The Membrane Glycoprotein Ia–IIa (VLA-2) Complex Mediates the Mg^{++}-dependent Adhesion of Platelets to Collagen

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Abstract. We have purified the platelet membrane glycoprotein Ia–IIa complex by detergent solubilization and sequential affinity chromatography on Concanavalin A-Sepharose and collagen-Sepharose. The complex, which is identical to the VLA-2 complex of lymphocytes and other cells and contains subunits of 160 and 130 kD on SDS-PAGE, was labeled with ^{125}I and incorporated into phosphatidyl choline liposomes. The liposomes, like intact platelets, adhered to collagenous substrates in an Mg^{++}-dependent manner with a $K'_{a(Mg^{++})}$ of 3.5 mM. Little adhesion of the liposomes to collagen occurred when Mg^{++} was replaced by Ca^{++} or EDTA. Calcium ions inhibited the Mg^{++}- dependent adhesion with a $K'_{i(Ca^{++})}$ of 5.5 mM. Liposomes containing the Ia–IIa complex adhered to substrates composed of types I, II, III, and IV collagen, but did not effectively adhere to substrates composed of type V collagen or gelatin. Adhesion to collagen was specific. The liposomes did not adhere to fibronectin, vitronectin, laminin, thrombospondin, fibrinogen, or von Willebrand factor substrates. The monoclonal antibody PlH5, which specifically immunoprecipitated the Ia–IIa complex, also specifically inhibited the Mg^{++}-dependent adhesion of both platelets and Ia–IIa-containing liposomes to collagen substrates. These findings provide additional evidence that the platelet membrane Ia–IIa complex is the mediator of Mg^{++}-dependent platelet adhesion to collagen and suggest that the VLA-2 complex may also function as an Mg^{++}-dependent collagen receptor in other cells.

THE adhesion of platelets to collagen plays a major role in thrombosis and hemostasis. When a vessel wall is damaged, platelets rapidly adhere to the exposed subendothelial components, of which fibrillar collagen is the most thrombogenic macromolecule (Baumgartner, 1977). Adherence to fibrillar collagen causes platelets to rapidly change their shape, secrete the contents of their alpha and dense granules and express on their surfaces activation-dependent receptors for adhesive glycoproteins (Hawiger, 1987). These events lead to platelet aggregation and the formation of the hemostatic plug.

Although the importance of the interactions between platelets and collagen is well recognized, there is no consensus either about the molecular mechanism(s) which mediate the interactions or about an approach to elucidating such a mechanism. Thus, while various proteins including fibronectin (Bensusan et al., 1978), a 65-kD protein (Chiang and Kang, 1982), the platelet glycoprotein IIb–IIIa complex (Tsunehisa et al., 1984; Kotite et al., 1984; Shadle et al., 1984), platelet factor XIII (Saito et al., 1986), platelet glycoprotein Ia (Nieuwenhuis et al., 1985; Santoro, 1986; Santoro et al., 1988; Kunicki et al., 1988), a 61-kD polypeptide (Kotite and Cunningham, 1986), and an 85-kD polypeptide (Lahav, 1987) have been proposed as platelet surface receptors for collagen, none has gained general acceptance as a physiologically relevant mediator of platelet adhesion to collagen.

We recently identified a process of platelet adhesion to collagen which exhibited many of the properties expected of a physiologically relevant mechanism (Santoro, 1986). In the presence of 2 mM Mg^{++}, platelets effectively adhered to substrates of both monomeric and fibrillar collagen, although only adhesion to the fibrillar collagen substrate resulted in platelet activation and secretion. Adhesion via this mechanism was dependent upon Mg^{++} and was inhibited by Ca^{++}. Whereas types I, III, and IV collagen supported adhesion via this mechanism, type V collagen and gelatin did not. An earlier study by Shadle and Barondes (1982) first suggested the existence of such an Mg^{++}-dependent mechanism of platelet-collagen adhesion. We have recently purified a heterodimeric glycoprotein complex from platelet membranes which bound to collagen in an Mg^{++}-dependent manner and appeared to be a likely mediator of Mg^{++}-dependent adhesion of platelets to collagen (Santoro et al., 1988). By biochemical and immunological criteria the protein complex appeared to be identical to both the platelet membrane glycoprotein Ia–IIa complex and the VLA-2 complex identified on lymphocytes, platelets, and other cell types.

In this paper we describe the insertion of the purified Ia–IIa complex into phosphatidyl choline liposomes and the characterization of the adhesive properties of these liposomes. The data indicate that the platelet surface glycoprotein Ia–IIa complex is the mediator of Mg^{++}-dependent platelet adhesion to collagen and raise the possibility that it functions similarly on other cells which express the VLA-2 antigen.

Materials and Methods

Proteins

Calf skin type I collagen and human collagen types I, III, IV, and V were purchased from Sigma Chemical Co. (St. Louis, MO). Purified human collagen types I, II, III, IV, and V were generously provided by Dr. Ed Crouch, Washington University School of Medicine. Fibrinogen was purchased from Kabi Diagnostica (Stockholm, Sweden). Human platelet thrombospondin was a gift of Dr. V. Dixit, University of Michigan Medical School; vitronectin was a gift from Dr. J. McDonald, Washington University School of Medicine. Laminin was obtained commercially from Bethesda Research Laboratories (Gaithersburg, MD).

Fibronectin was isolated from plasma by affinity chromatography on gelatin-Sepharose according to the method of Engvall and Ruoslahti (1977). von Willebrand factor was purified from the cryoprecipitate fraction of plasma according to a previously described procedure (Santoro and Cowan, 1982). The preparation and characterization of monoclonal antibodies PlH5 and PlD6 have been described elsewhere (Wayner and Carter, 1987).

Purification of Platelet Glycoprotein Ia–IIa Complex

The complex was purified from the particulate fraction of platelets produced by two cycles of freezing and thawing as recently described (Santoro et al., 1988). Particulate material was collected by centrifugation and solubilized in extraction buffer (0.05 M Tris-HCl [pH 7.4], 0.15 M NaCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 1% Lubrol-PX). Protease inhibitors (PMSF [2 mM], Trasylol [2 mM], and leupeptin [2 mM]) were included in the extraction buffer. After centrifugation at 20,000 *g* for 45 min (4°C), the soluble supernatant was applied to a Concanavalin A–Sepharose column equilibrated in the above buffer in which the Lubrol concentration had been reduced to 0.1%. After extensive washing, glycoproteins bound to the column were eluted with column buffer containing 0.5 M α-methyl mannoside. Eluted proteins were dialyzed extensively against 0.05 M Tris-HCl (pH 7.4), 0.15 M NaCl, 2 mM $MgCl_2$, and 0.1% Lubrol to remove the sugar and the Ca^{++}, and then subjected to affinity chromatography on a column of native triple helical collagen–Sepharose. This column was eluted by substitution of 2 mM EDTA for the $MgCl_2$ in the column buffer and the eluted fractions were collected into tubes containing sufficient 200 mM $MgCl_2$ to provide a final concentration of 5 mM free Mg^{++} in each fraction.

Radiolabeling

The purified Ia–IIa complex was radiolabeled with ^{125}I using 500 μCi of

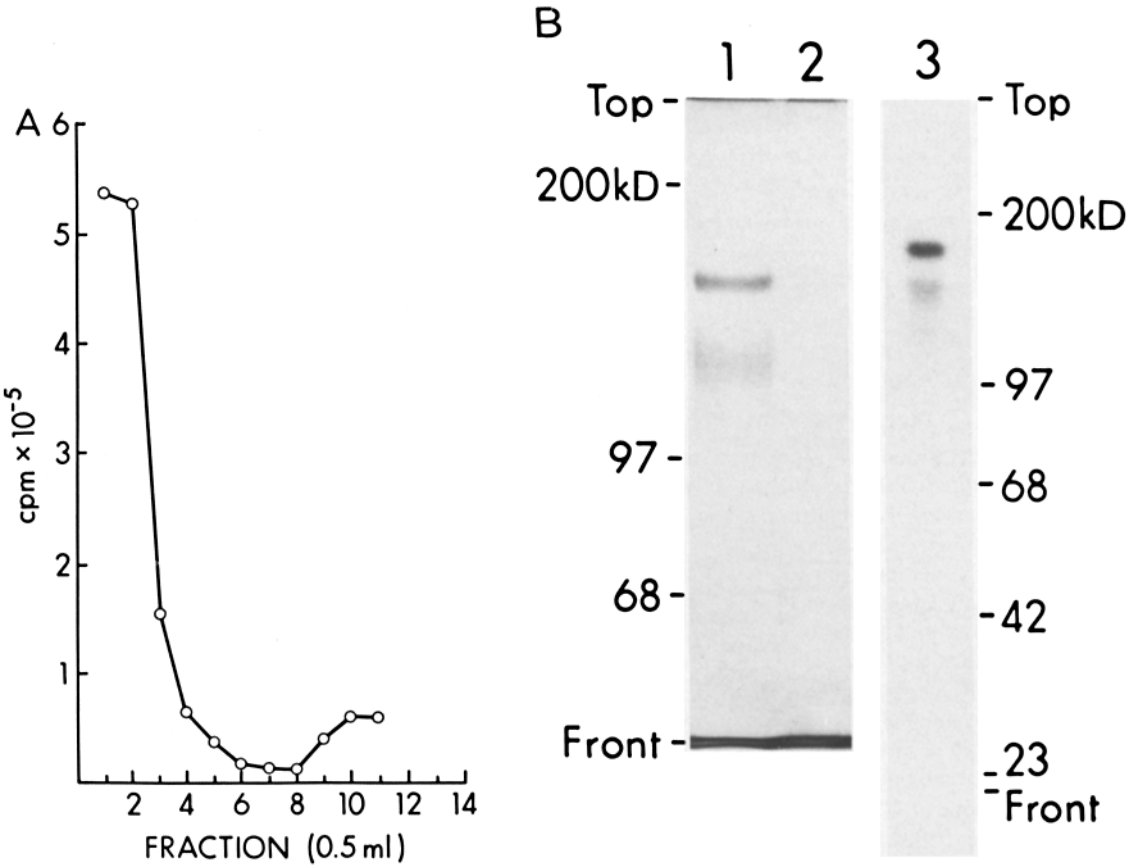

Figure 1. Preparation of ^{125}I-labeled glycoprotein Ia–IIa-containing liposomes by flotation on a discontinuous sucrose gradient. (*A*) Phosphatidyl choline liposomes, into which the purified ^{125}I-labeled Ia–IIa complex had been inserted, were purified by flotation through a discontinuous gradient composed of 50, 30, and 10% (wt/wt) sucrose as described in Materials and Methods. Fractions from the upper 1.5 ml were pooled for use in adhesion assays. (*B*) SDS-PAGE analysis of the purified glycoprotein Ia–IIa complex. (Lane *1*) Lane of purified Ia–IIa complex on a 6% polyacrylamide gel, run under reducing conditions, stained with silver. Glycoproteins Ia and IIa migrate at positions corresponding to 160 and 130 kD, respectively. The artefactual staining observed at the gel front was also present in the adjacent empty lane. (Lane 2) Silver-stained gel to which no sample was applied showing artefactual staining at the gel front. (Lane *3*) Autoradiogram of ^{125}I-labeled Ia–IIa complex used for incorporation into liposomes as described above.

^{125}I (Amersham Corp., Arlington Heights, IL) and two Iodobeads (Pierce Chemical Co., Rockford, IL) for 15 min according to the manufacturer's instructions. The labeled protein was separated from free $Na^{125}I$ by gel filtration on a column of Sephadex G-10 equilibrated with the above column buffer. To ensure that only functional complex was incorporated into the liposomes, the ^{125}I-labeled Ia-IIa complex was repurified by affinity chromatography on collagen-Sepharose, as described above, before incorporation into liposomes.

Analytical Procedures

Immunoprecipitations were performed as described by Mayes (1984) using anti-mouse IgG-conjugated agarose beads (Sigma Chemical Co.) to capture the immune complexes. SDS-PAGE was performed according to Laemmli (1970). The gels were stained for protein with silver nitrate (Merrill et al., 1981). For autoradiographic analysis, gels were dried under vacuum, overlaid with X-AR5 film (Eastman Kodak Co., Rochester, NY), and exposed at −70°C.

Liposomes

Radiolabeled Ia-IIa complex was incorporated into phosphatidyl choline liposomes using the octylglucoside method of Mimms et al. (1981). To separate the liposomes from unincorporated ^{125}I-labeled Ia-IIa complex, 0.5-ml aliquots of the liposome suspensions were made 50% (wt/wt) with respect to sucrose, then overlayered successively with 3.0 ml of 30% and 1.0 ml of 10% (wt/wt) sucrose in 0.05 M Tris-HCl, 0.15 M NaCl (pH 7.4), and then centrifuged at 275,000 *g* for 18 h in a rotor (model SW50.1; Beckman Instruments, Inc., Fullerton, CA). The purified liposomes were harvested from the top 1.0–1.5 ml of the gradients and used in adhesion assays performed as described by Pytela et al. (1985) using protein substrates prepared in 35-mm polystyrene petri dishes as previously described (Haverstick et al., 1985). Unless otherwise stated, substrates were coated with bovine type I collagen at 10 μg/ml. Control substrates were composed of BSA.

Platelet Adhesion

Platelets were radiolabeled by incubation with $Na^{51}CrO_4$ and washed by gel filtration as described by Haverstick et al. (1985). Assays of platelet adhesion to collagen were performed as previously described in detail (Santoro, 1986).

Results

Liposome Preparation

The platelet membrane glycoprotein Ia-IIa complex was purified from detergent-solubilized membranes by sequential affinity chromatography on Concanavalin A-Sepharose and collagen-Sepharose as recently described (Santoro et al., 1988). The purified material was essentially homogeneous and consisted of two prominent polypeptides of 160 and 130 kD when examined by SDS-PAGE under reducing conditions (Fig. 1 *B*, lane *1*). No other polypeptides were visible upon silver staining. The apparent band at 43 kD corresponds to the gel front and was present across the entire gel including lanes which did not contain sample (Fig. 1 *B*, lane *2*). The purified complex was radiolabeled with ^{125}I without any significant degradation during the radiolabeling procedure (Fig. 1 *B*, lane *3*). A minor contaminant of 105 kD, not detected by silver staining, became apparent after radioiodination and contained 2–6% of the label, depending upon the preparation, as judged by densitometric analysis of the autoradiograms. This polypeptide may have arisen from one of the parent polypeptides by proteolysis since it appears to be part of the Ia-IIa complex as judged by specific immunoprecipitation (Fig. 8). In some preparations, an additional trace radiolabeled contaminant of 213 kD was present. The radiolabeled Ia-IIa complex was then incorporated into phosphatidyl choline liposomes by the octylglucoside method of Mimms et al. (1981). The liposomes were then purified by flotation through a 10–50% (wt/wt) discontinuous sucrose gradient (Fig. 1 *A*). Proteins not associated with the liposomes remained at the bottom of the gradient, well separated from the liposome-containing fractions which were removed from the top 1.0–1.5 ml of the gradients.

Adhesion to Collagen

In the presence of 5 mM Mg^{++}, liposomes bearing the purified Ia-IIa complex adhered to bovine type I collagen substrates (Fig. 2). The extent of this adhesion increased with the concentration of collagen used to prepare the substrates up to a maximum level of adhesion which was observed on substrates prepared with 10 μg/ml of collagen. No such collagen-dependent increase in the extent of adhesion was observed when the assays were performed in EDTA-containing medium. The low degree of adhesion observed in EDTA, at all concentrations of collagen tested, was not different from the background extent of adhesion observed on BSA-coated substrates.

Substrate Specificity

Substrates were prepared from human collagen types I, II, III, IV, and V and examined for their ability to support the adhesion of liposomes containing the Ia-IIa complex. Assays were performed in both 2 mM EDTA and 5 mM Mg^{++}. As shown in Fig. 3, types I, II, and IV collagens supported significantly greater extents of adhesion of Ia-IIa-bearing liposomes in Mg^{++}-containing buffers than in EDTA-containing medium. In other similar experiments, we have observed that substrates of type III collagen also effectively supported Mg^{++}-dependent adhesion of the Ia-IIa-bearing liposomes (data not shown). In contrast to these findings, the presence of Mg^{++} did not significantly increase the low extent of liposome adhesion onto substrates of type V collagen. Thus, as observed with intact platelets in our earlier study (Santoro, 1986), collagen types I, II, III, and IV, but not type V, support the Mg^{++}-dependent adhesion of liposomes bearing the Ia-IIa complex. As also observed in our earlier study with intact platelets, substrates composed of gelatin did not support the adhesion of liposomes containing the Ia-IIa complex (data not shown).

The evidence presented above indicates that when the purified Ia-IIa complex is inserted into liposomes, it exhibits Mg^{++}-dependent collagen binding properties very similar to

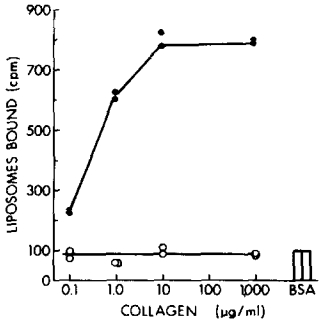

Figure 2. Adhesion of Ia-IIa-containing liposomes to substrates of type I calfskin collagen. Ia-IIa-containing liposomes were allowed to adhere to substrates prepared with the indicated concentrations of collagen. Adhesion of liposomes was determined in media containing either 5 mM $MgCl_2$ (●) or 2 mM EDTA (○). Control plates were coated with BSA and adhesion to them was determined in the presence of 5 mM $MgCl_2$.

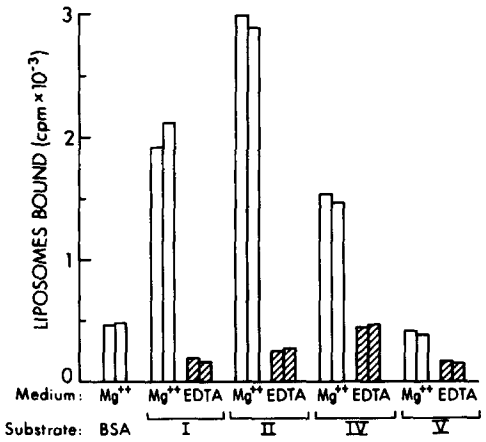

Figure 3. Adhesion of Ia–IIa-containing liposomes to types I, II, IV, and V human collagens. Substrates were prepared with the different collagens (200 μg/ml), and the extent of adhesion of liposomes containing the Ia–IIa complex was determined in media containing either 5 mM $MgCl_2$ (*open bars*) or 2 mM EDTA (*closed bars*). The results of duplicate determinations are shown.

those of nonactivated human platelets. The data do not, however, address the question of whether the binding is specific for collagen. Substrates were, therefore, prepared with a series of the major noncollagenous adhesive glycoproteins. The extent of adhesion of Ia–IIa-containing liposomes to these substrates in the presence of 5 mM Mg^{++} was determined and compared to the extent of adhesion to type I collagen and BSA substrates. As shown in Fig. 4, adhesion of the liposomes to fibronectin, laminin, thrombospondin, vitronectin, fibrinogen, and von Willebrand factor was in every case comparable to the low level of nonspecific adhesion onto BSA substrates. In no instance did the extent of adhesion of the liposomes to the noncollagenous substrates exceed the low level of adhesion to collagen observed in the presence of 2 mM EDTA. These observations suggest that the binding of the Ia–IIa complex to collagen is specific. Although fibronectin, laminin, thrombospondin, fibrinogen, and von Willebrand factor substrates did not support the adhesion of liposomes containing the Ia–IIa complex, these substrates were all capable of supporting the adhesion of intact platelets (data not shown).

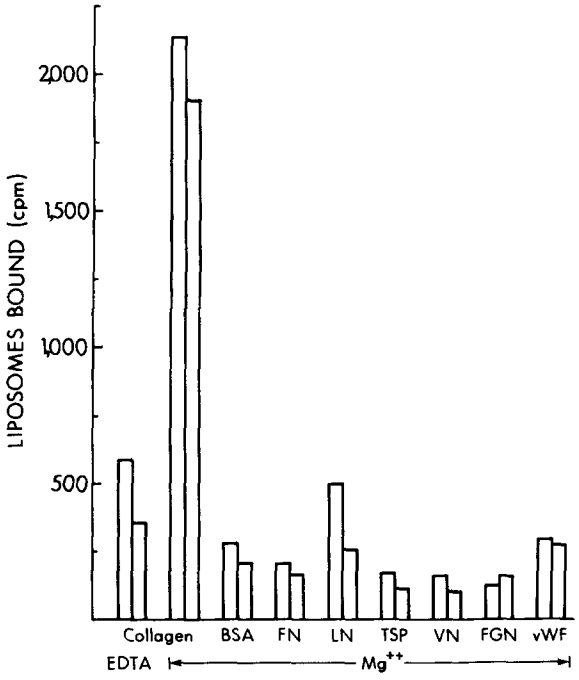

Figure 4. Substrate specificity of liposomes containing the Ia–IIa complex. Substrates were prepared with 25 μg/ml type I collagen, 5 mg/ml bovine serum albumin (*BSA*), 25 μg/ml fibronectin (*FN*), 20 μg/ml laminin (*LN*), 25 μg/ml thrombospondin (*TSP*), 25 μg/ml vitronectin (*VN*), 25 μg/ml fibrinogen (*FGN*), and 20 μg/ml von Willebrand factor (*vWf*). Adhesion to collagen substrates was determined in both 5 mM $MgCl_2$ and 2 mM EDTA. Adhesion to all other substrates was determined in 5 mM $MgCl_2$. The results of duplicate determinations are shown.

Divalent Cation Requirements

To more thoroughly examine the relationship between Mg^{++} concentration and the extent of adhesion, liposomes containing the Ia-IIa complex were allowed to adhere to type I collagen substrates in the presence of Mg^{++} concentrations ranging from 0.2 to 50 mM. The extent of liposome adhesion increased from the low levels observed below 0.5 mM to maximum levels observed at 20 mM Mg^{++} (Fig. 5 *A*). Further examination of the data in the double reciprocal format (Fig. 5 *B*) yielded a straight line with an intercept on the abscissa indicative of an apparent affinity constant for Mg^{++} $K'_{a(Mg^{++})}$ of 3.5 mM.

Our previous studies with platelets (Santoro, 1986) revealed the existence of an Mg^{++}-dependent mechanism of platelet–collagen adhesion through which Ca^{++} not only failed to support adhesion but inhibited Mg^{++}-dependent adhesion. In light of this, we examined the effects of Ca^{++} and Mg^{++} on the adhesion of liposomes containing the Ia–IIa complex to collagen. As shown in Fig. 6, in the presence of 5 mM Mg^{++}, the Ia–IIa-containing liposomes adhered to substrates of type I collagen. Adhesion to collagen was reduced to near the low levels observed on BSA substrates when either 5 mM Ca^{++} or 2 mM EDTA was substituted for the Mg^{++} in the assay buffer. Furthermore, the extent of adhesion was markedly reduced in buffer containing both Ca^{++} and Mg^{++} at 5 mM. This observation suggests that, as observed with intact platelets, the Mg^{++}-dependent adhesive process is antagonized by the presence of Ca^{++}. The effect of Ca^{++} on Mg^{++}-dependent adhesion of the Ia–IIa-bearing liposomes was examined in greater detail in a series of experiments in which the Mg^{++} concentration was varied in the presence of four different fixed concentrations of Ca^{++} (0, 0.5, 2, and 5 mM). As shown by the family of lines in Fig. 7 *A*, the extent of adhesion was progressively diminished as the concentration of Ca^{++} was increased. The common intercept of the lines on the abscissa indicates a $K'_{a(Mg^{++})}$ of 3.3–3.5 mM. The differing slopes of the lines suggest that Ca^{++} inhibits Mg^{++}-dependent adhesion via a simple linear noncompetitive mechanism (Plowman, 1972). The simplest interpretation of these data is that Ca^{++} and Mg^{++} bind to distinctly different sites on the protein complex. A similar pattern of straight lines converging on a common intercept along the abscissa was obtained when the data were replotted to obtain an estimate of $K'_{i(Ca^{++})}$ (Fig. 7 *B*) This analysis yielded a value of 5.5 mM for $K'_{i(Ca^{++})}$.

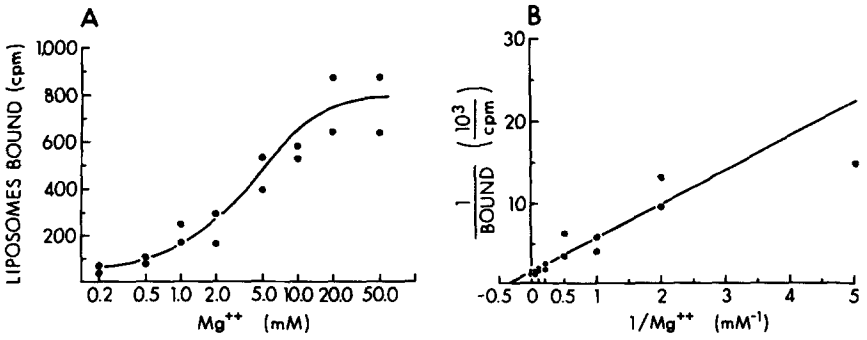

Figure 5. Mg^{++} dependence of the adhesion of liposomes containing the Ia–IIa complex to type I collagen. (*A*) Adhesion assays onto substrates prepared with 10 μg/ml of type I collagen were performed in the presence of the indicated concentrations of $MgCl_2$. (*B*) Double reciprocal plot of the data shown in *A*. The data indicate an apparent $K'_{a(Mg^{++})}$ of 3.8 mM.

Immunoinhibition of Adhesion to Collagen

Wayner and Carter (1987) recently described the development of a monoclonal antibody, PlH5, which inhibited the adhesion of fibroblasts to collagen. This antibody has also recently been shown to inhibit the adhesion of platelets to collagen and to react specifically with the platelet membrane glycoprotein Ia–IIa complex (Kunicki et al., 1988). As shown in Fig. 8, monoclonal antibody PlH5 specifically immunoprecipitated the purified, radiolabeled Mg^{++}-dependent collagen-binding complex used in the present studies. The complex was not immunoprecipitated by the control antibody, PlD6, which is specific for the Ic polypeptide of the platelet Ic–IIa complex. These observations confirm our earlier conclusion that the 160/130-kD heterodimeric Mg^{++}-dependent platelet surface collagen-binding complex is the Ia–IIa complex (Santoro et al., 1988).

As shown in Fig. 9 *A*, antibody PlH5, inhibited the Mg^{++}-dependent adhesion of intact washed platelets to substrates of type I collagen in a concentration-dependent manner. In the presence of 10 μg/ml of PlH5, the extent of platelet adhesion in the presence of 5 mM Mg^{++} was reduced to nearly the level of adhesion observed in EDTA. The low extent of adhesion observed in EDTA-containing buffers was not further reduced by the presence of PlH5. The control antibody, PlD6, had no inhibitory effect on Mg^{++}-dependent adhesion of platelets to collagen. However, 6 μg/ml of PlD6 inhibited platelet adhesion to fibronectin by 90%.

Monoclonal antibody PlH5 also reduced the Mg^{++}-dependent adhesion to collagen of liposomes containing the purified Ia–IIa complex to the low level observed in EDTA-containing media or to BSA substrates. These latter observations suggest that the observed Mg^{++}-dependent adhesion of the Ia–IIa-containing liposomes is in fact mediated by the Ia–IIa complex and not by a trace contaminant present in the Ia–IIa preparation which was subsequently incorporated into the liposomes. The ability of the antibody to inhibit essentially all of the observed Mg^{++}-dependent adhesion of intact platelets to collagen suggests that the Ia–IIa complex is the sole mediator of Mg^{++}-dependent adhesion of platelets to collagen.

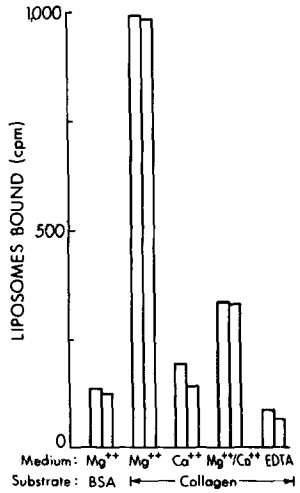

Figure 6. Effects of Mg^{++} and Ca^{++} on the adhesion to collagen of liposomes containing the Ia–IIa complex. Adhesion to substrates of type I collagen was determined in media containing 5 mM $MgCl_2$, 5 mM $CaCl_2$, 5 mM $MgCl_2$ and 5 mM $CaCl_2$, or 2 mM EDTA. Adhesion to BSA substrates was determined in 5 mM $MgCl_2$. The results of duplicate determinations are shown.

Discussion

The recognition of exposed subendothelial collagen by blood platelets is a key early step in the formation of a hemostatic plug after vascular injury. Although many different platelet surface and platelet surface-associated proteins have been proposed as mediators of platelet–collagen adhesion, none has yet gained general acceptance. We recently defined an Mg^{++}-dependent mechanism of platelet adhesion to collagen (Santoro, 1986) apparently identical to that observed by Shadle and Barondes (1982) and have isolated a platelet surface Mg^{++}-dependent heterodimeric collagen-binding complex composed of platelet membrane glycoproteins Ia and IIa (Santoro et al., 1988).

In the present report, we have described experiments in which we have examined the adhesive properties of liposomes into which the purified platelet membrane glycoprotein Ia–IIa complex has been incorporated. We have shown that these liposomes adhere to collagenous substrates in an Mg^{++}-dependent manner that precisely parallels that previously described for intact platelets (Santoro, 1986). Both liposomes containing the Ia–IIa complex and intact platelets required the presence of Mg^{++} in the low millimolar range for effective adhesion. For intact platelets K'_a was estimated as 0.5 mM whereas the liposomes exhibited a K'_a of 3.5 mM. Several possibilities may account for the difference: (*a*) the conformation of the complex may have been altered during the purification procedure, possibly at the time of elution from the collagen column with EDTA; (*b*) a subtle proteolytic cleavage undetected by electrophoretic analysis may have diminished the affinity of the purified complex for Mg^{++}, or (*c*) the lipid environment of the phosphatidyl choline liposomes may not optimally support Mg^{++} binding by the complex.

Ca^{++} was observed to inhibit the Mg^{++}-dependent adhesion of both platelets and Ia–IIa-bearing liposomes to collagen. The present studies with liposomes indicate that the in-

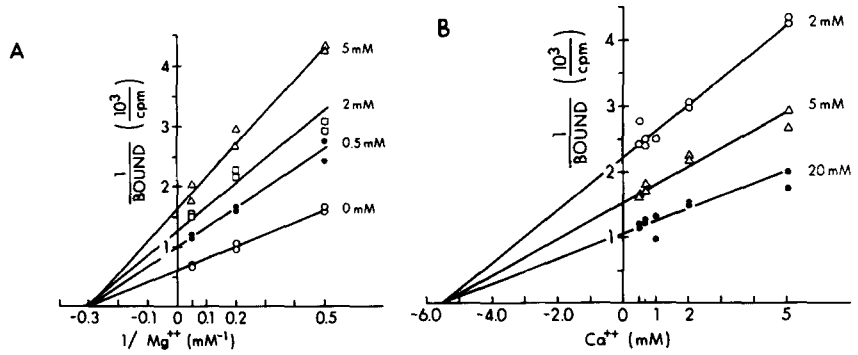

Figure 7. Inhibition of Mg^{++}-dependent adhesion to collagen of liposomes containing the Ia–IIa complex by Ca^{++}. (*A*) Double reciprocal plots of Mg^{++}-dependent adhesion performed in the presence of 0, 0.5, 2, and 5 mM $CaCl_2$. The data yield an estimate of $K'_{a(Mg^{++})}$ of 3.3–3.5 mM. (*B*) Plot of the data from *A* as the reciprocal of liposomes bound vs. Ca^{++} concentration at three different Mg^{++} concentrations (2, 5, and 20 mM). The data yield an estimate of 5.5 mM for $K'_{i(Ca^{++})}$.

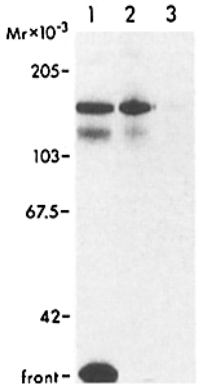

Figure 8. Monoclonal antibody PlH5 specifically immunoprecipitates the Ia–IIa complex. Purified ^{125}I-labeled glycoprotein Ia–IIa (lane *1*) was subjected to immunoprecipitation with monoclonal antibody PlH5 (lane 2) and PlD6 (lane *3*). Immunoprecipitates were collected and subjected to analysis by SDS-PAGE and autoradiography.

hibition is of the simple, linear noncompetitive type. The simplest interpretation of these data is that Ca^{++} and Mg^{++} bind to distinctly different sites on the Ia–IIa complex (Plowman, 1972). Presumably as the lower affinity Ca^{++} site becomes occupied, the conformation of the complex is altered so that it is no longer able to bind collagen. Although recent studies appear to support the supposition that Ca^{++} and Mg^{++} stabilize different conformations of the Ia–IIa complex (Staatz, W. D., K. J. Peters, and S. A. Santoro, unpublished observations), we cannot unequivocably exclude an effect of the ions on the collagen substrate.

The substrate specificity of the Ia–IIa-containing liposomes also mirrored that previously described for intact platelets (Santoro, 1986). The liposomes, like platelets, effectively adhered to collagen types I, II, III, and IV, but exhibited only low background levels of adhesion to substrates of type V collagen. Studies with the liposomes clearly indicated that the complex bound specifically to collagen and that substrates composed of other noncollagenous adhesive proteins (such as fibronectin, vitronectin, laminin, thrombospondin, von Willebrand factor, and fibrinogen) did not support adhesion of liposomes containing the Ia–IIa complex. Thus unlike such promiscuous receptors as the chicken CSAT complex (Damsky et al., 1981; Horwitz et al., 1985), the platelet IIb–IIIa complex (Pytela et al., 1986), or the vitronectin receptor (Charo et al., 1987; Cheresh, 1987), the specificity of the Ia–IIa complex appears to be restricted to collagen.

Additional evidence which supports the hypothesis that the Ia–IIa complex mediates the Mg^{++}-dependent adhesion of platelets to collagen was obtained from studies with monoclonal antibody PlH5 originally described by Wayner and Carter (1987). This antibody (*a*) binds to the platelet membrane Ia–IIa complex (Kunicki et al., 1988); (*b*) inhibits the Mg^{++}-dependent adhesion of platelets to collagen (Kunicki et al., 1988; and this report); (*c*) specifically immunoprecipitates the purified Mg^{++}-dependent 160/130-kD collagen-binding complex (glycoprotein Ia–IIa); and (*d*) specifically inhibits the Mg^{++}-dependent adhesion to collagen of liposomes bearing the purified Ia–IIa complex. The latter observation indicates that the adhesion of the Ia–IIa-containing liposomes to collagen is in fact mediated by the Ia–IIa complex and not by a trace contaminant, such as the minor 230- or 105-kD species detected after iodination, in the preparation.

Taken in the aggregate, these data lead us to the conclusion that the glycoprotein Ia–IIa complex mediates the Mg^{++}-dependent adhesion of platelets to collagen. The physiologic

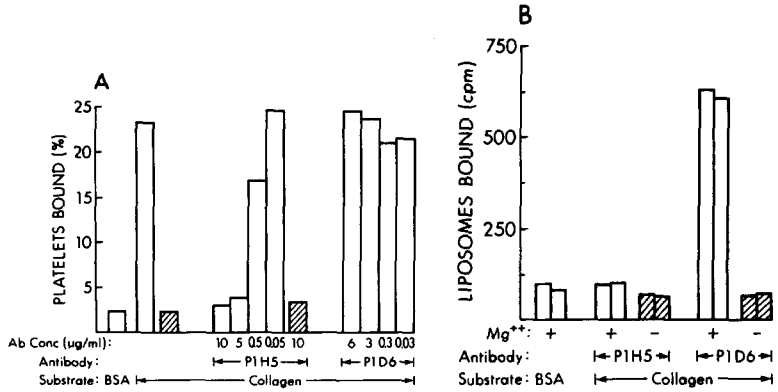

Figure 9. Inhibition of adhesion to collagen by monoclonal antibody PlH5. (*A*) Washed ^{51}Cr-labeled platelets were preincubated with either monoclonal antibody PlH5 or PlD6 at the indicated concentrations for 20 min. Adhesion to collagen substrates was then measured in the presence of 5 mM $MgCl_2$ (*open bars*) or 2 mM EDTA (*closed bars*). Adhesion to a BSA substrate was determined in the presence 5 mM $MgCl_2$. (*B*) Adhesion of liposomes to collagen or BSA substrates was determined in the presence of 5 mM $MgCl_2$ (*open bars*) or 2 mM EDTA (*closed bars*). Liposomes were preincubated for 2 h with either antibody PlH5 or PlD6, as indicated. The results of duplicate determinations are shown.

significance of this adhesive mechanism is suggested by a patient with a deficiency of glycoprotein Ia who exhibited a bleeding disorder and impaired platelet-collagen interaction (Nieuwenhuis et al., 1985). As previously discussed (Santoro, 1986), it appears that the Mg^{++}-dependent mechanism of adhesion does not operate at maximum efficiency in the presence of physiologic concentrations of divalent cations. However, both the presence of an impaired platelet-collagen interaction and a bleeding disorder in the individual deficient in the Ia-IIa complex and the fact that antibody PlH5 was selected for its ability to inhibit cell adhesion to collagen in a physiologic medium (Wayner and Carter, 1987) indicate the relevance of the adhesive mechanism described in this report.

It is unlikely that the Ia-IIa-mediated Mg^{++}-dependent adhesive mechanism represents the only mechanism by which platelets can adhere to collagen. Abundant evidence suggest that a divalent cation-independent mechanism of platelet-collagen adhesion also exists (for reviews see Santoro and Cunningham, 1981; Santoro, 1988). The identity of the platelet surface protein(s) which mediate the divalent cation-independent adhesive mechanism remains to be established.

In contrast to both the data presented above and the findings of Kunicki et al. (1988), which suggest that the glycoprotein Ia-IIa complex is the sole mediator of Mg^{++}-dependent platelet adhesion to collagen, is the possibility that more than a single molecular mechanism may mediate the initial Mg^{++}-dependent adhesion. Disruption of one component of such a two-component system could result in total, partial, or no inhibition of Mg^{++}-dependent adhesion depending upon the strength of adhesion supported by the remaining mechanism. Our data and those of Kunicki et al. (1988) indicate that, if this more complex model holds, inhibition of the function of the Ia-IIa complex results in essentially total inhibition of Mg^{++}-dependent adhesion. Shadle and Barondes (1984), however, have presented data which suggest that at least two distinct components contribute to Mg^{++}-dependent adhesion. On the basis of the ability of a monoclonal antibody directed against the IIb component of the glycoprotein IIb-IIIa complex to partially inhibit Mg^{++}-dependent platelet adhesion to collagen, Shadle et al. (1984) concluded that the IIb-IIIa complex was one component of such a mechanism.

More recent studies complicate their interpretation. As previously described (Santoro, 1986), the IIb-IIIa complex does not appear to bind to collagen in an Mg^{++}-dependent manner and, as discussed by Nieuwenhuis et al. (1985), platelets deficient in the IIb-IIIa complex undergo normal collagen-induced shape change and secretion. A recent study of the antibody used by Shadle et al. (1984) indicates that it recognizes an epitope on the IIb polypeptide which becomes expressed after occupancy of the adhesive protein binding site on the IIb-IIIa complex (Frelinger et al., 1988), an event which occurs after platelet activation. It, therefore, seems unlikely that the inhibitory activity of this antibody on platelet-collagen adhesion results from a direct effect on an initial adhesive mechanism. The mechanism by which an antibody that recognizes an epitope, expressed as a postactivation postreceptor occupancy event, inhibits the function of an apparently distinct receptor(s) is undoubtedly complex. The observations raise the possibility that the activity of one adhesive protein receptor may be modulated by interactions with other receptors.

It appears likely that the Mg^{++}-dependent adhesive mechanism we have elucidated is not unique to platelets but is also used by other cells to adhere to collagen. We recently demonstrated that the Mg^{++}-dependent 160/130-kD collagen-binding complex was immunochemically identical to the VLA-2 (very late activation) antigen (Santoro et al., 1988). This antigen, which was first described as appearing on activated T lymphocytes 9-10 d after activation with antigen or mitogen, is also expressed on fibroblasts, nerve cells, and platelets (Pischel et al., 1987; Takada et al., 1987; Hemler et al., 1987). Pischel et al. (1988) recently established the identity of the VLA-2 complex with the glycoprotein Ia-IIa complex on platelets. The results of the present study and other recent work in this laboratory (Santoro et al., 1988) suggest that the VLA-2 complex functions as a cell surface collagen-binding complex and hence may mediate the Mg^{++}-dependent adhesion of cells to collagen. In this regard it is noteworthy that antibody PlH5, which inhibits the collagen binding activity of the platelet membrane glycoprotein Ia-IIa complex, was originally selected for its ability to inhibit the adhesion of fibroblasts to collagen (Wayner and Carter, 1987).

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