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Inhibition of Polyethylene Glycol-Induced Cell Fusion of HEp2 Cells by Lectins (40579)

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Membrane fusion has an important biological role in cellular endocytosis (1), exocytosis (2), sperm-egg fertilization (3), and in the development of muscle tissues (4). Membrane fusion is also responsible for the appearance of multinucleate cells in certain pathological conditions (5). Despite the participation of membrane fusion in various membrane-related biological activities and numerous efforts to study the fusion process, the mechanism of membrane fusion is far from being understood.

In recent years, polyethylene glycol (PEG) has been shown to be effective in inducing cell fusion in plant cells (6, 7), animal cells (8, 9), microbial protoplasts (10, 11), and heterokaryon formation between human and plant cells (12). The ability to induce fusion of membranes from a wide variety of cells, the low cytotoxicity to cells treated (13), and the ability to initiate membrane fusion in synchronous fashion makes PEG an excellent fusogen for use in cell hybridization studies and in studying the mechanisms of membrane fusion (14, 15). In the present communication we report that two lectins, concanavalin A (Con A) and wheat germ agglutinin (WGA), are inhibitors of PEG-induced cell fusion.

Materials and methods. HEp2 cells (Flow Laboratory, Inglewood, Calif.) were propagated in Eagle's minimal essential medium (MEM) supplemented with 5% fetal bovine serum and 50 μg/ml of gentamicin (Schering Corp. Kenilworth, N.J.). Monolayer cells were dispersed with diaminoethanetetraacetic acid treatment and inoculated at 10^5 cells/0.2 ml on individual coverglasses (1.8 cm diam) in petri dishes. These were incubated for 2 hr at 37° to allow attachment of cells after which 4 ml of MEM was added to each petri dish. The cells were subsequently incubated for an additional 12 h.

Induction of cell fusion by PEG. PEG (Carbowax, MW 1000, Fisher Scientific Co., Pittsburgh, Pa.) was prepared as a 40% v/v solution in phosphate-buffered saline (PBS, pH 7.2). The induction of cell fusion by PEG is a temperature-dependent process. All solutions used were equilibrated to room temperature. The HEp2 cells were washed three times with PBS and then covered with 4 ml of PEG solution for 5 min. The cells were then washed with PBS placed in MEM, and incubated for 2 hr at 37°. Control cultures and PEG-treated cultures were fixed in neutral-buffered formalin and stained with hematoxylin and eosin.

Method of quantification of cell fusion. The number of cells that underwent fusion were enumerated by microscopic examination. The calculation of the cell fusion index (FI) was performed as described (16). The formula used is as follows:

$$\text{Fusion Index} = \left[\left(\frac{\text{number of nuclei in polykaryons}}{\text{number of cells}}\right)_{\text{expt.}} - \left(\frac{\text{number of nuclei in polykaryons}}{\text{number of cells}}\right)_{\text{control}}\right] \times 100\%.$$

For practical laboratory applications, the FI was calculated based on 30 randomly chosen microscopic fields from each coverglass examined.

Lectins and lectin treatment. WGA and Con A (Sigma Chemical Co., St. Louis, Mo.) stock solutions were prepared at 200 μg/ml in PBS. Dilutions in PBS were made immediately before each experiment. In experiments, where simultaneous lectin and PEG treatment were required, properly diluted lectin solutions were mixed with 80% PEG to yield the desired final lectin and PEG (40%) solutions. Four milliliters of the mixture was then added to each culture dish. For the lectin pretreatment experiments, cells were incubated for 5 min in the presence of various

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dilutions of Con A. Four milliliters of 80% PEG was then added to yield a final concentration of 40% PEG. Cells were further incubated for 5 min at room temperature to initiate fusion. In the post-PEG-induction experiments, lectins were added following PEG treatment. The PEG-treated cells were incubated with various concentrations of Con A solutions for 5 min. The Con A solutions were then removed and the cells incubated in MEM for 2 hr at 37°.

Binding of α-methyl-D-mannoside (α-MM, Pfanstiehl Laboratory, Waukegan, Il.) to Con A was carried out for 30 min at room temperature. The Con A/α-MM mixtures were then added with PEG to initiate the cell fusion process. A molecular weight of 26,500/sugar binding site was used to calculate the concentration of Con A used in the study.

Results. Inhibition of cell fusion by Con A or WGA. HEp2 cells treated simultaneously with PEG and Con A or WGA exhibited a reduction in cell fusion (Fig. 1) which was linearly related to concentrations of lectins used. Con A was a more potent inhibitor than WGA on a weight basis. Con A, 20 μg/ml, prevented about 64% of the treated cells from undergoing cell fusion while 80 μg/ml of WGA was required to reach a comparable level of reduction in cell fusion. The concentration of Con A required to inhibit 50% of the cells from undergoing fusion was estimated at 18.5 μg/ml whereas that of the WGA was 67 μg/ml.

The effect of α-MM on inhibition of cell fusion by Con A. α-Methyl-D-mannoside is a specific binding sugar for Con A. It was used to determine if α-MM-treated Con A would exhibit a similar or reduced ability to inhibit fusion of HEp2 cells. At the concentrations used, α-MM alone neither enhanced nor reduced cell fusion induced by PEG. In contrast, α-MM employed at 10^{-3} *M* effectively neutralized the inhibition by Con A at concentrations below 10 μg/ml (Fig. 2). A reduction in inhibition of cell fusion by Con A was seen when Con A was employed at 20 μg/ml. A fusion index of 70% was observed for the 10^{-2} *M* α-MM-pretreated Con A solution as compared to a fusion index of 42% when α-MM was omitted. The results showed that the inhibitory activity of Con A is related to the receptor binding capacity of Con A employed. Reduction of the available Con A for cell membrane binding reduced the inhibitory effect of Con A solution on the cell fusion process.

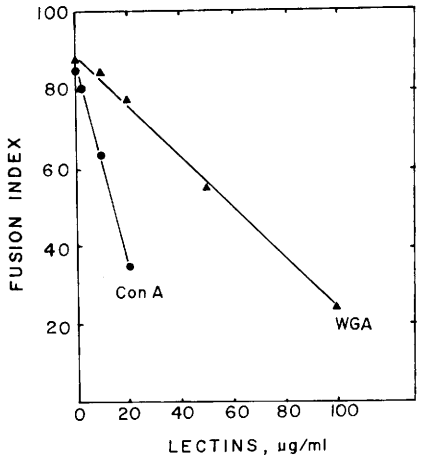

FIG. 1. The effect of simultaneous addition of lectins on PEG-induced cell fusion of HEp2 cells. HEp2 cells (10^5 cell/0.2 ml) grown on coverglasses (1.8 cm diam) were treated for 5 min at room temp with 4 ml of 40% PEG and various amounts of Con A or WGA. Fusion indices of the treated cells were determined from the stained cells fixed 2 hr following the treatment. ●——●, Con A-treated cultures; ▲——▲, WGA-treated cultures.

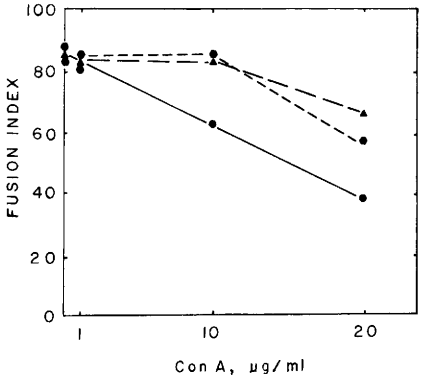

FIG. 2. Reversal of Con A inhibition of cell fusion by α-MM. Various concentrations of Con A were pretreated with either 10^{-2} or 10^{-3} *M* of α-MM at room temp for 30 min. The α-MM-treated Con A solutions were mixed with PEG and the mixed solutions were used to initiate cell fusion. Fusion indices were determined from the stained cells fixed 2 hr following the PEG treatment. ●——●, Untreated Con A; ▲——▲, 10^{-2} *M* α-MM-reacted Con A; ⬢——⬢, 10^{-3} *M* α-MM-reacted Con A.

Role of Con A-membrane interactions on

cell fusion. The interaction of Con A with membane surface receptors was examined by treating cells with Con A before, simultaneously, or after PEG treatment. In the Con A-pretreated cultures, while maintaining the same level of Con A during the PEG induction, there was an enhanced fusion inhibition observed (Fig. 3). Nearly 50% of cells were prevented from undergoing fusion at 10 μg/ml of Con A while at 20 μg/ml, nearly all of the cell fusion was inhibited. Addition of Con A to cells following PEG treatment was also inhibitory to cell fusion but the inhibition was less effective than if Con A and PEG were added simultaneously. The concentrations required to reduce the percentage of fusion by 50% for the pretreatment, simultaneous treatment, and posttreatment of Con A was calculated at 10.5 μg/ml, 16.5 μg/ml, and 21.5 μg/ml, respectively.

Discussion. Cell membrane fusion is a rapid and dynamic process which has been suggested to consist of several steps (17). The process of cell fusion which is inhibited by Con A or WGA appears to involve the induction and/or the fusion stage.

Aggregation of cells by Con A or WGA is a commonly observed phenomenon. Intercellular crosslinking of cell surface receptors by these lectins could lead to a regional fixation of the intercellular distance that prevents the close contact necessary to initiate fusion between two cell membranes. It is conceivable that cells crosslinked by Con A molecules would have a diminished ability to undergo fusion.

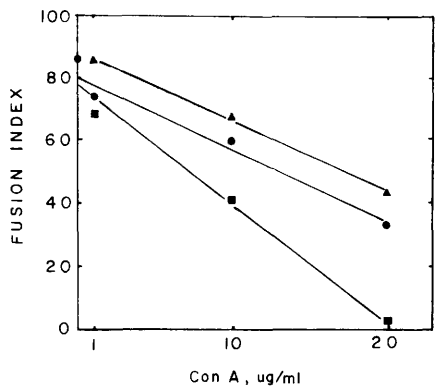

FIG. 3. The effect of addition of Con A prior to, simultaneous, and post-PEG-treatment on induced cell fusion process. HEp2 cells were treated with Con A solutions for 5 min prior to the PEG treatment simultaneously treated with Con A and PEG, or treated with Con A solution for 5 min following a 5-min PEG treatment. Fusion indices were determined from cells fixed and stained 2 hr after the PEG treatment. ■——■, Cells pretreated with Con A; ●——●, cells simultaneously treated with Con A and PEG; ▲——▲, cells treated with Con A following a 5-min treatment of PEG.

Another explanation for the lectin-mediated inhibition of cell fusion might be the steric hindrance imposed by these lectins on the approximation of cell membranes. Crystallographic studies indicate that each Con A subunit is globular in shape with molecular dimensions of 42 × 40 × 30 Å (18). The presence of lectin molecules at the cell surface could shield the lectin-coated cells from making close contact with their neighbors. A separation distance of 10 Å has been used by Poste and Allison (17) in their discussion of the short-range membrane interactions in fusion. Therefore, contact between cell membranes would be prevented by molecules that are much too large for such short-range interactions to initiate.

Intramembrane aggregation of proteins following lectin treatment might also be a contributory factor by which Con A or WGA interferes with the cell fusion processes. Aggregation of membrane proteins of cells by Con A and other lectins has been reported. (19). Con A has shown to be inhibitory to virus-induced cell fusion (20) and it has been suggested that it does so by inhibiting the mobility of membrane glycoproteins (21). The binding of Con A or WGA to respective membrane receptors and the subsequent aggregation of receptors could interfere with the PEG-induced membrane perturbation. Membrane perturbation is a necessary step in the cell fusion reaction (22, 23). The aggregation of cell membrane proteins has been reported to occur in PEG-treated L cells (24). Membrane perturbation has also been detected in HEp2 cells following PEG treatment (Fan, unpublished data). Ahkong *et al.* (22) proposed that fusion takes place between two adjacent perturbed membrane regions devoid of membrane proteins. The presence of lectin-aggregated membrane particles could then interfere with the PEG-induced process of membrane alterations and resulting in inhibition of membrane fusion.

The possibility of a direct interaction between lectin and PEG is not likely since it has been demonstrated that sugar-treated Con A exhibits a decreased ability to prevent cell fusion. The amount of Con A used in the experiment is also too small (7.5×10^{-7} *M*) as compared to PEG (4.5×10^{-1} *M*) employed.

The results of this study do not discriminate among any of the three possible modes of the inhibition of cell fusion. We feel that a combination of these suggested possibilities exists to modify the result of fusion. It is suggested that Con A and WGA inhibit cell fusion by interfering with the induction of cell fusion or preventing approximation of the perturbed membranes. Such modes of action by these lectins would prevent the fusion process from progressing into the actual physical fusion of membranes. Because of the ability of Con A and WGA to inhibit cell fusion, these lectins may be useful tools to be applied in membrane fusion studies.

Summary. Concanavalin A (Con A) and wheat germ agglutinin were tested for their ability to inhibit the polyethylene glycol-induced fusion of HEp2 cells. The inhibitory effect of these lectins was related to the concentration of lectins employed. Further studies with Con A revealed that the inhibition was also related to the available receptor binding capacity and the timing of the addition of the lectin. The presence of lectins in the early phase of the cell fusion process prevented the polyethylene glycol-treated cells from undergoing a fusion process.

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