Correlation Between the Mobility of Inner Plasma Membrane Structure and Agglutination by Concanavalin A in Two Cell Lines of MOPC 173 Plasmocytoma Cells

(intramembranous particles/binding sites/cell agglutination/murine plasmocytoma cell lines)

CLAUDINE GUÉRIN*, ALAIN ZACHOWSKI*, BERNADETTE PRIGENT*, ALAIN PARAF*†, IRÈNE DUNIAT†, MARIE-ALINE DIAWARA†, AND E. L. BENEDETTI†

*Laboratoire d’Immunodifférenciation et †Laboratoire de Microscopie Electronique, Institut de Biologie Moléculaire de l’Université Paris VII, 2, Place Jussieu, Paris 5e, France

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ABSTRACT Both the distribution of the concanavalin A-binding sites and the rearrangement of the intramembranous particles revealed by the freeze-etching technique, have been studied by means of two variants of the same cell line issued from MOPC 173 murine plasmocytoma. One variant does not agglutinate even in presence of high lectin concentration. It has been shown that the number of binding sites and affinity are almost the same in the two variants. The clustered distribution of intramembranous particles is induced by the interaction of the concanavalin A and the cell surface only in the variant which is agglutinable. From these results it becomes apparent that the clustered distribution of the membrane particulate components is an acquired feature of the plasma membrane accompanying cell agglutination.

It is becoming apparent that the agglutination by plant-lectins in normal and in transformed cells is related to the topographic distribution of the lectins receptors (1, 2), rather than to differences in the number or unmasking of available sites for binding (3, 4). The proper mechanism of lectins—plasma membrane-interactions triggering cell agglutination is, however, not yet fully understood. An enhancement of cell agglutinability could be related to an inherent clustered distribution of lectin sites. On the contrary, a random dispersion of the receptors should not favour the interaction of cell surfaces which leads to agglutination (1). Experimental data are also consistent with the hypothesis that, after or during the binding, a rearrangement of the plasma membrane architecture is induced which involves a redistribution of the agglutinin receptor sites (2, 5).

We have been able to establish, in tissue culture, various cell clones issued from MOPC 173 murine plasmocytoma (6). One of these clones, the ME2 line, is susceptible to density inhibition of growth and is not transplantable to mice. These cells can be agglutinated by as little as 5 μg/ml of concanavalin A (Con A) and the arrest of cell division may be induced by the addition of 25 μg/ml of the lectin to the medium (7). From the ME2 cell line, a variant (ME2C+) has been selected which is not agglutinated even by 100 μg/ml of Con A and grows normally in a medium containing the same amount of Con A (7).

The purpose of this note is to establish a possible correlation between the interaction of the cell surface with Con A and the rearrangement of the plasma membrane architecture. We studied the distribution of the binding sites and the dynamic aspects of the intramembranous particles using the freeze-etching technique after the binding and agglutination with Con A.

Although extensive studies have been already carried out on the effect of the plant agglutinins on the surface distribution of lectin receptors (2, 8) during the binding and/or cell agglutination, only in a few appropriate instances has it been shown that there is a topographic correlation between the distribution of lectins receptors sites exposed on the true cell surface and the intramembranous particles revealed by the fracture in freeze-etched preparations (9). Using two variants of the same cell line, we demonstrated that both cell types have almost the same number of sites and an equivalent-binding affinity. Moreover we found that the clustering of intramembranous particles seems to be an acquired membrane feature which occurs after the binding of Con A and is associated with cell agglutination ability.

MATERIALS AND METHODS

Cells. ME2 cells are growing in an Eagle’s medium supplemented with yeast extract, lactalbumin, vitamins, glutamin and 2% calf serum. Culture conditions have already been described (6) and the cells are collected 3–5 days after seeding, by treating the monolayer with EDTA (5 x 10^-4 M) in phosphate-buffered saline (pH 7.4) for 30 min and washing them three times at 20°C in the same buffer. Con A resistant variant (ME2C+) is cultivated and collected under the same conditions.

Concanavalin A. For routine studies purified Con A (Calbiochem) was used, whereas for labeling studies, the Con A was purified on affinity columns until only one band was found by acrylamid gel electrophoresis. Labeled Con A was made by acetylation of the purified protein with [¹⁴C]N-hydroxysuccinimide. After purifying the labeled lectin by affinity chromatography, the specific activity of the final product was 7000 cpm/μg of protein.

Binding Sites Estimation. All experiments were performed at pH 7.4. Cells are suspended in phosphate-buffered saline supplemented with 0.1% bovine-serum albumin to a final concentration of 10^9 cells per ml. Both lines, ME2 and ME2C+, are incubated with variable concentrations of Con A (5–75 μg/ml). Specific binding is determined by the difference between total binding of Con A [molecular weight considered to be 100,000 (10)] and binding obtained in presence of α-methyl mannopyronoside [technique of Betel and van den
TABLE 1. Number and affinity of the binding sites of ME₄ and ME₄Cr cells

<table>
<thead>
<tr>
<th>Cells</th>
<th>Sites/cell</th>
<th>Cell surface</th>
<th>Sites/μm²</th>
<th>Equilibrium constant</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME₄</td>
<td>3.72 × 10⁶</td>
<td>1160 μm²</td>
<td>3.2 × 10⁴</td>
<td>10.3 × 10⁴ M⁻¹</td>
</tr>
<tr>
<td>ME₄Cr</td>
<td>6.23 × 10⁶</td>
<td>1350 μm²</td>
<td>4.6 × 10⁴</td>
<td>9.7 × 10⁴ M⁻¹</td>
</tr>
</tbody>
</table>

The total number of Con A binding sites and their affinity were determined as described in Methods. Calculation of the cell surface was derived from direct micrometric measurements of the cells.

Berg (11]). All incubations are made in a water bath with agitation at 37°C. All results are plotted according to the Scatchard’s method (12).

Assay for Agglutination. The cell suspension is adjusted to a concentration of 10⁶ cells per ml in phosphate-buffered saline and the solution of Con A is added in concentrations varying from 2.5 to 900 μg/ml. The density and size of the aggregates were scored from 0 to ++++ after 40 min at 24°C.

Electron Microscopy. Cells were incubated at room temperature for 45 min in phosphate-buffered saline containing 100 μg/ml of Con A with 5 × 10⁶ cells. The cells were then fixed in 1% glutaraldehyde in cacodylate buffer (pH 7.2) for 30 min, washed three times, and impregnated in 20% glycerol in distilled water. Controls without Con A were processed similarly. Freeze-etching was performed in a Balzers apparatus. The etching time was 1–2 min at 100°C. Electron microscopic observations were made with an EM 300 Philips.

RESULTS

Binding and Equilibrium Constant. Table 1 presents the results of binding experiments. It should be noted that although the number of sites per cell varies from 3.72 × 10⁶ in ME₄ line to 6.23 × 10⁶ in ME₄Cr variant, the sites, densities per unit surface, are similar. Moreover, the equilibrium constant derived from Fig. 1 is almost identical for the two lines.

Agglutination. Agglutination of the ME₄ cells with Con A occurs at a concentration of as little as 2.5 μg/ml of purified lectin. In contrast, agglutination of the ME₄Cr variant is almost absent even in presence of high concentration of Con A (Table 2).

Electron Microscopic Observations. Fig. 2 shows large fracture faces of the plasma membrane of two adjoining ME₄ cells. The outwardly directed fracture face A of the inner leaflet of one plasma membrane is characterized by the presence of many (600/μm²) randomly distributed globular particles having an average diameter of 80 Å. The inwardly directed fracture face B of the outer leaflet of the adjoining plasma membrane shows only few particles (150/μm²). Randomly dispersed particles are also observed on fracture faces A and B in the ME₄Cr variant (Fig. 5 and 6). In the ME₄Cr variant, the number of particles found on face A (400/μm²) is closer to that on face B (250/μm²) than in the ME₄ cells where the asymmetrical distribution of particles in faces A and B is more pronounced. After incubation with Con A and when cell agglutination has taken place, the fracture face A in ME₄ cells is characterized by a marked clustering of particles (Fig.

DISCUSSION

Interaction between plant-lectins (such as Con A) and plasma membrane in animal cells, causes several effects including stimulation of cell mitosis (13, 14), restriction of the mobility of immunoglobulin receptors (15), rearrangement of lectin-binding sites in the plane of the plasma membrane (2, 5), and cell agglutination (3, 4). The present study was designed to determine if the rearrangement of the inner membrane structure which might follow the binding of the lectin, is an essential feature for cell agglutination. We were able to study this by means of two variants issued from MOPC 173 murine plasmocytoma. One of the two variants is not agglutinated even in presence of high concentration of Con A, although the number of binding sites and the affinity in the ME₄ and ME₄Cr are almost the same. We have shown that before the binding with Con A, the intramembranous particles are randomly dispersed on fracture faces A and

![Image](image_url)

Fig. 1. Specific binding and affinity for [1H]Con A of ME₄ and ME₄Cr cells plotted according to Scatchard (12); r is specific bound Con A and c, the free lectin. 1 × 10⁶ cells (A) or 8 × 10⁶ cells (B) were incubated with various amounts of [1H]Con A in a final volume of 2 ml. Specific binding was determined as described in Methods. Points are calculated from the mean of at least two experiments. Lines are fitted by the method of least squares.

![Table 2. The effect of various concentrations of Con A on the agglutination of ME₄ and ME₄Cr cells](table2)

<table>
<thead>
<tr>
<th>Concentration of Con A (μg/ml)</th>
<th>Degree of agglutination</th>
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<tbody>
<tr>
<td></td>
<td>ME₄</td>
</tr>
<tr>
<td>300</td>
<td>++++</td>
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<tr>
<td>100</td>
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<td>25</td>
<td>+++</td>
</tr>
<tr>
<td>5</td>
<td>++</td>
</tr>
<tr>
<td>2.5</td>
<td>++</td>
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</table>

Cells are harvested and incubated with Con A as described in Methods. Agglutination was scored from no agglutination (0) to complete agglutination (++++).
Figs. 2 through 7 illustrate the features observed in freeze-etched replicas. Bar indicates 0.1 μ (μ = 1 micrometer). Fig. 2: Typical aspect of the fracture faces A and B of an untreated ME2 cell. IS indicates the intercellular space. The particles on both fracture faces are randomly dispersed. Magnification ×120,000. Fig. 3: Clustered distribution of particles on fracture face A in a ME2 cell treated with 100 μg/ml of Con A. Magnification ×180,000. Fig 4: Small clusters of particles on fracture face B in a ME2 cell treated with 100 μg/ml of Con A. Magnification ×120,000. Figs. 5 and 6: Typical aspect of the random distribution of particles in fracture faces A and B of an untreated ME2C' cell. Magnification ×150,000. Fig. 7: The random distribution of particles is still present on fracture face A of a ME2C' cell after incubation with 100 μg/ml of Con A. Magnification ×180,000.
B for both variants. The clustered aspect of the intramembranous particles became apparent after the binding, only in the cell variant which agglutinates. In contrast, even after prolonged interaction of the resistant variant with high concentration of Con A, the intramembranous particles were still randomly dispersed. Thus, the clustered distribution of the intramembranous particles seems to be more an acquired membrane feature consequent to the binding, rather than a preexistent character of the plasma membrane in agglutinatable cells. The rearrangement of the lectin-binding sites has been investigated by Rosenblith et al. (5) who have found a clustered distribution of the receptors as a consequence of the interaction of the cell surface with Con A in protease-treated normal and in transformed cell. The latter phenomenon might reflect the mobility of intramembranous particles revealed in our observations; in fact many experimental data tend to prove that the membrane particulate components are topographically associated with lectins receptors (16–18; compare ref. 19).

Loor (16) has also reported a rearrangement of intramembranous particles into patches which is induced by a capping/mitogenic dose of phytohemagglutinin in lymphocyte-plasma membrane. This last result was interpreted to mean that there is a close association between the lectin-binding sites and intramembranous particles. It should be noted, however, that these observations may not necessarily reflect mobility of identical structures. It is conceivable that lectin receptors may be associated with heterogeneous membrane components either "peripheral" or "integral" (20). This "integral" component, an amphipathic glycoprotein likely associated with the intramembranous particles (21), might move quite independently from the former component. It is noteworthy that in thymocyte and lymphocyte, no correlation between agglutination (capping) of a peripheral membrane constituent such as mouse Ig, and clustering of intramembranous particles, could be detected (17, 22). If the clustering of intramembranous particles, observed by us, in any way reflects the rearrangement of the exposed lectin receptors, then the pertinence of the cluster formation to cell agglutination caused by Con A should be evaluated. The topographic distribution of the intramembranous particles resulting from the Con A binding in the agglutinable MEv variant brings to mind the clustered feature of the lectin receptors depicted by Singer and Nicolson (23). It should be stressed, however, that the clustered distribution of Con A binding sites as well as the intramembranous particles are not inherent characters of the agglutinatable cells, but are an acquired feature consequent to the lectin interaction. Pertinent to this conclusion are the concomitant loss of mobility of intramembranous particles and the absence of agglutinability in the MEvC' cells. The absence of cluster formation in MEvC' cannot be related to a lower density of the exposed receptors and/or intramembranous particles. In fact the affinity, the number of receptor sites, and the number of the intramembranous particles per unit surface on the two variants, are almost the same. Hence, the means by which Con A resistance occurs at the plasma membrane remains to be established. Mild trypsin digestion of MEvC' shows that the proteolysis immediately restores the cell agglutinability and the mobility of the intramembranous particles.

§ Cl. Guérin, A. Paraf, and J. M. Dubert, manuscript in preparation.

Mobility of Inner Plasma Membrane Structure

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* E. L. Benedetti, I. Dunia, and M. A. Diawara, manuscript in preparation.