Biosynthesis of Heparin

SOLUBILIZATION, PARTIAL SEPARATION, AND PURIFICATION OF URIDINE DIPHOSPHATE-GALACTOSE: ACCEPTOR GALACTOSYLTRANSFERASES FROM MOUSE MASTOCYTOMA*

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SUMMARY

The biosynthesis of the neutral trisaccharide, 3-O-β-D-galactosyl-4-O-β-D-galactosyl-D-xylose of the heparin-protein linkage region, has been investigated. A microsomal fraction of heparin-producing mouse mastocytoma catalyzed the incorporation of galactose from UDP-galactose into endogenous as well as exogenous substrates. Partial acid hydrolysis of 14C-galactose-labeled endogenous acceptor yielded several fragments with the characteristics of neutral oligosaccharides previously isolated from the heparin-protein linkage region and, in addition, a compound with the chromatographic properties of N-acetyllactosamine.

The stepwise synthesis of the neutral trisaccharide was investigated with separate assays for the two galactosyl transferase reactions, with exogenous acceptors such as D-xylose (product, 4-O-β-D-galactosyl-D-xylose) and 4-O-β-D-galactosyl-D-xylose (product, 3-O-β-D-galactosyl-4-O-β-D-galactosyl-D-xylose). Treatment of the particulate enzyme with ammonium hydroxide in the presence of Tween 20 resulted in the solubilization of considerable amounts of galactosyltransferase activities. Whereas only 4% of the UDP-galactose-D-xylose galactosyltransferase was brought into solution by treatment of the enzyme with detergent at neutral pH, about one-third of the transferase activity related to the synthesis of the second galactose moiety was recovered in the supernatant under similar conditions, indicating that the two reactions are catalyzed by two different enzymes.

A third galactosyltransferase which catalyzed the transfer of galactose to N-acetylglucosamine was also detected in the microsome particles. This enzyme was solubilized by the same procedure as that used for the two transferases mentioned above but was discriminated from the latter by its stability toward heat and by fractionation with ethanol.

MATERIALS AND METHODS

Most sulfated glycosaminoglycans are in their native state bound to protein or polypeptide via three neutral sugar residues

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1 The abbreviations used are: GlcUA, D-glucopyranosyluronic acid; GlcNAc, 2-acetamido-2-deoxy-D-glucose; Gal-4-Xyl, 4-O-β-D-galactopyranosyl-D-xylose; Gal-3-Gal4-Xyl, 3-O-β-D-galactopyranosyl-4-O-β-D-galactopyranosyl-D-xylose.

UDP-galactose-14C (240 to 280 μCi per μmole) was purchased from Amersham Nuclear Centre or from New England Nuclear. Tween 20 was obtained from Koch-Light Industries. A sample of Gal-4-Xyl was generously supplied by Professor Bengt Lindberg. β-Galactosidase (Escherichia coli) was a gift from Dr.

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Arne Dahlqvist. Other reference compounds mentioned in the text were prepared as described (4).

A heparin-producing FMS mast cell tumor (5, 6) was maintained in the solid state in (A/Sri × Leaden)F1 mice by subcutaneous and intramuscular transplantation in the hind legs every 10 to 14 days.

Analytical Methods—Colorimetric procedures for the determination of pentose, hexose, and protein have been described (7–9). Prior to protein determination, the samples were dialyzed against distilled water or, alternatively, the protein material was precipitated with cold 10% trichloracetic acid and taken up in 1 ml of distilled water or, alternatively, the protein material was subjected to centrifugation as depicted in Fig. 1. The microsomal fraction, sedimenting at 1 × 10^5 × g, was utilized in the initial experiments with exogenous substrates and for studies with the endogenous acceptors.

Preparation of Galactosyltransferases from Mouse Mastocytoma—All procedures were carried out at 0–4°C. Tumor-bearing mice were stunned by a blow and killed by cervical dislocation. The tumors, weighing 0.5 to 1.0 g, were removed and dissected free from adherent tissue. After homogenization in a Virtis 45 homogenizer with 2 volumes of a buffer containing Tris-acetate (50 mM, pH 7.4), KCl (70 mM), and EDTA (1 mM), the crude material was subjected to centrifugation as depicted in Fig. 1. The microsomal fraction, sedimenting at 1 × 10^5 × g, was utilized in the initial experiments with exogenous substrates and for studies with the endogenous acceptors.

Solubilization of Galactosyltransferases—The microsomal fraction was suspended in the buffer mentioned above and Tween 20 was added (final concentration, 2%). The solution was then brought to pH 10.4 to 10.6 by addition of concentrated ammonium hydroxide and quickly readjusted to pH 7.4 by addition of glacial acetic acid (cf. Reference 11). After centrifugation at 1.59 × 10^6 × g for 2 hours, the top 10 ml from each tube (total volume, 13 ml) were withdrawn and aliquots were tested for enzymatic activity as described below.

Transfer of Galactose to Endogenous Acceptor—The microsomal fraction derived from four tumors (0.5 ml; 8 mg of protein) was incubated with UDP-galactose-14C (1 μCi; 240 μCi per μmole) at 37°C for 3 hours. The reaction was stopped by the addition of 1 volume of cold 10% trichloroacetic acid and a neutral sugar fraction was isolated after partial acid hydrolysis of the washed precipitate essentially as described (4), with omission of the papain treatment.

Transfer of Galactose to Exogenous Acceptors—Transfer of galactose to low molecular weight substrates such as xylose or Gal-4-Xyl was studied by incubating the acceptor (0.5 to 5 μmole) at 37°C for 0.5 to 6 hours with UDP-galactose-14C, enzyme fraction (0.01 to 0.75 mg of protein in 0.05 ml of the Tris-acetate buffer mentioned above), and MnCl₂ (10 to 20 mM), in a total volume of 0.05 ml (for exact experimental conditions, see the legends to figures and tables). The kinetic parameters were determined with solubilized enzyme preparations which were obtained by treatment of the microsomal fraction with detergent and alkali as described above.

The reactions were stopped by heating the tubes in a boiling water bath for 2 to 3 min and the mixtures were spotted on paper strips (4 × 40 cm). After removal of anionic derivatives by electrophoresis (Buffer D), the papers were dried and cut at a distance of about 2 cm from the site of application, toward the anode. A wick of filter paper was attached with a sewing machine, and the neutral sugar fraction, which had migrated slightly toward the cathode, was subjected to chromatography in Solvent A. The radioactivity on the chromatograms was located with a strip scanner. Control incubations without
FIG. 2. Paper chromatography of deionized partial acid hydrolysate of \(^{14}C\)-galactose-labeled endogenous acceptors. For assay conditions, see "Materials and Methods." The standards shown on the guide strip are: I, galactose; II, N-acetyllactosamine; III, Gal-4-Xyl; IV, Gal-3-Gal; and V, Gal-3-Gal-4-Xyl.

FIG. 3. Radioscans illustrating the transfer of galactose to xylose (a) before and (b) after treatment of the 1 \(\times\) 10⁴ \(\times\) g pellet fraction (Fig. 1) with detergent and alkali. Incubation mixtures containing xylose (1 \(\mu\) mole), UDP-galactose-\(^{14}C\) (0.05 \(\mu\)Ci; 280 \(\mu\)Ci per \(\mu\)mole), and MnCl\(_2\) (20 mM) were kept at 37\(^\circ\) for 45 min and assayed as described under "Materials and Methods." The migration distances of the products may be correlated with those of the authentic standards shown below the tracings: I, galactose; II, Gal-4-Xyl; and III, Gal-3-Gal-4-Xyl.

The added acceptor were included in all experiments and indicated whether further purification from interfering, labeled substances was necessary. In such cases, the latter materials were adequately separated from the product by subjecting the eluted fraction to paper electrophoresis in Buffer E.

RESULTS

Transfer of Galactose to Endogenous Acceptor  Incubation of the particulate enzyme with UDP-galactose-\(^{14}C\) resulted in incorporation of 3.35 \(\times\) 10⁴ cpm (23\% of added radioactivity) into trichloroacetic acid-precipitable material. Following acid hydrolysis (1\(\text{m}\) HCl; 100\(^\circ\) for 3 hours) and passage of the neutralized solution through columns (2 \(\times\) 4 cm) of Dowex 1 and Dowex 50, all of the radioactivity in the eluate migrated as did galactose on paper chromatography (Solvent B) or paper electrophoresis (Buffer E). About one-third of the radioactivity was recovered as a neutral sugar fraction following partial acid hydrolysis (pH 1.5; 100\(^\circ\) for 5 hours) of the \(^{14}C\)-galactose-labeled trichloroacetic acid precipitate and passage through ion exchange columns. Paper chromatography (Solvent A) of this fraction gave the distribution of radioactivity shown in Fig. 2. The sections indicated were eluted and rechromatographed or further purified by paper electrophoresis in Buffer E. Section A yielded a fraction with the chromatographic properties (Solvents A and C) of N-acetyllactosamine (33 150 cpm; cf. Reference 12), Section B contained a fraction which migrated similar to Gal-4-Xyl (5280 cpm; Solvent A and Buffer E), and Sections C and D showed the presence of materials with mobilities similar to those of Gal-3-Gal (307 cpm) and Gal-3-Gal-4-Xyl (300 cpm) (paper chromatography, Solvent A and paper electrophoresis, Buffer D). Digestion of each fraction with \(\beta\)-galactosidase released all of the radioactivity as free galactose (paper chromatography, Solvent B). Treatment of Fraction C with lead tetraacetate resulted in the formation of a compound with the electrophoretic mobility of Gal-2-Lyx. Since Gal-3-Gal is converted into the latter disaccharide under similar conditions (13), this result, in conjunction with the chromatographic mobility, suggests the identity of Fraction C with the disaccharide, Gal-3-Gal.

Although a detailed structural study of Fractions B to D was not undertaken in the present investigation, the properties observed were in good accord with the characteristics of neutral oligosaccharides previously isolated from the protein-polysaccharide linkage regions of chondroitin sulfate and heparin (13, 14). The results lend support to the conclusion that a significant portion of the galactose incorporated into the trichloroacetic acid-precipitable material in the particulate mouse mastocytoma preparation is transferred to precursors of the heparin-protein linkage region.

The presence in the partial acid hydrolysate of a fragment with the properties of N-acetyllactosamine indicated that other
types of galactosyl transfer reactions also occurred in the enzyme preparation. This observation is further discussed below.

**Transfer of Galactose to Xylose and Gal-$Xy$**—Figs. 3a and 4a show the distributions of radioactivity obtained on paper chromatography after incubating the particulate enzyme with UDP-galactose-14C and xylose or Gal-4-Xyl. Since some interference with endogenous products was observed, the fractions migrating similarly to Gal-4-Xyl or Gal-3-Gal-4-Xyl were eluted with water and subjected to paper electrophoresis in Buffer E. Such a purification step is illustrated in Fig. 5, which shows the complete separation from Gal-3-Gal-4-Xyl of the interfering substances.

Further characterization of the products was obtained as follows. Treatment of the presumed Gal-4-Xyl with β-galactosidase or with 1 M HCl at 100° for 3 hours yielded galactose as the only product (paper chromatography, Solvent B and paper electrophoresis, Buffer E). On partial acid hydrolysis (HCl, pH 1.5; 100° for 5 hours) only two compounds, corresponding in migration to undegraded Gall-Xyl and to galactose, were observed (paper chromatography, Solvent A).

The product of incubation migrated as did authentic Gal-4-Xyl in chromatographic and electrophoretic systems which separate the position isomers of galactosylxylose (Solvents A and C; Buffer E; cf. Reference 13). When applied to a column (2 × 140 cm) of Sephadex G-25, eluted with 10% ethanol, the radioactivity appeared at the effluent volume of Gal-4-Xyl. Since the addition of xylose to the reaction mixture was a prerequisite for product formation, it is concluded that the radioactive substance was identical with Gal-4-Xyl.

The product obtained with Gal-4-Xyl and UDP-galactose-14C possessed the chromatographic (Solvent A) and electrophoretic (Buffer E; Fig. 5) properties of Gal-3-Gal-4-Xyl. Digestion with β-galactosidase released all of the radioactivity as galactose (Solvent B and Buffer E). Further characterization of the radioactive product was obtained by partial acid hydrolysis under the conditions described above for Gall-Xyl. As expected for a compound with the structure, Gal-3-Gal-4-Xyl, labeled in the nonreducing, terminal position, three hydrolytic fragments were observed on paper chromatography in Solvent A (Fig. 6): free galactose (9370 cpm), Gal-a-Gal (705 cpm), and Gal-3-Gal-4-Xyl (620 cpm). Treatment of a sample of the presumed Gal-3-Gal with lead tetraacetate resulted in the production of a compound which migrated similarly to Gal-2-Lyx, well separated from the unoxidized compound, which had moved to the position of Gal-3-Gal (paper electrophoresis, Buffer E). These observations clearly indicate the formation of a β-1 → 3 linkage between the two galactose moieties and the presence, in the mouse mastocytoma preparation, of a UDP-galactose-Gal-4-Xyl galactosyltransferase.

**Transfer of Galactose to N-Acetylglucosamine**—Since the partial acid hydrolysate of the 14C-galactose-labeled endogenous acceptors had shown the presence of a fragment with the mobility of N-acetyllactosamine, free N-acetylglucosamine was also tested as acceptor for galactose. The product formed showed migration characteristics identical with those of the endogenous fragment and yielded galactose on treatment with β-galactosidase. Of the three exogenous substrates tested in the present study, N-acetylglucosamine was the most active galactose acceptor (Table I). A similar result has been obtained in
studies on galactosyltransferase reactions in embryonic chick cartilage (12).

Solubilization and Partial Purification of Galactosyltransferases—Treatment of the particulate enzyme with alkali in the presence of Tween 20 caused a marked stimulation of product formation from UDP-galactose and xylose or Gal-4-Xyl (Figs. 3 and 4, respectively). Generally, a 2- to 4-fold increase of the total activity was observed, with the optimal value resulting from adjusting the pH to about 10.6 for approximately 20 sec. In contrast to the stimulatory effect, observed on the formation of product from xylose and Gal-4-Xyl, the transfer of galactose to the endogenous acceptors was impaired by this procedure, as evidenced by the diminished peaks located at the origins of the chromatograms shown in Figs. 3b and 4b.

The enzymes were quite stable toward prolonged treatment at alkaline pH. No appreciable loss of activity was observed after keeping the microsomal fraction at pH 9.8 for 18 hours at 4°C. The treatment of the particulate enzyme with Tween under alkaline conditions resulted in the solubilization of considerable amounts of galactosyltransferase activity from the particles, as seen by analysis of the supernatant fraction following centrifugation at 1.59 x 10^5 x g for 2 hours. With xylose and Gal-4-Xyl as substrates, analysis of the various enzyme fractions (Fig. 1) revealed that the 25 to 50% ammonium sulfate fraction contained 20 to 25% of the total activity of the crude homogenate, whereas the specific activities of the two reactions had increased 6- to 9-fold, respectively. Interestingly, the ratios of the two products formed varied for the solubilized and particulate enzyme fractions, indicating a preferential release from the microsomes of the UDP-galactose-Gal-4-Xyl galactosyltransferase.

Selective Solubilization of UDP-galactose-Gal-4-Xyl Galactosyltransferase—The findings described above prompted more detailed studies on the solubilization procedure in attempts to separate the two galactosyltransferases under investigation. In the absence of added detergent, no appreciable enzyme activity was found in the supernatant following centrifugation of the alkali-treated particles. However, a considerable portion of the UDP-galactose-Gal-4-Xyl galactosyltransferase activity was brought into solution by treatment with Tween 20 at pH 7.2. This is illustrated in Fig. 7, which shows the release of the transferase activities into the supernatant fluid as a function of pH. By contrast, more than 80% of the UDP-galactose-xylose galactosyltransferase activity was recovered in the particulate fraction in the pH range 7.2 to 9.2, only 4% being solubilized by the detergent at pH 7.2.

The selective solubilization of the UDP-galactose-Gal-4-Xyl galactosyltransferase by treatment with Tween at neutral pH provides strong evidence that the two galactosyl transfer reactions described above, which may represent the synthesis of the galactosamine moiety in the heparin-protein linkage region, are catalyzed by two different enzymes. A similar conclusion, on the basis of substrate competition experiments, was reached in studies on the biosynthesis of the chondroitin sulfate-protein linkage region (4).

Kinetic Studies—The two solubilized enzymes, obtained after treatment of the microsomal fraction at pH 10.6 in the presence of Tween 20 and centrifugation at 1.59 x 10^5 x g, were further characterized as follows. Product formation was linear with time for 3 hours but continued at a slightly reduced rate for at least 5 hours. Product formation was also proportional to the concentration of protein within the range tested (0.25 to 2.5 mg per ml). The enzymes were active over a wide pH range with maximal activity at pH 7.2 to 7.5 (Fig. 8).
The transfer of galactose to xylose as a function of the nucleotide concentration gave an approximate $K_m$ value of $0.3 \times 10^{-4}$ M for UDP-galactose (Fig. 9). At fixed nucleotide concentration, half-maximal velocity occurred at an acceptor concentration of $1.8 \times 10^{-5}$ M (Fig. 10). Similarly, the corresponding $K_m$ values for the UDP-galactose-Gal-4-Xyl galactosyltransferase were $2.5 \times 10^{-4}$ M and $2.1 \times 10^{-2}$ M for UDP-galactose and Gal-4-Xyl, respectively.

Addition of a divalent cation, notably manganese, was required for product formation. Under the conditions tested, Co$^{2+}$ could only partially replace Mn$^{2+}$ (about 10%; Fig. 11). The particulate enzymes, which had not been subjected to the treatment with detergent and alkali, were less specific as to metal requirements. Both Mn$^{2+}$ and Co$^{2+}$ gave comparable yields and the presence of Mg$^{2+}$ or Ca$^{2+}$ also stimulated the enzymes significantly. No product formation was detected with Cu$^{2+}$.

Other properties of the particulate enzyme system which were tested, notably pH dependence and the formation of product as a function of time, were similar to those observed for the solubilized galactosyltransferases.

**Ethanol fractionation of galactosyltransferases in mouse mastocytoma**

To 3 ml of a preparation of the solubilized enzyme (2.5 mg of protein per ml) in Tris-acetate buffer, pH 7.4, was added 1 ml of absolute ethanol at 0°. After 10 min, the precipitate was collected by centrifugation at $20,000 \times g$ for 10 min. The pellet and supernatant fractions (4 ml) were dialyzed against the Tris-acetate buffer, containing 10 mM MnCl$_2$, and assayed for galactosyl transfer activity as described under "Materials and Methods." To determine the activity prior to ethanol fractionation, 1 ml of the Tris-acetate buffer was added to 3 ml of the solubilized enzyme preparation, dialyzed, and incubated with exogenous substrates.

**TABLE II**

| Substrate           | $^3$H pro unit$^a$ |
|---------------------|-------------------|
| Before fractionation| Supernatant fraction | Pellet fraction |
| GlcNAc              | 88,006            | 39,952           | 46,146           |
| Xylose              | 24,810            | 37               | 7,274            |
| Gal-4-Xyl           | 6,793             | 275              | 1,180            |

* Enzymatic product from UDP-galactose-$^3$H (0.1 μCi; 240 μCi per pmole) and substrate after incubation at 37° for 90 min.
* One-half micromole per incubation.
* Two micromoles per incubation.

**Fractionation of UDP-galactose-GlcNAc Galactosyltransferase—** Experiments were carried out which were designed to distinguish between the two galactosyltransferases mentioned above and the UDP-galactose-GlcNAc galactosyltransferase. About 70% of the latter enzymatic activity was recovered in the supernatant after treatment of the microsomal fraction with Tween and alkali under standard conditions (see "Materials and Methods"). The enzyme was partly separated from the two other galactosyltransferases by fractionation with ethanol (Table II). Following centrifugation and dialysis, no synthesis of Gal-4-Xyl occurred in the 25% ethanol fraction, whereas almost half of the...
A solubilized enzyme preparation (2.5 mg of protein per ml) was first incubated as indicated below and cooled on ice. Aliquots were subsequently incubated for 90 min with UDP-galactose-\(^{14}C\) (0.1 \(\mu\)Ci; 210 \(\mu\)Ci per pmole), MnCl\(_2\) (10 mM), and appropriate acceptor. The products were determined by the standard assay procedure (see "Materials and Methods").

### DISCUSSION

The neutral trisaccharide, Gal-3-Gal-4-Xyl, occurs as a conspicuous link in several glycosaminoglycans between the alternating uronic acid and protein moieties of the complex. The synthesis of the two galactose units in this sequence has previously been investigated with a cell-free system from embryonic chick cartilage (3, 4), which has been shown to produce chondroitin sulfate (15, 19). Although an enzyme preparation from mouse mastocyteoma catalyzed the transfer of xylose from UDP-xylose to endogenous material, presumably to heparin precursors (2), no reports have appeared on the incorporation of galactose in such a system. This work has shown the transfer of galactose from UDP-galactose to endogenous acceptors in a particular enzyme preparation from mouse mastocyteoma. The fragments obtained on partial acid hydrolysis of galactose-\(^{14}C\)-labeled endogenous material were apparently analogous to those obtained under similar conditions from embryonic chick cartilage homogenates. The stepwise synthesis of the neutral trisaccharide mentioned above was also studied with exogenous acceptors derived from the protein-polysaccharide linkage region, such as xylose and Gal-4-Xyl. Whether or not these reactions may exclusively reflect the synthesis of the heparin-protein linkage region is unclear. Although heparin constitutes the major polysaccharide in the mast cell tumor, recent experiments in our laboratory have indicated that chondroitin sulfate comprises about 10% of the total polysaccharide synthesized in vivo and in vitro by the neoplastic cells. It is not possible at the present stage to determine whether the biosynthesis of heparin and chondroitin sulfate in the mastocyteoma involves similar or different galactosyltransferases. No evidence for the presence of two enzymes catalyzing the same reaction was obtained, however.

Since heparin is the major polysaccharide of the mast cell tumor used, it is concluded that the transfer of galactose to xylose and to Gal-4-Xyl mainly reflects the synthesis of the galactose residues of the heparin-protein linkage region. The solubilization and partial separation of the two galactosyltransferases afforded more conclusive evidence, in addition to that previously presented for the biosynthesis of chondroitin sulfate (4), for the presence of two independent enzymes completing the synthesis of the Gal-3-Gal-4-Xyl sequence. The properties of the solubilized enzymes differed in some respects from those observed with the chondroitin sulfate-producing system of chick embryo cartilage. Rather than exhibiting maximal activity in the pH range 5.4 to 5.7, the solubilized enzymes both showed a maximum above pH 7. Although stable for more than 2 months when stored at \(-20^\circ\), the solubilized transferases were more labile to heat inactivation than the membrane-bound chick cartilage analogues (Table III, cf. Reference 19). As expected for molecules which may only to some extent resemble the structure of the true substrate (conceivably a glycosyl-peptide precursor of heparin), the \(K_m\) values for xylose and Gal-4-Xyl were considerably higher than those determined for UDP-galactose.

In addition to the characteristic linkage region fragments observed, the partial acid hydrolysis of the galactose-\(^{14}C\)-labeled endogenous product also contained a compound with the chromatographic properties of \(N\)-acytlylgalactosamine as a main hydrolysie fragment. The subsequent demonstration of the UDP-galactose-\(N\)-acetylglucosaminyltransferase supports the conclusion that other types of galactosyl transfer reactions may occur in the mouse mastocyteoma preparation. A similar galactosyl transfer reaction has recently been shown in embryonic chick cartilage (12). Enzymes from several sources which catalyze the transfer

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**Table III**

| Temperature of preliminary incubation | Time of preliminary incubation | \(^{14}C\) product |
|--------------------------------------|--------------------------------|-------------------|
|                                      | min                            | From GlcNAc\(^a\) | From xylose\(^a\) | From Gal-4-Xyl\(^b\) |
| 50\(^o\)                            | 1                              | 92,880            | 30,608            | 9,150              |
| 55                                  | 1                              | 89,171            | 8,806             | 3,310              |
| 55                                  | 5                              | 75,886            | 409               | 1,008              |

*One-half micromole of acceptor per incubation.

*Two micromoles of acceptor per incubation.

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**Figures:**

- Figure 1: A solubilized enzyme preparation (2.5 mg of protein per ml) was first incubated as indicated below and cooled on ice. Aliquots were subsequently incubated for 90 min with UDP-galactose-\(^{14}C\) (0.1 \(\mu\)Ci; 210 \(\mu\)Ci per pmole), MnCl\(_2\) (10 mM), and appropriate acceptor. The products were determined by the standard assay procedure (see "Materials and Methods").

- Figure 2: For each reaction, only 20 to 30% of the total enzymatic activity was recovered. In contrast, essentially all of the UDP-galactose activities of these two enzymes were significantly impaired by the incubation with UDP-galactose and N-acetylglucosamine had decreased but slightly. By comparison, the UDP-galactose-GlcNAc galactosyltransferase showed intermediate characteristics when tested for its lability toward heat.

- Figure 3: These results clearly suggest that the three galactosyl transfer reactions detected in the mouse mastocyteoma preparation are mediated by different enzymes which may be discriminated from each other by fractionation as well as by inactivation procedures.

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2. The different properties observed for the particulate and solubilized enzyme activities, notably metal ion requirements, may not be taken as evidence for the presence of two enzymes catalyzing the same reaction; conceivably, such variations may be explained by conformational differences between the membrane-bound and solubilized enzyme.
of galactose from UDP-galactose to N-acetylglucosamine have been described (17-19).

The interpretation of the results obtained with the endogenous acceptors present in the tumor homogenate is hampered by the lack of knowledge of their structures. Possibly, the UDP-galactose-GlcNAc galactosyl transfer reaction may reflect the synthesis, under physiological conditions, of a similar linkage from UDP-galactose and endogenously bound N-acetylglucosamine residues. It is of note that the transfer of galactose to endogenous material was substantially impeded by the presence of N-acetylglucosamine in the reaction mixture. This result favors but does not conclusively establish a kinship between an enzyme catalyzing the transfer of galactose to a residue of N-acetylglucosamine present in the endogenous acceptor, and the UDP-galactose-GlcNAc galactosyltransferase. Since almost half of the galactose was transferred from UDP-galactose to free N-acetylglucosamine under the conditions used, a similar inhibitory effect might have been caused by depletion of the nucleotide pool.

The separation of the UDP-galactose-GlcNAc galactosyltransferase from the other two transferases under study is in agreement with the results obtained with a particulate enzyme system from embryonic chick cartilage which catalyzes the same reactions (12). In contrast, however, the latter enzyme was extremely labile toward heat, whereas the solubilized enzyme from the mast cell tumor exhibited a marked stability when tested under similar conditions. A more comprehensive characterization of the mouse mastocytoma enzyme was not undertaken in the present study and it is not known at present whether this discrepancy may indicate different physiological roles for the two UDP-galactose-GlcNAc galactosyltransferases investigated.

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