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THE EFFECTS OF VARIOUS LECTINS ON PLATELET AGGREGATION AND RELEASE*

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SUMMARY

1. The following lectins have been studied with regard to their effects on platelet functions: wheat germ agglutinin (*N*-acetylglucosamine determinant), *Phaseolus coccineus* (determinant unknown), *Ricinus communis* and *Agaricus bisporus* (galactose determinant), concanavalin A, *Lathyrus sativus*, *Lens culinaris* and *Pisum sativum* (glucose, mannose determinants), and soybean lectin (*N*-acetylgalactosamine determinant).

2. Soybean lectin (*N*-acetylgalactosamine) was virtually without effect on platelet aggregation or release.

3. Lectins with glucose and mannose specificities had little effect on platelets but, at high concentrations, did induce some release of serotonin but not of ADP, suggesting two different mechanisms for the release of these two constituents.

4. Lectins with specificities towards galactose (especially *R. communis*), *N*-acetylglucosamine (wheat germ agglutinin) and the lectin of *P. coccineus* (determinant unknown) were strong inducers of platelet aggregation and the release of both ADP and serotonin; the aggregation induced by *R. communis* was inhibited by the destruction of ADP while that induced by wheat germ agglutinin and *P. coccineus* was not greatly inhibited, suggesting that these may act as true agglutinins. At subthreshold concentrations, these lectins also inhibited aggregation induced by ADP, and serotonin release induced by collagen. These lectins also induced the agglutination of platelets and platelet membranes in a microtiter assay system.

5. Concanavalin A (glucose, mannose determinant) was unusual in that it caused agglutination only of membranes and not of intact platelets but did cause release of ADP and serotonin. At subthreshold concentrations, it also inhibited the release of serotonin by collagen.

6. The agglutination of platelet membranes by wheat germ agglutinin and *P. coccineus* was specifically inhibited by the macroglycopeptide (Glycopeptide I) of human platelets and by platelet Glycopeptide II, respectively. Platelet Glycopeptide III was without effect on any of the lectins studied.

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INTRODUCTION

Lectins bind specifically to carbohydrates on cell surfaces and cause agglutination of erythrocytes and of a variety of transformed cells. Because of these properties, lectins have been used to explore the structure of membranes in platelets [1, 2] and in other cells (for a recent review, see Lis and Sharon [3]).

On the basis of indirect evidence, it has recently been suggested that the adhesion of platelets to collagen may be mediated by galactosyl residues present on collagen and a specific glucosyltransferase present on the platelet surface [4]. One approach to a further test of this hypothesis was considered to be the inhibition of adhesion by the use of lectins specific for galactose to block the receptor sites on collagen. Although convincing patterns of lectin inhibition of collagen adhesion have not been obtained in the present work, certain patterns of lectin effects on platelet aggregation and release have emerged and have been related to the glycoprotein receptors of the platelet surface.

Nine lectins have been examined for their effect in inducing platelet aggregation, the platelet release reaction and membrane agglutination, and for their inhibitory effect on the initiation of these phenomena by collagen, ADP and thrombin. These lectins, and their probable carbohydrate specificities are as follows: wheat germ agglutinin (*N*-acetylglucosamine [5]), *Ricinus communis* (D-galactose [6]), *Agaricus bisporus* (D-galactose [7]), concanavalin A (D-glucose, D-mannose [8]), *Lathyrus sativus* (D-glucose, D-mannose; Kocourek, J., personal communication), *Lens culinaris* (D-glucose, D-mannose [9, 10]), *Pisum sativum* (D-glucose, D-mannose [9]), soybean (*N*-acetyl-D-galactosamine [11]) and *Phaseolus coccineus*, the carbohydrate specificity of which has not yet been determined (Kocourek, J., personal communication).

MATERIALS AND METHODS

The following reagents were obtained from Sigma Chemical Company, St. Louis, Mo.: bovine achilles tendon collagen, acid soluble calf skin collagen, adenosine-5′-diphosphate (Grade I), thrombin (from bovine plasma), phosphoenolpyruvate (trisodium salt), pyruvate kinase (Type II), lactate dehydrogenase (Type II), β-NADH (Grade III) and trypsin (Type III). 5-Hydroxytryptamine-3′-[^{14}C]creatinine sulfate (serotonin) (spec. act., 30 Ci/mole) was purchased from Amersham Searle Corporation, Arlington Heights, Ill.; concanavalin A from Miles Laboratories, Inc., Kankakee Ill.; and fibrinogen (Grade L) from Kabi, Stockholm, Sweden.

Lectins were kindly supplied by Dr J. Kocourek (*L. sativus*, *L. culinaris*, *P. coccineus*, *R. communis*), Dr R. Kornfeld (*A. bisporus*, *R. communis*), Dr N. Sharon (soybean), Dr T. Shinohara (*P. sativum*), and Dr E. Walborg (wheat germ agglutinin), and were used without further purification. They were prepared in stock solutions of 1 mg dry weight/ml.

Platelet concentrates and platelet-rich plasma were provided by the Washington Regional Red Cross Blood Center and were prepared from single units of blood (approximately 450 ml) collected from normal volunteer donors in plastic blood collection containers using acid–citrate–dextrose (N.I.H. solution A) as anticoagulant. Platelet membranes were prepared from platelet concentrates by the glycerol-lysis method [12]. The membrane fraction isolated following density step centrifugation

was used in these studies. These "step" membranes were washed with 0.05 M Tris maleate, pH 7.0, and suspended in 40% glycerol containing 0.15 M NaCl for storage at −40 °C.

Platelet glycopeptides were prepared according to the procedure of Pepper and Jamieson [13]. Glycopeptide I was supplied in a highly-purified form by Dr T. Okumura of this laboratory.

For the study of platelet aggregation, platelet-rich plasma obtained within 4 h of collection was freed of contaminating erythrocytes by centrifugation at $1500 \times g$ for 1 min and centrifuged at $130 \times g$ at room temperature for 15 min. The platelet pellet was resuspended by gentle vortexing to one-half the original volume in phosphate-buffered saline, pH 7.4, to give a suspension of approximately 10^8 platelets/ml. White blood cells were not observed, and erythrocytes represented less than $1/10^4$ platelets. Aggregation was measured by the turbidimetric method of Born and Cross [14] using a Chronolog aggregometer (Chronolog Corporation, Broomall, Pa.). The instrument was adjusted to 90% transmittance with phosphate-buffered saline and to 10% transmittance with the platelet suspension (0.9 ml) in a 1.5-ml cuvette, with stirring in the aggregometer.

Lectin solutions were prepared to concentrations of 1 mg/ml in water. The solution was added to the cuvette, and the change in percentage transmittance was displayed with a recorder. Aggregation was always verified by visual examination.

In order to determine whether the lectin-induced agglutination of platelets was mediated by ADP [15], or whether the agglutination was due to a bridging mechanism similar to that which presumably causes agglutination of erythrocytes and a variety of transformed cells, the platelets were incubated with a mixture of phosphoenolpyruvate and pyruvate kinase in order to destroy any ADP formed in the reaction. Washed platelets were resuspended in phosphate-buffered saline, pH 7.1, and incubated with 0.3 mM phosphoenolpyruvate, 10 units/ml pyruvate kinase, 1.5 mM Mg^{2+}, and 15 mM K^+, or with an equal volume of phosphate-buffered saline with Mg^{2+} and K^+ as a control.

Inhibition studies

When lectins were tested for their inhibitory effect, they were incubated with the platelet suspension for 5 min before addition of an aggregating agent. The lectin concentration used for these inhibition studies was the highest concentration which did not itself induce aggregation (subthreshold concentration); lectins which did not induce aggregation at all were tested at 100 μg/ml. The minimum concentration of a lectin necessary to induce aggregation varied with different units of platelets; threshold concentrations were therefore determined for each experiment. However, threshold concentrations rarely varied by more than a factor of 2.

Washed platelets were found not to aggregate in response to ADP unless Ca^{2+} and fibrinogen were present. For experiments with ADP-induced aggregation, 3 mM $CaCl_2$ and 100 μg/ml fibrinogen were added to the washed platelet suspension. After preincubating the lectin with the platelet suspension 10^{-5} M ADP was added. Control experiments used water instead of the same volume of lectin.

The inhibition by lectins of collagen-induced platelet aggregation was tested with two collagen preparations. For most experiments tendon collagen prepared by the method of Holmsen et al. [16] was used. For some experiments 2 mg of calf skin

acid-soluble collagen was suspended in 2 ml 0.15 M NaCl and homogenized on ice in a Ten Broeck homogenizer for about 3 min. The homogenate was centrifuged to remove undissolved particulate material. The bovine tendon collagen was tested at a final concentration of approximately 100 μg/ml, and the calf skin collagen at approximately 25 μg/ml. These represent the lowest concentrations which induced a reproducible level of aggregation.

For inhibition experiments using thrombin, bovine thrombin was used as an aggregating agent at 0.25 unit/ml, the lowest concentration to induce aggregation at a reproducible level.

Microtiter assays

In order to determine agglutination of platelets and platelet membranes, microtiter agglutination assays were devised using Microtest^{tm} tissue culture plates (Falcon Plastics, Oxnard, Calif.).

For the study of platelet agglutination, platelet-rich plasma was centrifuged at $130 \times g$ for 15 min, and the platelets were resuspended in phosphate-buffered saline to one-tenth the original volume of plasma. 5 μl of lectin from several dilutions of a 1 mg/ml stock solution, 5 μl of inhibitor or water, and 5 μl of washed platelets ($5 \cdot 10^8$ per ml) were added sequentially to wells of the test plate. After 1 h plates were examined by phase contrast microscopy at a magnification of 125 $\times$. Agglutination of platelet membranes was assayed by the same procedure as that used for intact platelets. Membranes were first dialyzed for 18 h against running water at 10 °C to remove glycerol and then centrifuged at 43 500 $\times g$ for 1 h. The membrane pellet was resuspended in distilled water to a concentration of approximately 1 mg protein per ml. Plates were examined after 1-1.5 h to determine agglutination of membranes.

Release reaction

For assay of the platelet release reaction, 50 ml platelet-rich plasma was incubated with 10 μCi [^{14}C]serotonin for 30 min at room temperature. Platelets were sedimented by centrifugation at $130 \times g$ for 15 min and resuspended in phosphate-buffered saline to a concentration of approximately $5 \cdot 10^7$ per ml. One ml samples of the platelet suspension were added to 12 mm $\times$ 75 mm disposable culture tubes containing 10–100 μg of lectin in a volume of 100 μl. In experiments designed to test whether lectins inhibit collagen- or thrombin-induced release, the collagen or thrombin was added last in a volume of 100 or 25 μl, respectively. The lectin concentration was the highest concentration which did not itself induce release. Because of variations in the platelets, this concentration was determined for each experiment. A control to which no aggregating agent or inhibitor was added was always carried out. Tubes were shaken on a Variable Speed Rotator (Clay-Adams, Parsippany, N. J.) for 10 min at 210 rev./min. The tubes were centrifuged at $180 \times g$ for 25 min. 100-μl aliquots of the supernatant solution were added to vials containing scintillation fluid [17] and counted in a Packard Tricarb Scintillation Spectrometer. The amount of radioactivity released from the platelets was calculated as a percentage of the radioactivity present in the original platelet suspension.

Release of nucleotide diphosphates was measured enzymatically by a procedure based on that of Adam [18]. 1-ml aliquots of a washed platelet suspension, concentrated to 20 times the original concentration in physiological saline ($5 \cdot 10^8$–

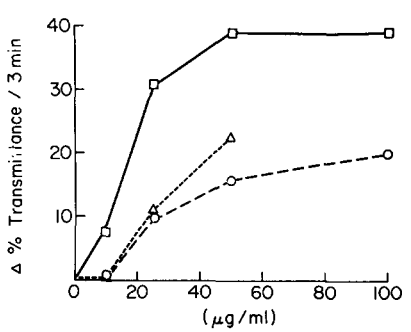
$10 \cdot 10^8$ platelets per ml) were added to 12 mm × 75 mm disposable culture tubes containing 0–100 μg of lectin in a volume of 100 μl. In some experiments, 100 μl of bovine tendon collagen (approximately 50 μg) was also added. The suspensions were mixed on a rotator for 10 min and then centrifuged at $180 \times g$ for 15 min. The supernatant solution was added to a tenth volume of cold 9 M $HClO_4$, mixed, and centrifuged. The supernatant portion was neutralized with dry $KHCO_3$ and centrifuged. 500 μl of the deproteinated extract was added to 500 μl of a solution containing 200 μg EDTA · $Na_2H_2 \cdot 2H_2O$, 1100 μg magnesium acetate · $4H_2O$, 300 μg phosphoenolpyruvate, 100 μg NADH, and 10 units lactate dehydrogenase per ml of triethanolamine buffer, pH 7.5, (5.6 g triethanolamine · HCl; 77 mg magnesium acetate · $4H_2O$ in 100 μl water). The reaction was started by addition of 2 units of pyruvate kinase. The decrease in absorbance at 3400 Å was monitored at 24 °C in a Beckman DUR Recording Spectrophotometer (Beckman Instruments, Fullerton, Calif.) with a Gilford 2000 Multiple Sample Absorbance Recorder (Gilford Instruments Laboratories, Inc., Oberlin, Ohio); nucleotide diphosphate concentration was calculated according to Adam [18]. In this report all nucleotide diphosphates released by the platelet are considered to be ADP.

RESULTS

Platelet aggregation

Of the nine lectins tested, only *P. coccineus*, *R. communis*, and wheat germ agglutinin caused platelets to aggregate when tested at concentrations of 25 μg/ml or less (Table I, Column 1). *A. bisporus* induced agglutination only at 100 μg/ml or higher. The other lectins were ineffective even at 100 μg/ml.

The change in percentage transmittance of the platelet suspension over the first 3 min was used to estimate the rate of platelet aggregation. The relationship between concentration and the response of platelets (Fig. 1) indicates that wheat germ agglutinin was the most effective lectin studied. Concentrations above 50 μg/ml did not seem to increase the rate of platelet aggregation in any case.



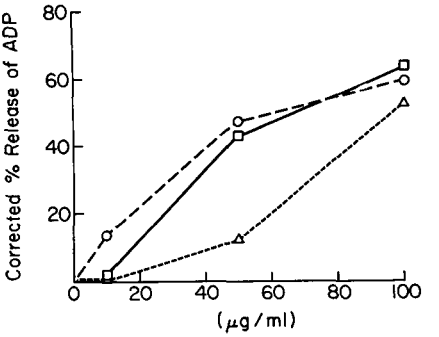


Fig. 1. Effect of lectin concentration on aggregation of platelets. □, wheat germ agglutinin; △, *R. communis*; ○, *P. coccineus*.

Fig. 2. Effect of lectin concentration on release of ADP from platelets. □, wheat germ agglutinin; △, *R. communis*; ○, *P. coccineus*. Corrected % release of ADP = (ADP released by lectin − ADP released by control)/(ADP released by thrombin − ADP released by control) × 100 %.

TABLE I

SUMMARY OF THE EFFECTS OF LECTINS ON PLATELET FUNCTIONS

Number in parentheses following + is the minimum concentration of lectin (μg/ml) required to produce the given effect. Values preceded by − were not effective at the highest concentrations tested. ± signifies variable effects. See text for details. The data presented are for representative experiments.

Specificity	Lectin	Induces aggregation	Induces release of ADP	Induces release of serotonin	Inhibits aggregation induced by:				Inhibits release of serotonin induced by:		Inhibits release of ADP by bovine tendon collagen	Agglutinates platelets (microtest plates)	Agglutinates membranes (microtest plates)
					ADP**	Bovine tendon collagen	Calf skin collagen	Thrombin	Bovine tendon collagen	Thrombin			
		1	2	3	4	5	6	7	8	9	10	11	12
Glc, man	Concanavalin A	−(100)	±(100)	+(50)	−(100)	−(100)	±(100)	−(100)	+(25)	−(25)	−(50)	−(600)	+(165)
	L. sativus	−(100)	−(100)	+(50)	−(100)	−(100)	±(100)	−(100)	−(25)	−(25)	−(100)	−(165)	−(165)
	L. culinaris	−(100)	−(100)	+(25)	−(100)	−(100)	±(100)	−(100)	−(25)	−(25)	−(100)	−(165)	−(165)
	P. sativum	−(100)	−(100)	+(50)	−(100)	−(100)	±(100)	−(100)	−(25)	−(25)	−(50)	−(165)	−(165)
GalNAc	Soybean	−(100)	*	−(100)	−(100)	−(100)	*	−(100)	+(100)	−(100)	*	−(165)	−(165)
Gal	*R. communis*	+(20)	+(50)	+(50)	+(50)	−(20)	*	−(50)	+(25)	−(25)	−(10)	+(83)	+(21)
	A. bisporus	±(100)	±(100)	+(25)	−(100)	−(50)	±(50)	−(50)	−(10)	−(10)	−(50)	+(165)	+(42)
GlcNAc	Wheat germ agglutinin	+(50)	+(50)	+(25)	+(50)	−(20)	±(10)	−(50)	+(25)	−(10)	−(10)	+(83)	+(5)
Unknown	*P. coccineus*	+(25)	+(25)	+(25)	+(20)	−(20)	±(20)	−(20)	+(10)	−(10)	−(10)	+(56)	+(42)

* Data not available.

** Concentration of lectin which inhibits by 75 % or more.

The aggregation induced by the lectin *R. communis* was inhibited by the phosphoenolpyruvate–pyruvate kinase system to about the same extent as collagen-induced aggregation, suggesting that this was mediated by ADP, while the agglutination induced by the lectin *P. coccineus* and by wheat germ agglutinin was only partially inhibited by this system (Table II).

TABLE II

INHIBITION BY PHOSPHOENOLPYRUVATE–PYRUVATE KINASE OF LECTIN-INDUCED AGGREGATION

Washed platelets (approx. 10^8 per ml) were suspended in phosphate-buffered saline, pH 7.1, and preincubated with 0.3 mM phosphoenolpyruvate, 10 units/ml pyruvate kinase, 1.5 mM Mg^{2+}, and 15 mM K^+, or with an equal volume of phosphate-buffered saline containing Mg^{2+} and K^+. Aggregation was performed in the aggregometer. Percentage inhibition was the change in percentage transmittance after 3 min of the phosphoenolpyruvate–pyruvate kinase-treated platelets divided by the change in percentage transmittance after 3 min of the control platelets. Values are for 3 separate experiments.

Lectin	Percentage inhibition
P. coccineus (50 μg/ml)	41; 44; 53
Wheat germ agglutinin (10 μg/ml)	38; 33; 50
R. communis (50 μg/ml)	68; 79; 50
Bovine tendon collagen (10 μg/ml)	74; 67
(5 μg/ml)	76

Release of nucleotide diphosphates

P. coccineus, *R. communis*, and wheat germ agglutinin caused release of nucleotide diphosphates when added to washed platelets in concentrations of 50 μg/ml or less (Table I, Column 2), the release being concentration dependent (Fig. 2). *A. bisporus* and concanavalin A caused nucleotide diphosphate release only at higher concentrations (100 μg/ml), although even at this concentration release was not always observed.

Release of serotonin

All the lectins tested, with the exception of soybean lectin, caused platelets to release [^{14}C]serotonin (Table I, Column 3). However, the percentage of radioactivity released, compared to the amount taken up, was related to the concentration of lectin and differed widely among the various lectins studied (Fig. 3). None of the lectins released more than about 50% of radioactive [^{14}C]serotonin compared with about 75% for a thrombin control. *R. communis*, *P. coccineus* and wheat germ agglutinin reached the 50% value in the concentration range of 10–20 μg/ml while levels approaching this were obtained with *P. sativum*, concanavalin A, *L. culinaris* and *A. bisporus* only at 100 μg/ml, the highest level tested; *L. sativus* and soybean lectin gave minimal release at all concentrations tested.

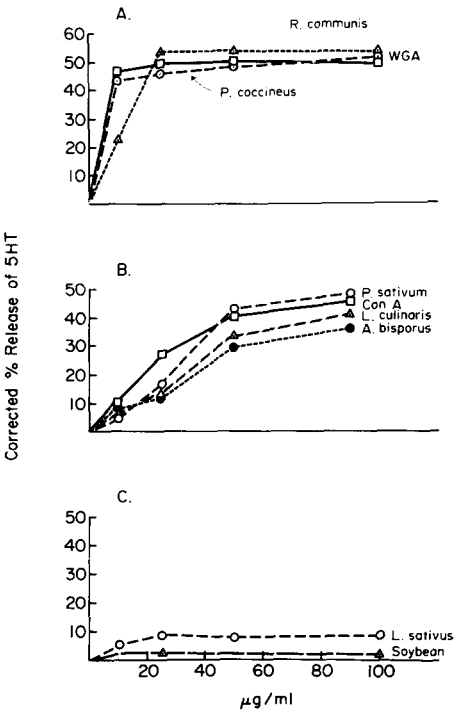


Fig. 3. Effect of lectin concentration on release of [^{14}C]serotonin from platelets. Corrected % release of serotonin = (dpm released by lectin − dpm released in control)/(dpm released + dpm retained) × 100 %. (a) □, wheat germ agglutinin; △, *R. communis*; ○, *P. coccineus*. (b) ○, *P. sativum*; □, concanavalin A; △, *L. culinaris*; ●, *A. bisporus*. (c) ○, *L. sativum*; △, soybean.

Inhibition of aggregation

Lectins were tested for their ability to inhibit the aggregation of platelets induced by ADP, collagen, or thrombin. Under the conditions outlined in Materials and Methods, ADP-induced aggregation was inhibited by nearly 100% by the lectins of *P. coccineus* and *R. communis* and by wheat germ agglutinin (Table I, Column 4). None of the lectins inhibited aggregation induced by bovine tendon collagen (Table I, Column 5) while all the lectins inhibited by approximately 40% the aggregation induced by calf skin collagen (Table I, Column 6). None of the lectins inhibited thrombin-induced aggregation (Table I, Column 7).

Inhibition of release reactions

Platelets were loaded with [^{14}C]serotonin and preincubated with lectins at concentrations which caused negligible release of radioactivity. When bovine tendon collagen was added in a concentration which caused maximal release of radioactivity (approximately 50 μg/ml), four of the lectins, *P. coccineus*, *R. communis*, wheat germ agglutinin, and concanavalin A, inhibited the release of radioactivity by 8–25% as compared to that induced by collagen in the absence of these lectins (Table I, Column 8).

None of the lectins tested at subthreshold levels inhibited the release of [^{14}C]-serotonin from platelets initiated by thrombin (Table I, Column 9) or the release of ADP initiated by bovine tendon collagen (Table I, Column 10).

Agglutination of platelets

When washed platelets were incubated with serial dilutions of lectins in wells of microtest plates, the lectins of *P. coccineus*, *A. bisporus*, *R. communis*, and wheat germ agglutinin caused agglutination at concentrations in the range of 5–42 μg/ml with wheat germ agglutinin being the most active (Table I, Column 11); the other lectins tested were without effect.

Agglutination of membranes

In the microtiter test system, the lectins which caused the agglutination of platelets (*P. coccineus*, *A. bisporus*, *R. communis* and wheat germ agglutinin) also caused the agglutination of membranes. In addition, concanavalin A caused membrane agglutination although it was without effect on intact platelets at concentrations as high as 600 μg/ml. The lectins of *L. sativus*, *L. culinaris*, *P. sativum* and soybean were without effect on membrane agglutination (Table I, Column 12).

Effect of carbohydrate haptens

Of the lectins which caused membrane agglutination, the effects of the lectins of *R. communis*, concanavalin A and wheat germ agglutinin could be inhibited by lactose, α-methyl-D-glucopyranoside, and *N*-acetylglucosamine, respectively, their known haptenic determinants. However, none of the more common monosaccharides inhibited agglutination induced by the lectin of *P. coccineus*, the specificity of which has not been ascertained (Kocourek, J., personal communication) nor by *A. bisporus* which is inhibited by the erythrocyte glycopeptide having the structure galactose $\overset{\beta 1\rightarrow 3}{\rightarrow}$ *N*-acetylgalactosamine → serine [7].

The effect of well characterized platelet glycopeptides [13] on lectin-induced membrane agglutination was also studied. In one particular experiment, wheat germ agglutinin agglutinated membranes at a minimum concentration of 83 μg/ml; at this concentration of wheat germ agglutinin membrane agglutination was inhibited in the presence of 10 μg/ml of platelet Glycopeptide I, as was membrane agglutination induced by 165 μg/ml wheat germ agglutinin. Glycopeptide I, in concentrations up to 100 μg/ml, had no inhibitory effect on agglutination induced by other lectins.

In another experiment, the lectin of *P. coccineus* agglutinated membranes at a concentration of 165 μg/ml. At this concentration of *P. coccineus*, 165 μg/ml of Glycopeptide II inhibited agglutination of platelet membranes. However, Glycopeptide II, in concentrations as high as 300 μg/ml, did not inhibit agglutination induced by other lectins.

Glycopeptide III, in concentrations up to 300 μg/ml, did not inhibit agglutination induced by any of the lectins tested.

DISCUSSION

The results presented here indicate that in general lectins with specificities directed towards α-D-mannopyranose or α-D-glucopyranose moieties do not have a

significant effect on platelets; these include the lectins of *L. culinaris*, *P. sativum* and *L. sativus*. In addition, the soybean lectin, specific for *N*-acetylgalactosamine, was also without detectable effect on platelets.

On the other hand, lectins with a galactose-directed specificity, such as those of *R. communis* and *A. bisporus*, interact with platelets to varying degrees. Wheat germ agglutinin, which is specific for *N*-acetylglucosamine, interacts strongly with platelets while the lectin of *P. coccineus*, the specificity of which is not yet known, is also active in modifying platelet function.

Concanavalin A seems to represent a special case insofar as it did not cause aggregation of washed platelets or the agglutination of intact platelets in the microtiter assay; however, it did cause agglutination of isolated platelet membranes. In contrast, Nachman et al. [2] found that concanavalin A induced platelet aggregation, but variations in the platelet preparations may account for this difference. It has been reported [19] that concanavalin A binds to non-terminal α-D-mannose and α-D-glucose residues. It is possible, therefore, that the isolation of platelet membranes may have exposed binding sites for concanavalin A which were not available on the surface of the intact platelet; a similar finding for liver cells has been reported by Henning and Uhlenbruck [20].

Calculations of the minimum number of lectin molecules required to cause platelet aggregation as measured in the aggregometer and membrane and platelet agglutination as measured in microtiter assays, agreed to within an order of magnitude (Table III); similar agreement is obtained for the concentrations required for *P. coccineus*, the molecular weight of which is not known. The number of concanavalin A molecules required for membrane agglutination also agrees with the values obtained for the lectins of *A. bisporus* and *R. communis* and for wheat germ agglutinin. These calculations are based on the data given in Table I and assume that these lectins do not contain contaminating inactive components.

The lectins which caused platelet aggregation also caused the release of both ADP and serotonin, as did concanavalin A which caused membrane agglutination,

TABLE III

MINIMUM CONCENTRATION OF LECTIN REQUIRED FOR AGGREGATION OR AGGLUTINATION

Lectin	Molecules/platelet · 10^{-6}		
	Platelet aggregation (aggregometer)	Platelet agglutination (microtest plates)	Membrane agglutination** (microtest plates)
Wheat germ agglutinin	13.2	0.8	6.0
A. bisporus	16.8	2.6	5.1
R. communis	2.0	1.3	2.5
*P. coccineus**	$25 \cdot 10^{-8}$ μg/platelet	$25 \cdot 10^{-8}$ μg/platelet	$17 \cdot 10^{-8}$ μg/platelet
Concanavalin A	–	–	5.4

* Mol. wt not determined.

** Based on assumption that 1 mg membrane protein is derived from 10^9 platelets.

only. Haslam [15] has demonstrated that aggregation induced by thrombin and collagen is mediated by ADP which is released from the platelets themselves. The agglutination induced by wheat germ agglutinin (*N*-acetylglucosamine determinant) and by *P. coccineus* (determinant unknown) were only partially inhibited by the use of the phosphoenolpyruvate–pyruvate kinase system to destroy ADP, while the agglutination induced by *R. communis* (galactose determinant) was strongly inhibited by this system. It seemed likely, therefore, that wheat germ agglutinin and *P. coccineus* act primarily as agglutinins while the effect of *R. communis* mimics that of the physiological aggregating agents and may be due to the release of ADP.

The above lectins, which were specific for either D-galactose or *N*-acetylglucosamine, and concanavalin A released both serotonin and ADP. On the other hand, the lectins of *L. sativus*, *L. culinaris* and *P. sativum*, which were specific for D-glucose and D-mannose, but which did not initiate aggregation, caused the release of only serotonin; this suggests that the release of serotonin and ADP from the platelet occurs independently of each other.

Those lectins which were most effective in inducing platelet aggregation and the agglutination of platelet membranes (*P. coccineus*, *R. communis*, and wheat germ agglutinin), when used in subthreshold concentrations, effectively inhibited ADP-induced platelet aggregation.

These same lectins, together with concanavalin A which caused the agglutination of membranes only, when used in subthreshold concentrations, partially inhibited the release of serotonin induced by collagen. Only soybean agglutinin is an exception in that it inhibited the release of serotonin without causing the aggregation of platelets or the agglutination of platelet membranes at any of the concentrations tested.

The results obtained from studying the effects of lectins on collagen-induced platelet aggregation suggest that these effects are non-specific. Aggregation induced by calfskin acid-soluble collagen was inhibited to approximately the same extent (about 40%) by all the lectins tested while the aggregation induced by bovine tendon collagen was not inhibited by any of the lectins.

Significant differences were found in the effects of platelet membrane glycopeptides in inhibiting the agglutination of membranes induced by the most active lectins, *P. coccineus*, *R. communis* and wheat germ agglutinin. Proteolytic digestion of intact platelets [13] or isolated membranes [21] yields three size classes of glycopeptide of molecular weights 120 000 (Glycopeptide I), 22 500 (Glycopeptide II) and 5000 (Glycopeptide III). The glycopeptides also differ in their composition and structure insofar as the carbohydrate–protein linkages in Glycopeptide III appear to be entirely of the *N*-asparaginyl type while those of Glycopeptide I contain both *N*- and *O*-glycosidic linkages characteristic of membrane glycoproteins; Glycopeptide I also appears to be related to the thrombocyte-specific antigen of the platelet surface.

The fact that Glycopeptide I specifically inhibited the agglutination of platelet membranes induced by wheat germ agglutinin, while Glycopeptide II specifically inhibited the agglutination induced by *P. coccineus* suggests a highly complementary specificity in these two systems and suggests that these glycopeptides may be sites on the membrane to which the lectins bind. It may also be noted that these lectins are the most active in inducing the release of ADP and serotonin from platelets and, at subthreshold concentrations, in inhibiting the aggregation induced by ADP. Using

platelet membranes in the microtiter system we have also found that the erythro-agglutinating lectin of *P. vulgaris* is inhibited by both Glycopeptide I and Glycopeptide II but that the glycopeptides are without effect on membrane agglutination induced by the leukoagglutinin although Majerus and Brodie [1] showed that both of these lectins can induce the aggregation of whole platelets.

NOTE ADDED IN PROOF (Received March 5th, 1974)

The hemagglutinating effect of the lectin of *P. coccineus* has been found to be nhibited by Glycopeptide I.3 isolated from the erythrocyte membrane, although no one of its monosaccharide components is active [22].

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