STUDIES ON LECTIN-INDUCED AGGLUTINATION OF *DROSOPHILA* EMBRYONIC CELL LINES

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Abstract—The agglutination responses of three *Drosophila* cell lines to concanavalin A and wheat germ agglutinin have been examined. Although the cell lines were originally derived from late embryonic stages of the *Ore-R* strain of *Drosophila melanogaster*, they show quantitative differences in lectin-induced agglutination. Line 1 cells were least agglutinable with both lectins. All three cell lines reached maximum agglutination with concanavalin A concentrations at 25 $\mu g/ml$, but the agglutination response to wheat germ agglutinin was biphasic such that an initial rapid increase in agglutination with concentrations up to 25 $\mu g/ml$ was followed by slower agglutination above this concentration. Cells of lines 1 and 2 from ten-day old cultures exhibited greater lectin-induced agglutination than cells from three-day old cultures. Age-dependent differences were not found for line 3 cells which gave maximum agglutination responses in both young and old cultures. Cell agglutination by concanavalin A was almost completely inhibited by pretreatment of the lectin with methyl-α-D-mannopyranoside, but preincubation of wheat germ agglutinin with *N*-acetyl-D-glucosamine caused only partial blockage. Lectin-induced agglutination was not reversible by treatment with the monosaccharide inhibitors. These observations have been discussed with reference to the origin of the three cell lines and their cell surface properties.

INTRODUCTION

PLANT lectins have been used extensively to study surface properties of cultured cells since the binding of lectin molecules to specific receptor sites on the cell surface initiates an agglutination reaction. Among the lectins that have been utilized in agglutination tests, wheat germ agglutinin (WGA) shows a specificity for *N*-acetyl-D-glucosamine and concanavalin A (Con A) selectively binds with α-D-glucose or α-D-mannose derivatives. Generally, lower concentrations of lectins agglutinate virus-transformed and malignant cells than the concentrations required to agglutinate normal cells (AUB *et al.*, 1963; BURGER and GOLDBERG, 1967; INBAR and SACHS, 1969), and it is these differential responses of cells to lectin treatment that have prompted several hypotheses concerning cell surface architecture. BURGER'S (1969) model proposes that agglutinin receptor sites are exposed in transformed cells whereas the receptor sites remain covered in normal cells. An alternate hypothesis suggests that differences in topographic distribution of lectin-binding sites on the cell surface may be related to agglutinability (NICOLSON, 1974).

The major focus of attention in agglutination studies with WGA and Con A has been vertebrate cells. Recently, the response of *Drosophila* cells to lectins was examined, and both WGA and Con A induced fusion of the cells as well as agglutination (BECKER, 1972; RIZKI *et al.*, 1975; HALFER and PETRELLA, 1976). These observations indicate that there may be cell surface differences between vertebrate and insect cells, since lectin-induced cell fusion has not been reported for vertebrate cells. The present study was therefore undertaken to examine lectin-induced agglutination of *Drosophila* cells under conditions similar to those employed for vertebrate cells.

MATERIALS AND METHODS

Drosophila melanogaster cell lines 1, 2, and 3 were obtained from Dr. Imogene Schneider in September, 1972, and have been maintained by weekly subculture in Schneider's medium (SCHNEIDER, 1964, 1966) supplemented with 15% heat-inactivated fetal calf serum (Rehatuin FS, Reheis Chemical Co.). Serum was tested and found to be free of bacterial virus contaminants according to the method of MERRIL *et al.* (1972) and Dr. T. B. FRIEDMAN (personal communication). The patterns of growth and morphology of the lines are similar to those originally described by SCHNEIDER (1972), although changes in karyotype have occurred (ANDREWS, 1975). Cells were grown in glass T-15 flasks containing 1.5 ml culture medium at 25°C with an initial inoculum of 1.0×10^6 cells per flask. Doubling times of lines 1, 2, and 3 were 19 to 20 hr, 16 hr, and 19 to 20 hr respectively.

The cells of lines 1 and 3 remain attached to the bottom of the flasks, whereas line 2 cells grow in suspension after the flask surface is covered with a monolayer of cells. Prior to harvesting cells of lines 1 and 3, they were rinsed three times with buffered saline solution (SEECOF, 1971) while they remained attached to the bottom of the flasks. The cells were then freed by gently flushing with a Pasteur pipette and suspended in saline solution at a concentration of 2.0×10^6 cells/ml. Line 2 cells were concentrated by centrifugation. The pellet of cells was washed twice with saline solution and the cells were then resuspended in saline solution. This procedure was repeated and the cells were finally dispersed in saline solution at a concentration of 2×10^6 cells/ml. An aliquot of 0.1 ml of cell suspension was used for each agglutination test.

Agglutination tests were performed at 25°C in one-

dram shell vials with 0.1 ml saline solution containing the appropriate concentration of WGA (Sigma Chemical Company) or Con A (Calbiochem). To each vial, 0.1 ml of cell suspension was added. Controls vials contained 0.1 ml of the cell suspension and 0.1 ml saline solution. The vials were placed on a rotary shaker (based on the design of HENKART and HUMPHREYS, 1970) with a radius of gyration of 5 cm, and rotated at 70 rev/min. At the end of the treatment period, the number of residual single cells in each vial was counted with a hemacytometer. The per cent cell agglutination was calculated as: (1—single cells in lectin-treated vial/single cells in control vial) × 100. Agglutination was confirmed by observation with a phase contrast microscope. All values for agglutination reported in tabular form represent the averages of triplicate culture flasks from representative experiments.

The inhibitory effects of methyl-α-D-mannopyranoside (MMP) and N-acetyl-D-glucosamine (NAGlu) on lectin-induced agglutination were studied by preincubating the inhibitors and lectins in saline solution for at least five minutes before the addition of cells. The mixtures were then rotated, and the cells counted as described above to determine the per cent agglutination.

RESULTS

Figure 1 summarizes the agglutination responses of the three cell lines as a function of lectin concentration. Agglutination of the cells of all three lines was maximal at a Con A concentration of approximately 25 μg/ml. However, differences among the three lines were apparent in the initial rise of the curves as well as the levels of agglutination achieved by 25 μg/ml Con A. Approximately 95% of the line 3 cells were agglutinated by the Con A treatment whereas only 55% of the line 1 cells were clumped by this same experimental procedure.

The response to WGA also revealed differences among the cell lines and, as in the case with Con A, fewer line 1 cells were agglutinated (Fig. 1). There was, however, a biphasic agglutination response to WGA with the amount of agglutination increasing rapidly up to a concentration of approximately 25 μg/ml and less rapidly above this concentration.
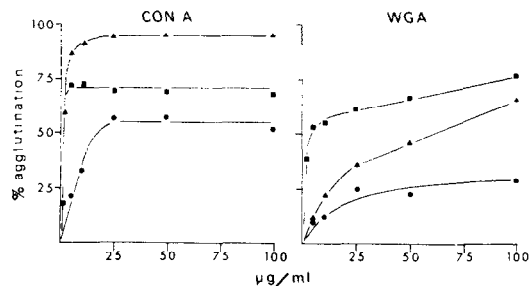

Fig. 1. Agglutination as a function of lectin concentration. Each point represents a single determination taken after 5 min reaction of cells and lectins. Cells were obtained from parallel 10-day cultures of line 1 (●), line 2 (■), and line 3 (▲).

To determine whether culture age affects agglutination responses, cells were harvested for agglutination on days three and ten after subculture, and subjected to the agglutination reaction for five minutes with 100 μg/ml Con A, a concentration assuring maximum agglutination. The cells of lines 1 and 2 from ten-day cultures exhibited greater agglutination than did cells from three-day cultures while line 3 cells showed maximum agglutination at both ages (Table 1). The effect of culture age on cell agglutination induced by WGA was also examined in a similar series of experiments using two concentrations of WGA. Again, the percentage of cells agglutinated in ten-day cultures of lines 1 and 2 was higher than that in three-day cultures (Table 1) whereas line 3 cells showed no change in agglutination with culture age. These data on WGA agglutination also confirm the observations depicted in Fig. 1 by showing that increased agglutination of the cells was obtained when the WGA concentration was raised to 500 μg/ml. The increase in agglutination observed with a WGA concentration of 500 μg/ml as compared to 100 μg/ml was significant at the 0.05 level in all instances except for line 1 cells from three-day cultures which showed about five per cent agglutination at both concentrations.

Preincubation of Con A with 0.1 M MMP, a monosaccharide which Con A reportedly binds (GOLDSTEIN et al., 1965), almost completely inhibited the agglutination of the cells of the three lines by Con A (Table 2). The agglutination of all three cell

Table 1. Effect of culture age and lectin concentration on agglutinability

	Culture age (days)	Con A 100 μg/ml	WGA 100 μg/ml	WGA 500 μg/ml
Line 1	3	35.6 ± 2.8	5.7 ± 6.7	6.2 ± 6.3
	10	79.4 ± 4.9	57.5 ± 6.8	73.8 ± 5.2
	$\overline{X}_{10} - \overline{X}_3$	43.8*	51.8*	67.6*
Line 2	3	60.9 ± 3.3	67.2 ± 5.0	85.6 ± 3.2
	10	89.5 ± 1.6	85.9 ± 2.9	92.7 ± 0.1
	$\overline{X}_{10} - \overline{X}_3$	28.6*	18.7*	7.1*
Line 3	3	94.7 ± 1.1	60.9 ± 2.7	92.9 ± 2.6
	10	94.4 ± 0.7	68.0 ± 6.3	89.0 ± 2.2
	$\overline{X}_{10} - \overline{X}_3$	−0.3	7.1	−3.9

Cell agglutination was scored after 5 min of reaction with lectins. The mean ± S.D. values are based on three samples, each taken from an independent culture. * indicates the differences between means $\overline{X}_{10} - \overline{X}_3$ are significant at $\infty 0.05$. The increased agglutination with 500 μg WGA is statistically significant in all cases except for line 1, 3-day old cultures.

Table 2. Blockage of lectin activity by monosaccharides: methyl-α-D-mannopyranoside (MMP) and N-acetyl-D-glucosamine (NAGlu)

	Con A		WGA	
	100 μg/ml	100 μg/ml +0.1 M MMP	100 μg/ml	100 μg/ml +0.1 M NAGlu
Line 1	81.2 ± 3.5	1.3 ± 6.9	60.9 ± 3.1	44.8 ± 6.9
Line 2	85.9 ± 1.0	3.3 ± 3.4	88.0 ± 3.1	77.9 ± 5.4
Line 3	94.6 ± 1.1	1.5 ± 1.4	72.1 ± 7.2	27.9 ± 6.9

Lectins were incubated with MMP or NAGlu for 5 min. Cells were then added and agglutination was scored after 5 min' reaction. The mean ± S.D. in each case is based on three samples from independent cultures. All differences between agglutination values of control and monosaccharide-treated lectins are statistically significant at α0.05.

lines was also inhibited by preincubation of WGA with 0.1 M of the monosaccharide it binds, NAGlu (BURGER and GOLDBERG, 1967), but to a lesser extent than that obtained in the Con A experiments. When the cells were first agglutinated by Con A or WGA, the agglutination was not reversed by the addition of 0.1 M MMP or NAGlu respectively, even after several hours of incubation.

DISCUSSION

The experiments involving the agglutination response of Schneider's *Drosophila* cell lines as a function of Con A concentration show that the rank order of the three lines is line 3 > line 2 > line 1; for the WGA response, the rank order is line 2 > line 3 > line 1. The fact that the two rank orders differ suggests that there are quantitative differences in the binding sites for the two lectins among the three cell lines. An additional discrimination between the cell lines can be generated if the agglutination response is examined as a function of subculture age. The lectin-mediated agglutination of the cells from ten-day cultures of lines 1 and 2 was higher than that from three-day cultures whereas line 3 cells showed maximum agglutination in both young and old cultures (Table 1). The differences between the agglutination values of young and old cultures of line 1, however, were higher than the differences for line 2.

The increased lectin-induced agglutination observed in older *Drosophila* cell cultures implies a change in cell surface properties as the cultures age. Agglutinability of other cell types by lectin treatment is also influenced by culture age (BEN-BASSAT et al., 1971; NOONAN and BURGER, 1973) and, in this respect, the behavior of these insect cells is comparable to that of transformed vertebrate cells, suggesting generalized age-dependent effects on cell surface. That the surface features of *Drosophila* cells change with increasing culture age has been verified by examination of Schneider's cell lines with the scanning electron microscope (RIZKI et al., in preparation). These changes are especially pronounced for line 1 cultures in which the cells form colonies on the substratum. In small colonies of young cultures the cells have a smooth surface texture whereas many of the cells in large colonies of older cultures possess numerous microvilli. An increase in surface area concomitant with development of microvilli and other surface projections suggests exposure of additional lectin-binding sites for cell–cell interactions, so the age-associated surface differences correlate well with the observed age-dependent agglutination responses for this line. Alternately, the same number of binding sites in sur-

faces covered by microvilli might provide better mechanical strength to cell–cell interactions due to interdigitation of the microvillar surfaces with bivalent Con A molecules. In this case, microvillar morphology would have an additive effect on cell clumping. The correlation between microvillar surfaces and the cell agglutination response is also consistent with the observations on line 3 cells. Line 3 cells are covered with numerous microvilli in both young and old cultures, and this line shows high agglutination responses to Con A and WGA at both ages. On the other hand, line 2 cells are relatively smooth-textured and pronounced differences in surface topography between two- and seven-day cultures were not observed. The three-day cell cultures of this line showed a higher agglutination response to Con A than the three-day line 1 cells which are also smooth-surfaced, suggesting that initially line 2 cells may have more binding sites than line 1 cells. The contribution of microvilli to agglutination can be assessed by comparing the relative increases in agglutination within the two lines. Agglutination of line 1 cells by Con A shows a 45% difference between day 3 and day 10 while line 2 shows a difference of 25% within the same interval. Compared in this way, we might assign the additional increase (45%–25% = 20%) in agglutination of line 1 to the development of microvilli and surface projections.

Another possible explanation for age-dependent effects on cell agglutination is suggested by the fact that dividing cells in these lines are insensitive to membrane perturbations normally induced by Con A and WGA (RIZKI et al., 1975; RIZKI et al., in preparation). One might expect, therefore, that a sample of cells from a culture in the log phase of growth would exhibit less agglutination than a population derived from older cultures. The final level of cell clumping attained for a given lectin treatment will be influenced by a number of factors that relate to the distribution of lectin-binding sites on the cell surface. NICOLSON (1974) has evaluated the rôles of cell surface and membrane structure in lectin-induced agglutination, but it should be stressed that cell fusion was not observed in the studies on which his discussions were based. Consideration of the factors that affect clumping of *Drosophila* cells must account for the aggregation of at least some of the cells by this process (BECKER, 1972; RIZKI et al., 1975; HALFER and PETRELLA, 1976).

The biphasic nature of the agglutination response of the cells of Schneider's *Drosophila* lines to WGA suggests a heterogeneity of cell surface binding sites for this lectin whereas Con A binding sites appear to be uniform and saturable at concentrations above

25 μg/ml. WGA specifically binds NAGlu but has been shown to bind with greater affinity to its di- and trisaccharides (ALLEN et al., 1973; NAGATA and BURGER, 1974; GOLDSTEIN et al., 1975). In addition, GREENAWAY and LEVINE (1973) reported nonspecific binding of WGA to sialic acid, so a number of factors may influence cell agglutination by WGA. The observation that cell agglutination by WGA was decreased in the presence of NAGlu but not completely inhibited also supports the contention that binding of WGA to the Drosophila cell surface may not be limited to NAGlu sites. On the other hand, Con A-induced agglutination of Drosophila cells was blocked by pretreatment of this lectin with its specific inhibitor, MMP. These results are consistent with those of RIZKI et al. (in preparation) who observed that Con A-induced fusion of the cells of Schneider's Drosophila lines was prevented by MMP whereas WGA-induced fusion was not inhibited by NAGlu (RIZKI et al., 1975). It appears that WGA has a greater affinity for receptor sites on Drosophila cell surfaces than it does for NAGlu. The Con A- and WGA-induced cell fusion reported by RIZKI et al. (1975) is also supported in the present study by the inability of monosaccharide hapten inhibitors to reverse lectin-induced agglutination.

SCHNEIDER'S (1972) cell lines were derived from 20 to 24 hr old embryos, so the tissue of their origin is unknown. During the primary culture phase of line 3, cell masses were transplanted into host larvae that subsequently metamorphosed and the implants that had differentiated as adult structures were recovered. Therefore, line 3 probably originated from imaginal disc cells. A similar test was not performed with lines 1 and 2, but spheres from other primary cultures that were tested in this manner underwent differentiation to give adult tissues, so Schneider assumed that the other two lines were also derived from imaginal discs. This potential, however was no longer demonstrable after subculture (SCHNEIDER, 1972; DÜBENDORFER and SHIELDS, 1972). More recently, ILLMENSEE (1976) tested the potentiality of the nuclei from the three cell lines to promote differentiation. He found that line 2 nuclei still retain the potential to differentiate larval tissues whereas line 1 nuclei can contribute to the differentiation of both larval and adult tissues. No tissues were derived from line 3 donor nuclei. It seems that the three cell lines had different embryonic origins or the lines have digressed during culture in vitro. SCHNEIDER (1972) noted differences in the morphological and growth characteristics of the three cell lines. The present study indicates additional distinctive features of the three lines; these differences revealed by the use of lectins must relate to the cell surface architecture and the prevalent determined state of the cells.

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