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DISCOIDIN I-MEMBRANE INTERACTIONS

I. DISCOIDIN I BINDS TO TWO TYPES OF RECEPTOR ON FIXED *DICTYOSTELIUM DISCOIDEUM* CELLS

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Under physiological buffer conditions (17 mM P_i, pH 6.3), the endogenous lectin of *Dictyostelium discoideum*, discoidin I, binds to two types of receptors on the surface of glutaraldehyde-fixed, wild-type (NC-4) *D. discoideum* cells. We have designated these two types of receptors the carbohydrate or C sites and the ionic or I sites. Binding to the C sites is saturable with respect to discoidin I and is inhibited by hapten sugars (such as *N*-acetyl-D-galactosamine), but not by increasing buffer ionic strength with NaCl or polyelectrolytes. The number of C sites increases about 4-fold during the first 8.5 h of suspension differentiation, reaching a capacity for about $2 \cdot 10^4$ discoidin I tetramers per cell. The binding activity of the C sites is reduced about 50% by sequential $NaIO_4$ oxidation/$NaBH_4$ reduction of the fixed cells, but it is not reduced by $CHCl_3$-CH_3OH extraction of the fixed cells. In marked contrast, binding to the I sites appears nonsaturable with respect to discoidin I, and it is inhibited by increasing buffer ionic strength with NaCl or polyelectrolytes (such as poly-L-glutamic acid or heparin), but not by hapten sugars. The I sites are present on both vegetative and differentiated fixed cells and can bind more than 10^6 discoidin I tetramers per cell. The binding activity of the I sites on fixed cells is not reduced by sequential $NaIO_4$ oxidation/$NaBH_4$ reduction, but is reduced 70 to 90% by $CHCl_3$-CH_3OH extraction. The data suggest that the I sites represent ionic lipids that bind discoidin I electrostatically.

Introduction

During the first several hours of their starvation-induced developmental cycle, amoebae of the cellular slime mold migrate chemotactically into mounds of about 10^5 cells and establish stable intercellular attachments, constructing a multicellular organism (for review, see Ref. 1). Endogenous slime mold lectins represent one of several types of molecules currently believed to play an important role in this cell cohesion process [2–4].

In an attempt to further define the function of the endogenous lectins, we have directly investigated the interaction of the major lectin, discoidin I, from the species *Dictyostelium discoideum* with its receptors on the surface of *D. discoideum* cells. In a previous study [5], we ex-

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Abbreviations: 150 mM NaCl/P_i, 58 mM Na_2HPO_4/18 mM KH_2PO_4/75 mM NaCl/3 mM NaN_3 (pH 7.2); 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.

amined the interaction of ^{125}I-discoidin I with glutaraldehyde-fixed strain A3 *D. discoideum* cells in a phosphate-buffered saline, pH 7.2, of mammalian tonicity (150 mM NaCl/P_i). The ^{125}I-discoidin I bound to the fixed A3 cells in a reversible and saturable fashion. The interaction was of high affinity ($K_d \simeq 3 \cdot 10^{-10}$ M) and demonstrated the expected carbohydrate specificity, with *N*-acetyl-D-galactosamine and D-galactosides being best inhibitory saccharides. The receptor showed the expected developmental regulation, with the cellular binding capacity for discoidin I increasing about 5-fold during the first 10 to 15 h of development. From the sensitivity of the receptor activity to various chemical and enzymatic treatments, we were able to tentatively identify it as a glycoprotein.

This paper represents an extension of this earlier binding study in which we examine the interaction of ^{125}I-discoidin I with glutaraldehyde-fixed wild type (NC-4). *D. discoideum* cells under the physiological buffer conditions routinely employed for the growth [6] and differentiation [7] of these cells in suspension, i.e. in dilute phosphate buffer of pH 6.3 (17 mM P_i). We compare these results to those obtained for binding to these NC-4 cells in the higher ionic strength 150 mM NaCl/P_i employed previously [5]. The NC-4 cells have been used in this study because the axenic mutant strain A3 cells used previously [5] have been found to: (i) internalize a discoidin I-binding proteoglycan from their HL-5 growth medium and then subsequently excrete it while differentiating in suspension [8], and (ii) excrete as much as 40-times more discoidin I than the wild type NC-4 cells during suspension differentiation (unpublished data).

Materials and Methods

Materials. Most materials were obtained from the sources designated previously [5]. Casein hydrolysate (C-9386), heparin (sodium salt H-3125), EDTA (disodium salt, ED2SS), phenylmethylsulfonyl fluoride (P-7626), penicillin G (potassium salt, PEN-K), poly(L-glutamic acid) (sodium salt, P-4761) and streptomycin sulfate (S-6501) were from Sigma (St. Louis, MO). Dermatan sulfate (reference standard grade from hog mucosa) was kindly provided by Drs. M.B. Mathews and J.A. Cifonelli (The University of Chicago). All other chemicals were reagent grade from Sigma or Mallinckrodt (St. Louis, MO). Spore stocks of wild type (NC-4) and axenic mutant strain A3 *D. discoideum* cells were kindly provided by Dr. S.D. Rosen (University of California, San Francisco). Stocks of *Escherichia coli* strain B/r were kindly provided by M.B. Kastan and Dr. M.W. Lieberman.

Growth, differentiation and fixation of cells. E. coli strain B/r were grown to stationary phase on autoclaved modified M9 minimal culture medium [9] (containing 3 g casein hydrolysate, 10 g D-glucose, 7 g K_2HPO_4, 3 g KH_2PO_4, 0.5 g NaCl, 1 g NH_4Cl, 120 mg $MgSO_4$, 15 mg $CaCl_2 \cdot 2\,H_2O$, 2 μg $MnCl_2 \cdot 4\,H_2O$, 27 μg $FeCl_3 \cdot 6\,H_2O$ and 0.1 μmol HCl per liter of distilled H_2O), harvested by centrifugation (4000 × *g*, 8 min, 4°C), washed once with 17 mM P_i by centrifugation and resuspended in 17 mM P_i to 3-times their stationary phase density. This bacterial suspension was then inoculated with wild type (NC-4) *D. discoideum* spores or cells from an existing suspension culture and gyrated (approx. 200 rev./min, New Brunswick model G-2 shaker) at 22°C until reaching a density of $(1–4) \cdot 10^6$ cells/ml (vegetative cells), at which time cells were transferred at $(1–2) \cdot 10^4$ cells/ml into a fresh bacterial suspension. The NC-4 cells were serially transferred no more than 15 times for use in experiments. After the first two transfers from spores, the NC-4 cells doubled every 3 or 4 h under these conditions until reaching $(0.8–1.0) \cdot 10^7$ cells/ml.

After reaching $(1–4) \cdot 10^6$ cells/ml, the vegetative NC-4 cells were washed five or six times with 17 mM P_i by centrifugation (800 × *g*, 3 min, 4°C) and fixed with glutaraldehyde (see below) or gyrated (approx. 200 rev./min) at 10^7/ml in 17 mM P_i at 22°C for 8.5 h (differentiated cells). Vegetative and differentiated NC-4 cells were fixed with glutaraldehyde exactly as described elsewhere [5].

Axenic mutant strain A3 *D. discoideum* cells were grown on HL-5 medium [6] prepared using Oxoid (U.K.) yeast extract and proteose peptone.

Discoidin I. Discoidin I was isolated by affinity chromatography of crude soluble extracts of axenic mutant strain A3 *D. discoideum* cells on columns of acid-treated Sepharose 6B in 150 mM NaCl/P_i as described [5], except that $4 \cdot 10^{-4}$ M phenyl-

methylsulfonyl fluoride was present during the freeze-thaw cell lysis procedure.

Affinity-purified discoidin I was radioiodinated with [^{125}I]Bolton-Hunter reagent, repurified and stored as described [5], except that the reaction was performed on 15–20 μg of discoidin I at pH 8.5 instead of on 10 μg at pH 7.5. The resulting ^{125}I-discoidin I had a specific radioactivity of 55–80 μCi/μg.

Prior to use in experiments, unlabeled and ^{125}I-labeled discoidin I were dialyzed 10–15 h against at least 900 vol. of the appropriate buffer (17 mM P_i or 150 mM NaCl/P_i) at 4°C and centrifuged (13000 × *g*, 5 min, 4°C) or Millipore-filtered (3.0 μm) to remove any precipitate.

Binding assay. The binding assays were performed by the centrifugation method used in our earlier study [5]. All solutions were prepared in the appropriate buffer (17 mM P_i or 150 mM NaCl/P_i) containing 1 mg/ml of albumin and centrifuged (2000 × *g*, 3 min, 4°C) prior to use. Typically, the binding assays were performed in albumin pre-equilibrated 12 × 75 mm Falcon tubes in a total volume of 300 μl containing $(0.7-1.5) \cdot 10^6$ cpm/ml of ^{125}I-discoidin I, $1.0 \cdot 10^7$ cells/ml fixed NC-4 cells and the indicated final concentrations of inhibitors or unlabeled discoidin I all in buffer (17 mM P_i or 15 mM NaCl/P_i) containing 1 mg/ml of albumin. The tubes were shaken at approx. 380 strokes/min on a New Brunswick model R-2 reciprocating shaker for the indicated times at 23 ± 1°C, and then the cells were washed twice by centrifugation (2000 × *g*, 3 min, 4°C) with 5 ml with 4°C buffer (17 mM P_i or 150 mM NaCl/P_i) containing 1 mg/ml of albumin as described [5]. Pelleted radioactivity was determined in a Beckman 300 γ counter. The binding data represent the means of triplicate or quadruplicate determinations, which varied by less than ±5% from their reported means. In all cases, the data have been corrected for the tube binding observed in parallel incubations in the absence of cells. Tube binding was typically 1–5% of the added cpm.

Treatment of fixed cells. For $CHCl_3$-CH_3OH extraction, the fixed, differentiated NC-4 cells were suspended at $3 \cdot 10^7$ cells/ml in $CHCl_3$-CH_3OH (1:1, v/v) for 30 min at 23°C with periodic, vigorous blending on a Vortex mixer. The cells were washed extensively with 150 mM NaCl/P_i by centrifugation (2500 × *g*, 5 min, 4°C). Control cells were extracted with 150 mM NaCl/P_i.

For $NaIO_4$ oxidation/$NaBH_4$ reduction, the fixed, differentiated NC-4 cells were suspended at $3 \cdot 10^7$ cells/ml in 30 mM $NaIO_4$/50 mM sodium acetate/3 mM NaN_3 (pH 4.5) and incubated in the dark at 4°C for 72 h with daily resuspension. Control cells were incubated in 50 mM sodium acetate/3 mM NaN_3 (pH 4.5). The oxidized and control cells were washed extensively with distilled H_2O by centrifugation (2500 × *g*, 5 min, 4°C) and then resuspended to $3 \cdot 10^7$ cells/ml in 60 mM $NaBH_4$ in 150 mM NaCl/P_i. The suspensions were triturated periodically with Pasteur pipets for 30 min at 23°C. After 30 min, an additional volume of $NaBH_4$ solution was added, followed by 5 vol. of 150 mM NaCl/P_i. The cells were pelleted by centrifugation (2500 × *g*, 5 min, 4°C) and then washed extensively with 150 mM NaCl/P_i by centrifugation.

The fixed cells survived the $CHCl_3$-CH_3OH extraction and $NaIO_4$ oxidation/$NaBH_4$ reduction in high yield (> 80% recovery of cells) and showed no drastic changes in shape or size as assessed by phase contrast microscopy.

Sedimentation equilibrium analysis. Unlabeled discoidin I (approx. 1 mg/ml) was dialyzed for 10–15 h against at least 900 vol. of the appropriate buffer (17 mM P_i or 150 mM NaCl/P_i) at 4°C, Millipore-filtered (3.0 μm), diluted to a final concentration of 50 μg/ml into buffer (17 mM P_i) or 150 mM NaCl/P_i) and subjected to sedimentation equilibrium analysis as described previously [5].

Other methods. Discoidin I concentration was routinely estimated by absorbance at 280 nm; a 0.1 mg/ml solution of discoidin I has an A_{280} of 0.3. All other methods were performed as described previously [5].

Results

The binding of ^{125}I-discoidin I to glutaraldehyde-fixed NC-4 *D. discoideum* cells was examined under two different buffer conditions, 17 mM P_i (pH 6.3) and 150 mM NaCl/P_i (pH 7.2). The 17 mM P_i is considered to be physiological for cellular slime mold growth [6] and differentiation [7] and is of lower ionic strength and pH than 150 mM NaCl/P_i, which was used exclusively in all

earlier binding studies [5,10] *.

Under both buffer conditions, the binding of tracer concentrations (10 ng/ml) of ^{125}I-discoidin I to fixed, differentiated cells required more than 2.5 h to reach apparent equilibrium at 4°C and at 23°C. Depending on the batch of fixed cells, the level of equilibrium binding observed in 17 mM P_i was always 2- to 5-fold greater than that observed in 150 mM NaCl/P_i. The higher ionic strength appeared to readily account for the reduced equilibrium binding observed in 150 mM NaCl/P_i. Binding to the fixed cells at low (10 ng/ml) and high (10 μg/ml) discoidin I concentrations could be reduced by adding increasing amounts of NaCl to the 17 mM P_i while maintaining a constant pH of 6.3. The binding was reduced even further by the increased ionic strength of the phosphate buffers at pH 7.2 compared to pH 6.3.

Specificity of discoidin I binding

Binding to the fixed cells under the two buffer conditions showed differences in sensitivity to hapten sugar inhibitors. For example, the best monosaccharide inhibitor of discoidin I binding, *N*-acetyl-D-galactosamine [5], was capable of totally inhibiting the binding observed in 150 mM NaCl/P_i (Fig. 1, ●). In contrast, over the same sugar concentration range, the binding in 17 mM P_i was only partially inhibited (Fig. 1, ○). However, the number of discoidin I molecules inhibited from binding at each sugar concentration was about the same under both buffer conditions. This suggested that the binding observed in 17 mM P_i represented two different components. One component corresponded to the binding observed in 150 mM NaCl/P_i, which was totally inhibited by *N*-acetyl-D-galactosamine; and the other component corresponded to that additional binding observed in 17 mM P_i, which was not inhibited by *N*-acetyl-D-galactosamine, but was inhibited by increased ionic strength.

These two binding components observed in 17 mM P_i were again readily distinguishable by their sensitivity to inhibition by polyelectrolytes. Fig. 2A shows that increasing concentrations of poly(L-glutamic acid) or heparin (which has no galactose or galactosamine residues) inhibited the additional, ionic strength-sensitive component of the binding observed in 17 mM P_i (open symbols), while having no effect on the binding observed in 150 mM NaCl/P_i (closed symbols). At concentrations as high as 1 mM, EDTA had no effect on either of the binding components.

Based on the differential specificities of these two binding components, we postulated that discoidin I binding to fixed cells could be mediated by two types of receptor sites, the carbohydrate or 'C sites' and the ionic or 'I sites'. Binding to the C sites was carbohydrate specific and was observed in both 17 mM P_i and 150 mM NaCl/P_i, whereas the I sites were available only under the physiological conditions of low ionic strength (17 mM P_i). Binding to the I sites was not specific for carbohydrate, but was inhibited by increasing ionic strength with salts or polyelectrolytes. Binding to the I sites is readily quantitated as the difference between total binding observed in 17 mM P_i and 150 mM NaCl/P_i.

Interestingly, polyelectrolytes that contain *N*-

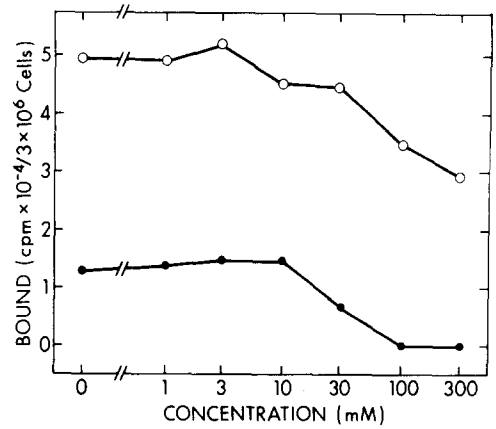

Fig. 1. *N*-Acetyl-D-galactosamine inhibition of binding in 17 mM P_i and 150 mM NaCl/P_i. The binding assay (see Materials and Methods) was performed for 2.5 h at 23°C on 300-μl samples containing the indicated final concentrations of *N*-acetyl-D-galactosamine; $3 \cdot 10^6$ fixed, differentiated NC-4 cells; and $2.6 \cdot 10^5$ cpm (2.6 ng) of ^{125}I-discoidin I in 17 mM P_i (○) or 150 mM NaCl/P_i (●).

* The penicillin/streptomycin combination of antibacterial agents was used in 17 mM P_i so that this buffer would be compatible with later studies using living cells. Identical results were obtained when 3 mM NaN_3 was substituted for penicillin/streptomycin in the 17 mM Na_2HPO_4-KH_2PO_4 (pH 6.3) in these binding studies using fixed cells.

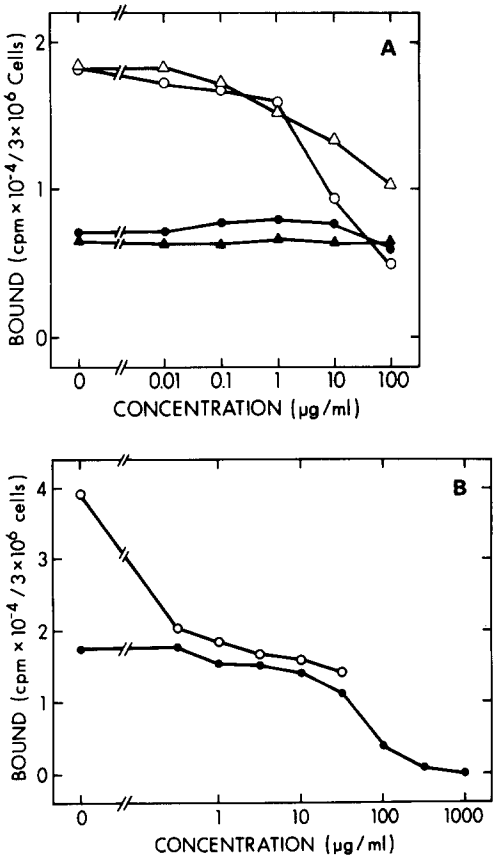

Fig. 2. (A) Polyelectrolyte inhibition of binding in 17 mM P_i and 150 mM NaCl/P_i. The binding assay (see Materials and Methods) was performed for 2.5 h at 23°C on 300-μl samples containing the indicated final concentrations of poly(L-glutamic acid) (△, ▲) or heparin (○, ●); $3 \cdot 10^6$ fixed, differentiated NC-4 cells; and $9.8 \cdot 10^4$ cpm (0.82 ng) of ^{125}I-discoidin I in 17 mM P_i (△, ○) or 150 mM NaCl/P_i (▲, ●). (B) Dermatan sulfate inhibition of binding in 17 mM P_i and 150 mM NaCl/P_i. The binding assay was performed as in (A); $2.7 \cdot 10^5$ cpm (2.7 ng) of ^{125}I-discoidin I in 17 mM P_i (○) or 150 mM NaCl/P_i (●).

acetylgalactosamine or galactose residues were much more effective at inhibiting discoidin I binding to the I sites than were those polyelectrolytes that do not contain these residues. For example, Fig. 2B (○) indicates that the *N*-acetylgalactosamine-containing glycosaminoglycan, dermatan sulfate, is at least 300-times more potent than heparin (Fig. 2A, ○) as an I site binding inhibitor. At higher concentrations, dermatan sulfate inhibits binding to the C sites (Fig. 2B, ● and Ref. 8), suggesting that it does interact with carbohydrate-binding sites of discoidin I.

Effects of treatments on the discoidin I receptors

The two types of discoidin I receptor sites were clearly recognized as distinct based on their differential sensitivities to certain treatments of the fixed cells. The activity of the I sites for binding tracer levels (10 ng/ml) of ^{125}I-discoidin I could be reduced 70–90% by prior extraction of the fixed cells with $CHCl_3$-CH_3OH. The binding activity of the C sites on these $CHCl_3$-CH_3OH extracted cells was increased 10–30%. Conversely, $NaIO_4$ oxidation followed by $NaBH_4$ reduction decreased the C site binding activity of the fixed cells by about 50% while causing a 20% increase in the I site binding activity.

Discoidin I concentration dependence of binding

Binding to the fixed, differentiated cells was examined as a function of discoidin I concentration by including increasing amounts of unlabeled discoidin I in the incubation mixture with a fixed amount of ^{125}I-discoidin I. As expected [5], in 150 mM NaCl/P_i the unlabeled discoidin I competed with tracer for binding to these fixed cells*. Specific binding saturated at about $2 \cdot 10^4$ tetramers per cell (Fig. 3, ▲). This specific binding capacity is almost identical to that determined for fixed, differentiated strain A3 cells in our previous binding study using the 150 mM NaCl/P_i buffer [5], while the affinity (calculated from the slope of a Scatchard plot, not shown) is about 15-times lower ($K_d \simeq 5 \cdot 10^{-9}$ M).

In contrast, the unlabeled discoidin I did not compete with ^{125}I-discoidin I for binding to the I sites. In fact, unlabeled discoidin I enhanced binding to the I sites. That is, the binding appeared to exhibit positive cooperativity. When these data were plotted as the number of discoidin I tetramers bound per cell, binding to the I sites appeared nonsaturable with a capacity of greater than 10^6 tetramers per cell (Fig. 3, ○). The total binding observed in 17 mM P_i at any discoidin I

* As was the case for binding to the fixed A3 cells [5], binding to the fixed NC-4 cells in 150 mM NaCl/P_i shows an apparent positively cooperative phase at low discoidin I concentration (<0.04 μg/ml). However, for simplicity this phase of binding curve has been avoided in the experiment shown in Fig. 3 by using a discoidin I tracer concentration greater than 0.04 μg/ml.

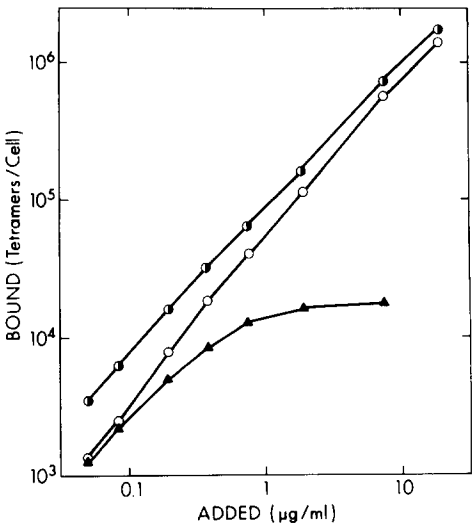

Fig. 3. Discoidin I concentration dependence of binding to the C and I sites. The binding assay (see Materials and Methods) was performed for 2.5 h at 23°C on 300-μl samples containing increasing concentrations of unlabeled discoidin I; $3 \cdot 10^6$ fixed, differentiated NC-4 cells; and $2.5 \cdot 10^5$ cpm (20 ng) of ^{125}I-discoidin I in 17 mM P_i or 150 mM NaCl/P_i. The data are plotted to show the number of discoidin I tetramers bound per cell to the I sites (○), the C sites (▲), and to the I sites plus C sites (◑) as a function of the total discoidin I concentration. C site binding has been corrected for the 'nonspecific' binding observed in 150 mM NaCl/P_i in the presence of >10 μg/ml of unlabeled discoidin I.

concentration is the sum of that bound to the I and C sites. Thus, the net result seen for total binding in Fig. 3 (◑) indicates no apparent competition between unlabeled and ^{125}I-labeled discoidin I. Fig. 3 emphasizes the difference in the discoidin I concentration dependence of binding to the two types of receptor sites. At higher discoidin I concentrations, the I site binding predominates, and thus, total binding in 17 mM P_i (Fig. 3, ◑) is also nonsaturable.

To eliminate the possibility that the nonsaturable binding to the I sites results from polymerization of discoidin I at low ionic strength sedimentation equilibrium ultracentrifugation was performed in 17 mM P_i and 150 mM NaCl/P_i. The slopes of the log A_{280} vs. R^2 plots (not shown) were identical in the two buffers. Furthermore, these slopes were not changed when 50 mM *N*-acetyl-D-galactosamine was included. Under all conditions the slopes were consistent with the $M_r \simeq 100000$ tetrameric quaternary structure reported [5,11] for native discoidin I.

Developmental regulation of the discoidin I receptors

We compared the binding of ^{125}I-discoidin I to fixed vegetative and differentiated NC-4 cells under both buffer conditions. The vegetative and differentiated cells exhibited similar total discoidin I binding activities in 17 mM P_i over a wide range of cell density. However, the binding was not equally distributed between the I and C sites. Scatchard analysis indicated that the vegetative cells have about 4-times fewer C sites per cell than differentiated cells. Binding to the I sites at each cell density is represented by the difference between total binding in 17 mM P_i and binding to the C sites. Both vegetative and differentiated cells possess I sites. Binding to the I sites, and hence total binding in 17 mM P_i, did not saturate in the tested cell density range.

Discussion

Two types of receptors for discoidin I have been identified on the surface of glutaraldehyde-fixed *D. discoideum* cells. We have designated these receptors the ionic or 'I sites' and the carbohydrate or 'C sites'. As is summarized in Table I, the I and C sites are clearly identified as distinct by their differential sensitivities to various inhibitors and treatments.

The C sites appear to correspond to the developmentally regulated, carbohydrate-containing discoidin I receptors previously identified [5] on glutaraldehyde-fixed strain A3 *D. discoideum* cells, despite their 15-fold lower affinity on these NC-4 cells. The C sites are partially sensitive to $NaIO_4$ oxidation/$NaBH_4$ reduction. This result appears to contradict the apparent insensitivity of the carbohydrate receptor to $NaIO_4$ oxidation alone reported in our earlier study [5]. This difference is explained by the fact that ^{125}I-discoidin I becomes covalently crosslinked to fixed cells that have been oxidized by $NaIO_4$ without a subsequent $NaBH_4$ reduction (unpublished data).

By performing our binding assay in the physiological buffer 17 mM P_i, we have been able to detect a second type of discoidin I receptor, designated the I site. This receptor has gone undetected

TABLE I

A COMPARISON OF THE PROPERTIES OF THE TWO TYPES OF DISCOIDIN I RECEPTORS

Property	Binding to	
	C sites	I sites
Inhibition by hapten sugars	Yes	No
Inhibition by salts	No	Yes
Inhibition by polyelectrolytes	No	Yes
Sensitivity to $CHCl_3$-CH_3OH extraction	No	Yes
Sensitivity to $NaIO_4$ oxidation/$NaBH_4$ reduction	Yes (partial)	No
Apparent saturability with discoidin I	Yes	No
Capacity for discoidin I (tetramers/cell)	$2 \cdot 10^4$ (differentiated)	$>10^6$
Developmental regulation	Yes (vegetative<differentiated)	No

in all previous binding studies because they employed higher ionic strength buffers [5,10]. The sensitivity of the I sites to inhibition by high salt concentrations and polyelectrolytes suggests that their interaction with discoidin I is electrostatic in nature. However, this electrostatic interaction appears not to result from the gross attraction of oppositely charged entities because discoidin I (^{125}I-labeled or unlabeled) has little or no net charge at pH 6.3, its apparent isoelectric point when denatured in urea (unpublished data). In addition, this electrostatic interaction does not appear to be mediated by divalent cations because: (i) divalent cations should only be present as trace contaminants in the buffers employed, and (ii) concentrations of EDTA as high as 1 mM have no effect on binding to the I site.

The I sites appear to be extracted from the fixed cells with $CHCl_3$-CH_3OH. Thin-layer chromatography indicates that this treatment extracts phospholipids, neutral lipids and fatty acids from the fixed cells, leaving most of the protein components behind (unpublished data). Thus, the I sites may be ionic lipids. We have previously shown that anionic lipids dramatically enhance the hemagglutination activity of purified discoidin I [12], and in the following paper [13], we demonstrate that discoidin I binds to negatively charged phospholipid vesicles.

Binding to the I sites exhibits an unusual discoidin I concentration dependence (Fig. 3). The positive cooperative nature of this binding interaction causes the I sites to appear nonsaturable with respect to discoidin I and also precludes calculation of the affinity of the interaction. The same positive cooperativity is observed for discoidin I binding to negatively charged phospholipid vesicles [13] and living *D. discoideum* cells [14]. In fact, cell surface labeling experiments [14] have shown that unlabeled discoidin I indeed binds to the I sites of living *D. discoideum* cells in the nonsaturable fashion predicted from the binding studies using ^{125}I-labeled discoidin I.

The differential effects of hapten sugars and polyelectrolyte inhibitors on the binding of discoidin I suggest that the I and C site receptors on fixed cells bind to different sites on the discoidin I molecule. Binding to the I sites is inhibited by polyelectrolytes that do not contain moieties capable of interacting with the carbohydrate binding site of discoidin I such as poly(L-glutamic acid) and heparin. However, a polyelectrolyte that does inhibit binding to the C sites, dermatan sulfate, is at least 300-times more potent than heparin as an inhibitor of binding to the I sites. This effect is suggestive of some type of interplay between the two types of binding sites on the discoidin I molecule. The lectin concanavalin A appears to bind polyelectrolytes, such as heparin, by an electrostatic mechanism that can be modulated by hapten sugars [15,16]. In addition, the agglutination of vesicles containing a mixture of glycolipids and negatively charged phospholipids by concanavalin A is extremely sensitive to increased ionic

strength [17]. This phenomenon has led Hampton et al. [17] to postulate the existence of a positively charged region on concanavalin A that interacts with negatively-charged lipids. Our data suggest that such a positively charged domain is also present on discoidin I.

In the following paper [13], we further characterize the interaction of discoidin I with lipids using vesicles prepared from purified phospholipids. In the next paper [14], we extend our binding studies to living NC-4 *D. discoideum* cells to examine the relevance of discoidin I-lipid interactions in vivo.

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