CELL AGGLUTINATION MEDIATED BY CONCANAVALIN A AND THE DYNAMIC STATE OF THE CELL SURFACE

M. INOUE

Department of Pathology, Okayama University Medical School, Okayama 700, Japan

SUMMARY

The binding of \(^{131}\text{I}\)-labelled concanavalin A (\(^{131}\text{I}-\text{Con A}\)) to the cell surface has been studied in Ehrlich ascites tumour cells (EATC) and beef erythrocytes under various conditions. The binding of concanavalin A (Con A) to the cell surface was very specific and the available binding sites were saturated within a few minutes. The amount of \(^{131}\text{I}-\text{Con A}\) bound to EATC was 4.14 \times 10^7 molecules/cell at 37 °C and 2.12 \times 10^7 molecules/cell at 0 °C. Under these conditions, cell agglutination was observed only at 37 °C and not at 0 °C. However, the binding sites measured at 0 °C were also effective for agglutination at 37 °C. Beef erythrocytes were agglutinated by Con A only after treatment of cells with papain. The number of binding sites for Con A on the cell surface was decreased by this treatment to about half the number present on untreated cells. Various reagents such as colchicine, monoiodoacetic acid, dinitrophenol, rotenone, sodium azide and carboxyl cyanide-m-fluorophenylhydrazone (FCCP) had no effect on Con A-mediated cell agglutination. In contrast, periodate treatment produced a remarkable decrease in the agglutinability of cells. From these data, it is concluded that the cell agglutination induced by Con A was due to the topographical distribution of the surface receptors for the lectin, and not the result of energy-dependent or microtubule-dependent reaction processes. The number and the state of Con A receptors on the cell surface were in a dynamic condition, their conformation, orientation, and/or topographical distribution changing under different conditions.

INTRODUCTION

Certain lectins selectively agglutinate transformed cells or protease-treated normal cells (Inbar & Sachs, 1969). Various factors are important in cell agglutination, including the binding forces of the agglutinating molecules, the number of molecules involved in agglutination, the surface net charge, configuration and flexibility of the cell surface. It has been postulated that the difference in agglutinability by lectins between protease-treated and untreated normal cells or normal and transformed cells is due to unmasking of cryptic agglutinin-binding sites on the membrane (Burger, 1969). This explanation is not in accord with the recent finding that normal cells bind radioisotope-labelled agglutinins to approximately the same extent as transformed or protease-treated normal cells (Cline & Livingston, 1971; Ozanne & Sambrook, 1971; Arndt-Jovin & Berg, 1971; Sela, Lis, Sharon & Sachs, 1971). Recently, Inbar, Ben-Bassat & Sachs (1971a, b) reported that cell agglutination mediated by Con A was temperature-dependent, and that it was probably due to structural or metabolic change on the surface membrane. It is conceivable that the mechanism of cell agglutination induced by Con A does not follow a common pattern.
in every case. With this consideration in mind the author has attempted to clarify
the mechanism of cell agglutination induced by Con A and found several interesting
phenomena.

MATERIALS AND METHODS

Ehrlich ascites tumour cells (EATC) were transplanted in the peritoneal cavity of ddN mice
and harvested from ascites fluid 7 days after transplantation. Tumour cells or beef erythrocytes
were washed 3 times with glucose-free Hanks's balanced salt solution (HBS) by centrifuging
at 2000 rev/min for 30 s. Concanavalin A (Con A) was purified from Jack bean meal (Sigma
Chemical Co.) by the method of Agrawal & Goldstein (1967), and stored at −20 °C in HBS
containing 1 mM MnCl₂ as a stabilizer. Sialidase (Type V), hyaluronidase (Type I) and papain
were obtained from Sigma Chemical Co. Other reagents used were of analytical grade. All
experiments were carried out in HBS. The number of tumour cells used was 10⁷/ml, and of
beef erythrocytes, 10⁸/ml. Agglutination tests were carried out after incubating the cells with
agglutinin for 10 min at room temperature, following the method of Burger & Goldberg (1967).

The binding of ¹³¹I-Con A was carried out at 0 and 37 °C. Cells suspended in 3 ml of HBS
were incubated with different concentrations of ¹³¹I-Con A, with or without 100 mM α-methyl-
D-glucoside (α-MG), a haptenic inhibitor of Con A. After various periods of incubation they
were washed 3 times with 10 ml of cold HBS, and cell-bound radioactivity was then determined
by use of a well-type scintillation counter (Toshiba Medical Co. Ltd, Type UDS-24210). The
binding number of Con A was calculated by subtracting cell-bound radioactivity in the
presence of α-MG (100 mM) from the total radioactivity of bound Con A in the absence of
the haptenic inhibitor. The molecular weight of Con A used for the calculation of the binding
number was 55000. ¹³¹I-Con A used in this experiment was prepared by iodination of native
Con A with Na¹³¹I after the method described by Marchalonis (1969). The labelled lectin was
further purified by Sephadex G-200 column chromatography in the presence of 100 mM α-MG.
¹³¹I-Con A (10⁶ cpm/mmol Con A) was found to be identical with the native agglutinin as
regards agglutination activity. Protein concentration was determined by the method of Lowry,
Rosebrough, Farr & Randall (1951) using bovine serum albumin as a standard.

RESULTS

Binding of ¹³¹I-Con A to the surface of EATC

Fig. 1 shows the time course of binding of ¹³¹I-Con A to the surface of EATC at
37 °C; binding was complete within about 5 min. Fig. 2 shows the binding of
¹³¹I-Con A to EATC at 0 and 37 °C. The amount of Con A bound to the cell increased
until saturation as a function of Con A concentration. The amount of ¹³¹I-Con A
bound was 4.14 × 10⁷ molecules/cell at 37 °C and 2.12 × 10⁷ molecules/cell at 0 °C.
However, cell agglutination was observed only at 37 °C and not at 0 °C. About twice
as much Con A was bound to the cell surface at 37 °C as at 0 °C. If cells pretreated
with ¹³¹I-Con A at 0 °C were washed with cold HBS to remove free Con A from the
reaction mixture and the temperature was raised from 0 to 37 °C, then cell agglutina-
tion was observed without addition of any more lectin to the system. The results
suggest that the binding sites for Con A on the cell surface changed their state
depending on temperature. Fig. 3 shows the changes in agglutinability and the number
of Con A binding sites of beef erythrocytes after treatment of the cells with papain,
which rendered them agglutinable with Con A. However, the number of binding
sites for Con A on the erythrocyte surface was decreased to half the original number
by the enzyme treatment. The data suggest that there was no direct relationship
Fig. 1. Binding of $^{131}$I-concanavalin A to Ehrlich ascites tumour cells. The cells (10$^7$ cells/ml) were incubated with $^{131}$I-Con A (123 µg/ml) at 37 °C for various times, and cell-bound radioactivity of $^{131}$I-Con A determined as described in text.

Fig. 2. Binding of $^{131}$I-concanavalin A to Ehrlich ascites tumour cells at 0 and 37 °C. EATC (10$^7$ cells/ml) were incubated with various concentrations of $^{131}$I-Con A at 0 and 37 °C for 30 min, and cell-bound radioactivity of $^{131}$I-Con A determined as described in text. ○—○, 0 °C; ●—●, 37 °C.
between the number of binding sites available on the cell surface and the agglutinability of cells, and it therefore appears that other factors are important for inducing cell agglutination.

Fig. 3. Effect of papain treatment of beef erythrocytes on agglutinability (○—○) and number of concanavalin A-binding sites (●—●). Beef erythrocytes (10⁸ cells/ml) were pretreated with 0.1% papain at 37 °C for various periods. The concentration of ¹³¹I-Con A used for determining the amount of cell-bound radioactivity and the agglutinability of cells was 83 μg/ml. For further details, see text.

**Effect of various reagents on cell agglutination induced by Con A**

It has been reported that certain reagents such as colchicine or vinblastine inhibit the agglutination of polymorphonuclear leukocytes induced by a low concentration of Con A (Berlin & Ukena, 1972). Table 1 shows the effect of various reagents on Con A-mediated cell agglutination of EATC. A metabolic inhibitor of glycolysis such as monoiodoacetic acid, and inhibitors of the energy transfer reaction of mitochondria such as rotenone, FCCP, dinitrophenol and sodium azide had no effect on the agglutination. Furthermore, colchicine, a specific inhibitor of microtubule action, had no effect on agglutination under the conditions of this experiment. In contrast, treatment with potassium periodate produced a remarkable decrease in the agglutinability of cells by Con A. All these reagents had the same effect on the agglutination of papain-treated beef erythrocytes. Table 2 shows the effect of chemical fixation of EATC on the agglutinability of cells by Con A. Native EATC were agglutinated by Con A at 37 °C and not at 0 °C. However, the cells were agglutinated by Con A at both temperatures if they had been fixed by glutaraldehyde. The data suggest that energy-transducing reactions or the action of microtubules are excluded from a leading role in cell agglutination mediated by Con A.
Cell agglutination and surface receptor

Table 1. Effect of various reagents on cell agglutination induced by concanavalin A

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Agglutination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>++</td>
</tr>
<tr>
<td>Monooiodoacetic acid, 1 mM</td>
<td>++</td>
</tr>
<tr>
<td>Rotenone, 0.2 mM</td>
<td>++</td>
</tr>
<tr>
<td>FCCP, 0.1 mM</td>
<td>++</td>
</tr>
<tr>
<td>Dinitrophenol, 1 mM</td>
<td>++</td>
</tr>
<tr>
<td>Sodium azide, 10 mM</td>
<td>++</td>
</tr>
<tr>
<td>Colchicine, 0.1 mg/ml</td>
<td>++</td>
</tr>
<tr>
<td>Potassium periodate, 0.5 %</td>
<td>—</td>
</tr>
</tbody>
</table>

Cells were pretreated with each reagent for 30 min at room temperature. Agglutination was carried out with 200 μg/ml of Con A for 10 min in the presence of the reagent.

Table 2. Effect of chemical fixation of EAT cells on agglutination induced by concanavalin A

<table>
<thead>
<tr>
<th>Temperature</th>
<th>0 °C</th>
<th>37 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Glutaraldehyde-fixed cells</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

EATC (10⁶ cells/ml) were fixed with 0.25% glutaraldehyde for 30 min at room temperature, then washed 3 times with 10 ml of cold HBS. Other conditions as in Table 1.

DISCUSSION

In this report, evidence has been obtained which indicates that there is no correlation between the number of lectin-receptor sites available and agglutination behaviour. The agglutinability of beef erythrocytes increased remarkably even when the number of binding sites for Con A on the cell surface was reduced by papain treatment, which liberated Con A-binding substances from the surface (Inoue, Mori & Seno, 1972; Inoue, Mori, Utsumi & Seno, 1972). There was no further increase in the amount of Con A bound to the cell surface at 37 °C after the receptors on the cell surface were saturated by Con A. Therefore, pinocytosis of Con A molecules by the cells is unlikely to account for the difference between the amount of Con A bound to the cell surface at 0 and at 37 °C. Rather, it seems to show a difference between the number of exposed binding sites for Con A available at the 2 temperatures. It is also suggested that receptors for Con A may change their state of molecular conformation and/or of orientation on the cell surface. In the case of the action of inhibitors or uncouplers of the energy-transducing reaction, they showed no effect on agglutination. Therefore, energy-dependent processes were excluded as essential factors in this agglutination. Cell agglutination was also induced even when EATC was pretreated by glutaraldehyde. Furthermore, EATC or papain-treated beef erythrocytes adhered
to the surface of Con A-treated Sephadex particles (Inoue et al. 1972). Among various reagents used for treating the cells, only potassium periodate was effective in inhibiting the agglutination of cells mediated by Con A. The data suggest that the agglutination of cells induced by Con A depends on the topographical or micro-environment arrangement of glycoprotein receptors for Con A on the cell surface which are dynamically changing their molecular conformation or orientation on the cell surface according to the physicochemical environment of the cells.

The author would like to express deep thanks to Dr. G. M. W. Cook, Strangeways Research Laboratory, Cambridge, England, and Dr. K. Utsumi, Department of Biochemistry, Cancer Institute, Okayama University Medical School, Okayama, Japan, for many stimulating discussions and suggestions. The author also thanks Professor S. Seno, Department of Pathology, Okayama University Medical School, Okayama, Japan, for his kind guidance and many helpful discussions during this work.

REFERENCES


(Received 25 April 1973)