Collagen-platelet interaction: platelet non-integrin receptors

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Summary. Platelet-collagen interaction is a complex event that involves ligand-receptor interaction. There are many adhesive non-integrin receptors for platelets to interact with various types of collagens. These non-integrin receptors also serve as signal transducers both from the outside of platelets to the inside and possibly vice versa. The present review covers basic aspects of non-integrin receptor function and various signal transduction pathways.

Key words: Receptor, Platelet, Collagen, Signal transduction, Protein phosphorylation

Introduction

Platelets play an important role in the process of hemostasis and thrombosis. Following injury to blood vessels and in certain pathologic conditions, platelets adhere to the exposed subendothelial connective tissue, collagen in particular, and aggregate, releasing several biological active substances. The adherence of platelets to the endothelial component-collagen has been demonstrated to be a receptor mediated event (Puett et al, 1973; Brass and Bensusan, 1974; Chiang et al., 1975).

The addition of collagen to human platelets leads to platelet protein phosphorylation either on tyrosine and serine/threonine residues, the activation of phospholipase A2 that generates thromboxane A2, or phospholipase C that generates tri-s-inositol phosphate and mobilize calcium. These biochemical reactions are closely associated with platelet shape change, release granular contents, and platelet aggregation. Collagen also stimulates protein phosphatases 1 and 2A to dephosphorylate platelet proteins to modify platelet reactivity. Okadaic acid, a protein phosphatase 1 and 2A inhibitor, and phenyl arsine oxide, a tyrosine phosphatase inhibitor, both inhibit collagen-induced platelet aggregation and the release reaction.

Platelet collagen receptors

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 heterodimeric proteins, includes VLA2 (very late activation antigen, α2β1) (Kumicki et al., 1988; Staatz et al., 1989) and glycoproteins (GP) IIb-IIIa (αIIbβ3), GPⅡa-Ⅲa, and GPⅠc-Ⅱa (Body, 1996). Other investigators (Springer, 1990; Santoro and Zutter, 1995) have reviewed the platelet integrin receptor for collagen. The other group, composed of monomer proteins or two identical subunit proteins, includes collagen glycosyl transferase (Barber and Jameison, 1971), membrane-bound fibronectin (Bensusan et al., 1978), a 65 kDa protein described by our laboratory (Chiang and Kang, 1982), GPIIb (Shadle et al., 1984), a 61 kDa protein (Kotite and Cunningham, 1986), a 160 kDa protein (Santoro, 1986), a 80 KDa protein (Lahav, 1987), a 85/90 KDa protein (Deckmyn et al., 1992), GPⅡa (Takada and Helmer, 1989), factor XIII (Saito et al., 1986), GP IV (Tandon et al., 1989), GP VI (Moroi et al., 1989), factor VIII (Aihara et al., 1984), and 62 kDa protein (Ryo et al., 1992) (Table 1). It is currently accepted that GPIIb and GPⅡa are integrin subunits αIIb and α2, respectively, and that they exist only as heterodimers with integrin β subunits. Both integrin and non-integrin receptors have been proposed as having a role in platelet-collagen interaction. The role of the integrin receptors has been well worked out, but considerable number of non-integrin receptors i.e. (glycoprotein IV, glycoprotein VI, 80/90 kDa protein, 62 kDa protein, and 65 kDa protein) have been studies extensively and proposed as having a role in this interaction.

Platelet receptor(s) for type III collagen

It has been reported that different peptides identified in types I and III collagens are involved with platelet interaction. A nona peptide [located at α1(III)-CB4] was reported to be specific for type III collagen interaction.
Table 1. Platelet proteins responsible for binding of collagen

<table>
<thead>
<tr>
<th>PLATELET PROTEIN</th>
<th>HOW ISOLATED</th>
<th>REFERENCE</th>
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<tbody>
<tr>
<td>Glucosyl transferase</td>
<td>enzyme substrate</td>
<td>Böhrer and Jameson, 1971</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>binding and antibody</td>
<td>Bersusan et al., 1978</td>
</tr>
<tr>
<td>65 kDa protein</td>
<td>affinity column, adherence and polyclonal</td>
<td>Chang and Kang, 1982</td>
</tr>
<tr>
<td>Glycoprotein Ib</td>
<td>antibody study</td>
<td>Shadle et al., 1984</td>
</tr>
<tr>
<td>von Willebrand factor</td>
<td>binding study</td>
<td>Ahara et al., 1984</td>
</tr>
<tr>
<td>61 kDa protein</td>
<td>affinity column</td>
<td>Kohle and Cunningham, 1986</td>
</tr>
<tr>
<td>Factor XIII</td>
<td>binding</td>
<td>Salib et al., 1986</td>
</tr>
<tr>
<td>80 kDa glycoprotein</td>
<td>affinity column</td>
<td>Lahav, 1997</td>
</tr>
<tr>
<td>Glycoprotein IV</td>
<td>patient study</td>
<td>Tandon et al., 1989</td>
</tr>
<tr>
<td>Glycoprotein la</td>
<td>patient study</td>
<td>Takada and Helmer, 1989</td>
</tr>
<tr>
<td>Glycoprotein VI</td>
<td>patient study</td>
<td>Mori et al., 1989</td>
</tr>
<tr>
<td>85-90 kDa glycoprotein</td>
<td>patient study</td>
<td>Deshmukh et al., 1992</td>
</tr>
<tr>
<td>62 kDa glycoprotein</td>
<td>patient study</td>
<td>Ryo et al., 1992</td>
</tr>
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All of these proteins bind to type I collagen.

with platelets (Legrand et al., 1980). Two CNBr peptides, α1(I)-CB3 and α1(I)-CB5 were also reported to be reactive sites for type I collagen interaction with platelets (Katzman et al., 1973; Fauvel et al., 1978). In addition, Balleisen et al. (1979) suggest that collagen-induced platelet aggregation is inhibited by antibodies to distinct types of collagen. Furthermore, the complexity of collagens has become increasingly apparent. More than 19 genetically distinct types of collagen have been described. The walls of vessels contain relatively large amounts of type I and type III collagens which can aggregate human platelets either in the soluble or the fibril form (Balleisen et al., 1976; Hughes et al., 1976). Types IV, V, and XI collagen also can cause platelets to aggregate but only in the fibril form (Fitzsimmons et al., 1986; Morton et al., 1987). Because types I and III collagen are the predominant collagen types in vascular walls, most investigators have investigated the mechanism of interaction of platelets with types I and III collagen. Reports suggest that there are multiple reactive sites on type III collagen to interact with platelet (Morton et al., 1987; Chiang et al., 1993a,b; Glattau et al., 1997), but the reactive site(s) on platelets has not been determined. We have purified a protein from isolated platelet membranes (Mr 47 kDa) that binds to type III collagen-Sepharose 2B column. A polyclonal antibody (anti-47p) was raised against the purified protein. The anti-47p inhibits type III collagen but not type I collagen-induced platelet aggregation and the release of ATP (Chiang et al., 1993a,b). These results suggest that there is (are) platelet protein(s) to interact with other types of collagen.

Reactive site(s) of collagen

It has been established that a tetrapeptide segment, Arg-Gly-Asp-Ser (RGDS), of fibronectin is the cell attachment site for cell-matrix interaction (Pytel et al., 1986; Ruoslahti and Pierschbacher, 1987; Yamada, 1991). This sequence has been found in other proteins including type I collagen, von Willebrand factor, and the α chain of fibrinogen. Gartner and Bennett (1985) have reported that this tetrapeptide sequence inhibits platelet aggregation stimulated by ADP, collagen, and thrombin without inhibiting platelet shape change, or serotonin release and does not interfere with platelet adhesion, perhaps because the tetra peptide inhibits the GPIIb/IIIa-fibrinogen interaction. The tetra peptide and its cyclo derivative have also been reported to inhibit the binding of platelet to fibronectin, von Willebrand factor (Plow et al., 1985) and platelet aggregation (Coller et al., 1995; Norikaz et al., 1996). Another active site for integrin-matrix interaction is Asp-Gly-Glu-Ala (DGEA), defined from collagen sequence (Staatz et al., 1990, 1991). In addition, Morton et al. (1995a,b) have reported that peptides containing a repeat of Gly-Pro-HyPro sequence are extremely platelet reactive, more active than collagen fibers in inducing platelet aggregation. These peptides express α2β1-independent activity and it is believed that these peptides recognize a crucial signaling collagen receptor directly that is not the integrin α2β1. The relationship among these proteins is unknown, but different experimental approaches may be the cause of the large number of collagen receptors reported. For example, in the presence of Mg²⁺, the adherence of platelets to collagen is mediated by platelet VLA2 (Staatz et al., 1989), but in the absence of metal ions, platelets adhere to many non-integrin proteins as described above. In an in vitro study, Mg²⁺ inhibits platelet reactivity to collagen- and ADP-induced platelet aggregation and increases bleeding time in healthy volunteers (Ravn et al., 1995). The same laboratory also reports that aspirin and Mg²⁺ have synergistic inhibitory effects on collagen-induced platelet aggregation. Thus far, we have isolated and purified a receptor for type I collagen (Mr 65 kDa) from isolated platelet membranes using collagen affinity column chromatography and preparative gel electrophoresis. Type I collagen-induced platelet aggregation is inhibited by preincubation of platelets with anti-65m and anti-65p reactive to our receptor (Chiang and Kang, 1982; Chiang et al., 1984, 1988). The purified receptor does not react with either
anti-fibronectin or anti- GPIIb/IIIa, suggesting that the receptor is not a part of fibronectin or GPIIb/IIIa (Chiang et al., 1984). Other unpublished data also suggest that the 65 kDa protein does not react with anti-von Willebrand factor or anti-GPIa. In addition, collagen-receptor interaction causes phosphoinositide hydrolysis and mobilization of calcium (Chiang et al., 1988). Flow cytometry studies show that anti-65m and polyclonal antibody raised against 47 kDa bind to the platelet surface (Chiang and Kang, 1982; Chiang et al., 1993a,b). The 65 kDa receptor has been cloned and sequenced by our laboratory (Chiang et al., 1997). Database (gene and protein) searches failed to find homology. The recombinant protein was expressed in prokaryotic and eukaryotic vectors. The recombinant protein blocks type I collagen but not type III collagen, ADP-, and thrombin-induced platelet aggregation and the release of ATP (Chiang et al., 1997).

**Inhibitors of collagen-induced platelet aggregation**

Along with a large number of compounds such as aspirin, prostaglandin I₂ (PGI₂), heparin, etc., antibodies of collagen receptors (GPIIb/IIIa, fibronectin, 65 kDa, 85/90 kDa, 47 kDa, α2β1, GPIV, and factor VIII) are also inhibitors of collagen-platelet interactions in addition to the peptides RGD and DGEA mentioned above. Other inhibitors are leech protein (Henrita van Zanten, 1995), hemorrhagin catrocollastatin (snake-venom) (Rahman et al., 1995, Zhou et al., 1996), RGI3965, a novel platelet fibrinogen receptor antagonist (Bostwick et al., 1996), a mutant of echistatin (Yamada and Kidera, 1996), a non-peptide GPIIb/IIIa inhibitor, L-734217 (Cook et al., 1996), and a mimetic of the RGDF-peptide (Pueyo et al., 1996). Each of these proteins and peptides is required at high concentrations to inhibit collagen-induced aggregation. For example, complete inhibition of collagen-induced platelet aggregation requires 250 μM RGDS (Gartner and Bennett, 1985). Other inhibitors require high concentrations resulting in adverse effects, such as leech protein. The DGEA peptide requires 5 mM to completely inhibit platelet adhesion to collagen and its derivative, (GPHyPro)₅-GADGEA(GPHyPro)₅ which is 10 to 30 fold more effective than the parent peptide (Santoro et al., 1994). In contrast, our peptide-1 (18 amino acids) completely inhibits type I collagen-induced platelet aggregation at 20 to 40 μM (Chiang and Kang, 1997). Recently, I have defined the peptide-1 to a smaller fragment of 5 amino acids (unpublished data).

**Two distinct pathways of collagen-induced signal transduction**

Collagen-platelet interaction involves specific receptors as mentioned in the earlier section. The participation of such specific molecules in physiological collagen-platelet interaction remains largely unclassified and the molecular mechanism by which collagen induces platelet activation has yet to be elucidated. Evidence indicates that the integrin α2β1 is the principal platelet adhesion receptor for collagen (Kunicki et al., 1988; Staatz et al., 1989). Collagen binding domain is located within the α2 subunit (Takada and Helmer, 1989). Clinical deficiency of platelet α2β1 results in a mild to severe bleeding tendency, accomplished by defective platelet aggregation in response only to collagen (Nieuwenhuis et al., 1985; Kehrel et al., 1988). It is still not established whether the binding of collagen to the receptor is sufficient for stimulating a full picture of collagen-induced platelet activation. Using a patient's platelets that lack GP IV with normal integrin α2β1 content, Ichinohe et al. (1997) have demonstrated and established that signaling pathways between the integrin and the non-integrin (GP IV) are different.

Protein tyrosine phosphorylation is now considered the key signaling event in the activation of platelets (Clark et al., 1994). Collagen is known to stimulate tyrosine protein kinases, c-Src (Liebenthoff et al., 1993), Syk (Clark et al., 1994), and focal adhesion kinase (Lipfert et al., 1992), and to promote rapid tyrosine phosphorylation of platelet proteins (Blake et al., 1994; Clark et al., 1994; Cichowski et al., 1996). Collagen-stimulated protein tyrosine phosphorylation is unique in that it is insensitive to inhibition by cAMP-increasing agents (Ichinohe et al., 1995) which are known to inhibit platelet activation by most agonists. Although integrin α2β1-mediated cell adhesion is believed to be essential for the induction of tyrosine phosphorylation (Shattil et al., 1993), other described receptors may also represent candidates responsible for the collagen-stimulated protein tyrosine phosphorylation (Suigijama et al., 1987; Moroi et al., 1989).

Presently, the precise role of specific receptors for the various collagen types in platelet adhesion has not yet been fully established. Some insight has been obtained regarding the platelet receptors (integrin and non-integrin), their reactive sites, the role of divalent ions, the signal generated, and the contribution of cofactors (von Willebrand factor and fibronectin) in collagen-platelet interaction. Much remains to be studied, particularly regarding the details of molecular interactions that occur. Investigations may lead to selective approaches to inhibit platelet function and adhesion to collagen in atherosclerotic plaques without interfering with hemostasis.

**Receptor phosphorylation and phosphatidylinositol turnover**

Protein phosphorylation in relation to its physiological function has been studied in many systems (for detailed review - reference Krebs and Beavo, 1979). Receptor phosphorylation and its role on the ligand binding are altered in several systems (Kasuga et al., 1982; Roth and Karlsson, 1982; Garcia et al., 1983; Huganir et al., 1986; Yarden and Schlessinger, 1987). We noted that the response of platelets to collagen in terms of thromboxane A2 production was enhanced by...
pretreatment with protein kinase purified from human plasma. In addition, these kinases were shown to catalyze phosphorylation of platelet outer surface proteins including the collagen receptor (Chiang et al., 1988). Phosphorylation of platelet surface proteins enhances the response of platelets to subthreshold amount of collagen and also increases the content of thromboxane A2 (Chiang et al., 1991). Type I collagen can be phosphorylated by the purified human plasma protein kinases (Fig. 1).

Collagen stimulates phospholipase A2 activity

The precise mechanism by which collagen leads to the generation of free arachidonic acid, presumably by activation of phospholipase A2, is not clear. Although it has generally been assumed that increases in intracellular Ca^{2+} during platelet stimulation are responsible for phospholipase A2 activation, there is no definite evidence to support this hypothesis (Siess et al., 1983). It has been reported that phospholipase A2 requires high, non-physiological concentration of Ca^{2+} for activation (Rittenhouse and Sasson, 1985). Recently, observation of a novel phospholipase A2 from human and sheep platelet is a Ca^{2+} independent (Irvine, 1982) or exquisitely sensitive to physiological concentration of Ca^{2+} (Rittenhouse and Sasson, 1985); it may be responsible for the initial mobilization of arachidonic acid.

Various physiological stimuli have been suggested to activate phospholipase A2; angiotensin, bradykinin, prolactin, and thrombin, for example, release arachidonic acid when added to responsive cells (Aarsman et al., 1985; Balfou et al., 1986). In human platelets, collagen and thrombin have been known to activate phospholipase A2 to release arachidonic acid and subsequently to form thromboxane A2 (B2). The release of arachidonic acid is tacitly assumed to be an indication of phospholipase A2 action. This may or may not be the case since arachidonic acid can also be derived from non-phospholipase A2 mediated phospholipid hydrolysis, as previously reported. A better proof on phospholipase A2 activation occurs after physiological stimulation was obtained in chondrocytes and synovial fibroblasts (Loeb and Gross, 1986). Recently, we have observed that 0.09N H_2SO_4 extracts from collagen-treated platelets or platelet membrane enhances 2-fold arachidonic acid release from phosphatidylincholine (Chang et al., 1986).

Role of protein phosphatase in collagen-platelet interaction

There are three protein phosphatases in human platelets (Gratescu et al., 1977; Burchell et al., 1987; Edelman et al., 1987). The role of each protein phosphatase has not yet to be defined. It has been reported that okadiac acid inhibits phosphoprotein phosphatases (Biolojan and Takai, 1988). Lerea et al. (1989) has reported that vanadate and molybdate increase a specific platelet protein phosphorylation (Lerea, 1991). He further demonstrated that thrombin can be selectively inhibited by okadiac acid in electropermeabilized platelets (Chiang, 1992). I have reported that okadiac acid and vanadate also inhibit collagen-induced platelet aggregation and the release of ATP. These reports suggest that phosphoprotein

--- a1
--- a2

![Fig. 1. Radioautogram of the phosphorylated soluble collagen type I collagen. Soluble type I collagen (10 μg) was incubated with the purified human plasma protein kinases (10 μg of fraction I, lane 1 and fraction II, lane 2), in a reaction mixture contained: 2 mM MgCl_2, 2 mM NaF, and 10 μM (β,γ-32P)ATP in a final volume of 0.025 ml. Control experiments were: collagen (10 μg) plus reaction mixture (lane 3), human plasma protein kinase fraction I (10 μg) plus reaction mixture (lane 5), and human plasma protein kinase fraction II (10 μg), plus reaction mixture (lane 5). These samples were incubated at 30°C for 5 minutes and were stopped with adding equal volume of SDS-PAGE sample buffer and boiled for 3 minutes. These samples were analyzed with 7.5% SDS-PAGE and radioautography.](image-url)
phosphatases are involved in platelet function. In subsequent study, we have observed that collagen stimulates phosphoprotein phosphatase 1 and 2A (Chiang et al., 1993a, b). Recently, we have observed that protein phosphatase 1 is coprecipitated with anti-type I collagen receptor (65 kDa) (Chiang, 1993). The inhibitory effect of okadaic acid and phenylarsine oxide may be mediated by inhibiting phosphorylation/dephosphorylation of platelet protein phosphatase 1 (Chiang, 1998). I am not attempting to cover all of the related literatures. There is a more detailed review on protein kinases and phosphatases in platelet activation (Watson et al., 1993).

Conclusion remarks

Collagen-platelet interaction is a complex process. It involves various signal transduction components existed in platelets. Most studies are focused on receptors, platelet proteins phosphorylation, phospholipase A2, and phospholipase C. The role of protein phosphatase in this interaction has not yet established. There are several platelet proteins; glycoprotein IIIA, annexin, moesin, talin, etc are substrates for protein kinases. Collagen is also a substrate for cAMP-dependent protein kinase. The function of the phosphorylation and/or dephosphorylation of these proteins needs to be elucidated.

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References


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different aggregatory sites. Biochem. J. 248, 483-487.
Rahman S., Lu X., Kakkar V.V. and Aurh K. (1995). The integrin αIIbβ3 contains distinct and interacting binding sites for snake-venom RGD proteins: Evidence that the receptor-binding characteristics of snake-venom RGD proteins are related to the amino acid environment flanking the sequence of RGD. Biochem. J. 312, 223-232.