Molecular Basis of Platelet Granule Secretion

Robert Flaumenhaft

Abstract—The energy-dependent release of granule contents from activated platelets is a well-established component of normal hemostasis and thrombosis. A role for membrane fusion in this process has been presumed for decades, but only recently have the mechanisms of platelet membrane fusion been investigated at the molecular level. Such studies have demonstrated that platelet membrane fusion is controlled by lipid components of the membrane bilayer, by transmembrane proteins termed SNARE proteins, and by chaperone proteins that interact with SNARE proteins. This core membrane fusion machinery is controlled by activation-dependent changes in cytoskeletal organization, intracellular calcium levels, kinase activity, and intracellular protease activity. Through these mechanisms, interactions of ligands with their cognate cell-surface receptors are transmitted to the membrane fusion machinery to facilitate membrane fusion and secretion of granule contents from platelets. (Arterioscler Thromb Vasc Biol. 2003;23:1152-1160.)

Key Words: platelets | secretion | membrane fusion | SNARE protein | signal transduction

Early accounts of platelet secretion describe a process of viscous metamorphosis involving loss of individual platelet contents into thrombi by a route believed to involve fusion and lysis of platelets.1 Subsequent experiments showed differential loss of platelet proteins and maintenance of membrane integrity after stimulation of platelets.2,3 These observations led to the supposition that platelets secrete their contents without cell lysis by a process of granule extrusion. This process was termed the release reaction.3 Morphologic studies revealed that the release of granules from activated platelets is unusual in that platelet granules become centralized upon platelet activation,4 whereas classic exocytosis by nucleated cells occurs via fusion of granules with the plasma membrane. The observation that the cytoskeleton directs granule centralization led to speculation that it provides a contractile force that facilitates the release of granule contents through the open canalicular system.5 Several lines of evidence subsequently suggested that granule secretion was not dependent on platelet shape change.6-11 In more recent years, membrane fusion has been emphasized as an event critical for the release of platelet granular contents into the extracellular environment.12,13 Membrane fusion is regulated at several different levels. At the membrane level, specific lipids have been shown to contribute to the membrane fusion event required for granule secretion in platelets. At the membrane protein level, membrane fusion is orchestrated by a superfamily of proteins, termed soluble NEM-sensitive attachment protein receptors (SNARE) proteins, that form a universal membrane fusion machinery.14 A third level of regulation is provided by a set of chaperone proteins that bind to and modulate the activity of the SNARE protein core to facilitate membrane fusion. In turn, membrane lipid components, SNARE proteins, and their chaperones are regulated by a variety of signaling pathways. These signals lead to posttrans-
The level of regulation of membrane fusion to consider is the role granules are fused to form a large vacuole). The first incorporated into the surface-connected membrane (or several). This fusion pore rapidly expands event itself. Membrane fusion involves the formation of a pore for platelet granule secretion necessitates. **Lipid Components**

Formation of a pore for platelet granule secretion necessitates the fusion of 2 lipid bilayers. The fusion of opposing lipid bilayers in an aqueous environment requires sufficient energy to overcome electrostatic repulsive and hydration forces between the 2 membranes. Given this requirement, one would anticipate that the lipid composition at the site of fusion would be a critical determinant of the fusion process. Relatively little is known about the specific lipids required for platelet membrane fusion. Two lipid components, phosphatidic acid (PA) and phosphatidylinositol 4,5-bisphosphate (PIP2), have been studied in this context. Experimental evidence demonstrates functions for both lipid components in platelet membrane fusion. A role for PA in platelet granule secretion is suggested by several observations. Synthesis of PA after stimulation of permeabilized platelets with PMA and GTP-γ-S correlates with increased granule secretion. Furthermore, inhibition of PA synthesis by ethanol, which affects phospholipase D activity, inhibits both PA production and dense granule secretion. In addition, PA and PA analogues augment dense granule secretion induced by activation of protein kinase C (PKC) and by GTP-γ-S. These results are consistent with studies of PA performed in other established secretory cells, such as chromaffin cells, PC12 cells, and neurons. The exact role of PA in potentiating membrane fusion has not been defined. However, altering membrane curvature, serving as protein attachment sites, and signaling are proposed functions for PA in membrane fusion.

A role for PIP2 in regulated granule secretion was first demonstrated in neuroendocrine cells. Maintenance of polyphosphoinositides was found to be crucial for vesicle secretion from chromaffin cells. Subsequently, type I phosphatidylinositol phosphate and phosphatidylinositol transfer protein were determined to be cytosolic factors capable of reconstituting Ca2+-induced secretion in PC12 cells. In platelets, PIP2 is synthesized in an activation-dependent manner by both type I and type II PIPKs (Figure 2). A role for PIP2 in platelet granule secretion is evidenced by several observations in permeabilized platelets. When phosphatidylinositol-specific phospholipase-C is infused into permeabilized platelets, it cleaves PIP2 and inhibits α-granule secretion. Exogenously added PIP2 also inhibits platelet α-granule secretion, presumably by competing with endogenous PIP2 localized in platelet membranes. Antibodies directed at type II PIPKs inhibit PIP2 synthesis and interfere with α-granule secretion mediated by Ca2+. A thrombin receptor agonist peptide (TRAP), or the PKC agonist PMA, further, recombinant type II PIPK augments TRAP- or PMA-induced α-granule secretion. These experiments demonstrate that PIP2 synthesis resulting from the activity of type II PIPK contributes to agonist-induced granule secretion.

Several potential roles for PIP2 in platelet granule secretion have been proposed. Several proteins involved in membrane trafficking and cytoskeletal reorganization contain PIP2-binding domains (Figure 2). Proteins may interact with PIP2 via linear cationic sequences or specific protein interaction domains. Examples of PIP2-interacting linear sequences are found in gelsolin family proteins, which contain PIP2-binding sequences consisting of positively charged residues interspersed with hydrophobic residues. The best characterized PIP2 protein interaction domains are the pleckstrin homology domains like those found in phospholipase D, phospholipase C isoforms, and many regulators of small GTP-binding proteins (Figure 2). The C2 domain of synaptotagmin binds PIP2 preferentially to other phosphoinositides after exposure to Ca2+. The synthesis of PIP2 microdomains adjacent to membrane-associated PIPKs may enable the recruitment of proteins containing PIP2-binding domains. PIP2 also inhibits the lateral diffusion of SNARE proteins in lipid membranes, which could facilitate the recruitment of SNARE proteins to raft-like domains. The function for PIP2 in directing the localization and organization of the secretory machinery, however, remains to be established. The central
role of PIP₂ in actin remodeling may also contribute to its influence on granule secretion. In addition, PIP₂ stimulates phospholipase D activity, leading to increased synthesis of PA. In turn, PA stimulates type I PIPK, leading to increased PIP₂ synthesis. Thus, PA and PIP₂ may act in a coordinated manner to facilitate membrane fusion.

SNARE Proteins and the Exocytotic Core Complex

It is clear that the regulation of membrane fusion in the platelet, or in any other cell, is not controlled entirely at the level of the lipid membrane. Over the last decade, the protein machinery responsible for controlling the formation of the fusion pore in cells has been studied in detail. In particular, a critical component of this machinery, the SNARE proteins, has been demonstrated to play a central role in platelet membrane fusion.

SNARE proteins are membrane-associated proteins oriented such that most of the protein is cytosolic. The original SNARE hypothesis stated that vesicular SNARE proteins (termed v-SNAREs) located on vesicular or granular membranes interact with SNARE proteins located on target membranes (termed t-SNAREs). Both v-SNAREs and t-SNAREs contain heptad-repeat regions that assemble into helical bundles involving coiled-coil interactions (Figure 3). These interactions occur in a parallel manner to form a 4-helix bundle termed the exocytic core complex that brings granular and plasma membranes into close apposition. One family of SNARE proteins termed the vesicle-associated membrane protein (VAMP) or synaptobrevin family of gene products was originally described as v-SNAREs. The syntaxin and SNAP-23 family of gene products was originally described as t-SNAREs. VAMPs and syntaxins contribute 1 coiled-coil domain to the exocytic core complex, whereas SNAP-23 contributes 2 coiled-coil domains (Figure 3). It is now recognized that v-SNAREs and t-SNAREs are not restricted to vesicles and plasma membranes, respectively. However, the formation of an exocytic core complex by SNARE proteins on opposing membranes (ie, in a trans conformation) remains a well-recognized feature of membrane fusion.
isoforms, tetanus toxin, a metalloproteinase that specifically cleaves VAMP.

Investigators have evaluated the subcellular localization of SNARE proteins in various platelet membranes using immunonanogold labeling of electron micrographs. These experiments demonstrate that approximately 80% of VAMP-3 is associated with granule membranes (Table 2). In contrast, approximately 60% of SNAP-23 is associated with plasma membranes. Syntaxin-2 and -4 are more evenly distributed among platelet membranes. These results have been confirmed by subcellular fractionation. This arrangement of SNARE proteins provides a molecular basis for fusion of granule membranes with either plasma membrane, membranes of the open canalicular system, or other granule membranes.

Defining the organization of the membrane fusion machinery within platelet membranes is an essential aspect in understanding fusion events. A detailed ultrastructural analysis of dense granules using serial thin sections demonstrated that approximately 70% of dense granule membranes were within 12.5 nm of surface-connected membranes and connected by a bridge-like structure. This study also showed that the population of dense granules in close apposition with surface-connected membranes was preferentially secreted on platelet activation. This morphologic evidence suggests that this population of granules is docked (ie, anchored to surface-connected membranes). Whether this population is primed (ie, competent to undergo rapid fusion in response to stimulation) in the resting platelet is unknown. Trimeric SNARE protein complexes have been detected on α-granules (unpublished results, 2002), raising the question of whether granule-associated SNARE complexes exist in a cis or trans conformation (Figure 4). The fact that multiple antibodies and inhibitory peptides inhibit activation-induced granule secretion speaks against a tightly bound, preformed complex that would sterically hinder access to inhibitors. A cis conformation of SNARE proteins in resting platelets is also consistent with the observation that N-ethylmaleimide–sensitive fusion protein (NSF, see below for description) is required for platelet granule fusion. Thus, stimulation of platelets may lead to activation of NSF that catalyzes the dissociation of these cis complexes, allowing for formation of trans complexes in membranes that are closely associated. Of course, SNARE protein complexes may exist in both cis and trans states in the resting platelet.

### Chaperone Proteins

Many chaperone proteins that bind to and direct the function of SNARE proteins have been described. A small but important subset of these proteins has been found in platelets, and many of these have been shown to function in granule secretion (Figure 4). NSF is a hexameric ATPase that is essential for most forms of membrane-trafficking, including regulated granule secretion. Both inhibitory peptides and antibodies to NSF have been demonstrated to interfere with

### TABLE 1. SNARE Proteins Mediate Secretion of Platelet α-Granules, Dense Granules, and Lysosomes

<table>
<thead>
<tr>
<th>Secretory Event</th>
<th>VAMP-3</th>
<th>VAMP-8</th>
<th>Syntaxin-2</th>
<th>Syntaxin-4</th>
<th>SNAP-23</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Granule secretion</td>
<td>Inhibitory antibodies, tetanus toxin</td>
<td>Blocking peptide</td>
<td>Inhibitory antibody</td>
<td>Inhibitory antibody</td>
<td>Inhibitory antibody</td>
</tr>
<tr>
<td>Dense granule secretion</td>
<td>Blocking peptide</td>
<td>Blocking peptide</td>
<td>Inhibitory antibody</td>
<td>Inhibitory antibody</td>
<td>Blocking peptide</td>
</tr>
<tr>
<td>Lysosome secretion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Tetanus toxin is a metalloproteinase that specifically cleaves isoforms of VAMP.

### TABLE 2. Localization of SNARE Proteins in Platelet Membranes as Determined by Immunonanogold Labeling

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Total Particles Counted</th>
<th>Plasma Membrane, %</th>
<th>OCS Membrane, %</th>
<th>Granule Membrane, %</th>
<th>Other Membrane, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAMP-3†</td>
<td>1014</td>
<td>4.6</td>
<td>9.1</td>
<td>80.5</td>
<td>5.8</td>
</tr>
<tr>
<td>Syntaxin-2†</td>
<td>1004</td>
<td>24.6</td>
<td>28.8</td>
<td>35.9</td>
<td>10.8</td>
</tr>
<tr>
<td>Syntaxin-4†</td>
<td>1163</td>
<td>6</td>
<td>33</td>
<td>25</td>
<td>35</td>
</tr>
<tr>
<td>SNAP-23†</td>
<td>1069</td>
<td>63.3</td>
<td>15.8</td>
<td>20.3</td>
<td>0.6</td>
</tr>
</tbody>
</table>

*All other platelet organelle membranes.
†See Reference 37. ‡See Reference 41.
dense granule, α-granule, and lysosome release from platelets. Although alternative functions have been proposed, an essential role of NSF in facilitating granule secretion is to serve as a molecular chaperone that disassembles cis SNARE complexes so that they can interact in a trans conformation. Thus, upon inhibition of NSF, SNARE proteins are sequestered in cis complexes and unavailable to interact with SNARE proteins on opposing membranes. The soluble NSF-attachment protein (SNAP)-α-SNAP binds and activates NSF. In platelets, wild-type α-SNAP augments Ca²⁺-induced granule secretion, whereas a dominant-negative α-SNAP mutant (α-SNAPL294A) and antibodies directed at α-SNAP inhibit granule secretion. Munc-18c is a 67-kDa protein that binds syntaxin. An ortholog of Munc-18c is found in platelets, binds syntaxin-4, and is phosphorylated upon platelet activation with thrombin or PMA. Phosphorylation of this Munc-18c ortholog by PKC decreases its affinity for syntaxin-4, raising the possibility that activation-induced release of syntaxin-4 from Munc-18c contributes to regulated granule release (Figure 4). CDCrel-1 is another syntaxin-4-binding protein found in platelets that is phosphorylated upon platelet activation. Genetically engineered mice that lack CDCrel-1 demonstrate enhanced dense granule release after stimulation with a variety of agonists, demonstrating that CDCrel-1 regulates granule secretion. Thus, SNARE-binding proteins serve an important modulatory role in platelet granule secretion.

Regulation of Platelet Granule Secretion

Granule Secretion and the Cytoskeleton

Ultrastructural studies have shown that platelet granule secretion is fundamentally different from that observed in nucleated cells. Such studies have revealed that platelets contain an elaborate membrane system, termed the open canalicular system, which consists of tunneling invaginations of the cell membrane. In the resting state, platelet α-granules and dense granules are distributed in an apparently random fashion throughout the platelet. On platelet activation, however, centralization of platelets occurs concurrently

Figure 4. Potential mechanisms for regulation of the formation of the platelet exocytic core complex. In the resting state, platelet
SNARE proteins may be sequestered either in cis complexes or by binding to chaperone proteins such as Munc-18c (brown). Interactions of Rab proteins (pink) with their effector proteins (aqua) may facilitate initial tethering to membranes. ATP-dependent activation of NSF (gray) by α-SNAP (green) leads to the disassembly of cis complexes. Released from the cis complex, SNARE proteins from closely apposed membranes are able to interact in a trans complex. Platelet activation also leads to posttranslational modifications of chaperone proteins. For example, phosphorylation of Munc-18c may free syntaxin (red), enabling trans complex formation with SNAP-23 (white) and VAMP (blue). Formation of trans SNARE protein complexes facilitates membrane fusion.
with activation-induced shape change and precedes granule release. Thus, it is possible that the morphologic changes induced by cytoskeletal reorganization contribute to granule secretion.

The influence of the cytoskeleton on granule secretion has been a subject of debate. One study demonstrated that inhibition of tubulin using monoclonal antibodies inhibited platelet granule secretion.66 Studies using the microtubule stabilizing agent taxol, however, suggested that microtubule reorganization does not influence granule secretion.67 The role of actin polymerization in granule secretion has also been scrutinized. Morphologic observations led some investigators to suggest that actomyosin-dependent granule centralization and membrane fusion act synergistically to facilitate granule secretion.5,67 Several other studies, however, suggest that the cytoskeleton does not facilitate granule secretion and that F-actin disassembly might actually be required for normal granule secretion.6,7 Several studies using various cytochalasins to prevent activation-induced actin polymerization have demonstrated that these inhibitors do not block agonist-mediated granule secretion.8–10 Under some experimental conditions, cytochalasins actually augment dense granule release.9 This result raises the possibility that actin may serve as a barrier that must be overcome for platelets to release their granules. Molecular evidence for such a mechanism was provided by investigators working with a Ca2+-dependent, F-actin severing protein termed scinderin.11 Recombinant scinderin was found to augment dense granule release, whereas inhibitory peptides derived from scinderin diminished Ca2+-induced granule secretion from permeabilized platelets.11 In addition, myristoylated alanine-rich C kinase substrate (MARCKS), which binds and crosslinks actin,68 is inactivated by PKC phosphorylation after platelet activation. Inactivation of MARCKS may also contribute to disassembly of an F-actin barrier that regulates secretion.69 Thus, actin polymerization seems to act as a barrier to membrane fusion rather than as a facilitator.

Yet the cytoskeleton serves as more than just a barrier in granule secretion. Kinases involved in the signaling pathway leading to actomyosin contraction may contribute to granule secretion. For example, inhibition of Rho kinase by the small molecule inhibitor Y-27632 inhibits phosphorylation of myosin-binding subunit and myosin light chain as well as granule secretion induced by ADP and a thromboxane analogue.70,71 Similarly, inhibitors of myosin light chain kinase such as W-7, ML-9, and GMCHA have been demonstrated to inhibit platelet granule secretion.72-75 Phosphorylation of myosin light chain increases the activity of actin-activated myosin ATPase and stimulates movement of myosin along polymerized actin. In neuroendocrine cells, myosin is proposed to mediate movement of vesicles from a reserve pool to a release ready vesicle pool.76,77 The role of actomyosin contraction in platelet granule secretion, however, remains to be elucidated.

Calcium-Binding Proteins

A large body of evidence from multiple cell types supports a role for Ca2+ in granule secretion. A rise in [Ca2+]i accompanies platelet granule secretion, and secretion can be triggered in permeabilized platelets by elevating [Ca2+]i.78 A current problem in the field is the identification of the binding proteins that mediate the effects of [Ca2+]i on platelet granule secretion. Ca2+-binding proteins involved in secretion fall into 2 general categories, EF hand proteins and Ca2+/phospholipid-binding proteins.79 Examples of EF hand proteins that have been involved in secretion and are found in platelets include calmodulin and calcyclin.80 Calmodulin binds to platelet α-granules.81 Pharmacologic evidence suggests that Ca2+/calmodulin-dependent phosphorylation of myosin light chain contributes to platelet granule secretion.72-75 The mechanism by which Ca2+/calmodulin-induced phosphorylation of myosin light chain mediates secretion was initially thought to be via activation of myosin light chain with subsequent contraction of the actomyosin. More recent data from nucleated cells suggest that calmodulin binds specifically to VAMP82 and mediates granule secretion by directly affecting the exocytic core complex.83 A Ca2+/
phospholipid-binding protein termed syntaptotagmin that acts as a Ca^{2+} sensor is found in platelets (unpublished observation, 2001), but its activity in platelets has not been evaluated. The Ca^{2+}-binding proteins scinderin and calpain also seem to function in platelet granule secretion.11,84–86

Protein Kinase C
A role for PKC in platelet granule secretion has been appreciated for decades. Earlier studies suggested that stimulation of platelets with phorbol esters led to platelet granule secretion without an increase in intracellular Ca^{2+}.87 A brain protein found to augment Ca^{2+}-dependent granule secretion from permeabilized platelets was determined to be PKCα.88 Thus, PKC is involved in both Ca^{2+}-dependent and Ca^{2+}-independent granule secretion. The signaling events leading from engagement of cell surface receptors with their cognate ligands to activation of PKC are well-established.89 The current challenge is to identify downstream effectors of PKC (Figure 5). One set of potential downstream effectors of PKC is the SNARE proteins and their chaperones. Munc-18, syntaxin-4, and CDCrel-1 are phosphorylated by PKC in platelets.39,54,90 Furthermore, there is some evidence that PKC phosphorylation of Munc-18c interferes with its binding to syntaxin-499 and that PKC phosphorylation of syntaxin-4 inhibits its binding to SNAP-23 (Figure 5A).90 MARCKS protein is another potential effector (Figure 5B). Kinetic studies have demonstrated that phosphorylation of MARCKS by PKC proceeds platelet granule secretion.91 Furthermore, inhibition of phosphorylation of MARCKS by PKC using a pseudosubstrate based on the phosphorylation site within MARCKS inhibits granule secretion. Unphosphorylated MARCKS binds tightly to PIP2 in membranes and protects it from degradation by phospholipase C (PLC).92 On phosphorylation by PKC, the affinity of MARCKS for PIP2 decreases substantially,93 allowing for other PIP2-binding proteins to bind PIP2.94 Because PIP2 mediates platelet granule secretion,24,26 exposure of PIP2 after MARCKS phosphorylation may contribute to granule secretion. Type II PIPK also represents a potential downstream effector of PKC (Figure 5C). Activation of platelets with PMA results in translocation of type II PIPK from the platelet cytosol and enhances the binding of a PIP2-binding domain to platelets.28 Inhibition of PMA-induced type II PIPK activity inhibits platelet granule secretion. Whether PKC directly phosphorylates type II PIPK, however, has not been determined. Identification of these many downstream effectors of PKC emphasizes the importance of this kinase in platelet granule secretion.

Intracellular Proteases
Several lines of evidence suggest that intracellular proteases influence platelet granule secretion. SNARE proteins are susceptible to proteolysis. SNAP-23 is cleaved by calpain on platelet activation.85,86 VAMP-3 is also cleaved by calpain but seems to be susceptible to cleavage by other platelet proteases as well.86 In contrast, neither syntaxin-2 nor -4 is cleaved by on platelet activation.55,86 In addition, calpain cleaves several signaling molecules, such as PKC and PLC, that influence signaling of granule secretion. Protease-induced downregulation of PKC has been shown to interfere with dense granule secretion from intact platelets.86 Inhibition of calpain using either a specific, cell-permeant inhibitor of calpain derived from the natural inhibitor calpastatin or peptidyl inhibitors of calpain inhibits agonist-induced P-selectin surface expression from intact platelets.84 In contrast, inhibition of calpain does not substantially affect release of granule contents from permeabilized platelets induced by 100 μmol/L Ca^{2+}.85 Thus, the effects of proteolysis of intracellular proteins on granule secretion vary depending on the conditions of the experiment.

Conclusion
A detailed understanding of platelet membrane fusion will involve knowledge of the protein and lipid components that contribute to formation of the fusion pore, the organization of the components within platelet membranes, the sequence of interactions that enable these components to facilitate membrane fusion, and the manner in which these components are regulated to affect secretion rapidly after platelet activation. The past 6 years of research in this area have identified several of the proteins involved in platelet membrane fusion, such as SNARE proteins and their chaperones. Although there is some information regarding the role of platelet lipids such as PA and PIP2 in the fusion process, this critical aspect of membrane fusion remains less well-studied. The subcellular localization of SNARE proteins in platelets has been investigated. Yet the organization of the secretory machinery in platelet membranes is not well understood. It is unknown, for example, whether a pool of platelet granules exists that is primed to secrete rapidly on platelet activation. In addition, there are few data describing the sequence of SNARE protein interactions that translate activation-dependent signals into a physical force capable of fusing membranes. Some posttranslational modifications of the membrane fusion machinery resulting from activation-induced signals have been identified. Yet a richer understanding of how platelet activation elicits membrane fusion will evolve through elucidation of the function of the cytoskeleton in granule secretion, identification of Ca^{2+}-binding proteins that direct membrane fusion, and additional characterization of downstream effectors of kinases such as PKC. This fundamental knowledge will impact attempts to modify platelet granule secretion for therapeutic benefit.

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References
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42. Polgar J, Chung SH, Reed GL. Vesicle-associated membrane protein 3 (VAMP-3) and VAMP-8 are present in human platelets and are required for granule secretion. Blood. 2002;100:1081–1083.


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