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DISCOIDIN I-MEMBRANE INTERACTIONS

II. DISCOIDIN I BINDS TO AND AGGLUTINATES NEGATIVELY CHARGED PHOSPHOLIPID VESICLES

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The endogenous lectin of *Dictyostelium discoideum*, discoidin I, binds to multilamellar phosphatidylcholine vesicles containing a net negatively charged lipid constituent. This constituent can be a phospholipid or a fatty acid. The amount of binding observed correlates directly with the magnitude of the net charge on the negatively charged constituent and its mole fraction in the vesicles. The binding exhibits a time course and a specificity similar to those observed previously (Bartles, J.R. and Frazier, W.A. (1982) Biochim. Biophys. Acta 687, 121–128) for binding to the I sites on fixed *D. discoideum* cells. The best inhibitors of binding are those components, such as NaCl and polyelectrolytes, which serve to increase the effective ionic strength of the buffer and thereby attenuate the electrostatic interaction between positively charged domains on the discoidin I tetramer and the negatively charged vesicle surface. Binding to the vesicles is multivalent, exhibits apparent positive cooperativity with respect to discoidin I, and appears to be modulated allosterically by hapten sugars of discoidin I. Discoidin I can cause the agglutination of sonicated negatively charged phospholipid vesicles. Vesicle agglutination requires a concentration of discoidin I above a certain threshold, which is dictated by vesicle composition and concentration. Vesicle agglutination is not observed with equivalent or higher concentrations of concanavalin A or bovine serum albumin. The rate and extent of vesicle agglutination are reduced by inhibitors of discoidin I binding to the phospholipid vesicles. Thus, discoidin I has the ability to bind to and to agglutinate negatively charged membranous structures by an electrostatic mechanism that does not rely on the carbohydrate binding activity of the lectin.

Introduction

The endogenous lectin discoidin I is believed to be required for developmental cell cohesion in *Dictyostelium discoideum* [1–3]. In the previous paper [4], we demonstrated the existence of two types of receptors for discoidin I on the surface of glutaraldehyde-fixed *D. discoideum* cells: the carbohydrate or C sites and the ionic or I sites. The C sites correspond to the developmentally regulated carbohydrate-containing receptor characterized previously [5] on fixed *D. discoideum* cells. The I sites bind discoidin I electrostatically, and hence, are detected only under the physiological condition of relatively low ionic strength (17 mM P_i, pH 6.3). Based on the enormous number of I sites

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Abbreviations: 17 mM P_i, 17 mM Na_2HPO_4-KH_2PO_4/100 U penicillin G per ml/100 μg streptomycin sulfate per ml (pH 6.3); albumin, bovine serum albumin; PC, phosphatidylcholine; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol.

found on the fixed cells (greater than 10^6/cell) and their extractability with chloroform/methanol, we suggested that the I sites represent ionic lipids [4]. In order to test this hypothesis, we have investigated the interaction of discoidin I with vesicles prepared from purified phospholipids.

Materials and Methods

Materials. Concanavalin A (twice crystallized, 79-001) was from Miles (Elkhart, IN). Phosphotungstic acid (P-4006), phosphatidylcholine (PC) (type V-E from egg yolk, P-5763), phosphatidylglycerol (PG) (ammonium salt, grade I from egg lecithin, P-0514), phosphatidic acid (sodium salt, from egg lecithin, P-9511), phosphatidylethanolamine (PE) (type III from egg yolk, P-6386), phosphatidylserine (PS) (from bovine brain, P-8515), phosphatidylinositol (PI) (sodium salt, grade I from soybean, P-0639), cardiolipin (sodium salt, from bovine heart, C-3760) and *cis*-vaccenic acid (grade I, V-6251) were obtained from Sigma (St. Louis, MO). Lipid purity was verified by thin-layer chromatography on 0.25 mm Silica gel 60 plates (Merck) in $CHCl_3/CH_3OH/H_2O$ (65:25:4, v/v) with I_2 vapor and Phospray (Supelco) visualization. The phospholipids were essentially free of lysophospholipid and fatty acid contaminants, but PC, phosphatidic acid and PS did contain trace amounts of diacylglycerol impurities. All other materials were obtained from the sources designated previously [4]. Discoidin I was isolated, radioiodinated and prepared for use in experiments as described previously [4].

General methods. All methods were performed at 23 ± 1°C unless otherwise indicated. Phospholipid concentration was estimated as the number of equivalents of carboxylate ester (reported as equiv. ester) using the colorimetric assay of Snyder and Stevens [6] on 50- to 100-μl aliquots of vesicle suspensions in 17 mM P_i using PC as a standard. Protein concentration was routinely estimated by absorbance at 280 nm; 0.1 mg/ml solutions of discoidin I, concanavalin A and albumin have A_{280} values of 0.3, 0.14 and 0.07, respectively.

Vesicle preparation. Vesicles were prepared using PC and the indicated mol% of another phospholipid or fatty acid. Vesicle components were mixed in the appropriate ratios from their organic solvent stock solutions and rotary evaporated to dryness twice from $CHCl_3$ in a round bottom flask at 30°C, forming a thin uniform layer of dried lipid on the walls of the flask. The flasks were then lyophilized for 6 to 15 h to remove residual organic solvents.

To prepare multilamellar vesicles, 17 mM P_i was added to the lyophilized lipid to give a final concentration of about 20 μequiv./ml ester. The flask was swirled for 1 min until all the lipid was removed from the walls of the flask, three 1-mm diameter glass beads were added, and the suspension was vigorously blended on a Vortex mixer for 30 s. For use in binding assays, the multilamellar vesicles were washed twice with 17 mM P_i containing 1 mg/ml of albumin by centrifugation (48000 $\times g$, 10 min, 4°C), resuspended in 17 mM P_i containing 1 mg/ml of albumin by vigorous blending on a Vortex mixer and used within 1 h.

For use in vesicle agglutination assays, the unwashed multilamellar vesicle preparation was diluted with 17 mM P_i to about 5 μequiv./ml ester and sonicated in four or five 15-s bursts in a glass test tube submerged in an ice-water bath using the tapered microtip of a Branson model W-185 probe-type sonicator (40 to 50 watts output). The sonicated vesicles had an A_{550} of less than 0.2 and were used within 1 h.

Binding assay. The binding assays were performed in albumin pre-equilibrated polycarbonate centrifuge tubes containing $(0.5–1.0) \cdot 10^6$ cpm/ml (10–25 ng/ml) of ^{125}I-discoidin I, 0–3 μequiv./ml ester of the washed multilamellar phospholipid vesicles (see above) and the indicated final concentrations of inhibitors or unlabeled discoidin I in 17 mM P_i (pH 6.3) containing 1 mg/ml of albumin. The tubes were shaken at about 380 strokes/min on a New Brunswick model R-2 reciprocating shaker to keep the vesicles suspended. After the indicated time intervals, 200- or 300-μl samples of the incubation mixture were washed twice by centrifugation (48000 $\times g$, 10 min, 4°C) in albumin pre-equilibrated polycarbonate centrifuge tubes with at least 10 vol. of 4°C 17 mM P_i containing 1 mg/ml of albumin.

For dissociation experiments, portions of the binding incubation mixture were centrifuged (48000 $\times g$, 10 min, 4°C), and the supernatant containing the unbound ^{125}I-discoidin I was de-

canted. The pelleted vesicles were resuspended to one-fiftieth their initial (incubation mixture) concentration in albumin pre-equilibrated polycarbonate centrifuge tubes with 17 mM P_i containing 1 mg/ml of albumin and the indicated final concentrations of other components, and the suspensions were periodically agitated to keep the vesicles suspended. After the indicated times of dissociation, 10-ml samples of the diluted vesicle suspension were centrifuged (48000 × *g*, 10 min, 4°C), and the pellets were washed twice with 10 ml of 4°C 17 mM P_i containing 1 mg/ml of albumin by centrifugation as before.

Pelleted radioactivity was determined on the ^{125}I channel of a Beckman 300 γ counter, and pelleted phospholipid was determined colorimetrically as described above. The binding data represent the means of four or five determinations, which varied by less than ±6% from their reported means. In all cases, the data have been corrected for the tube binding observed in parallel incubations in the absence of vesicles (typically 2–5% of the added cpm) and for the amount of phospholipid pelleted after the binding incubation (typically > 90% of the added μequivalents ester).

Vesicle agglutination assay. 600 μl of the sonicated 5 μequiv./ml ester suspension of phospholipid vesicles (see above) and 200 μl of 17 mM P_i were added to a 1 cm path length quartz cuvette, and the turbidity of the samples was monitored continuously as A_{550} using a Gilford 240 spectrophotometer equipped with a recorder. Next, 200 μl of 17 mM P_i or a 6-fold concentrated solution of inhibitor in 17 mM P_i was added. None of the inhibitors employed caused a significant change in the A_{550} of the suspension beyond that expected from dilution alone. At time zero, and again at later times when so designated in the individual figures, the indicated amounts of discoidin I, concanavalin A or albumin were added from precentrifuged (48000 × *g*, 10 min, 4°C) 1.5 mg/ml solutions in 17 mM P_i. An equal volume of 17 mM P_i was added simultaneously to control samples. The reported A_{550} traces are corrected for the dilutions accompanying the various additions.

Electron microscopy. One part of a 2.5 μequiv./ml ester suspension of sonicated phospholipid vesicles in 17 mM P_i (containing any added proteins) was mixed with two parts of an aqueous 1% (w/v) solution of phosphotungstic acid in a glass test tube for 30 s at 23°C. A drop of this suspension was allowed to stand on a Formvar-coated 200-mesh copper grid for 30 s before drawing off the excess fluid with a piece of filter paper. The grids were rinsed once for 30 s with a drop of water and examined in a Zeiss EM9 S-2 electron microscope.

Results

Direct binding studies

Multilamellar vesicles of different composition were assayed for binding at subsaturating vesicle concentrations. Table I indicates that significant ^{125}I-discoidin I binding was observed only to those vesicles containing net negatively charged components; vesicles containing electrically neutral components only, such as the PC or PC-PE vesicles, did not bind significant amounts of ^{125}I-discoidin I. With the exception of the PC-PI vesicles, those vesicles containing negatively charged phospholipid components with one net negative charge/headgroup phosphate (PC-PS, PC-PG and PC-cardiolipin) bound roughly equal amounts of ^{125}I-discoidin I. Consistent with a requirement for net negative charge on the vesicles, the PC-phosphatidic acid vesicles (with phosphatidic acid having two net negative charges per headgroup

TABLE I

EFFECTS OF VESICLE COMPOSITION ON BINDING

The binding assay (see Materials and Methods) was performed for 2.5 h at 23°C on 300-μl samples containing 0.75 μequivalent ester of vesicles of the indicated composition and $2.7 \cdot 10^5$ cpm (5.4 ng) of ^{125}I-discoidin I.

Vesicle composition	Bound (cpm/μequiv. ester)
PC	80
PC-PE (20 mol%)	10
PC-*cis*-vaccenic acid (20 mol%)	2100
PC-PS (20 mol%)	3000
PC-PG (20 mol%)	3200
PC-cardiolipin (11 mol%) [a]	3800
PC-PI (20 mol%)	6500
PC-phosphatidic acid	9600

[a] Equivalent to 20 mol% in cardiolipin headgroup phosphate.

phosphate) bound about 3-times this much ^{125}I-discoidin I. The PC-PI vesicles bound about 2-times more ^{125}I-discoidin I than expected based solely on their net charge. The net negative charge on the vesicles need not be contributed by a phospholipid, because the PC vesicles containing the fatty acid *cis*-vaccenic acid also bound significant amounts of ^{125}I-discoidin I.

The amount of ^{125}I-discoidin I bound to PC-cardiolipin vesicles increased dramatically as the mol% of cardiolipin in the vesicles increased. We selected PC-cardiolipin vesicles containing 33 mol% cardiolipin (PC-cardiolipin (33 mol%) vesicles) for use in the subsequent experiments to further characterize the discoidin I-vesicle interaction. Binding to the PC-cardiolipin (33 mol%) vesicles increased in a hyperbolic fashion with increasing vesicle concentration. The binding saturated at about 2.2 μequiv./ml of ester, which amounted to about 0.33 μmol/ml of net negatively charged headgroups for these PC-cardiolipin (33 mol%) vesicles. About 20% of the added ^{125}I-discoidin was bound at this saturating vesicle concentration. Binding appeared to take more than 2 h to approach steady state. Unless otherwise indicated, all subsequent experiments examining the specificity and reversibility of the discoidin I-phospholipid vesicle interaction employed 1.5 μequiv./ml of PC-cardiolipin (33 mol%) vesicles and 2.5 h incubations.

Table II indicates that binding to the PC-cardiolipin (33 mol%) vesicles was inhibited by those salts (150 mM NaCl) and polyelectrolytes (10 μg/ml dermatan sulfate or 100 μg/ml heparin) that were shown previously [4] to inhibit the binding of ^{125}I-discoidin I to the ionic or I sites on fixed NC-4 *D. discoideum* cells. Low temperature (4°C) reduced binding to the levels observed in high salt concentrations, and concentrations of EDTA as high as 1 mM inhibited binding by 32%. It is difficult to interpret this inhibitory effect of EDTA because of the ionic nature of this compound. Interestingly, 50 mM *N*-acetyl-D-galactosamine caused a slight (15 to 35%) but significant ($P < 0.01$ and $P < 0.05$ in two separate experiments) inhibition of binding relative to 50 mM *N*-acetyl-D-glucosamine controls.

Binding to the PC-cardiolipin (33 mol%) vesicles exhibited the same type of apparent positive cooperativity with respect to discoidin I as did binding to the I sites of fixed NC-4 *D. discoidum* cells [4]. Fig. 1A shows that unlabeled discoidin I enhanced ^{125}I-discoidin I binding to the vesicles. This behavior generates the apparent nonsaturable discoidin I concentration dependence illustrated in Fig. 1B. In general, albumin (1 mg/ml) was included in all of the ^{125}I-discoidin I binding experiments in order to reduce tube binding to acceptable levels (2–5% of the added cpm). The presence of albumin in the assays did not cause the apparent positively cooperative discoidin I binding, but it did seem to enhance the magnitude of the cooperative effect. When the binding assays were performed in the presence and absence of 1 mg/ml albumin, in tubes that had been pre-equilibrated with 1 mg/ml of albumin and then rinsed with buffer, the albumin caused a 30% decrease in binding at tracer concentrations of ^{125}I-discoidin I (10 ng/ml) and a 40% increase in binding at high discoidin I concentrations (10 μg/ml). Thus, the unlabeled discoidin I enhanced binding by 250% in the absence of albumin and by 500% in its presence.

TABLE II

INHIBITORS OF DISCOIDIN I-VESICLE ASSOCIATION

The binding assay (see Materials and Methods) was performed for 2.5 h at 23°C (except for 4°C entry) on 300-μl samples containing 0.45 μequivalent ester of PC-cardiolipin (33 mol%) vesicles, $2.5 \cdot 10^5$ cpm (3.8 ng) of ^{125}I-discoidin I and the indicated final concentrations of inhibitors. The data are tabulated as a percentage of the corresponding control binding observed in the absence of inhibitor ($4.5 \cdot 10^4$ cpm/μequiv. ester).

Inhibitor	Bound (% of control)
None	100
4°C	22
150 mM NaCl	23
10 μg/ml dermatan sulfate	33
100 μg/ml heparin	34
1 mM EDTA	68
50 mM *N*-acetyl-D-galactosamine	62–83 [a]
50 mM *N*-acetyl-D-glucosamine	96–97 [a]

[a] Range of two separate experiments. Bound values in the presence of 50 mM *N*-acetyl-D-galactosamine were significantly less than those in the presence of 50 mM *N*-acetyl-D-glucosamine ($P<0.01$ and $P<0.05$) by Student's *t*-test for the two experiments.

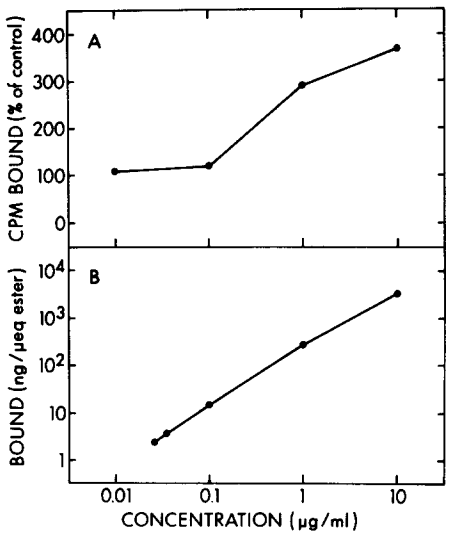

Fig. 1. Discoidin I concentration dependence of binding. The binding assay (see Materials and Methods) was performed for 2.5 h at 23°C on 300-μl samples containing 0.45 μequivalent ester of PC-cardiolipin (33 mol%) vesicles, $3.0 \cdot 10^5$ cpm (7.5 ng) of ^{125}I-discoidin I and the final concentration of unlabeled discoidin I indicated in A, where the data are plotted as a % of the cpm bound in the absence of unlabeled discoidin I ($5.2 \cdot 10^4$ cpm/μequiv. ester). B, The data in A have been replotted to show the number of ng of discoidin I bound/μequivalent of ester as a function of the total discoidin concentration.

To investigate the reversibility of the discoidin I-vesicle interaction, the ^{125}I-discoidin I was permitted to bind to the PC-cardiolipin (33 mol%) vesicles, and the unbound ^{125}I-discoidin I was removed by centrifugation. The vesicles, with approx. 1 ng of ^{125}I-discoidin I bound per μ equivalent of ester, were then diluted to one-fiftieth their initial concentration in 17 mM P_i containing 1 mg/ml of albumin, and the amount of ^{125}I-discoidin I remaining bound was measured as a function of time. Upon dilution, the ^{125}I-discoidin I dissociated in two kinetic phases: an initial, rapid phase ($t_{1/2} < 30$ min) and a subsequent slower phase ($t_{1/2} \simeq 300$ min). About 30% of the ^{125}I-discoidin I dissociated in the rapid phase when the dilution was performed in the absence of binding inhibitors. However, when the vesicles were diluted in the presence of 100 μg/ml of heparin, the amount of ^{125}I-discoidin I dissociating in the rapid phase was increased from 30% to 50%. Dilution of the vesicles into 10 mM *N*-acetyl-D-galactosamine caused a slight (about 10%), but significant ($P < 0.05$) increase of the amount dissociating in the rapid phase, while 10 mM *N*-acetyl -D-glucosamine had no effect. The dissociation behavior was identical at 4°C and 23°C.

Vesicle agglutination studies

The addition of relatively large quantities of discoidin I (>0.2 mg/ml) to a suspension of sonicated PC-cardiolipin (33 mol%) vesicles (2.5 μequiv./ml ester) caused a dramatic time-dependent increase in the turbidity of the suspension. To the naked eye, this turbidity increase was reflected in a change in the appearance of the suspension from translucent to opaque and milky white, like that of the corresponding multilamellar PC-cardiolipin (33 mol%) vesicles. Most of the observed increase in turbidity (measured as A_{550}) occurred relatively rapidly within the first 10 min of discoidin I addition, with a much slower rate of

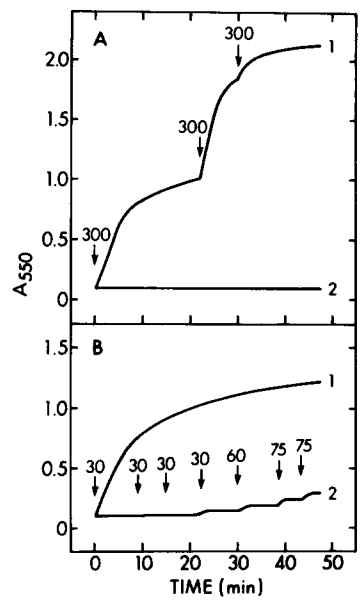

Fig. 2. Discoidin I concentration dependence of vesicle agglutination. Vesicle agglutination was performed using sonicated PC-cardiolipin (33 mol%) vesicles at 23°C as described in Materials and Methods. (A) The indicated numbers of micrograms of discoidin I (curve 1) or equivalent volumes of buffer (curve 2) were added to the vesicles at the times indicated by the arrows. (B) The indicated numbers of micrograms of discoidin I were added to the vesicles at the times indicated by the arrows (curve 2) or 300 μg of discoidin I was added simultaneously to the vesicles at time zero (curve 1).

increase being observed at later times (e.g., see Fig. 2B, curve 1). These turbidity increases eventually saturated with successive discoidin I additions (Fig. 2A, curve 1). Additions of buffer alone to the sonicated PC-cardiolipin (33 mol%) vesicles caused no turbidity increases (Fig. 2A, curve 2).

Light and electron microscopic analyses suggested that the discoidin I-induced turbidity increase was the result of phospholipid vesicle agglutination. When examined under the oil immersion lens in the light microscope, these turbid vesicle suspensions were seen to consist of relatively large (2–5 μm in diameter), irregularly shaped clusters of Oil Red O- and OsO_4-positive material, whereas nothing was observed in comparably stained sonicated vesicle preparations without prior discoidin I addition. Fig. 3 compares the electron microscopic appearance of the sonicated PC-cardiolipin (33 mol%) vesicle suspensions prior to and after the addition of discoidin I. Prior to discoidin I addition, the sonicated vesicles existed primarily as individuals with diameters ranging from 150 to 400 nm (Fig. 3A). After discoidin I addition, most of the vesicles were found to be clumped together or agglutinated into relatively large (2–5 μm in diameter), irregularly shaped structures (Fig. 3B). The agglutinated vesicles were on the average 2- to 3-fold smaller in diameter and appeared to be embedded in a lightly stained matrix (Fig. 3B). The vesicle shrinkage appeared to result from osmotic effects because it is observed with other proteins that do not agglutinate the vesicles (see below).

Fig. 3. Electron micrographs. Vesicle agglutination was performed on sonicated PC-cardiolipin (33 mol%) vesicles at 23°C as described in Materials and Methods. One hour after the addition of 300 μg of discoidin I or an equivalent volume of buffer to the vesicles, the suspensions were stained with phosphotungstic acid and examined by electron microscopy as described in Materials and Methods. (A) Unagglutinated control vesicles. (B) Discoidin I-agglutinated vesicles. The bar indicates 2 μm.

Maximal vesicle agglutination appeared to require the simultaneous addition of relatively large suprathreshold quantities of discoidin I to the sonicated vesicles. Curve 2 in Fig. 2B shows that slight step-wise increases in turbidity are observed when >300 μg of discoidin I were added to the sonicated PC-cardiolipin (33 mol%) vesicles in successive small aliquots (30 to 75 μg). However, the cumulative turbidity increase observed was $<20\%$ of that observed when 300 μg of discoidin I was added simultaneously to the vesicles (Fig. 2B, curve 1). The threshold discoidin I concentration appeared to be a function of the discoidin I to lipid ratio because maximal agglutination of lower concentrations of sonicated PC-cardiolipin (33 mol%) vesicles required proportionately smaller amounts of discoidin I (data not shown).

The vesicle agglutination reaction was not specific for PC-cardiolipin (33 mol%) vesicles, but was observed for other negatively charged phospholipid vesicles as well. Discoidin I agglutinated sonicated PC-PG (50 mol%) vesicles even more rapidly than the PC-cardiolipin (33 mol%) vesicles, whereas PC-PS (50 mol%) required more discoidin I for maximal agglutination. Sonicated PC-PE (50 mol%) vesicles are electrically neutral and were not

agglutinated by discoidin I. The agglutination of PC-cardiolipin vesicles observed for discoidin I was not observed with equivalent or higher concentrations of the lectin concanavalin A or bovine serum albumin.

Inhibitors of ^{125}I-discoidin I binding to multilamellar PC-cardiolipin (33 mol%) vesicles listed in Table II reduced both the rate and the extent of sonicated PC-cardiolipin (33 mol%) vesicle agglutination. 150 mM NaCl totally blocks agglutination while 100 μg/ml of dermatan sulfate and 100 μg/ml of heparin reduce turbidity by 60 and 30%, respectively. Curiously, 1 mg/ml of albumin was more effective than 100 μg/ml of dermatan sulfate at blocking vesicle agglutination, while it actually enhanced steady state discoidin I binding to multilamellar PC-cardiolipin (33 mol%) vesicles at these high discoidin I concentrations. Interestingly, 50 mM *N*-acetyl-D-galactosamine actually enhanced the rate and extent of sonicated PC-cardiolipin (33 mol%) vesicle agglutination relative to a 50 mM *N*-acetyl-D-glucosamine control.

Discoidin I-mediated phospholipid vesicle agglutination appeared to be irreversible because the addition of inhibitors of vesicle agglutination to the agglutinated vesicles did not cause their disaggregation. In addition, the agglutinated vesicles could be diluted as much as 10-fold into buffer without noticeable agglutinate disruption.

Discussion

The data reported here represent the first direct demonstration of a binding interaction between the purified soluble endogenous lectin of *D. discoideum* and vesicles prepared from purified phospholipids. The binding of discoidin I to the negatively charged phospholipid vesicles exhibits properties similar to those observed for binding to the ionic or I sites of fixed *D. discoideum* cells [4], thus providing further evidence in support of the identification of the I sites as anionic lipids. The discoidin I-vesicle interaction appears to result from the direct electrostatic attraction of the negatively-charged lipid vesicle surface for a positively-charged domain on the discoidin I molecule.

In the previous paper [4], we suggested that carbohydrate-containing receptors and ionic receptors interact with different binding sites on the discoidin I molecule. However, these two types of binding sites may not act independently of one another. As was the case for binding to the I sites of fixed *D. discoideum* cells [4], a polyelectrolyte that can bind to the carbohydrate binding site of discoidin I, such as dermatan sulfate, is effective at inhibiting the discoidin I-vesicle interaction at lower concentrations than a polyelectrolyte which does not bind to the carbohydrate binding site of discoidin I, such as heparin. This effect could arise due to increased local concentration of the polyanion in the vicinity of the positively charged domain. Alternatively, an allosteric interaction between the carbohydrate binding sites and the positively charged domain of discoidin I may occur. The fact that *N*-acetyl-D-galactosamine exerts a small, but significant inhibitory effect on discoidin I-vesicle association and a small, but significant enhancement of discoidin I-vesicle dissociation suggests that such an allosteric mechanism is operative. We have already shown that certain negatively charged lipids can serve to enhance erythrocyte agglutination mediated by the carbohydrate binding sites of discoidin I [7].

Interestingly, lowering the temperature to 4°C is as effective as increasing buffer ionic strength at reducing discoidin I binding to the vesicles. This suggests that there is some requirement for vesicle bilayer fluidity for maximal discoidin I binding. This fluidity requirement appears to exist only for discoidin I-vesicle association, because dissociation of the discoidin I from the vesicles appears to be the same at 4°C and 23°C.

The binding of discoidin I to the negatively charged phospholipid vesicles exhibits apparent positive cooperativity. This type of cooperative behavior was also observed for the binding of discoidin I to the I sites of fixed *D. discoideum* cells [4]. In the following paper [8], we demonstrate by cell surface labeling that unlabeled discoidin I actually does bind to the I sites of living *D. discoideum* cells in the nonsaturable fashion predicted by this apparent positive cooperativity in ^{125}I-discoidin I binding.

Quite surprisingly, we find that relatively high concentrations of discoidin I can agglutinate sonicated negatively charged phospholipid vesicles with a specificity similar to that observed for binding to the corresponding multilamellar

vesicles. Those concentrations required for vesicle agglutination are less than the estimated cytosolic concentration of discoidin I in cohesive *D. discoideum* cells. [8]. There are two presently inexplicable differences between the effects of inhibitors on vesicle agglutination and ^{125}I-discoidin I binding. First, 1 mg/ml of albumin appears to be a quite effective inhibitor of vesicle agglutination, while it actually enhances ^{125}I-discoidin I binding to the vesicles at high discoidin I concentrations. Secondly, *N*-acetyl-D-galactosamine enhances the rate and extent of vesicle agglutination relative to an *N*-acetyl-D-glucosamine control. While this effect provides additional evidence for an allosteric effect of hapten sugars on the positively charged lipid binding domains of discoidin I, this is the opposite of the effect that was expected based on the inhibitory effect of *N*-acetyl-D-galactosamine on ^{125}I-discoidin I binding to the vesicles. Perhaps both of these discrepancies result from the fact that vesicle agglutination is a measure of intervesicle crosslinking rather than binding per se.

Other lectins, such as concanavalin A and *Ricinus communis* agglutinin I, have been shown to cause the agglutination of phospholipid vesicles containing certain glycolipids [9,10]. However, in these cases, the agglutination reaction required the presence of glycolipid in the vesicles and clearly reflected the specificity of the carbohydrate binding sites of lectin. Concanavalin A has been shown to agglutinate α-D-glucosylglycolipid-containing phospholipid vesicles so long as they contain negatively charged phospholipids [9]. This fact and the sensitivity of this agglutination to increased buffer ionic strength led Hampton et al. [9] to postulate the existence of a positively-charged domain on concanavalin A, which interacts electrostatically with the vesicle surface.

Considering the ubiquitous distribution of negatively charged lipids in cellular membranes, it seems quite likely that discoidin I can bind to and agglutinate cellular membranous structures, and even cells, by an electrostatic mechanism. The ability to bind to phospholipid vesicles may be a general property of slime mold lectins. Preliminary experiments on the lectin pallidin from *Polysphondylium pallidum* indicate that it too binds to phospholipid vesicles, but with a different phospholipid head group specificity than was observed for discoidin I (Chung, K.-N., Bartles, J.R. and Frazier, W.A., unpublished data). In the following paper [8], we apply the results of our studies using phospholipid vesicles and fixed *D. discoideum* cells [4] to an analysis of the interaction of discoidin I with living *D. discoideum* cells and address the more general question of the function of discoidin I in cell cohesion.

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