CHAPTER 5

Platelet Glycoprotein VI
Stephanie M. Jung and Masaaki Moroi*

Abstract

Glycoprotein VI (GPVI) is a membrane glycoprotein unique to platelets and has been identified as a physiological receptor for collagen. Damage to a vessel wall exposes the subendothelial component collagen to platelets in the blood flow. Interaction of platelets with collagen via the receptor GPVI results in platelet activation and adhesion—the processes that are essential for thrombus formation. On the platelet surface, GPVI is present as a complex with the homodimeric Fc receptor γ-chain (FcRγ with a possible stoichiometry of two GPVI molecules and one FcRγ dimer). When collagen binds to GPVI, a platelet activation cascade is initiated by tyrosine phosphorylation of the immunoreceptor tyrosine-based activation motif of FcRγ and this phosphorylation induces the formation of a large complex composed from many signal-transducing proteins. In flow adhesion experiments that closely approximate physiological conditions, GPVI is essential for the formation of large platelet aggregates on collagen. However, GPVI-deficient patients or mice do not show any severe bleeding tendency. This suggests that a GPVI inhibitor would be able to inhibit thrombus formation but still not cause a significant bleeding tendency. Such an inhibitor would show promise as an anti-thrombotic agent for clinical use.

Introduction

Platelet glycoprotein VI (GPVI) is a platelet-specific collagen receptor with a molecular weight of about 62 kDa. Structural model for the interaction of GPVI with collagen based on the crystal structure of GPVI has been recently suggested.1 This receptor was first identified as a protein deficient in patients' platelets that did not aggregate in response to the physiologically important agonist collagen.2,6 The GPVI-deficient platelets, however, aggregated normally when induced by other agonists, including thrombin, ADP and ristocetin. These observations suggested that GPVI acts in a critical step specifically related to the reaction with collagen. Although the initial findings on GPVI were tantalizing because of its possible role in thrombotic processes, for the first five years or so research progressed slowly because the only means to identify this glycoprotein was to use the autoantibody from one patient. In addition, platelets also contain another, well-established collagen receptor, integrin α2β1 (α2β1). By using functional studies, such as aggregometry, to assess collagen responsiveness, it is difficult to differentiate the effects of GPVI from those of α2β1. However, the contribution of GPVI could be differentiated from that of α2β1 by using of an autoantibody against GPVI or GPVI-deficient patients' platelets, indicating that GPVI is a platelet collagen-specific receptor involved in platelet activation.7,9 In 1995-97, research on GPVI was greatly accelerated by the discovery of GPVI-specific agonists. Searching for the specific structure of collagen that induces platelet activation, Dr. Barnes' group at Cambridge found that a triple helical collagen-mimetic peptide containing 10 repeats of the Gly-Pro-Hyp sequence induces platelet

*Corresponding Author: Masaaki Moroi—Department of Protein Biochemistry, Institute of Life Science, Kurume University, 1-1 Hyakunen Koen, Kurume, Fukuoka, 839-0864, Japan. Email: mmoroi@lsi.kurume-u.ac.jp

aggregation independently of $\alpha_\beta_1$, which the authors called collagen-related peptide (CRP). CRP strongly activates platelets, particularly when it is cross-linked. The CRP-induced activation is inhibited by the Fab fragment of an antibody against GPVI, verifying that CRP activates platelets by specifically interacting with GPVI. The tropical rattlesnake *Crotalus durissus terrificus* was found to have a platelet activating venom protein, Convulxin (Cvx), that is a specific agonist of GPVI. Cvx is a C-type lectin composed of two subunits ($\alpha$ and $\beta$) cross-linked by disulfide bonds to form a heterotetramer ($\alpha\beta$)$_4$. Similar to collagen, these two agonists (CRP and Cvx) induce many of the platelet activation reactions, including secretion, phosphorylation and intracellular Ca$^{2+}$ release. However, they react only with GPVI, strongly suggesting that collagen-induced platelet activation is mediated mainly by GPVI rather than other collagen receptor, integrin $\alpha_\beta_1$. One of the most interesting findings is that these agonists induce a strong tyrosine phosphorylation of platelet proteins such as the tyrosine kinase Syk, phospholipase $\gamma_2$ (PLC$\gamma_2$) and the scaffolding protein, linker for activation of T-cells (LAT). This raises questions about possible signal-transducing mechanisms of platelet activation initiated by the collagen binding to GPVI. The relevant studies will be discussed later in this chapter.

Discovery of the GPVI-specific agonists facilitated the cloning and elucidation of the structure of this receptor. Low content of GPVI in platelet membranes had made it difficult to purify and study the protein without these molecular and structural biology tools. In 1999, Clemetson's group reported the first successful purification and cloning of GPVI. The deduced structure of GPVI indicates that GPVI is a member of the immunoglobulin (Ig) superfamily with two Ig-like domains. The Arg residue in its transmembrane domain has been shown to interact with an Asp residue of the Fc receptor $\gamma$-chain (Fc$\gamma$), forming a receptor complex on the platelet surface. After gene cloning, it became possible to perform detailed analyses of GPVI function at the molecular level by using a recombinant protein, GPVI-expressing cells and monoclonal antibodies against GPVI. Finally, the crystal structure of the GPVI extracellular Ig-like domain has been recently reported, thus providing a reasonable explanation for the structural and functional features of this receptor.

In contrast to ubiquitous integrin $\alpha_\beta_1$, GPVI is a platelet-specific receptor that has a unique physiological function in thrombus formation. GPVI reacts specifically with collagen fibrils and activates platelets through a tyrosine phosphorylation-dependent pathway. Clinical and animal studies indicate that the deficiency in GPVI does not induce any severe bleeding tendency, although the thrombus formation in GPVI-deficient mice is impaired. Thus, specific inhibitors against GPVI are potentially ideal anti-thrombotic drugs that would not induce significant bleeding as a side effect and nowadays, much effort is being put into finding a specific GPVI inhibitor suitable for clinical use.

**Structure of GPVI**

From its cDNA sequence, GPVI is identified as a glycoprotein composed of 319 amino acid residues and a signal sequence of 20 amino acids. The GPVI gene consists of eight exons and is located on chromosome 19q13.4 of the human genome. The extracellular region of GPVI contains two Ig-like domains and a mucin-like Ser/Thr-rich domain is present between the Ig-like and transmembrane domains. The Ig-like domain is responsible for the binding of GPVI to collagen. Also, many O-linked carbohydrate chains are expected to conjugate to the Ser/Thr residues of the Ser/Thr-rich domain. One putative glycosylation site for the N-linked carbohydrate chain is identified at Asn72. Thus, it might be hypothesized that the Ig-like domain extends out from the polysaccharide layer over the platelet surface, forming a structure similar to platelet GPIb. Another characteristic structural feature of GPVI is the presence of the charged residue in its transmembrane domain (Arg252). The presence of the charged amino acid residue in the transmembrane domain is known to be characteristic for the proteins that associate with Fc$\gamma$ containing a negatively charged Asp residue in its transmembrane domain. Thus, a salt bridge formed between GPVI and Fc$\gamma$, stabilizes the receptor complex. The importance of this salt bridge was confirmed by expression experiments. The model of the GPVI-Fc$\gamma$ complex presented in Figure 1 is consistent with the experimental data.