D-GALACTOSE-BINDING LECTINS INDUCE A DIFFERENTIAL RESPONSE OF BLOOD PLATELETS

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SUMMARY. Platelets are strongly aggregated by 50 µg/ml of soybean agglutinin (SBA), Cytisus scoparius agglutinin (CSA I), and peanut agglutinin (PNA). The effects of SBA, CSA I, and PNA require pretreatment of the platelets with neuraminidase and are inhibited by D-galactose. PNA is the only one of these lectins which simultaneously induces the secretion and the concomitant shape change of platelets. Cytochalasin B enhances the effect of PNA but is inactive with SBA and CSA I. Thus the saccharide specificity of the lectins does not determine the kind of the platelet response for which additional binding properties of these lectins may be crucial.

INTRODUCTION. Evidence is accumulating showing that crosslinkage and redistribution of cell-surface receptors are prerequisites for various effects of lectins on cells (review article 1). Properties of lectin and its receptors may be considered to determine the potency of a lectin to crosslink sugar receptors on a cell surface. Recently, Goldman et al. (2) investigated the vacuolating effects of various lectins on macrophages. Soybean agglutinin and peanut agglutinin which bind to terminal D-galactose residues were nonvacuolating. However lectins (wheat germ, wax bean, concanavalin A) which bind to sugar residues closer to the peptide backbone induced vacuolation. Therefore, it has been suggested that one of the reasons for the differential response of cells to lectins is the location of their specific sugar receptors of a carbohydrate side chain of membrane glycoproteins (2). By binding close to the peptide backbone the lectin may crosslink its receptors more easily than binding to terminal sugar residues located further away (2). Our

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observations on the lectin stimulation of blood platelets argue against this assumption. These results demonstrate that a differential response of platelets may be triggered at terminal D-galactose residues.

MATERIAL AND METHODS. Washed platelets. Human platelets were isolated from EDTA platelet-rich plasma (PRP) (3) at 20°C and washed with EDTA-containing Tris/HCl-buffered saline, pH 7.4, supplemented with 5 mM KCl, 0.5 mM glucose, and 50 µg/ml albumin. Platelet serotonin was previously labelled by preincubating the PRP with 0.3 µM ^{3}H-serotonin (Amersham Buchler TRA 223) for 15 min at 20°C. The concentrated washed platelets ($1x10^7$ μl^{-1}) were maintained at 4°C until aliquots were transferred to cuvettes in which the test medium had been warmed to 37°C.

Aggregation assay. One ml samples ($2x10^5$ platelets μl^{-1}) were analyzed in a six-channel aggregometer (modified model UDC 1A, Kontron/Labotron, Munich, Germany) equipped with interference filters 600 nm. Two min after addition of the concentrated platelets to the test medium (which had the same composition as the washing solution except that EDTA was exchanged by 0.5 mM CaCl) the stirring mechanism was switched on and the lectins were applied to the cuvettes.

Shape-change studies were performed with the same equipment as used in aggregation experiments. To avoid aggregation, 1 mM EDTA was added to the test medium and the stirring mechanism was switched on for less than 1 sec on the addition of a lectin. In order to amplify the small changes in optical density during the shape change of the platelets the recorder was set to the very sensitive absorbance range log 0.25.

Release of serotonin. At the end of a 5-min experiment (aggregation or shape change) the sample was poured into an ice-cooled vial containing EDTA and centrifuged for 30 sec at 12,000 x g (Eppendorf 3200) at 4°C. 500 µl supernatant were checked for radioactivity. The total ^{3}H and the radioactivity of the supernatant were concurrently determined in 500 µl control suspension of labelled platelets. The percentage release of serotonin was calculated by the quotient: ^{3}H sample supernatant minus ^{3}H control supernatant per total ^{3}H.

Lectins. Soybean agglutinin (SBA) and peanut agglutinin (PNA) were isolated according to procedures described in the literature (4,5) and checked for homogeneity by polyacrylamide-disc electrophoresis. Cytisus scoparius agglutinin I (CSA I) was purified by affinity chromatography on Sepharose-N- ε -aminocaproyl-β-D-galactopyranosylamine (6).

Cytochalasin B was purchased from Aldrich Chem. Co, Milwaukee, USA, and solved in dimethylsulfoxide. The final concentration of dimethylsulfoxide in the platelet experiments was $\leq$ 0.1 % .

Neuraminidase treatment. The platelet suspensions ($2x10^5$ μl^{-1}) were incubated with 25 U/ml of neuraminidase from Vibrio cholerae (E.C. 3.2.1.18) (Behringwerke) for 20 min immediately before the addition of the lectins.

RESULTS AND DISCUSSION. Platelets respond to certain lectins with aggregation and/or a discharge of secretory organelles (= the release reaction) (7-10). There are some lectins which selectively induce aggregation and others which cause a strong release reaction without concomitant aggregation (unpublished results). Therefore, it is clear that aggregation and release reaction of platelets are based on different triggering mechanisms. This study shows that the differential response to lectins may, however, be triggered by binding to the same sugar residues of cell-surface glycoproteins. A comparison is made between the effects on aggregation and the release reaction of three lectins which have in common their binding capacity for galactose-like saccharide sites on cell membranes: soybean agglutinin (SBA) (11), peanut agglutinin (PNA) (5), and Cytisus scoparius agglutinin I (CSA I). The other criterion for our assumption that these lectins bind to the same target sugar(s) is the requirement for a pretreatment of the platelets with neuraminidase to render the platelets responsive to these lectins. SBA, PNA, and CSA I induce neither aggregation nor the release reaction of native platelets even at 200 µg/ml (the highest concentration applied). After pretreating the platelets with neuraminidase these lectins produce a strong but differential response at lectin concentrations of about 50 µg/ml.

Figure 1 shows the platelet aggregation induced by the three lectins at two concentrations. The main result is the fact that PNA-induced aggregation is accompanied by a release of serotonin while almost no release of serotonin is found in samples with SBA- or CSA I-induced platelet aggregations of similar strength. PNA-induced aggregation shows a distinct lag phase until the downward deflection of the curve indicates aggregation. The lag

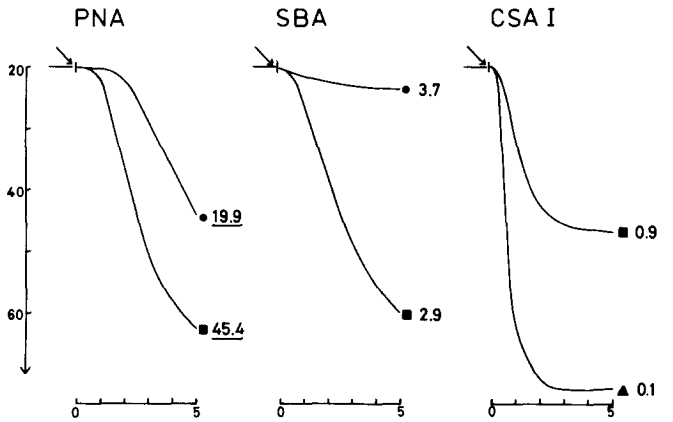

Fig. 1 Abscissa: time (min); ordinate: light transmission (%). Platelet aggregation induced by two concentrations of peanut agglutinin (PNA), soybean agglutinin (SBA), and Cytisus scoparius agglutinin (CSA I). The arrows indicate the point of addition of the lectin. Lectin concentrations: ● 20 µg/ml, ■ 50 µg/ml, and ▲ 100 µg/ml. The numbers at the end of each curve show the percentage of serotonin released during the experiment (5 min). The platelet suspensions were preincubated for 20 min with neuraminidase (25 U/ml).

phase is particularly pronounced at lower lectin concentrations (20 µg/ml, see fig. 1). The occurrence of a lag phase leads to the assumption that PNA-triggered aggregation requires previous propagation of the release reaction. This requirement is well known for physiological stimuli of platelet aggregation, e.g. thrombin (12) or collagen (13). In addition, the three lectins exhibit different dose-response relationships. These appear to be steep for SBA and CSA I and quite shallow for PNA, a fact which may point to the different mechanisms for their aggregating effects.

Evidence for the ability of PNA to induce the secretion is obtained from experiments with nonstirred (nonaggregating) platelet suspension. It has previously been demonstrated that lectins which directly induce the platelet secretion (e.g. concanavalin

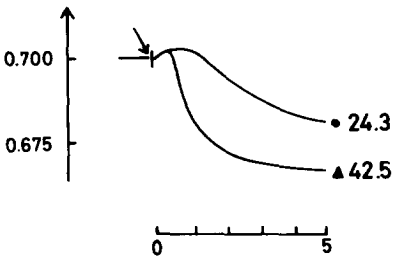

Fig. 2 Abscissa: time (min); ordinate: light absorbance. The shape change and the serotonin release of platelets exposed to 50 µg/ml peanut agglutinin (the arrow indicates the point of its addition) • without cytochalasin B, ▲ with 1 µg/ml cytochalasin B added 2 min prior to the addition of the lectin. The shape change is indicated as the change in absorbance of the unstirred platelet suspension. The numbers at the end of each curve show the percentage of serotonin released during the experiment. The platelet suspensions were preincubated for 2o min with neuraminidase (25 U/ml).

A) cause a characteristic biphasic change in optical density of the platelet suspension (shape change) (10). The final downward deflection of the shape-change curve corresponds to the extrusion phase of the release reaction (14).

Figure 2 demonstrates that PNA evokes a biphasic shape change and the release of serotonin. The extrusion phase may be stimulated by cytochalasin B and the release of serotonin increases simultaneously (Fig. 2). It has recently been shown that the stimulative effect of cytochalasin B is restricted to the extrusion phase of the release reaction (15) and requires the initiation of the release reaction by an appropriate lectin (14). SBA and CSA I are inactive even at 200 µg/ml and CB consistently fails to evolve its stimulative effect. The differential response to these lectins is confirmed by electron micrographs which show with PNA the typical morphologic alterations linked to the secretion response and the absence of these changes with SBA and CSA I (unpublished results). The aggregating effects of

SBA and CSA I are blocked by 6 mM D-galactose. This hapten concentration is, however, not sufficient to suppress the effects of PNA. The finding that about 30 mM D-galactose is neccessary to block the effect of 50/ug/ml PNA suggests that PNA has a particularly high affinity to its natural receptors. N-acetyl-galactosamine is a much weaker inhibitor than D-galactose with the three lectins.

It has repeatedly been shown that SBA and PNA bind only with neuraminidase-treated cells (2,5). The ability of CSA I to agglutinate erythrocytes was markedly enhanced by treating the cells with neuraminidase (6). These findings agree with our results on platelets. Treatment of platelets with neuraminidase was necessary to expose binding sites by which the differential response of the platelets may be triggered. Since these lectins possess the same binding capacity for D-galactose-like residues it is quite probable that the D-galactose residues penultimate to sialic acid (16) are their common saccharide receptors. The inhibitory effect of D-galactose on the activity of the three lectins support this conclusion. Assuming that terminal D-galactose is the essential component of their binding sites, the differential response of platelets to these lectins point to the importance of certain additional properties of a lectin in determining the direction of the cellular response. It is not known whether crosslinkage of surface receptors is the prerequisite either for platelet aggregation or (and) the release reaction induced by lectins. Therefore, one can only speculate what properties are characteristic for a lectin which induces not only aggregation but also the secretion response, e.g. PNA. The number and the steric arrangement of the carbohydrate-binding sites on the lectin molecule may account for a higher affinity to

cellular receptors of the secretion-inducing PNA. These factors more than the saccharide-binding specificity appear to be relevant to the differential response to lectins. Goldman et al. already assumed from results with chemically modified lectins that the sugar specificity is not the only reason for the difference in the effects of lectins on cells (2).

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