CELL STRUCTURE AND FUNCTION 7, 145–153 (1982)

Lectin Binding Studies on the Slime Mold, *Physarum polycephalum*

Haruko Kitagaki[1], Yumiko Hatakeyama[1], Isamu Matsumoto[1], Nobuko Seno[1], Machiko Taniguchi[2] and Jiro Ohta[2]

Departments of Chemistry[1] and Biology[2], Faculty of Science, Ochanomizu University, Ohtsuka Bunkyo-ku, Tokyo 112, Japan

ABSTRACT. Cell surface glycoconjugate receptors for wheat germ agglutinin, *Ricinus communis* agglutinin, concanavalin A and soybean agglutinin on two strains of myxoamoebae of *Physarum polycephalum* with fluorescein isothiocynate-labelled lectins were observed microscopically, but no receptors for *Dolichos biflorus* agglutinin, *Ulex europeus* agglutinin-I and *Arachis hypogaea* agglutinin were found. These results suggest that the myxoamoebae have cell surface glycoproteins with *N*-glycosidically linked hetero-saccharide chains, but not oligosaccharides with blood group A and H determinants. The number of lectin receptor sites and their affinity constants for lectins were obtained for the myxoamoebae and microplasmodia of *P. polycephalum* from binding assays with ^{125}I-lectins. The densities of these lectin receptors and the magnitudes of the affinity constants were similar to those of mammalian cells. Neuraminidase treatment of the myxoamoebae and microplasmodia induced neither a decrease in WGA receptors nor an increase in RCA receptors. These results are strong evidence that sialyl residues are absent in glycoconjugates on the surface of the myxoamoebae and microplasmodia of *P. polycephalum*.

The slime molds are generally classified into two groups, cellular and acellular slime molds. The lectins on the cell surface of cellular slime molds, which may function in the aggregation of cells to form the pseudoplasmodium (25, 28), have been purified and characterized (26, 27, 28, 30). These lectin receptors have been reported to be glycoconjugates located on the cell membrane (23). But little is known about the surface carbohydrates of the myxoamoebae and microplasmodium cells of acellular slime molds, although a galactan phosphosulfate has been found in their nuclei (6, 8) and secreted fluid (5, 15).

We here report on the surface glycoconjugates of two conjugatable, different mating-types of myxoamoebae (strains J and F in the haploid vegetative phase of the life cycle) and those of the microplasmodium (multinucleated diploid vegetative phase) studied in binding experiments with various exogenous plant lectins.

Abbreviations used: WGA, wheat germ agglutinin; RCA, *Ricinus communis* agglutinin; Con A, concanavalin A; SBA, soybean agglutinin; DBA, *Dolichos biflorus* agglutinin; UEA-I, *Ulex europeus* agglutinin-I; AHA, *Arachis hypogaea* agglutinin; SJA, *Sophora japonica* agglutinin; FITC, fluorescein isothiocyanate; PBS, phosphate buffered saline; BSA, bovine serum albumin.

MATERIALS AND METHODS

Organisms. Strains J and F of the myxoamoebae from *P. polycephalum* were grown with *Aerobacter aerogenes* on agar medium in the dark at 24°C as reported previously (33). The microplasmodia were grown in an axenic semi-defined culture medium in shaken flasks as previously reported (21). The microplasmodium is multinucleated, but is surrounded by a single cell membrane. Therefore, it can be considered a single cell so far as its surface is concerned, and will be called a cell hereafter.

The cells (myxoamoebae and microplasmodia) were collected by low speed centrifugation and washed 3 times with 0.01 M phosphate buffer (pH 6.0) containing 0.05 M NaCl to remove bacteria and slime.

Lectins and other chemicals. The neuraminidase of *Arthrobacter ureafaciens* was obtained from Nakarai Chemicals Ltd., Kyoto, Japan, and $Na^{125}I$ (370 mCi/ml) from New England Nuclear, Boston, Mass.

Crude lectin preparations of *Ricinus communis* agglutinin (castor bean agglutinin, specific to β-D-galactosyl residue; RCA) and soybean agglutinin (specific to α(β)-*N*-acetyl-D-galactosaminyl; SBA) and concanavalin A (specific to α-D-mannosyl; Con A) were purified by affinity chromatography as described previously (13). Crude *Sophora japonica* agglutinin (specific to β-*N*-acetyl-D-galactosaminyl; SJA) was purified according to the method of Allen *et al.* (2). Wheat germ agglutinin (specific to α-sialyl or β-*N*-acetyl-D-glucosaminyl; WGA) and fluorescein isothiocyanate (FITC)-labelled RCA, SBA, Con A, WGA, *Arachis hypogaea* agglutinin (peanut agglutinin, specific to β-D-galactosyl-(1→3)-*N*-acetyl-D-galactosaminyl; AHA), *Dolichos biflorus* agglutinin (horse gram agglutinin, specific to α-*N*-acetyl-D-galactosaminyl; DBA), and *Ulex europeus* agglutinin I (gorse seed agglutinin, specific to α-L-fucosyl; UEA-I) were purchased from Maruzen Sekiyu Ltd., Tokyo, Japan. Iodination of the purified lectins was performed by the chloramine-T method with $Na^{125}I$ (14). The specific activities of the iodinated lectins were 10^{13} to 10^{15} cpm/mole.

Enzyme treatment of cells. Cells were suspended at a final concentration of $1–2 \times 10^8$ cells/ml of 0.02 M phosphate buffered saline (PBS, pH 6.0) for the myxoamoebae and 1.5×10^5 cells/ml for the microplasmodia. Each cell suspension was incubated with one unit of neuraminidase for 60 min at 25°C under gentle shaking (4). These treated cells were washed 3 times by centrifugation with PBS containing 0.25% bovine serum albumin (PBS-BSA) then resuspended in the same buffer for the binding assay. As the control, cells were incubated without the enzyme and washed by the same procedure.

Binding studies with FITC-lectins. Cells were washed with PBS (pH 7.2) and suspended at a final concentration of 10^5 cells/ml in the same buffer (9). After incubation of 100 μl of the cell suspension with 10 μg of each FITC-lectin (WGA, RCA, Con A, SBA, DBA, UEA-I or AHA) at room temperature for 15 min, the cells were washed by centrifugation 3 times with PBS. The washed cells were kept at 0°C and examined within 1 h under a Nikon EF fluorescent microscope equipped with a blue excitation filter system (for FITC) and illuminated by a 50 W halogen lamp. Photomicrographs were taken with Kodak Ektachrome 400 (ED-Daylight) and were processed to ASA 400.

Binding studies with ^{125}I-lectins. Binding studies with ^{125}I-lectins essentially were made according to the method described previously (12). The suspension (100 μl) containing $10^6–10^7$ myxoamoebae or 10^4 microplasmodia cells was incubated for 60 min at 0°C with 100 μl of a solution of lectins at various concentrations (30–800 μg/ml). After the cells had been washed 3 times with PBS-BSA by centrifugation, the amount of bound ^{125}I-lectin was determined with an Aloka autowell gamma system ARC-500. Corrections were made for the nonspecific binding of lectin and inactivated lectin. Data for the binding studies were plotted

by the method of Steck-Wallach (31).

Hemagglutination inhibition test with the slime polysaccharide fraction. Extracellular polysaccharide was precipitated with ethanol from the slime of the microplasmodia then fractionated with cetyl pyridinium chloride. The cetyl pyridinium complex was extracted sequentially with 0.2, 0.4 and 0.7 M NaCl. The major part of the slime polysaccharide was recovered in the 0.4 M NaCl fraction (36%). This fraction was purified further by ethanol precipitation, after which it was used for the inhibition test.

The slime polysaccharide solution (27.9 mg/ml) was diluted serially with PBS, then 50 μl of lectin solutions were added to 50 μl of each of these solutions. The mixtures were allowed to stand for 1 h at room temperature, after which 50 μl of trypsin-treated human erythrocyte suspension was added to each one. After incubation for 30 min at room temperature, the agglutinability of the erythrocytes was observed macroscopically.

Mixed agglutination test with human erythrocytes. Cells of strain J or F of the myxoamoeba, or the microplasmodium, were suspended in PBS (10^5 cells/ml). A portion of this suspension was mixed with an equal volume of 5% human erythrocyte suspension and incubated for 30 min at 0°C, then it was observed microscopically.

RESULTS

Binding of FITC-lectins. The lectin-treated cells were often agglutinated, but no morphological changes were observed in them under microscopy.

Results of the binding of FITC-lectins to the myxoamoebae are shown in Fig. 1a–e. Cell-bound fluorescence was seen on both the J and F strains treated with FITC-WGA -RCA, -Con A or -SBA, but none was seen on cells treated with FITC-AHA, -DBA or -UEA-I.

Of the lectins used, FITC-WGA gave the strongest fluorescence followed by FITC-RCA and -Con A. FITC-RCA showed only localized fluorescence along the cell fringe. No differences were found between strains J and F in their binding abilities for all the lectins used.

Because the microplasmodium has a strong fluorescence (Fig. 1f), the binding of FITC-lectins could not be distinguished from its self-fluorescence.

Binding of ^{125}I-lectins. Results of the binding of ^{125}I-lectins to strains J and F of the myxoamoebae and to the microplasmodium were plotted by the method of Steck and Wallach (Fig. 2). In this experiment, four lectins (WGA, RCA, Con A and SBA) which bound with the myxoamoebae in the experiment with FITC-lectins were used. The number of lectin binding sites and the association constants are given in Table 1.

The order for the number of binding sites on the myxoamoebae was WGA>RCA>Con A>SBA (Table 1), which is consistent with the results obtained with FITC-lectins. The number of binding sites on strain F was somewhat larger than the number on strain J, but no other differences were found between these strains.

The order for the number of receptor sites on the microplasmodium, however, differed from that of the myxoamoebae, WGA=RCA>SBA>Con A. The number of receptor sites on the microplasmodium was about three to four orders of magnitude higher than the number on the myxoamoebae. But, given the size of the microplasmodia (100–200 μm in diameter) and the myxoamoebae (10 μm in diameter), the calculated densities of the lectin binding sites on the myxoamoebae and microplasmodia were almost the same, 10^{16-17} sites/cm^2 on the cell surface.

Effect of neuraminidase treatment on lectin receptors. To obtain further details of

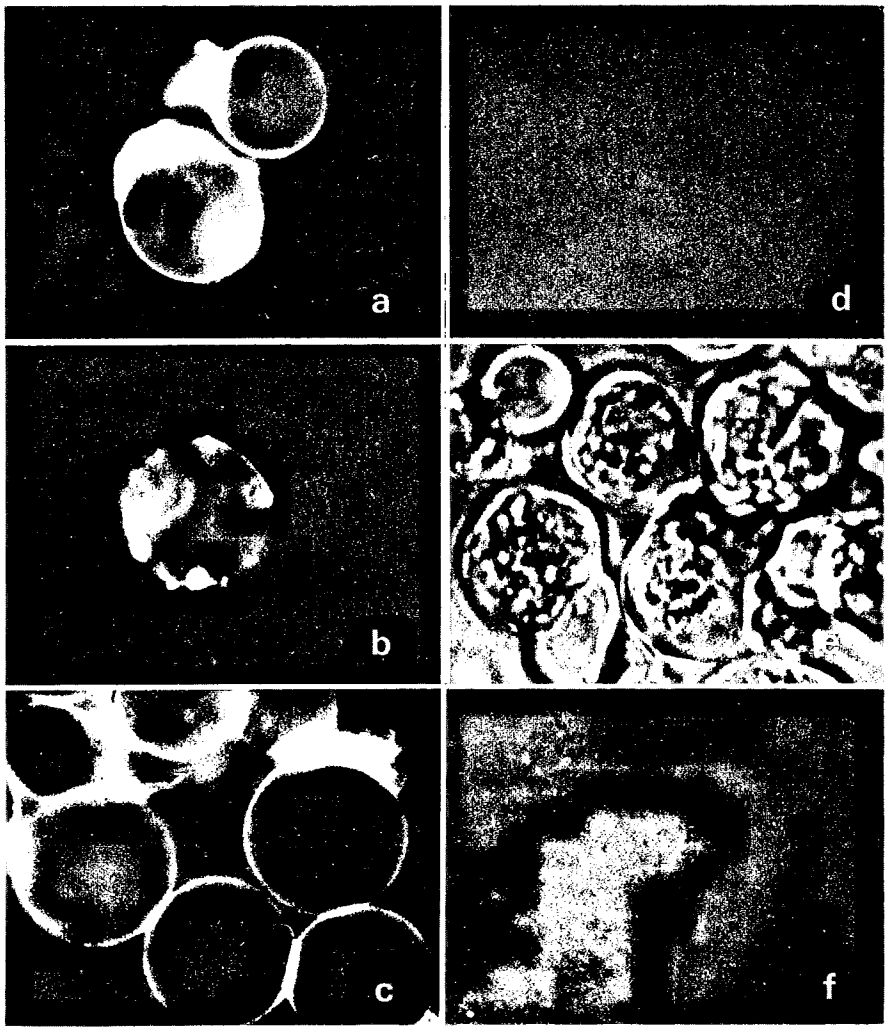

Fig. 1. Photomicrographs of myxoamoebae cells (a–e) and the microplasmodium (f) of *P. polycephalum*. Strain F is labelled with FITC-WGA, ×1000, 60 sec exposure (a); strain F is labelled with FITC-RCA (b); strain J is labelled with FITC-Con A (c); strain F is labelled with FITC-AHA (d); the same as (d), but photographed in a light field, ×1000, 5 sec exposure (e); and unlabelled microplasmodium, ×100, 60 sec exposure (f).

the nature of the lectin receptors, WGA and RCA were bound to cells treated with neuraminidase. This enzyme treatment, however, had practically no effect on the FITC-lectin binding to both strains of myxoamoebae.

Neuraminidase treatment did not change the number of WGA receptor sites on the myxoamoebae nor on the microplasmodium, but it did tend to increase the number of RCA sites slightly. These increases in the RCA receptor sites were not significant, however, nor were the affinity constants changed (Table 1).

Hemagglutination inhibition test with the slime polysaccharide fraction. The slime

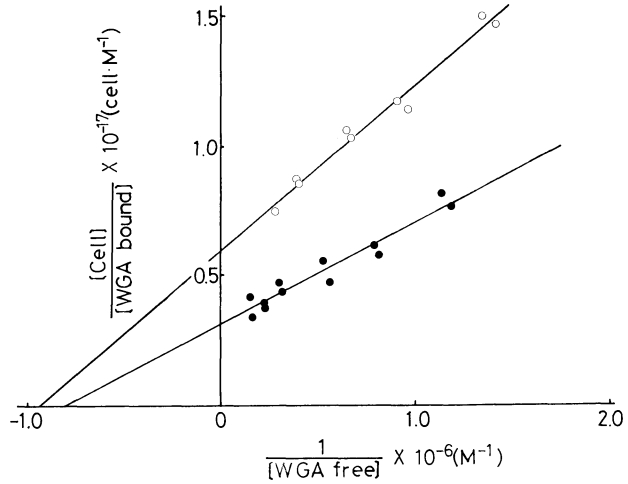

Fig. 2. Binding of ^{125}I-labelled WGA to strains J (○) and F (●) myxoamoebae cells. Data for the binding studies were plotted by the method of Steck and Wallach (31) with the equation:

$$\frac{[\text{Cell}]}{[\text{WGA bound}]} = \frac{1}{\text{Ka}\cdot\text{n}} \cdot \frac{1}{[\text{WGA free}]} + \frac{1}{\text{n}}$$

in which [WGA free]=the concentration of free WGA (M); [WGA bound]=the concentration of bound WGA (M); [Cell]=the number of cells per liter; n=the number of lectin receptor sites per cell; and Ka=the affinity constant (M^{-1}).

polysaccharide fraction slightly inhibited the agglutination of RCA, but not that of SBA or SJA at the concentrations examined (Table 2).

Mixed agglutination test with human erythrocytes. Myxoamoebae and microplasmodia cells did not bind with human erythrocytes (data not shown). No agglutination of these cells was observed under microscopy, which suggests that there is no lectin on the cell surface of *Physarum*.

TABLE 1. LECTIN BINDING ASSAYS FOR MYXOAMOEBAE AND MICROPLASMODIUM CELLS

Cell type	WGA		RCA		Con A		SBA	
	n	Ka	n	Ka	n	Ka	n	Ka
Myxoamoeba, J	10×10^6	0.90	6.4×10^6	0.54	3.7×10^6	12	0.45×10^6	3.0
Neuraminidase-treated J	10×10^6	0.78	7.5×10^6	0.52	—	—	—	—
Myxoamoeba, F	18×10^6	0.84	6.9×10^6	1.2	3.8×10^6	19	0.94×10^6	3.2
Neuraminidase-treated F	18×10^6	0.84	9.0×10^6	0.74	—	—	—	—
Microplasmodium	6.8×10^9	0.52	7.0×10^9	0.53	2×10^9	0.3	5.2×10^9	0.04
Neuraminidase-treated microplasmodium	6.6×10^9	0.41	6.1×10^9	0.64	—	—	—	—

n: number of lectin receptor sites per cell.
Ka: affinity constant ($\times10^6$ M^{-1}).
Each value is the average of duplicate or triplicate experiments.

TABLE 2. HEMAGGLUTINATION INHIBITION TEST WITH THE SLIME POLYSACCHARIDE FRACTION

Lectin	Concentration of Inhibitor (mg/ml)				
	0	3.5	7.0	14.0	27.9
RCA	++++	+++	+++	+	−
SBA	++++	++++	++++	++++	++++
SJA	++++	++++	++++	++++	++++

The 0.4 M NaCl fraction from the slime polysaccharide fraction was used in the hemagglutination inhibition test as described in Methods. The degree of agglutination of the erythrocytes was determined macroscopically after centrifugation at 500×g for 1 min.

DISCUSSION

Plant lectins have been widely used to detect certain structures of glycoconjugates on the cell membranes of mammals (1, 12) and invertebrates (22, 24). In our study, the combined use of fluorescein-conjugated lectin and ^{125}I-labelled lectin enabled us to determine some characters of the cell surface glycoconjugates with small amounts of material.

Two major findings were made: strains J and F of the myxoamoebae and the microplasmodium of *P. polycephalum* have glycoconjugate receptors for the plant lectins WGA, RCA, Con A and SBA, but do not have receptors for DBA, UEA-I and AHA, and the WGA receptors on these cells are resistant to neuraminidase treatment.

The order of the number of receptor sites for each lectin (WGA>RCA>Con A, SBA) was similar to that for mammalian cells (1, 12). The numbers of lectin receptor sites per unit area on the cell surface of *Physarum* were an order of magnitude lower than those of rat ascites hepatoma cells (12), but were the same order as those of human erythrocytes (1, 11). The affinity constants were almost the same in all cases.

RCA, SBA and Con A, respectively, are known to be specific for Gal$^{\beta}$GlcNAc structure, Gal$^{\beta}$GlcNA$^{c\beta}$Man structure and mannosyl cores of the *N*-glycoside-linked hetero-saccharide chains of glycoprotein (10, 20). Therefore, cells of the myxoamoebae and microplasmodium were assumed to have glycoproteins with *N*-glycoside-linked hetero-saccharide chains on their surfaces.

We found the polygalactan phosphosulfate excreted from *Physarum* to be a very weak inhibitor of RCA, as reported by Horisberger *et al.* (8); whereas, the affinity constant of RCA to the microplasmodium was relatively high (0.5×10^{6} M^{-1}). These results rule out the possibility that the polygalactan phosphosulfate is an RCA receptor on the microplasmodial surface.

The WGA receptors on *Physarum* differed from those of mammalian cells. These receptors on mammalian cells decrease remarkably with neuraminidase treatment because they are composed of both *N*-acetyl-β-D-glucosaminyl residues and α-sialyl residues (16, 3). In many cases (12, 19, 29), the decrease in WGA receptors that is caused by neuraminidase treatment is accompanied by a marked increase in RCA receptors because most of the sialyl residues are linked to β-D-galactosyl residues. But, neither a decrease in WGA receptors nor a significant increase in RCA receptors was found when *Physarum* cells were treated with neuraminidase.

These results are a strong indication that cells of *Physarum* have no sialyl residues on their surface, as has been reported for mosquito cells tested by the lectin binding

assay (4) and chemical analysis (32). The absence of DBA and UEA-1 receptors on the myxoamoebae of *Physarum* that is shown by the FITC-lectin binding studies suggests that these cells have no oligosaccharides with blood group A and H determinants.

Recently, cell surface glycoconjugates have been considered to function in cell to cell recognition and adhesion (7). The cellular slime mold provides a good example of this because the formation of pseudoplasmodia is due to the glycoprotein called contact site A (17, 18) and/or the specific interaction between endogenous lectins and the cell surface receptors of the glycoconjugates. These lectins, the discoidin from *Dictyostelium discoideum* (26, 30) and the pallidin from *Polysphondylium pallidum* (27, 28), as well as their glycoconjugate receptors (23), have been studied extensively.

In contrast, the acellular slime mold develops a multinucleated plasmodium without a cell-aggregation process. Therefore, the lectin that functions in the aggregation of cells may be unnecessary to acellular slime molds. But, the presence of glycoconjugate receptors for exogenous lectins was demonstrated in this study.

Strains J and F of the myxoamoebae conjugate with each other, but there is no conjugation within each strain. Glycoconjugates also may participate in this recognition system although no remarkable differences in lectin receptors were found between the two strains of myxoamoebae used.

The cell surface receptors for each lectin on the myxoamoebae seem to be heterogeneous because each lectin can bind with a limitted number of, but different, glycoconjugates that have a particular sugar structure. Therefore, the possibility that only part of a lectin receptor participates in the recognition mechanism of the myxoamoebae can not be excluded. This possibility is being investigated with lectins used as the blocking agent in the conjugation of these cells.

REFERENCES

1. ADAIR, W.L. and S. KORNFELD. Isolation of the receptors for wheat germ agglutinins and the *Ricinus communis* lectins from human erythrocytes using affinity chromatography. *J. Biol. Chem.* **249**, 4696–4704, 1974
2. ALLEN, H.J. and E.A.Z. JOHNSON. The isolation of lectins on acid-treated agarose. *Carbohyd. Res.* **50**, 121–131, 1976
3. AUBERY, M., M. DODEUR, R. ROGUET and R. BOURRILLON. Specific modifications of hepatoma cell surface glycoproteins with enzymes. *Exp. Cell Res.* **113**, 303–310, 1978
4. BUTTERS, T.D. and R.C. HUGHES. Lectin binding to mosquito *Aedes aegyptii* and human KB cells: Structural comparisons of membrane oligosaccharides. *Carbohyd. Res.* **61**, 159–168, 1978
5. FARR, D.R., H. AMSTER and M. HORISBERGER. Composition and partial structure of the extracellular polysaccharide of *Physarum polycephalum. Carbohyd. Res.* **24**, 207–209, 1972
6. FARR, D.R. and M. HORISBERGER. Structure of a β-galactan isolated from the nuclei of *Physarum polycephalum. Biochim. Biophys. Acta* **539**, 37–40, 1978
7. GLICK, M.C. and H. FLOWERS. Surface membranes. in *The Glycoconjugates*, Vol. II, ed. Horowitz, M.I. and W. Pigman, Academic Press, New York, pp. 337–384, 1978
8. HORISBERGER, M., D.R. FARR and M. VONLANTHEN. Ultrastructural localization of β-D-galactan in the nuclei of the myxomycete *Physarum polycephalum. Biophim. Biophys. Acta* **542**, 308–314, 1978
9. INBAR, M., H. BEN-BASSAT and L. SACHS. Difference in the mobility of lectin sites on the surface membrane of normal lymphocytes and malignant lymphoma cells. *Int. J. Cancer* **12**, 93–99, 1973

10. IRIMURA, T., T. KAWAGUCHI, T. TERAO and T. OSAWA. Carbohydrate-binding specificity of the so-called galactose-specific phytohemagglutinins. *Carbohyd. Res.* **39**, 317–327, 1975
11. KAWAGUCHI, T., I. MATSUMOTO and T. OSAWA. Studies on competitive binding of lectins to human erythrocytes. *Biochemistry* **13**, 3169–3173, 1974
12. KITAGAKI, H., I. MATSUMOTO, N. SENO and S. NAGASE. Difference of lectin receptors between the free cell type and island forming cell type of rat ascites hepatoma cells. *Nat. Sci. Rept. Ochanomizu Univ. Tokyo* **32**, 115–126, 1981
13. MATSUMOTO, I., H. KITAGAKI, Y. AKAI, Y. ITO and N. SENO. Derivatization of epoxy-activated agarose with various carbohydrates for the preparation of stable and high capacity affinity adsorbents. *Anal. Biochem.* **116**, 103–110, 1981
14. MATSUMOTO, I. and T. OSAWA. Specific purification of eel serum and *Cytisus sessilifolius* anti-H hemagglutinins by affinity chromatography and their binding to human erythrocytes. *Biochemistry* **13**, 582–588, 1974
15. MCCORMICK, J.J., J.C. BLOMQUIST and H.P. RUSCH. Isolation and characterization of an extracellular polysaccharide from *Physarum polycephalum*. *J. Bacteriol.* **104**, 1110–1118, 1970
16. MONSIGNY, M., A.C. ROCHE, C. SENE, R. MAGET-DANA and F. DELMOTTE. Sugar-lectin interactions: How does wheat-germ agglutinin bind sialoglycoconjugates? *Eur. J. Biochem.* **104**, 147–153, 1980
17. MÜLLER, K. and G. GERISCH. A specific glycoprotein as the target site of adhesion blocking Fab in aggregating *Dictyostelium* cells. *Nature* **274**, 445–449, 1978
18. MÜLLER, K., G. GERISCH, I. FROMME, H. MAYER and A. TSUGITA. A membrane glycoprotein of aggregating *Dictyostelium* cells with the properties of contact sites. *Eur. J. Biochem.* **99**, 419–426, 1979
19. NICOLSON, G.L., M. LACOBIERE and W. ECKHART. Qualitative and quantitative interactions of lectins with untreated and neuraminidase-treated normal, wild-type, and temperature-sensitive polyoma-transformed fibroblasts. *Biochemistry* **14**, 172–179, 1975
20. Ogata, S., T. MURAMATSU and A. KOBATA. Fractionation of glycopeptides by affinity column chromatography on concanavalin A-Sepharose. *J. Biochem.* **78**, 687–696, 1975
21. OKADA, N. and J. OHTA. The fruiting of a myxomycete, *Physarum polycephalum*. *Bot. Mag. (Tokyo)* **76**, 342–348, 1963
22. PETERS, B.P. and I.J. GOLDSTEIN. The use of fluorescein-conjugated *Bandeiraea simplicifolia* B_4-isolectin as a histochemical reagent for the detection of α-D-galactopyranosyl groups. *Exp. Cell Res.* **120**, 321–334, 1979
23. REITHERMAN, R.W., S.D. ROSEN, W.A. FRAZIER and S.H. BARONDES. Cell surface species-specific high affinity receptors for discoidin: Developmental regulation in *Dictyostelium discoideum*. *Proc. Natl. Acad. Sci. U.S.A.* **72**, 3541–3545 ,1975
24. ROSATI, F., R. DE SANTIS and A. MONROY. Studies on fertilization in the ascidians. *Exp. Cell Res.* **116**, 419–427, 1978
25. ROSEN, S.D., P.L. HAYWOOD and S.H. BARONDES. Inhibition of intercellular adhesion in a cellular slime mould by univalent antibody against a cell-surface lectin. *Nature* **263**, 425–427, 1976
26. ROSEN, S.D., J.A. KAFKA, D.L. SIMPSON and S.H. BARONDES. Developmentally regulated, carbohydrate-binding protein in *Dictyostelium discoideum*. *Proc. Natl. Acad. Sci. U.S.A.* **70**, 2554–2557, 1973
27. ROSEN, S.D., J. KAUR, D.L. CLARK, B.T. PARDOS and W.A. FRAZIER. Purification and characterization of multiple species (isolectins) of a slime mold lectin implicated in intercellular adhesion. *J. Biol. Chem.* **254**, 9408–9415, 1979
28. ROSEN, S.D., D.L. SIMPSON, J.E. ROSE and S.H. BARONDES. Carbohydrate-binding protein from *Polysphondylium pallidum* implicated in intercellular adhesion. *Nature* **252**, 128–151, 1974
29. ROSEN, S.W. and R.C. HUGHES. Effects of neuraminidase on lectin binding by wild-type and ricin-resistant strains of hamster fibroblasts. *Biochemistry* **16**, 4908–4915, 1977
30. SIMPSON, D.L., D.S. ROSEN and S.H. BARONDES. Discoidin, a developmentally regulated carbohydrate-binding protein from *Dictyostelium discoideum*. Purification and characterization. *Biochemistry* **13**, 3487–3493, 1974

31. STECK, T.L. and D.F.H. WALLACH. The binding of kidney-bean phytohemagglutinin by Ehrlich ascites carcinoma. *Biochim. Biophys. Acta* **97**, 510–522, 1965
32. STOLLAR, V., B.D. STOLLAR, R. KOO, K.A. HARRAP and R.W. SCHLESINGER. Sialic acid contents of sindbis virus from vertebrate and mosquito cells. *Virology* **69**, 104–115, 1976
33. TANIGUCHI, M., K. YAMAZAKI and J. OHTA. Extraction of contractile protein from myxoamoebae of *Physarum polycephalum*. *Cell Struc. Funct.* **3**, 181–190, 1978

(*Received for publication, April 5, 1982*)