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Abstract

Human placenta alkaline phosphatase (HP-ALP), a glycoprotein, was stained histochemically for the purpose of examining the concanavalin A (Con A) binding sites on the cell surface. HP-ALP was bound to the cell surface by Con A. This simple method successfully detected Con A binding sites on the cell surface.

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— BRIEF NOTE —

A NEW HISTOCHEMICAL METHOD USING HUMAN PLACENTA ALKALINE PHOSPHATASE FOR DEMONSTRATING CONCANAVALIN A BINDING SITES ON CELL SURFACES

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Abstract: Human placenta alkaline phosphatase (HP-ALP), a glycoprotein, was stained histochemically for the purpose of examining the concanavalin A (Con A) binding sites on the cell surface. HP-ALP was bound to the cell surface by Con A. This simple method successfully detected Con A binding sites on the cell surface.

It is well known that concanavalin A (Con A) binds to glycoproteins on mammalian cell surfaces. Several methods have been reported for detecting the Con A binding sites (1-3). The present paper reports on a new method for microscopic demonstration of Con A binding sites on cell surfaces using histochemical staining of purified human placenta alkaline phosphatase (HP-ALP) which binds to Con A (4). Con A acts as a bridge between the cell surface binding sites and HP-ALP.

Cells used for the present study were Rous sarcoma virus-induced mouse ascites sarcoma (SR-C3H/He) cell established by Yamamoto and Takeuchi (5). HP-ALP was solubilized from mature human placenta with papain, precipitated with ammonium sulfate, and purified by Sephadex G-200 and Deae-cellulose column chromatography. The details will be published elsewhere (6). Purified HP-ALP was almost homogenous electrophoretically. The procedure was as follows: HP-ALP (46 μg of protein) and 10^7 cells were mixed in 1.0 ml of ST medium (0.15 M NaCl, 10 mM Tris-Cl pH 7.2), and 1.0 ml of Con A solution (200 μg/ml of ST medium) was added; this mixture was incubated at 36°C for 15 min. After the reaction, the product was centrifuged at 1000 rpm for 5 min; the precipitated cells were collected; and the cells were washed with ST medium at room temperature. The cells thus obtained were smeared on glass slides, and HP-ALP was histo-

chemically stained according to the method of Burstone (7). As a control for histochemical staining and enzymatic assay, a mixture of HP-ALP and cells without Con A was prepared; another control used for enzymatic assay was a mixture of HP-ALP and Con A solution without cells. The experimental procedures for the controls were the same as the experimental group. All procedures were performed at room temperature. HP-ALP activity of each supernatant of the first centrifugation was measured by the King and Kind method (8).

TABLE 1 ALKALINE PHOSPHATASE ACTIVITY IN THE SUPERNATANT

Mixture				
Cell	+	+		+
AL-P	+	+	+	
Con A	+		+	
AL-P activity	56	86	68	≒0 *

* Endogenous activity of cell alkaline phosphatase was measured.

Plus (+) indicates addition into mixture. Each mixture was centrifuged, and the alkaline phosphatase activity in the supernatant was measured by the method of King-Kind (8). The numbers represent activity in each supernatant expressed in King-Armstrong units/100ml.

Table 1 shows the alkaline phosphatase activity of the supernatants. The lowest activity was found in the mixture composed of HP-ALP, cells and Con A. This indicates that HP-ALP binds to cell surfaces using Con A molecules as a bridge. The activity was reduced in the presence of Con A even without cells.

Fig. 1 reveals the binding sites of Con A on the cell surface by histochemical staining of HP-ALP. The sites were granular. Fig. 2 shows cells of the control group, where Con A was not added. Granules were not observed on these cell surfaces; this result indicates that HP-ALP does not attach to cell surfaces without Con A and that endogenous alkaline phosphatase is not contained in this type of cell. Since HP-ALP is stable at room temperature and the procedures are relatively easy to perform, this method is convenient for demonstrating Con A binding sites. In the case of alkaline phosphatase-containing cells, however, the interpretation of the results is rather complicated, but this problem may be resolved by taking advantage of the heat stability of HP-ALP (9). The application of this method for electron microscopy is presently under investigation.

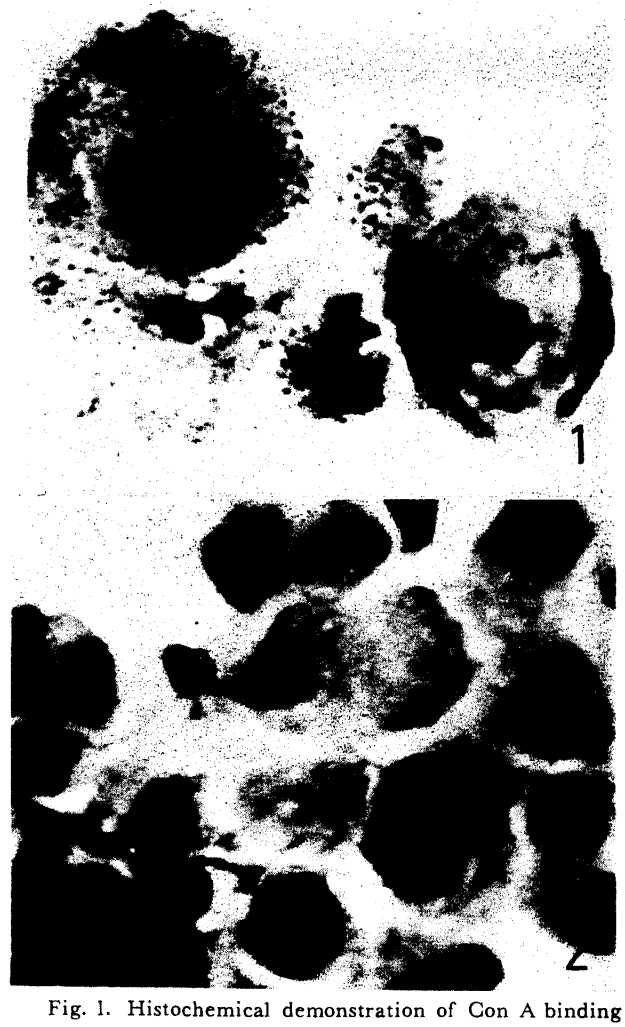

Fig. 1. Histochemical demonstration of Con A binding sites on the cell surface using human placenta alkaline phosphatase. Note that the binding sites are granular. ×1000

Fig. 2. Control (cells and alkaline phosphatase mixed without Con A) Histochemical staining of alkaline phosphatase was the same as the cells in Fig. 1. Note that granules are not present. ×1000

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