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Cell Biology

Role of a 16S glycoprotein complex in cellular adhesion

(myoblast/glycosaminoglycans/aggregation/nerve cells)

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ABSTRACT **Myogenic cells release into their culture medium a glycoprotein complex that mediates cellular adhesion. In the absence of calcium this complex has a sedimentation value of 16 S; it aggregates in the presence of calcium. The 16S material both agglutinates and increases the rate of cell–substratum adhesion of a myoblast variant and *inhibits* the adhesion of a nerve-like cell line to culture dishes. It is also a hemagglutinin. The 16S particle is composed of glycosaminoglycans and several proteins, including fibronectin and collagen.**

Macromolecules responsible for cellular adhesion are released from cells into their environment. For example, glycoproteins involved in the aggregation of retinal cells have been isolated from growth-conditioned medium (1, 2), and molecules secreted from fibroblast-like cells promote cell–substratum adhesion (3, 4). Because many glycoproteins found in the growth medium of cultured cells are derived from the cell surface (for example, see refs. 5–8), examination of these extracellular molecules may help to characterize the cell's outer plasma membrane and the cell's adhesive mechanisms.

Adhesion-defective variants of anchorage-dependent cells have been described (9–11) and are being used to define the processes involved in adhesion. One example is a variant of the anchorage-dependent skeletal muscle myoblast line L6 (12). The variant, designated M3A, grows as single cells in suspension on plastic petri dishes (9). The parental L6 cell line releases macromolecules into its culture medium which can adhere to the surface of petri dishes and promote the adhesion of M3A cells to the substratum (unpublished data). The cell–substratum adhesive interaction between M3A cells and substrate-attached material (SAM) (13) from L6 cells is inhibited by exogenous glycosaminoglycans (GAGs), suggesting that GAGs are involved in the adhesive interactions of these cells. In addition, growth-conditioned medium from L6 cells rapidly aggregates M3A cells. The following data show that the adhesion- and aggregation-promoting activities reside in glycoprotein particles of a defined size.

MATERIALS AND METHODS

Cells and Culture. The L6 myoblast line was obtained from Yaffe (12) and maintained in 10% fetal calf serum in Dulbecco's modified Eagle's medium (14). The M3A variant of L6 was selected for growth in suspension over agar and grown in Dulbecco's modified Eagle's medium containing 10% fetal calf serum (9). The PC12 clone was obtained from Greene (15) and grown in the same medium plus 10% fetal calf serum and 5% horse serum.

Protein and Glycosaminoglycan Assays. Cells were labeled with [^{3}H]leucine as described (6). Tube gel electrophoresis was done in 5% acrylamide and 0.1% $NaDodSO_4$ according to Maizel (16). GAG analysis was carried out by labeling cells with [^{3}H]glucosamine or [^{35}S]sulfate, chromatographing the sample on a DEAE-cellulose column (17), and identifying the GAGs in the individual peaks by enzymatic methods (18). Hydroxyproline was determined by using cells labeled with [^{14}C]proline. The fractions of interest were isolated, hydrolyzed in 6 M HCl, and chromatographed on an amino acid analyzer; the radioactivity in the 4-hydroxyproline and proline peaks was determined.

Preparation of Conditioned Medium and SAM. Conditioned medium was prepared from exponentially dividing cells by washing the cells twice in serum-free Hepes-buffered medium and incubating the cells in the same medium for 15 hr at 37°C. To prepare SAM-coated dishes, growth-conditioned medium was placed in 35-mm plastic petri dishes (Falcon) for 18 hr, and then the dishes were washed twice with 0.5 mM ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid (EGTA), three times with water, and once with Hepes medium. After the washing, 2 ml of Hepes medium containing 0.2% bovine serum albumin was added.

Adhesion Assays. To assay cell–substratum adhesion, early stationary phase M3A or PC12 cells were labeled with [^{3}H]-leucine (5 μCi/ml; 1 Ci = 3.7×10^{10} becquerels) for 15 hr. The cells were washed three times with Hepes medium containing 0.2% albumin and 0.2-ml aliquots were pipetted into 35-mm SAM-coated plastic petri dishes containing 2 ml of the same medium. At indicated times the dishes were swirled 10 times, the medium was aspirated, and the remaining attached cells were dissolved in Triton X-100 and their isotope content was determined. The data were plotted as the fraction of input cells that adhered as a function of time. Variation between duplicates was less than 5% (19).

Cell aggregation was measured by the disappearance of single cells from an agitated suspension (19, 20). Cells were washed twice in Hepes buffer containing 0.2% bovine serum albumin and added to 0.5-ml aliquots of the test medium made 0.2% in albumin. The cells were agitated on a rotary shaker at 100 rpm and the disappearance of single cells was monitored with a Coulter Counter.

The rabbit erythrocytes used for the hemagglutination assays were prepared in two ways. Some were trypsin-treated and fixed with glutaraldehyde; others were trypsin-treated, fixed with glutaraldehyde, and treated with ethanol (21). Assays were done in microtiter plates in 150 mM sodium chloride/10 mM phosphate, pH 7.1. Titers were determined at 1 hr and the specific activity was defined as titer^{-1} divided by mg of protein used in the assay.

Poly(A)$^-$ RNA was obtained from T. Hunter (Salk Institute), highly purified *Clostridium histolyticum* collagenase was from E. Harper (University of California at San Diego), anti-human cold-insoluble globulin was from S. J. Singer (University of California at San Diego), and the chondroitinases and hyaluronidase were from Miles.

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Abbreviations: SAM, substrate-attached material; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid; GAG, glycosaminoglycan.

RESULTS

SAM-Induced Adhesive Responses. Because M3A and PC12 grow as single cells loosely associated with the culture dishes, their adhesive properties can be assayed without the use of proteolytic enzymes or chelating agents to dissociate them. When isotopically labeled M3A or PC12 cells were added to the SAM-coated culture dishes, L6 SAM stimulated the adhesion of M3A cells but completely blocked the binding of PC12 cells (Fig. 1*A*). M3A cells exposed to serum-free conditioned medium from L6 were rapidly aggregated; L6 conditioned medium did not aggregate PC12 cells (Fig. 1*B*).

Identification of Particle Involved in Adhesion. When the growth-conditioned medium of L6 cells was centrifuged for 2 hr at 100,000 × *g*, all of the adhesion and aggregation activities were found in the pellet. If the adhesion-promoting material is a defined molecule or macromolecular complex, it should sediment in a sucrose gradient and the activity mediating adhesion should be associated with this entity. Our unpublished work has suggested that GAGs are involved in the adhesion of M3A cells to L6 SAM. Therefore, cells were incubated for 18 hr in serum-free medium with [^{35}S]sulfate, which labels sulfated GAGs; unlabeled serum-free L6 conditioned medium was also prepared. The conditioned media were centrifuged at 100,000 × *g* for 3 hr, the supernatant was aspirated, and the pellet was washed twice with Hepes medium and resuspended in the same medium. About half of the sulfated GAGs were recovered in the pellet. The samples were then sedimented on 5–20% (wt/vol) sucrose gradients for 18 hr. The $^{35}SO_4$ label defined a peak that sedimented about one-third of the way into the gradient (Fig. 2). When the ability of each gradient fraction of the unlabeled material to mediate cell–substratum adhesion and cell aggregation was assayed, an increase in adhesion of M3A cells and a decrease in adhesion of PC12 cells were effected only by the gradient fractions that coincided with the presence of the isotope.

In another experiment to test the correlation between the peak of sulfate-labeled material on the sucrose gradient and cell–substratum adhesion, L6-secreted macromolecules were labeled with [^{35}S]sulfate, and the gradient was collected directly into petri dishes. The adhesion of [^{3}H]leucine-labeled M3A cells to SAM prepared from each gradient fraction was determined and both isotopes were counted. Again, the sulfate labeling and adhesion were coincidental, and all of the sulfate label from the gradient peak adhered to the petri dish. Finally, when the gradient fractions were tested for their ability to stimulate the aggregation of M3A cells, this activity cosedimented with that of the sulfate label (Fig. 2).

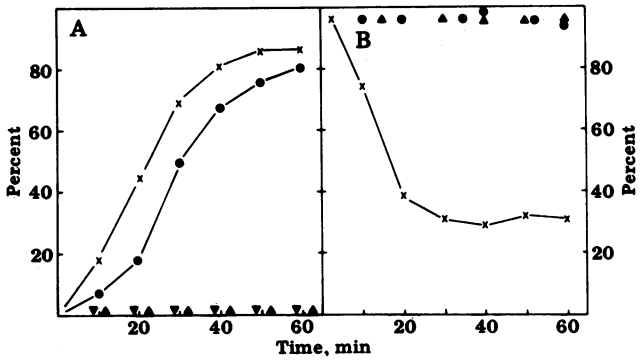

FIG. 1. Kinetics of adhesion of M3A and PC12 cells mediated by L6 conditioned medium. Exponentially growing L6 cells were incubated overnight in serum-free Hepes medium. (*A*) Some of this growth-conditioned medium was then placed in petri dishes overnight, and the resultant SAM-coated dishes were washed. Early stationary phase M3A and PC12 cells were isotopically labeled and plated (3 × 10^6 cells per 60-mm dish) into the SAM-coated dishes. The data are presented as the percentage of input cells that had adhered at each time point. ×, M3A cells plated on dishes containing L6 SAM; ●, PC12 cells plated on new petri dishes; ▲, M3A cells on new petri dishes; ▼, PC12 cells on dishes containing L6 SAM. (*B*) The remainder of the growth conditioned medium was diluted 1:4 into Hepes medium and its effect on the aggregation of M3A cells was determined. The aggregation data are presented as the fraction of input cells remaining as single cells. ●, M3A aggregation, fresh medium; ×, M3A aggregation, L6 growth conditioned medium; ▲, PC12 aggregation, L6 conditioned medium.

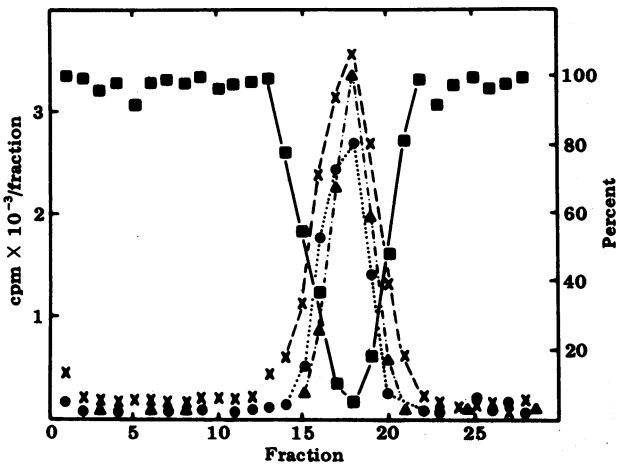

FIG. 2. Cosedimentation of L6 GAGs and adhesion activities in sucrose gradients. L6 cells were labeled with $^{35}SO_4$ in serum-free medium (5 × 10^6 cells) or were incubated in serum-free medium alone (2 × 10^7 cells). After 18 hr the conditioned media were centrifuged at 100,000 × *g* for 2 hr. The two 100,000 × *g* pellets were then centrifuged for 18 hr at 35,000 rpm at 4°C in an SW 41 Beckman rotor on 5–20% linear sucrose gradients containing 0.01 M Hepes buffer (pH 7.1). The data are presented as cpm per fraction: 96% of the input counts were recovered. Each gradient fraction with unlabeled cellular material was divided into three aliquots. One was diluted into Hepes medium to determine its effect on M3A agglutination. These data are presented as the percentage of maximal aggregation at 40 min (see Fig. 1). The remaining two were each diluted into Hepes medium and incubated in petri dishes overnight at 37°C. The following day the adhesion of isotopically labeled M3A and PC12 cells was determined and these data are presented as the percentage of input cells that adhered at 50 min. The top of the gradient is at the right. ×, $^{35}SO_4$-Labeled material; ▲, aggregation of M3A; ●, cell–substratum adhesion of M3A; ■, cell–substratum adhesion of PC12.

Table 1. Inhibition of M3A adhesion

Reagent	1(50%) Conc. (M) to give 50% inhibition: L6 conditioned medium	L6 particle
Chondroitin sulfate A	5×10^{-4}	4×10^{-4}
Chondroitin sulfate B	$>10^{-3}$	$>10^{-3}$
Chondroitin sulfate C	2×10^{-5}	2×10^{-5}
Heparin	$>10^{-4}$	$>10^{-4}$
Heparan sulfate	$>10^{-4}$	$>10^{-4}$
Hyaluronic acid	1×10^{-6}	1×10^{-6}

SAMs from L6 conditioned medium and SAM from the sulfate-labeled peak on sucrose gradients (Fig. 2) of L6 conditioned media were prepared as described in Fig. 2, and the kinetics of M3A adhesion were determined as in Fig. 1. For each concentration of carbohydrate, the percentage of input cells attached to the dish was determined in a 1-hr period. From these data, the percentage of input cells attached at 30 min was plotted against the carbohydrate concentration, and the concentration of carbohydrate that gave 50% inhibition was determined. The concentrations are shown in terms of the molarity of glucuronic acid, assuming it is a free molecule.

The interaction between M3A cells and SAM prepared from L6 conditioned medium can be inhibited by exogenous GAGs (unpublished data). These GAGs presumably compete for binding sites on the cell surface or in the SAM which are involved in the adhesion reaction, and the amount and kind of GAGs required to inhibit adhesion are unique for each cell line and the SAM to which it is adhering. If the L6 particle is responsible for the adhesion of M3A cells to SAM from L6 conditioned medium, then the specificities of inhibition by GAGs should be the same for each. The adhesion of M3A cells to L6 SAM was inhibited by chondroitin sulfates A and C and hyaluronic acid. The same GAGs inhibited the binding of M3A cells to the sulfate-labeled gradient fractions derived from L6 conditioned medium (Table 1).

Calcium-Dependent Aggregation of the Adhesion Activity. The sedimentation profiles presented in Fig. 2 were obtained when the 100,000 × *g* pellets of L6 growth conditioned medium were resuspended in 0.01 M Hepes buffer and centrifuged into sucrose gradients made in the same buffer. However, when the pellets were dissolved in Hepes buffer containing 2 mM calcium, incubated at room temperature for 2 hr, and centrifuged into sucrose gradients containing 2 mM calcium, 85% of the sulfate-labeled material was recovered at the bottom of the gradient along with the cell–substratum adhesion- and M3A

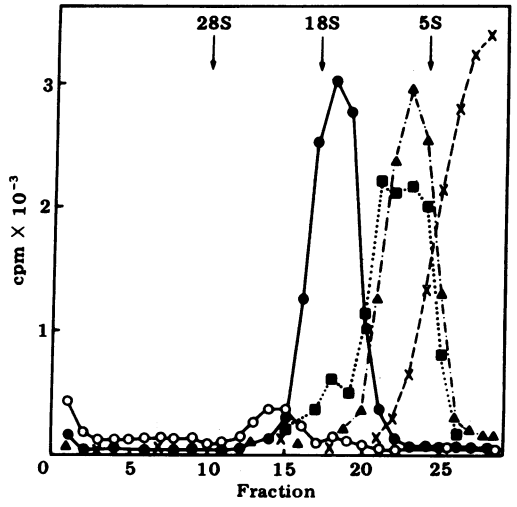

FIG. 3. Calcium-induced aggregation and enzymatic digestion of 16S particle. Two sets of exponentially dividing L6 cells were labeled with [^{35}S]sulfate and [^{3}H]glucosamine for 18 hr, the culture medium was centrifuged at 100,000 × *g* for 3 hr, and the pellet was washed twice with Hepes medium. The pellet of the first set was resuspended in 0.01 M Hepes buffer at pH 7.1 and divided into two equal aliquots. One was made 2 mM in calcium chloride. The tubes were gently agitated for 2 hr at 22°C and then sedimented on a 5–20% sucrose gradient in either Hepes buffer or Hepes buffer plus 2 mM calcium. In the absence of calcium, only 6% of the input counts was associated with the bottom of the tube, but in 2 mM calcium, 87% of the input counts was found at the bottom. The second pellet was divided into five equal aliquots. One was digested with fungal hyaluronidase (50 units per ml in 0.01 M sodium acetate/0.15 M sodium chloride, pH 5.1) for 18 hr at 37°C; others were digested with chondroitinase AC or ABC (0.5 unit per ml in 0.01 M sodium acetate/0.15 M sodium chloride, pH 7.9) for 18 hr at 37°C, with collagenase for 4 hr (24), or in pH 7.9 buffer alone. The samples were then centrifuged into sucrose gradients in 0.01 M Hepes buffer as described above. The arrows mark the positions of 28, 18, and 5S poly(A)$^-$ RNAs used as internal markers. The results with chondroitinase AC and ABC were identical; only the chondroitinase AC results are presented. All of the cell–substratum adhesion promoting activities, assayed as in Fig. 2, were lost after treatment with fungal hyaluronidase or chondroitinase AC. ●, Gradient in 0.01 M Hepes; ○, gradient in 0.01 M Hepes plus 2 mM calcium; ▲, hyaluronidase digest; ×, chondroitinase AC digest; ■, collagenase digest.

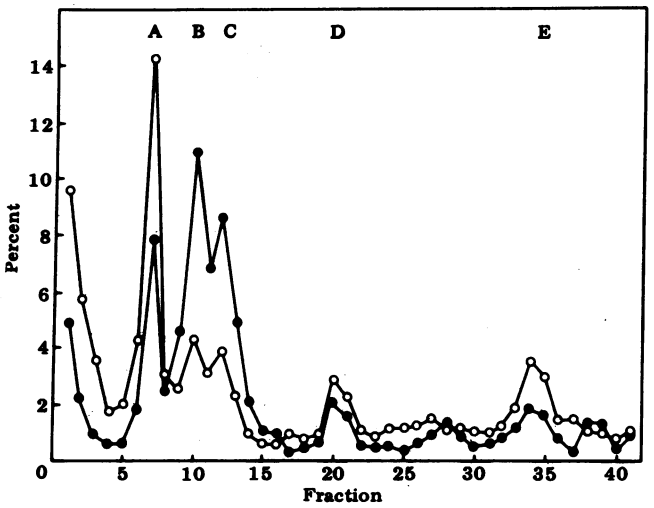

FIG. 4. Protein composition of 16S particle. Exponentially dividing L6 myoblasts were labeled with [^{3}H]leucine and [^{14}C]proline for 18 hr and the culture supernatant was centrifuged (100,000 × *g* for 3 hr). The pellet was then sedimented on a 5–20% sucrose gradient as described in Fig. 2. The 16S peak was isolated and electrophoresed in 5% polyacrylamide gels containing $NaDodSO_4$. The data are presented as the percentage of the total counts recovered in the gel at each point. The results were the same when the label was reversed. Although 92% of the input isotope was recovered, between 10% and 20% of this isotope was retained at the top of the gel. ○, [^{3}H]Leucine; ●, [^{14}C]proline.

aggregation-promoting activities (Fig. 3). In the absence of calcium this material could be resedimented as a single peak, indicating that it was aggregated by calcium. The material in calcium-free gradients had a sedimentation coefficient of approximately 16 S.

Hemagglutinating Activity of the L6 Particle. Hemagglutinating activities have been described in muscle cultures (22, 23). To test the possibility that the L6 particle is also a hemagglutinin, it was sedimented in a sucrose gradient as described in Fig. 2 along with sulfate-labeled 16S material. Each gradient fraction was then tested on either trypsinized glutaraldehyde-fixed or alcohol-washed trypsinized glutaraldehyde-fixed rabbit erythrocytes (21). Hemagglutinating activity was found only for alcohol-washed cells, and it cosedimented with the 16S particle. The specific activity (titer^{-1} per mg of protein) of the particle from L6 myoblasts was approximately 16,000. Various sugars and GAGs were examined for their ability to inhibit the hemagglutination reaction. Of the sugars tested (*N*-acetyl-D-glucosamine, *N*-acetyl-D-galactosamine, D-galactose, lactose, D-fucose, α-methyl mannoside, D-glucose, thiodigalactoside), none inhibited agglutination at concentrations as high as 50 mM. Individual GAGs listed in Table 1 also were inactive in blocking hemagglutination at concentrations up to 2 mg/ml.

Composition of 16S Particle. Because previous results suggested that L6 GAGs were involved in cell–substratum interactions and that the adhesion-promoting activities were trypsin sensitive, it was likely that L6 16S particles contained both protein and GAGs. Therefore, L6 cells were labeled with [^{3}H]leucine, [^{14}C]proline, [^{3}H]glucosamine, or $^{35}SO_4$, the culture medium was centrifuged at 100,000 × *g* for 2 hr, the pellets were washed twice, and the pellets were sedimented on sucrose gradients. The isotopically labeled 16S peak was collected, made 2 mM in calcium to aggregate it, and recentrifuged at 100,000 × *g* for 2 hr. The pellet was assayed for protein on polyacrylamide gels, and the GAG content was determined by column chromatography and enzymatic analysis. When particles la-

beled with [^{3}H]leucine and [^{14}C]proline were electrophoresed on NaDodSO$_4$/polyacrylamide gels, five major protein bands were resolved (Fig. 4). Several minor protein bands were detected by radioautography. Protein A is fibronectin because it was specifically precipitated with anti-human cold-insoluble globulin and comigrated on NaDodSO$_4$/polyacrylamide gels with fibronectin isolated from secreted proteins. Proteins B and C are collagens by several criteria: (*i*) they comigrated on NaDodSO$_4$/polyacrylamide gels with two of the three secreted L6 collagens (24); (*ii*) they contained high ratios of proline, alanine, and glycine relative to leucine; (*iii*) when isolated from the gels and assayed for hydroxyproline, the combined peaks of bands B and C contained 6.2% 4-hydroxyproline (peak A and combined peaks D and E contained less than 0.1% hydroxyproline); (*iv*) treating the particle with highly purified collagenase destroyed only peaks B and C. The identities of the proteins in peaks D (M_r, 100,000) and E (M_r, 56,000) are unknown. Each of these proteins comigrated with an L6 secreted protein (24). Together they constitute a subset of the total proteins released into the culture medium.

To determine the nature of the GAGs associated with the 16S particle of L6, the ^{35}SO$_4$- and [^{3}H]glucosamine-labeled 16S peak from sucrose gradients was digested with Pronase and chromatographed on a DEAE-cellulose column to separate the classes of GAGs (Fig. 5). The elution profile was similar to that previously described (25). Peak A is hyaluronic acid because it contained no sulfate, was degraded by fungal hyaluronidase, and was not degraded by nitrous acid. Peak B is unknown, but it is probably unsulfated chondroitin because it lacked sulfate, was eliminated by chondroitinase ABC, and was resistant to fungal hyaluronidase. Peak C was degraded by nitrous acid and it contained sulfate, making it heparan sulfate. Peak D contained sulfate and was degraded by chondroitinase ABC but not by fungal hyaluronidase or nitrous acid and thus is chondroitin sulfate. It contained approximately 10% chondroitin sulfate B as defined by the method of Saito *et al.* (18).

Gaussian sedimentation of a particle on a sucrose gradient is necessary but not sufficient to establish its homogeneity. For example, a series of noninteracting molecules could have the same size and could sediment together on the gradient. However, if all of a particle's constituents are required for its structural integrity, then the elimination of one component should shift the sedimentation velocity of the remaining molecules. This can be accomplished with the 16S L6 particle by treating it with fungal hyaluronidase, chondroitinase AC, or collagenase. Fig. 3 shows that hyaluronidase and collagenase shifted the sedimentation of the particle; after chondroitinase AC digestion, no material entered the gradient. Although the data in Fig. 3 represent sulfate-labeled material, the 16S particle was labeled at the same time with [^{3}H]glucosamine and [^{3}H]-leucine. After enzymatic digestion, the remaining leucine-labeled material and [^{3}H]glucosamine-labeled GAGs migrated to the same positions on the gradients as did the sulfate-labeled GAGs. Protein and GAG analysis of these fractions showed the protein composition to be intact but hyaluronic acid and chondroitin sulfate were eliminated; collagenase selectively eliminated collagen. Finally, when leading and trailing edges of the ^{35}S-labeled 16S peak were resedimented on sucrose gradients, they cosedimented with [^{3}H]glucosamine-labeled molecules from the center of the peak. It follows that the 16S peak is homogeneous with respect to sedimentation velocity, and that chondroitin sulfate, hyaluronic acid, and collagen are required for its structural integrity.

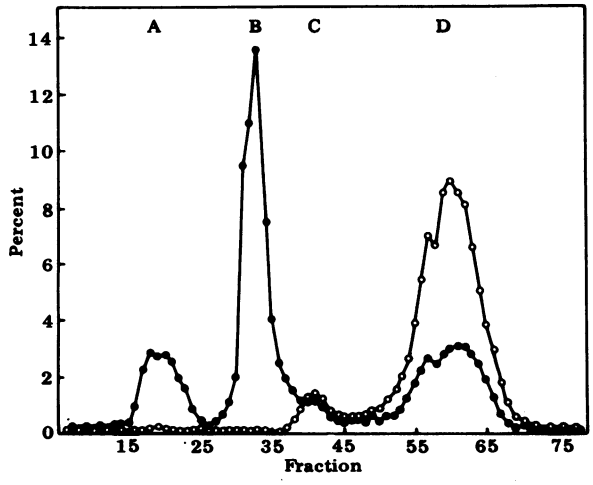

FIG. 5. Elution profile of GAGs from L6 16S particle. Exponentially dividing cells were labeled with [^{3}H]glucosamine and ^{35}SO$_4$ for 18 hr and the culture medium supernatant was centrifuged (100,000 × *g* for 3 hr). The pellet was applied to a 5–20% sucrose gradient in the absence of calcium. The 16S peak was isolated and digested with Pronase, and the GAGs run on a DE-52 column. The data are expressed as the percentage of the total recovered counts in each fraction. ○, [^{35}S]Sulfate label; ●, [^{3}H]glucosamine label.

DISCUSSION

The following conclusions may be made from the above data. (*i*) Muscle cells release material into their culture medium which induces the aggregation and cell–substratum adhesion of myoblast variant M3A (Figs. 1–3). (*ii*) Myoblasts also secrete material which decreases the adhesion of the nerve-like cell line PC12 to culture dishes (Figs. 1 and 2). (*iii*) The entity responsible for these adhesive responses sediments as a 16S peak in calcium-free sucrose gradients (Fig. 2; Table 1). (*iv*) There are several proteins in the 16S particle, including collagen and fibronectin (Fig. 4). (*v*) L6 particles also contain hyaluronic acid, heparan sulfate, chondroitin sulfate, and an undefined GAG (Fig. 5). (*vi*) This adhesion-mediating particle from L6 cells aggregates in the presence of calcium (Fig. 3). (*vii*) The L6 16S particle is a hemagglutinin. It is concluded that myoblast-conditioned medium contains macromolecular complexes of defined size and composition that can mediate the adhesion of cells.

Some of the previously described adhesion-promoting activities isolated from growth-conditioned medium may be related to the 16S glycoprotein complex. Clonal cell lines of central nervous system nerve or glia and of muscle all release GAG-containing material that cosediments with the L6 16S particle (unpublished data). Lymphomas and plasmacytomas (which grow as single cells in suspension culture) and the peripheral nerve lines PC12 and C1300 do not secrete 16S glycoprotein complexes, and the adhesion-deficient variant of L6, M3A, synthesizes an aberrant particle with a reduced sedimentation coefficient (unpublished data). Because both the molecular composition and the adhesive properties of 16S particles differ between cell lines, a spectrum of adhesive specificities can be generated by glycoprotein complexes of the same sedimentation value.

Although activities promoting myoblast adhesion have not been previously isolated from skeletal muscle cells, activities that agglutinate erythrocytes have been described (22, 23). The 16S particle isolated from L6 myoblasts is also a hemagglutinin. Although the specificity with respect to saccharide inhibition of L6 particles is different from any of the published activities, it is most analogous to that of the lectin-2 of chicken skeletal muscle (21, 23). Like lectin-2, L6 16S particles agglutinated ethanol-treated, glutaraldehyde-fixed rabbit erythrocytes but not glutaraldehyde-fixed cells. Lectin-2 was also removed from

conditioned medium by centrifugation. Hemagglutination by the 16S particle is not inhibitable by GAGs, but the cell–substratum adhesion of M3A cells to L6 SAM and the 16S particle is inhibitable by GAGs (Table 1). In addition, the L6 particles are at least 2 orders of magnitude more active in promoting M3A aggregation and cell–substratum adhesion than they are in hemagglutination. This difference in activity may be due to a greater specificity for the binding sites on the myoblast cells.

The L6 16S particle contains GAGs. GAGs have been implicated in cell–substratum and cell–cell adhesion (26–28). Other components of the L6 16S particle may have a less direct role in the adhesion of M3A cells. For example, fibronectin mediates cell–substratum adhesion in some fibroblast-like cells, primarily through an interaction with substrate collagen. However, the adhesion of M3A cells to SAM derived from L6 is completely inhibited by exogenous GAGs (Table 1). It is thus unlikely that this interaction involves a direct binding of fibronectin to collagen.

In contrast to M3A cells, PC12 cells bind to petri dish surfaces but do not adhere to L6 SAM or L6 16S particle-coated substrata (Figs. 1 and 2). Coating petri dishes with GAGs or collagen does not inhibit PC12 adhesion. SAM from cells that do not secrete fibronectin is also ineffective at inhibiting PC12 adhesion. However, coating dishes with fibronectin inhibits the binding of PC12 cells to the same extent as does coating with L6 SAM (unpublished data). Because L6 particles contain fibronectin, it is likely that this molecule is involved in the inhibition of PC12 binding by L6 SAM.

Cell–substratum adhesive interactions, cell motility, and cell–cell agglutinations are calcium dependent (for example, see refs. 29 and 30). Fig. 3 shows that, when L6 conditioned medium is centrifuged in a sucrose gradient in the absence of calcium, a 16S peak of adhesion-promoting activity is found. In the presence of calcium the activity aggregates and sediments to the bottom of the centrifuge tube. Because cells can regulate the amount of bound calcium (31, 32), the calcium sensitivity of particle aggregation could account for the modulation of the cell–substratum interactions required for division and locomotion.

The multimeric structure of the 16S particle allows for an almost unlimited range of binding specificities. The particle utilizes basic cellular components (collagens, fibronectin, GAGs) to generate its adhesive capacity. The GAG components are required for adhesion because when the L6 16S particles are treated with fungal hyaluronidase or chondroitinase AC they lose their binding activity as they alter their sedimentation velocity (Fig. 3). The particles are also sensitive to collagenase (Fig. 3). These observations, together with the Gaussian sedimentation profile, suggest that the 16S material is a structurally limited particle requiring at least three of its major components for its integrity. Because the 16S particles seem to have an overall nonspecific adhesiveness (e.g., to petri dishes) in addition to a specific binding affinity for cells, the particles will probably remain within the area of the cell of origin. However, once they are fixed in an extracellular location such as the cell surface or basement membrane they will be able to sort out cells by their specific interactions. The particles may then become stabilized and contribute to the overall integrity of the tissue.

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1. McClay, D. R. & Moscona, A. A. (1974) *Exp. Cell Res.* **87**, 438–441.
2. Thiery, J., Brackenbury, R., Rutioshauser, U. & Edelman, J. (1977) *J. Biol. Chem.* **252**, 6041–6845.
3. Moore, E. G. (1976) *J. Cell Biol.* **70**, 634–647.
4. Millis, A. & Hoyle, M. (1978) *Nature (London)* **271**, 668–670.
5. Doljanski, F. & Kapeller, M. (1976) *J. Theor. Biol.* **62**, 253–257.
6. Schubert, D. (1976) *Exp. Cell Res.* **102**, 329–340.
7. Baum, B. J., McDonald, J. A. & Crystal, R. G. (1977) *Biochem. Biophys. Res. Commun.* **79**, 8–14.
8. Kao, W., Berg, R. & Prockop, D. (1977) *J. Biol. Chem.* **252**, 8391–8397.
9. Tarikas, H. & Schubert, D. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 2377–2381.
10. Pouyssegur, J. M. & Pastan, I. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 544–548.
11. Whipple, A. P. & Millis, J. T. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 2838–2842.
12. Yaffe, D. (1968) *Proc. Natl. Acad. Sci. USA* **61**, 477–481.
13. Culp, L. (1974) *J. Cell Biol.* **63**, 71–83.
14. Vogt, N. & Dulbecco, R. (1963) *Proc. Natl. Acad. Sci. USA* **49**, 171–179.
15. Greene, L. & Tischler, M. (1976) *Proc. Natl. Acad. Sci. USA* **75**, 4587–4591.
16. Maizel, J. (1971) in *Methods in Virology*, eds. Maramorosch, K. & Koprowski, H. (Academic, New York), Vol. 5, pp. 179–193.
17. Underhill, C. B. & Keller, J. M. (1976) *J. Cell. Physiol.* **89**, 53–64.
18. Saito, H., Yamagato, T. & Suzuki, S. (1968) *J. Biol. Chem.* **243**, 1536–1546.
19. Schubert, D. & Whitlock, C. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 4055–4058.
20. Obrink, B., Kuhlenschmidt, M. S. & Roseman, S. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 1077–1081.
21. Kobiler, D. & Barondes, S. H. (1979) *FEBS Lett.* **101**, 257–259.
22. Podleski, T. R., Greenberg, I. & Nichols, S. C. (1979) *Exp. Cell Res.* **122**, 305–316.
23. Ceri, H., Shadle, P. J., Kobiler, D. & Barondes, S. (1979) *J. Supramol. Struct.* **11**, 61–67.
24. Schubert, D., Tarikas, H., Humphreys, S., Heinemann, S. & Patrick, J. (1973) *Dev. Biol.* **33**, 18–37.
25. Kraemer, P. M. & Barnhart, B. J. (1978) *Exp. Cell Res.* **114**, 153–160.
26. Rollins, B. J. & Culp, L. (1979) *Biochemistry* **18**, 5621–5630.
27. Barnhart, B. J., Cox, S. H. & Kraemer, P. M. (1979) *Exp. Cell Res.* **119**, 327–332.
28. Perkins, M. E., Ji, T. H. & Hynes, R. O. (1979) *Cell* **16**, 941–952.
29. McGuire, E. J. (1976) *J. Cell Biol.* **68**, 90–100.
30. Gail, M. H., Boone, C. W. & Thompson, C. S. (1973) *Exp. Cell Res.* **72**, 386–390.
31. Henkart, M. P. & Nelson, P. G. (1979) *J. Gen. Physiol.* **73**, 655–673.
32. Tupper, J. T., del Russo, M., Hazelton, B. & Zorgniotti, F. (1978) *J. Cell. Physiol.* **95**, 71–84.