Purification and Comparison of Two Developmentally Regulated Lectins from *Dictyostelium discoideum*

DISCOIDIN I AND II

William A. Frazier, Steven D. Rosen, Richard W. Reitherman, and Samuel H. Barondes

From the Department of Psychiatry, University of California, San Diego, School of Medicine, La Jolla, California 92037

When cells of the cellular slime mold *Dictyostelium discoideum* differentiate from a nonsocial amoeboid form to a cohesive, aggregating form, they synthesize a lectin-like protein called discoidin, which is present on the cell surface. It is now reported that discoidin consists of two distinct lectins, designated discoidin I and discoidin II, which, although similar in some respects, differ in their electrophoretic mobilities, isoelectric points, subunit molecular weights, amino acid compositions, tryptic peptide maps, the erythrocyte species which they agglutinate, and the sensitivity of their agglutination activity to inhibition by monosaccharides. Furthermore, discoidins I and II differ in their developmental regulation as evidenced by the distinct time courses of their appearance during differentiation.

The cellular slime mold, *Dictyostelium discoideum*, grows and multiplies as unicellular amoebae. Upon starvation, these cells differentiate over a period of 12 hours into cells which can form stable intercellular contacts and thus construct a multicellular organism (1-3). Concomitant with the development of this cohesiveness, the cells synthesize a protein which is a potent agglutinin of sheep erythrocytes, and whose activity is inhibited by several sugars which are derivatives of β-galactose (4). This lectin-like protein, which is present on the cell surface (4, 5), may mediate the intercellular cohesiveness of *D. discoideum* cells, linking them by binding to surface oligosaccharides on adjacent cells.

The protein responsible for agglutination of sheep erythrocytes by crude extracts of axenically grown, growth phase *D. discoideum* cells (strain A-3) has been purified by affinity chromatography on Sepharose 4B and named discoidin. It has an apparent molecular weight of 100,000 under nondenaturing conditions and a molecular weight of 26,000 to 28,000 under denaturing conditions (6). On electrophoresis in polyacrylamide gels containing sodium dodecyl sulfate, it gave rise to a major band which accounted for more than 90% of the Coomassie blue-staining material and a band of slightly higher mobility accounting for less than 10% of the Coomassie blue-staining material (6).

This minor component of lower molecular weight has now been purified and found to be a distinct developmentally regulated lectin distinguished from the major lectin by its isoelectric point, amino acid composition, tryptic peptide map, the types of erythrocytes which it agglutinates, and the inhibition of this agglutination by sugars. Furthermore, this newly identified lectin, designated discoidin II, is also found in differentiating wild type NC-4 slime mold cells where its developmental regulation is different from that of the major lectin, now called discoidin I. Discoidin II comprises greater than 30% of the total lectin of wild type NC-4 cells at early stages of differentiation, and its relative proportion declines to that found in axenically grown cells later in differentiation.

**EXPERIMENTAL PROCEDURES**

*Cell Culture—*Dictyostelium discoideum* strains A-3 (axenic) and NC-4 (wild type) were grown as previously described (4). For large scale preparations, NC-4 was grown on 1-cm deep agar layers in disposable aluminum foil pans (25 x 30 x 4 cm) (Ekco Products, Inc.) covered with sterile aluminum foil in association with *Aerobacter aerogenes*. Growth phase NC-4 cells were differentiated, after separation from bacteria, by swirling on a gyratory shaker in 16.7 mM sodium-potassium phosphate buffer, pH 6.0, at a density of 1 to 3 x 10⁷ cells/ml (7), at room temperature. Cells were collected after specific periods of differentiation by centrifugation for 5 min at 1,500 x g. The preparation of crude extracts and the purification of discoidin on columns of Sepharose 4B (Pharmacia) were modified for large scale procedures from those of Simpson et al. (6).

**Purification of Discoidin. Preparation of Crude Extracts and Affinity Chromatography on Sepharose 4B—**In four preparations, 5 to 8 x 10¹⁴ axenically grown growth phase cells were sonicated (Bronwill Sonifier) in 400 ml of phosphate-buffered saline (4), pH 6.4, for a total of 5 min at a setting of 70. The sonicate was centrifuged 75 min at 35,000 rpm in a type 35 rotor (Beckman) and the clear yellow supernatant, volume 350 to 375 ml, had an activity of 640 units in the standard V-plate sheep red blood cell agglutination assay described below.

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*This work was supported by United States Public Health Service Grant MH18282 and a grant from the Alfred P. Sloan Foundation.

†Supported by the Alfred P. Sloan Foundation.
Cells of the wild type strain NC-4 were differentiated 10 to 12 hours, suspended in 400 ml of phosphate-buffered saline containing 0.3 M \( \beta \)-galactose, and broken by quick freezing in liquid N, and thawing in cold running tap water (8). The lysate was centrifuged as above and the supernatants (350 ml) were mixed with 150 ml of Sepharose 4B and then dialyzed against three changes of 9 liters each of phosphate-buffered saline, pH 6.4. Including 0.3 M \( \beta \)-galactose in the buffer in which the cells were broken greatly increased the yield of discoidin from NC-4 cells, but not from A-3 cells.

For affinity chromatography, the supernatants from axenic A-3 cells were pumped onto columns (6 x 50 cm) (1,000 ml bed volume) of Sepharose 4B equilibrated in phosphate-buffered saline, pH 6.4, at 72 ml/hour. The dialyzed NC-4 supernatant-Sepharose 4B mixtures were pumped onto columns (5 x 50 cm) (1,000 ml bed volume) of Sepharose 4B and the liquid allowed to drain onto the column. The column was sealed and pumped with phosphate-buffered saline, pH 6.4, at 72 ml/hour. The column was eluted with this buffer until the recording of absorbance at 280 nm returned to base-line, at which time 500 ml of 0.3 M \( \beta \)-galactose in the same buffer were pumped through the column followed by phosphate-buffered saline.

Ion Exchange Chromatography of Sepharose 4B Pools on DEAE-cellulose—The DEAE 4B pools (about 200 ml) were dialyzed against a total of 29 liters of 5 mM Tris-HCl buffer at pH 8.0 (three changes over 36 hours). When this material was pumped onto a column (1.5 x 30 cm) of DE52 cellulose (Whatman) equilibrated with the same buffer (60 ml/hour), all of the protein bound to the ion exchange resin. The column was developed with a three-chambered parabolic gradient (total volume = 300 ml) from 0 to 0.25 M NaCl in the starting buffer.

Hemagglutination Assay—Hemagglutination activity of crude extract, isolated discoidin, was measured with formalinized sheep erythrocytes as described by Rosen et al. (4) or with a modification of this procedure in which dilutions of lectin and formalinized erythrocytes (sheep or rabbit) were incubated for 1 hour at 37°C in 0.14 M saline and 5% (w/v) Ficoll as a gel stabilizer. The assay was carried out at 8°C in microtiter plates (Nunc). Gels were stained in 0.2% toluidine blue for 6 hours at 2 ma/gel. Protein samples were prepared for electrophoresis in the absence of sodium dodecyl sulfate was done by the method of Davis (11). Isoelectric focusing in 4% polyacrylamide gels was carried out by the procedure of Righetti and Drysdale (12) with 2% ampholyte, pH range 6.0 to 8.0 (LKB Produkter).

RESULTS

Affinity Chromatography of Crude Extracts—Fig. 1 shows an elution profile representative of the chromatography of crude extracts of either axenic A-3 cells or NC-4 wild type cells on columns of Sepharose 4B. The solid line represents the profile of absorbance at 280 nm and the points and dashed line indicate the units of agglutination activity in the V-plate assay with rabbit erythrocytes. Note that no hemagglutination activity eluted with the major protein peak, but approximately one column volume after the galactose is introduced, a protein peak coincident with a peak of hemagglutination activity is eluted. The plateau of absorbance following this peak was shown to be due to the galactose solution. Identical elution profiles were obtained in all seven preparations of discoidin from axenic and wild type cells, the area of the second protein peak varying with the amount of activity in the crude extract applied to the column. Recovery of hemagglutination activity at this stage were 50% to greater than 100%, this large variation being due to the 2-fold serial dilution procedure employed in the assay. The active material was pooled as indicated by the bar in Fig. 1. Electrophoresis of 200 µg of the Sepharose 4B pool material on 7% polyacrylamide gels at pH 8.9 revealed a diffuse intense band of low mobility, a fainter band of higher mobility and material at the top of the gel (Fig. 2A). As previously reported for discoidin purified from A-3 cells, the Sepharose 4B pool from both A-3 and NC-4 cell preparations contained a major polypeptide component with a molecular weight of about 26,000 and a minor component of lower apparent molecular weight as judged by electrophoresis on 10% polyacrylamide gels in sodium dodecyl sulfate (Fig. 2B). It has been observed that the major and minor components could be separated by preparative isoelectric focusing under non-denaturing conditions, having pI values equal to 6.1 and about 6.8, respectively (6). An ion exchange column method was devised, based on this information for the rapid, large scale separation of the two components of the Sepharose 4B pool.

Ion Exchange Chromatography of Sepharose 4B Pool—All of the protein of the Sepharose 4B pool of axenic cells was found to bind to DE52 cellulose eluted as described under "Experimental Procedures." The column was developed with a gradient of sodium chloride, and the resulting elution profile of absorbance at 280 nm is shown in Fig. 3 (solid line). The dashed line in Fig. 3 indicates the hemagglutination activity assayed in V-plates with sheep erythrocytes. The major protein peak coincides with this activity, while the first peak is devoid of activity. However, when fractions of the first peak which have no hemagglutination activity against sheep erythrocytes were
FIG. 1 (left). Affinity chromatography of crude extracts on a Sepharose 4B column (1,000 ml bed volume, flow rate 72 ml/hour). The solid line is the recording of absorbance at 280 nm and the points and dashed line indicate the hemagglutination activity measured with rabbit erythrocytes in the V-plate assay. Fractions of 12 ml were collected starting when the sample and rinse (~400 ml) had been loaded on the column. Active fractions were pooled as indicated by the horizontal bar.

FIG. 2 (right). Polyacrylamide disc gel electrophoresis of the Sepharose 4B pool. Gel A, 200 µg of protein on a 7% polyacrylamide gel run at pH 8.9 and stained with Coomassie blue (see text). Gel B, 100 µg of protein on a 10% polyacrylamide sodium dodecyl sulfate gel stained with Coomassie blue. The major and minor bands are near the center of the gel and the band of higher mobility is cytochrome c (100 µg).

Fig. 3. Ion exchange chromatography of the Sepharose 4B pool on DEAE-cellulose (DE52). The starting buffer was 5 mM Tris-HCl, pH 8.0, and the flow rate was 60 ml/hour. Under these conditions all material of the Sepharose 4B pool absorbing at 280 nm was bound to the column. Elution was accomplished with a three-chambered parabolic gradient (300 ml) from 0 to 0.25 M NaCl in the starting buffer applied at Fraction 1 and extending to Fraction 96. The open circles and solid line indicate the absorbance at 280 nm and the closed circle and dashed line indicate the hemagglutination activity measured with formalinized sheep erythrocytes in the V-plate assay. Fractions marked A and B were those assayed with three species of erythrocytes (Table I). Fractions were pooled as indicated by the horizontal bars.

TABLE I

| Fraction* | Erythrocyte species† |
|-----------|---------------------|
|           | Sheep | Rabbit | Human | O |
| A         | 0     | 512    | 64    |
| B         | 128   | 296    | 32    |

* Fractions are those marked A and B in Fig. 3.
† Agglutination activity in units per ml assayed in the V-plate assay using formalinized erythrocytes of the species indicated.

Pools 1 and 3 were characterized by electrophoresis on polyacrylamide gels in the presence and absence of sodium dodecyl sulfate and by isoelectric focusing in polyacrylamide gels. Fig. 4, A and B, shows representative 7% polyacrylamide gels run at pH 8.9 in the absence of sodium dodecyl sulfate for Pools 1 and 3, respectively. The material in Pool 1 runs as a compact band near the top of the gel while Pool 3 appears as diffuse stained material throughout the upper two-thirds of the gel with a substantial amount of material at the top of the gel. Fig. 4, C and D, shows the results of isoelectric focusing of Pools 1 and 3, respectively, in 4% polyacrylamide gels with 2% ampholytes in the pH range 6 to 8. Determination of pH gradients on parallel gels indicates that the band in Gel 4C (Pool 1) is at a region of pH 6.8 to 7.0 and the band in Gel D (Pool 3) is in the region of pH 6.0. Fig. 5 indicates the behavior of material from Pools 1 and 3 on 10% polyacrylamide gels in sodium dodecyl sulfate. The sharp band near the bottom of each gel is cytochrome c included as an internal standard. The...
discoidin I and II are identical with these proteins on the basis of their mobilities on polyacrylamide gels in the presence and absence of sodium dodecyl sulfate, differential agglutination of sheep and rabbit erythrocytes and amino acid compositions. Thus, both the axenic strain A-3 and wild type NC-4 strains of *D. discoideum* contain discoidins I and II. With several preparations obtained from NC-4 cells differentiated for only 9 hours some protein failed to bind to DE52 cellulose columns. This material was shown to be identical with discoidin I on the basis of electrophoretic migration on sodium dodecyl sulfate polyacrylamide gels and amino acid composition. It was never observed in extracts of A-3 cells and was absent in several preparations from NC-4 cells obtained after 12 hours of differentiation.

**Amino Acid Compositions and Tryptic Peptide Maps of Discodins I and II**—To assess the extent of chemical relatedness of discodins I and II and to determine whether discoidin II might be derived from discoidin I, the amino acid composition of each protein was determined. Table II summarizes the amino acid compositions (residues per polypeptide chain) obtained for four different preparations of discoidin I and three preparations of discoidin II. The most notable differences between the two proteins occur in the hydroxyamino acids. Discoidin I has 24 threonine residues and 15 serine residues per peptide chain, while discoidin II has only 12 threonine residues and nearly twice as many serine residues (23 residues). Glutamic acid is less prominent in discoidin II (13 residues) than in discoidin I (20 residues). Other major differences include the content of alanine, tyrosine, lysine, and arginine (Table II).

These differences in amino acid composition make it unlikely that discoidin II is derived from discoidin I by proteolysis. More direct evidence that this is not the case is found in comparative tryptic peptide maps of the two proteins. Equivalent amounts (determined by amino acid analysis) of each protein were reduced, carboxymethylated with [1-14C]iodoacetic acid and digested with trypsin under identical conditions. The lyophilized digests were spotted on paper, chromatographed in the first dimension, and, after drying, electrophoretically treated in the second dimension. Peptides were visualized with ninhydrin. After tracing the ninhydrin-positive spots, the papers were autoradiographed to locate radioactive S-14C-carboxymethylcysteine-containing peptides.

Photographs of these two-dimensional tryptic peptide maps and their autoradiographs are shown in Fig. 6. The map and autoradiograph for discoidin I is seen in Fig. 6, A and B, and that for discoidin II is shown in Fig. 6, C and D. Peptides in the ninhydrin patterns for discoidin I (Fig. 6A) and discoidin II (Fig. 6C) which appear to be in identical positions are indicated by dotted circles and ninhydrin-positive spots which are radioactive as indicated by the corresponding autoradiographs are enclosed by dashed circles. Of the approximately 20 peptides visualized in the maps of discoidin I and II (Fig. 6A, A and C), only three (dotted circle) appear to occupy identical positions. The most striking difference in ninhydrin patterns between the two tryptic peptide maps is in the bottom third of each. The map for discoidin I (Fig. 6A) exhibits major peptides which run as smears near the electrophoretic origin indicating that they are probably quite large. These peptides are not in evidence in the map for discoidin II (Fig. 6C). The dashed lines in Fig. 6A indicate that four ninhydrin-positive spots also contain radioactivity, consistent with four discrete 14C-labeled peptides in the discoidin I map. The smear of radioactivity
TABLE II

Amino acid compositions of discoidins I and II

| Amino acids      | Discoidin I | Discoidin II |
|------------------|-------------|--------------|
|                  | Mean residue no. | Integral no. | Mean residue no. | Integral no. |
| Aspartic acid    | 34.6        | 35           | 36.1            | 36           |
| Threonine        | 24.0        | 24           | 12.4            | 12           |
| Serine           | 15.0        | 15           | 23.3            | 23           |
| Glutamic acid    | 20.1        | 20           | 13.0            | 13           |
| Proline          | 9.9         | 10           | 7.8             | 8            |
| Glycine          | 15.0        | 15           | 18.8            | 14           |
| Alanine          | 17.6        | 18           | 12.2            | 12           |
| Cysteine         | (3.6)       | (4)          | ND              | ND           |
| Valine           | 18.0        | 18           | 17.4            | 17           |
| Methionine       | 1.0         | 1            | 0.7             | 1            |
| Isoleucine       | 12.8        | 13           | 12.7            | 13           |
| Leucine          | 13.0        | 13           | 13.6            | 14           |
| Tyrosine         | 10.8        | 11           | 7.9             | 8            |
| Phenylosalamine  | 11.3        | 11           | 10.0            | 10           |
| Histidine        | 4.9         | 5            | 6.1             | 6            |
| Lysine           | 8.0         | 8            | 11.9            | 12           |
| Arginine         | 13.1        | 13           | 10.4            | 10           |
| Glucosamine      | <0.2        | 0            | <0.2            | 0            |
| Galactosamine    | <0.2        | 0            | <0.2            | 0            |
| Total residues   | 234         |              | 209             |              |
| Calculated molecular weight | 26,301       |              | 23,099          |              |

* Based on duplicate analyses of four preparations of discoidin I and three preparations of discoidin II.

* From Ref. 6.

* Estimated detection limit.

* Does not include tryptophan.

along the chromatographic dimension which remains at the electrophoretic origin (Fig. 6B) may be due to S-[14C]carboxymethylmercaptoethanol or a mixture of heterogeneously labeled peptides incompletely cleaved by trypsin. The latter explanation is the more likely one, since virtually no radioactivity is seen in this position in the autoradiograph of the map of discoidin II (Fig. 6D). In fact, the complete absence of radioactive spots seen in Fig. 6D may indicate that discoidin II contains no cysteine. The 4 residues of cysteine per peptide chain reported previously for the naturally occurring mixture of discoidin I and II (6) may all reside in the discoidin I polypeptide chain. Even though this technique of trypsin peptide mapping is not quantitative in that peptides are not recovered and compared by their amino acid compositions, the striking difference in both the patterns of ninhydrin-positive spots and radioactively labeled spots revealed by autoradiography between the peptide maps for discoidins I and II, indicate that discoidin II is a distinct protein and not a proteolytic derivative of discoidin I.

Comparison of Hemagglutination Properties of Discoidins I and II—It has already been noted that discoidins I and II differ in their hemagglutination properties with respect to the fixed erythrocytes used in the V-plate assay (Table I). These differences in erythrocyte species specificity were further investigated using fresh sheep and rabbit erythrocytes in the quantitative hemagglutination assay. As seen in Fig. 7, both discoidin I (●) and discoidin II (A) agglutinate fresh rabbit erythrocytes (solid lines) at lower concentration and to a greater extent than fresh sheep erythrocytes (dashed lines). The half-maximal agglutination of rabbit erythrocytes is achieved by discoidin I at 1.1 μg of protein per assay vial while...
less discoidin II (0.7 μg) is required to reach this level of agglutination. With sheep erythrocytes, however, the order of potency of the two proteins is reversed. Discoidin I again produces half-maximal agglutination at 1.1 μg, but discoidin II is considerably less potent, requiring 4.4 μg to reach the half-maximal level. The maximum level of agglutination achieved with the two proteins is comparable for each cell type.

Another means of comparing the hemagglutination properties of discoidins I and II is to investigate the inhibition of the agglutination activity of each protein by simple sugars. Fresh rabbit erythrocytes were chosen as the assay cell type, since both discoidin I and II agglutinate these cells in a similar fashion (Fig. 7). A series of standardized quantitative assays were set up with each protein such that the level of agglutination in the absence of any added sugar was about 70%, near the top of the linear range of the per cent agglutination versus concentration of discoidin curve (Fig. 7). Addition of sugars which inhibit the interaction of the discoidins with the rabbit erythrocytes will then lower the observed agglutination along this linear region of the plot. Ten different sugars at a final concentration of 5 mM were tested with discoidins I and II. These results are presented in Fig. 8 as the per cent inhibition given by a 5 mM concentration of each sugar. The pattern of inhibition by these sugars is clearly different for discoidins I and II. Lactose, β-D-galactose, α-D-methyl-β-D-galactose, and 3-O-methyl-β-D-glucose are good inhibitors of the activity of discoidin II and poor inhibitors of discoidin I at this concentration. N-Acetyl-D-galactosamine, L-fucose, and α-D-methyl-β-D-glucose are the only sugars which inhibit the two proteins to about the same extent (Fig. 8).

Developmental Time Course of Discoidins I and II—The differences in structural and functional properties of discoidins I and II presented above clearly indicate that they are distinct proteins. Since discoidin I, the major lectin of differentiating *D. discoideum* cells, has been shown to be regulated developmentally (4), it is important, in terms of elucidating its function, to determine whether discoidin II is also regulated developmentally. To do this, NC-4 cells differentiating in suspension culture were harvested at successive time intervals, and the cells were extracted in galactose. The dialyzed crude extracts were assayed for agglutination activity and discoidins I and II were purified by affinity chromatography on Sepharose 4B. Aliquots of the concentrated Sepharose 4B pools were electrophoretically treated on 15% polyacrylamide gels in sodium dodecyl sulfate. After staining with Coomassie blue, the gels were scanned and the relative areas of the peaks corresponding to discoidins I and II were determined.

These data for the relative amount of each protein present in differentiating NC-4 cells at 3-hour intervals up to 12 hours of differentiation are shown in Fig. 9. The area of the peak of Coomassie blue stain for each protein at each time point is expressed as the percentage of the area of the discoidin I peak present at 12 hours. The filled squares in Fig. 9 represent the hemagglutination activity of each extract measured with rabbit erythrocytes (V-plate assay) which detect both discoidin I and II (see above). This result is in good agreement with the developmental time course of hemagglutination activity measured with sheep erythrocytes (4). From the start of differentiation until about 6 hours there is a lag in the appearance of both discoidins I and II. After 6 hours discoidin I increases linearly to 12 hours while discoidin II increases most between 6 and 9 hours and tends to level off between 9 and 12 hours. This same result was obtained for discoidin II in a similar experiment in which discoidin II was measured with rabbit erythrocytes after absorption of discoidin I in the extracts with formalized sheep erythrocytes (data not shown). Of particular significance is the fact that the relative proportions of discoidins I and II change during differentiation. For example, at 6 hours there is...
50% as much discoidin II as I and this value declines with time to 16% at 9 hours and 10% at 12 hours. This is shown more clearly in Fig. 10 in which the densitometry scanning patterns are presented for the regions of interest of 10% polyacrylamide sodium dodecyl sulfate gels of the Sepharose 4B pools from axenic cells differentiated for 6, 9, and 12 hours. The amplitudes of the scans have been adjusted so that the peaks for discoidin I (mobility \( \approx 0.649 \)) are of about the same height. It is clearly seen that the discoidin II peak (mobility \( \approx 0.687 \)) decreases markedly in size relative to the discoidin I peak at increasing times of differentiation. Thus it appears that discoidins I and II are subject to different regulation during the course of differentiation.

**DISCUSSION**

The data presented above indicate that there are two distinct developmentally regulated lectin-like proteins or agglutinins in cohesive cells of *D. discoideum*. These proteins are now designated discoidin I and discoidin II. Discoidin I is the major component of the agglutination activity of extracts of both axenically grown A-3 cells and the wild type strain NC-4. Discoidin II, although present at less than 10% of the level of discoidin I in axenic cells, comprises about 35% of the total lectin at early stages of differentiation of wild type cells (Figs. 9 and 10). At later stages of differentiation, the ratio of the two lectins approaches their relative proportion in axenic cells.

Although discoidin II was first identified as a separate protein band of lower molecular weight than discoidin I on polyacrylamide gels in sodium dodecyl sulfate, the fact that the two proteins can be separated under nondenaturing conditions (Fig. 3 to 5) suggests that they do not form stable mixed subunit oligomers. Discoidin I is a tetramer of subunits having a molecular weight of 26,000 (6). The tryptic peptide map of this protein (Fig. 6, A and B) is consistent with a homotetrameric structure of identical subunits. The total number of peptides detected is close to that predicted from the number of lysine and arginine residues (21 residues) per \( M_r = 26,000 \) peptide chain. Although the multimeric stoichiometry of discoidin II has not been determined, the tryptic peptide map indicates that if it is also an oligomer, it is probably a homomultimer (Fig. 6, C and D).

The amino acid compositions of discoidins I and II shown in Table II have been calculated to yield molecular weights in agreement with the peptide chain molecular weights determined by polyacrylamide gel electrophoresis in sodium dodecyl sulfate. Since discoidin II contains substantially more serine and lysine residues per polypeptide chain than discoidin I it appears very unlikely that it is derived from discoidin I by proteolysis. The low number of potentially identical peptides in the tryptic peptide maps of the two proteins (Fig. 6, A and C) further substantiates this conclusion, as does the complete disparity in the pattern of radioactively labeled peptides seen in Fig. 6, (B and D). The difference in isoelectric points of the two proteins noted previously (6) and confirmed in this study (Fig. 4, C and D) is consistent with the presence of 6 less acidic amino acid residues in discoidin II (Table II).

The distinct structures of discoidins I and II are reflected in their distinct functional properties. Table I indicates that while both lectins agglutinate fixed rabbit and human erythrocytes, discoidin I agglutinates fixed sheep erythrocytes, but discoidin II does not. With fresh sheep erythrocytes the distinction between discoidins I and II is not as sharp. However, discoidin I is clearly a better agglutinin of these cells than discoidin II (Fig. 7).

The inhibition of cell agglutination by simple sugars is a device that has been used widely to probe the specificity of the carbohydrate binding sites of lectins (16). The relative inhibitory potencies of the sugars tested for inhibition of rabbit erythrocyte agglutination by discoidins I and II indicate that the carbohydrate binding sites of these two lectins may preferentially recognize different domains of saccharide structure. The ten sugars tested may be grouped into three classes based on the degree of their differential effect on discoidins I and II. The first class (class 1), comprised of sugars which inhibit discoidin II more than twice as effectively as discoidin I, includes D-galactose, \( \alpha \)-methyl-D-galactoside, \( \beta \)-methyl-D-galactoside, and lactose (4-O-\( \beta \)-D-galactopyranosyl-D-glucose). A second class (class 2) inhibits discoidin II only about 50% better than discoidin I and includes 3-O-methyl-D-glucose, D-fucose, and melibiose (6-O-\( \alpha \)-D-galactopyranosyl-D-glucose). The third class (class 3), which inhibits both lectins to about the same extent, contains \( \alpha \)-methyl-D-glucoside, L-fucose, and N-acetyl-D-galactosamine. Of these three sugars, only N-acetyl-D-galactosamine is a good inhibitor. Class I sugars, good inhibitors of discoidin II and relatively poor inhibitors of discoidin I, are all derivatives of D-galactose at position 1 of the pyranose ring. Class 2 sugars, good inhibitors of discoidin II and moderately good inhibitors of discoidin I, all have substituents on the \( \beta \) side of a pyranose ring, a methyl group in the case of D-fucose and 3-O-methylglucose and a galactose in the case of melibiose. The only class 3 sugar which is a good inhibitor of discoidin I is N-acetyl-D-galactosamine, having the acetyl function on the \( \alpha \) side of the ring. This suggests that discoidin II recognizes positions 3, 4, 5, and 6 of the pyranose ring, thus differentiating between galactose and glucose, which is not an inhibitor, while discoidin I appears to recognize primarily the acetyl group at position 2 of galactosamine and perhaps some aspect of the \( \beta \) face of the pyranose ring and the presence of a substituent at position 1. It does not differentiate well between \( \alpha \)-methyl-D-glucoside and \( \alpha \)-methyl-D-galactoside, indicating that position 4 of the ring is not recognized well. It should be remembered that these comparisons are
based on inhibitory potency relative to the receptor for these
lectins on erythrocytes. The relevance of these observations
to the interaction of discoidins I and II with their natural
receptors on slime mold cells will require further investigation.

In a recent study, Rosen et al. (9) determined the relative
inhibitory potencies of five sugars against the rabbit erythro-
cyte agglutination activity of crude extracts of six species of
slime mold. The rank order of inhibitory potency determined in
this study for discoidin I is in excellent agreement with the
data obtained with crude extracts of D. discoideum, reflecting
the fact that discoidin I is the major component of lectin
activity (see above).

A potentially important difference between discoidins I and
II is their distinct developmental regulation. Neither protein is
present in vegetative (feeding) wild type cells (Fig. 9). When
food is removed to initiate differentiation to a cohesive state,
both increase over a 12-hour period but at different rates.
Discoidin I increases linearly from 6 to 12 hours of develop-
ment, while discoidin II reaches a higher percentage of its
12-hour level at earlier times. The net result of these two
patterns of development is that, at earlier times of differentia-
tion, the ratio of discoidin II to discoidin I is greater than at
later times (Fig. 10).

Evidence has previously been presented that in two species
of cellular slime mold, D. discoideum (4 6) and Polysphon-
dylium pallidum (17) developmentally regulated carbohydrate-
binding proteins (lectins) present on the cell surface may
mediate species-specific cell cohesion. At present, it is not
known whether the component of the lectin activity of D.
discoideum, discoidin II, described in this study, is present
on the cell surface along with discoidin I (4, 5). However,
the possibility of differential times of appearance or differ-
etial spatial distributions on the cell surface, of two carbo-
hydrate-binding proteins with different specificity provides
the potential for both spatial and temporal regulation of cell
cohesion during the formation of a multicellular organism
from single cells.

Acknowledgments—We thank Dr. Ralph A. Bradshaw for
providing facilities for amino acid analysis and other aspects of
the chemical characterization and Dr. Ruth Hogue-Angeletti
and Dr. Russell Doolittle for performing amino acid analyses.
The help of Dr. Irving Boime and Linda Boyd in preparing the
tryptic peptide maps is gratefully acknowledged.

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