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Research article

Cell spreading on laminin substrate involves Con A-binding proteins

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Summary — Concanavalin A (Con A), a tetravalent lectin, was shown to impair 8 chick embryo fibroblast (8 d CEF) spreading on a laminin (LM) substrate but not on a fibronectin substrate (FN), suggesting that cell surface Con A binding proteins could be involved in 8 d CEF spreading on a LM substrate. The interaction of Con A-binding proteins with Con A is dependent upon the carbohydrate moieties of the isolated glycoproteins; since they interact strongly with Con A-Sepharose and are eluted with 0.3 Mol/l α-methylmannopyranoside, the isolated Con A binding-proteins inhibit 8 d CEF adhesion to a Con A substrate to the same extent as α-methylmannopyranoside. Furthermore, the isolated Con A binding proteins specifically inhibit in a dose-dependent manner 8 d CEF spreading on LM but not on FN.

fibroblast / Con A-binding site / adhesion / laminin / fibronectin

Résumé — L'étalement des cellules sur la laminine implique des protéines fixant la Con A. *La concanavaline A (Con A), lectine tétramérique, empêche l'étalement des fibroblastes d'embryons de poulet âgés de 8 jours (8d CEF) sur un support de laminine, mais pas sur un support de fibronectine. Ceci suggère que les protéines membranaires fixant la Con A seraient impliquées dans l'étalement des fibroblastes sur la laminine. L'intéraction de ces protéines avec la Con A dépend des chaînes oligosaccharidiques des glycoprotéines isolées qui réagissent fortement avec la Con A-Sépharose et sont éluées par l'α-méthylmannopyranoside (0,3 mol/l). Les protéines fixant la Con A. Ces protéines fixant la Con A inhibent spécifiquement l'étalement des fibroblastes sur laminine mais pas sur fibronectine.*

fibroblaste / site Con A / adhésion / laminine / fibronectine

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INTRODUCTION

The interaction between cells and the extracellular matrix is a multiple step phenomenon (Aplin and Hughes, 1981). The initial attachment of cells to the extracellular matrix is followed by a spreading period. This latter process requires a rearrangement of intracellular cytoskeletal components which leads to a drastic change in cell morphology together with topological modifications of the cell surface. Results recently obtained in our laboratory show that 8-d-old chick embryo fibroblasts (8 d CEF) use different mechanisms to attach and spread on fibronectin (FN) and laminin (LM). We have shown that the lectin concanavalin A (Con A) impaired spreading on a LM but not on a FN substrate (Codogno *et al*, 1988a). To ascertain the role of some cell surface glycoproteins in the spreading of CEF onto LM, we used the tetrameric lectin Con A as a tool. Therefore, in this study, we carried out experiments to investigate the nature of the interaction between Con A and 8 d CEF Con A-binding sites, and the role of Con A binding sites during the cell spreading on laminin.

MATERIALS AND METHODS

Cell culture

Primary monolayer cultures of fibroblasts were established from 8-d-old chick embryos. Cells were used before confluence and were obtained by TPCK-trypsin treatment, as previously reported (Codogno *et al*, 1988a).

Adhesion assay and cell spreading

FN and LM substrates were prepared as detailed previously (Codogno *et al*, 1987). Negative controls of adhesion were performed using plates coated with 2% bovine serum albumin (BSA). At the end of the adhesion assay (Routinely 2 h), the plates were washed 3 times with PBS. Cell attachment and spreading were determined by direct microscopic observation of different fields.

Cell surface Con A-binding protein isolation

Con A binding protein isolation was conducted after metabolic labelling with [^{35}–S]methionine (50 μCi/ml, sp act 1 200 Ci/mmole, CEA Saclay) for 16 h, as previously described (Nowakoski *et al*, 1987; Codogno *et al*, 1988a). Labelled Con A-binding proteins were diluted in PBS, pH 7.4, at the required concentration and added at the beginning of the adhesion assay. Similarly, α-methylmannopyranoside (0.1 mol/l) and ovalbumin (5–40 μg/ml) from Sigman, La Verpillère were added at the beginning of the adhesion assay.

Con A-Sepharose chromatography of isolated Con A binding proteins

Chromatography on Con A-Sepharose (Pharmacia, Bois d'Arcy) was conducted at room temperature, as previously reported (Codogno *et al*, 1985), on a 1 x 5 cm column. Con A binding proteins (30 000 cpm) were loaded on a column equilibrated with 0.025 mol/l Tris-HCl, pH 7.5 containing 0.1 mol/l NaCl and 10^{-3} mol/l $CaCl_2$, $MgCl_2$ and $MnCl_2$. Elution was performed with the same buffer containing successively: 0.01 mol/l α-methylglucopyranoside and 0.3 mol/l α-methylmannopyranoside. The radio-activity was determined in a counter Minaxi β 4 000 (Packard) equipped with a dpm calculation program. More than 90% of the radioactivity loaded was recovered.

RESULTS

Isolation of Con A binding sites

Con A-binding sites were isolated from CEF labelled with [^{35}S]methionine incubat-

ed with biotinylated-Con A, and then extracted with n-octylglucoside. The resulting soluble material was subsequently subjected to chromotography on avidin-Agarose. The radioactivity recovered in the eluted fraction accounted for 17% of the loaded radioactivity. Polyacrylamide gel electrophoresis and fluorography of the Con A-binding proteins revealed glycoproteins ranging from 30-72 kDa.

Relation between cell surface Con A binding sites and Con A specificity

Two sets of experiments were designed to determine the specificity of the Con A binding sites for the Con A lectin.

– When isolated, Con A binding sites were subjected to chromatography on a Con A-Sepharose column; all the radioactivity was eluted with 0.3 M α-methylmannopyranoside,

– When CEF were plated on a Con A substrate, the cell adhesion curve reached a plateau when 50 µg/ml of Con A were used (data not shown). The adhesion could be almost completely inhibited with 0.1 mol/l α-methylmannopyranoside (90% inhibition). Con A-binding sites blocked, in a dose-dependent manner, CEF adhesion to Con A (fig 1). This inhibition was not related to the presence of detergent since dialyzed extract, under the same conditions and added to a cell suspension in a control experiment, did not alter CEF adhesion capacities. These 2 experiments argue for the specificity of the Con A-binding glycoproteins for the Con A lectin.

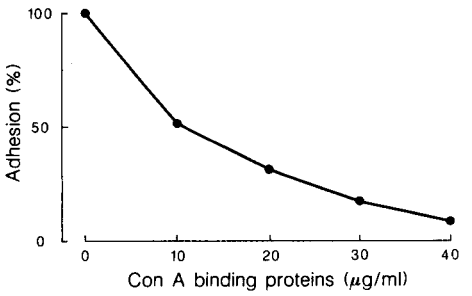

Fig 1. Effect of Con A-binding proteins on CEF adhesion to a Con A substrate. Con A was coated at a concentration of 50 µg/ml as described under *Materials and Methods*. Con A-binding proteins were added at the beginning of the adhesion assay. Cells were incubated for 2 h at 37 °C and the number of cells attached were scored after 3 washes to remove non-adherent cells and fixation with 2.5% glutaraldehyde.

Isolated Con A binding sites inhibit CEF spreading on LM

Con A was shown to specifically impair CEF spreading on LM, whereas no such phenomenon was observed on FN. Isolated Con A-binding proteins did not affect either CEF attachment on an LM or FN substrate in the range of concentrations used (0.5–5 µg/ml). The presence of free Con A-binding proteins did not modify the spreading of CEF on an FN substrate even when lower quantities of coating FN were used (data not shown). Conversely, Con A-binding proteins inhibited CEF spreading on LM in a dose-dependent manner (table I). The inhibition of CEF spreading was complete when 3 µg/ml of Con A-binding proteins were added to the adhesion medium. The induced effect was not due to the presence of small quantities of detergent associated with the Con A-binding proteins since the control buffer (see above) did not perturb CEF spreading on LM (table I). The specificity of the effect was verified using an irrevalent Con A-binding protein, *ie*, ovalbumin, in the concentration range used from 5–40 µg/ml, which did not modify the CEF spreading on LM (table I).

Table I. Effect of Con A-binding proteins on 8 d CEF spreading on LM and FN substrates. Routinely, spreading was visualized after 2 h of incubation at 37 °C. Under these conditions, the same number of cells were attached to LM and FN substrates. Spreading was quantified as follows: 100 – 200 cells were scored under microscopic observation. +: 100% of spreading; +/–: 50% of spreading; – –: no spreading, *ie*, round cells.

	Con A-binding proteins µg/ml			*Dialysed extract*	*Ovalbumin 40 µg/ml*
	1	*2*	*5*		
Spreading on LM	+	+/–	–	+	+
Spreading on FN	+	+	+	+	+

DISCUSSION

The nature of molecular events leading to cell spreading, after the initial interaction between extracellular matrix receptors and their ligands, remains poorly understood. Using Con A, we have shown that this lectin specifically impaired 8 d CEF spreading on LM substrate (Codogno *et al,* 1988a). Although, Dean *et al* (1988) have shown that Con A binding to LM impaired $B_{16}F_1$ cells spreading, the results presented here showed that isolated Con A binding glycoproteins inhibited CEF spreading onto LM, thus arguing for the role of these membrane associated glycoproteins in this phenomenon. These receptors are a heterogeneous population with molecular mass ranging from 30–72 kDa.

The Con A-binding sites interact strongly with the lectin *via* their carbohydrate moieties, suggesting that these receptors are decorated with N-linked high mannose type chains. To our knowledge, these isolate Con A binding proteins are unrelated to the 69K LM receptor (Lesot *et al,* 1983) and to the extracellular matrix receptor belonging to the integrin family (Ruoslahti, 1988). Nevertheless, we cannot exclude the presence of 5 nucleotidase among the Con A binding proteins isolated which interact with LM (Dieckhoff *et al,* 1986) and was shown to inhibit CEF spreading on LM (Codogno *et al,* 1988b). During the isolation of WGA- and *Ricinus communis* (RCA)-binding molecules from BHK cells, it was recently reported (Tuan and Grinnell, 1988) that these receptors account for 1.5 and 0.15%, respectively, of the radioactivity recovered after detergent extraction. Herein, we show that Con A-binding molecules represent 17% of the detergent extracted material. In spite of differences in the procedures used to extract lectin-binding sites in these 2 different studies, the high quantity of Con A binding proteins isolated is in good agreement with the large number of Con A binding sites on 8 d CEF (Bernard *et al,* 1982).

The isolated Con A-binding proteins inhibit in a dose-dependent manner, the spreading of 8 d CEF on LM but not on FN. A recent ultrastructural study reports that Con A-binding proteins are in close proximity to the cytoskeletal components and their distribution is regulated by the actin meshwork (Katsumoto and Kurimura, 1988). Furthermore, the results reported herein show direct competition between "soluble" Con A-binding proteins and mem-

brane bound Con A-binding proteins, suggesting that these glycoproteins could interact with LM. Further studies will be addressed at elucidating the relationship between Con A-binding proteins, LM and cytoskeletal proteins.

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