PLATELET ACTIVATION AND WHEAT GERM AGGLUTININ-BINDING

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1. Introduction

Platelet membrane contains a few glycoproteins, which are important to the aggregation phenomena [1,2]. Platelets interact with wheat germ agglutinin (WGA), possibly through glycoprotein I [3–5], and are agglutinated by this lectin. A glycoprotein extract, isolated from human platelet plasma membrane, exhibits haemagglutination and, in addition, causes agglutination of platelets [6]. We have postulated two possible mechanisms to explain this activity:

(1) A lectin is buried in the membrane which, upon activation, becomes exposed.
(2) Both lectin and the receptor may be present at the surface of the cell, but are self-neutralized in the freely-circulating platelet.

Once the cell is activated, the pair-complex dissociates and intercellular interaction occurs outside the plane of the surface membrane. To further examine the availability of WGA receptors on the platelet membrane, and their possible exposure due to activation, we studied the binding of WGA to platelets before and during aggregation.

We demonstrate here that there is no change in the binding of ^{125}I-labeled WGA to washed human platelets, as a result of thrombin or ADP-induced aggregation. On the contrary, a marked decrease in the lectin binding was observed when the aggregation was performed in platelet-rich plasma (PRP).

2. Materials and methods

Washed platelets were obtained either as a 24 h old platelet concentrate from the Central Blood Bank in Tel Aviv, or fresh, prepared as in [7]. Briefly, blood was collected into 1/10 vol. sodium citrate 3.2% and 1/20 vol. aspirin 1 mM. After 15 min incubation at 37°C, the blood was centrifuged at 300 × *g* for 10 min to yield the PRP. The PRP was incubated with 1 μM prostaglandin E_1 (PGE_1) for 20 min and adjusted to pH 6.5 with 0.1 M citric acid. This mixture was centrifuged at 1000 × *g* for 20 min and the platelets were resuspended in 1–2 ml Tyrode's solution, containing 2 mg/ml albumin, 0.02 M Hepes and 0.3 μM PGE_1. The platelets were separated by gel filtration on Sepharose 2B column, equilibrated with the above buffer not containing PGE_1.

Platelet-rich plasma (PRP) was obtained by collecting the blood into 1/10 vol. 3.8% sodium citrate and centrifuging 3 times at 300 × *g* for 10 min each. WGA was purchased from Makor Chem., Jerusalem, and iodinated as follows: 1 mg lectin was dissolved in 200 μl borate buffer 0.1 M (pH 8.5) containing 50 mM *N*-acetyl-D-glucosamine. This solution was added to 50 μCi Bolton and Hunter reagent [8] (Radiochemical Centre Amersham) and the mixture was incubated at room temperature for 30 min. Glycine was added to 1 mM final conc. and after a further 15 min incubation the mixture was dialyzed against cold water for 3 days. Binding of WGA to platelets (washed or PRP) was studied in the following way: 450 μl PRP or washed platelets were stirred in the Chronolog cuvette and 10–15 μl ^{125}I-labeled WGA in saline (usually 4–8 × 10^4 cpm) were added at different stages of the aggregation (initiated by thrombin, ADP or epinephrine, as in table 2). After 3 min the platelet aggregate was transferred to an Eppendorf tube and centrifuged. The platelet pellet was washed with saline and counted. Non-specific binding was determined by adding the same amount of ^{125}I-labeled WGA to platelets containing 10 mM *N*-acetyl-D-glucosamine.

Abbreviations: WGA, wheat germ agglutinin; PRP, platelet-rich plasma; PGE_1, prostaglandin E_1; ADP, adenosine diphosphate

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3. Results and discussion

3.1. *Binding of ^{125}I-labeled WGA to platelets*

Time and concentration curves of ^{125}I-labeled WGA to platelets are shown in fig.1,2. The specific binding of WGA to platelets was linear up to 0.1 μg lectin/ml, saturation was reached at 1–2 μg/ml. From table 1, it can be seen that full agglutination of platelets occurred only at ⩾0.5 μg WGA/ml, while at <0.05 μg WGA/ml there was no agglutination at all. It seems, therefore, that at low lectin concentrations, where the binding is linear, there is no agglutination, probably because of the low WGA concentration. At higher concentrations the platelets tend to be agglutinated by the lectin with continued binding, but deviation from linearity.

The time curve of WGA binding to platelets shows that maximal binding is obtained after <1 min (fig.2).

3.2. *Effect of platelet aggregation on WGA binding*

The effect of aggregation on the binding of WGA was studied. ^{125}I-Labeled WGA was added to platelets during aggregation induced by various reagents, at 4 different points (see legend to table 2), and at the end of the aggregation the platelet pellet was isolated and the degree of specific binding was determined (see section 2). Results are shown in table 2. The binding of WGA to washed platelets is not affected by aggregation initiated by any of the agents used (thrombin, ADP, epinephrine), no matter at what stage the lectin

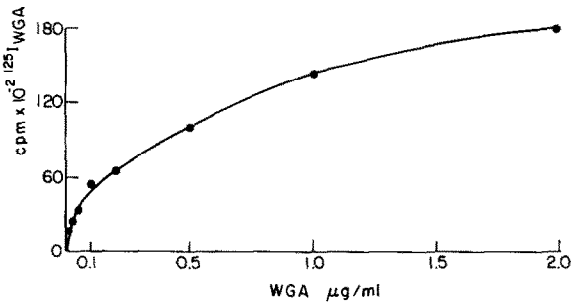

Fig.1. Concentration curve of wheat germ agglutinin (WGA) binding to washed human platelets.

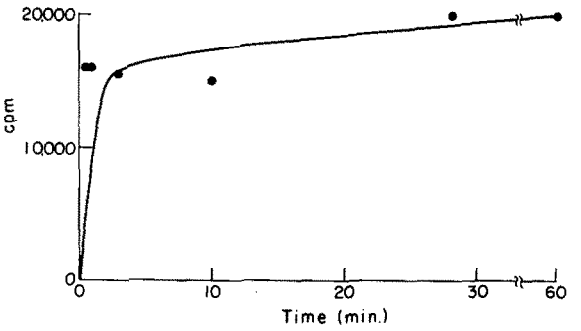

Fig.2. Time curve of wheat germ agglutinin (WGA) binding to human platelets (in platelet plasma).

Table 1
Agglutination of human washed platelets by WGA

WGA (μg/ml)	Agglutination
10	+++
1	+++
0.5	++
0.05	+
0.005	–

Table 2
Change in binding of WGA to human platelets

Washed platelets		Platelet-rich plasma		
Aggregation by thrombin	Aggregation by ADP	Aggregation by thrombin	Aggregation by ADP	Aggregation by epinephrine
a +10%	+8%	–275%	–210%	–60%
b + 8%	–2%	–100%	–360%	–60%
c – 6%	n.d.	– 2%	–120%	–17%
d + 6%	n.d.	+ 10%	– 50%	0%

The change in binding of WGA is expressed in % compared to the binding of the lectin to resting platelets: a, WGA added together with aggregation agent; b, WGA added at the end of aggregation (usually 1–1.5 min after the addition of aggregating agent); c, WGA added 30 s before aggregating agent; d, WGA added 2 min before aggregating agent; n.d., not determined

was added. On the contrary, when WGA was added to PRP together with or after the aggregating agent, a marked decrease in the lectin binding to the platelets was observed. This reduction in the specific binding of WGA was up to 700% in a few cases and always >100%. The fact that this effect was observed only in PRP and not in washed platelets indicates that a plasma component participates in this phenomenon. It seems, that, as a result of platelet activation (in PRP), either by thrombin, epinephrine or ADP, a factor present in the plasma is activated and competes with WGA on binding to the platelets. Glycoprotein I may be the binding site for WGA on platelet membrane [2,3,5]. However, glycoprotein I may be also involved in the interaction of platelets with plasma von Willebrand factor [9,10]. It is, therefore, possible that the binding sites of WGA and von Willebrand factor are identical or at least similar. Another possible explanation, which cannot be ruled out at this stage, is the possible release of a specific component from the platelet membrane during activation. This component would be part of the WGA binding site and present in PRP, but probably lost or inactivated during the preparation of washed platelets.

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