

# Platelet Physiology

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## Abstract

### Keywords

- ▶ platelet function
- ▶ platelet structure
- ▶ platelet granules
- ▶ platelet surface glycoproteins
- ▶ platelet activation pathways
- ▶ platelet physiology
- ▶ review

Platelets are the smallest blood cells, numbering 150 to 350 × 10<sup>9</sup>/L in healthy individuals. The ability of activated platelets to adhere to an injured vessel wall and form aggregates was first described in the 19th century. Besides their long-established roles in thrombosis and hemostasis, platelets are increasingly recognized as pivotal players in numerous other pathophysiological processes including inflammation and atherogenesis, antimicrobial host defense, and tumor growth and metastasis. Consequently, profound knowledge of platelet structure and function is becoming more important in research and in many fields of modern medicine. This review provides an overview of platelet physiology focusing particularly on the structure, granules, surface glycoproteins, and activation pathways of platelets.

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## Platelet Structure

Platelets have an average diameter of 2 to 5 μm, a thickness of 0.5 μm, and a mean cell volume of 6 to 10 fl.<sup>9</sup> For convenience, the structure of the platelet can be conceptually divided in a peripheral zone, a sol-gel zone, an organelle zone, and membrane systems.<sup>10</sup>

## Peripheral Zone

The platelet plasma membrane is relatively smooth and has a thicker glycocalyx (GP-polysaccharide covering) than other blood cells. In high-resolution electron microscopy, it shows a wrinkled appearance with many tiny folds and the randomly distributed apertures of the open canalicular system.<sup>10,11</sup> The glycocalyx as the platelet's exterior coat is a dynamic structure and the site of first contact with the surrounding milieu. It contains surface GPs required for the interaction of platelets with subendothelial structures of the injured vessel wall, platelet activation, platelet adhesion and aggregation, as well as clot retraction.<sup>12</sup> In particular, the mobile receptor complexes GPIb-IX-V and integrin αIIbβ3 are abundantly expressed on the surface of resting platelets and are of great importance in hemostasis (see below).<sup>13,14</sup>

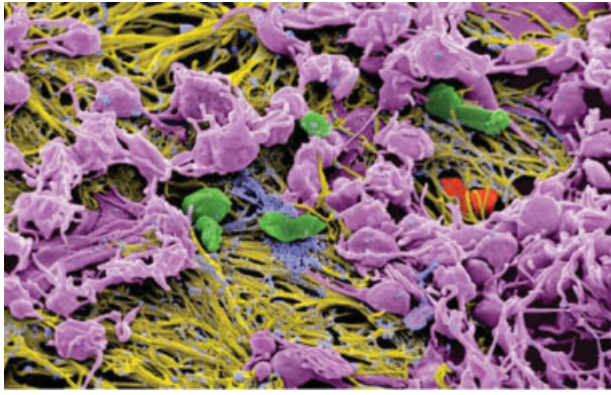
Below the glycocalyx is the lipid bilayer,<sup>15</sup> which is incompressible and unstretchable. Consequently, additional membrane needed for platelet spreading must be provided by the tiny folds of the platelet surface and the internalized membrane parts of the open canalicular system.<sup>16</sup> The lipid bilayer appears morphologically similar to the unit membranes of other cell types but plays an important role in blood coagulation. It contains tissue factor (TF), which is exposed on

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**Fig. 1** Platelets in thrombosis. Scanning electron micrograph of a portion of a human coronary artery thrombus, showing many activated platelets adhering to a bed of fibrin strands. Also visible are groups of microparticles and a few cholesterol crystals and erythrocytes. (Image by John W. Weisel and Chandrasekaran Nagaswami, Department of Cell and Developmental Biology, University of Pennsylvania School of Medicine, Philadelphia, PA. Reprinted with permission from Michelson.<sup>1</sup>)

the platelet surface in its inactive form along with negatively charged phosphatidylserine following platelet activation.<sup>17</sup> Subsequently, activated platelets release TF-bearing microparticles (MP) capable of binding coagulation factors Va, Vila, and Xa to their surface phosphatidylserine. Through the interaction of these coagulation factors with the meanwhile decrypted TF, thrombin generation is enhanced on the surface of activated platelets as well as on platelet-derived MP.<sup>10</sup>

The platelet's submembrane area lies directly under the lipid bilayer and is of great importance for platelet function. It contains a system of thin actin filaments—the membrane contractile cytoskeleton—which is required for platelet shape change and the translocation of receptors and particles over the platelet's surface.<sup>18</sup> In the submembrane compartment, the cytoplasmic domains of all transmembrane receptors interact with proteins, many of which are associated with calmodulin, myosin, and actin filaments that constitute the above-mentioned cytoskeleton.<sup>19</sup> Thereby, they regulate the signaling processes required for platelet activation.

### Sol-Gel Zone

The transparent yet viscous matrix inside platelets is labeled the sol-gel zone. It resembles liquid gel and contains organized microtubules and microfilaments, randomly distributed glycogen, a few smooth and clathrin-coated vesicles, as well as secretory organelles. Microtubules are arranged in circumferential coils close to the cell wall, thereby forming a system that supports the membrane contractile cytoskeleton.<sup>20–22</sup> Various experimental approaches strongly suggest that microtubules are needed for maintaining the discoid shape of human platelets.<sup>23,24</sup> Actin microfilaments in the sol-gel zone form the cytoplasmic actin filament cytoskeleton, the matrix in which all organelles are suspended and which keeps organelles apart from each other and from the cell wall in the resting platelet.<sup>10,25</sup> Following platelet activation, the cytoplasmic actin system constricts

the microtubule coils moving  $\alpha$ -granules and dense bodies to the platelet center,<sup>26</sup> which may ultimately result in the secretion of their contents through the open canalicular system.<sup>25,27</sup>

### Organelle Zone

Three major types of secretory organelles are present in platelets:  $\alpha$ -granules, dense granules, and lysosomes (►Table 1). In addition, platelets contain simple mitochondria, which are important for their energy metabolism, glycosomes,<sup>28</sup> electron dense chains and clusters,<sup>29</sup> and tubular inclusions.<sup>30</sup>

$\alpha$ -granules have a round to oval shape with a diameter of 200 to 500 nm. An average human platelet contains 50 to 80  $\alpha$ -granules, which makes them the most frequent organelles.<sup>31,32</sup> In resting platelets,  $\alpha$ -granules are separated from each other by the cytoplasmic actin filament cytoskeleton. The fusion of  $\alpha$ -granules during long-term platelet storage is a first sign of cell damage.<sup>33</sup> In vivo, fusion of  $\alpha$ -granules resulting in giant  $\alpha$ -granules is seen in patients with Paris-Trousseau-Jacobsen syndrome,<sup>34</sup> White platelet syndrome,<sup>35</sup> and Medich giant platelet disorder.<sup>30</sup> While the submembrane zone of  $\alpha$ -granules contains von Willebrand factor (VWF) in tube-like structures,<sup>36</sup> various proteins are found in their peripheral zone including megakaryocyte-synthesized proteins such as coagulation factor V, thrombospondin, P-selectin, and VWF, as well as externally synthesized proteins taken up by platelets (e.g. fibrinogen). The  $\alpha$ -granule's central zone appears denser than its peripheral zone potentially indicating the presence of proteins with binding sites for heavy metals.<sup>10</sup>

The three to eight dense granules per normal human platelet are smaller than  $\alpha$ -granules and display great morphological variability.<sup>37</sup> Their most prominent feature is an electron-opaque spherical structure, which is usually surrounded by an empty space. However, in some dense granules, this space is traversed by filaments or filled with a granule-like substance.<sup>10</sup> Besides adenine nucleotides such as adenosine triphosphate (ATP) and adenosine diphosphate (ADP), dense granules contain serotonin, pyrophosphate, calcium, and magnesium (see below).

Other electron-opaque structures in the cytoplasm are chains and clusters of hexagonal beads which are present in 2 to 22% of human platelets and seem to increase with age.<sup>29</sup> The origin and function of these electron-dense formations remains unknown. It was previously speculated that electron-dense chains and clusters represent precursors of dense granules, but this hypothesis was abandoned after studying patients with storage pool diseases, whose platelets lacked dense granules while containing the usual amount of chains and clusters.<sup>38</sup>

Human platelets also contain 0 to 2 spherical lysosomes, which are slightly smaller than  $\alpha$ -granules. Their content comprises at least 13 acid hydrolases, cathepsin D and E, lysosomal-associated membrane protein (LAMP)-2, and CD63, and can be released in response to strong platelet stimulation in vitro. However, the role of lysosomes in platelet function and hemostasis remains largely unknown.<sup>10</sup>

**Table 1** General features of platelet granule types

	Number/platelet	Diameter (nm)	Surface area ( $\mu\text{m}^2$ )/platelet	Common markers	General function
$\alpha$ -granules	50–80	200–500	14	VWF CXCL4 (PF4) P-selectin	Hemostasis/thrombosis Inflammation Angiogenesis Host defense Mitogenesis
Dense granules	3–8	150	< 1	CD63 Serotonin	Hemostasis/thrombosis Inflammation
Lysosomes	< 3	200–250	< 1	Acid phosphatase	Endosomal digestion

Abbreviations: CXCL4, chemokine (C–X–C motif) ligand 4; PF4, platelet factor 4; VWF, von Willebrand factor.

Source: Reprinted with permission from Flaumenhaft.<sup>51</sup>

The glycogen-containing platelet glycosome is another component of the platelet's organelle zone.<sup>28</sup> Glycosomes have a round or oval shape, and a similar size to  $\alpha$ -granules so that they can easily be confused with glycogen-bearing  $\alpha$ -granules. Tubular inclusions, which often contain glycogen as well, can be discriminated from glycosomes by their multi-lamellar membrane.

Finally, mitochondria are seen in the organelle zone. Despite their low number and simple structure, they provide the platelet's energy requirements, and make sure that a blockade of anaerobic glycolysis does not impair platelet function. Although mitochondria are also seen as important providers of calcium by some authors, other investigations favor the dense tubular system and extracellular calcium as the major calcium sources in platelet activation.<sup>39</sup>

### Membrane Systems

Besides the outer plasma membrane, the membrane systems in human platelets comprise Golgi complexes, the surface-connected open canalicular system, the dense tubular system, and the rough endoplasmic reticulum.

Residues of megakaryocytic Golgi complexes are observed in less than 1% of normal human platelets but can be found more frequently in patients with certain hypogranular platelet disorders.<sup>35,40</sup> The presence of Golgi complexes in platelets from patients with hypogranular syndromes such as White platelet syndrome may indicate ongoing granulopoiesis.<sup>35</sup>

The open canalicular system is a part of the platelet's surface membrane, which extends toward the interior of the platelet and in doing so forms a tubular structure,<sup>41–43</sup> which exerts three major functions. Its channels can be used for the transport of plasma components such as fibrinogen to  $\alpha$ -granules<sup>44–46</sup> and can also serve as route for the release of granular contents during platelet activation.<sup>27</sup> Moreover, the channels of the open canalicular system can be evaginated and thereby provide membrane parts needed for platelet spreading following platelet adhesion to an injured vessel wall.<sup>47</sup> Through this mechanism, activated platelets are able to increase their surface area more than fourfold compared with resting discoid platelets.<sup>48</sup>

The dense tubular system is a residuum of the parent megakaryocyte's smooth endoplasmic reticulum and consists

of channels randomly dispersed in the platelet cytoplasm. The channels are separated from the canaliculi of the open canalicular system which appear empty by electron microscopy and—in contrast to them—contain an amorphous substance resembling the surrounding cytoplasm in opacity.<sup>41</sup>

Channels of rough endoplasmic reticulum are only seen in patients with fast platelet turnover due to immune thrombocytopenia and are then usually studded with ribosomes.<sup>10</sup>

## Platelet Granules

Platelet granules were first described in the late 19th century, but it took until 1966 to differentiate dense granules from  $\alpha$ -granules,<sup>49</sup> and another year to distinguish the latter from lysosomes by the then newly developed method of electron microscopy (–Table 1).<sup>50</sup>

### Formation of Platelet Granules

The formation of platelet granules is initiated in the megakaryocyte, but their maturation continues in the circulating platelet.<sup>51</sup>

### $\alpha$ -Granules

The proteins stored in  $\alpha$ -granules are provided by synthesis and endocytosis. While synthesized proteins are transported from the endoplasmic reticulum to the trans-Golgi network, where they are packaged in immature granules,<sup>52,53</sup> plasma proteins are taken up by megakaryocytes via the endocytotic pathway and uptake of plasma proteins via this pathway continues in the mature platelet.<sup>51</sup> Membrane trafficking required for both pathways is mediated by coat proteins such as clathrin, adaptor proteins (AP)-1, AP-2, AP-3, and other vesicle trafficking proteins, for example, soluble N-ethylmaleimide sensitive fusion protein (NSF) attachment protein receptors (SNAREs) and monomeric GTPases such as Rabs. Thus, for both pathways, clathrin-coated vesicles are formed through membrane invagination under the influence of AP.<sup>51</sup> However, differences exist: AP-1 appears to play a pivotal role in the synthetic pathway,<sup>31</sup> whereas AP-2 mediates endocytosis.<sup>54</sup> The resulting vesicles from the trans-Golgi network or the plasma membrane are then moved to multivesicular bodies, which represent transient structures at an intermediate stage of

granule production in megakaryocytes.<sup>55</sup> Multivesicular bodies are involved in  $\alpha$ -granule and dense granule sorting,<sup>55,56</sup> and kinetic studies have shown that endocytosed proteins are transported from endosomes to immature multivesicular bodies to mature multivesicular bodies to  $\alpha$ -granules.<sup>51</sup> The latter contain small vesicles called exosomes,<sup>55</sup> which in part persist in mature  $\alpha$ -granules and can be released following platelet activation.<sup>57</sup> Megakaryocyte-derived granules are transferred to the nascent platelets on microtubule tracks during proplatelet formation.<sup>51</sup>

### Dense Granules

Dense granules are lysosome-related organelles, which means that they originate from the endosomal system rather than from the trans-Golgi network.<sup>58</sup> In the endosomal compartment, biogenesis of lysosome-related organelle complexes (BLOCs) are involved in vesicle trafficking required for dense granule formation.<sup>51</sup> Besides BLOCs-1, -2, and -3,<sup>59-62</sup> AP-3 plays an important role in dense granule formation, and like defects in BLOC-2 or -3,<sup>63</sup> certain mutations in the AP-3 gene are associated with dense granule deficiency in Hermansky-Pudlak syndrome. During megakaryopoiesis, dense granules appear concomitantly with  $\alpha$ -granules, and like  $\alpha$ -granules, early dense granules are also sorted in multivesicular bodies.<sup>56</sup> Their content becomes denser as they mature, most likely due to increased membrane pump activity.<sup>51</sup>

### Granule Content

#### $\alpha$ -Granules

$\alpha$ -granules contain membrane-associated and soluble proteins, which are involved in various processes including

cell adhesion, coagulation, inflammation, cell growth, and host defense (► **Table 2**). Following platelet activation, membrane-bound granule proteins are expressed on the platelet surface, whereas soluble granule proteins are released into the extracellular compartment. Most of the membrane-bound proteins are already present on the surface of resting platelets,<sup>37</sup> for example, integrins such as  $\alpha$ IIb $\beta$ 3, immunoglobulin family receptors such as GPVI, Fc receptors (FcR), platelet endothelial cell adhesion molecule, the GPIb-IX-V complex, tetraspanins, CD36, and Glut-3.<sup>64,65</sup> However, some membrane-associated proteins including fibrocytin L, CD109, and P-selectin are exclusively expressed on the surface of activated, rather than resting, platelets.<sup>64</sup> In particular, platelet surface P-selectin expression is therefore widely used as a sensitive flow cytometric marker of platelet activation (► **Fig. 2**).<sup>66-68</sup>

Proteins in platelet releasate can derive from different platelet granules, exosomes, and from cleavage of initially surface-bound proteins. However, proteomic analyses have identified more than 300 soluble proteins released by  $\alpha$ -granules.<sup>64,69</sup> Many of the released proteins are also found in human plasma, prompting questions as to how the  $\alpha$ -granule constituents differ from their plasma counterparts in structure or function.

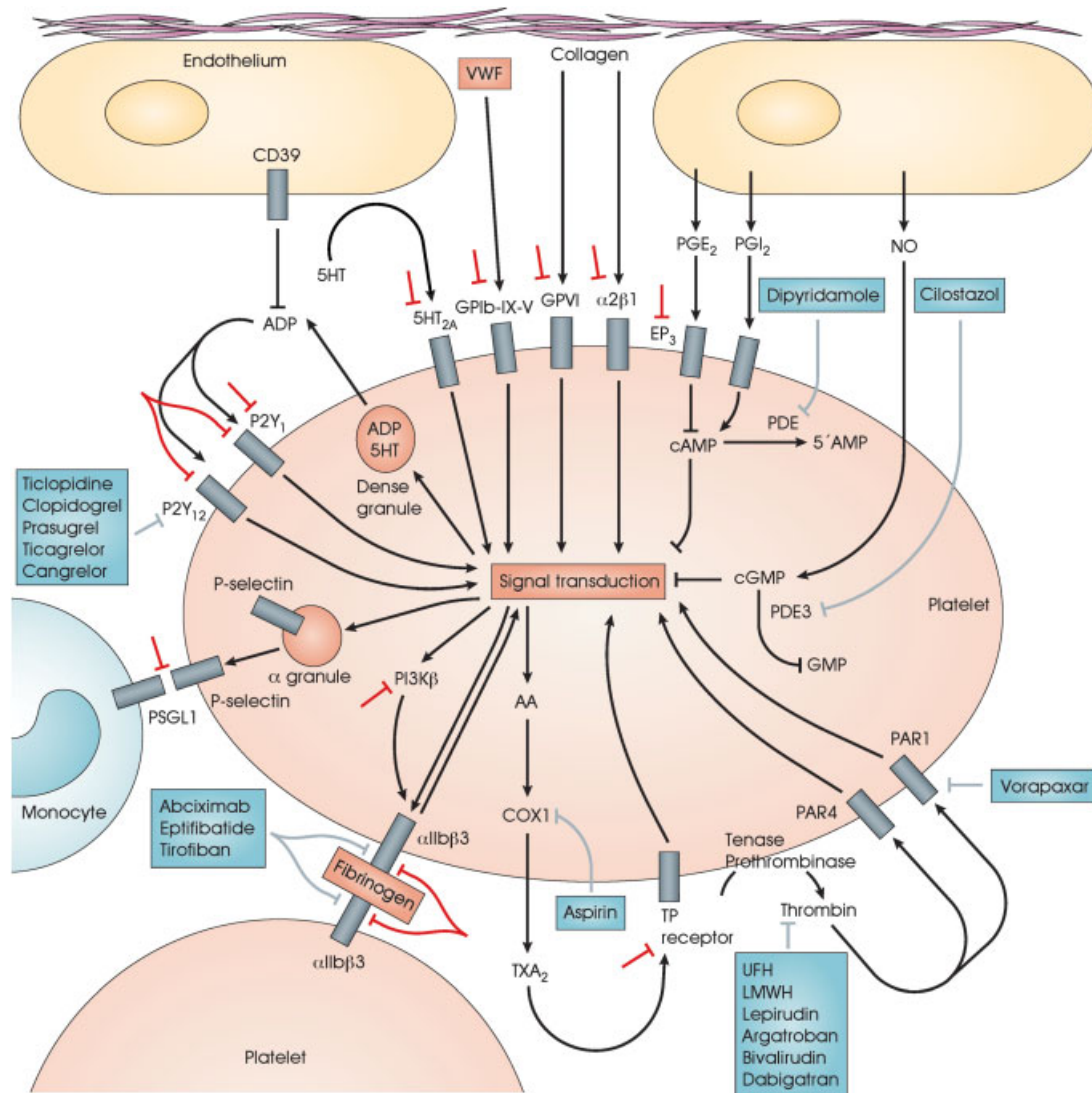
#### Dense Granules

Platelet dense granules contain high concentrations of adenine nucleotides, namely, ADP and ATP,<sup>70</sup> uracil and guanine nucleotides, calcium, and potassium (► **Table 3**). Moreover, polyphosphates and bioactive amines such as serotonin and histamine are present in platelet dense granules.<sup>71,72</sup> The milieu within platelet dense granules is kept at a pH of approximately 5.4 by a H<sup>+</sup>-ATPase

**Table 2**  $\alpha$ -granule contents

Type	Examples
Integral membrane proteins	$\alpha$ IIb $\beta$ 3, GPIb-IX-V, GPVI, P-selectin
Coagulants, anticoagulants, and fibrinolytic proteins	Factors V, IX, XIII, antithrombin, protein S, tissue factor pathway inhibitor, plasminogen, $\alpha$ <sub>2</sub> -macroglobulin
Adhesion proteins	Fibrinogen, von Willebrand factor, thrombospondin
Chemokines	CXCL1 (GRO- $\alpha$ ), CXCL4 (PF4), CXCL5 (ENA-78), CXCL8 (IL8), CCL2 (MCP-1), CCL3 (MIP-1 $\alpha$ ), CCL5 (RANTES)
Growth factors	Epidermal growth factor, hepatocyte growth factor, insulin-like growth factor, transforming growth factor $\beta$
Angiogenic factors and inhibitors	Vascular endothelium growth factor, fibroblast growth factor, platelet-derived growth factor, angiostatin, endostatin
Microbicidal proteins	Thymosin- $\beta$ 4, thrombocidins1 and 2
Immune mediators	Complement C3 precursor, complement C4 precursor, IgG

Abbreviations: CCL, chemokine (C-C motif) ligand; CXCL, chemokine (C-X-C motif) ligand; ENA-78, epithelial-derived neutrophil-activating peptide 78; GP, glycoprotein; GRO- $\alpha$ , growth-regulated oncogene  $\alpha$ ; IgG, immunoglobulin G; IL8, interleukin 8; MCP-1, monocyte chemoattractant protein 1; MIP-1 $\alpha$ , macrophage inflammatory protein 1 $\alpha$ ; PF4, platelet factor 4; RANTES, regulated on activation normal T cell expressed and secreted. Source: Reprinted with permission from Flaumenhaft.<sup>51</sup>



**Fig. 2** Platelet function and molecular targets of antiplatelet agents. Initial platelet adhesion to damaged vessel walls is mediated by the binding of exposed collagen to platelet surface GPIIb/IIIa and integrin  $\alpha 2\beta 1$  and by the binding of VWF to the platelet surface GPIb-IX-V complex. This complex is also a receptor for other platelet ligands (thrombospondin, collagen, and P-selectin), leukocyte integrin  $\alpha \text{M}\beta 2$ , and procoagulant factors (thrombin, kininogen, factor XI, and factor XII). Thrombin, generated by the coagulation cascade, is a potent activator of human platelets through two platelet surface receptors: PAR-1 and PAR-4. Three groups of platelet surface receptors provide important positive feedback loops for platelet activation: P2Y<sub>1</sub> and P2Y<sub>12</sub> are stimulated by ADP released from platelet dense granules; 5HT<sub>2A</sub> receptors (5HT<sub>2A</sub>) are stimulated by 5HT (also known as serotonin) released from platelet dense granules; and the thromboxane prostanoid (TP) receptor is stimulated by TXA<sub>2</sub> generated by the platelet COX1-dependent signaling pathway. Platelet-to-platelet aggregation is mediated by fibrinogen and, at high shear flow, by VWF binding to activated integrin  $\alpha \text{IIb}\beta 3$ . Perpetuation of platelet-to-platelet aggregation is augmented by other receptors, including JAMA and JAMC, growth-arrest specific gene 6 receptor and ephrin. Platelet-monocyte adhesion is initially mediated by the binding of platelet surface P-selectin to its constitutively expressed cognate receptor, PSGL1, on the monocyte surface. Activated platelets, monocytes, and microparticles bind coagulation factors and provide a surface for the generation of a fibrin clot. Approved antiplatelet agents and their molecular targets are shown in boxes. Indirect inhibitors (UFH, LMWH) and direct inhibitors (lepirudin, argatroban, bivalirudin, and dabigatran) of thrombin, unlike PAR-1 antagonists, are anticoagulants rather than specific antiplatelet drugs. However, their inhibition of thrombin results in reduced platelet activation. Investigational strategies for novel antiplatelet agents are shown by the symbols adjacent to: GPIb-IX-V, GPIIb/IIIa,  $\alpha 2\beta 1$ , EP<sub>3</sub>, 5HT<sub>2A</sub>, P2Y<sub>1</sub>, P2Y<sub>12</sub>, PSGL1, PI3K $\beta$ ,  $\alpha \text{IIb}\beta 3$ , and the TP receptor. AA, arachidonic acid; COX1, cyclooxygenase 1; EP<sub>3</sub>, prostaglandin E<sub>2</sub> receptor EP<sub>3</sub> subtype; GP, glycoprotein; JAMA, junctional adhesion molecule A; JAMC, junctional adhesion molecule C; LMWH, low-molecular-weight heparin; NO, nitric oxide; PAR, protease-activated receptor; PDE, phosphodiesterase; PG, prostaglandin; PI3K $\beta$ , phosphoinositide 3-kinase  $\beta$ -isoform; PSGL1, P-selectin glycoprotein ligand-1; TXA<sub>2</sub>, thromboxane A<sub>2</sub>; UFH, unfractionated heparin; VWF, von Willebrand factor; 5HT, 5-hydroxytryptamine. (Modified with permission from Michelson.<sup>68</sup>)

proton pump.<sup>73</sup> Furthermore, the multidrug resistance protein 4 has been described on platelet dense granules and is considered responsible for the uptake of adenine nucleotides,<sup>74</sup> whereas serotonin is trafficked from

platelet cytoplasm into dense granules by the vesicular monoamine transporter 2. The latter may also concentrate histamine into platelet dense granules.<sup>75</sup> GPIb, integrin  $\alpha \text{IIb}\beta 3$ , CD63 (granulophysin), and LAMP-2 are among the

**Table 3** Dense granule contents

Type	Examples
Cations	Ca <sup>2+</sup> , Mg <sup>2+</sup> , K <sup>+</sup>
Phosphates	Polyphosphate, pyrophosphate
Bioactive amines	Serotonin, histamine
Nucleotides	ADP, ATP, UTP, GTP

Abbreviations: ADP, adenosine diphosphate; ATP, adenosine triphosphate; GTP, guanosine triphosphate; UTP, uridine triphosphate.

Source: Reprinted with permission from Flaumenhaft.<sup>51</sup>

membrane-associated proteins found in platelet dense granules.<sup>76</sup>

### Lysosomes

Platelet lysosomes bear protein degrading enzymes such as cathepsins, elastase, and collagenase; carbohydrate degrading enzymes such as glucosidase and galactosidase; and acid phosphatase as phosphate ester cleaving enzyme (→ **Table 4**). LAMP-1, LAMP-2, and CD63 are found in the lysosomal membrane in a highly glycosylated state and support its protective function.<sup>51</sup>

## Granule Secretion

### Mechanisms of Platelet Granule Secretion

Membrane fusion plays a key role in platelet granule secretion. Following platelet activation, platelet granules accumulate in the cell center during platelet shape change, and may fuse with one another in homotypic fusion.<sup>77</sup> In a further step, granules fuse with the open canalicular system releasing their contents into its channels and thereby finally to the extracellular space.<sup>45,78</sup> Another mechanism of granule release is the direct fusion of platelet granules with the plasma membrane.<sup>79</sup> Soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs) on platelet granules, namely, vesicular SNAREs (vSNAREs) and so-called target SNAREs (tSNAREs), associated with the plasma membrane and the open canalicular system mediate the fusion of platelet granules with one another, the open canalicular system and the plasma membrane.<sup>80,81</sup> Vesicle-associated membrane protein (VAMP)-8 is considered the most important vSNARE for platelet granule release, whereas VAMPs-

**Table 4** Lysosomal contents

Type	Examples
Protein degrading enzymes	Cathepsins, elastase, collagenase, carboxypeptidase
Carbohydrate degrading enzymes	Glucosidase, galactosidase, mannosidase
Phosphate ester cleaving enzymes	Acid phosphatase

Source: Reprinted with permission from Flaumenhaft.<sup>51</sup>

2 and -3 may play minor roles.<sup>80,82,83</sup> Syntaxins 2, 4, 7, 11, and 12 and SNAP-23, -25, and -29 have been described as tSNAREs.<sup>82,84–86</sup> The function of SNAREs in platelet granule secretion is regulated by chaperone proteins such as the Mg<sup>2+</sup>-dependent ATPase NSF.<sup>87</sup> NSF disassembles membrane-associated SNARE complexes thereby enabling their interaction with cognate SNAREs on opposing membranes. Its important role in the platelet release reaction is exemplified by studies showing that inhibitory peptides and antibodies to NSF impair platelet  $\alpha$ -granule release.<sup>87,88</sup> Other important players in platelet granule release are Sec1/Munc proteins and Rab proteins, which can influence the function of SNAREs.<sup>89–94</sup>

In addition, the membrane's lipid composition affects its ability to fuse.<sup>95</sup> The platelet cytoskeleton is also involved in granule secretion. Although actin polymerization seems to inhibit  $\alpha$ -granule and dense granule release in the resting platelet,<sup>96</sup> it facilitates granule secretion during platelet activation.<sup>97</sup>

Furthermore, actomyosin contraction may foster granule secretion.<sup>98–100</sup> Microtubules are considered a minor player in platelet granule release since mice with microtubule deficiencies exhibit only a modest impairment of granule secretion.<sup>101,102</sup>

Similar to other cells, the increase of intracellular Ca<sup>++</sup> supports granule secretion in platelets.<sup>103</sup> Finally, several protein C kinase isoforms take part in the platelet release reaction. In particular, protein C kinase isoforms  $\alpha$  and  $\beta$  support granule secretion,<sup>104</sup> while others differentially affect granule release.<sup>104–106</sup>

### Functions of Platelet Granule Secretion

Platelet granule secretion is involved in hemostasis and thrombosis, inflammation, atherogenesis, antimicrobial host defense, and mitogenesis.<sup>51</sup>

Upon platelet activation,  $\alpha$ -granules release fibrinogen and VWF, which promote platelet-platelet and platelet-endothelial cell interactions. Furthermore, the fibrinogen receptor  $\alpha$ Ib $\beta$ 3, the collagen receptor GPVI, and components of the VWF receptor complex GPIb-IX-V, which are found in  $\alpha$ -granules, are expressed on the platelet surface and subsequently support platelet adhesion.<sup>37,107</sup> By releasing coagulation factors such as factors V and IX,<sup>108</sup>  $\alpha$ -granules also participate in secondary hemostasis. Finally,  $\alpha$ -granules may be involved in the maintenance of hemostatic balance by secreting proteins that limit coagulation including antithrombin, protein S, and TF pathway inhibitor.<sup>109,110</sup> The contribution of  $\alpha$ -granules to normal hemostasis is evidenced by the bleeding tendency in patients with gray platelet syndrome.<sup>111</sup> Based on their content, an involvement of  $\alpha$ -granules in thrombosis is expected,<sup>64,69</sup> although their exact role remains to be determined.

Dense granules participate in hemostasis and thrombosis as the primary source of ADP, which acts as a strong platelet agonist at sites of vascular injury. Moreover, the secretion of serotonin by dense granules supports platelet aggregation and promotes vascular tone,<sup>112</sup> while released Ca<sup>++</sup> and polyphosphates contribute to clot formation.<sup>51</sup> On the contrary, some of the released diadenosine polyphosphates are

partial antagonists of the ADP receptors and may be involved in limiting platelet activation once it has begun.<sup>113,114</sup> The importance of dense granules in normal hemostasis is exemplified by the bleeding diathesis in patients with Hermansky-Pudlak syndrome or Chediak-Higashi syndrome, whereas their involvement in thrombus formation has been proven by *in vitro* and *in vivo* experiments.<sup>115,116</sup>

Both  $\alpha$ -granules and dense granules are involved in inflammatory processes.  $\alpha$ -granules provide platelet surface receptors enabling the interaction with leukocytes and endothelial cells,<sup>117,118</sup> thereby leading to mutual activation, cell recruitment, and propagation of their inflammatory phenotype.<sup>119</sup> Furthermore,  $\alpha$ -granules release numerous proinflammatory and immune-modulating factors fostering recruitment and activation of inflammatory cells, chemokine secretion, as well as cell differentiation.<sup>120–123</sup> The role of  $\alpha$ -granules in atherosclerosis is mostly attributable to their proinflammatory actions.<sup>124</sup>

Dense granules can secrete polyphosphates and thus initiate the generation of bradykinin,<sup>112</sup> which supports vascular permeability and edema *in vivo*.<sup>125</sup> An involvement of dense granules in atherogenesis has been shown in mice with dense granule deficiency.<sup>126</sup>

$\alpha$ -granules participate in host defense by providing various antimicrobial proteins, for example, chemokine (C-X-C motif) ligand 4 (CXCL4), derivatives of CXCL7, CCL5 (RANTES), and thymosin- $\beta$ 4,<sup>127</sup> as well as complement and complement-binding proteins.<sup>64</sup>

Proangiogenic proteins such as vascular endothelial growth factor, platelet-derived growth factor, fibroblast growth factor, epidermal and insulin-like growth factor,<sup>128</sup> as well as inhibitors of angiogenesis including thrombospondin-1, CXCL4, angiostatin, and endostatin have been identified in  $\alpha$ -granules.<sup>129,130</sup> Recent studies suggest that pro- and anti-angiogenic factors are released agonist specifically.<sup>130–132</sup> Besides angiogenesis,  $\alpha$ -granule secretion may play a role in tumor growth and stability,<sup>133</sup> metastasis,<sup>134,135</sup> and wound healing.<sup>136,137</sup>

## Platelet Surface Glycoproteins

Although there are many types of platelet surface GPs,<sup>138</sup> the GPIb-IX-V complex, GPVI, and integrin  $\alpha$ IIb $\beta$ 3 (also known as GPIIb/IIIa) are considered the most important platelet surface GPs mediating platelet adhesion, activation, and aggregation, respectively, and their structures and functions will therefore be described in more detail below.

### GPIb-IX-V Complex

The GPIb-IX-V complex acts as platelet surface receptor and is heavily involved in normal hemostasis as well as in arterial thrombosis.<sup>139,140</sup>

#### Structure

Human GPIb $\alpha$  is a type-I, membrane-spanning GP with an N-terminal, ligand-binding domain, a sialomucin core, a transmembrane region, and a cytoplasmic tail.<sup>141</sup> Its major ligand-binding domain comprises seven tandem leucine-rich

repeats, an N-terminal capping sequence, a C-terminal flanking sequence, and an anionic sequence.<sup>139,142–144</sup> GPIb $\alpha$  and GPIX are present at approximately 25,000 copies per platelet, whereas GPV is present at approximately 12,500 copies.<sup>145</sup>

#### Function

GPIb-IX-V propagates the adhesion of activated platelets to endothelial cells and subendothelial structures of the injured vessel wall, mainly by binding its most important ligand VWF, which is itself able to bind collagen (–Fig. 2).<sup>146</sup> Another ligand for GPIb-IX-V is thrombospondin, which seems to mediate platelet adhesion at high shear rates in the absence of VWF.<sup>147</sup> It has been shown that GPIb $\alpha$  can also bind P-selectin, thereby offering another mechanism of platelet-endothelial cell and platelet-platelet interactions.<sup>148</sup> Furthermore,  $\alpha$ M $\beta$ 2 (Mac-1) serves as a counter receptor for GPIb-IX-V, enabling the attachment of platelets to leukocytes.<sup>149</sup>

Besides its role in platelet adhesion, GPIb-IX-V assembles procoagulant activity on activated platelets by providing binding sites for  $\alpha$ -thrombin, factor XI, and high-molecular-weight kininogen.<sup>142,150,151</sup> On the contrary, the binding of factor XII to GPIb $\alpha$  competes with kininogen binding and inhibits thrombin-dependent platelet aggregation associated with thrombin binding to GPIb $\alpha$ .<sup>152</sup>

Finally, complex signaling processes are initiated by cross-linking of GPIb-IX-V by VWF or other multivalent ligands, ultimately resulting in the activation of  $\alpha$ IIb $\beta$ 3 and ectodomain shedding of GPIb $\alpha$ .<sup>140,141,153–155</sup>

### Glycoprotein VI

GPVI is the major signaling receptor for collagen on human platelets, and exerts functions in hemostasis and other platelet-mediated processes.<sup>156–159</sup>

#### Structure

GPVI belongs to the immunoglobulin superfamily of receptors. It consists of 319 amino acids and is present at approximately 3,700 copies per platelet.<sup>145,160</sup> GPVI comprises two extracellular immunoglobulin domains, D1 and D2, which are connected by a peptide strand and linked to the transmembrane domain via a glycosylated stem.<sup>160</sup> The cytoplasmic domain of human GPVI consists of 51 amino acids and shows an amino acid-rich area near the transmembrane region and a proline-rich area.<sup>160–162</sup> GPVI exists as a complex with the FcR  $\gamma$ -chain, which is expressed on platelets in monomeric and dimeric forms.<sup>163,164</sup> The monomeric form is particularly present on unactivated platelets and its affinity for collagen is too low to allow activation in response to physiological concentrations of collagen.<sup>165,166</sup> In contrast, the dimeric form has an increased affinity for collagen and binding of collagen to the dimeric complex may result in intracellular signals leading to the generation of further dimers. Without the FcR  $\gamma$ -chain, GPVI does not reach the platelet surface and collagen-induced platelet activation is not initiated.<sup>167</sup>

#### Function

Platelets adhere to exposed collagen fibers by binding of immobilized VWF to GPIb-IX-V.<sup>160</sup> This allows binding of collagen to

low-affinity GPVI and results in intracellular signals with subsequent inside-out activation of integrins including  $\alpha 2\beta 1$  and  $\alpha \text{IIb}\beta 3$  as well as further clustering of GPVI (**► Fig. 2**). Thereby, GPVI activation is reinforced, and stable platelet adhesion and spreading are promoted through binding of  $\alpha 2\beta 1$  and  $\alpha \text{IIb}\beta 3$  to collagen and VWF, respectively.<sup>168,169</sup>

Following activation by collagen and other agonists, GPVI is rapidly shed from platelet surface, most likely to prevent excessive collagen-stimulated megakaryocyte and platelet activation in the bone marrow and after minor damages to the vasculature.<sup>160</sup>

Inherited defects in GPVI in two patients were only associated with a mild bleeding syndrome suggesting that hemostasis may not be the primary role of the GPVI receptor complex.<sup>160,170,171</sup> GPVI may also be involved in processes beyond hemostasis,<sup>172</sup> for example, in the pathogenesis of rheumatoid arthritis and the development of the cardiovascular system.<sup>173,174</sup>

### Integrin $\alpha \text{IIb}\beta 3$

Platelet surface integrin  $\alpha \text{IIb}\beta 3$  (previously termed GPIIb/IIIa) is transformed from its resting low-affinity state to a high-affinity receptor as the final step of platelet activation and subsequently mediates platelet aggregation at a molecular level (**► Fig. 2**).<sup>175,176</sup>

#### Structure

$\alpha \text{IIb}\beta 3$  belongs to the integrin family of cell adhesion molecules<sup>177-179</sup> and is found on platelets, megakaryocytes, mast cells, basophils, and some tumor cells.<sup>180-185</sup> With 80,000 to 100,000 copies per platelet,<sup>186,187</sup> it constitutes the major integral plasma membrane protein on human platelets accounting for 17% of the platelet membrane protein mass.<sup>188</sup> Moreover,  $\alpha \text{IIb}\beta 3$  is present in platelet  $\alpha$ -granule membranes and can become expressed following platelet activation.<sup>189,190</sup>

$\alpha \text{IIb}\beta 3$  is a heterodimer consisting of an  $\alpha \text{IIb}$  and  $\beta 3$  subunit, both synthesized as single glycosylated polypeptide chains.<sup>191</sup>  $\alpha \text{IIb}$  consists of 1,008 amino acids,<sup>192</sup> whereas  $\beta 3$  is composed of 762 amino acids.<sup>193,194</sup> Both subunits comprise a large extracellular domain, a transmembrane segment, and a short cytoplasmic tail,<sup>193-196</sup> and are arranged on the platelet surface in a type-1 orientation with the N-terminus residing in the extracellular region and the C-terminus within the cytosol.<sup>197</sup>

#### Function

Agonist-induced platelet activation triggers intracellular signaling events that converge at the cytoplasmic tails of  $\alpha \text{IIb}\beta 3$  and are then transmitted across the platelet membrane via inside-out signaling ultimately resulting in the transformation of the extracellular domain of  $\alpha \text{IIb}\beta 3$  into a high-affinity receptor for fibrinogen and VWF.<sup>176,197,198</sup> By binding divalent fibrinogen or multivalent VWF,<sup>176,198</sup> activated  $\alpha \text{IIb}\beta 3$  enables platelet-platelet interactions and consequently the formation of platelet aggregates. Moreover, by binding vitronectin, fibronectin, or thrombospondin-1,<sup>199-201</sup> activated  $\alpha \text{IIb}\beta 3$  may also mediate platelet

adhesion to subendothelial structures and regulate platelet aggregation.<sup>197</sup>

The role of activated  $\alpha \text{IIb}\beta 3$  in platelet aggregation makes it a prime target for antithrombotic therapy (**► Fig. 2**). Indeed, antibodies, peptides, and nonpeptides binding to  $\alpha \text{IIb}\beta 3$  have been shown to effectively block  $\alpha \text{IIb}\beta 3$ -mediated platelet-platelet bridging, and three GPIIb/IIIa receptor antagonists are currently approved to prevent and treat detrimental platelet aggregation in patients undergoing percutaneous coronary interventions.<sup>202,203</sup>

## Platelet Activation Pathways

Human platelets can be activated by numerous agonists via different pathways.<sup>204</sup> Besides the above-discussed processes of VWF- and collagen-induced platelet activation, in particular, thrombin and ADP play major roles in human platelet activation (**► Fig. 2**).

### Thrombin

The serine protease thrombin is the most potent platelet agonist and activates platelets via protease-activated receptors (PARs) and GPIb-IX-V.<sup>205-209</sup> The four PARs belong to the superfamily of G-protein-coupled receptors with seven transmembrane-spanning  $\alpha$ -helices, four extracellular loops and domains, and four intracellular loops and domains.<sup>210</sup> PAR-1 and PAR-4 mediate most of the platelet response to thrombin on human platelets,<sup>205,211</sup> whereas PAR-2 is not expressed on platelets and PAR-3 functions only as a cofactor for thrombin activation of PAR-4.<sup>212</sup> While PAR-1 is sensitive to low levels of thrombin, PAR-4 triggers platelet activation and aggregation only at high thrombin concentrations, and cleavage of PAR-4 by thrombin occurs 20- to 70-fold slower than cleavage of PAR-1.<sup>213</sup> Moreover, anti-PAR-1 blocking antibodies and PAR-1 antagonists blocked the activation of platelets by low concentrations of thrombin, whereas anti-PAR-4 blocking antibodies did not affect thrombin-inducibile platelet activation.<sup>211</sup> Therefore, PAR-1 is the most important receptor for the activation of human platelets by thrombin. In 2014, the first PAR-1 receptor antagonist (**► Fig. 2**) was approved for clinical use in patients with a history of myocardial infarction or peripheral arterial disease to prevent thrombotic cardiovascular events based on the results of two large clinical trials.<sup>214,215</sup>

### Adenosine Diphosphate

ADP is one of the major components of the releasate from activated platelets, and its critical role in the process of platelet activation and aggregation was recognized more than 50 years ago.<sup>216</sup> It acts as an agonist at two platelet purinergic G-protein coupled receptors—the Gq-coupled P2Y<sub>1</sub> and the Gi-coupled P2Y<sub>12</sub> receptor. Like other P2Y receptors, P2Y<sub>1</sub> and P2Y<sub>12</sub> are seven-membrane-spanning proteins with a carboxyl terminal domain on the cytoplasmic side and an amino terminal domain being exposed to the extracellular environment.<sup>217</sup> P2Y<sub>1</sub> activation initiates ADP-induced platelet aggregation and is responsible for platelet shape change.<sup>218</sup> However, without



P2Y<sub>12</sub> activation, the result is a small and reversible platelet aggregation. P2Y<sub>12</sub> stimulation results in amplification and stabilization of the aggregation response. There is a complex interplay between P2Y<sub>1</sub> and P2Y<sub>12</sub>,<sup>219</sup> and coactivation of both is necessary for full platelet aggregation.<sup>220</sup> Due to its prominent role in platelet aggregation, the P2Y<sub>12</sub> receptor has become a major target of antiplatelet therapy (►Fig. 2),<sup>68,221,222</sup> and the prescription of a P2Y<sub>12</sub> receptor antagonist in addition to aspirin is the current standard of care in patients with acute coronary syndromes and in those undergoing percutaneous cardiovascular interventions with stent implantation.<sup>223–225</sup> In contrast, no antagonists of the P2Y<sub>1</sub> receptor have been approved for clinical use.

## Conclusion

New imaging techniques as well as in vitro and in vivo studies have resulted in a comprehensive view of platelet structure, secretion, adhesion, and activation, thereby providing the foundation of today's understanding of the role of platelets in health and disease. Nevertheless, there still remain numerous knowledge gaps with regard to platelet physiology and pathophysiology, which offer promising targets for further investigations. Most important, evolving knowledge on platelets needs to be integrated in future research efforts with the ultimate goal of improving patient care.

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