Biochimica et Biophysica Acta 812 (1985) 512–522
Elsevier

BBA 72446

Role of the membrane concanavalin A binding site in platelet-fibrin interactions

Thomas W. Chow, Larry V. McIntire * and Dolores M. Peterson

Chemical Engineering Department, Rice University, P O Box 1892, Houston, TX 77251 (U S A)

(Received May 4th, 1984)

Key words: Concanavalin A; Platelet membrane, Platelet-fibrin interaction; Rheological technique, Platelet contractile force, Blood clotting

Concanavalin A was employed to study the role of platelet membrane glycoproteins in platelet-fibrin interactions during clot formation. A rheological technique was used to study the interactions, measuring the clot rigidity and platelet contractile force simultaneously during the formation of network structure. Concanavalin A lowered the clot rigidity and contractile force of a platelet-rich plasma clot by a small extent. Plasma glycoproteins probably compete with platelet membranes for concanavalin A binding in platelet-rich plasma. Both native concanavalin A (tetrameric) and succinyl concanavalin A (dimeric) lowered the clot rigidity and contractile force of a washed platelet-fibrin clot dramatically, almost down to those values found for fibrin clots. Inhibition studies with α-methyl-D-mannoside indicated that the concanavalin A effects were specific for the concanavalin A binding capacity to platelets. The effects of native concanavalin A on platelet-fibrin clots were only partially reversible, while the succinyl concanavalin A effects were completely reversible. The observed concanavalin A effects are probably mainly due to concanavalin A binding to platelet membrane glycoproteins. The concanavalin A binding site appears to play an important role in the fibrin binding to platelets.

Introduction

Platelets and fibrin are the two major components of a blood clot, the results of hemostasis or thrombosis. A fibrin network is formed by the proteolytic action of the enzyme thrombin on the fibrin precursor, fibrinogen. Polymerizing fibrin strands attach to the platelet membrane [1–3] to make a clot stable by pulling in and tightening the network [4–6]. The molecular basis for this platelet-fibrin binding is, however, not known. Knowledge of this is crucial in understanding hemostasis, thrombosis and the related pathogenic mechanisms involved in atherogenesis and embolic phenomena.

In this paper data are presented that indicate platelet surface glycoproteins may play a role in fibrin binding to the membrane. Concanavalin A, a lectin, was employed in the study. Concanavalin A was chosen for this work because of the following observations. ^{125}I-labelled concanavalin A can intensely label glycoproteins IIb and III [7]. Glanzmann's thrombasthenia platelets, however, have a greatly reduced concanavalin A binding capacity [8]. Thrombasthenic platelets are greatly deficient in glycoproteins IIb and III [9]. Also the clot rigidities of platelet-rich plasma samples from thrombasthenic patients are much lower than those of normal platelet-rich plasma samples [4]. Furthermore, in intact platelets, concanavalin A can induce physical interaction between surface glycoproteins IIb and III and the internal cytoskeleton [10,11].

* To whom correspondence should be addressed.

It is hypothesized that concanavalin A may block fibrin receptors. If this is true, then in the presence of concanavalin A, the clot rigidity as well as the platelet generated contractile force will be reduced dramatically. Concanavalin A effects on clot rigidity as well as platelet contractile force were measured simultaneously using a rheological technique. Both native concanavalin A (tetrameric) as well as succinyl concanavalin A (dimeric) were employed. Clots from plasma samples as well as from a purified fibrinogen-washed platelet system were used.

Materials and Methods

Plasma samples. Blood samples were collected by venipuncture from healthy donors and anticoagulated with 0.1 volume of 3.8% sodium citrate solution. Platelet-poor plasma and platelet-rich plasma were obtained by differential centrifugation [4]. The platelet counts of platelet-rich plasma samples were adjusted with platelet-poor plasma using a Coulter counter Model ZBI (Coulter Electronics Inc., Hialeah, FL). To study the effects of concanavalin A (native ConA from Sigma Chemical Co., St. Louis, MO, and succinyl ConA from Polysciences, Warrington, PA), plasma samples incubated with the specified amount of concanavalin A were clotted using recalcification.

Purified samples. Washed platelets were prepared using the differential centrifugation procedure of Mustard [12]. The washed platelets were resuspended in Hepes buffer (12 mM Hepes/137 mM NaCl/2.7 mM KCl/2 mM $CaCl_2$/1 mM $MgCl_2$/5.6 mM glucose/0.1 mM bovine albumin). Purified Kabi fibrinogen (Helena Laboratories, Beaumont, TX) was also dissolved in Hepes buffer. To study the effects of concanavalin A, samples of washed platelets were incubated with concanavalin A and then clotted with the addition of fibrinogen and human thrombin (Sigma). Another lectin, *Lens culinaris* (Sigma), was also employed in this study for comparison of the effects of different lectins with similar sugar binding characteristics.

Rheological measurements. The clot rigidity and contractile force were measured using a Fluids Rheometer (Rheometrics Inc., Union, NJ). The details of this rheological testing have been described earlier [5,13]. Clotting of a 2 ml test sample was initiated either by recalcification or by thrombin addition and the sample was immediately loaded between two parallel plate platens. A small amplitude, forced-sinusoidal oscillation was then applied continuously to the lower platen. The frequency of the oscillation was 6 Hz and the peak torsional shear strain was 5%. Stress developed by this movement was transmitted to the upper platen by the test sample and the resultant shear strain on the upper platen was monitored by a transducer. From the phase difference and the amplitude ratio (analyzed at 1-min intervals during clot formation) the clot rigidity (dynamic shear elastic modulus (G')) could be calculated, using linear viscoelasticity theory. The contractile force generated by the clot was measured directly by a normal force transducer. All measurements were done at 25°C.

Biochemical assays. Assays for platelet aggregation, release and lysis in the presence of concanavalin A were carried out under conditions similar to those in which the rheological measurements were made — no agitation of the samples. Aggregation was monitored by decrease in platelet count. Release was measured by using ^{14}C-radiolabelled serotonin which was actively taken up into platelet dense granules [14]. Lysis was measured by monitoring level of a cytoplasmic enzyme, lactate dehydrogenase, in the platelet suspending buffer.

Concanavalin A effects on fibrin cross-linking mediated by factor XIII were studied using the 5 M urea clot solubility test [15] and SDS-polyacrylamide gel electrophoresis [16].

Results

Typical rheological data

Normal plasma samples. Typical rheological data of plasma are presented in Fig. 1. The data shown are the clot rigidity (dynamic shear elastic modulus (G')) as well as the contractile force developed by normal platelet-poor plasma and platelet-rich plasma samples during the clotting process.

The initial G' for either a platelet-poor plasma or platelet-rich plasma sample is about 1 dyn/cm^2 (Fig. 1A). The G' value will rise abruptly after a lag time following recalcification, with the onset of

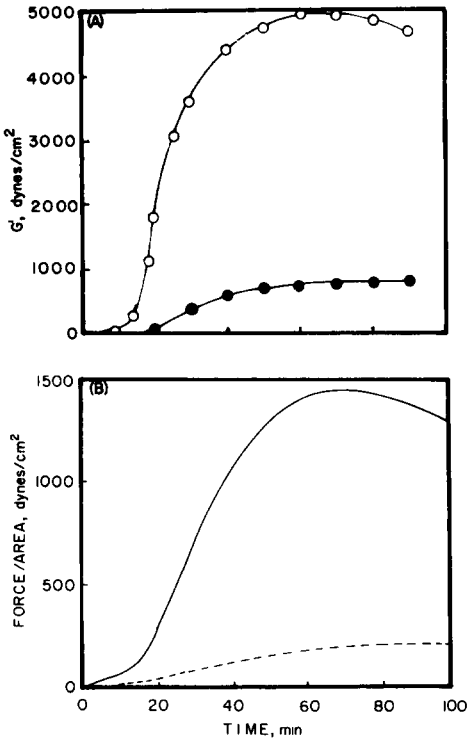

Fig. 1. Clotting curves of normal plasma for both the clot rigidity modulus (G') and contractile force/unit area Platelet-rich plasma with 300000 cells/μl and platelet-poor plasma with less than 2000 cells/μl (the lowest possible count by a Coulter Counter) were prepared from citrated plasma. At time zero, 2 ml samples of platelet-rich plasma or platelet-poor plasma were recalcified with 22 mM $CaCl_2$ (final concentration) to initiate clotting for the rheological measurements The instrument settings were 0 58 mm gap width, 37 7 rad/s oscillation frequency and 5% maximum shear strain The samples were kept at a constant temperature of 25°C throughout the clotting process. The plasma samples are indicated as follows ○ and ———, plate-rich plasma, ● and - - - - - -, platelet-poor plasma

the fibrin polymerization. The lag time is shorter for a platelet-rich plasma sample than that for a platelet-poor plasma sample. The platelet-poor plasma sample in Fig. 1A attained a plateau maximum G' (G'_{max}) of 800 dyn/cm^2. The G'_{max} of platelet-poor plasma samples studied ranged from 500 to about 1000 dyn/cm^2, depending on the fibrinogen concentration. The platelet-rich plasma sample in Fig. 1A attained a G'_{max} of 5000 dyn/cm^2 and then declined slowly. The platelet-rich plasma sample had a platelet count of 300000/μl. The G'_{max} of platelet-rich plasma samples studied ranged from 4000 to 6000 dyn/cm^2, depending mainly on the platelet concentration.

The shapes of the contractile force curves are similar to the G' curves (Fig. 1B). The platelet-rich plasma sample attained a maximum contractile force of about 1500 dyn/cm^2 and then declined. The value of this maximum contractile force depends on the platelet concentration. The platelet-poor plasma sample, on the other hand, had a baseline contractile force of about 100 dyn/cm^2. This value is close to the sensitivity and stability limit of the normal force transducer employed in the measurements. The G' and contractile force data are strongly coupled [5].

Thrombasthenic plasma samples. Rheological data were taken on plasma samples from a Glanzmann's thrombasthenia patient whose platelets have earlier been shown to be greatly deficient in glycoproteins IIb and III [17]. The results are presented in Fig. 2. The thrombasthenic platelet-

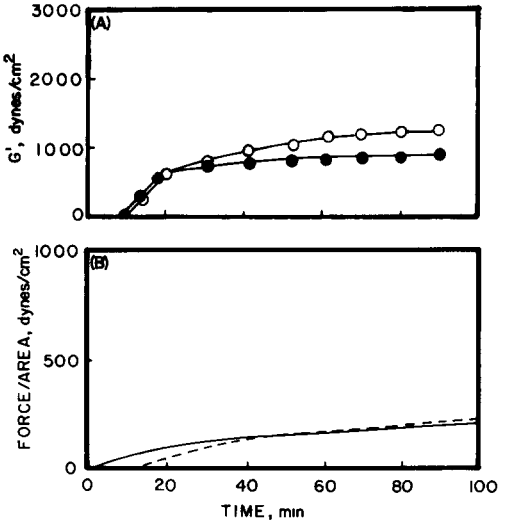

Fig. 2. Clotting curves of Glanzmann's thrombasthenia plasma clot rigidity modulus (G') and contractile force/unit area The platelet count was 300000/μl for the platelet-rich plasma sample and less than 2000/μl for the platelet-poor plasma sample. Time zero was the instant of recalcification with 22 mM $CaCl_2$ (final concentration). The instrument settings for the rheological measurements were 0 58 mm gap width, 37.7 rad/s oscillation frequency and 5% maximum strain. The plasma samples are indicated as follows: ○ and ———, platelet-rich plasma, ● and - - - - - -, platelet-poor plasma

rich plasma sample had a platelet count of 300000/μl, the same as the normal platelet-rich plasma sample in Fig. 1.

The thrombasthenic platelet-poor plasma sample had a G'_{max} of 880 dyn/cm^2 and a baseline maximum contractile force of about 100 dyn/cm^2. These data do not differ significantly from those of the normal platelet-poor plasma sample. The thrombasthenic platelet-rich plasma sample, however, is greatly different from the normal platelet-rich plasma sample. The thrombasthenic platelet-rich plasma attained a G'_{max} of 1300 dyn/cm^2 and only baseline contractile force, compared to the G'_{max} of 5000 dyn/cm^2 and maximum contractile force of 1500 dyn/cm^2 for the normal plasma sample in Fig. 1. Actually, the rheological data of thrombasthenic platelet-rich plasma are not much different from those of platelet-poor plasma.

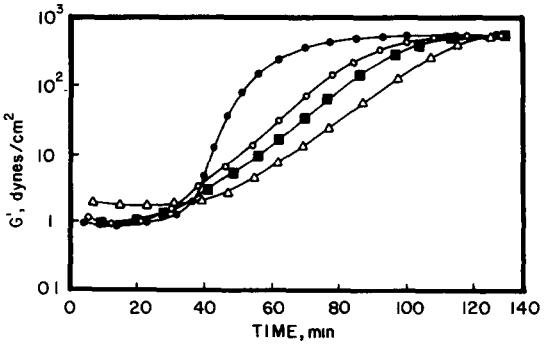

Fig. 4. Effects of tetrameric concanavalin A on platelet-poor plasma clot rigidity (G'). 1.9 ml of platelet-poor plasma samples were incubated with 0.1 ml of concanavalin A in Hepes buffer for 2 min. At time zero, the samples were recalcified with 22 mM $CaCl_2$ (final concentration) to start the clotting for rheological measurements. The tetrameric concanavalin A concentrations are indicated as follows: ●, 0 μg/ml; ○, 100 μg/ml, ■, 250 μg/ml; △, 500 μg/ml.

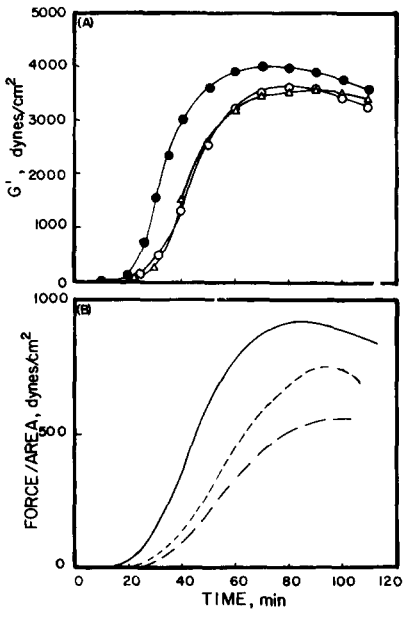

Fig. 3. Effects of tetrameric concanavalin A on the platelet-rich plasma clot rigidity (G') and contractile force/unit area. 1.9 ml of platelet-rich plasma samples were incubated with 0.1 ml of varying amount of concanavalin A in Hepes buffer for 2 min. The samples were then recalcified with 22 mM $CaCl_2$ (final concentration) to start the clotting for rheological measurements. All platelet-rich plasma samples had a platelet count of 300000/μl. The concanavalin A concentrations are indicated as follows. ● and ———, 0 μg/ml, ○ and - - - - - -, 250 μg/ml, △ and ·—·—·, 500 μg/ml

Effects of concanavalin A on plasma samples

Figs. 3 and 4 show the effects of native concanavalin A (tetrameric) on plasma clots. Tetrameric concanavalin A lowered the maximum clot rigidity (G'_{max}) of platelet-rich plasma samples (Fig. 3A). Concanavalin A concentrations of 250 μg/ml and 500 μg/ml lowered the G'_{max} from 4000 to 3620 and 3580 dyn/cm^2 (10% and 13% reduction). Concanavalin A effects on the contractile force were more significant, leading to 20 and 40% reduction in the maximum contractile force for the two concanavalin A concentrations (Fig. 3B).

Tetrameric concanavalin A decreased the rate of platelet-poor plasma clotting kinetics (Fig. 4). However, concanavalin A did not appear to affect the extent of fibrin polymerization as concanavalin A had no effect on the final value of G'_{max}. All samples in Fig. 4 attained a G'_{max} of 550 dyn/cm^2. Also all samples had a negligible baseline contractile force of about 100 dyn/cm^2 (data not shown).

Effect of tetrameric concanavalin A on purified samples

Since most coagulation factors and many other proteins in the plasma are glycoproteins [18], they may compete with the platelets for the binding of concanavalin A. Fig. 4 strongly supports this possibility. To avoid this competitive binding prob-

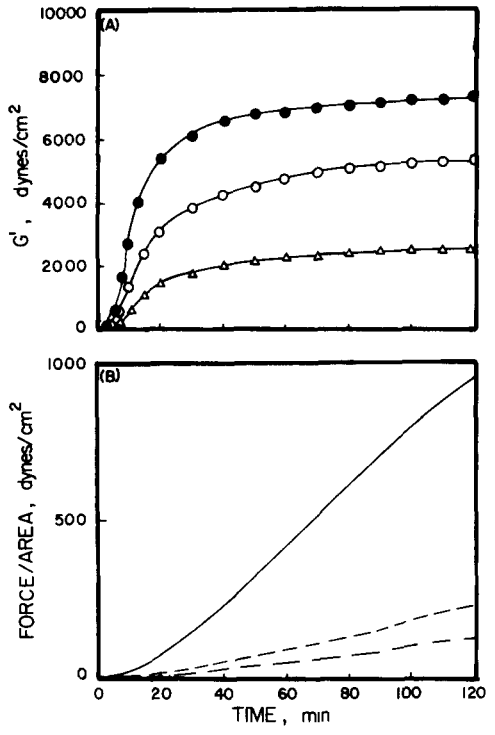

Fig 5 Effects of tetrameric concanavalin A on the clot rigidity (G') and contractile force/unit area of washed platelet-fibrin clots 1 ml of washed platelets was incubated with 0 1 ml of concanavalin A in Hepes buffer for 2 min At time zero, 0 9 ml of fibrinogen in Hepes buffer and 10 μl thrombin were added to start the clotting for rheological measurements All samples had a final platelet count of 300000/μl, fibrinogen concentration of 3 mg/ml and thrombin concentration of 0.25 NIH unit/ml. The tetrameric concanavalin A concentrations are indicated as follows. ● and ———, 0 μg/ml; ○ and - - - - - -, 50 μg/ml, △ and ·–·–, 100 μg/ml

lem, rheological measurements were done with purified samples of fibrinogen and washed platelets instead of plasma samples.

Fig. 5 shows the effects of tetrameric concanavalin A on these platelet-fibrin clots. The G'_{max} of a platelet-fibrin clot decreased with increasing concanavalin A concentration (Fig. 5A). A concanavalin A concentration of 100 μg/ml lowered the G'_{max} from 7300 to 2500 dyn/cm^2. The G'_{max} of a corresponding purified fibrin clot without platelets was about 1800 dyn/cm^2.

An even more dramatic effect on the contractile force was observed (Fig. 5B). At a concanavalin A concentration of 100 μg/ml, the contractile force at 120 min after the initiation of clotting was lowered from about 1000 dyn/cm^2 to about 200 dyn/cm^2. The contractile force at 120 min was chosen for convenience; the maximum force was attained at about 200 min, and not all samples were run this long. The actual maximum contractile force of the platelet-fibrin clot in Fig. 5 was about 1700 dyn/cm^2. The baseline contractile force for a corresponding fibrin clot was about 100 dyn/cm^2.

A three-dimensional fibrin network formation is necessary for the development of rigidity and contractile force of a platelet-fibrin clot. The G' of a washed platelet sample without fibrinogen is about 1 dyn/cm^2 either in the presence or absence of thrombin (0.25 NIH unit/ml). The corresponding contractile force is of baseline value.

The results of experiments similar to Fig. 5 are summarized in Table I. A dose-response effect of tetrameric concanavalin A on platelet-fibrin clots is indicated. A 100% decrease means that the G'_{max} and contractile force of the platelet-fibrin clots are equal to those values for a fibrin clot without

TABLE I

TETRAMERIC CONCANAVALIN A EFFECTS ON THE MAXIMUM G' AND CONTRACTILE FORCE OF PLATELET-FIBRIN CLOTS

The definition of % decrease in G'_{max} and contractile force are as follows

$$\% \text{ decrease in } G'_{max} = \left(1 - \frac{G'_{ConA} - G'_{Fg}}{G'_{control} - G'_{Fg}}\right) \times 100$$

$$\% \text{ decrease in force} = \left(1 - \frac{F_{ConA} - F_{Fg}}{F_{Control} - F_{Fg}}\right) \times 100$$

The subscripts Control, Fg, and ConA indicate the control platelet-fibrin sample, fibrin sample and platelet-fibrin sample with concanavalin A present, respectively G' and F indicate the sample G'_{max} and contractile force at 120 min The contractile force at 120 min after initiation of clotting is chosen for convenience; the maximum force takes about 200 min to attain

ConA concn (μg/ml)	% decrease in G'_{max}	% decrease in contractile force (F)
0	0	0
50	30	64
100	86 ± 9	96 ± 3

platelets (G'_{Fg}, F_{Fg}). At a concanavalin A concentration of 100 μg/ml, the G'_{max} and contractile force were reduced to values close to those of a fibrin clot without platelets.

A variation of concanavalin A incubation time from 0 to 10 min was carried out. No noticeable difference in the concanavalin A effects on platelets was found due to incubation time; the extent of the reduction of clot rigidity and contractile force was about the same. This agrees with the observation [10] that the redistribution of glycoproteins IIb and III is partially completed within 15 s after concanavalin A addition. An incubation time of 2 min was chosen for most experiments presented here to allow for more than enough time for maximal concanavalin A effects on platelets.

Possible concanavalin A effects on thrombin were also investigated. Incubation of platelets with concanavalin A was done in the presence as well as in the absence of thrombin. Also concanavalin A incubation after the platelet incubation with thrombin (under no agitation) for 1 min was also examined. No differences in the concanavalin A effects on G'_{max} and contractile force were found in any of these cases.

Assays for platelet aggregation, release and lysis were carried out under conditions similar to our rheological experiments — no agitation after the initial mixing. Aggregation, release and lysis were negligible for all tetrameric concanavalin A and dimeric concanavalin A concentrations used in this paper (data not shown). Also at these concentrations, concanavalin A had no effect on ADP-induced aggregation (2 μM ADP final concentration).

Succinyl concanavalin A effects

Succinyl concanavalin A (dimeric) was also employed in this study. The succinyl concanavalin A (from Polysciences, Warrington, PA) was prepared by modification of concanavalin A with succinyl anhydride, and the purity was confirmed by appearance of a single component in disc gel electrophoresis [19]. Comparing the effects of the two forms of concanavalin A is interesting because tetrameric concanavalin A induces membrane glycoprotein rearrangement while dimeric concanavalin A does not [19].

The G'_{max} of a platelet-fibrin sample decreased

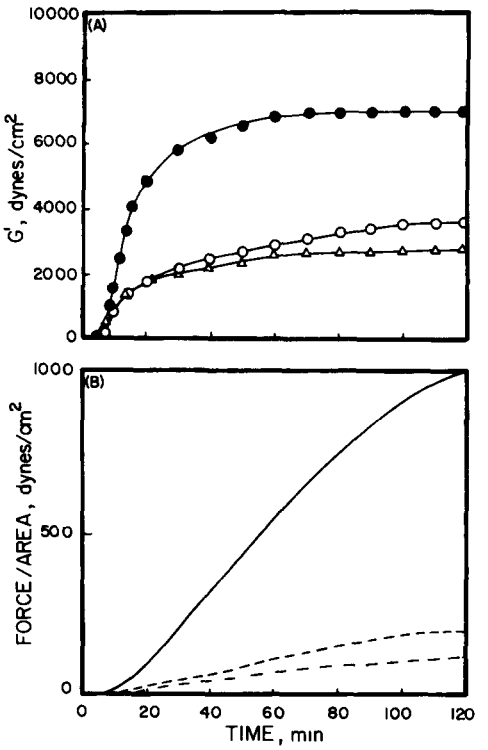

Fig. 6. Effects of succinyl concanavalin A on the clot rigidity (G') and contractile force/unit area of washed platelet-fibrin clots 1 ml of washed platelets (300000 cells/μl final concentration) was incubated with 0 1 ml of succinyl concanavalin A for 2 min. At time zero, 0.9 ml of fibrinogen (3 mg/ml) and 10 μl thrombin (0.25 NIH unit/ml) were added to start the clotting for rheological measurements. The succinyl concanavalin A concentrations are indicated as follows: ● and ———, 0 μg/ml; ○ and - - - - - -, 400 μg/ml, △ and ·– –·, 800 μg/ml.

with increasing dimeric concanavalin A concentration (Fig. 6A). A dimeric concanavalin A concentration of 800 μg/ml lowered the G'_{max} from about 7100 to about 2700 dyn/cm^2. The corresponding fibrinogen solution attained a G'_{max} of 1800 dyn. A similar effect on the contractile force was seen (Fig. 6B). A dimeric concanavalin A concentration of 800 μg/ml lowered the contractile force from 1000 to 120 dyn/cm^2 at 120 min after clotting was initiated.

Result of similar experiments are summarized in Table II. A concentration dependent effect of dimeric concanavalin A is indicated. A dimeric concanavalin A concentration of 800 μg/ml reduced the G'_{max} and contractile force of a platelet-

TABLE II

DIMERIC CONCANAVALIN A EFFECTS ON THE MAXIMUM G' AND CONTRACTILE FORCE OF PLATELET-FIBRIN CLOTS

The definitions of % decrease in G'_{max} and contractile force are the same as those in Table I

Dimeric concanavalin A concn. (μg/ml)	% decrease in G'_{max}	% decrease in contractile force (F)
0	0	0
100	6	36
300	51	66
400	63	85
500	60	86
800	79	94

fibrin clot close to those values found for a fibrin clot.

Effects of concanavalin A on fibrinogen samples

Concanavalin A effects on fibrin clots without platelets were also measured. 0.1 ml of concanavalin A in Hepes buffer was added to 1.9 ml of purified fibrinogen solution and the sample was clotted immediately by 10 μl of thrombin. The final fibrinogen concentration was 3 mg/ml and the thrombin concentration was 0.25 NIH unit/ml. Varying amounts of concanavalin A were used. Tetrameric as well as dimeric ConA was employed.

Tetrameric concanavalin A concentrations of 50 and 100 μg/ml lowered the G'_{max} of a fibrin clot by 2% and 36%, respectively. A dimeric concanavalin A concentration of 250 μg/ml lowered the G'_{max} of a fibrin clot by 8% but a dimeric concanavalin A concentration of 500 μg/ml increased the G'_{max} by 18%. Concanavalin A had no effect on the fibrin clot contractile force, as all fibrin samples had a baseline contractile force of about 100 dyn/cm^2.

Also concanavalin A effects on fibrin cross-linking mediated by factor XIII were studied by SDS-polyacrylamide gel electrophoresis. The amounts of γ-dimers and α-polymers present were approximately the same, regardless the amount of tetrameric concanavalin A present (Fig. 7). As much as 200 μg/ml of concanavalin A was used

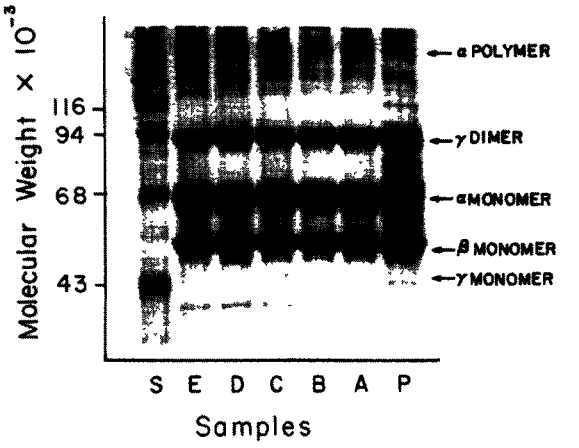

Fig 7. Effects of tetrameric concanavalin A on the cross-linking of fibrin clots 2 ml samples of fibrinogen (3 mg/ml) were clotted with thrombin (0 25 NIH unit/ml) in the presence of varying amount of concanavalin A for 4 h The clots were washed with saline and centrifuged They were then dissolved by overnight incubation in 9 M urea, 3% SDS, 3% mercaptoethanol, 10% glycerol solution at 37°C 10-μl samples were electrophoresed for 4 h in 7 5% polyacrylamide gels and stained with Coomassie blue Lane S, molecular weight standards, lane P, platelet-poor plasma sample; lane A–E, fibrin samples with the following concanavalin A concentrations. lane A, 0 μg/ml, lane B, 50 μg/ml; lane C, 100 μg/ml, lane D, 150 μg/ml; lane E, 200 μg/ml.

in the experiments shown. Concanavalin A appears to have little effect on the extent of cross-linking of a fibrin clot. This was further confirmed by 5 M urea clot solubility test — the fibrin clot did not dissolve in 5 M urea even in presence of the highest concentration of concanavalin A.

Inhibition studies

α-Methyl-D-mannoside binds to concanavalin A and can be used as an inhibitor of concanavalin A effects on platelets. Reversal of either dimeric or tetrameric concanavalin A effects on rigidity increased with increasing concentration of mannoside (Fig. 8). The tetrameric concanavalin A effects on platelets were only partially reversible; the % inhibition was always higher for the experiments when the mannoside incubation was carried out before the concanavalin incubation. The dimeric concanavalin A effects on platelets were, however, completely reversible. The inhibition curves were the same regardless of the incubation sequence of

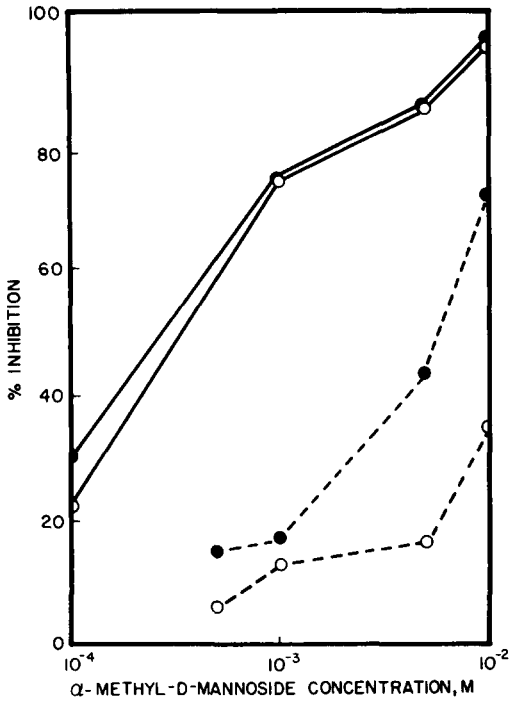

Fig 8 Inhibition of the concanavalin A effects on the washed platelet-fibrin clot rigidity by α-methy-D-mannoside 1 ml samples of platelets (300000 cells/μl final concentration) were incubated with 0 1 ml of concanavalin A for 2 min and 0 1 ml of mannoside for 2 min with the incubation sequence indicated as follows. ●, mannoside first, concanavalin A second, ○, concanavalin A first, mannoside second 0 8 ml of fibrinogen (3 mg/ml) and 10 μl thrombin (0 25 NIH unit/ml) were then added to initiate the clotting for rheological measurements. The tetrameric concanavalin A concentration was 100 μg/ml and dimeric concanavalin A concentration was 500 μg/ml for all inhibition experiments Tetrameric concanavalin A experiments are indicated by (- - - - -), and dimeric concanavalin A experiments are indicated by (———) The % inhibition is defined as follows,

$$\% \text{ inhibition} = (G'_{man} - G'_{ConA})/(G'_{Control} - G'_{ConA}) \times 100$$

where

$G'_{conA} = G'_{max}$ of platelet-fibrin clots with either tetrameric concanavalin A (100 μg/ml) or dimeric concanavalin A (500 μg/ml) present,

$G'_{man} = G'_{max}$ of clots with both concanavalin A and mannoside present,

$G'_{Control} = G'_{max}$ of control clots (no concanavalin A, no mannoside)

dimeric concanavalin A and the mannoside. Also for the same mannoside concentration, tetrameric concanavalin A effects were inhibited to a lesser extent than dimeric concanavalin A effects. Similar inhibition curves of concanavalin A effects on the contractile force of washed platelet-fibrin clots were observed (data not shown).

Similar inhibition experiments were done for samples of fibrinogen only. In the presence of 0.01 M α-methyl-D-mannoside, the small dimeric concanavalin A effect as well as the tetrameric concanavalin A effect on fibrin clot G'_{max} were completely eliminated. The reversal of concanavalin A effects was equally effective regardless of the incubation sequence of concanavalin A and mannoside with the fibrinogen solution.

Effect of Lens culinaris on purified samples

Lens culinaris has the same sugar specificity as concanavalin A [20]. Experiments similar to those used for studying concanavalin A effects were carried out. *Lens culinaris* (up to 1000 μg/ml) had no noticeable effect on the G'_{max} and contractile force of either platelet-fibrin clots or fibrin clots.

Discussion

The data presented here indicate that concanavalin A receptor on platelet membranes may be important in the platelet-fibrin interactions. Tetrameric concanavalin A lowers the G'_{max} and contractile force of a platelet-rich plasma sample significantly (Fig. 3). The effects, however, were not as dramatic as expected. This may be due to the possibility that other plasma glycoproteins may compete for the concanavalin A in its binding to platelet membranes. Most coagulation factors are glycoproteins [18]. It has been observed that the amount of [^{3}H]concanavalin A which combines with platelets in plasma is about 200-times less than that binding to washed platelets [21]. The data on concanavalin A effects on platelet-poor plasma strongly support this possibility of competitive binding; the kinetics of platelet-poor plasma clotting are delayed (Fig. 4). This also agrees well with the observation [22] that concanavalin A can delay the partial thromboplastin time of normal human control plasma. This inhibitory effect is due to its capacity to interact with the carbohydrate portion of blood clotting factors [22].

To bypass this problem of competitive binding by plasma factors, a purified system of washed

platelets and purified fibrinogen was used for the study. Both tetrameric and dimeric concanavalin A greatly lower the clot rigidity as well as the contractile force generation of a platelet-fibrin clot, almost down to those values of a purified fibrin clot (Tables I and II).

The changes in the rheological parameters of washed platelet-fibrin clots may be due to concanavalin A effects on any one of the three major components of the purified clots — thrombin, fibrin, and platelets. Concanavalin A effects on thrombin are unlikely. No difference in the clot rigidity and contractile force was observed when concanavalin A incubation of platelets was done either in the presence or the absence of thrombin. Also concanavalin A does not affect the thrombin time of a purified fibrinogen clot [22]. Furthermore, it has been demonstrated that partial removal of the oligosaccharides of the thrombin molecules by exoglycosidases has no apparent effect on the following thrombin activities: fibrinogen clotting, binding to polymerized fibrin and stimulation of platelets [23].

Concanavalin A effects on fibrin network formation appear to be minimal. Concanavalin A does not have any significant effect on the extent of formation of γ-dimers and α-polymers of fibrin clots (Fig. 7). This agrees well with the observation [24] that the thrombin time is not affected by the presence or absence of the carbohydrate moieties of fibrinogen. Also, tetrameric and dimeric concanavalin A do not alter the baseline contractile force of purified fibrin clots. However, these lectins do have small effects on the clot rigidity. Tetrameric concanavalin A slightly lowers the G'_{max} while dimeric concanavalin A slightly increases the G'_{max} of a fibrin clot. These effects can be completely reversed by 0.01 M α-methyl-D-mannoside. These small concanavalin A effects on the fibrin clot rigidity can be explained by the concanavalin A binding to the carbohydrate moiety of fibrinogen and subsequent slight alteration of the fibrin conformation or its ability to interact with other fibrin molecules to form fibrin strands. The increased negative charge on succinyl concanavalin A may be the reason for the different effects on the fibrin clot rigidity by the two forms of concanavalin A.

Both tetrameric and dimeric concanavalin A lower the G'_{max} and contractile force of a platelet-fibrin clot dramatically The effects of tetrameric concanavalin A are only partially reversed by 0.01 M α-methyl-D-mannoside. Therefore it seems the effects observed in platelet-fibrin clots are mainly due to concanavalin A effects on platelets. The mannoside inhibitions of concanavalin A effects on platelet-fibrin clots indicate that the concanavalin A effects are specific to binding interactions. Nonspecific effects, such as cell poisoning, are unlikely. It has been observed by gel overlay experiments [11] that tetrameric concanavalin A and succinyl concanavalin A bind to the same receptor on the platelet surface. Tables I and II indicate that a higher succinyl concanavalin A concentration is required to attain the same effects on platelet-fibrin clot rigidity and contractile force generation when compared to tetrameric concanavalin A This can be explained by the slightly lower binding capability of succinyl concanavalin A, probably because of its decreased valence and increased negative charge [11].

The tetrameric concanavalin A effects on platelet-fibrin clot rigidity and contractile force are only partially reversible. Since tetrameric concanavalin A is a multimeric ligand, it can cause glycoprotein cross-linking. The observed irreversibility of tetrameric concanavalin A effects is probably partially due to post-binding glycoprotein rearrangement. Succinyl concanavalin A, with a lower valence, has similar effects on platelet-fibrin clot rigidity and contractile force to those of tetrameric concanavalin A except that they are completely reversible. Rearrangement of surface glycoproteins after binding of succinyl concanavalin A is, therefore, unlikely. This agrees with the observation [19] that succinyl concanavalin A does not induce cap formation of its receptors in lymphocytes. Hence the observed concanavalin A effects on platelet-fibrin binding cannot be attributed purely to rearrangement of glycoproteins and subsequent masking of the fibrin receptor.

Another possible explanation for the observed concanavalin A effects is that concanavalin A activates the platelet contractile mechanism before the fibrin binding. This is unlikely because of the following observations. Both tetrameric concanavalin A and succinyl concanavalin A effects can be reversed by an inhibitor (α-methyl-D-man-

noside) even when the concanavalin A incubation is before the mannoside incubation. Furthermore, succinyl concanavalin A does not induce physical interaction between surface glycoproteins and the internal cytoskeleton (contractile mechanism), even though tetrameric concanavalin A can [11]. Therefore the observed concanavalin A effects are probably mainly due to its binding to the surface glycoproteins. It appears as if the concanavalin A receptor and the fibrin receptor are the same one, or very closely related on the platelet surface. Therefore the concanavalin A receptor may be important in platelet-fibrin interactions.

Even though *Lens culinaris* has a similar carbohydrate specificity to that of concanavalin A, it does not have any noticeable effect on either the clot rigidity or the contractile force generation of a platelet-fibrin clot. The difference between *Lens culinaris* and concanavalin A effects may be due to the fact that the *Lens culinaris* binding affinity is about 50-times smaller than that of succinyl concanavalin A [20]. Another possibility for the difference between the two lectins may be due to a subtle difference in their binding specificities [25,26]; they probably recognize distinct glycoproteins on platelet membranes. *Lens culinaris* was found to bind preferentially to glycoprotein IIb as identified by SDS-polyacrylamide gel electrophoresis of platelet membranes followed by staining with ^{125}I-labelled *Lens culinaris* [27]. On the other hand, ^{125}I-labelled concanavalin A was found to label glycoproteins IIb and III intensely [7].

Even though it cannot be deduced solely from the concanavalin A data presented here, the hypothesis that the glycoprotein complex IIb and III is the fibrin receptor is appealing because of the following observations. Concanavalin A binds to the platelet surface glycoproteins IIb and III [7] and this in turn can induce physical interaction between the surface glycoproteins and the internal cytoskeleton [10,11]. Glanzmann's thrombasthenia platelets have a greatly reduced binding capacity for concanavalin A, when compared to normal platelets [8]. Thrombasthenic platelets are greatly deficient in glycoproteins IIb and III [9,17]. Rheological measurements indicated the thrombasthenic platelet-rich plasma samples had greatly reduced clot rigidity (G'_{max}) and baseline contractile force (Fig. 2). Hence it appears that glycoproteins IIb and III may be the bridge between the fibrin strands and the internal platelet contractile apparatus necessary to transmit a force from the actin filament to the fibrin network. These glycoproteins may be the fibrin receptor on the platelet membrane exterior surface. It has been reported that the glycoprotein IIb and III complex acts as a fibrinogen receptor [28,29] and it is often assumed that the membrane fibrinogen receptor also served as the mediator between platelet and fibrin.

In conclusion, the concanavalin A receptor on platelet membranes appears to play a role in platelet-fibrin binding. Whether glycoproteins IIb and III play any role in platelet-fibrin binding cannot be answered until further work with monoclonal antibodies to glycoproteins IIb and III is carried out. However, the use of rheological techniques such as those described above will prove to be a useful additional tool to investigate the details of the complex interactions between internal platelet microfilaments and the external fibrin network.

Acknowledgements

This research was partially supported by grant HL 18672 from the NHLBI and Grant C-938 from the Robert A. Welch Foundation. We also wish to thank Dr. C.J. Jen for the many helpful discussions and suggestions.

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