

ated with an epitope that induces protective immunity against HTLV-III is not known. These studies suggest that peptides of conserved epitopes of the envelope glycoprotein should be evaluated for diagnostic or immunoprotective purposes in the control of the HTLV-III infection.

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11 September 1985; accepted 27 December 1985

Platelet Membrane Glycoprotein IIb/IIIa: Member of a Family of Arg-Gly-Asp-Specific Adhesion Receptors

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Adhesive interactions of the platelet surface with plasma proteins such as fibrinogen and fibronectin play an important role in thrombosis and hemostasis. The binding of both of these proteins to platelets is inhibited by synthetic peptides containing the sequence Arg-Gly-Asp, which corresponds to the cell adhesion site in fibronectin and is also present in the α chain of fibrinogen. An affinity matrix made of an insolubilized heptapeptide containing the Arg-Gly-Asp sequence selectively binds the platelet membrane glycoprotein IIb/IIIa from detergent extracts of platelets. When incorporated into liposome membranes, the isolated protein confers to the liposomes the ability to bind to surfaces coated with fibrinogen, fibronectin, and vitronectin but not to surfaces coated with thrombospondin or albumin. This platelet receptor is related to the previously identified fibronectin and vitronectin receptors in that it recognizes an Arg-Gly-Asp sequence but differs from the other receptors in its wider specificity toward various adhesive proteins. These results establish the existence of a family of adhesion receptors that recognize the sequence Arg-Gly-Asp.

IN RECENT YEARS CONSIDERABLE progress has been made in identifying components that mediate the interaction between cells and extracellular matrix proteins. It has been shown that a tripeptide sequence, Arg-Gly-Asp, which is present in both fibronectin (1) and vitronectin (2), mediates the attachment of cultured fibroblasts to these two adhesive proteins (1-3). Distinct cell surface receptors for the Arg-Gly-Asp sequence in fibronectin (4) and vitronectin (5) have been identified in both fibroblastic and osteosarcoma cells. The Arg-Gly-Asp sequence is important in adhesive interactions at the platelet surface as well, since platelets also attach to fibronectin (6) and synthetic peptides containing Arg-Gly-Asp inhibit such attachment (7). More-

over, these peptides inhibit the binding of fibronectin and fibrinogen to platelets from solution and prevent platelet aggregation (8). This suggests that the Arg-Gly-Asp sequence, which occurs twice in fibrinogen (9), may serve as a platelet recognition site on this protein. Fibrinogen, however, does not promote the adhesion of fibroblastic cells (1). These findings suggest that platelets have a receptor capable of recognizing the Arg-Gly-Asp sequence in fibrinogen while fibroblasts do not. We report here that platelets have a receptor that recognizes the Arg-Gly-Asp sequence in several adhesive proteins, including fibrinogen, fibronectin, and vitronectin. We also report that this receptor is indistinguishable from the platelet membrane glycoprotein gpIIb/IIIa, but

differs from the previously identified Arg-Gly-Asp receptors of fibroblastic cells that recognize only fibronectin or vitronectin.

The platelet receptor was identified by affinity chromatography on the heptapeptide Gly-Arg-Gly-Asp-Ser-Pro-(Lys) coupled to Sepharose (GRGDSP-Sepharose); the lysine residue was added to optimize coupling to the Sepharose. This peptide corresponds in sequence to the cell attachment site in fibronectin. A detergent extract of washed human platelets was applied to this affinity matrix, and specifically bound components were eluted with a solution of the GRGDSP peptide. As shown in Fig. 1, this resulted in the binding and selective release of two polypeptides, which, when reduced, migrated in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with mobilities corresponding to molecular weights of 124,000 (124K) and 108K. These polypeptides were not released by a control elution with an inactive variant of the peptide in which the aspartic acid is substituted by a glutamic acid residue.

The sizes of the polypeptides of the putative Arg-Gly-Asp receptor from platelets are compatible with this protein being gpIIb/IIIa. The latter exists in platelets as a noncovalent heterodimer composed of gpIIb with a disulfide-linked heavy and light chain at about 120K and 20K and gpIIIa of about

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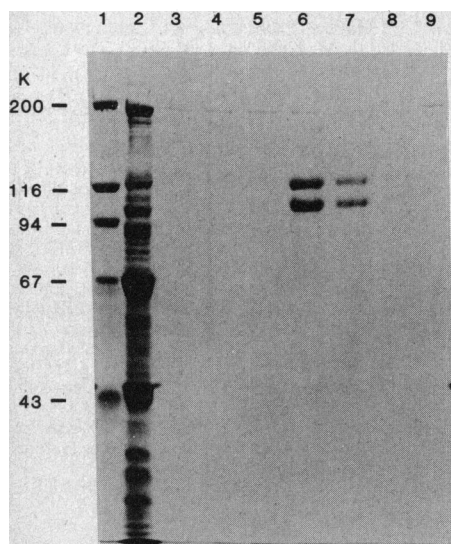


Fig. 1. Affinity chromatography of a platelet extract on GRGDSP-Sepharose. Outdated human platelets (5 U) were washed with Tyrode's buffer containing 1 mM CaCl_2 and were collected by centrifugation at 2200 rev/min. To this pellet (5 ml) was added 5 ml of cold 10 mM phosphate, 150 mM NaCl, 1 mM CaCl_2 , and 1 mM MgCl_2 [phosphate-buffered saline (PBS), pH 7.3] containing 50 mM octylthiogluco- side (Behring Diagnostics) and 3 mM phenylmethylsulfonyl fluoride (PMSF). After resuspension of the pellet and incubation at 4°C for 15 minutes, the lysate was centrifuged at 30,000g for 20 minutes (4°C) and the supernatant (8 ml) was applied to a GRGDSP-Sepharose column (bed volume, 10 ml). The affinity matrix had been prepared by incubating 120 mg of GRGDSP(K)-peptide [the lysine residue (K) was added to optimize coupling] with 10 ml of CNBr-Sepharose (Pharmacia). The extract was incubated with the affinity matrix overnight at 4°C and then the column was washed with 50 ml of PBS containing 25 mM octylthiogluco- side and 1 mM PMSF (column buffer). Elution of specifically bound components was accomplished by washing the column with 10 ml of column buffer containing GRGDSP-peptide (1 mg/ml) and then with another 10 ml of column buffer. Fractions (2.5 ml) were collected after the peptide solution was applied. Aliquots (60 μl) of each fraction were subjected to electrophoresis on a 7.5 percent polyacrylamide gel (19). Protein bands were made visible by staining with Coomassie blue. The amount of protein recovered from the column was about 0.5 mg, as estimated by densitometric scanning of the gel bands. Lane 1, molecular weight markers: myosin, 200K; β -galactosidase, 116K; phosphorylase b, 94K; bovine serum albumin, 67K; and ovalbumin, 43K. Lane 2, platelet extract that was applied to the column. Lanes 3 to 9, aliquots of fractions 1 to 7, respectively, collected after application of GRGDSP-peptide.

100K. These subunits exhibit characteristic properties in SDS-PAGE; reduction of gpIIb decreases its size whereas gpIIIa appears to increase in size (10). The platelet Arg-Gly-Asp receptor and gpIIb/IIIa (10) behaved identically when their electrophoretic mobilities were compared in SDS-PAGE under reducing and nonreducing conditions (lanes 4, 5, and 9 in Fig. 2, A and B). The platelet Arg-Gly-Asp receptor also cross-reacts in immunoblottings with two monoclonal antibodies, one (PMI-1) (11) reactive with gpIIb and the other (22C4) (11) with gpIIIa, and a third antibody against the gpIIb/IIIa complex inhibited the function of the isolated receptor (Table 1).

These results indicate that the platelet Arg-Gly-Asp receptor and gpIIb/IIIa are identical.

Figure 2 also shows the electrophoretic properties of the fibronectin and vitronectin receptors (4, 5), isolated from human placenta. The platelet receptor (lane 4) is clearly different from the fibronectin receptor (lane 2), whereas its two polypeptides differ only slightly from the vitronectin receptor (lanes 3 and 6). However, the monoclonal antibody to gpIIb failed to react with the vitronectin and fibronectin receptors in immunoblotting of reduced proteins, suggesting that each receptor is a distinct molecular species. That the platelet receptor is a unique pro-

tein, different from the previously isolated vitronectin and fibronectin receptors, is also strongly suggested by the binding specificities of the three receptors.

To analyze the binding properties of the receptors, the platelet protein obtained by affinity chromatography on the Arg-Gly-Asp-containing heptapeptide was incorporated into phosphatidylcholine liposomes, and the binding of the resulting liposomes to various adhesive proteins was measured in a solid-phase assay (4). For comparison, liposomes containing the fibronectin receptor or vitronectin receptor were assayed in parallel. Liposomes containing the platelet receptor bound to fibronectin, vitronectin, and fibrinogen but not to thrombospondin (Fig. 3A) or to albumin. In contrast, the other two receptors bound to fibronectin or vitronectin, respectively, but not to fibrinogen or thrombospondin (Fig. 3, B and C). The GRGDSP peptide inhibited the binding of platelet receptor liposomes to each protein, while its inactive variant containing a glutamic acid instead of the aspartic acid residue had no effect on this assay (Table 1). The binding of platelet receptor liposomes to fibrinogen was inhibited by a monoclonal antibody (10E5) directed against the gpIIb/IIIa complex (Table 1). Coller *et al.* (12) have shown that this antibody interferes with the binding of fibrinogen to platelets. Therefore, the platelet Arg-Gly-Asp receptor appears to be identical to gpIIb/IIIa. As expected, this antibody had no effect on the binding of vitronectin receptor liposomes to vitronectin.

There is considerable evidence that fibrinogen, fibronectin, and von Willebrand factor (vWF) interact with a shared recognition site on platelets. Thrombasthenic platelets, which lack gpIIb/IIIa, are deficient with respect to the binding of these ligands (13), and certain monoclonal antibodies to gpIIb/

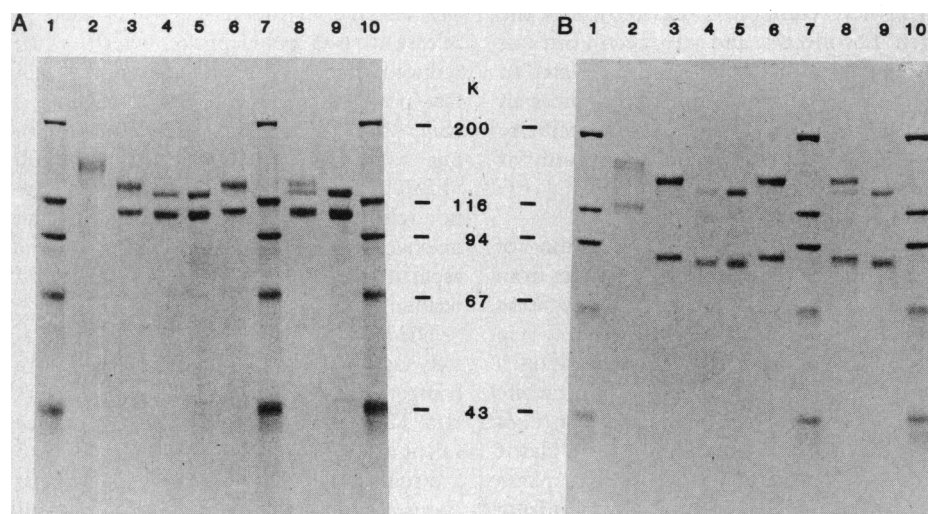


Fig. 2. Electrophoretic analysis of receptors by SDS-PAGE under reducing (A) or nonreducing (B) conditions. Platelet receptor was from the preparation represented in lane 6 in Fig. 1. gpIIb/IIIa was prepared by established methods (10), as were fibronectin and vitronectin receptors (4, 5). The procedure was scaled up so that 300 ml of placental extract was fractionated on a 10-ml affinity column. Both receptors were further purified by affinity chromatography on wheat germ agglutinin-Sepharose (5). Samples were run on a 7.5 percent gel (19) and protein bands were made visible by Coomassie blue staining. Lanes 1, 7 and 10, molecular weight markers; lane 2, fibronectin receptor; lanes 3 and 6, vitronectin receptor; lane 4, platelet receptor; lane 5, gpIIb/IIIa; lane 8, mixture of vitronectin receptor and platelet receptor; and lane 9, mixture of platelet receptor and gpIIb/IIIa.

IIIa produce a similar binding deficiency (12) in normal platelets. Moreover, fibrinogen can inhibit the binding of fibronectin and vWF to platelets (14), and a family of peptides from the γ chain of fibrinogen coordinately inhibit the platelet binding of fibrinogen, fibronectin, and vWF (15). Each of these three ligands contains an Arg-Gly-Asp sequence (1, 9, 16), and Arg-Gly-Asp-containing peptides also inhibit their binding to platelets (8). The results reported here provide direct evidence for an interaction between gpIIb/IIIa and the sequence Arg-Gly-Asp in a synthetic heptapeptide as well as in fibrinogen, fibronectin, and vitronectin. The vWF, which was not tested, probably interacts with gpIIb/IIIa by the same mechanism as fibrinogen and fibronectin. It is most likely that the gpIIb/IIIa polypeptides interact directly with the Arg-Gly-Asp sequence; however, it remains possible that

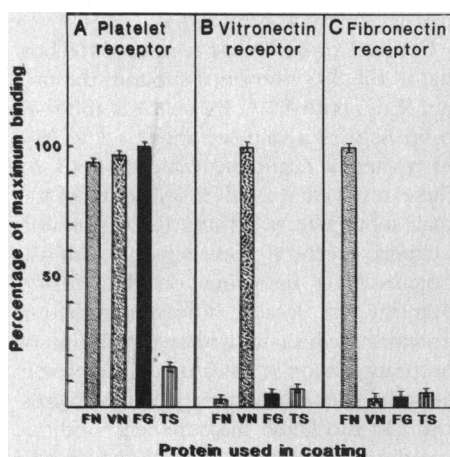


Fig. 3. Binding of receptor-containing liposomes to various protein substrates. Preparation of substrates was carried out by coating 96-well microtiter plates with proteins (20 μ g/ml in PBS) overnight at room temperature. Unoccupied binding sites on the plastic were then blocked by incubation with 4 mg/ml of bovine serum albumin. The proteins used for the coating were prepared and tested as follows. Fibronectin and vitronectin were isolated from human plasma (20, 21) and were free of any contamination by fibrinogen detectable by enzyme-linked immunosorbent assay. Fibrinogen was isolated (22) and freed of any fibrinogen or cell attachment-promoting fragments of fibrinogen by further fractionation on gelatin-Sepharose and on Sepharose linked to a monoclonal antibody that reacts with the cell attachment domain of fibronectin. Thrombospondin was isolated from platelets (23). To prepare receptor liposomes (4), fraction 4 (Fig. 1) was used as the platelet receptor. Fibronectin and vitronectin receptors were prepared (4, 5) and the binding of receptor liposomes to protein-coated microtiter wells was assayed (4). Maximum binding was 94 ng per well for the platelet receptor, 60 ng per well for the vitronectin receptor, and 43 ng per well for the fibronectin receptor. The mean and range of four determinations are given. Abbreviations: FN, fibronectin; VN, vitronectin; FG, fibrinogen; and TS, thrombospondin.

additional components, which may be undetectable by SDS-PAGE and Coomassie blue staining, are involved in this interaction.

We do not detect significant binding of platelet receptor liposomes to thrombospondin (Fig. 3). This may appear to contradict previous results of Plow *et al.* (12) showing inhibition of thrombospondin binding to platelets by monoclonal antibodies to gpIIb/IIIa. However, the binding site for thrombospondin is present on thrombasthenic platelets (17) and therefore seems to be only related, but not identical, to gpIIb/IIIa.

In addition to providing information about receptor specificity, the affinity chromatography procedure represented in Fig. 1 provides a convenient way to isolate the receptor, because a high degree of purity and a reasonable yield are achieved in a single step and a total detergent extract of platelets can be used as starting material. Moreover, the isolated receptor retains biological activity with regard to binding to surface-associated ligands.

Our results establish the existence of at least three different receptor molecules that recognize Arg-Gly-Asp sequences in different proteins. One of these recognizes the Arg-Gly-Asp sequence only in fibronectin (4) and another only in vitronectin (5), while the platelet receptor interacts with Arg-Gly-Asp sequences in both fibronectin and vitronectin as well as in fibrinogen. The significance of the relaxed specificity of the platelet receptor may be that it provides platelets with a simple mechanism to interact with multiple adhesive proteins. Platelets may require less specificity in their binding to adhesive proteins than do other cells because platelets have to respond to a variety of exposed extracellular matrices; other types of cells would probably have to have evolved more specific adhesion mechanisms to derive more precise positional information from matrix contacts (18).

The Arg-Gly-Asp receptors may be of a common evolutionary origin and structurally related. This is suggested by their related specificities as well as by their common features in SDS-PAGE. All three receptors appear to be composed of two subunits that show a characteristic response to reduction; one band increases in electrophoretic mobility while the other decreases (Fig. 2), suggesting a related pattern of intrachain disulfide bonding. Receptors such as the three described here may all have evolved from one primordial adhesion receptor, so that they now form a group of receptors specifically recognizing Arg-Gly-Asp sequences that differ slightly from one another due to differences in the neighboring amino acid sequences. Future studies may elucidate

Table 1. Inhibition of binding of platelet receptor liposomes to various substrates by synthetic peptides or monoclonal antibodies. The liposome binding assay was carried out as described in the legend to Fig. 3. Synthetic peptides (250 μ g/ml) or monoclonal antibodies (35 μ g/ml) were added to the liposome suspension before application to microtiter wells (GRGESP, Gly-Arg-Gly-Glu-Ser-Pro). Monoclonal antibody 4B2 reacts with fibronectin (24) and 10E5 is an antibody to IIb/IIIa that inhibits the interaction of fibrinogen with platelets (12). The data are means \pm standard errors for four determinations performed with one preparation of platelet receptor.

Coating	Inhibitor	Phosphatidylcholine bound per well (ng)
<i>Synthetic peptides</i>		
Fibronectin	None	88 \pm 2
	GRGDSP	6 \pm 1
	GRGESP	70 \pm 4
Vitronectin	None	90 \pm 6
	GRGDSP	10 \pm 2
	GRGESP	74 \pm 6
Fibrinogen	None	94 \pm 5
	GRGDSP	21 \pm 3
	GRGESP	92 \pm 3
<i>Monoclonal antibodies</i>		
Fibrinogen	None	81 \pm 3
	10E5	4 \pm 1
	4B2	78 \pm 2

how this variability of one basic adhesive signal relates to the structure of the adhesion receptors at the levels of the protein and the gene.

Note added in proof: After this report had been submitted, a paper related to ours (25) showed that gpIIb/IIIa binds to the cell attachment site of fibronectin.

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1 August 1985; accepted 15 November 1985

Distribution of Protein and RNA in the 30S Ribosomal Subunit

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In *Escherichia coli*, the small ribosomal subunit has a sedimentation coefficient of 30S, and consists of a 16S RNA molecule of 1541 nucleotides complexed with 21 proteins. Over the last few years, a controversy has emerged regarding the spatial distribution of RNA and protein in the 30S subunit. Contrast variation with neutron scattering was used to suggest that the RNA was located in a central core of the subunit and the proteins mainly in the periphery, with virtually no separation between the centers of mass of protein and RNA. However, these findings are incompatible with the results of efforts to locate individual ribosomal proteins by immune electron microscopy and triangulation with interprotein distance measurements. The conflict between these two views is resolved in this report of small-angle neutron scattering measurements on 30S subunits with and without protein S1, and on subunits reconstituted from deuterated 16S RNA and unlabeled proteins. The results show that (i) the proteins and RNA are intermingled, with neither component dominating at the core or the periphery, and (ii) the spatial distribution of protein and RNA is asymmetrical, with a separation between their centers of mass of about 25 angstroms.

THE TECHNIQUE OF CONTRAST VARIATION in neutron scattering has been particularly useful in the study of ribosome structure because of the different scattering length densities of protein and nucleic acid. For a particle of volume V the forward scattered intensity $I(0)$ and amplitude $A(0)$, and the radius of gyration R_g are functions of the contrast $\bar{\rho} = \rho_p - \rho_s$ between the mean scattering length density ρ_p of the particle and the scattering length density ρ_s of the solvent (1):

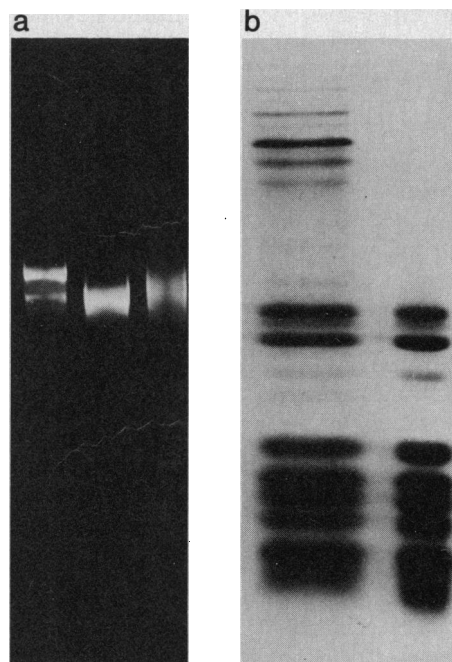
$$I(0) = [A(0)]^2 = (\bar{\rho} V)^2 \quad (1)$$

and

$$R_g^2 = R_c^2 + \frac{\alpha}{\bar{\rho}} - \frac{\beta}{\bar{\rho}^2} \quad (2)$$

Here R_c is the radius of gyration of a homogeneous particle that has the same shape as the particle under consideration. The parameter α is the second moment of the fluctuation of the scattering length density of the particle about its mean density,

which indicates whether the core of the particle is more or less dense than the periphery. In a two-component system, β is



related to the separation d between the centers of mass of the two components by the relation

$$d = \sqrt{\beta} \left(\frac{1}{|\bar{\rho}_1|} + \frac{1}{|\bar{\rho}_2|} \right)$$

where $\bar{\rho}_1$ and $\bar{\rho}_2$ are the contrasts of the particle at which the two components are matched by the solvent (2).

Contrast variation has been used to show that in the 30S ribosomal subunit, the protein R_g is about 80 Å, the RNA is about 61 to 66 Å, with a value of about -1×10^{-3} for α , and a negligible value of β (3-6). These results have led to a model of the small subunit in which the RNA primarily occupies a central core region, with the proteins lying uniformly on the outside. Over the last decade, however, individual proteins have been located on the subunit by the triangulation of measured interprotein distances (7) and immune electron microscopy (8, 9). These measurements indicate that the radius of gyration of the protein component is 68 Å and that the proteins are not uniformly distributed about the RNA in the 30S subunit. This report describes a series of experiments to resolve the conflict between these contradictory views of the structure of the 30S ribosomal subunit.

Unlabeled and deuterated 16S RNA, 30S ribosomal subunits, and subunits reconstituted from deuterated RNA and unlabeled proteins were prepared (10). Subunits depleted of protein S1 were prepared by the method of Steitz *et al.* (11). All subunits studied were fully active in polyuridylic acid-directed phenylalanine incorporation (12). The subunits were characterized on gels (Fig. 1). Scattering measurements were

Fig. 1. Ribosomal subunits used for neutron scattering. (a) Gels of 3 percent polyacrylamide, 0.5 percent agarose on the different species studied. Lane 1, 30S subunits without salt wash; lane 2, S1-depleted 30S subunits; lane 3, 30S reconstituted from deuterated 16S RNA. (b) Sodium dodecyl sulfate gels (15 percent) on 30S ribosomal proteins. Lane 1, subunits with protein S1; lane 2, reconstituted subunits.