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Regular Article

A defined peptide that inhibits the formation of the glycoprotein IIb and IIIa complex

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Abstract Collagen—platelet interaction plays an important role in hemostasis and pathological thrombosis. The proposed mechanism of the interaction was the activation of platelets→releasing of contents from granules→aggregation. The common end point is the platelets and fibrin aggregates. Platelet glycoprotein (GP) IIb/IIIa (the αIIbβ3 integrin) complexes serve as a receptor for the binding of fibrinogen to form firmed aggregates. Blockading of GP IIb/IIIa has been proposed to prevent platelet aggregation independent of the substance(s) responsible for activating the platelets. The development of various forms of GP IIb/IIIa inhibitor has resulted in the inhibition of platelet aggregation, although studies of αIIbβ3 receptor function and various GP IIb/IIIa inhibitors have demonstrated the potential for these agents to produce effects on other aspects of platelet function as well as having nonplatelet effects. This study investigated platelet inhibition provided by blocking the GP IIb/IIIa complex formation by using a peptide derived from the GP IIIa molecule. The peptide inhibits both types I and III collagen-induced platelet aggregation in a dose-dependent manner. The defined peptide interferes with the formation of the GP IIb/IIIa complex by inhibiting the binding of FITC—PAC-1 onto ADP-, type I collagen-, and type III collagen-activated platelets. However, P-selectin secretion is not affected by the peptide. In addition, the peptide is not interfering with the binding of FITC—PAC-1 to platelets that were preincubated with indomethacin. Results from this study may suggest that the defined peptide is an effective agent to block the interaction of types I and III collagen with platelets.

Abbreviations: GP, glycoprotein; IPTG, isopropylthio-β-D-galactoside; LB, liquid broth; TBS, 20 mM Tris—500 mM NaCl—Tween 20 (0.25 ml/l), pH 7.4; PBS, 20 mM phosphate—130 mM NaCl, pH 7.4; PRP, platelet-rich plasma; Tris-EDTA, 20 mM Tris—130 mM NaCl—1 mM EDTA, pH 7.4; Anti-90 m, monoclonal antibody raised against the purified platelet 90-kDa glycoprotein; PAC-1, an antibody raised against the activated platelets that specific recognized GP IIb/IIIa.

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Introduction

Interaction of the platelet receptors (integrin and nonintegrin) for various types of collagens (especially types I and III) in the exposed sub endothelial vessel walls as a result of on-going microvascular injury results in platelet activation and aggregation with the release of mediators. Collagen—platelet interaction has been proposed to play a primary role in the initiation of hemostasis and pathological thrombosis. An understanding of the mechanism by which platelets adhere to collagen is important for the development of therapeutic agents that could prevent pathological thrombosis.

Two groups of platelet proteins have been proposed as collagen receptors, which can initiate platelet aggregation through collagen receptor interaction. One group, represented by members of the integrin family, in which the receptor contains heterodimers, includes glycoprotein Ia—IIa ($\alpha 2\beta 1$) [1] and glycoprotein (GP) IIb/IIIa ($\alpha IIb\beta 3$) [2]. The other group, composed of single chain or proteins with homodimers, includes a 65-kDa protein described by our laboratory [3], an 85/90-kDa protein [4], GP IV [5], GP II [6], and GP VI [7]. The functional relationship among these proteins is unknown, but different experimental approaches may have led to descriptions of a large number of putative collagen receptors. For example, in the presence of Mg^{++}, the adherence of platelets to collagen is mediated by platelet glycoprotein Ia—IIa [1], but in the absence of metal ions, platelets adhere to many non integrin proteins as described above.

Glycoprotein (GP) IIb/IIIa is one of the members of the platelet integrin family and is thought to play a very important role in the final stage of platelet aggregation. The binding of fibrinogen and von Willebrand factor to GP IIb/IIIa on the activated platelets mediates the aggregation of platelets, thereby providing a fast-acting hemostatic mechanism at the site of vascular injury. The binding of fibrinogen on the activated platelets reside in a tetra peptide sequence, Arg-Gly-Asp-Ser (RGDS) of fibrinogen [8]. The RGDS sequence can also be found in type I collagen, but it is cryptic in native collagen and exposed only on proteolytic degradation and denaturation [8]. In addition, the tetra peptide sequence is also shared with other adhesion molecules such as vitronectin, fibronectin, and laminin.

Previously, we have purified and characterized a platelet membrane glycoprotein with an *M*r of 90-kDa that functions as a receptor for types I and III collagen [9]. The monoclonal antibody raised against the purified 90-kDa proteins also inhibits α-thrombin- and epinephrine-induced platelet aggregation [9]. The protein has been identified as glycoprotein IIIa [10]. In the present study, we have chemically synthesized several peptides of the outer domains of the protein. One of the peptides inhibits both type I and type III collagen-induced platelet aggregation. The same peptide also blocks the binding of FITC—PAC-1 binding to the ADP-, type I collagen-, and type III collagen-activated platelets.

Materials and methods

Preparation of platelet-rich plasma (PRP)

Human blood (9 parts) was collected from normal volunteers after an overnight fast in polypropylene tubes containing 1 part 3.8% sodium citrate. PRP was prepared by centrifuging the citrated blood at room temperature for 10 min at $226\times g$ [11]. Whole blood and PRP were exposed to plastic surfaces or siliconized vessels only. Platelet counts of the PRP ranged from 200,000 to 300,000 per mm^3.

Platelet aggregometry

Platelet aggregation was assayed by the turbidimetric method of Born [12] using a ChronoLog Lumi aggregometer (Chrono Log, Havertown, PA). An aliquot of 0.45 ml PRP was pipetted into a siliconized cuvette and stirred at a constant speed of 1100 rpm at 37 °C. The changes in percent transmission were recorded continuously.

Preparation of washed platelets

Equal volumes of PRP and 20 mM Tris—HCl—130 mM NaCl—1 mM EDTA, pH 7.4 (Tris—NaCl—EDTA) were mixed together and centrifuged at $1000\times g$ for 5 min. The pellet formed was washed once with Tris—NaCl—EDTA and then suspended in the same buffer at a concentration of 400,000—600,000 platelets/mm^3.

Preparation of types I and III collagen

Neutral salt soluble collagen (type I and type III) was extracted from human placenta (collected from local hospital after full-term delivery). The extracted collagens were purified by different salt concentration fractionation as previously described [13,14].

Flow cytometry

Washed platelets [in the presence and absence of the GP IIIa-4 (80 μg/ml)] were incubated with