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On the subunit composition of discoidins

Akira HASE and Ken-ichi ONO

Carbohydrate-binding proteins (discoidins) were isolated from the cellular slime mold *Dictyostelium discoideum* and examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and two-dimensional gel electrophoresis.

Discoidin II consisted of a single species of subunit of approximate molecular weight of 24,000 daltons and its isoelectric point was pI 7.7 On the contrary, discoidin I was composed of at least two species of subunit, which had the same molecular weight of 26,000 daltons but differed in their isoelectric points (pI 7.2 and pI 7.6). However, these two discoidin I subunits were equivalent in their carbohydrate-binding activities.

The cellular slime mold *Dictyostelium discoideum* has two proteins which have lectin-like activities and can agglutinate sheep erythrocytes (ROSEN *et al.*, 1973). These two proteins are called "discoidins-discoidin I and II" (SIMPSON *et al.*, 1974), or carbohydrate-binding proteins (CBPs) (SIU *et al.*, 1976). Discoidin I and II have been reported to be homotetramers of subunits of 26,000 and 24,000 daltons, respectively (FRAZIER *et al.*, 1975). The majority of discoidins present in slime mold cells is discoidin I (ROSEN *et al.*, 1973). And their localization on the cell surface has been shown by several techniques (CHANG *et al.*, 1975; SIU *et al.*, 1976). Since discoidin I was shown to be a cell-surface lectin and the increase of its lectin-activity was correlated with the development of cohesiveness of cells, BARONDES and his co-workers proposed that discoidin plays a significant role to the cell-cell cohesion in aggregates of *D. discoideum* (ROSEN *et al.*, 1976).

We have studied the role of several components of plasma membranes on the development of *D. discoideum* and during the course of investigation we found that discoidin I was a heteropolymer consisting of at least two different subunit species, whereas discoidin II was a homopolymer. The carbohydrate-binding activities of these two subunits were also examined.

Materials and Methods

Organism and culture conditions

Dictyostelium discoideum strain NC4 was grown in association with *E. coli* on the SM agar plates (SUSSMAN, 1966).

At the aggregation phase, cells were harvested in the cold SM buffer, and washed several times in the same buffer by centrifugation.

Isolation of discoidins

Discoidins were isolated by a modification of the method of FRAZIER *et al.* (1975). The washed, aggregation-phase cells were resuspended in 75 mM sodium phosphate buffer, pH 6.4, containing 75 mM NaCl (buffered saline). D-Galactose was added to 0.3 M as a final concentration. Cells were disrupted by freeze-thowing and the homogenate was centrifuged at 40,000 rpm ($120{,}000 \times g$) for 60 min in a Hitachi RP 50-2 rotor. The supernatant was stirred for 30 min at 4°C and then centrifuged at $12{,}000 \times g$ for 20 min. The soluble protein fraction of the supernatant was next dialyzed overnight against a large volume of the buffered saline and loaded on a Sepharose 4 B column which had been equilibrated with the buffer. The unbound material was washed out with the excess volume of the buffer and discoidins were eluted with the same solution containing 0.3 M D-galactose. This discoidin fraction was dialyzed overnight against cold distilled water, lyophilized, and stored in a deep freezer (−28°C).

SDS-polyacrylamide gel electrophoresis

Sodium dodecyl sulfate (SDS)-polyacrylamide gel was prepared according to the method of LAEMMLI (1970). The concentrations of acrylamide in stacking and separating gels were 3% and 10%, respectively.

Fifty μg of discoidins were dissolved in 100 μl of 1% SDS and 2% β-mercaptoethanol and heated at 100°C for 2 min. Electrophoresis was performed with a constant current of 1 mA per gel for 4 h. Gels were fixed and stained according to the method of FAIRBANKS *et al.* (1971).

Two-dimensional polyacrylamide gel electrophoresis

Two-dimensional gel electrophoresis of discoidins was performed by a slight modification made by AMES and NIKAIDO of the O'FARRELL method (AMES and NIKAIDO, 1976; O'FARRELL, 1975).

The pH range of the isoelectric focusing gels was pH 5~8. The acrylamide concentration of the separating slab gel was 12.5%. For detection of spots, the second-dimension gel was fixed in 50% trichloroacetic acid and stained in 0.1% Coomassie Blue R-250 in 50% trichloroacetic acid.

Measurement of carbohydrate-binding activity of discoidin subunits

Discoidins were dissolved in the buffered saline. To aliquots of the solution, SDS was added to 0.1, 0.25, 0.5, 1.0, or 2.0% as a final concentration, respectively. Each solution was loaded on a Sepharose 4 B column

which has been equilibrated with the buffered saline containing SDS at the same concentration as that of the applied sample solution. The solution free of SDS was also applied to the column. The unbound proteins were washed out by 10 column volumes of the buffered saline containing SDS at various levels. Then the bound discoidins were eluted with the buffer containing 0.3 M D-galactose. The relative carbohydrate-binding activity (r. c. b. a.) of discoidin monomers was calculated using the following equation.

$$\text{r. c. b. a.}\,(\%) = \frac{E_x}{A_x} \times \frac{A_o}{E_o} \times 100$$

A_x and A_o are the amounts of applied proteins to the Sepharose 4 B columns at the SDS concentrations of $x\%$ and 0%, respectively. E_x and E_o are the amounts of proteins eluted from the columns with the buffered saline containing 0.3 M D-galactose at the SDS concentrations of $x\%$ and 0%, respectively.

Protein amounts were determined by the method of LOWRY *et al.* (1951).

Results and Discussion

Fig. 1 shows the result of a SDS-polyacrylamide gel electrophoresis of isolated discoidins. The major band of 26,000 daltons is the subunit of discoidin I and the acceding minor band of 24,000 daltons is that of discoidin II. Under these conditions of electrophoresis, either discoidin I or discoidin II showed a single band, only.

However, when discoidins were analyzed by a two-dimensional electrophoresis, discoidin II subunits appeared as a single spot, but at least two spots having the same intensity of staining were observed with discoidin I subunits at the same molecular-weight line (Fig. 2). The subunit having a higher isoelectric point (pI) was designated as subunit Ia and the other having a lower pI as subunit Ib. The isoelectric point of subunit Ia was pI 7.6, while that of subunit Ib was pI 7.2. It seems likely that subunit Ia still consists of some components but the resolution is not enough to make a definite conclusion. Furthermore, the relative intensity of stained subunit Ia to Ib did not change during the early stages of development of *D. discoideum* (data not shown).

To know whether or not subunits Ia and Ib are equivalent in their biological activities, we examined changes in the carbohydrate-binding activity of discoidins after dissociation into subunits. As shown in Fig. 3, the agarose-binding activity of discoidins did not decrease at all in the presence of 0.1 or 0.25% SDS. Even at 0.5% level, a rather high binding value was ob-

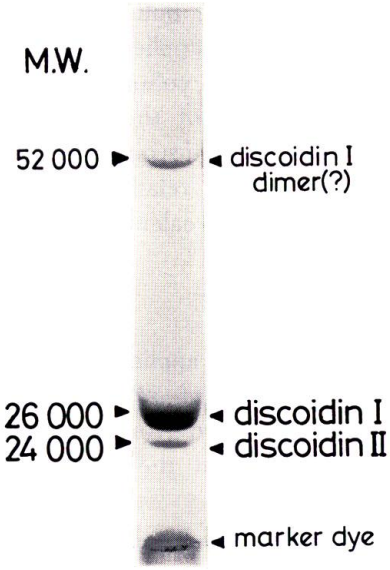

Fig. 1. Electrophoretogram of discoidin subunits in a 10% polyacrylamide gel. Discoidins were isolated and purified by adsorption on a Sepharose 4B column and elution with a buffer containing 0.3 M D-galactose as described in "Materials and Methods". The protein of about 26,000 daltons is the subunit of discoidin I. A minor band of 24,000 daltons is the subunit protein of discoidin II.

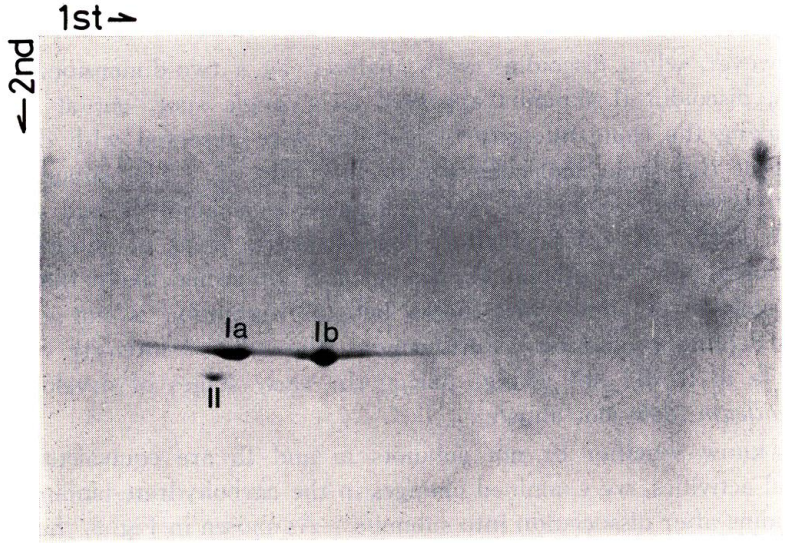

Fig. 2. Two-dimensional electrophoretogram of discoidin subunits. The isoelectric focusing (first dimension) was from the cathode on the left to the anode on the right. The separation by molecular weight (second dimension) was from the cathode at the top to the anode at the bottom. Two discoidin I subunits are indicated as Ia and Ib, and the II indicates the subunit of discoidin II.

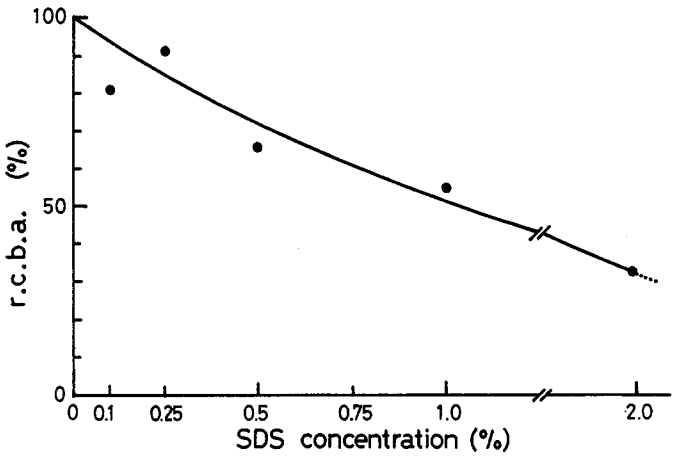

Fig. 3. Effect of SDS on the relative carbohydrate-binding activity of discoidin subunits. The relative carbohydrate-binding activity (r. c. b. a.) was estimated from the protein amount of discoidin subunits specifically bound to Sepharose 4B.

tained. At the SDS concentrations of 0.1% or more native discoidins dissociated almost completely to their subunits (HASE, unpublished). From these results, we conclude that subunits Ia and Ib both can bind to agarose independently. WEST and MCMAHON (1977) reported that discoidin which had been denatured by boiling in a solution containing 1% SDS and β-mercaptoethanol could recover its native activity of binding to agarose after the removal of SDS by dialysis. Our results showing the persistence of the carbohydrate-binding activity at rather lower concentrations of SDS give further information about the stability of this activity of discoidins.

The architecture of discoidin I is unclear, but, since native discoidin I consisting of four subunits showed only a band of pI 6.1 on an isoelectric focusing as reported by SIMPSON *et al.* (1974) (data not shown) and the staining intensities of subunits Ia and Ib were the same, both subunits may be contained in the equimolar ratio in the native protein. However, if we estimate the isoelectric point of native discoidin I as the heterotetramer mentioned above on the basis of those of its subunits, the value of pI 7.4 is obtained. This value differes from the pI value observed by SIMPSON *et al.* (1974). This discrepancy may be due to the difference in the tertiary structure between the native form and the denatured one.

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