Biol Cell (1991) 71, 289–291

Original article

Adhesion of sea-urchin embryonic cells to substrata coated with cell adhesion molecules

Valeria Matranga [1], Daniela Di Ferro [1], Melchiorre Cervello [1], Francesca Zito [1], Eizo Nakano [2]

[1] *Istituto di Biologia dello Sviluppo del CNR, Via Archirafi 20, 90123 Palermo, Italy;*
[2] *Department of Biology, Nagoya University, Chikusa, Nagoya 464-01, Japan*

(Received 14 January 1991; accepted 20 February 1991)

Summary – A cell-to-substratum adhesion assay is developed to study the adhesion of sea-urchin embryonic cells to coated substrata. The involvement in this process of both carbohydrate and protein molecules is reported. Concanavalin A (Con A) increases the attachment of cells to the substratum in a dose-dependent manner and this effect is completely abolished when the incubation is carried out in the presence of the specific monocarbohydrate Con A-inhibitor, α-methyl-D-mannoside. A Con A-mediated enhancement of cell-to-substratum adhesion was also detected on cells deprived of toposome, a glycoprotein complex responsible for cell-to-cell adhesion. The involvement of other molecules as well as toposome in the process of cell-to-substratum adhesion is also investigated. Results of these *in vitro* experiments indicate that all the molecules tested contribute to the process of cell-to-substratum adhesion.

cell adhesion / sea-urchin / concanavalin A

Introduction

Interactions of cells with extracellular matrix (ECM) components are critically important events during embryonic development. The establishment of the correct assembly of the embryo greatly depends on morphogenetic movements that lead embryonic cells through ECM to their final destination. The use of plant lectins to study the interactions between cells and ECM has been of great advantage in many experimental systems. In the sea-urchin embryo, pioneering experiments by Lallier [6] showed that culturing embryos in the presence of Con A causes an abnormal embryonic development with an overexpression of ectodermal structures. On the other hand, low concentrations of Con A produce agglutination of cells, possibly due to the interaction of the lectin with carbohydrates carried by cell surface molecules. More recently the involvement of Con A in mediating cell-to-substratum adhesion and differentiation has been extensively described [1, 12, 13]. In the sea urchin embryo, scanning electron microscopic observations showed that cells, cultured in the presence of Con A, secrete extracellular fibrous material that may be involved in the process of cell-to-substratum adhesion [3].

In addition to ECM molecules, it has been postulated that molecules mediating cell-to-cell adhesion regulate morphogenetic movements. A putative role in position-dependent interactions during embryogenesis has been proposed for the molecule responsible for cell-to-cell adhesion in the sea-urchin embryo [11]. The molecule has been isolated as a 22S glycoprotein complex, named toposome, and its biological activity was assessed by a morphogenetic cell aggregation assay [7]. Although its role in mediating cell-to-cell adhesion has been well established, nothing is known on its role in the process of cell-to-substratum adhesion.

A variety of different assays used to measure the adhesion of many cell types to substrata have been giving rise to different interpretations. In some cases, cells are settled down by gravity while in others cell-to-substratum adhesion is initiated by centrifugation. Removal of cells that do not adhere to the substratum is also a crucial step that could produce different results. In the present study, we utilized a cell-to-substratum adhesion assay originally developed by McClay and Fink [8] and found that it is particularly suitable to study the adhesion of sea urchin embryonic cells to the substratum. Furthermore, we describe the Con A-mediated enhancement and the involvement of toposome and ECM molecules in the process of cell-to-substratum adhesion.

Materials and Methods

Paracentrotus lividus eggs were fertilized with a dilute sperm suspension and grown in Millipore filtered sea water (MFSW), containing penicillin (60 μg/ml) and streptomycin sulfate (50 μg/ml) at 15°C until the mesenchyme blastula stage in the presence of ^{3}H-lysine (1 μCi/ml). Dissociation of embryos into single cells was achieved by the technique documented by Matranga *et al* [7]. Toposome-deprived cells were obtained by resuspending 1×10^6 dissociated cells with 5 ml of 2.5% *n*-butanol in MFSW [2]. Cells were then immediately centrifuged to discard the supernatant.

The substrata were prepared by adsorbing different adhesion molecules on microtiter plates (Dynatech Laboratories, Inc). The plates were pre-coated with 100 μl of denaturated bovine serum albumin (10 mg/ml) at 37°C for 3 h. Coating of the plates with adhesion molecules was then performed by incubation with 100 μl of the tested molecules at 37°C for 3 h. In the experiments in which α-methyl-D-mannoside was used to block Con A, the lectin was added to the plates together with 100 mM α-methyl-D-mannoside and incubated as above. Assays were started by adding 1×10^5 cells labelled with ^{3}H-lysine in 100 μl of MFSW to each well. This resulted in a monolayer of single cells. The plates were then sealed and incubated at room temperature (18°C). After 1 h, the plates were inverted and centrifuged at 650 *g* in a swing-out rotor at 4°C for 8 min. Plates were quickly

frozen in an ethanol-dry ice bath, the bottoms of the wells were cut off and the radioactivity associated with cells was counted in a scintillation counter. The percentage of adhered cells was calculated assuming as 100% the radioactivity associated to 1 × 10^5 labelled cells counted separately. Results shown are the mean of eight replicates for which the standard error is calculated.

Toposome was prepared as previously described by Cervello and Matranga [2]. Con A, α-D-mannoside, chondroitin sulfate, penicillin and streptomycin were obtained from Sigma Chemicals Co (USA). Porcine fibronectin was from Ito Ham Co (Japan) and ^{3}H-lysine was from Amersham (UK).

Results

Using the cell-to-substratum adhesion assay described in *Materials and Methods,* a dose-dependent effect of Con A in mediating the adhesion of sea-urchin embryonic cells to the substratum was found (fig 1). The enhancement of cell adhesion can be observed at doses as low as 0.05 μg/ml of Con A coating and no measurable increase in the adhesion was observed for doses higher than 50 μg/ml. The effect was completely abolished if coating was carried out in the presence of α-methyl-D-mannoside at a concentration of 100 mM.

It has been shown that a mild treatment of cells with diluted *n*-butanol in sea water removes cell surface molecules without affecting the viability of cells, but causing their incompetence to aggregate [10]. In order to establish whether Con A-mediated cell-to-substratum adhesion was affected by the removal of toposome from the cell surface, the adhesion of toposome-deprived cells to Con A-coated substratum was examined. Figure 2 shows that Con A promotes the adhesion of toposome-deprived cells to the substratum with an increase from 5 to 68%. When plates were coated with Con A in the presence of α-methyl-D-mannoside, no enhancement in cell adhesion was observed. This result seems to indicate that Con A binds to cell surface molecules other than toposome.

In the present study, ECM molecules known to play an important role in cell-to-substratum adhesion [15] have been also tested. In table I, the adhesion-promoting activity of Con A is compared with that of fibronectin and chondroitin sulfate. It was found that the highest enhancement of cell-to-substratum adhesion was obtained when Con A-coated substrata were used. Analogous results were obtained using toposome-deprived cells. Each value in table I refers to the concentration at which the highest cell adhesion was observed.

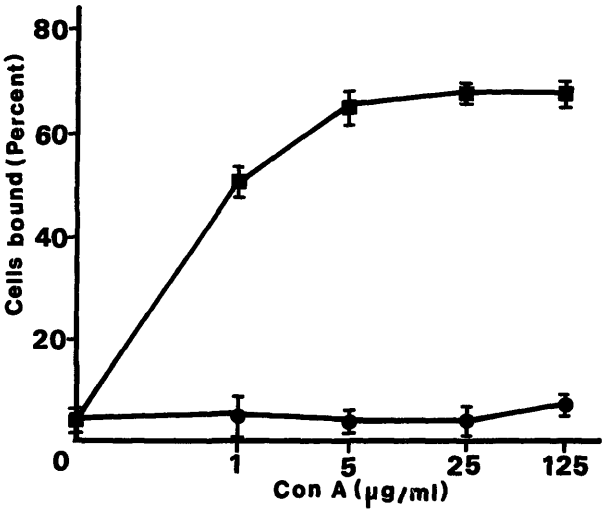

Fig 2. Adhesion of toposome-deprived blastula cells to Con A-coated substrata in the absence (■) or in the presence (●) or α-methyl-D-mannoside.

Table I. Adhesion-promoting activity of different cell adhesion molecules on sea-urchin embryonic cells. Coating was carried out by incubation with 100 μg/ml of tested molecules for 3 h. Values are mean ± SE.

Coating	*Percentage of cell binding*	
	Toposome-bearing cells	*Toposome-deprived cells*
None	40 ± 3	8 ± 3
Con A	82 ± 5	62 ± 2
Fibronectin	59 ± 3	23 ± 8
Chondroitin sulfate	48 ± 9	

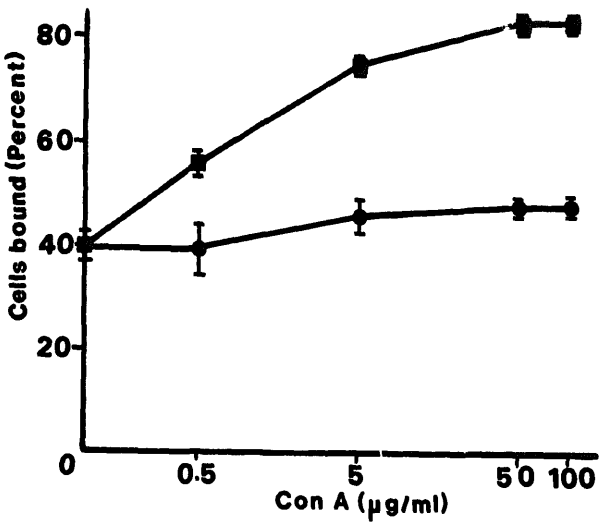

Fig 1. Adhesion of blastula cells to Con A-coated substrata in the absence (■) or in the presence (●) or α-methyl-D-mannoside.

The observation that toposome-deprived cells had *per se* a reduced binding (fig 2 and table I) prompted us to investigate on the possible involvement of toposome in cell-to-substratum adhesion. The adhesion of mesenchyme blastula cells to substrata coated with purified toposome was measured. Results in figure 3 show that toposome enhances cell-to-substratum adhesion in a dose-dependent manner, indicating its participation in the phenomenon.

Discussion

Cell-to-cell and cell-to-substratum adhesions are crucial interactions in the maintenance of the structural integrity of embryos, tissues, organs and organisms. Carbohydrate residues are certainly involved in these phenomena since

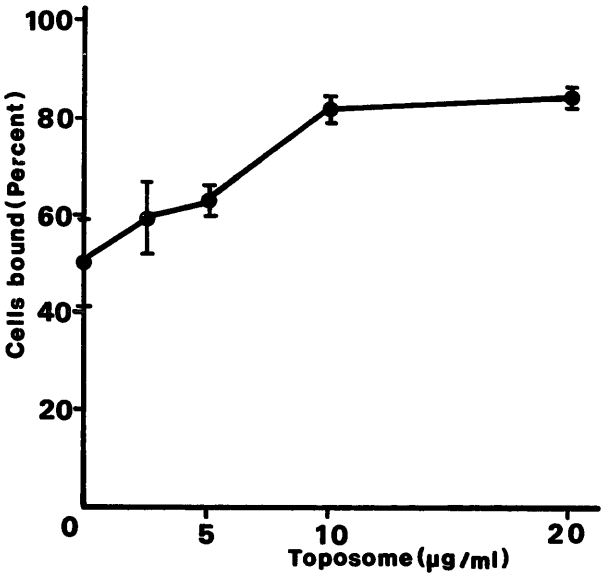

Fig 3. Enhancement of cell-to-substratum adhesion of blastula cells by toposome.

they are carried by cell surface glycoproteins that may play a role in cell recognition and cell migration. For this reason, lectins have been widely used in the sea-urchin embryo as tools to study the interaction of cells with substrata or with other cells [3–6, 14]. In measuring cell-to-substratum adhesion, particular attention should be also paid to the experimental conditions and to the embryonal stage of the cells used. In fact, it has been shown that the adhesion of cells from sea-urchin embryos to Con A [5], hyalin [8] and fibronectin [9] depends on the developmental stage at which embryos were dissociated.

In this report, we compared under the same experimental conditions the adhesion of cells dissociated from sea-urchin embryos at the mesenchyme blastula stage to substrata coated with cell adhesion molecules. The assay method we used offers many advantages: a) highly reproducible results, b) a large number of replicates, c) chemically defined substrata, d) gentle cell-to-substratum contact, e) known cell dislodgment force. Under these conditions, the adhesion of embryonic cells to substrata is mediated by Con A in a dose-dependent manner. Cell adhesion appears to involve a carbonydrate-Con A interaction since it is inhibited by the addition of α-methyl-D-mannoside. These results are in agreement with previous reports on hamster embryonic fibroblasts [13] and sea-urchin embryonic cells [3].

It is worth noticing that toposome-deprived cells are still able to bind to Con A-coated substrata in a dose-dependent manner. However, when toposome was used as coating molecule, it mediated cell-to-substratum adhesion of mesenchyme blastula cells. Furthermore, using the *in vitro* assay, other molecules, such as fibronectin and chondroitin sulfate, contribute to the adhesion of cells to the substratum. It seems therefore that many molecules are involved in the process of cell-to-substratum adhesion in the sea-urchin system. The differential expression of both carbohydrate and proteins, either at the cell surface or in the ECM, could therefore modulate the extent and the specificity of cell-ECM interactions during morphogenesis.

Acknowledgments

The authors extend their thanks to Mr Scaturro for sea-urchin collection and care. This work was partially supported by the "Progetto Bilaterale" Italy-Japan (VM and EN) of the Italian National Research Council.

References

1 Carter WG, Rauvala H, Hakomori S (1981) Studies on cell adhesion and recognition. II. The kinetics of cell adhesion and cell spreading on cell surface coated with carbohydarate-reactive proteins (glycosidases and lectins) and fibronectin. *J Cell Biol* 88, 138–148
2 Cervello M, Matranga V (1989) Evidence of a precursor-product relation-ship between vitellogenin and toposome, a glycoprotein complex mediating cell adhesion. *Cell Differ Dev* 26, 67–76
3 Iwata M, Nakano E (1984) Cell-to-substratum adhesion of dissociated embryonic cells of the sea urchin, *Pseudocentrotus depressus*. *Roux' Arch Dev Biol* 193, 71–77
4 Katow H, Solursh M (1982) *In situ* distribution of Con A-binding sites in mesenchyme blastula and early gastrula of the sea urchin *Lytechinus pictus*. *Exp Cell Res* 139, 171–180
5 Krach SW, Green A, Nicolson GL, Oppenheimer SB (1974) Cell surface changes occurring during sea urchin embryonic development monitored by quantitative agglutination with plant lectins. *Exp Cell Res* 84, 191–198
6 Lallier R (1972) Effects of concanavalin A on the development of sea urchin egg. *Exp Cell Res* 72, 157–163
7 Matranga V, Kuwasaki B, Noll H (1986) Functional characterization of toposomes from sea urchin blastula embryos by a morphogenetic cell aggregation assay. *EMBO J* 5, 3125–3132
8 McClay DR, Fink RD (1982) Sea urchin hyalin: appearance and function in development. *Dev Biol* 92, 285–293
9 Miyachi Y, Iwata M, Sato H, Nakano E (1984) Effect of fibronectin on cultured cells derived from isolated micromores of the sea urchin, *Hemicentrotus pulcherrimus*. *Zool Sci* 1, 265–271
10 Noll H, Matranga V, Cascino D, Vittorelli ML (1979) Reconstruction of membranes and embryonic development in dissociated blastula cells of the sea urchin by reinsertion of aggregation-promoting membrane proteins extracted with butanol. *Proc Natl Acad Sci USA* 76, 288–292
11 Noll H, Matranga V, Cervello M, Humphreys T, Kuwasaki B, Adelson D (1985) Characterization of toposomes from sea urchin blastula cells: a cell organelle mediating cell adhesion and expressing positional information. *Proc Natl Acad Sci USA* 82, 8062–8066
12 Rauvala H, Carter W, Hakomori S (1981) Studies on cell adhesion and recognition. I. Extent and specificity of cell adhesion triggered by carbohydrate-reactive proteins (glycosidases and lectins) and by fibronectin. *J Cell Biol* 88, 127–137
13 Rauvala H, Hakomori S (1981) Studies on cell adhesion and recognition. III. The occurrence of α-mannosidase at the fibroblast cell surface and its possible role in cell recognition. *J Cell Biol* 88, 149–159
14 Roberson M, Oppenheimer SB (1975) Quantitative agglutination of specific populations of sea urchin embryo cells with concanavalin A. *Exp Cell Res* 91, 263–268
15 Yamada KM, Olden K (1978) Fibronectins: adhesive glycoproteins of cell surface and blood. *Nature* 275, 179–184