Purification of the Major Sialoglycoproteins of 13762 MAT-B1 and MAT-C1 Rat Ascites Mammary Adenocarcinoma Cells by Density Gradient Centrifugation in Cesium Chloride and Guanidine Hydrochloride*

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Both MAT-B1 (nontransplantable) and MAT-C1 (xenotransplantable) ascites sublines of the 13762 rat mammary adenocarcinoma contain a major sialoglycoprotein (ASGP-1) with low electrophoretic mobility in dodecyl sulfate. ASGP-1 from each subline is rapidly purified by density gradient centrifugation in cesium chloride containing 4 M guanidine hydrochloride. The products give single bands on dodecyl sulfate-polyacrylamide gel electrophoresis and single peaks by gel filtration in dodecyl sulfate or guanidine hydrochloride. The composition of ASGP-1 from each subline is high in serine, threonine, galactosamine, glucosamine, galactose, and sialic acid, with carbohydrate compositions of 67 and 72% for MAT-B1 and MAT-C1, respectively. Amino acid compositions of ASGP-1 from the sublines are essentially the same. The major difference between them is a 3-fold greater content of sialic acid/unit of protein for MAT-C1 ASGP-1 than MAT-B1 ASGP-1. Molecular weights of 570,000 (MAT-B1) and 690,000 (MAT-C1) are estimated by sedimentation velocity analysis and gel filtration in 4 M guanidine hydrochloride. Fractionation of oligosaccharides from alkaline borohydride-treated ASGP-1 on Bio-Gel P-4 gives patterns that are quantitatively different for MAT-B1 and MAT-C1. The larger of the two major oligosaccharides from MAT-C1 (IIc) is larger than either of the two major oligosaccharides from MAT-B1, while the smaller of the two major MAT-B1 oligosaccharides (IVB) is smaller than either of the major MAT-C1 oligosaccharides. The size differences are consistent with the oligosaccharide compositions, showing increased amounts of sialic acid for the larger oligosaccharides. Differences in MAT-B1 and MAT-C1 ASGP-1 at the oligosaccharide level may account for the differences in ability of the sublines to be transplanted across species histocompatibility barriers.

It has been suggested that cell surface glycoproteins may play important roles in the escape of tumors from immune surveillance (1). For example, two sublines of the TA3 mouse mammary adenocarcinoma have been studied which differ in morphology, in agglutinability by concanavalin A, and in their ability to be transplanted across strain or species histocompatibility barriers (2). By proteolytic release (3) or lectin binding analyses (4) the allo- and xenotransplantable TA3-Ha ascites cells show large amounts of a major sialoglycoprotein, termed epiglycanin. The strain-specific TA3-St ascites cells have essentially none of this sialoglycoprotein (2, 4). It has been suggested that the presence of this sialoglycoprotein at the cell surface permits the TA3-Ha cells to survive immune destruction in the foreign host either by "masking" the histocompatibility antigens (2, 5) or by being released from the cell surface (6) to block immune destruction of the tumor (7, 8).

MAT-B1 and MAT-C1 ascites sublines of the 13762 rat mammary adenocarcinoma resemble the sublines of the mouse TA3 tumor in several respects. MAT-B1 and MAT-C1 cells differ in morphology (9), agglutinability by concanavalin A (9), cell surface sialic acid content, and xenotransplantability (1). MAT-C1 cells transplant into mice; MAT-B1 do not. However, MAT-B1 and MAT-C1 differ from the corresponding TA3 sublines in one important respect. Both of them have large amounts of a major cell surface sialoglycoprotein (ASGP-1) identified by chemical, enzymatic and metabolic labeling. Upon labeling with [3H]glucosamine ASGP-1 contains >70% of the label incorporated into trichloroacetic acid-precipitable radioactivity in both MAT-B1 and MAT-C1 cells. Thus, the differences in transplantability must be due to factors other than the presence of ASGP-1. Included among these might be the structure of the sialoglycoprotein, its organization at the cell surface, shedding from the cell surface, and morphology of the cells. The predominance of ASGP-1 at the cell surface suggests that it plays a role in the cell surface properties which are important to survival of the tumor cells.

To evaluate possible functions of ASGP-1, it is necessary to have more information about the structure of the intact glycoprotein. However, no general procedure for isolating such glycoproteins has been developed. Studies on epiglycanin have used fragments released from cells by proteolysis. In the present paper we describe the isolation of intact ASGP-1 by centrifugation of membranes in a CsCl gradient containing 4 M GdnHCl. CsCl gradients have been used previously to isolate proteoglycans from cartilage extracts (10, 11), glyco-

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2. The abbreviations used are: GdnHCl, guanidine hydrochloride; SDS, sodium dodecyl sulfate.
proteins from ovarian cyst fluids (12), and ovomucin from egg white (13). CsCl gradients are effective in separating molecules with a high carbohydrate content from protein, particularly when run in the presence of GdnHCl (11, 13). The glycoprotein purified from membranes by centrifugation co-migrated with MAT-B1 and MAT-C1 cellular ASGP-1 on gel electrophoresis in SDS. Compositions and some physical properties of ASGP-1 isolated from the MAT-B1 and MAT-C1 sublines are reported together with analyses of oligosaccharides released from the glycoprotein by alkaline borohydride treatment. The results indicate that, although MAT-B1 and MAT-C1 ASGP-1 are similar in composition and physical properties, there are distinct differences in the oligosaccharides attached to the polypeptide.

**EXPERIMENTAL PROCEDURES**

**Materials—**4-[(1-C)Glucosamine (90 to 60 mCi/mmol), N-[4,5-3H]glucosamine (2 to 6 Ci/mmol), and N-acetylglucosamine (100 Ci/mmol) were from Amersham; Instagel was from Packard; CsCl (99.99%) was from Merck. Carbohydrate standards included inositol (Supelco), N-acetylgalactosamine from Sigma, mannose from Mann Research Laboratories (9), and fucose (Fluka, Bailliere Laboratories); N-acetylgalactosaminyltransferase was prepared from sheep asialo-glycoprotein (11, 14).

**Cells—**MAT-B and MAT-C ascites sublines of the 13762 mammary tumor were obtained from Mason Research Laboratories and were maintained as described previously (15). MAT-B1 and MAT-C1 designate the sublines which arose from the MAT-B and MAT-C lines, respectively, by routine weekly passage for about 1 year in our laboratory (9). The distinguishing features of these sublines (9) have been stable for over 2 years since the original observations. Cells are carried by weekly transfers and as frozen stocks. Cells used for these studies were washed three times with cold Dulbecco's phosphate-buffered saline without calcium or magnesium after recovery from the peritoneal cavity.

**Cell Labeling—**Metabolic labeling with [3H]glucosamine (100 μCi), [14C]glucosamine (50 μCi), or [3H]leucine (100 μCi) was accomplished by injection of the compound in 0.5 ml of 0.9% NaCl into the peritoneal cavity of a tumor-bearing rat approximately 16 h prior to killing and recovery of the cells. This procedure has been shown to incorporate >70% of trichloroacetic acid-precipitable counts from [3H]glucosamine into the major sialoglycoprotein of both MAT-B1 and MAT-C1 cells.

**Preparation of Membranes—**Membranes were prepared by homogenization of hypot尼亚tically treated cells in the presence (15–17) or absence (17) of ZnCl2. Although the ZnCl2-stabilization procedure allowed isolation of envelopes discernible by phase contrast microscopy, a much higher yield of membrane and membrane glycoprotein was obtained by isolation of membrane fragments in the absence of ZnCl2. Thus, the following method was used for all preparative work. Washed cells were suspended in 10 volumes of 10 mM Tris, pH 8.0, and allowed to stand on ice for 2 min prior to centrifugation at 100,000 g for 2 min. The swollen cell pellet was suspended in 10 volumes of 10 mM Tris, pH 8.0, and homogenized by four to five strokes of a Dounce homogenizer with a tight pestle. Immediately after homogenization the suspension was brought to a concentration of 3 mm in MgCl2 by addition of 30 mm MgCl2, 100 mM NaCl. The homogenate was centrifuged at 10,000 g for 1 min and the supernatant was centrifuged at 100,000 g for 10 min. Membrane fragments were collected by centrifugation at 100,000 g for 90 min in an SW 27 rotor.

**Isolation of ASGP-1 from Membrane Fragments—**Preformed CsCl gradients were prepared by gently layering under each other 2 ml each of 4 M GdnHCl, 10 mM Tris, pH 7.4, containing 1.5, 2.0, 2.37, 2.79, and 3.15 mM CsCl in centrifuge tubes (5 X 3 inches). Membrane fragments were suspended in 10 mM Tris, pH 8.0, with 10 mM GdnHCl and 4 X 107 cells in 1 ml in 4 ml GdnHCl and 4 X 107 cells in 1 ml in 4 ml GdnHCl. The mixture was blended vigorously on a Vortex mixer, 1 ml (approximately 5 mg of membrane protein) was layered onto the CsCl gradient, and the gradients were centrifuged in a Beckman Ti75 rotor at 3°C for 4 h at 55,000 rpm. Fractions were pooled as indicated in Fig. 3A to constitute ASGP-1. CsCl and GdnHCl were removed by dialysis/concentration with a collodion bag apparatus. Gel Electrophoresis—SDS-polyacrylamide gel electrophoresis was performed using the system of King and Laemmli (18). Agarose gels consisted of 1% agarose in 90 mM Tris, 2.5 mM EDTA, 90 mM sodium borate, pH 8.2, cast to a height of 10 mm in 5-mm (inside diameter) glass tubes. Samples were solubilized with electrophoresis sample buffer (12) and diluted with the Tris/borate buffer prior to electrophoresis.

**Radioactivity Determinations—**Aliquots of whole cell, membrane, or gradient or column fractions were incubated with 3H]glucosamine and counted in a Packard Tri-Carb scintillation counter. The 3H counts were corrected for 14C spillover when both isotopes were used. Agarose gels slices (1.5 mm) were incubated overnight with 0.2 ml of Soluene 350, mixed with 3 ml of Instagel, and placed in the dark for at least 6 h prior to counting.

**Gel Filtration with 1% SDS—**ASGP-1 was prepared from [3H]glucosamine-labeled MAT-B1 and [14C]glucosamine-labeled MAT-C1 cells as described above, dialyzed and concentrated into distilled water using a collage bag apparatus, and mixed and heated with SDS-solubilized whole cells. Alternatively ASGP-1 from the two cell types was mixed prior to dialysis and was solubilized with 2% SDS, 0.063 μM Tris (pH 6.8), 0.25% mercaptoethanol. A volume of about 0.5 ml was applied to a Sepharose 2B column (0.9 X 50 cm) equilibrated with 1% SDS, 50 mM Tris, pH 8.0, and eluted with the same buffer. Void volume and retained volume were estimated from A280 peaks produced by protein in the solubilized cell samples.

**Sedimentation Velocity—**ASGP-1 was concentrated/dialyzed into 4 ml GdnHCl, 10 mM Tris, pH 6.8, with a collodion bag apparatus. Sedimentation velocity was performed in a Beckman model E ultracentrifuge with schlieren optics at a temperature of 21°C and a speed of 38,440 rpm.

**Isopycnic Centrifugation—**Cells which had been labeled with [3H]glucosamine were used to prepare ASGP-1. A volume of 100 μl of ASGP-1 (approximately 2 X 10^6 cpm) was mixed with 10 ml of either 2.37 M CsCl, 4 M GdnHCl, 10 mM Tris (pH 7.4) or 4.42 M CsCl, 10 mM Tris (pH 7.4) in a centrifuge tube (6 X 3 inches), and the tubes were filled and balanced with the same buffer. The samples were centrifuged at 4°C for 40 h at 55,000 rpm in a Ti 75 rotor, and fractionated as described above.

**Alkaline Borohydride Treatment—**A sample of ASGP-1 which had been dialyzed against distilled water was incubated at 45°C with 1.0 M NaBH4, 0.05 M NaOH for 16 h and neutralized with acetic acid. The mixture was chromatographed on Bio-Gel P-4 (200 to 400 mesh) equilibrated with 0.1 M pyridine/acetic acid, pH 6.0. The column was calibrated with stachyose, raffinose, maltose, and galactose. Oligomers of hyaluronic acid (umbilical cord), obtained by digestion with hyaluronidase (19), provided additional calibration.

**Amino Acid Analysis—**Aliquots of ASGP-1 were lyophilized in borosilicate ampules and hydrolyzed with 6 N HCl for 17, 24, or 48 h at 110°C. Analysis was performed on a single-column system equipped with an Autolab integration module; norleucine was used as an internal standard.

**Carbohydrate Analysis—**ASGP-1 isolated from approximately 3 X 10^9 ascites cells which had been labeled with [3H]glucosamine was treated and chromatographed on a column (1.0 X 114 cm) of Bio-Gel P-4 as described above. The chromatograms for MAT-B1 and MAT-C1 were not significantly different from the double-labeled profile shown in Fig. 7. Fractions comprising each peak were pooled as indicated in Fig. 7, lyophilized, and suspended in distilled water. Aliquots of oligosaccharides or ASGP-1 were hydrolyzed in borosicatic amic and hydrolyzed with 0.5 ml of 0.5 M methanolic HCl for 1/4 or 1/6 h at 65°C. Carbohydrate analysis by gas chromatography of trimethylsilyl derivatives was performed by the method of Reinhold (20) with inositol as the internal standard.

**RESULTS**

**Incorporation of [3H]Glucosamine by MAT-B1 and MAT-C1 Cells—**Following a 16-h labeling period, MAT-B1 and MAT-C1 cells incorporated 100 to 150 cpm/μg of protein of [3H]glucosamine into trichloroacetic acid-precipitable material. SDS-gel electrophoresis revealed >70% of this radioactivity was associated with a component of low electrophoretic mobility (Fig. 1, E and F), termed ASGP-1 [3H]Glucosamine, therefore, was used routinely in the isolation of ASGP-1 and radioactivity was monitored during the isolation procedure.

To determine the per cent of [3H]glucosamine which was incorporated as sialic acid, samples of MAT-B1 and MAT-C1 ASGP-1 were hydrolyzed with 0.5 M H2SO4 at 80°C for 1 h
Centrifugation of supernatants from trypsin-treated cells (Fig. 2B) shows a comparable peak, demonstrating that ASGP-1 can be released from whole cells by trypsin and, thus, is present at the external surface of the cell. The peak is at the same density as that for whole cells but appears broadened, indicating some degradation.

**Isolation of ASGP-1**—These results suggested that it might be possible to purify ASGP-1 from the ascites cells by a one-step gradient procedure. In practice this proved difficult because the presence of DNA complicated the fractionation procedure. For this reason, only about $2 \times 10^7$ cells could be fractionated on each gradient. Therefore, plasma membranes were isolated prior to centrifugation. Originally membrane envelopes were prepared by a Zn$^{2+}$-stabilization procedure. Typically 5 to 10% of whole cell radioactivity was recovered in the envelopes and less than 5% in a fraction of density 1.38 to 1.42 g/ml following CsCl density gradient centrifugation. Much higher yields were possible by isolation of membrane fragments (Table I). Density gradient profiles in 4 M GdnHCl and CsCl for membrane fragments from cells labeled with $[^3H]$glucosamine and $[^3H]$leucine are shown in Fig. 3. The bars in Fig. 3A denote the fractions which are pooled to constitute ASGP-1. The profile in Fig. 3B demonstrates that

![Fig. 2 (left). Centrifugation of $[^3H]$glucosamine-labeled cells and trypsin-treated cells in CsCl gradients containing 4 M GdnHCl. Labeling and conditions of centrifugation are described under "Experimental Procedures." Profiles of MAT-B1 (---) and MAT-C1 (----) are plotted on the same graph. Density curves (- - - -) coincide within experimental uncertainty. A, cells which had been solubilized with electrophoresis sample buffer containing SDS, B, cells (2 $\times$ 10$^7$/ml) were incubated at 37°C for 30 min with 0.1 mg/ml of trypsin and 0.1 mg/ml of soybean trypsin inhibitor was added. Cells were removed by centrifugation at 120 $\times$ g for 30 min and the supernatant was recentrifuged at 27,000 $\times$ g for 15 min. A volume of 100 $\mu$l of supernatant was applied to each gradient.

![Fig. 3 (right). Centrifugation of membrane fragments from $[^3H]$glucosamine and $[^3H]$leucine-labeled cells in CsCl gradients containing 4 M GdnHCl. Conditions for labeling, membrane isolation, and centrifugation are described under "Experimental Procedures." Profiles from MAT-B1 (---) and MAT-C1 (----) are plotted on the same graph. Density curves (- - - -) coincided within experimental uncertainty. A, membrane fragments from $[^3H]$glucosamine-labeled cells. B, membrane fragments from $[^3H]$leucine-labeled cells. The fraction at the top of the gradient contained 5000 cpm.](http://www.jbc.org/)

and chromatographed on Bio-Gel P-4. Approximately 17% of MAT-B1 and 41% of MAT-C1 radioactivity was released from ASGP-1.

**Density Gradient Centrifugation**—Preliminary experiments were performed by isopycnic centrifugation of SDS-solubilized cells in CsCl gradients containing 4 M GdnHCl to estimate the carbohydrate composition of ASGP-1 from its density. A broad band of radioactivity was observed at a density of about 1.4 g/ml when the samples were centrifuged at 38,000 rpm for 40 h in a Beckman SW 65 rotor (data not shown). Electrophoresis indicated that this band co-migrated with the major glycoprotein, ASGP-1, from SDS-solubilized cells. By using preformed gradients in a high speed angle-head rotor, a much sharper band was observed at the same density, and separation of ASGP-1 from protein and lipid could be achieved in less time. The gradients combine the fractionation of rate-zonal and isopycnic centrifugation. Although sucrose gradients generally combine these features and preformed CsCl gradients have not generally been used as a preparative tool.

Centrifugation of SDS-solubilized cells which had been labeled with $[^3H]$glucosamine gave the radioactivity profiles in Fig. 2A. Both sublines have a dominant peak centered at a density of 1.40 $\pm$ 0.02 g/ml. The time of centrifugation was altered from 16 to 40 h without apparent effect on the radioactivity profile. Fresh cells gave the same profile as SDS-solubilized or Triton X-100-treated cells. Guanidine HCl was required, however, since the radioactivity remained at the top of the gradient with protein and lipid when it was omitted.

![Fig. 1. SDS-polyacrylamide gel electrophoresis of MAT-B1 and MAT-C1 cells and purified ASGP-1. Conditions of labeling with $[^3H]$glucosamine, isolation of ASGP-1, and electrophoresis are described under "Experimental Procedures." For each set, MAT-B1 is on the left and MAT-C1 is on the right. A and B, cells, Coomassie blue stain; C and D, purified ASGP-1, Coomassie blue stain; E and F, cells, fluorogram; G and H, ASGP-1, fluorogram.](http://www.jbc.org/)
most of the protein from membrane fragments remains at the top of the gradient. Approximately 15% of whole cell [3H]-glucosamine is recovered in ASGP-1 (Table I). Recovery of [3H]leucine suggests that at least 0.5% of the total cell protein is associated with ASGP-1.

Characterization of Purified ASGP-1 by SDS Gel Electrophoresis—When the labeled material purified from the gradient was compared with labeled cells solubilized in SDS by gel electrophoresis, the radioactivity from the gradient was observed to migrate in the position of the component designated previously ASGP-1 (Fig. 1). The minor bands apparent in fluorograms of SDS-solubilized cells are absent from the ASGP-1 preparation. Coomassie blue stain revealed only one band for the purified glycoprotein (Fig. 1, C and D) which barely penetrated a 5% polyacrylamide gel. No evidence for protein contamination was detected in fluorograms of ASGP-1 from [3H]leucine-labeled cells or in electrophoretic profiles of ASGP-1 treated with 125I and chloramine-T in SDS (data not shown).

Since the glycoprotein does not appear to migrate well on 5% polyacrylamide gels, electrophoresis was also performed on 1% agarose gels. A single band of Coomassie blue stain and a single peak of radioactivity was observed for both MAT-B1 and MAT-C1 ASGP-1 (data not shown).

Characterization of ASGP-1 by Gel Chromatography—MAT-B1 and MAT-C1 cells were labeled metabolically with both [3H]- and [14C]glucosamine and ASGP-1 containing each isotope was isolated from each subline. This enabled us to compare ASGP-1 directly solubilized from cells by SDS with ASGP-1 purified from membranes of each cell type. We also compared ASGP-1 purified from membranes of MAT-B1 and MAT-C1 cells. Gel filtration in SDS showed that gradient-purified ASGP-1 is as large as the largest labeled species of SDS-solubilized cells for both sublines (Fig. 4, A and B). Low molecular weight, labeled material has been removed by the purification procedure. The comparison of ASGP-1 from MAT-B1 and MAT-C1 cells (Fig. 4C) indicates that the MAT-C1 glycoprotein may be slightly larger than ASGP-1 from MAT-B1 cells. However, both the electrophoretic and gel chromatographic results in SDS should be viewed with some caution, as glycoproteins are known to behave anomalously in both systems (22, 23).

Sedimentation Velocity of ASGP-1—Purified ASGP-1 from each cell line gave a single peak in the ultracentrifuge when sedimented in 4 M GdnHCl, 10 mM Tris, pH 6.8 (Fig. 5). MAT-C1 ASGP-1 appeared more heterogeneous than the MAT-B1 glycoprotein, and the sedimentation coefficient was larger for the MAT-C1 (8.0 S) compared with the MAT-B1 (6.2 S) glycoprotein. The effect of concentration of the glycoprotein

<p>| Table 1 |
|-----------------|-----------------|-----------------|
| Isolation of ASGP-1 from [3H]glucosamine and [3H]leucine-labeled cells |
| Conditions of labeling and isolation are described under “Experimental Procedures.” Values in the table represent per cent radioactivity recovered with respect to washed cells. |</p>
<table>
<thead>
<tr>
<th>Cells</th>
<th>[%] Glucosamine</th>
<th>[%] Leucine</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAT-B1</td>
<td>Membrane fragments</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>ASGP-1</td>
<td>15</td>
</tr>
<tr>
<td>MAT-C1</td>
<td>Membrane fragments</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>ASGP-1</td>
<td>15</td>
</tr>
</tbody>
</table>

Fig. 4. Gel filtration of solubilized whole cells and ASGP-1 on Sepharose 2B with 1% SDS. Preparation of samples and conditions of chromatography are described under “Experimental Procedures.” A, [14C]glucosamine-labeled MAT-B1 cells (■) solubilized in SDS and mixed with [3H]glucosamine-labeled MAT-B1 ASGP-1 (○); B, [3H]glucosamine-labeled MAT-C1 cells (■) solubilized in SDS and mixed with [14C]glucosamine-labeled purified MAT-C1 ASGP-1 (■); C, [3H]glucosamine-labeled, purified MAT-B1 ASGP-1 (○) mixed with [14C]glucosamine-labeled, purified MAT-C1 ASGP-1 (■).
on the sedimentation coefficient was not examined for the MAT-C1 ASGP-1 due to the limited amount of material. The sedimentation coefficient of the MAT-B1 glycoprotein was found to increase with decreasing concentration, although in no case was it as high as 8.0 S.

When ASGP-1 from either subline is dialyzed into 0.9% NaCl, 10 mM Tris, pH 7.4, the glycoprotein appears to aggregate. Schlieren patterns (not shown) indicate considerable heterogeneity with apparent sedimentation coefficients of 35 S and 48 S for MAT-B1 and MAT-C1 ASGP-1, respectively. The heterogeneity with apparent sedimentation coefficient of the MAT-B1 glycoprotein was found to increase with decreasing concentration, although in no case was it as high as 8.0 S.

Isopycnic Centrifugation of ASGP-1—Gel chromatography in both SDS and 4 M GdnHCl suggests there is an apparent size difference between ASGP-1 isolated from MAT-B1 and MAT-C1 cells, which might indicate a difference in composition between the glycoproteins from the two cell lines. The profiles of [3H]glucosamine-labeled ASGP-1 after isopycnic centrifugation (Fig. 6) indicate a slight density difference between the two species when centrifuged in the presence of 4 M GdnHCl (MAT-B1, 1.406 g/dl; MAT-C1, 1.411 g/ml). When centrifuged with a low concentration (0.1 M) of GdnHCl, the apparent density of both species is increased. A similar phenomenon has been reported for acidic molecules such as hyaluronic acid (24). The MAT-C1 glycoprotein again appears denser than MAT-B1 ASGP-1 (MAT-B1, 1.482 g/ml; MAT-C1, 1.506 g/ml). This suggests that MAT-C1 ASGP-1 may have a higher carbohydrate/protein ratio or may contain a higher percentage of acidic residues, which bind Cs⁺.

Composition of ASGP-1—The amino acid compositions of MAT-B1 and MAT-C1 ASGP-1 (Table II) are remarkably similar. The composition of each appears dominated by the amino acids serine, threonine, and glutamic acid. The high content of galactosamine, serine, and threonine suggests a mucin type glycoprotein in which a majority of the serine and threonine residues are glycosylated. Alkaline borohydride treatment destroys >50% and >60% of MAT-B1 and >55% and >75% of MAT-C1 ASGP-1 serine and threonine, respectively.

The carbohydrate compositions of MAT-B1 and MAT-C1 ASGP-1 (Table II) indicate that they are mucin-type glycoproteins. The low content of mannose suggests that few or no nitrogen-linked complex oligosaccharides are present. Glucose is probably a contaminant introduced during the dialysis/concentration step. Although the MAT-B1 glycoprotein appears to have higher amounts of galactose and glucosamine than MAT-C1 ASGP-1, the most dramatic differences appear in the content of sialic acid. The sialic acid content is 3-fold higher/unit of protein in MAT-C1 ASGP-1 than MAT-B1 ASGP-1. The total carbohydrate composition of MAT-C1 is also slightly higher (73%) than MAT-B1 ASGP-1 (67%).

Characterization of Oligosaccharides Released by Alkaline Borohydride Treatment—There are distinct differences in the oligosaccharides released by alkaline borohydride treatment of purified MAT-B1 and MAT-C1 ASGP-1. Labeling studies reveal that the MAT-C1 oligosaccharides appear predominantly in peaks II and III (Fig. 7) upon gel filtration. The majority of MAT-B1 oligosaccharides are found in peaks III

![Fig. 6. Isopycnic centrifugation of ASGP-1 from [3H]glucosamine-labeled cells. ASGP-1 prepared from cells labeled with [3H]glucosamine was subjected to isopycnic centrifugation as described under "Experimental Procedures." Profiles from MAT-B1 (----) and MAT-C1 (-----) ASGP-1 are plotted on the same graph A, CsCl with 4 M GdnHCl; B, CsCl with 0.1 M GdnHCl. Density curves (-----) coincided within experimental uncertainty.](http://www.jbc.org/)

### Table II

**Composition of ASGP-1**

<table>
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<th>Amino acid</th>
<th>MAT-B1</th>
<th>MAT-C1</th>
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<tbody>
<tr>
<td>Aspartic acid</td>
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<tr>
<td>Threonine</td>
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<td>175</td>
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<td>Serine</td>
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<td>Glutamic acid</td>
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<td>Glycine</td>
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<tr>
<td>Alanine</td>
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<td>Valine</td>
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<td>Methionine</td>
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<td>Isoleucine</td>
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<td>Leucine</td>
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<tr>
<td>Glucosamine</td>
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<td>Galactosamine</td>
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<tr>
<td>Galactose</td>
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</table>

Amino sugar values were obtained by extrapolation of values from amino acid analysis to zero time of hydrolysis. The glucosamine/galactosamine ratios from amino acid analysis and carbohydrate analysis agreed within 2%. Galactosamine was assumed to be the same in both analyses in order to express the values of the other sugars in moles/1000 amino acid residues.
Experimental Procedures." The samples were mixed, alkaline borohydride-treated, neutralized with acetic acid, and chromatographed on a Bio-Gel P-4 column (0.9 cm × 30 cm) equilibrated with 0.9% NaCl, 10 mM Tris, pH 7.4. Fractions were assayed for determination of 3H (MAT-B1) and 14C (MAT-C1) radioactivity. Arrows indicate elution positions of dextran (void volume), stachyose, and galactose.

and IV (Fig. 7). The fact that the 14C and 3H peaks are not coincident at peak III suggests that the MAT-B1 and MAT-C1 oligosaccharides may not be the same. The approximate size of each fraction was estimated to be 2400 (II), 1800 (III), 1100 (IV), and 600 (V).

Compositional analysis of the fractions was informative, even though the oligosaccharides may not be homogeneous. The relative amount of each oligosaccharide fraction observed by glucosamine labeling was supported by the recovery of (galactosamine + galactosaminol) reported in Table III. Alkaline borohydride treatment resulted in conversion of approximately 75% of galactosamine into galactosaminol for both MAT-B1 and MAT-C1 ASGP-1. No galactosaminol was detected in the void volume, suggesting that there are no large oligosaccharides as reported in other systems (25). Essentially no mannose was detected in any of the oligosaccharides, and most of the glucose eluted in the void volume.

For the MAT-B1 oligosaccharides, the ratio of glucosamine and galactose to galactosaminol decreased, whereas fucose increased as the size of the oligosaccharide decreased. For the MAT-C1 oligosaccharides, glucosamine and galactose remained constant and fucose increased as the size of the oligosaccharide decreased. A dramatic drop in sialic acid content occurred between Fractions III and IV for both MAT-B1 and MAT-C1 oligosaccharides. The average sizes of the oligosaccharides tabulated from the composition data (assuming 1 galactosaminol) are consistent with those estimated by gel filtration: VB (700 versus 600), IVB and IVC (930 and 1260 versus 1100), IIB and IIC (1590 and 1720 versus 1800), and IIB and IIC (3240 and 1920 versus 2400). Fraction IIB was a relatively minor component and, thus, the apparent discrepancy in composition and size between fractions IIB and IIC may not be real. The compositions of peak III from MAT-B1 and MAT-C1 glycoproteins are very similar, although the fucose appears higher in the MAT-C1 oligosaccharide.

The major two oligosaccharides (II, III) of the MAT-C1 ASGP-1 are relatively large and rich in sialic acid. In contrast, the MAT-B1 ASGP-1 has a small amount of the large oligosaccharide (II), a comparable amount of fraction III, and a substantial amount of fraction IV, a smaller oligosaccharide which is low in sialic acid.

**DISCUSSION**

Our previous studies have shown that MAT-B1 and MAT-C1 cells differ in morphology (9), concanavalin A receptor mobility (9), and xenotransplantability. Based on studies on the TA3 mouse mammary adenocarcinoma, other workers have postulated that such transplantability differences are the result of masking of histocompatibility antigens by the major cell surface sialoglycoprotein (2, 5). In TA3 cells the transplantable and nontransplantable sublines were distinguished by the presence or absence, respectively, of the sialoglycoprotein epiglycanin (2-4). In 13762 cells the surfaces of both transplantable and nontransplantable sublines appear dominated by a high molecular weight mucin-type glycoprotein similar to epiglycanin. Thus, we have studied the chemical and physical properties of the purified glycoproteins in an effort to detect more subtle differences which might relate to transplantability.

The isolation procedure which we describe has, to our knowledge, never been applied to a membrane glycoprotein. The procedure is simple and may have great potential in isolation of glycoproteins from other membrane sources. The technique, which is based on the density of the molecule, effectively separates protein and lipid from heavily glycosylated molecules. The use of 4 M GdnHCl appears to minimize binding of the detergents SDS and Triton X-100 to the glycoprotein since the densities of ASGP-1 from samples which have been treated with detergent are indistinguishable from

**Table III**

Carbohydrate compositions of oligosaccharides from MAT-B1 and MAT-C1 ASGP-1

<table>
<thead>
<tr>
<th>Oligosaccharide</th>
<th>MAT-B1</th>
<th>MAT-C1</th>
</tr>
</thead>
<tbody>
<tr>
<td>GlcNAcOH</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>GlcNAc</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Galactose</td>
<td>0.86</td>
<td>3.00</td>
</tr>
<tr>
<td>Sialic acid</td>
<td>0.91</td>
<td>5.23</td>
</tr>
<tr>
<td>Fucose</td>
<td>0.27</td>
<td>0.20</td>
</tr>
<tr>
<td>% (GlcNAcOH + GlcNAc) recovered</td>
<td>24.7</td>
<td>7.0</td>
</tr>
</tbody>
</table>

*Values are moles/mole of N-acetylgalactosamine.*
those which have not. In gradients containing low amounts of GdnHCl (<0.1 M), however, Triton X-100 extracts exhibited reduced densities compared with gradient-purified ASGP-1 (data not shown). If both detergent and GdnHCl are omitted, the glycoprotein exhibits a density of about 1.3 g/ml and cannot be separated from other proteins. This indicates that GdnHCl may interact with hydrophobic regions of the molecule and that effective separation may be contingent on suc- GdnHCl may interact with hydrophobic regions of the mole- brane was not successful, even though this molecule contains

However, it may be possible to use this procedure as a more
general glycoprotein isolation method (e.g. to isolate eryth- rhocyte glycoproteins) by first solubilizing the glycoproteins in

SDS and then dialyzing into GdnHCl.

The advantages of the CsCl isolation procedure are evident in the product. Purified ASGP-1 appears to be free of contami-
nating protein or glycoprotein and is homogeneous by sedi-
mentation velocity analysis in 4 M GdnHCl. That intact gly-
coprotein has been isolated is indicated by gel filtration in

SDS showing no degradation of the purified ASGP-1 relative to

that solubilized directly from cells. ASGP-1 released from
cells by Triton X-100 appears somewhat smaller than purified

ASGP-1 (26), showing that gel filtration is capable of

successing the glycoprotein oligosaccharides. Additional studies are

underway to determine the structures of the oligosaccharides

as a prelude to investigating these biosynthetic mechanisms.

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Purification of the major sialoglycoproteins of 13762 MAT-B1 and MAT-C1 rat ascites mammary adenocarcinoma cells by density gradient centrifugation in cesium chloride and guanidine hydrochloride.

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