Specific Adhesion of Rat Hepatocytes to β-Galactosides Linked to Polyacrylamide Gels*

(Received for publication, October 21, 1977)

PAUL H. WEIGEL,† ELI SCHMELL,‡ YUAN C. LEE,§ AND SATUL ROSEMAN¶

From the Department of Biology and the McCollum-Pratt Institute, The Johns Hopkins University, Baltimore, Maryland 21218

SUMMARY

Rat hepatocytes, isolated by a collagenase perfusion technique, specifically bind to polyacrylamide gels containing covalently immobilized 6-aminohexyl β-D-galactopyranosyl groups. Less than 5% of these cells bind to polyacrylamide or to gels with the following covalently linked ligands: 6-aminohexanol, or the 6-aminohexyl 6-pyranosides of α-mannose, β-glucose, β-2-acetamido-2-deoxyglucose, β-cellulbiose, β-maltose, or β-melibiose. Cell binding to β-D-galactoside gels occurs after a lag period at 37° and 65 to 100% of the cells adhere. The duration of the lag period is inversely related to the β-D-galactoside content of the gel but preincubation of the cells at 37° reduces the lag period. Cell-gel binding is a threshold phenomenon. Adhesion of cells to gels does not occur when the glycoside concentration is less than about 900 nmol per cm² × 0.25 mm thick gel piece. Above this critical concentration, cell-gel binding occurs and becomes maximal when the concentration is increased by only 20%. If these in vitro results apply to cellular interactions in vivo, they suggest that slight changes in the levels of cell surface or extracellular matrix carbohydrates may profoundly influence the behavior of neighboring cells.

Several cell processes, such as morphogenesis, tumorigenesis, and metastasis, may involve recognition and/or adhesion events between neighboring cells (1, 2). Since these cellular interactions are presumably mediated by cell surface components, and since these cell surfaces are coated with complex carbohydrates (3), we have suggested that carbohydrates are involved in these processes (4). In support of this suggestion, several recent investigations (5-8) have indicated that cell surface carbohydrates may play an important role in intercellular adhesion in lower organisms.

The hypothesis that cells can recognize and adhere to specific glycosides was first tested with suspensions of single cells and Sephadex beads containing covalently linked monosaccharides (9). These Sephadex bead derivatives were prepared by cyanogen bromide activation, followed by coupling to various 6-aminohexyl glycosides SV40-transformed 3T3 cells adhered specifically to the Gal¹ derivative (9). However, numerous problems were encountered with the cyanogen bromide activation method (10) and a new approach was devised based on the use of cross-linked polyacrylamide gels as the insoluble matrix (11-13). In the initial studies with these polymers (14), chicken hepatocytes, isolated by a collagenase perfusion technique developed by Dr. M. Kuhlenschmidt, were found to adhere specifically to polyacrylamide gels containing covalently linked GlcNAc groups. These cells did not bind appreciably to other glycoside or control gels. The binding of cells to the gel required a threshold or critical concentration of GlcNAc in the gel (15) below which there was no binding. At concentrations 10 to 20% above this value or higher, maximal binding occurred; usually 70 to 100% of the cells bound tightly to the gel.

In the present studies, rat hepatocytes were prepared by a similar perfusion method and found to adhere specifically to polyacrylamide gels containing Gal groups. When the Gal concentration in the gel exceeded a critical concentration by about 20%, most of the rat hepatocytes bound to the gel after an appreciable lag period at 37°. The results suggest that changes in the hepatocytes, essential to the adhesion process, occur during the lag period and that these cellular changes may take place more rapidly when the cells are in contact with the Gal gels.

EXPERIMENTAL PROCEDURES

Suspensions of rat hepatocytes containing primarily single cells were prepared by a modification of the perfusion method of Seglen (16) as previously described (17). The cell suspensions were stored on ice in Medium A (17) and all experiments were performed with this medium. Cell preparations were routinely 85 to 95% viable and contained 80 to 90% single cells; viability was assessed by trypan blue exclusion and by the distribution of lactate dehydrogenase activity between the extracellular medium and cells (14, 18).

The carbohydrate ligands used in these experiments were the following 6-aminohexyl α-glycopyranosides: β-glucose, β-galactose, α-mannose, β-2-acetamido-2-deoxyglucose, β-cellulbiose, β-maltose, and β-melibiose. Details of the synthesis and properties of the ligands will be reported elsewhere. Procedures used to synthesize and purify the α-acrylamido hexyl glycopyranosides and to co-polymerize them into thin (0.25 mm) gels have been described (13). Briefly, the free amino group of the aglycon was acylated with acryloyl chloride under alkaline conditions at 0°, and the product was purified by passage through a mixed bed ion exchange column. The product, an acrylamide derivative, was then co-polymerized with acrylamide (20%, w/v) and N,N'-methylenebisacrylamide (1%), w/v, cast in flat sheets, 0.25-mm-thick, and cut into pieces of the desired size and shape. After thorough washing, gel pieces were stored at 4° in 0.15 M NaCl with 30% ethanol. These gels contained 1 All sugar derivatives used in this study were α-pyranosides.

2 R. L. Schnaar, P. H. Weigel, M. S. Kuhlenschmidt, Y. C. Lee, and S. Roseman, manuscript in preparation.

3 "Maximum cell binding" means that the percentage of input cells bound is not changed by using higher concentrations of glycoside in the gels.

4 P. H. Weigel, M. Naoi, S. Roseman, and Y. C. Lee, manuscript in preparation.

5 "Maximum cell binding" means that the percentage of input cells bound is not changed by using higher concentrations of glycoside in the gels.
results and discussion

When freshly isolated rat hepatocytes were tested for their ability to bind to polyacrylamide gels containing covalently immobilized glycosides, a striking sugar specificity was seen (Fig. 1). Cells adhered specifically to the Gal gels, while there was essentially no binding to any of the other gels tested. The cells remained fully viable during the course of the experiment; less than 5% of the total lactate dehydrogenase activity was recovered in the extracellular medium after 60 min of incubation.

Binding of the cells to the Gal gels began after a lag period of about 10 min; after 30 min of incubation, most of the added cells (65 to 100% depending on the cell preparation) adhered to the gels (Fig. 1). The reason for the lag period is unknown. It was not related to the time required for cells to settle onto the gels or for the cell suspension droplet to equilibrate to temperature (2 to 4 min and 2 min, respectively, in these experiments). Presumably, the cells and/or the gels are modified during the lag period and these modifications are required for the formation of stable cell-gel bonds. The cells, for example, may be altered morphologically, by a redistribution of cell surface receptors, or by the insertion of new receptors into the plasma membrane. Another possibility is that cell-gel binding requires a cooperative process involving cell surface receptors and Gal in the gel. Nucleation events necessary for such associations may occur during the lag period.

If the lag period is a result of cellular modifications which are independent of contact with the gels, then this lag should be eliminated by preincubating the cells at 37° prior to contact with the gels. In fact, preincubation for 30 min at 37° resulted in a reduction of the lag period (Fig. 2) and the lag was essentially eliminated after a 45-min preincubation (Fig. 3). This effect was intrinsic to the cells per se, not to the formation of extracellular soluble factors (Fig. 3).

These preincubation experiments suggested that cell modification was necessary before stable binding of cells to gels could occur. However, two aspects of the kinetic results indicated that the events leading to cell-gel binding were more complex. (i) The length of the lag period was inversely related to the concentration of Gal in the gel (Fig. 2). For example, the lag increased from about 10 to 20 min when the Gal content of the gel was decreased from 1180 to 1024 nmol/cm² gel piece (Fig. 2, A and B); with the cell preparation used in Fig. 4, a decrease in Gal content from 970 to 934 nmol/cm² gel piece increased the lag period from about 10 to at least 20 min. Thus, the lag period appeared to depend, at least in part, on the glycoside content of the gel and this effect was very sensitive to small changes in Gal concentration. (ii) Cells in contact with the Gal gels during the lag period attached more rapidly to the gels, once binding began, than did cells preincubated in the absence of gels (Fig. 3).

Since chicken liver cells required a minimum or critical level of GlcNAc linked to the polyacrylamide gel before the cells adhered to these gels (15), the ability of rat hepatocytes to bind to gels containing different concentrations of Gal was therefore studied (Fig. 4). During a 60-min incubation at 37° there was almost no cell binding to gels containing 871 nmol of Gal (or less) per cm². When the Gal content was increased from 871 to 934 nmol/cm² gel piece, however, about 50% of the added cells were bound. Cell binding to the gels was further increased by raising the Gal content of the gels, so that maximum cell binding was observed at 1066 nmol/cm². This experiment (Fig. 4) clearly shows that rat hepatocyte binding to the Gal gels is a threshold phenomenon; when the Gal content of the gel was raised by approximately 20% above the critical level (about 900 nmol/cm²), cell binding increased from almost 0 to about 90% of the added cells. We emphasize that the sugar specificity of cell binding (Fig. 1) was obtained with gels containing from 1100 to 2400 nmol of ligand/cm² gel.
37° preincubated cells, 0° incubated medium.

The cell surface receptors responsible for the binding of chicken hepatocytes to GlcNAc gels and rat hepatocytes to Gal gels have not yet been identified. Ashwell and co-workers (20-24) have isolated binding proteins from liver membranes which may be similar or identical to the receptors responsible for binding cells to gels. These membrane proteins bind to glycoproteins having specific terminal glycose residues; the binding protein isolated from chicken liver binds to terminal GlcNAc groups (23, 24) while the binding protein from rat liver binds to terminal Gal groups (20, 21). Furthermore, these binding proteins function only in the presence of Ca"+ and this ion is also required for the binding of chicken hepatocytes to GlcNAc gels (14, 15) and for the rat hepatoocyte

piece, concentrations well above the critical level found for Gal. 6

The basis for the threshold behavior is unknown, although several explanations are possible. A particular arrangement of sugars may be necessary in order to bind cell receptors, or a certain number of binding events between the cell and the gel may be needed for stable cell binding. Increasing the glycoside concentration of the gel should enhance the probability of forming a particular sugar arrangement or of forming the number of linkages necessary for cell binding. Another possibility, mentioned previously, is that cell-gel binding depends on a cooperative interaction between cell surface components and gel ligands. Cooperative associations have been well characterized in a variety of systems. In general, the kinetics of such interactions involves a slow phase, during which a nucleation process occurs, followed by a rapid phase (19). Cooperative systems also exhibit critical concentration phenomena of the type seen in Fig. 4 (19). Whatever the mechanisms underlying the threshold concentration phenomenon described here, these in vitro results suggest that slight changes in the levels of cell surface or extracellular carbohydrates could have profound effects on the behavior of neighboring cells.

Fig. 2. Effect of preincubation at 37° on the binding of rat hepatocytes to polyacrylamide gels of different \( \beta \)-galactoside content. Gel pieces (0.64 cm² squares) were washed and transferred to four dishes as described in the legend to Fig. 1. A fresh cell suspension (83% singles and 93% viable at 2 \times 10^6 cells/ml) was split into two 4-ml portions in capped polystyrene tubes (9.5 \times 1.1 cm). One tube was stored on ice (open symbols) and the other was placed in a 37° air incubator for 30 min (closed symbols). The cells at 37° were prevented from aggregating by rotation at 6 rpm on a rotating drum 9 cm in diameter (the tube was perpendicular to the axis of rotation); this treatment has little affect on cell viability. A 60-μl droplet of the appropriate cell suspension was then placed on each gel. The incubation, wash, and determination of the number of cells bound to each gel were performed as described in Fig. 1, except that a single wash was performed, using a gyrotary shaker to swirl the dish (88 rpm for 0.4 min), to remove nonadherent cells from the gels. In a separate experiment, the time required for temperature equilibration of a cell suspension droplet initially on ice was found to be about 2 min under these conditions. The number in parentheses in each panel is the \( \beta \)-galactoside content of the gel in nanomoles/cm² x 0.25 mm gel piece.

Fig. 3. The effect of medium from preincubated cells on the kinetics of rat hepatocyte binding to \( \beta \)-galactoside polyacrylamide gels. Gel pieces (0.64 cm² squares; 1047 nmol/cm²) were washed and placed in four dishes as described in Fig. 1. Two tubes were prepared, as in Fig. 2, each with 4 ml of a fresh cell suspension (2 \times 10^6 cells/ml, 85% viability, 85% single cells); one was preincubated at 37° for 45 min as described in Fig. 2 and the other was incubated on ice. The cell suspension at 37° was then chilled on ice for 5 min and half of each cell suspension was removed to another tube. All four tubes were centrifuged at 45 \times g for 2 min to pellet cells. The medium was removed and exchanged between one of the 37° preincubated tubes and one of the ° incubated tubes. All four cell pellets were then resuspended on ice, placed on gels, and processed at the indicated times as described in Figs. 1 and 2. The symbols are as follows: ○, ° incubated cells and medium; □, 37° preincubated cells and medium; ●, ° incubated cells, 37° preincubated medium; ○, 37° preincubated cells, ° incubated medium.

Fig. 4. Effect of \( \beta \)-galactoside content of polyacrylamide gels on the kinetics and extent of rat hepatocyte binding. Gel pieces (0.64 cm² squares) were washed and placed in four dishes as described in Fig. 1. A 60-μl droplet of cell suspension (90% viable, 86% single cells and 2 \times 10^6 cells/ml) was placed on each gel. The incubation, wash, and determination of the number of adherent cells were performed as described in Fig. 2. The numbers to the right of the figure ("Galactose Content") refer to the \( \beta \)-galactoside content of the gels used, expressed as nanomoles per cm² x 0.25 mm.

It is, of course, possible that cells could bind to gels with higher concentrations of these "inactive" glycosides; that is, the threshold concentration for cell binding to an "inactive" ligand may be above the concentration used in the assay. 6

\[ \text{CONTENT} \]

\[ \text{TIME (MINUTES)} \]

\[ \text{CELLS BOUND (\% of cells added)} \]

\[ \text{TIME (MINUTES)} \]

\[ \text{CELLS BOUND (\% of cells added)} \]

\[ \text{TIME (MINUTES)} \]

\[ \text{CELLS BOUND (\% of cells added)} \]
binding to the Gal gels described in the present study (data not shown). The similarities between the properties of the liver binding proteins and those of the cell receptors responsible for cell-gel binding are striking. However, preliminary experiments provide evidence both for and against the conclusion that these two binding activities are indeed identical.

Acknowledgments—We gratefully acknowledge Dr. Fritz Sieber for providing us with the method for maintaining cell viability while preventing intercellular adhesion at 37°, Dr. Bjorn Öbrink who prepared cells for some of the initial experiments in this study, and Mrs. Dorothy Regula for help in preparing the manuscript. We also thank Dr. William Harrington for helpful and stimulating discussions.

REFERENCES
1. Revel, J. P. (1974) in The Cell Surface in Development (Moscona, A. A., ed) pp. 51-65, J. Wiley and Sons, New York
2. Nicholson, G. L. (1976) Biochim. Biophys. Acta 458, 1-72
3. Leblond, C. P., and Bennett, G. (1974) in The Cell Surface in Development (Moscona, A. A., ed) pp. 29-49, J. Wiley and Sons, New York
4. Roseman, S. (1970) Chem. Phys. Lipids 5, 270-297
5. Yen, P. H., and Ballou, C. E. (1974) Biochemistry 13, 2428-2437
6. Wiese, L., and Wiese, W. (1975) Exp. Cell Res. 82, 391-398
7. Turner, R. S., and Burger, M. M. (1973) Nature 244, 509-510
8. Chipowsky, S., Lee, Y. C., and Roseman, S. (1973) Proc. Natl. Acad. Sci. U. S. A. 70, 2309-2312
9. Schnaar, R. L., Sparks, T. F., and Roseman, S. (1977) Anal. Biochem. 79, 513-525
10. Schnaar, R. L., and Lee, Y. C. (1975) Biochemistry 14, 1535-1541
11. Schnaar, R. L., Weigel, P. H., Roseman, S., and Lee, Y. C. (1978) Methods Carbohydr. Chem. 8, in press
12. Weigel, P. H., Schnaar, R. L., Roseman, S., and Lee, Y. C. (1978) Methods Carbohydr. Chem. 8, in press
13. Schnaar, R. L. (1976) Ph.D. dissertation, The Johns Hopkins University, Baltimore, Md.
14. Weigel, P. H., Schnaar, R. L., and Kuhlenschmidt, M. S. (1977) Fed. Proc. 36, 553
15. Seglen, P. O. (1973) Exp. Cell Res. 82, 391-398
16. Schnaar, R. L. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 1077-1081
17. Öbrink, B., Kuhlenschmidt, M. S., and Roseman, S. (1977) Fed. Proc. 36, 553
18. Berg, T., Boman, D., and Seglen, P. O. (1972) Exp. Cell Res. 72, 571-574
19. Engle, J., and Winklmait, D. (1972) in Protein-Protein Interactions (Jencks, B., and Helmreich, E., eds) pp. 159-181, Springer-Verlag, New York
20. Hudgin, R. L., Pricer, W. E., Jr., Ashwell, G., Stockert, R. J., and Morelli, A. C. (1974) J. Biol. Chem. 249, 5536-5548
21. Kawasaki, T., and Ashwell, G. (1976) J. Biol. Chem. 251, 1296-1302
22. Prisco, W. E., Jr., and Ashwell, G. (1976) J. Biol. Chem. 251, 7539-7544
23. Lunney, J., and Ashwell, G. (1976) Proc. Natl. Acad. Sci. U. S. A. 73, 341-345
24. Kawasaki, T., and Ashwell, G. (1977) J. Biol. Chem. 252, 6536-6543
Specific adhesion of rat hepatocytes to beta-galactosides linked to polyacrylamide gels.

P H Weigel, E Schmell, Y C Lee and S Roseman

J. Biol. Chem. 1978, 253:330-333.

Access the most updated version of this article at http://www.jbc.org/content/253/2/330

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/253/2/330.full.html#ref-list-1