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ON THE SUSCEPTIBILITY OF HUMAN PLATELETS TO AGGREGATION BY CONCANAVALIN A AND THE EFFECT OF THIS LECTIN ON THEIR RESPONSE TO ADP

B. M. JONES and P. M. EVANS

Department of Zoology, University College of Wales, Aberystwyth (U.K.)

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SUMMARY

Concanavalin A aggregated gel-filtered platelets in 0.9% NaCl solution signifying cross-bridging by the lectin. Aggregation of these platelets by concanavalin A was temperature dependent; it did not occur at 0–4 °C unless the platelets were previously trypsinized. The level of aggregation of trypsinized platelets by concanavalin A at 0–4 °C was similar to that of untreated platelets at 37 °C. It is suggested that trypsin facilitates platelet aggregation by concanavalin A at 0–4 °C by causing a configurational change in membrane glycoproteins which orientates concanavalin A receptor sites into positions that favour lectin cross-bridging. Concanavalin A failed to aggregate platelets in plasma. Radioisotope studies showed that the amount of [^{3}H]concanavalin A which combined with platelets in plasma was extremely low compared with gel-filtered platelets in saline. The aggregation of Ehrlich ascites cells by concanavalin A was considerably reduced when platelet-free plasma was added to the medium suggesting that it was due to the presence of concanavalin A-reactive components in the plasma.

Concanavalin A inhibited the ADP-induced aggregation of platelets suspended in plasma or in a salts solution supplemented with calcium and fibrinogen, although the inhibitory effect was more conspicuous in the latter case. The results suggests that concanavalin A produces its inhibitory effect on ADP-induced platelet aggregation by interacting with membrane glycoproteins, and this further suggests their involvement in aggregation.

INTRODUCTION

Concanavalin A a lectin isolated from jack beans [1] binds specifically to carbohydrates [2, 3] and glycoproteins [4], hence its value as a research tool for exploring carbohydrate groupings at the cell surface. Recent studies have shown that three major glycoproteins are exposed on the surface of the platelet [5] and there is some evidence for the involvement of carbohydrate groupings [6] and at least one of the major glycoproteins [7] in ADP-induced platelet adhesion and aggrega-

tion. It is not known whether these major glycoproteins provide receptor sites for ADP [8] or have some other function in the process of platelet aggregation. If these glycoproteins contained receptor sites for concanavalin A, their interaction with the lectin could conceivably have some measureable effect on ADP-induced platelet aggregation.

It has been discovered that concanavalin A produces interesting effects on lymphocytes [9–11] and it will aggregate erythrocytes, malignant cells [12] and embryonic cells [13, 14], but not certain cultured cells, for example 3T3 cells, unless they have been virally transformed or previously trypsinized [15]. Reports on the effects of concanavalin A on platelets are conflicting; there is uncertainty about whether concanavalin A can [16, 17] or cannot [18] aggregate washed platelets, and whether the aggregation observed in some cases is a secondary effect due to ADP released in response to concanavalin A [17, 18]. It was also conceivable that platelets resembled 3T3 cells in that they would aggregate readily only after treatment with trypsin which could bring about configurational changes in platelet membrane glycoproteins [5]. Such changes may underlie not only the "stickiness" displayed by trypsinized platelets [19] but also the mechanism of ADP aggregation.

Our studies show that the aggregation of platelets by concanavalin A is due directly to lectin cross-bridging and not to ADP released in reponse to concanavalin A, since concanavalin A-induced aggregation occurred under conditions that did not support ADP-induced aggregation. Aggregation by concanavalin A is temperature-dependent and is considerably enhanced by trypsin treatment of the platelets. Radioisotope studies showed that concanavalin A binds to platelets in a balanced salts solution in considerably larger amounts than to platelets in plasma; this differential binding of concanavalin A and the failure of concanavalin A to aggregate platelets in plasma are probably due to the presence of concanavalin A-reactive components in the plasma. We also report that concanavalin A inhibited ADP-induced platelet aggregation.

MATERIALS AND METHODS

Platelet-rich plasma. Platelet-rich plasma was obtained from human blood. Sodium citrate at a final concentration of 3.8 mg/ml blood served as the anticoagulant. Platelet-rich plasma was separated from red cells by differential centrifugation at $250 \times g$ at room temperature (about 23 °C) and transferred to clean glass tubes. All glassware was siliconised to prevent platelets sticking to the glass. Platelet-free plasma was obtained by centrifuging platelet-rich plasma at $2500 \times g$.

Separation of platelets from plasma by gel filtration. A siliconised glass column containing Sepharose 2B (Pharmacia Fine Chemicals) was equilibrated in 0.9 % NaCl solution. Platelet-rich plasma (2–3 ml) was added to the top of the gel bed (15×2.5 cm) and eluted with the 0.9 % NaCl solution at a flow rate of about 40 ml/h. The eluate was monitored at 280 nm using a Uvicord II ultraviolet monitor with attached chart recorder. The platelets passed through the column at a faster rate than the plasma [20] and were collected as a suspension in the 0.9 % NaCl eluting solution. When preparations of washed platelets were required for aggregation experiments using ADP, a more complex eluting and isolation medium was used. The composition of this medium was NaCl, 9.0 g/l; KCl, 0.42 g/l; $MgCl_2 \cdot 6\,H_2O$,

1.01 g/l; glucose, 1.0 g/l; Tris, 1.45 g/l; the pH was adjusted to 7.4 with HCl. After collection the platelet suspension was stored in an ice-bath until required.

Chemicals. Concanavalin A was obtained from Calbiochem; α-methyl-D-glucopyranoside, a haptenic inhibitor of the interaction of concanavalin A with cell surface receptor sites [21] was used in control tests. Adenosine, adenosine diphosphate (ADP) and bovine fibrinogen (fraction 1) were obtained from Sigma and crystalline trypsin was obtained from B.D.H. Chemicals Ltd. All substances were added in a 0.9 % NaCl solution to platelet-rich plasma or to washed platelet suspensions.

Trypsinisation of washed platelets. Gel-filtered platelets were incubated at 37 °C for 10 min in 0.9 % NaCl solution containing 0.01 % crystalline trypsin. At the end of the incubation period soybean trypsin inhibitor was added to the platelet suspension to give a concentration of 0.025 % (w/v). Control preparations for aggregation experiments were also treated with trypsin inhibitor.

Aggregation. Platelet aggregation was measured quantitatively using a Corning-EEL Aggregometer model 169 with attached flatbed chart recorder. In experiments using platelet-rich plasma or washed platelets suspended in a physiological salts solution, the meter was set to zero using platelet-free plasma and distilled water, respectively. The density of platelets in the test preparations was adjusted by the addition of platelet-free plasma or the appropriate salts solution to give a reading on the aggregometer scale at a point 10 divisions from the left side (signified approx. $3 \cdot 10^8$ platelets/ml). Progress in platelet aggregation was indicated by an increase in light transmitted through the test preparation. The recorded measurements were supplemented by examining aggregates and the numbers of single cells in samples of preparations under the light microscope; well-formed aggregates were also clearly visible in the test tube. Experiments at 37 °C were conducted by utilising the aggregometer heating block maintained by thermostatic control at 37 °C; experiments at room temperature (20–23 °C) were performed with the heating device switched off; experiments at 4 and 12 °C were carried out in thermostatically controlled cold rooms. Platelet preparations and the necessary solutions were equilibrated before use to the test temperature. During the experiments the platelet suspensions were continuously agitated using the aggregometer top-stirrer set at 80 on the control panel.

In experiments designed to test the effect of concanavalin A on ADP-induced platelet aggregation, platelet-rich plasma and washed platelet suspensions were used. The aggregation of platelets in plasma was monitored at 37 °C. The washed platelet suspensions were used within 1 h after gel filtration; before use the suspending medium was supplemented with fibrinogen (0.3 mg/ml) and calcium (0.25 mg/ml) and incubated at 37 °C for 10 min. The suspension was then rapidly cooled to room temperature (20–23 °C) and cell aggregation in this mixture was monitored at this same temperature for two reasons. First, aggregation caused directly by concanavalin A occurs at room temperature at a slower rate than it does at 37 °C, thus changes in light transmission due to the presence of the lectin are minimised; and, secondly, at room temperature the washed platelets were found to be considerably more sensitive to ADP. Concanavalin A was added to the stirred mixture 2 min before the addition of ADP at $2.5 \cdot 10^{-6}$ M. Preliminary experiments showed that the addition of concanavalin A to the platelet suspension made an insignificant change (1–2 %) in light transmitted through the preparation,

compared with a change of 30–40 % caused by ADP aggregation of the platelets during the 2 min experimental period.

Studies with radioactively labelled concanavalin A. These studies were made to investigate the binding of concanavalin A to the surfaces of platelets in plasma and in a physiological salts solution. The [^{3}H]concanavalin A used was obtained from the Radiochemical Centre, Amersham, where it was prepared by acetylation of native concanavalin A with [^{3}H]acetic anhydride, 3.9 Ci/mmol [22]. Prior to use in experimental systems the [^{3}H]concanavalin A suspension was dialysed extensively against distilled water and lyophilised. When required the [^{3}H]concanavalin A was made up in NaCl solution containing 1 mM $MnCl_2$. [^{3}H]concanavalin A prepared in this way has been found to be identical to concanavalin A on the basis of agglutination, cell binding assays and by chromatography on Sephadex G-50 in 0.1 M glucose [22].

In the experiments, the platelets were exposed to [^{3}H]concanavalin A, while suspended in plasma or in isolation medium consisting of NaCl (8.0 g/l), KCl (0.2 g/l) and glucose (1.0 g/l). Washed platelet suspensions were prepared as follows: A 0.6 % (w/v) solution of ethylenediaminetetraacetic acid (EDTA) in calcium- and magnesium-free Tyrode was added to platelet-rich plasma in the ratio 1 part EDTA solution: 3 parts platelet-rich plasma. The platelets were then sedimented by centrifugation at $800 \times g$ for 12 min at 4 °C and washed in calcium- and magnesium-free Tyrode containing EDTA (0.15 %, w/v). The platelets were finally resuspended in isolation medium. To 2 ml of the washed platelet suspension was added 125 μg of [^{3}H]concanavalin A and the mixture was incubated for 1 h at 22 °C and then centrifuged at $800 \times g$ for 12 min at 4 °C. The platelets were washed in isolation medium containing 0.15 % (w/v) EDTA and finally suspended in the same solution.

Each platelet suspension, which contained platelets previously exposed to [^{3}H]concanavalin A while in plasma or in isolation medium, was divided equally to provide a test and control sample. The platelets in the two samples were washed several times in isolation medium by centrifugation and each supernatant was assayed for radioactivity on a Beckman LS 100 liquid scintillation spectrometer. When the ^{3}H count for successive supernatants became relatively constant (usually between the third and fourth wash), the control sample was washed finally in the isolation medium, and the test sample in medium containing 0.2 M α-methyl-D-glucopyranoside. A rise in the supernatant count for the test sample compared with the control sample supernatant would signify the removal of specifically bound [^{3}H]concanavalin A from the surfaces of the platelets.

RESULTS

Aggregation of gel-filtered platelets in saline by concanavalin A

To eliminate calcium, which is known to be a requirement for ADP aggregation, we prepared gel-filtered human platelets suspended in a 0.9 % NaCl solution. We found that the addition of concanavalin A (1 mg/ml) to this stirred preparation at 37 °C resulted in the formation of large aggregates of platelets that were plainly visible in the test tube. These gel-filtered platelets were also aggregated readily by concanavalin A at 0.25 mg/ml. Light transmission increased with progress in aggregation as indicated by the curve obtained at 37 °C (Fig. 1). When concanavalin A

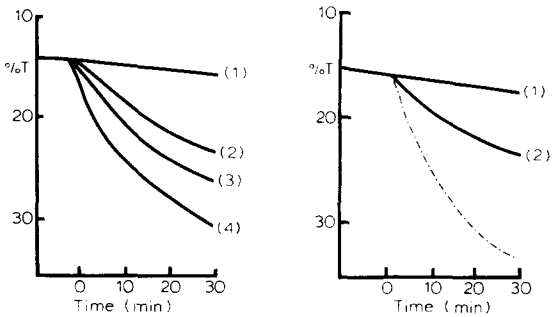

Fig. 1. Aggregation of gel-filtered platelets suspended in 0.9 % NaCl solution by concanavalin A (1 mg/ml) denoted by increase in light transmitted through the stirred preparation at 4 °C, curve (1); 12 °C, (2); 22 °C, (3); and 37 °C, (4). Concanavalin A added at zero time.

Fig. 2. Enhanced aggregation of pretrypsinized gel-filtered platelets suspended in 0.9 % NaCl solution by concanavalin A (1 mg/ml) at 4 or 12 °C (broken line; no significant difference between these two samples). Aggregation of untreated platelets in saline (control preparation) at 4 °C, curve (1); and 12 °C, (2). Concanavalin A added at zero time.

at 1 mg/ml plus 0.2 M α-methyl-D-glucopyranoside were added to the preparation at 37 °C the platelets did not aggregate.

Concanavalin A at 1 mg/ml failed completely to aggregate platelets in plasma. It was of interest that Ehrlich ascites cells aggregated readily in saline in the presence of concanavalin A at concentrations ranging from 1 μg to 1.0 mg/ml. But the level of aggregation of these cells by concanavalin A was conspicuously reduced when they were suspended in a solution to which platelet-free plasma had been added.

The addition of ADP ($2.5 \cdot 10^{-6}$ M) to gel-filtered platelets in saline did not cause their aggregation unless calcium and fibrinogen were also added. It could therefore be concluded from these findings that concanavalin A aggregated the gel-filtered platelets in saline by forming cross-bridges, and not by inducing a release of ADP from the platelets.

Effects of temperature and trypsinisation on the aggregation of gel-filtered platelets by concanavalin A

In studies of the effect of temperature on the aggregation of gel-filtered platelets in saline by concanavalin A, it was clear that aggregation was temperature dependent. The rate of aggregation of these platelets by concanavalin A at 1 mg/ml decreased as the temperature was reduced. This is reflected in the varying steepness of the downward slope of the light transmission curves traced at different temperatures over a 30 min period (Fig. 1). It will be seen that the slope of the curve becomes less steep as the temperature is reduced from 37 to 22 °C and 12 °C, There was also a distinct correlation between the size of the aggregates produced and temperature; the aggregates became smaller as the temperature was reduced. At 4 °C the platelets remained separate in the presence of concanavalin A at 1 mg/ml. This low temperature inhibition of aggregation was reversed when the temperature was raised to 37 °C.

Mild trypsin treatment (0.01 %(w/v) solution of trypsin in saline) of the gel-filtered platelets counteracted the inhibitory effect of low temperature on concanavalin A-mediated aggregation of platelets. At 4 and 12 °C the level of platelet

aggregation reached at the end of the 30 min experimental period after trypsin treatment (Fig. 2) was comparable with that recorded for untreated washed platelets rotated at 37 °C (Fig. 1).

At the different temperatures tested, untreated and trypsin-treated gel-filtered platelets in saline remained separate when rotated in the absence of concanavalin A. Aggregation of gel-filtered platelets in saline did not accur at 37 °C when concanavalin A at 1 mg/ml and α-methyl-D-glucopyranoside at 0.2 M were added simultaneously.

Binding of [^{3}H]concanavalin A to platelets

A previous suggestion that the inability of concanavalin A to aggregate platelets in plasma [16, 17] was due to the presence of concanavalin A-reactive glycoproteins in the plasma [16] was supported by our finding, described earlier, that the presence of platelet-free plasma in the medium considerably reduced the level of aggregation of Ehrlich ascites cells. But there remained the question of whether concanavalin A succeeded in binding at all to the platelet surface when the platelets were suspended in plasma, and whether concanavalin A sites were made available to concanavalin A only when the platelets had been isolated from the plasma and suspended in saline. Experiments were therefore designed to measure the amounts of [^{3}H]concanavalin A that binded to platelets while suspended either in plasma or in an isolation medium.

After exposure to [^{3}H]concanavalin A, the platelets were washed by centrifugation to remove non-bound label. This process was considered to be complete when

TABLE I

BINDING OF [^{3}H]CONCANAVALIN A TO PLATELETS SUSPENDED IN EITHER PLASMA OR ISOLATION MEDIUM

Each preparation of platelets which had been treated with [^{3}H]concanavalin A was divided equally into test and control samples. The platelets of both samples were washed several times and the radioactivity of the successive supernatants was measured. The counts for both samples were virtually constant after the third wash. The test sample was finally washed in isolation medium containing 0.2 M α-methyl-D-glucopyranoside and the control sample in isolation medium only. The results presented here were quantitatively reproducible.

Medium in which platelets were suspended when exposed to [^{3}H]concanavalin A	Sample	Number of washes	Counts ($\times 10^3$) per min for supernatant
Plasma	Test	4	1.9
		5 (with α-methyl-D-glucopyranoside)	4.1
	Control	4	2.7
		5	2.1
Isolation medium	Test	4	12.0
		5 (with α-methyl-D-glucopyranoside)	385.0
	Control	4	12.0
		5	12.2

the counts for the supernatant remained constant (this usually occurred between the third and fourth washing). An insignificant amount of radioactive label was invariably recovered in the supernatants at this time, though whether this was due to partial dissociation of platelet-bound [^{3}H]concanavalin A or the release during washing of unbound [^{3}H]concanavalin A that was simply trapped within platelet aggregates was not determined.

In experiments in which [^{3}H]concanavalin A was added to platelet-rich plasma, the supernatant ^{3}H count for the control sample remained constant between the fourth and fifth wash (Table I). However, the supernatant count for the test sample in which the platelets were washed finally (5th time) in a solution containing 0.2 M α-methyl-D-glucopyranoside showed an increase between the fourth and fifth washing. In the series of tests in which [^{3}H]concanavalin A was added to a suspension of washed platelets in isolation medium, the end results were similar except that after the test sample had been washed in the solution containing 0.2 M α-methyl-D-glucopyranoside, the amount of label recovered in the supernatant was much higher (Table I).

The results showed that [^{3}H]concanavalin A was capable of binding to platelets in plasma, but the amount of [^{3}H]concanavalin A binding to platelets in plasma was approx. 200 times less than that which binded to platelets suspended in a physiological solution.

Inhibition of ADP-induced platelet aggregation by concanavalin A

ADP added at low concentrations to platelet-rich plasma will induce rapid reversible aggregation of the platelets. The human platelets in plasma used in our studies aggregated extensively at 37 °C in the presence of ADP at $2.5 \cdot 10^{-6}$ M, reaching a peak in 1 min. The aggregates then dispersed rapidly during the following 2 min (Fig. 3A).

When concanavalin A (1 mg/ml) was added to platelet-rich plasma 1 min before the introduction of ADP at $2.5 \cdot 10^{-6}$ M platelet aggregation was reduced

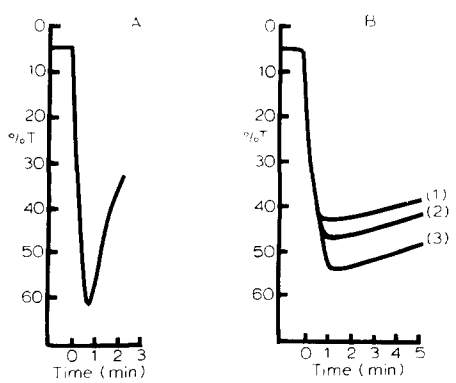

Fig. 3. Inhibition by concanavalin A of ADP-mediated aggregation of platelets and the reversal process in plasma. (A) Reversible aggregation of platelets in plasma by ADP ($2.5 \cdot 10^{-6}$ M), control, added at zero time. (B) Inhibition of ADP-mediated aggregation of platelets in plasma and of the reversal process by concanavalin A at 1 mg/ml, curve (1); at 0.5 mg/ml, (2); and at 0.25 mg/ml, (3). Concanavalin A added 1 min before ADP.

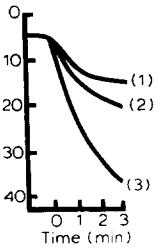

Fig. 4. Inhibition of ADP-mediated aggregation of gel-filtered platelets suspended in 0.9 % NaCl solution supplemented with calcium and fibrinogen. Curve (1), ADP ($2.5 \cdot 10^{-6}$ M)+concanavalin A (1 mg/ml); curve (2), ADP+concanavalin A (100 μg/ml); and curve (3), ADP alone (control). Concanavalin A introduced 1 min before ADP which was added at zero time.

(Fig. 3B) compared with that recorded for the control (Fig. 3A). The level of inhibition of ADP aggregation was dependent on the concentration of concanavalin A (Fig. 3B). Concanavalin A also succeeded in blocking the dispersal of the aggregates formed in the presence of ADP in platelet-rich plasma (Fig. 3B). The addition of α-methyl-D-glucopyranoside failed to disperse the aggregates; neither did the addition of adenosine (10^{-5} M) at the time when maximal aggregation had been reached result in dispersal.

Since there were grounds for believing that the inhibitory effect of concanavalin A on the aggregation of platelets in the plasma might be constrained by the presence of concanavalin A-reactive glycoproteins in the plasma, experiments were conducted with gel-filtered platelets in a salts solution supplemented with calcium and fibrinogen. The gel-filtered platelets in this solution aggregated readily at 22 °C in the presence of ADP at $2.5 \cdot 10^{-6}$ M; this is reflected in the steepness of the slope of the light transmission curve (Fig. 4). The aggregation of gel-filtered platelets was markedly inhibited if the platelets were incubated for 1 min with concanavalin A (1 or 0.1 mg/ml) prior to the addition of ADP (Fig. 4). Although concanavalin A alone was capable of inducing a significant degree of aggregation of platelets in 0.9 % NaCl solution or in this solution supplemented with calcium and fibrinogen over a prolonged 30 min experimental period, the amount of aggregation it could cause in 1 min was, according to the change in light transmission, about 1–2 % compared with the 30–40 % caused by ADP in the same period. The aggregation promotory effect of concanavalin A was therefore relatively very slight and was obscured by its pronounced inhibitory effect on ADP-induced platelet aggregation.

It was therefore concluded that concanavalin A was capable of inhibiting ADP-induced platelet aggregation.

DISCUSSION

In previous studies [16, 17] it was observed that concanavalin A caused the aggregation of washed platelets in buffered solutions containing ionic calcium. Since concanavalin A can induce washed platelets to release ADP [17, 18], which can, in turn, cause washed platelets to aggregate in the presence of calcium provided that fibrinogen remains bound to platelet membrane [23], the possibility of the aggregation effect being attributable to released ADP could not be dismissed. In the current

investigation it was shown that concanavalin A induced gel-filtered platelets to aggregate in a calcium- and fibrinogen-free solution which leads one to conclude that this aggregation was due to concanavalin A forming cross-bridges between surface receptors of adjacent platelets. This conclusion is reinforced by the failure of ADP to aggregate gel-filtered platelets in saline unless calcium and fibrinogen were added.

Aggregation of platelets by concanavalin A was a temperature-sensitive process. Aggregation was maximal at 37 °C, but as the temperature was reduced the level of aggregation progressively decreased, and, at 4 °C, aggregation did not occur. The inhibitory effect of low temperatures on aggregation induced by concanavalin A has been noted in experiments with other cells and it has been suggested that temperature affects cell aggregation by concanavalin A through its effect on the lateral diffusion rates of membrane receptors [24]. Low temperatures would be expected to increase the viscosity of the lipid matrix of the membrane and this would also tend to restrict the mobility of concanavalin A receptors, thereby hindering their 'clustering', a condition which has been regarded [24, 25] as a requirement for cell aggregation by lectins. However, other results do not concur with the suggestion that there is a causal relationship between clustering of receptors and cell aggregation. The demonstration that ferritin-conjugated concanavalin A induces clustering of membrane receptors on cells which are not readily aggregated by the lectin [26] suggests that a clustered distribution of receptors is not the sole requirement for aggregation to occur.

We therefore hypothesize that the orientation of lectin receptor sites may be an important factor in lectin-mediated secretion aggregation and that the inability of concanavalin A to aggregate platelets and other cells at low temperatures is due to the movement of receptor sites into positions which are unfavourable to the formation of intercellular cross-bridges by lectin molecules. If we accept the suggestion that receptor-containing macromolecules are directly or indirectly connected to submembrane microfilamentous and microtubular systems [11, 27], which are affected by low temperatures, it is possible that the altered state of these systems may result in concanavalin A receptor sites being placed in positions that are not conducive to cell aggregation by concanavalin A. According to our hypothesis the aggregation of gel-filtered platelets by concanavalin A at low temperatures after exposure to trypsin could be explained in terms of a configurational change in the receptor-containing glycoprotein brought about by the trypsin. In keeping with this suggestion is the demonstration that trypsinisation of platelets brings about a conformational change in the surface membrane, possibly through a reorientation of the glycopeptide hydrolytic products [5].

Our studies on the capacity of [^{3}H]concanavalin A to bind to platelets suspended in either plasma or a physiological salts solution showed that in both cases concanavalin A receptor sites were available to interact with the lectin but that the amount of concanavalin A that could bind to washed platelets was 200 times greater than that binding to platelets in plasma. It has been shown that thrombin treatment of platelets increased the number of lentil-PHA binding sites [28]; the additional binding sites were located on the membranes of the canalicular system and these probably arose from the fusion of granule membranes with the canalicular membranes during the thrombin-induced process. We do not therefore exclude the possi-

bility that the washing procedure employed in this study may induce the release of some platelet granule contents, and that extension of the canalicular membrane system by the insertion of granule membranes may provide some additional concanavalin A binding sites. However, changes of this kind in the interior of the platelet would not account for the fact that the washed platelets were aggregated by concanavalin A while platelets in plasma were not. The marked inhibitory effect of plasma on the aggregation of Ehrlich ascites cells by concanavalin A tends to support the view that this effect is due to the presence of concanavalin A-reactive glycoproteins in the plasma [16]. It would seem that the removal of these glycoproteins by resuspending the platelets in a salts solution enhances the binding of [^{3}H]concanavalin A to platelets. The amount of concanavalin A which binded to platelets in plasma is presumably insufficient to effect aggregation either by lectin cross-bridging or by stimulating a release of ADP.

High concentrations of concanavalin A produced a slight inhibition of the ADP-induced aggregation of platelets in plasma and, paradoxically, impaired the subsequent dispersal of aggregates which normally occurs when low concentrations of ADP are used to stimulate aggregation. When the platelets were suspended in a salts solution supplemented with calcium and fibrinogen, inhibition of aggregation was more pronounced, and the effect was achieved at lower lectin concentrations than those required to produce inhibition in the presence of plasma. It is curious that Greenberg and Jamieson [18], using the same concentration of concanavalin A (100 μg/ml) as in our studies, did not find that concanavalin A inhibited ADP aggregation. These conflicting results may be due to differences in the procedures employed to separate the platelets from the plasma. It is significant that the washed platelets by Greenberg and Jamieson [18] were not aggregated by concanavalin A, whereas those prepared by ourselves and other workers [16, 17] formed aggregates when exposed to concanavalin A.

The mechanism by which concanavalin A modifies the response of platelets to ADP is unclear. The impairment of aggregation reversal by concanavalin A is particularly perplexing since aggregates formed in the presence of both ADP and concanavalin A were not dispersed by the addition of α-methyl-D-glucopyranoside or adenosine, implying that neither lectin cross-briding nor physiological-type aggregation mediated by released ADP were responsible for stabilizing these aggregates. Our finding that concanavalin A inhibits ADP aggregation is consistent with the inhibition of thrombin-mediated aggregation by concanavalin A [17] since it is thought that this type of aggregation is secondarily induced by ADP released from platelets under the influence of thrombin [29]. Our results show that concanavalin A inhibited ADP aggregation more effectively under conditions which favoured a high level of binding of concanavalin A to the platelet surface. Although we do not discount the possibility of concanavalin A interacting with an extracellular factor which may be implicated with ADP aggregation, our evidence points to the inhibition of ADP aggregation being caused by the interaction of the lectin with platelet membrane glycoproteins. Thus it seems reasonable to regard these complexes as being involved in some way in the process of ADP-induced platelet aggregation.

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