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SYNERGISTIC EFFECTS OF LECTINS IN THE INTERACTION OF THROMBIN WITH HUMAN PLATELETS

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Summary

(1) Several lectins have been studied for their effects on the interaction of thrombin with human platelets. Wheat germ agglutinin, concanavalin A and *Ricinus communis* lectin increased the number of high affinity sites for diisopropylphosphothrombin on washed platelets from 3000 to about 12 000 but the binding affinities were unchanged (K_d approx 4 nM). Two other lectins, *Lens culinaris* and *Bandieria simplicifolia*, were without effect.

(2) Using formalinized platelets to avoid possible complications of the platelet release reaction, wheat germ agglutinin showed a marked increase (5-fold) in the binding of active thrombin, peanut agglutinin had no effect while *Ricinus communis* and *Bandieria simplicifolia* showed marginal increases (2-fold). Thrombin binding was decreased to about one quarter with *Lens culinaris*, *Phaseolus vulgaris* and concanavalin A.

(3) Wheat germ agglutinin caused a synergistic increase of platelet aggregation at low concentrations of thrombin (12.5 mU/ml) and ADP (1 μM), both in the absence and presence of added fibrinogen, but had no effect on ristocetin-induced aggregation.

Introduction

Thrombin binds to, and stimulates, a variety of different cell types including platelets [1—3], endothelial cells [4] and fibroblasts [5]. In the case of platelets, the binding of thrombin exhibits complex kinetics indicative of negative

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cooperativity. However, it is not known whether there is a single molecular class of binding sites or, alternatively, whether two distinct molecular species of binding site may be present [6]. In any event, about 500 sites of high affinity for thrombin (K_d 3 nM) have been detected, although this can be increased to about 3000 under appropriate conditions [7], together with 30 000 sites of lower affinity (K_d 30 nM).

A glycoprotein which appears to contain the major thrombin-binding activity of human platelets has been isolated and purified to homogeneity [8]. This glycoprotein, termed glycocalicin, is released from the platelet surface in soluble form following platelet homogenization and appears to have close similarities to platelet glycoprotein I, which remains bound to the membrane, both in its orientation at the platelet surface and in its immunological and functional properties [8—10]. Purified glycocalicin inhibits thrombin-induced aggregation [11] and is a competitive inhibitor of the binding of thrombin to the platelet surface [12]. Furthermore, the amount of thrombin bound is proportional to the amount of glycocalicin/glycoprotein I present on the surface of normal platelets [12] and in platelets from patients with a variety of bleeding disorders [13—15].

We have addressed the question of whether the amount of thrombin bound to the platelet surface with high affinity could be a reflection of the degree of clustering of binding sites within the platelet membrane. Structural studies have shown that the thrombin-binding segment of glycocalicin/glycoprotein I resides in the carbohydrate-poor peptide portion (M_r 45 000) of the molecule [12]. We reasoned that lectins might cause the association of molecules of glycocalicin/glycoprotein I within the plane of the membrane by interacting with the macroglycopeptide without inhibiting the reversible access of thrombin to the binding site in the carbohydrate-poor tail portion of the membrane glycoprotein.

The present data show that, of all the lectins examined, only wheat germ agglutinin can increase significantly the amount of thrombin bound to both normal and formalinized platelets. In addition, this increased binding results in increased platelet aggregation with normal platelets with respect to both thrombin and ADP.

Materials and Methods

The lectins from *Bandieria simplicifolia*, *Ricinus communis*, *Phaseolus vulgaris*, and peanut agglutinin were obtained from Vector Laboratories, Burlingame, CA; concanavalin A from Pharmacia Fine Chemicals, Piscataway, NJ; and wheat germ agglutinin from Miles-Yeda, Ltd. Elkhart, IN. Lectins were used as purchased without further purification. $Na^{125}I$ (carrier-free) was obtained from New England Nuclear Co. Boston, MA. Sucrose (density gradient grade) was purchased from Schwartz-Mann, Spring Valley, NY. The bovine serum albumin used in the binding buffer was from Sigma Chemical Company, St. Louis, MI. Cohn Fraction III was a gift from the Michigan State Department of Health Laboratories, East Lansing, MI. All other chemicals were of analytical grade.

Human thrombin of spec. act. 2500—2900 NIH U/mg was prepared from

Cohn Fraction III by the method of Martin et al. [17]. Thrombin activity was determined by the plasma clotting time on a Fibrometer (BBL, Cockeysville, MD, Model 5). An extinction coefficient for thrombin ($E^{1\%}_{280} = 16.2$) measured in 0.21 M NaCl/0.02 M sodium phosphate, pH 7.2 at 280 nm was used to determine thrombin concentrations [18]. Esterase activity of thrombin was measured with the chromogenic substrate A2238 (Kabi, Stockholm, Sweden).

^{125}I-labeled thrombin. Thrombin was iodinated by the chloramine-T method [19]. The presence of benzamidine during the iodination procedure was found to enhance stability during storage [17].

^{125}I-labelled diisopropylphosphothrombin. Radioiodinated thrombin was reacted with diisopropylfluorophosphate [1]. The reaction was allowed to continue until complete loss of activity was indicated by the plasma clotting time. The ^{125}I-labelled diisopropylphosphothrombin was dialyzed for 6 h at 4°C against 3 × 500 ml changes of 0.75 M NaCl in 0.05 M sodium phosphate, (pH 7.2). The final product did not clot fibrinogen and no release was observed in the Lumiaggregometer with gel filtered platelets at a concentration of 3 U/ml.

Washed platelets. Platelet-rich plasma, anticoagulated with citrate phosphate-dextrose, was prepared from fresh human blood obtained from the Washington Region, American Red Cross Blood Services. Platelets were isolated from the platelet-rich plasma by centrifugation ($800 \times g$ for 15 min), the pellet was resuspended in the Tris-EDTA buffer of Phillips [20] (hereafter referred to as Phillips' buffer) and contaminating red cells were removed by further centrifugation for 1 min at $1600 \times g$. Platelets were then centrifuged at $800 \times g$ for 15 min, and the pellet resuspended and washed twice with Tris-cacodylate binding buffer (0.15 M Tris/0.14 M sodium cacodylate/5 mg/ml bovine serum albumin, pH 7.4) and diluted to a final concentration of $0.5—4 \cdot 10^9$ platelets/ml.

Formalinized platelets. After the second washing, the platelet pellet was resuspended in Phillips' buffer and formalinized by the method of Allain et al. [21]. The formalinized platelets were stored at 4°C in 0.05 M imidazole/0.11 M NaCl and 0.02% sodium azide. Before use in the binding studies, the platelets were centrifuged and washed once with Tris-cacodylate binding buffer.

Measurement of thrombin binding. Washed platelets, at a final concentration of 10^9/ml were incubated in the presence or absence of the various lectins for 20 min at room temperature in Tris-cacodylate buffer containing 1% bovine serum albumin. ^{125}I-labelled diisopropylphosphothrombin was added to a final concentration range of 3—250 mU/ml (0.3—2.5 nM). The total volume of the reaction mixture was 0.1 ml. After 20 min incubation, the platelets were separated from the suspending solution by centrifugation through a discontinuous sucrose gradient in 1 ml polypropylene conical centrifuge tubes as follows [22]: the reaction mixture was layered onto 0.7 ml of a sucrose gradient composed of 0.1 ml of 262 mM sucrose beneath 0.6 ml of 131 mM sucrose, both solutions being 25 mM in Tris-HCl at pH 7.4, 56 mM in NaCl, and 1% in bovine serum albumin. Following centrifugation at $5000 \times g$ for 5 min, the supernatant solution was removed and counted for the determination of unbound thrombin. The tip of the centrifuge tube was cut off and radioactivity determined as the measure of bound thrombin. Nonspecific binding of thrombin was

determined in identical mixtures where a 2000-fold excess of unlabelled thrombin was added after 20 min incubation. Radioactivity was measured on an LKB 1280 gamma counter.

Measurement of thrombin binding to formalinized washed platelets in the presence of lectins was performed with ^{125}I-labelled thrombin employing the same technique.

All data were plotted on double reciprocal plots according to the method of Steck and Wallach [23]. However, numerical values for binding parameters were calculated directly using a programmable calculator (Hewlett Packard, Model 65).

Aggregation studies. Aggregation of platelet suspensions was analyzed at 37°C in a Payton aggregometer. Platelet suspensions were prepared by gel filtration of platelet-rich plasma over Sepharose 2B 24 using modified Tyrode's elution buffer (136 mM NaCl/2.7 mM KCl/0.47 mM NaH_2PO_4/0.01 M $NaHCO_3$/2 mM $MgCl_2$, and 0.2% bovine serum albumin, pH 7.4). Lectin solution (10 μl) or buffer was then added to 0.5 ml of the platelet suspension ($2 \cdot 10^8$ cells/ml) made to 1.5 mM with respect to Ca^{2+} and the mixture incubated for 1 min with stirring in the aggregometer cell. Aggregation was initiated by the addition of thrombin or ADP at final concentrations as described. Ristocetin-induced aggregation was studied at a concentration of 1.5 mg/ml.

Results

Binding of thrombin to washed platelets

The effect of lectins on the binding of ^{125}I-labelled thrombin to washed platelets was monitored using disopropylphosphothrombin, which is catalytically inactive but which binds normally to platelets [1], to eliminate complications due to induction of platelet aggregation and release. Concentrations of lectins were chosen which did not themselves induce agglutination of washed platelets.

In the absence of added lectins, a linear double-reciprocal plot was obtained for the binding of thrombin to washed human platelets (Fig. 1). From these data, a value of 3300 high affinity sites with K_d 4.4 nM was calculated (Table I); these values are in good agreement with standard values under these conditions (7,9).

Marked increases in the amount of thrombin bound were found in the presence of *Ricinus communis* lectin, wheat germ agglutinin and concanavalin A with values for the number of high affinity sites being approx. 11 000, 14 000 and 14 000, respectively (Fig. 1; Table I). In the presence of *Bandieria simplicifolia* and *Lens culinaris* lectins, the amount of thrombin bound (5000—6000 sites/cell) was marginally above that of the control.

The dissociation constants for the binding of thrombin to lectin-treated platelets did not show significant differences from controls (range 2.3—5.0 nM), even for those lectins which showed the greatest increase in the amount of thrombin bound (Table I).

Binding of thrombin to formalinized platelets

Since formalinized platelets can bind thrombin normally but do not aggre-

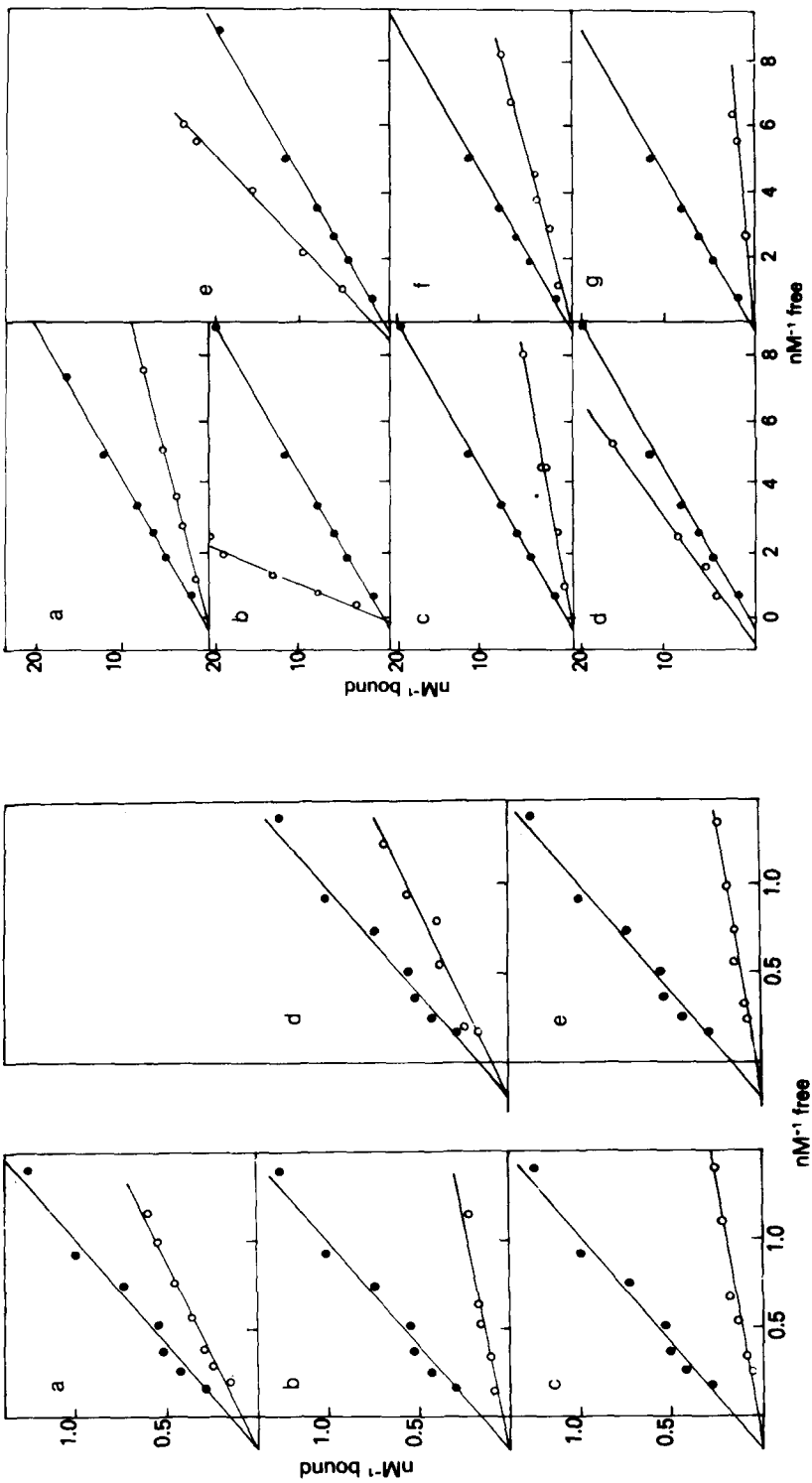

Fig. 1. High affinity binding of diisopropylphosphothrombin (3—250 mU/ml, 0.03—2.5 nM) to washed platelets in the presence of different lectins (25 μg/ml): Closed circles are control values in each case. (a) *Bandieria simplicifolia* lectin; (b) Concanavalin A; (c) *Lens culinaris* lectin; (d) Wheat germ agglutinin; (e) *Ricinus communis* lectin.

Fig. 2. High affinity binding of thrombin (3—250 mU/ml, 0.03—2.5 nM) to formalinized platelets in the presence of different lectins (25 μg/ml). Controls are closed circles in each case. (a) *Bandieria simplicifolia* lectin; (b) Concanavalin A; (c) *Lens culinaris* lectin; (d) Wheat germ agglutinin; (e) *Ricinus communis* lectin; (f) *Phaseolus vulgaris* hemagglutinin-E; (g) peanut agglutinin.

TABLE I

HIGH AFFINITY BINDING OF THROMBIN TO HUMAN PLATELETS

Platelets were washed with diisopropylphosphothrombin. n.d., not determined.

Lectin added	Specificity	Washed platelets		Fixed platelets	
		Sites/platelet	K_d (nM)	Sites/platelets	K_d (nM)
None	—	3300	4.4	1100	4.0
Wheat germ agglutinin	GlcNAc	14 100	3.9	5800	6.9
Concanavalin A	Glc, Man	14 200	5.0	450	6.8
R. communis	Gal	11 200	2.3	2500	4.3
B. simplicifolia	Gal, GalNAc	5200	3.6	3000	5.0
L. culinaris	Glc, Man	5800	4.3	300	1.8
Phytohemagglutin E	Gal-GlcNAc	n.d.	n.d.	300	1.5
Peanut agglutinin	Gal-GlcNAc, Gal	n.d.	n.d.	1200	4.6

gate in response to it [12], the effect of lectins was examined in this system in order to avoid complications from platelet stimulation by the lectins in the unfixed platelet system. In the absence of added lectins, the binding of ^{125}I-labelled thrombin to formalinized platelets gave a value of about 1100 sites (K_d 4 nM), values similar to that of the unformalinized control within the limitations of this type of assay (Fig. 2, Table I).

In the presence of wheat germ agglutinin the number of high affinity sites for thrombin increased to 6000 while with the lectins of *Ricinus communis* and *Bandieria simplicifolia* there was a marginal increase in high affinity thrombin binding to 2500—3000, but dissociation constants remained in the normal range in each case (4—6 nM) (Fig. 2; Table II). In contrast to the results with unfixed platelets, the addition of *Lens culinaris* and concanavalin A to fixed platelets resulted in a decrease in high affinity binding of thrombin to values of about 3—400 sites per platelets, but with dissociation constants of about 2 nM in the former case and 7 nM in the latter (Fig. 2d,e; Table I). Because of this unexpected decrease with fixed platelets we examined the binding of thrombin with two other lectins to determine whether a consistent pattern could be observed: *Phaseolus vulgaris* agglutinin also caused a reduction to one fourth in the amount of thrombin bound while peanut agglutinin showed no effect (Fig. 2f,g; Table I). This may indicate an inability of peanut agglutinin to bind to platelets since this lectin will cause agglutination of platelets only after they have been treated with neuraminidase to expose haptenic residues [25].

Effect of lectins on platelet aggregation

The binding studies showed that only wheat germ agglutinin caused an increase in high affinity thrombin binding in both normal and fixed platelets. However, in order to evaluate both binding and activation, aggregation studies with thrombin were carried out using gel filtered platelets in the presence of each of the lectins. In each case, the lectin concentration used did not itself induce either agglutination or the platelet release reaction within the time course of the experiment. The lectins from *Bandieria simplicifolia*, *Phaseolus vulgaris*, *Lens culinaris* and peanut agglutinin, which did not cause a synergistic

increase in thrombin binding, were also without effect on thrombin-induced platelet aggregation. *Ricinus communis* lectin, which had caused a slight increase in thrombin binding to normal platelets, but not to fixed platelets, also did not affect thrombin-induced platelet aggregation. As expected from previous work [26], thrombin-induced aggregation was completely inhibited by concanavalin A although the platelet shape change and release reaction were unaffected (data not shown).

Only in the case of wheat germ agglutinin was a synergistic response observed in thrombin-induced platelet aggregation (Fig. 3A). Wheat germ agglutinin (10 μg/ml) alone, added at zero time, did not cause platelet agglutination (curve 1). Similarly, the addition of low concentrations of thrombin (12.54 μ/ml, 0.125 nM) to gel filtered platelets (at the arrow, ↓) did not cause significant aggregation (curve 2). However, when thrombin at the same concentration was added to gel filtered platelets containing wheat germ agglutinin, there was a marked synergism and maximum platelet aggregation was observed (curve 6). This synergistic effect was completely inhibited by EDTA (4 nM), by the use of platelets which had been treated with aspirin in vitro or in vivo, or if the haptenic sugar, *N*-acetylglucosamine (10 mM), was added prior to the addition of thrombin. Furthermore, addition of *N*-acetylglucosamine during the course of the aggregation decreased the synergistic effect but the amount of the decrease became less marked as the aggregation proceeded (curves 3, 4 and 5).

ADP-induced aggregation was also studied in order to determine whether synergism was unique to thrombin or was a relatively non-specific effect of

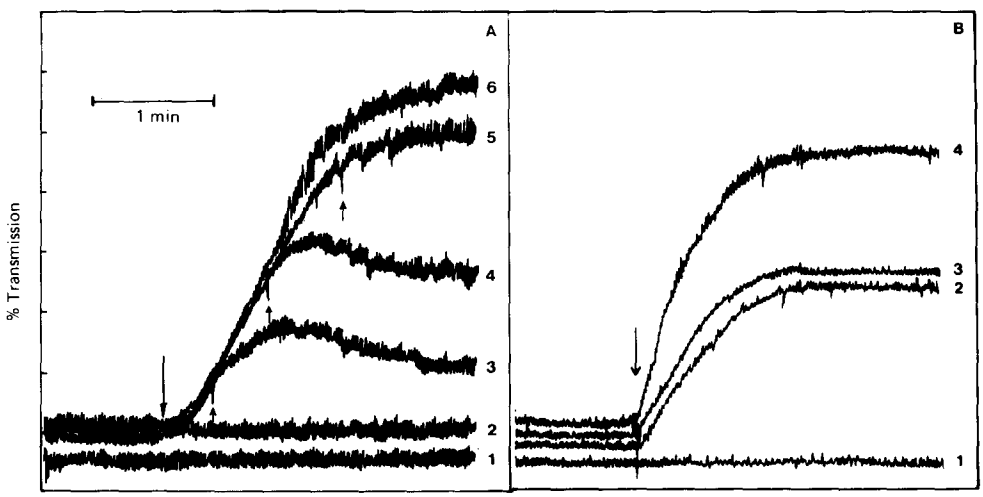

Fig. 3. Effect of wheat germ agglutinin on aggregation of gel filtered platelets. Wheat germ agglutinin (5 μg/ml) was added at zero time in each case. (A) With thrombin. Curve 1 is wheat germ agglutinin alone; curve 2 is thrombin alone (12.5 mU/ml) added at the arrow (↓); curve 6 shows the synergistic effect of wheat germ agglutinin but without added fibrinogen; curve 4 is ADP plus wheat germ agglutinin in the presthe arrows (↑) during the course of the synergistic aggregation. (B) With ADP (1 μM). Curve 1 is the control with ADP added at the arrow (↓) in the absence of added wheat germ agglutinin; curve 2 is with ADP plus fibrinogen (1 mg/ml) in the absence of wheat germ agglutinin; curve 3 is ADP in the presence of wheat germ agglutinin but without added fibrinogen; curve 4 is ADP plus wheat germ agglutinin in the presence of added fibrinogen. Comparison is between curves 1 and 3, and between 2 and 4.

wheat germ agglutinin. Wheat germ agglutinin also caused a synergistic stimulation of ADP-induced aggregation of gel-filtered platelets. This stimulation occurred even in the absence of added fibrinogen but was marked when exogenous fibrinogen was added. These results are shown in Fig. 3B in which curves 1 and 3 show that synergistic effect of wheat germ agglutinin in the absence of added fibrinogen while curves 2 and 4 show the effect in its presence.

Ristocetin agglutinates platelets by a different mechanism from other aggregating agents and its action does not require viable platelets [21]. At the concentrations that induced synergistic aggregation with ADP or thrombin, wheat germ agglutinin (5 μg/ml) was without effect on ristocetin-induced aggregation. Concanavalin A (50 μg/ml) also did not affect ristocetin-induced aggregation.

Discussion

Plant lectins have been shown to induce platelet aggregation and release [27] and quantitative measurements have been carried out on the direct binding of various lectins to intact platelets [28—31]. In the present work, the addition of plant lectins to washed platelets has been shown to have an indirect effect in causing a 4-fold increase in the amount of thrombin bound in the case of three of the five lectins examined. However, the affinity of binding of thrombin to platelets in the presence of these lectins, as distinct from the amount bound, was not greatly affected by any of the lectins and dissociation constants were in a similar range to control values (K_d approx. 4 nM). On the other hand, the stimulation of thrombin binding did not appear to be directly related to the haptenic sugar specificity of the individual lectin: for example, binding was increased by concanavalin A, with specificity for glucose and mannose, but not by *Lens culinaris* lectin, which has the same specificity. Similarly, thrombin binding was increased by *Bandieria simplicifolia* lectin (galactose, *N*-galactosamine). Interestingly, treatment of platelets with thrombin doubles the amount of lentil lectin bound [28] even though this lectin does not affect the amount of thrombin bound.

The effects of lectins on the binding of thrombin to washed platelets, even using blocked thrombin, might be complicated by the induction of low levels of platelet release which is known to occur with a variety of lectins [27]. However, we have previously shown that the binding of thrombin to formalinized platelets gives identical values for the number of high affinity sites (~3000) and for their dissociation constant (~3 nM) to that found in unformalinized controls. In addition, over a period of 7 days there was a doubling in the number of high affinity sites (3000 → 6000), but no change in the affinity at these sites, suggesting a residual mobility of binding sites within the membrane [12]. For these reasons, lectin effects on thrombin binding were studied with fixed platelets. In this case, binding was markedly increased (5-fold) only by wheat germ agglutinin while the increase (2-fold) by *Ricinus communis* and *Bandieria simplicifolia* are probably not significant considering the limitations of this type of assay. Surprisingly, three of the lectins (concanavalin A, *Lens culinaris* and *Phaseolus vulgaris*) caused a reduction to about one-fourth of control with fixed platelets: the reason for this reduction is not known. The affinities of

binding in the presence of lectins with fixed platelets were similar to control values in the absence of lectins irrespective of the amount of thrombin bound.

These results were all obtained in the concentration range for high affinity binding of thrombin to platelets (1—3 nM). At higher thrombin concentrations (>3 nM) both washed platelets and fixed platelets were agglutinated in the presence of lectins even without added calcium suggesting that different mechanisms were involved from those observed at low thrombin concentrations. Because of this agglutination, binding studies were not carried out at the high thrombin concentrations necessary to determine binding in the low affinity range.

The activity of thrombin is known to be inhibited by concanavalin A [32]. One possible explanation of the increased binding of thrombin in the presence of lectins was that it could be binding to unfilled carbohydrate valences of a lectin already bound to the platelet. Because of the possibility of lectin interactions with the glycoprotein components of fibrinogen, we used the chromogenic substrate S2238 to measure thrombin activity. None of the lectins affected the thrombin-induced hydrolysis of S2238 at the concentrations used (25 μg/ml) suggesting that platelet-bound lectin did not contribute significantly to the observed lectin effects.

Of the various lectins examined, only wheat germ agglutinin showed a synergistic increase in thrombin binding in both the washed (diisopropylphosphothrombin) and fixed platelet systems. This suggested that the proposed interactions with glycocalicin/glycoprotein I might be of importance in the high affinity binding of thrombin since wheat germ agglutinin is known to react directly with the macroglycopeptide portion of the molecule [8]. On the other hand, these observations could be due, for example, to a generalized effect on membrane microviscosity which has been shown to occur with lymphocyte membranes in the presence of various lectins [33,34]: this aspect is currently under investigation. Since wheat germ agglutinin reacts with terminal sialic acid in glycoproteins and glycolipids [35], it might be expected to have broad effects on platelet membrane function. Using gel filtered platelets, it was found that only wheat germ agglutinin caused a synergistic increase in the aggregation response with thrombin and that the other lectins were without effect, except for the known inhibition by concanavalin A due to secondary effects [26]. This synergism by wheat germ agglutinin was inhibited by subsequent addition of GlcNAc but the inhibition was not due to any toxic effects of the haptenic sugar since normal aggregation with thrombin was observed in its presence.

The stimulation of ADP-induced aggregation demonstrated that the synergistic effects of wheat germ agglutinin were not confined to thrombin. The nature of the ADP receptors of platelets is not known: if they are glycoprotein or glycolipid in nature, it is possible that the wheat germ agglutinin effects could be due to the clustering mechanism proposed for glycocalicin/glycoprotein I.

During the course of this work a report has appeared on the binding of thrombin to fixed platelets in the presence of two of the seven lectins studied here; namely, wheat germ agglutinin and *Lens culinaris* lectin [36]. The two studies are in general agreement with regard to the binding of these two lectins. However, we found that wheat germ agglutinin, at the low concentrations

(5 μg/ml) necessary to show the synergistic response with thrombin and ADP in the washed platelet system, did not affect aggregation induced by ristocetin although higher concentrations (50 μg/ml) have been reported to block the agglutination of fixed platelets [36,37].

Certain correlations can be made in the present study on the basis of the number of lectins employed, the use of both washed platelets (with diisopropylphosphothrombin) and fixed platelets in the binding assays and the study of aggregation responses to the thrombin. First, quantitative data obtained on thrombin binding with fixed platelets cannot automatically be extended to the interpretation of mechanisms in untreated platelets because of unexplained variations between the two systems; second, only in the case of wheat germ agglutinin can elevated binding of thrombin be observed in both the fixed and washed platelet systems; third, in the case of gel-filtered platelets this enhanced binding of thrombin was associated with enhanced aggregation; fourth, this enhanced aggregation was dependent on the continued presence of wheat germ agglutinin since it reverted to base line levels in the presence of the haptenic sugar, *N*-acetylglucosamine; fifth, the synergistic effect on platelet stimulation is not unique to thrombin but is observed with other aggregating agents such as ADP.

It will be of interest to determine whether similar synergistic effects of wheat germ agglutinin are seen with other cells which bind thrombin such as endothelial cells and fibroblasts.

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References

1 Tollefsen, D.M., Feagler, J.R. and Majerus, P.W. (1974) J. Biol. Chem. 249, 2646—2651
2 Martin, B.M., Feinman, R.D. and Detwiler, T.C. (1975) Biochemistry 14, 1308—1314
3 Ganguly, P. (1974) Nature 247, 306—307
4 Awbrey, B.J., Hoak, J.C. and Owen, W.G. (1979) J. Biol. Chem. 254, 4092—4095
5 Carney, D.H. and Cunningham, D.D. (1978) J. Supramol. Struct. 9, 337—350
6 Workman, E.F., White, G.C. and Lundblad, R.L. (1977) J. Biol. Chem. 252, 7118—7123
7 Shuman, M.A. and Majerus, P.W. (1975) J. Clin. Invest. 56, 945—950
8 Okumura, T., Lombart, C. and Jamieson, G.A. (1976) J. Biol. Chem. 251, 5950—5955
9 Okumura, T. and Jamieson, G.A. (1976) J. Biol. Chem. 251, 5944—5949
10 Solum, N.O., Hagen, I., Filion-Myklebust, C. and Stabaek, T. (1980) Biochim. Biophys. Acta 597, 235—246
11 Okumura, T. and Jamieson, G.A. (1976) Thromb. Res. 8, 701—706
12 Okumura, T., Hasitz, M. and Jamieson, G.A. (1978) J. Biol. Chem. 253, 3435—3443
13 Bolin, R.B., Okumura, T. and Jamieson, G.A. (1977) Natire 269, 69—70
14 Jamieson, G.A. and Okumura, T. (1978) J. Clin. Invest. 61, 861—864
15 Bolin, R.B., Okumura, T. and Jamieson, G.A. (1977) Am. J. Hematol. 3, 63—71
16 Ganguly, P., Sutherland, S.B. and Bradford, H.R. (1978) Br. J. Haematol. 39, 599—605
17 Martin, B.M., Wasieski, W.W., Fenton, II, J.W. and Detwiler, T.C. (1976) Biochemistry 15, 4886—4893
18 Kezdy, F.J., Lorand, L. and Miller, K.D. (1965) Biochemistry 4, 2302—2308
19 Hunter, W.M. (1967) in Handbook of Experimental Immunology (Weir, D.M., ed.), p. 608, Blackwell, Oxford

20 Phillips, D.R. (1972) Biochemistry 11, 4582—4588
21 Allain, J.P., Cooper, H.A., Wagner, R.H. and Brinkhous, K.M. (1975) J. Lab. Med. 85, 318—328
22 Chuang, H.Y., Mohammad, S.F., Crowther, P.E. and Mason, R.C. (1977) Rep. Commun. Pathol. Pharmacol. 18, 291—301
23 Steck, T.L. and Wallach, D.F.H. (1965) Biochim. Biophys. Acta 97, 510—522
24 Tangen, O., Berman, H.J. and Marfey, P. (1971) Thromb. Diath. Haemorrh. 25, 268—278
25 Patscheke, H. and Worner, P. (1977) Thromb. Res. 11, 391—402
26 Schmukler, M. and Zieve, P.D. (1974) J. Lab. Clin. Med. 83, 887—895
27 Greenberg, J.H. and Jamieson, G.A. (1974) Biochim. Biophys. Acta 345, 231—242
28 Majerus, P.W. and Brodie, G.N. (1972) J. Biol. Chem. 247, 4253—4257
29 Ganguly, P. and Fossett, N.J. (1979) Biochem. Biophys. Res. Commun. 89, 1154—1160
30 Ganguly, P. and Fossett, N.J. (1980) Biochem. Biophys. Res. Commun. 92, 499—504
31 Rotman, A. and Heldman, J. (1980) FEBS Lett. 110, 330—332
32 Karpatkin, S. and Karpatkin, M. (1974) Biochem. Biophys. Res. Commun. 57, 1111—1118
33 Toyoshima, S. and Osawa, T. (1975) J. Biol. Chem. 250, 1655—1660
34 Barnett, R.E., Scott, R.E., Furcht, L.T. and Kersey, J.H. (1974) Nature 249, 465—466
35 Bhavanadan, V.P. and Katlic, A.W. (1979) J. Biol. Chem. 254, 4000—4008
36 Ganguly, P., Gould, N.J. and Sidhu, P. (1979) Biochim. Biophys. Acta 586, 574—583
37 Nachman, R.L., Tarasov, E., Weksler, B.B. and Ferris, B. (1977) Thromb. Res. 12, 91—104