Purification and Characterization of UDP-N-Acetylgalactosamine: Polypeptide N-Acetylglactosaminytransferase from Bovine Colostrum and Murine Lymphoma BW5147 Cells*

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UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferase has been purified from two sources. A soluble form, purified 517,000-fold to homogeneity from bovine colostrum, has a molecular mass of 70,000 daltons by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and 69,000 daltons by gel filtration. A membrane-bound form, partially purified 2,500-fold from BW5147 mouse lymphoma cells, has a molecular mass of 70,000 daltons by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and 71,500 daltons by gel filtration. The purified colostrum enzyme exhibits specificity for UDP-GalNAc, has its pH optimum between pH 7.2 and 8.6, and requires Mn²⁺ for activity. The Kₘ is 8 μM for UDP-GalNAc and 2.5 mg/ml for deglycosylated bovine submaxillary mucin. Treatments with endo-β-N-acetylglucosaminidases H and F indicate that the colostrum enzyme is a glycoprotein containing two N-linked oligosaccharides. On most enzyme molecules, both oligosaccharides are of the complex type, but some molecules contain one complex type and one high mannose type. Antibodies raised against homogenous bovine enzyme cross-react on immunoblots with a single protein of 71,000 daltons in the partially purified preparation and in a crude microsomal extract from BW5147 cells.

A variety of proteins, including mucins, other secretory proteins, and integral membrane proteins, contain O-glycosidically linked oligosaccharides (see Ref. 1 for a recent review). In contrast to Asn-linked oligosaccharide synthesis which is initiated by the en bloc transfer of a preassembled oligosaccharide from a lipid carrier to the nascent protein (2), O-linked oligosaccharide synthesis begins with the transfer of N-acetylgalactosamine from its nucleotide sugar donor to serine or threonine residues on the protein (3). Additional sugars may then be added, one at a time, to form a great variety of oligosaccharide structures. The enzyme UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferase has a key role in O-linked glycosylation, catalyzing the first step in the assembly of these structures. Although a number of kinetic studies have been performed with partially purified enzyme preparations from bovine, ovine, and porcine submaxillary glands, the enzyme has not been fully characterized (4-8). More recently, Sugiu et al. (9), using affinity chromatography on apomucin-Sepharose, reported a purification of the transferase to near homogeneity from ascites hepatoma AH66 cells. A number of the properties of this enzyme were studied.

In this paper we have used a slight modification of the procedure of Sugiu et al. to purify the soluble N-acetylgalactosaminyltransferase of bovine colostrum to electrophoretic homogeneity. Our preparation differs from the ascites hepatoma enzyme in several aspects. We also report a 2500-fold purification of an intracellular membrane-bound form of the enzyme from murine lymphoma BW5149 cells and describe an antibody raised against the purified colostrum transferase that cross-reacts with the intracellular murine lymphoma BW5149 enzyme.

**EXPERIMENTAL PROCEDURES**

**RESULTS**

The procedures used to purify the N-acetylgalactosaminyltransferase from bovine colostrum and BW5147 lymphoma cells are described in the Miniprint Section. The following sections deal with some of the properties of the enzymes.

**Enzymatic Properties of the Bovine Colostrum Transferase**

**Donor Specificity**—The purified N-acetylgalactosaminyltransferase is highly specific for UDP-GalNAc, as shown in Fig. 6. Of the nucleotide sugars tested, only UDP-GalNAc competed effectively with the radiolabeled sugar nucleotide under the conditions used. A 50% decrease in counts incorporated was achieved at an added nucleotide concentration of ~18 μM similar to the theoretically expected value of 15 μM, the donor concentration used in the assay.

**Effect of Substrate Concentration**—The N-acetylgalactosaminyltransferase has an apparent Kₘ for UDP-GalNAc of 8 μM. The concentration of apomucin required for half-maximal

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reaction velocity was 2.5 mg/ml. The acceptor concentration is expressed as mg of protein/ml rather than M due to the heterogeneity in the apomucin preparation.

**pH Dependence**—The assays to determine the pH optimum were performed in the absence of added Mn²⁺, since this cation caused precipitates at pH >8. Three different buffers were used covering a pH range from 5.0 to 10.6. Under these conditions the purified enzyme was found to have a broad pH optimum between 7.2 and 8.6 (Fig. 7).

**Ion Dependence**—The purified transferase is dependent on divalent cations (Table II). The presence of a 0.5 mM EDTA completely inhibited the enzyme. This effect was suppressed by performing the assay in the presence of excess Mn²⁺. In the absence of added Mn²⁺, the enzyme retained ~60% of its activity, indicating that it contains some endogenous divalent cations (Fig. 8). Therefore, when the effect of different cations was measured, the enzyme was preincubated in 0.5 mM EDTA to chelate any endogenously bound cations. Table II shows that of the 9 cations tested, Mn²⁺ was the most effective in restoring the activity. Co²⁺ was almost as effective as Mn²⁺, and Cd²⁺ and Ni²⁺ were able to restore the activity partially. Fig. 8 shows that maximum enzyme activity is reached at ~2.5 mM Mn²⁺.

**Physical Properties of the Bovine Colostrum Transferase**

**Amino Acid Composition**—The amino acid composition of the bovine colostrum enzyme is shown in Table III. Since the analytical method used deaminates asparagine and glutamine, the values for aspartic acid and glutamic acid include also asparagine and glutamine, respectively. The overall composition agrees fairly well with the average composition of proteins as defined by Dayhoff (20). The major differences in glycine, glutamic acid, and cysteine contents which are 208, 65, and 62%, respectively, of the average values.

**Molecular Size**—Comparison of the Sephadex G-100 superfine elution position of the purified N-acetylgalactosaminyltransferase with the elution positions of 5 protein standards yielded a molecular size of approximately 69,000 daltons (Fig. 4A). This is in good agreement with the molecular size of 70,000 daltons calculated from the migration distance of the enzyme on SDS-PAGE (Fig. 5A).

**Evidence for N-Linked Glycosylation**—Studies using affinity chromatography on various lectin columns indicated that the purified N-acetylgalactosaminyltransferase probably contained N-linked oligosaccharide units. The protein bound to

**Table II**

| Assay system | pmol transferred | % of complete |
|--------------|-----------------|---------------|
| Complete     | 111             | 100           |
| +EDTA, -Mn²⁺ | 0               | 0             |
| +EDTA, +Mn²⁺ | 111             | 100           |
| +EDTA, +Co²⁺ | 96.4            | 87            |
| +EDTA, +Ca²⁺ | 8.5             | 7.7           |
| +EDTA, +Mg²⁺ | 0.4             | 0             |
| +EDTA, +Cd²⁺ | 50.0            | 45.1          |
| +EDTA, +Zn²⁺ | 0               | 0             |
| +EDTA, +Cu²⁺ | 12.3            | 11.1          |
| +EDTA, +Hg²⁺ | 0               | 0             |
| +EDTA, +Ni²⁺ | 33.6            | 30.3          |

and could be partially eluted with the appropriate hapten sugars from concanavalin A-Sepharose and lentil lectin-Sepharose (data not shown). When the ¹²⁵I-labeled enzyme was treated with endo-β-N-acetylglucosaminidases H and F and analyzed by SDS-PAGE/autoradiography, a distinct shift in migration position was observed for the endo F-treated material (Fig. 9, lanes 3, 4, 8, and 9). The magnitude of the shift corresponds to a M, difference of approximately 5000. By contrast, most of the material was resistant to endo H with only a small fraction shifting to a lower M, (Fig. 9, lanes 2 and 7). This pattern was not altered by increasing the incubation time and/or the amount of added endo H (data not shown). The faint 60,000-dalton band is a contaminant, most likely immunoglobulin heavy chain, present in the preparation used for these experiments.

**Immunological Properties of the Transferase from Bovine Colostrum and Murine Lymphoma BW5147 Cells**

**Characterization of Antiserum**—Rabbit antibodies raised against purified bovine colostrum N-acetylgalactosaminyltransferase precipitate transferase activity from a partially purified (Apomucin Sepharose II eluate) preparation of the colostrum enzyme (Fig. 10A). In this experiment, 72 µg of IgG (corresponding to 50 µl of antiserum) added to an incubation mixture containing 69 units of N-acetylgalactosaminyltransferase precipitated 81% of the activity or approximately 30 ng of transferase (based on a specific activity of 1860 units/mg). A purified IgG fraction was used rather than whole antiserum since rabbit serum contains interfering N-acetylgalactosaminyltransferase activity. When titrating the antiserum with purified ¹²⁵I-labeled bovine colostrum transferase and could be partially eluted with the appropriate hapten sugars from concanavalin A-Sepharose and lentil lectin-Sepharose (data not shown). When the ¹²⁵I-labeled enzyme was treated with endo-β-N-acetylglucosaminidases H and F and analyzed by SDS-PAGE/autoradiography, a distinct shift in migration position was observed for the endo F-treated material (Fig. 9, lanes 3, 4, 8, and 9). The magnitude of the shift corresponds to a M, difference of approximately 5000. By contrast, most of the material was resistant to endo H with only a small fraction shifting to a lower M, (Fig. 9, lanes 2 and 7). This pattern was not altered by increasing the incubation time and/or the amount of added endo H (data not shown). The faint 60,000-dalton band is a contaminant, most likely immunoglobulin heavy chain, present in the preparation used for these experiments.

**Table III**

| Amino acid | residues/mol² |
|------------|--------------|
| Aspartic acid | 47           |
| Threonine   | 27           |
| Serine      | 55           |
| Glutamic acid | 68          |
| Proline     | 27           |
| Glycine     | 83           |
| Alanine     | 31           |
| Cysteine    | 8            |
| Valine      | 29           |
| Methionine  | 8            |
| Isoleucine  | 21           |
| Leucine     | 35           |
| Tyrosine    | 14           |
| Phenylalanine | 15          |
| Histidine   | 12           |
| Lysine      | 24           |
| Arginine    | 22           |
| Tryptophan  | ND           |

² Values corrected for 7% carbohydrate content.
³ Includes asparagine.
⁴ Includes glutamine.
⁵ Determined as cysteic acid after performic acid oxidation.
⁶ Determined as methionine sulfone after performic acid oxidation.
⁷ ND, not determined.

The abbreviations used are: SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; PMSF, phenylmethylsulfonyl fluoride; α-MEM, minimal essential medium; Endo F and H, endo-β-N-acetylglucosaminidase F and H, respectively; l, liter.
precipitable with trichloroacetic acid. Expected, based on the previous characterization of the purified gel was sedimented at 10,000 g for 30 min. Unreacted iodine was removed by gel filtration on a Sephadex G-25 column equilibrated with 20 mM imidazole, pH 7.2, 150 mM NaCl, 1 mg/ml bovine serum albumin, 0.1% taurodeoxycholate, 1 mg/ml KI followed by extensive dialysis against 20 mM imidazole, pH 7.2, 150 mM NaCl, 0.1% taurodeoxycholate. Aliquots of the iodinated enzyme (45 ng, ~100,000 cpm) were digested with endo H or endo F and analyzed on SDS-PAGE followed by autoradiography as described under "Experimental Procedures." Samples loaded on the gel contained 3600–4800 cpm. Autoradiographic exposure time was 5 h. The migration positions of molecular weight standards are indicated to the right. Lanes 1, 5, 6, and 10, transferase incubated with endo H buffer but without endo H. Lanes 2 and 7, SDS-denatured (see "Experimental Procedures") transferase digested with endo H. Lanes 3 and 8, native transferase digested with endo F. Lanes 4 and 9, SDS-denatured (see "Experimental Procedures") transferase digested with endo F. Lanes 1 through 5, samples run under nonreducing conditions. Lanes 6 through 10, samples run under reducing conditions.

**FIG. 10. Immunoprecipitation of bovine colostrum N-acetylgalactosaminyltransferase.** A. 68 milliliters of purified bovine colostrum N-acetylgalactosaminyltransferase was incubated with increasing amounts of anti-N-acetylgalactosaminyltransferase IgG in PBS for 18 h on ice in a final volume of 50 μl. 100 μl of a 1:1 suspension of protein A-Sepharose in PBS was then added and the incubation continued for an additional 50 min on ice during which time the tube was vortexed every 5 min. Following incubation, the gel was sedimented at 10,000 × g and the supernatant was assayed for remaining N-acetylgalactosaminyltransferase activity. All values have been corrected for nonspecific adsorption. B, 4 ng (~9000 cpm) of 125I-conjugated bovine colostrum N-acetylgalactosaminyltransferase was incubated with increasing amounts of anti-N-acetylgalactosaminyltransferase antiserum for 18 h on ice. Final volume was adjusted to 50 μl with PBS, and the incubation mixture also contained 0.1% taurodeoxycholate. 100 μl of a 1:1 suspension of protein A-Sepharose in PBS was added to precipitate antigen-antibody complexes and the incubation was continued on ice for 30 min with vortexing every 5 min. The gel was then sedimented at 10,000 × g for 5 min, washed 3 times with PBS containing 0.1% taurodeoxycholate, and counted. Radioactivity precipitable with preimmune serum has been subtracted from each value.

**FIG. 11. SDS-polyacrylamide gel electrophoresis and immunoblotting of UDP-GalNAC-polypeptide N-acetylgalactosaminyltransferase from bovine colostrum and murine lymphoma BW5147 cells.** Triton X-114 extracts from microsomes isolated from murine lymphoma BW5147 cells, partially purified N-acetylgalactosaminyltransferase from murine lymphoma BW5147 cells, and purified N-acetylgalactosaminyltransferase from bovine colostrum were subjected to SDS-PAGE in 10% gels (16). The proteins were transferred electrophoretically to nitrocellulose membranes and incubated with 17 μl/ml anti-N-acetylgalactosaminyltransferase antiserum followed by 100 ng/ml (5.6 × 10^6 cpm) 125I-Protein A. The nitrocellulose membranes were washed, dried, and processed for autoradiography as described under "Experimental Procedures." Lane 1, detergent phase from Triton X-114 extraction of 80 μg of mouse lymphoma BW5147 microsomes; lane 2, 0.22 unit of partially purified N-acetylgalactosaminyltransferase from murine lymphoma BW5147 cells; lane 3, 1.8 units of purified bovine colostrum N-acetylgalactosaminyltransferase.

**DISCUSSION**

UDP-GalNAc-polypeptide N-acetylgalactosaminyltransferase purified to homogeneity from bovine colostrum is a monomer of approximately 70,000 daltons as determined by both SDS-PAGE and gel filtration chromatography. An antibody raised against the purified 70,000-dalton enzyme precipitates the enzyme activity as well as the radiolabeled protein. The difference in the precipitation capacity of the antibody between the two experiments (~0.7 ng of enzyme precipitated/μl when calculated on the basis of activity versus ~1.8 ng/μl when calculated on the basis of the iodinated enzyme protein) is probably due to the presence of inactive enzyme. This would be expected since the enzyme loses activity upon storage. Isolation of the intracellular form of this enzyme from mouse lymphoma BW5147 cells yielded a preparation which, although not homogenous, eluted at a position corresponding to a molecular mass of 71,500 on gel filtration chromatography and contained a band of approximately 70,000 daltons on SDS-PAGE (Figs. 4B and 5B). Blotting of this preparation with anti-bovine colostrum N-acetylgalactosaminyltransferase antibody resulted in a band of approx-
N-Acetylgalactosaminyltransferase

imatively 71,000 daltons. If one assumes that the soluble trans-
ferase is derived from a membrane-bound intracellular form
by proteolytic cleavage, these results indicate either that the
membrane-anchoring portion of the intracellular transferase
is relatively small or that this part of the enzyme is very
sensitive to proteases and is readily cleaved during the puri-
fication procedure. The former possibility seems more likely
since even when a crude membrane extract prepared in the
presence of protease inhibitors is blotted, no species of higher
molecular mass could be detected. The fact that antibody
raised against the soluble bovine enzyme cross-reacts with the
membrane-derived murine enzyme lends further support to
the conclusion that the soluble enzyme is a cleavage product.

In the purification of N-acetylgalactosaminyltransferase
from ascites hepatoma AH66 cells, Sugiura et al. (9) reported
an apparent $M_r$ of 54,000–56,000. The specific activity of this
enzyme was 390 units/mg as compared to 1,860 units/mg for
the bovine colostrum enzyme. This indicates that the soluble
trans-
ferase is a cleavage product, thereby providing direct evi-
dence for its subcellular location.

An intriguing finding is the presence of complex-type Asn-
linked oligosaccharides on the bovine enzyme. This indicates
that the enzyme has been exposed to the late stage oligosac-
charide processing occurs after the enzyme is released into
the colostrum. The availability of the antiserum described in
this report will hopefully enable us to study further the relation-
ship between the intracellular and secreted forms of this enzyme
also to immunolocalize it, thereby providing direct evi-
dence for its subcellular location.

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SUPPLEMENTARY MATERIAL: RECOMMENDATIONS FOR PRODUCTION AND PURIFICATION OF N-ACETYLGALECTOSAMINYLTRANSFERASE FROM Bovine Colostrum and Mammalian Lymphoid Cell Extracts 

**Experimental Procedures**

Materials

1-UDP-GalNAc (10.7 Ci/mmol) was from New England Nuclear (Boston, MA). N-acetylglucosamine (NAG) was from Mann Research Laboratories (San Diego, CA). N-ethylmaleimide was from Sigma Chemical Co. (St. Louis, MO). UDP was from Sigma Chemical Co. (St. Louis, MO). 

Methods

The procedure for N-acetylgalactosamine (GalNAc) transferase assay is described below. The activity of the enzyme was measured by determining the incorporation of 1-UDP-GalNAc into N-acetylgalactosamine by the procedure of Manley and Wolin (10). The reaction mixture contained at 37°C for 30 minutes. 

**Results**

The results of the N-acetylgalactosamine transferase assay are shown in Table 1. The enzyme was purified from bovine colostrum and human cell extracts by the procedures described below. 

**Discussion**

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**Conclusion**

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N-Acetylgalactosaminytransferase

Step 1: Separation of lipid globules and particles. The crude colostrum was first deproteinized by an initial low speed centrifugation. The main purpose of the following dialysis was to adjust the ionic content of the preparation for DEAE-Sephacel chromatography. This procedure however, also appeared to destabilize the remaining particles in the preparation, and thus facilitated their sedimentation during the subsequent high speed centrifugation. The product from step 1 was a clear yellowish solution which could be directly loaded on the DEAE-Sephacel column. The step typically resulted in a 2-3-fold purification with a yield exceeding 90%.

Step 2: DEAE-Sephacel chromatography. Although the bovine colostrum N-acetylgalactosaminyltransferase is not adsorbed to DEAE-Sephacel, even at pH 8.3, this procedure served as a negative purification step, i.e., to remove contaminating proteins which bound to the column. This is of great importance since the capacity of the aquaparin columns used in the subsequent affinity steps appears to be inversely proportional to the amount of contaminating protein loaded.

Step 3: Affinity chromatography on apomucin-Sepharose. This step and the following one are the major bases for the purification. The procedure is adapted from that used by Sugiura, 85, 86, 87 (9). The capacity of the apomucin gel is low (3 U/ml gel) in this first affinity step due to the large amounts of contaminating proteins. In addition, since the enzyme binds poorly at this step, the column was washed with a low ionic strength buffer containing UOP and NaCl which are essential for enzyme binding. The enzyme elutes in a broad peak (low column volumes). The subsequent dialysis against buffer containing NaCl and gel causes UOP to be lost from the elution (which is necessary to be able to assay the peak) and also concentrates the eluate. The concentration of UOP when eluting is 0.2 M, NaCl is 1 M which is suboptimal for the purification of UOP-GalNAc:polypeptide. Therefore the emya preparation is not stable. Therefore the enzyme preparation was first dialyzed against a sodium deoxycholate solution (0.1% final concentration) before concentration and final purification. The dialyzed enzyme was then adsorbed on apomucin-Sepharose 1. This step and the subsequent dialysis caused the product to be resolved into two distinct peaks which were pooled to give a specific activity of 800 units/mg and a yield of 5%.

Step 4: Affinity chromatography on apomucin-Sepharose II. At this point in the purification, the capacity of the affinity column is at least 20 times greater than that of the first affinity step. Furthermore, since the interaction of the enzyme with the bound apomucin is stronger, the column can be washed with up to 10 M NaCl (in the presence of NaCl and UOP) with less than 10% loss of the adsorbed enzyme. The eluted fractions must be dialyzed as soon as possible since the partially purified enzyme rapidly loses activity in the presence of high salt and in the absence of gel. Even with these changes the preparation losses about 20% of its activity per week, particularly after concentration, unless a further stabilizing agent is added. To remedy this problem, a variety of proteins, reducing agents and concentrations of NaCl and MgCl were tried. We found that the addition of a low concentration of a non-denaturing detergent like Triton X-100 or sodium deoxycholate had the most stabilizing effect. Therefore the enzyme preparation was routinely supplemented with 0.02% sodium deoxycholate (0.1% final concentration) during the affinity chromatography. Sodium deoxycholate was chosen because it forms stable solutions at 4°C over a wide pH range and because it is dialytable. Typical elution profiles for apomucin affinity steps are shown in the figure below.

Step 5: Gel filtration chromatography on Sephadex G-100 superfine. The concentrated eluate from the second affinity chromatography step contains two proteins: the N-acetylgalactosaminyltransferase and a larger protein which, based on its behavior on SDS-PAGE in the presence and absence of sodium deoxycholate, is most likely an immunoglobulin. It runs large on SDS-PAGE. A broad lane and splits into smaller peptides. Mr 60,000 and 25,000 daltons respectively, under reducing conditions (see Figure 2 and 3). The G-100 superfine chromatography provides baseline separation between these two proteins. The conditions used (see Experimental Procedures) and the elution profile is shown as a single symmetric peak (Figure 4A). When material from this peak was analyzed by SDS-PAGE only one single protein band could be detected by silver staining (Figure 5A).

Purification of UOP-GalNAc:polypeptide N-Acetylgalactosaminytransferase from Mouse IgG

| Step | Total Protein | Activity | Specific Activity | Purification | Yield |
|------|---------------|----------|-------------------|--------------|-------|
| 1    | 762,300       | 2,758    | 0.0366            | 100          | 1/100 |
| 2    | 270,047       | 0.9487   | 0.0275            | 2.75         | 0.976 |
| 3    | 55,361        | 2,462    | 0.0445            | 12.4         | 89.3  |
| 4    | 703           | 1,896    | 2.637             | 60.7         | 60.7  |
| 5    | 1,152         | 205.6    | 57.11             | 59.0         | 59.0  |
| 6    | 1,880         | 516.67   | 280.1             | 18.9         | 18.9  |

| a | 1 unit equals 1 mol N-acetylgalactosamine transferred to apomucin per minute under assay conditions |
| b | Total initial volume of colloidal 3 x 1 |

Figure 1: Elution profiles (transferase activity) from apomucin-Sepharose. A, Apomucin-Sepharose I. B, Apomucin-Sepharose II. The columns were loaded, washed, and eluted as described in Experimental Procedures. Application of wash and elution buffers are indicated with arrows. 1, 1; wash with buffer containing 0.25 M UOP; 2, elution with buffer D; 3, 1; wash with buffer containing 0.25 M UOP alone and supplemented with NaCl to 0.15 and 0.5 M respectively; 4, elution with buffer D containing 100 M NaCl.

Figure 2: SDS-Polyacrylamide gel electrophoresis of bovine colostrum UOP-GalNAc:polypeptide N-Acetylgalactosaminytransferase. Fractions containing enzyme activity from apomucin-Sepharose I and Sepharose G-100 superfine were concentrated as outlined in Experimental Procedures, mixed with an equal volume of SDS-PAGE sample buffer with or without sodium deoxycholate, and for 1 hour before analysis on a 18 SDS-polyacrylamide gel. After electrophoresis for 1 hour, the gel was stained using the silver staining procedure. The migration positions of five molecular weight standards are shown to the right. Lane 1: sample run in the absence of sodium deoxycholate; Lane 2: sample run in the presence of sodium deoxycholate. A, Material eluted from apomucin-Sepharose I (50 mg); B, Alents of the pooled activity fractions from Sepharose G-100 superfine (1 mg).

Figure 3: SDS-polyacrylamide gel electrophoresis of affinity purified UOP-GalNAc:polypeptide N-Acetylgalactosaminytransferase from mouse IgG. A, IgG. Transferrin eluted from the second affinity step was concentrated as follows: 15 x 1 elute was dialyzed against 20 volumes of 10 M Tris, 0.05 M NaCl, 0.1% Triton X-100, 20% glycerol for 1 hours (2 buffer changes). The dialyzed sample was then loaded on a CM-cellulose column, 0.5 ml bed volume, equilibrated in the buffer used for dialysis. After washing with 5 column volumes of equilibration buffer, the column was eluted with 25 M, 0.05 M, 0.1% Triton X-100 in 10 M Tris, 0.05 M NaCl, 10% glycerol. Fractions were collected, dialyzed against buffer E and assayed for activity. 40% of the fraction containing the highest activity was mixed with an equal volume SDS-PAGE sample buffer and analyzed on a 12 SDS-polyacrylamide gel.
N-Acetylgalactosaminyltransferase

**Figure 1:** Determination of molecular weight of N-acetylgalactosaminyltransferase on gel filtration. Cell extract from bovine colostrum was subjected to analytical gel filtration as described in Experimental Procedures. Standard proteins were: alcohol dehydrogenase (150,000), aldolase (160,000), alkaline phosphatase (80,000), inorganic pyrophosphatase (63,000), peroxidase (40,000), carbonic anhydrase (29,000), and cytochrome c (12,400). A. Bovine colostrum transferase chromatographed on Sephadex G-150. B. Mouse lymphoma 56147 transferase chromatographed on Sephadex G-150.

**Figure 2:** Donor specificity of bovine colostrum N-acetylgalactosaminyltransferase. The nucleotide sugar specificity was determined using the standard assay procedure, but in the presence of different concentrations of unlabeled sugar nucleotides. 0.5 ml of enzyme was used and the assay time was 10 min. The competing effect of UDP-galactose (A), UDP-glucose (B), UDP-α-N-acetylgalactosamine (C), and UDP-α-N-acetylgalactosamine (D) was determined.

**Figure 3:** Determination of pH optimum for bovine colostrum N-acetylgalactosaminyltransferase. Transferrase activity was assayed for 10 min using 0.5 ml of enzyme and the standard assay procedure but in the absence of Mn²⁺ and with buffers of equal ionic strength but different pH substituted for the assay buffer. The buffers used were pH 5.4-7.4, cacodylate; pH 6.0-8.0, Mops; and pH 7.0-9.0, glycine-NaOH.

**Figure 4:** Effect of increasing concentrations of Mn²⁺ on bovine colostrum N-acetylgalactosaminyltransferase. Transferrase activity was assayed using the standard assay procedure but varying the concentrations of added Mn²⁺. 0.5 ml of enzyme was used and the assay time was 10 min.