PLATELET PLASMA MEMBRANE LECTIN ACTIVITY

T. Kent Gartner*, Danny C. Williams* and David R. Phillips+

*Department of Biology
Memphis State University
Memphis, Tennessee 38152

and

+Department of Biochemistry
St. Jude Children's Research Hospital
Memphis, Tennessee 38101

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SUMMARY

The lectin activity of human platelet and erythrocyte membranes was evaluated using trypsinized, formalinized erythrocytes from eight species. Platelet membranes had the greatest lectin activity against cow erythrocytes, but also had significant activity against human, sheep, electric eel, and rabbit erythrocytes. In contrast, erythrocyte membranes only had low lectin activity against electric eel erythrocytes with no activity against the other types of erythrocytes tested. The platelet membrane lectin activity was found to reside in protein molecules on the external surface of the platelet plasma membrane. The lectin activity of platelet membranes was inhibited by amino sugars and some basic amino acids: N-acetylated amino sugars and other neutral sugars were without effect. These results demonstrate that the external surface of the platelet plasma membrane has a specific lectin activity.

Blood platelets are responsible for the primary arrest of bleeding. In performing this function, the plasma membrane surfaces of platelets undergo change which causes them to become mutually cohesive and aggregate. Numerous proposals have been made concerning the molecular entities which mediate platelet aggregation; however, a unifying hypothesis has not been postulated (see (1) for a review).

Although a variety of approaches have been used to try to elucidate the mechanism(s) of cell-cell recognition and direct interaction for different cell types, perhaps the most provocative has developed from demonstrations of lectin activity on external cell surfaces (2-5). These demonstra-

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tions led to the idea that a primary force causing intracellular cohesion is lectin-lectin receptor interactions. This model specifies that lectins on one cell bind to receptors on an adjacent cell (5, 6). As a first step towards determining the basis of the membrane-membrane interactions which result in the aggregation of platelets, we have evaluated the lectin activity of platelet membranes. Here we report that the external surface of platelet plasma membranes have marked lectin activity which is specifically blocked by certain compounds.

MATERIALS AND METHODS

Isolation of Membranes. Human platelet plasma membranes were isolated from washed platelets (7) by the modification of the glycerol lysis technique (8) and suspended in ETS buffer (1 mM EDTA, 10 mM Tris, 154 mM sodium chloride, pH 7.4). In our initial experiments, platelet membranes were prepared within three hours of veinipuncture; however, subsequent experiments showed that outdated platelet concentrates prepared for clinical transfusion gave similar results, therefore, membranes prepared from these concentrates were used for most experiments. Human erythrocyte membranes isolated from freshly drawn blood as reported by Dodge, *et al.* (9) were suspended in the same ETS buffer.

Agglutination of Erythrocytes. Erythrocytes from eight species (cow, human, sheep, electric eel, rabbit, goat, hog and catfish) were used to assay the lectin activity of human platelet and erythrocyte membranes. The erythrocytes from the various species were washed and treated first with trypsin using the method of Lis and Sharon (10) and then fixed with formaldehyde as described by Butler (11). The treated cells were stored in phosphate buffered saline (PBS) containing 100 ppm of merthiolate (10). Agglutination of erythrocytes was assayed in microtiter "V" plates (Cooke Engineering Laboratory Products) (2, 4). To each well, we first added 25 µl of PBS buffer. Next, twenty-five µl of test membranes (containing 50-60 µg protein for platelet membranes) were added to the first well of a row of eight wells and was serially diluted (2) in a series of 6 wells. The remaining two wells containing no membranes served as controls. Finally, 25 µl of a 2.5% suspension of the trypsin treated, formalinized erythrocytes were added to each well. The contents of the wells were agitated. Each test was performed in triplicate. The contents of the wells were evaluated for agglutination after 1.5 hours at room temperature (2, 4). The definition of an agglutination unit described by Rosen, *et al.* (2) was used in this study. The number of agglutination units of an extract is defined as the reciprocal of the endpoint dilution (first dilution which gives no agglutination). Thus, a sample that had to be diluted 1:16 to attain an endpoint contained 16 agglutination units.

RESULTS AND DISCUSSION

The lectin activity of human platelet and erythrocyte membranes was evaluated by testing the ability of these membranes to agglutinate trypsinized, formalinized erythrocytes from different species. The basis of this assay is that the surface of fixed erythrocytes, which themselves lack lectin

TABLE I

LECTIN ACTIVITY OF HUMAN PLATELET AND ERYTHROCYTE MEMBRANES AGAINST ERYTHROCYTES FROM EIGHT SPECIES

Erythrocyte Source	Lectin Activity (units/mg protein)*	
	Platelet Membranes	Erythrocyte Membranes
Cow	270	ND+
Human	67	ND
Sheep	33	ND
Electric Eel	44	20
Rabbit	21	ND
Goat	ND	ND
Hog	ND	ND
Catfish	ND	ND

* Determined by dividing the number of agglutination units by the amount of protein (60 µg platelet membranes or 220 µg erythrocyte membranes) in the 25 µl sample.

+ N.D. = none detectable. The limit of detection was 17 units/mg protein for platelet membranes and 5 units/mg protein for erythrocyte membranes.

activity, have lectin receptors which can bind to lectins. Lectin activity of the sample is revealed by its ability to crosslink polyvalently and thereby agglutinate the fixed erythrocytes. As shown in Table I, human platelet plasma membranes demonstrated marked lectin activity against erythrocytes from certain species. The platelet membranes were most active against cow erythrocytes; indeed, erythrocytes from four bovine breeds were examined and all were very active with platelet membranes. Platelet membranes could not agglutinate washed, untreated human erythrocytes; however, formaldehyde fixation of the erythrocytes made them reactive in the lectin assay. The

TABLE II

SUBCELLULAR DISTRIBUTION AND TRYPSIN INACTIVATION OF PLATELET MEMBRANE LECTIN ACTIVITY

Source of Lectin:	Lectin Activity (units/mg protein)
Intact Platelets	58
Plasma Membranes	300
Membranes Incubated without Trypsin*	190
Trypsin-Treated Membranes*	ND+

* Platelet membranes (2.4 mg membrane protein per ml) were incubated for 15 min at 37°C in ETS buffer with 20 µg per ml of trypsin.

+ N.D. = none detectable.

combination of trypsinization and formaldehyde fixation caused maximum activation of the human erythrocytes for the lectin assay. In contrast, human erythrocyte membranes did not have any demonstrable activity against the erythrocytes tested, except for a weak but reproducible activity against erythrocytes from electric eel (*Electrophorus electricus*).

The lectin activity of human platelets appeared to reside in proteins located in the plasma membrane (Table II). The plasma membrane location of the lectin was demonstrated by comparing the lectin activity of isolated membranes to intact platelets. Accordingly, isolated membranes had a 6-fold increase in specific lectin activity. This increase in activity is similar to increases observed with other platelet membrane markers (12, 13). It appeared that the lectin activity of platelet membranes resides in protein molecules because trypsin, at 20 µg/ml, completely eliminated the activity (Table II). In contrast, incubation of the membranes in the absence of

TABLE III

EFFECTS OF REAGENTS ON PLATELET MEMBRANE LECTIN ACTIVITY

INHIBITION:

Sugars:	Amino Acids:
α-D-mannosamine (50%)	L-arginine (50%)
α-D-galactosamine (25%)	L-lysine (25%)
α-D-glucosamine (25%)	L-histidine (25%)

NO EFFECT:

Sugars:	Amino Acids:	Miscellaneous:
α-D-mannose	L-asparagine	Urea
α-D-galactose	L-glutamine	EDTA
α-D-glucose	Glycine	
N-acetyl-D-galactosamine	L-methionene	
N-acetyl-D-glucosamine	L-phenyl alanine	
α-D-fucose	L-valine	
α-L-fucose		
α-lactose		
Maltose		
Sucrose		

The numbers in parentheses signify the extent of inhibition of agglutination caused by a final concentration of 30 mM of the test substances. Urea at 30 mM and 200 mM caused no inhibition, however, at 1.0 M, urea caused 75% inhibition of hemagglutination. NaCl had no inhibitory effect at 30 mM, but caused 25% inhibition at 60 mM. EDTA was tested in phosphate buffered saline at a final concentration of 5 mM.

added protease caused only 40% loss in activity. This loss could have been due to one of the endogenous platelet proteases (14).

Lectin-mediated agglutinations of fixed erythrocytes typically can be inhibited by compounds which compete with receptors on the fixed erythrocytes for the active sites of the lectins (15, 16). For example, disaccharides, hexoses, hexosamines and amino acids have been found to specifically inhibit different plasma membrane-bound lectins (2, 4, 17). As shown in Table III,

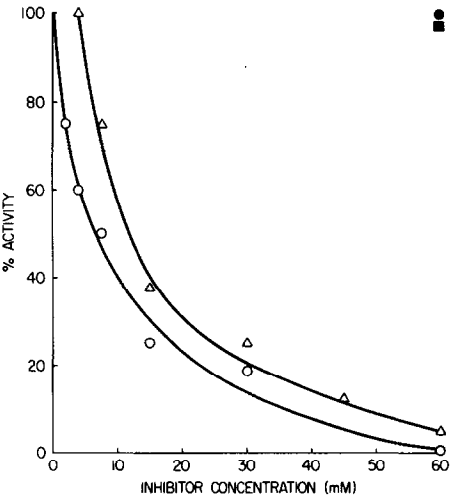

FIGURE 1 - Effects of inhibitors on human platelet membrane hemagglutination of trypsinized, formaldehyde treated cow erythrocytes. The inhibitors used were: O - arginine, Δ - mannosamine, ● - glutamine, and ■ - mannose.

we found that hexosamines specifically inhibited the human platelet membrane lectin activity towards cow erythrocytes. Hexoses and N-acetylated derivatives of the hexosamines were without effect. The inhibitory effect was not limited to the basic sugars. Some basic amino acids, e.g., arginine, lysine and histidine, also inhibited the lectin activity of human platelet membranes. This effect, however, was not the direct result of ionic strength since asparagine and glutamine as well as ionic strength adjustments with sodium chloride had respectively little or no effect on the membrane-induced agglutinations. Glycine, methionine, phenylalanine, and valine were also without effect.

Figure 1 shows the effects of concentration of two inhibitors, mannosamine and arginine on platelet membrane lectin activity. Fifty percent of the lectin activity was inhibited by 8 mM arginine or 12 mM mannosamine. By contrast, 60 mM mannose or glutamine was without effect indicating specific inhibition of the platelet lectin activity.

The platelet plasma membrane lectin activity appears to be specific: it is not found on human erythrocyte membranes; it is only active against

erythrocytes from certain species and it is specifically blocked by certain sugars and amino acids. In this regard, it is interesting that most of the compounds which inhibit the hemagglutinating activity of purified chick embryo fibroblast LETS protein (17) also inhibit the lectin activity of human platelet membranes. The primary discrepancy in the behavior of the two lectin activities appears to be the insensitivity of the platelet lectin activity to EDTA, whereas the LETS protein lectin activity is inactivated by as little as .01 mM EDTA, the platelet lectin activity is unaffected by this reagent. However, despite these apparent similarities, we do not have sufficient information about either the platelet plasma membrane lectin nor the platelet LETS protein to make a meaningful comparison of the two proteins.

To date, the functional groups on the external surface of the plasma membrane responsible for platelet aggregation have not been defined. Since the lectins on platelets can crosslink the receptors on cow erythrocytes, it may be that the lectin components of the platelet membrane mediate platelet aggregation. If this is the case, a detailed characterization of the lectin activity described here could result in elucidation of the membrane-membrane interactions which result in aggregation of platelets and simultaneously reveal a mechanism of general significance for the cohesion and adhesion of metazoan cells.

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