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INTERACTION OF LENTIL LECTIN WITH HUMAN PLATELETS. EVIDENCE AGAINST GLYCOPROTEIN II AS AGGREGATION MEDIATOR

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SUMMARY

Human platelets bind on an average of 5×10^5 molecules of lentil lectin/cell with an apparent dissociation constant of 3×10^{-7} M. The lectin binds mainly to surface glycoprotein II with an apparent molecular weight of 125,000. Lentil lectin neither caused aggregation nor did it inhibit platelet aggregation by other agents. It had no influence on the binding of thrombin to platelets or on thrombin-induced clot retraction. The hypothesis that glycoprotein II mediates platelet aggregation needs reevaluation.

Platelets are known to play an important role in hemostasis and thrombosis. When stimulated, platelets undergo a complicated series of morphological and biochemical changes leading to the formation of a hemostatic plug. Recent studies suggest that some aspects of this process might be mediated by platelet surface components, particularly the glycoproteins (reviewed in 1). Lectins are known to bind to surface receptors of a variety of cells and lead to their agglutination. Lectins also precipitate polysaccharides and glycoproteins which, like cell agglutination, may be inhibited with specific sugars (2). Because of these properties, the interaction of lectins with glycoproteins is, in many ways, analogous to antigen-antibody reaction and serves as useful probes to explore structure-function relationship of cell surface glycoproteins. An added advantage of lectins is that their combining sites are small allowing for a more specific type of reaction (2). In this study, we have explored the interaction of lentil lectin with human platelets. We show that lentil lectin binds tightly to platelets but it neither causes aggregation nor does it affect aggregation by other agents.

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MATERIAL AND METHODS

Blood was collected from healthy volunteers in plastic bags or syringes utilizing 0.1 vol of 3.8% sodium citrate as anticoagulant. The red cells were removed by differential centrifugation and the platelet-rich plasma (PRP) was collected. If necessary, platelets were washed as described and resuspended in phosphate-buffered saline (PBS) (3).

Lentil lectin was purchased from Sigma Chemical Co., St. Louis, Missouri. It contained both lentil A and lentil B which are known to be identical in terms of erythrocyte binding and mitogenicity for lymphocytes (4). The lectin was labeled with ^{125}I with chloramine T. The specific radioactivity of the lectin was in the range of 3.0 to 8.0 x 10^5 cpm/μg. The binding of the lectin to platelets was determined at least in duplicates by Millipore filtration (5). Different amounts of the labeled lectin was incubated with a constant number of washed platelets for 15 min at room temperature in a total volume of 1 ml. The samples were then filtered under reduced pressure and the filters washed with 4 ml of PBS. Nonspecific binding was determined by filtering equal amounts of lectin in the absence of platelets. The filters were finally counted in a Packard autogamma spectrometer.

Platelet aggregation was measured in a dual channel aggregometer (Payton, Buffalo, New York) (6). The experimental sample was analyzed in one channel while a control was run in the other. The amounts of stimulants shown in Table I are final concentrations in a total volume of 0.5 ml. The procedures for gel electrophoresis and lectin staining of the separated platelet components have been described (5). After electrophoresis, the gel lane was fixed and washed repeatedly until the pH of the washing buffer (PBS) was 7.0. The gel was then incubated overnight with the labeled lectin containing 0.5% albumin. Excess, unbound lectin was removed by repeated washing of the gel with PBS until the radioactivity in the washing fluid reached a constant, low value. The gels were dried under vacuum and left in contact with Kodak x-ray films in the cold. The films were developed after 1-3 days. Other details are provided in figure legends.

RESULTS AND DISCUSSION

The binding of ^{125}I-labeled lentil lectin to fresh platelets is shown in Fig. 1. The binding curve showed saturation kinetics similar to those observed in studies on the binding of phytohemagglutinins to platelets or erythrocytes (4). The amount of lectin bound increased with increasing concentration of lectin added with a distinct break at about 30 to 40 μg/ml of the lectin. Analysis of double reciprocal plots of the data indicated that there are about 5 x 10^5 sites/platelet for lentil lectin with an apparent dissociation constant of 4 x 10^{-7} M. These data compare favorably with the values of 8 x 10^5 sites/cell and an apparent dissociation constant of 3.2 x 10^{-7} M reported for the binding of lentil lectin to formaldehyde-fixed platelets (5). Thus, both fresh and fixed platelets are capable of binding lentil lectin with similar parameters.

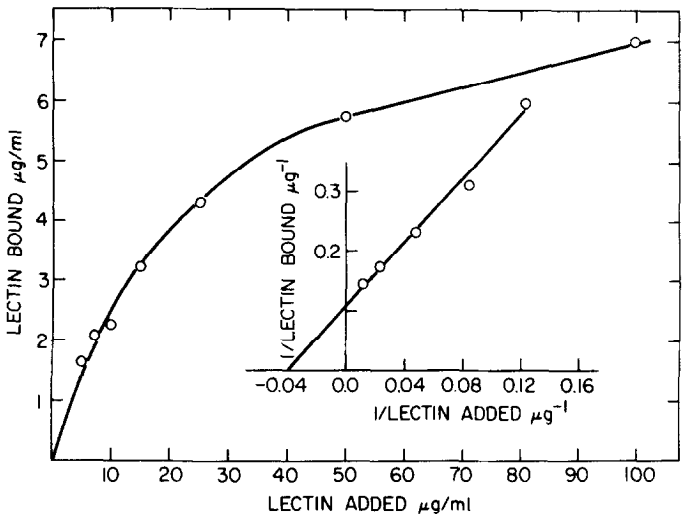

Fig. 1. Binding of lentil lectin to washed platelets in phosphate-buffered saline. Platelets (2.5×10^8) were incubated with different amounts of [^{125}I]lentil lectin for 15 min at room temperature in a total volume of 1 ml. The amount of lectin bound to platelets was determined by millipore filtration. The insert shows a double reciprocal plot of the binding data for the determination of the number of binding sites and the apparent dissociation constant.

Platelet membranes were isolated by density gradient centrifugation and analyzed by SDS gel electrophoresis. Staining for protein revealed a number of bands ranging in molecular weight from 200,000 to 25,000. Staining with [^{125}I]lentil lectin showed a prominent component with an apparent molecular weight of 125,000. A number of other faint bands were also observed (Fig. 2a). Densitometric scanning of autoradiographs again showed a prominent peak and other smaller peaks (Fig. 2b). These data confirm previous reports based on affinity chromatography that lentil lectin binds mainly to platelet GP-II and more weakly to other surface components (7, 8).

Platelets in plasma were incubated with different amounts of lentil lectin at 37° for 10 min. The lectin at saturating concentrations neither aggregated platelets nor did it affect platelet aggregation by thrombin or wheat germ agglutinin or ADP (Table I). In contrast, wheat germ agglutinin at these concentrations caused 50% to 80% aggregation of platelets. Since plasma contains glycoproteins, the experiments were repeated with washed platelets and the results were the same as with PRP. Furthermore, lentil lectin did

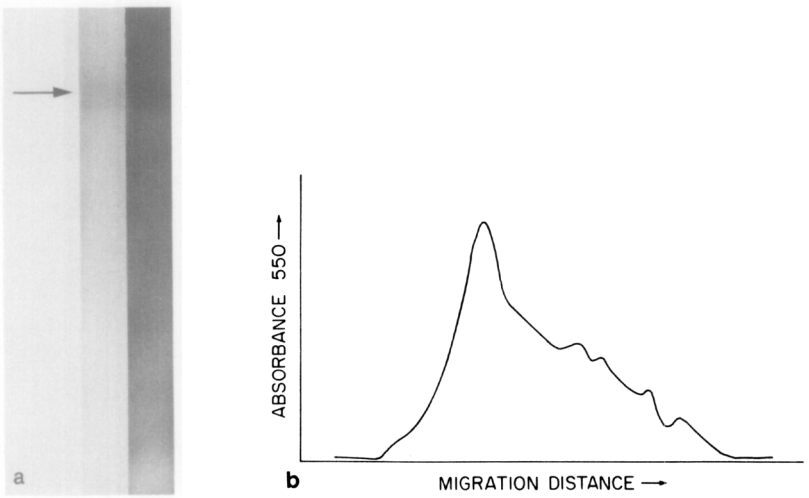

Fig. 2. Specificity of binding of lentil lectin to platelets. Isolated platelet membranes were solubilized and the components separated by SDS gel electrophoresis. Gel lanes were stained with [^{125}I]lentil lectin and autoradiographs were developed after 24 hrs. (a) Autoradiographs of two different gels are shown. The most prominent band (arrow) has an apparent molecular weight of 125,000. (b) Densitometer scan of an autoradiograph which showed one prominent peak and other smaller peaks.

TABLE I. Effect of Lentil Lectin on Platelet Aggregation

Lectin µg	Stimulant	Aggregation %*
50	0	0
100	0	0
0	Thrombin 80 mU	70
30	Thrombin 80 mU	70
50	Thrombin 80 mU	70
0	Adenosine diphosphate 10^{-5} M	50
30	Adenosine diphosphate 10^{-5} M	55
50	Adenosine diphosphate 10^{-5} M	55
0	Wheat germ agglutinin 40 µg	50
50	Wheat germ agglutinin 40 µg	50

*Average of four determinations

neither block clot retraction of PRP by thrombin (Fig. 3) nor did it affect the binding of labeled thrombin to platelets. These results show that lentil lectin binds to platelets but has no influence on platelet aggregation or clot retraction.

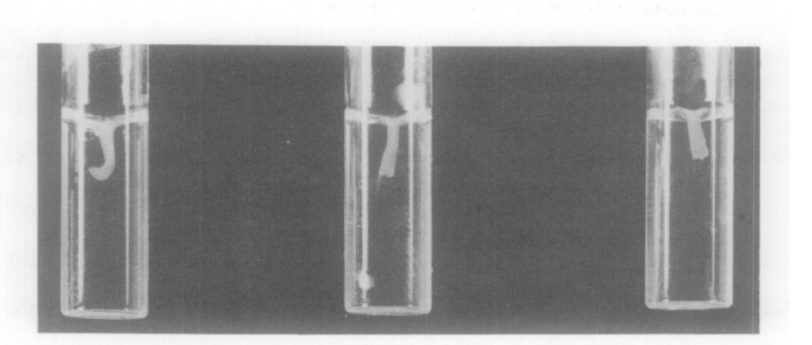

Fig. 3. Lack of effect of lentil lectin on clot retraction of platelet-rich plasma by thrombin (0.4 U/ml). The first tube on the left is control without lectin, the next two tubes contained 30 µg/ml and 50 µg/ml of the lectin respectively.

Recent studies with platelets from patients with different pathological conditions have revealed specific defects in their surface glycoproteins (1). A number of investigators has shown that thrombasthenic platelets are deficient in GP-II and GP-III (or GP-II_b and GP-III_a) (1, 8). Thrombasthenia is a congenital bleeding disorder in which the platelet count as well as the initial response of platelets (binding, shape change, secretion) to stimuli are normal but they fail to aggregate and do not show clot retraction. The crucial question is whether these glycoprotein deficiencies are responsible for the functional defects observed in thrombasthenic platelets. Monovalent fragments of an antibody to GP-III (or GP-III_a) did not affect platelet aggregation by any of the agents tested suggesting that this glycoprotein may not be involved in platelet aggregation (9). This finding has led to the hypothesis that GP-II plays a vital role in platelet aggregation. This concept was supported by the observation that normal platelets treated with subagglutinating concentrations of an alloantibody (IgG) detected in a thrombasthenic patient did not aggregate by a variety of agents (1). Double antibody precipitation studies suggested that the antibody may be directed to a platelet surface glycoprotein of molecular weight 120,000 (1). It is known that the platelet surface contains F_c receptors and results obtained with intact IgG are difficult to interpret (10). Heterologous antibodies to platelets or fibrinogen antibodies induce defects in platelet aggregation (11,

12). It is essential to show that normal platelets treated with monovalent Fab fragments of the alloantibody will not aggregate but will undergo normal release reaction when stimulated. The results of the present study show that lentil lectin binds tightly to platelets, primarily to GP-II. However, the lectin at saturating concentrations neither caused platelet aggregation nor did it affect aggregation by other agents or clot retraction by thrombin. The hypothesis that GP-II plays a crucial role in platelet aggregation by all physiological agents should be reevaluated.

ACKNOWLEDGMENTS

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REFERENCES

1. Nurden, A. T., and Caen, J. P. (1979) Semin. Hematol. 16, 234-250.
2. Sharon, N., and Lis, H. (1972) Science 177, 949-959.
3. Ganguly, P. (1972) J. Biol. Chem. 247, 1809-1816.
4. Majerus, P. W., and Brodie, G. N. (1972) J. Biol. Chem. 247, 4253-4257.
5. Ganguly, P., Gould, N. L., and Sidhu, P. (1979) Biochim. Biophys. Acta 586, 574-583.
6. Ganguly, P. (1977) Biochim. Biophys. Acta 498, 21-27.
7. Clemetson, K. J., Pfueller, S. L., Luscher, E. F., and Jenkins, C.. S. P. (1977) Biochim. Biophys. Acta 464, 493-508.
8. Phillips, D. R., and Agin, P. P. (1977) J. Clin. Invest. 60, 535-545.
9. Kaplan, K. L., and Nachman, R. L. (1974) Br. J. Haematol. 28, 551-560.
10. Moore, A., Ross, G. D., and Nachman, R. L. (1978) J. Clin. Invest. 62, 1053-1060.
11. Clancey, R., and Firkin, B. (1973) Thromb. Res. 3, 375-381.
12. Tollefsen, D. M., and Majerus, P. W. (1975) J. Clin. Invest. 55, 1259-1268.