observe more than a 5-10% de novo increase in filamentous actin in the whole platelet lysates under these conditions. The actin content of the core collected by Con A in the presence of PGE1 is, most likely, the preexisting pool of 30-40% filamentous actin present in the resting platelet. This would be consistent with a recent finding that a subpopulation of gycoprotein Ib-IIIa is associated with actin in the resting platelet (42).

Actin-binding protein, although phosphorylated by cyclic AMP elevation independent of activation, was not extensively phosphorylated during Con A activation despite almost normal levels of 40P and myosin light chain phosphorylation. Since actin-binding protein was retained in the isolated cytoskeletal core to the same extent as with other activators causing extensive actin-binding protein phosphorylation (9, 16), these results may argue against an obligatory role for such phosphorylation during activation to initiate cytoskeletal interactions. However, it should be noted that the mechanism by which Con A is collecting the actin-binding protein may not be the same as activation-dependent formation of the pseudopodal cytoskeleton. With respect to this possibility, it has been recently reported that actin-binding protein may be associated in the unactivated platelet with gycoprotein Ib (43), a possible component of the Con A cytoskeletal cores.

The behavior of the apparent pseudopodal core from Con A–activated platelets also differs from thrombin- or arachidonate-treated platelets with respect to sensitivity to CB. With these typical activators, CB may inhibit the formation of long actin filaments necessary for pseudopodal formation (9, 10). The cytoskeletal cores contain actin (presumably short filaments) and myosin, but the content of actin-binding protein is substantially reduced. However, in Con A–activated platelets, the extensive Con A cross-linking of the gycoprotein Ib-IIIa complex on the surface allows the actin-binding protein–alpha-actinin–filamentous actin–tropomyosin–membrane complex to be precipitated despite CB pretreatment. The simplest explanation for this result is our previous suggestion (10) that the gycoprotein Ib-IIIa linkage to this cytoskeletal complex remains intact in the presence of CB. While Con A may not organize these complexes into the same pseudopodal cytoskeletal organization, its external cross-linking of these complexes substitutes for the lack of long actin filaments and insufficient cross-linking by actin-binding protein and alpha-actinin.

The contribution of the surface cross-linking to the recovery of gycoprotein Ib-IIIa and actin-binding protein in the core is further illustrated by the increased retention of the actin-binding protein–filamentous actin–gycoprotein Ib-IIIa membrane complex in cytoskeletal cores from platelets activated by thrombin in the presence of the forming fibrin strands despite CB pretreatment. The fibrinogen–fibrin clot provides the equivalent of an external network of multivalent ligands (44). In contrast to Con A multivalent binding, the formation of the gycoprotein Ib-IIIa binding sites for fibrinogen/fibrin is dependent on activation and sensitive to cyclic AMP inhibition (45).

The identity of the cytoskeletal component(s) linking the gycoprotein Ib-IIIa to the cytoskeleton is as yet unknown (46). That extensive cross-linking of the gycoprotein Ib-IIIa complex by an external ligand network, generated by two very different multivalent ligands, retains the actin-binding protein–alpha-actinin–filamentous actin–tropomyosin complex in the cytoskeletal core despite the disruption by CB of the internal cytoskeletal network illustrates the reciprocal nature of these multivalent interactions and the specificity of the gycoprotein Ib-IIIa link to the platelet cytoskeleton.

This work was supported in part by National Institutes of Health Grant No. HL34445-01 (to Dr. Carroll), National Institutes of Health Grant No. HL07430 (to Dr. Wheeler), and Medical Research Council Grant No. MA 7396 (to Dr. Gerrard).

Received for publication 27 August 1985, and in revised form 3 May 1985.

REFERENCES

1. Ash, J. F., and S. J. Singer. 1976. Concanavalin A-induced transmembrane linkage of concanavalin A surface receptors to intracellular myosin-containing filaments. Proc. Natl. Acad. Sci. USA. 73:4574-4579.
2. Sheterline, P., and C. R. Hopkins. 1981. Transmembrane linkage between surface glycoproteins and the platelet cytoskeleton in neoplastic leukocytes. J. Cell Biol. 90:743-754.
3. Jung, G., R. M. Helm, C. A. C. Carraway, and K. k Carraway. 1984. Mechanism of concanavalin A–induced anchorage of the major cell surface glycoproteins to the submembrane cytoskeleton in 13762 ascites mammary adenocarcinoma cells. J. Cell Biol. 98:179-187.
4. McGregor, J. L., K. J. Clemetson, E. James, T. Greenland, and M. Dechavanne. 1979. Identification of human platelet glycoproteins in SDS-polyacrylamide gels using [125I]labelled lectins. Thromb. Res. 16:825-831.
5. Pitchford, J. L., M. Metz, W. G. Forsmann, P. Worsner, and R. Bovensman. 1979. Unstructural changes of lectin-stimulated platelets analyzed by thin sections and freeze-fracturing. J. Ultrastruct. Res. 65:234-245.
6. Bennett, W. F., J. S. Belville, and G. Lynch. 1979. A study of protein phosphorylation in shape change and Ca2+-dependent serotonin release by blood platelets. Cell. 18:1015-1023.
7. Painter, R. G., and M. Ginsberg. 1982. Concanavalin A induces interactions between surface glycoproteins and the platelet cytoskeleton. J. Cell Biol. 92:365-373.
8. Phillips, D. R., K. J. Jennings, and H. H. Edwards. 1982. Identification of membrane proteins mediating the interaction of human platelets. J. Cell Biol. 86:77-86.
9. Carroll, R. C., R. G. Butler, P. A. Morris, and J. M. Gerrard. 1982. Separability assembly of platelet pseudopodal and contractile cytoskeletons. Cell. 30:285-292.

WHEELER ET AL. Transmembranous Cytoskeletal Interactions in Platelets
10. Wheeler, M. E., A. C. Cox, and R. C. Carroll. 1984. Retention of the glycoprotein IIb-IIIa complex in the isolated platelet cytoskeleton: effects of separable assembly of platelet pseudopod and contractile cytoskeletons. J. Clin. Invest. 74:1080–1089.

11. Cox, A. C., R. C. Carroll, J. G. White, and G. H. R. Rao. 1984. Recycling of platelet phosphorylation and cytoskeletal assembly. J. Cell Biol. 98:8–15.

12. Feinstein, M. B., J. J. Egan, and E. E. Opas. 1983. Reversal of thrombin-induced myosin phosphorylation and the assembly of cytoskeletal structures in platelets by adenosine 3′,5′-cyclic monophosphate. J. Biol. Chem. 258:1260–1267.

13. Zavattiero, G. B., and M. B. Feinstein. 1984. Cytoplasmic Ca2+ in platelets is controlled by cyclic AMP: antagonism between stimulators and inhibitors of adenylyl cyclase. Biochem. Biophys. Res. Commun. 120:579–585.

14. Studer, F. W. 1978. Analysis of bacteriophage T3 early RNAs and proteins on slab gels. J. Mol. Biol. 123:237–248.

15. Gerrard, J. M., S. E. Kindom, D. A. Peterson, J. Poller, K. E. Krantz, and J. G. White. 1984. Platelet proteins phosphorylated on exposure of intact platelets to ionophore A23187 or to prostaglandin E2. Possible role of a membrane phosphopolypeptide in the regulation of calcium-ion transport. J. Biol. Chem. 259:7510–7514.

16. Daniel, J. L., J. R. Molish, and H. Holmsen. 1984. Calcium flux. FEBS Lett. 170:64–68.

17. Lyons, R. M., and R. M. Atherton. 1979. Characterization of a platelet protein phosphorylated during the thrombin-induced release reaction. Biochemistry. 18:544–552.

18. Fox, J. E. B., A. K. Say, and R. J. Haslam. 1979. Subcellular distribution of the different platelet proteins phosphorylated on exposure of intact platelets to ionophore A23187 or to prostaglandin E2. Possible role of a membrane phosphoprotein in the regulation of calcium flux. J. Biol. Chem. 254:1260–1267.

19. Feinman, M., J. J. Egan, and E. E. Opas. 1983. Reversal of thrombin-induced myosin phosphorylation and the assembly of cytoskeletal structures in platelets by adenosine 3′,5′-cyclic monophosphate. J. Biol. Chem. 258:1260–1267.

20. Fox, J. E. B., J. K. Boyles, C. C. Reynolds, and D. R. Phillips. 1984. Actin filament content and organization in unstimulated platelets. J. Cell Biol. 98:1935–1941.

21. Rybicki, J. P., D. L. Venton, and G. C. Breton. 1983. The thromboxane antagonist, 13-azaprostanoic acid, inhibits arachidonic acid-induced Ca2+ release from isolated platelet membranes. J. Biol. Chem. 258:5663–5669.

22. Simon, M. F., H. Chap, and L. Douste-Blazy. 1984. Activation of phospholipase C in human platelet activation. Comparison of human platelet activation. J. Biol. Chem. 259:7510–7514.

23. Kawamoto, S., and H. Hidaka. 1984. Ca2+-activated, phospholipid-dependent protein kinase in human platelet activation. Comparison of human platelet activation. J. Biol. Chem. 259:7510–7514.

24. Qing-Qi, Z., S. Rosenberg, J. Lawrence, and A. Stracher. 1984. Role of actin binding proteins in the regulation of calcium-ion transport. Biochem. J. 214:631–661.

25. Marcus, A. J. 1978. The role of lipids in platelet function: with particular reference to the arachidonic acid pathway. J. Lipid Res. 19:793–826.

26. Feinstein, M. B., J. J. Egan, and E. E. Opas. 1983. Reversal of thrombin-induced myosin phosphorylation and the assembly of cytoskeletal structures in platelets by adenosine 3′,5′-cyclic monophosphate. J. Biol. Chem. 258:1260–1267.

27. Studer, F. W. 1978. Analysis of bacteriophage T3 early RNAs and proteins on slab gels. J. Mol. Biol. 123:237–248.

28. Wheeler, M. E., A. C. Cox, and R. C. Carroll. 1984. Retention of the glycoprotein IIb-IIIa complex in the isolated platelet cytoskeleton: effects of separable assembly of platelet pseudopod and contractile cytoskeletons. J. Clin. Invest. 74:1080–1089.