Co-purification of galactosyltransferases from chick-embryo liver

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Two galactosyltransferases with nearly identical $M_r$ values were purified 5000–7000-fold from microsomal membranes of chick-embryo livers by using several affinity columns. One enzyme transfers galactose from UDP-galactose to form a $\beta$-(1$\rightarrow$4)-linkage to GlcNAc ($N$-acetylgalactosamine) or AsAgAGP [asialoagalacto-($\alpha_1$-acid glycoprotein)]. The other enzyme forms a $\beta$-(1$\rightarrow$3)-linkage to AsOSM [asialo-(ovine submaxillary mucin)]. Both enzymes were solubilized (85%) from a microsomal pellet by using 1% Triton X-100 in 0.1 M NaCl. The supernatant activities were subjected to DEAE-Sepharose chromatography and four affinity columns: UDP-hexanolamine–Sepharose, $\alpha$-lactalbumin–Sepharose, GlcNAc–Sepharose and either AsAgAGP–Sepharose or AsOSM–Sepharose. The AsAgAGP enzyme [(1$\rightarrow$4)-transferase] and the AsOSM enzyme [(1$\rightarrow$3)-transferase] behave identically on the DEAE-Sepharose and UDP-hexanolamine–Sepharose columns, and similarly on the $\alpha$-lactalbumin–Sepharose column. Final separation of the two enzymes, however, could only be achieved on affinity columns of their immobilized respective acceptors. Both purified enzymes migrate as a single band on sodium dodecyl sulphate/polyacrylamide-gel electrophoresis after silver staining, and both have an apparent $M_r$ of 68 000. The enzymes were radioiodinated and again subjected to sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. Radioautographic analyses showed only one, intensely radioactive, band. Activity stains performed for both transferases after cellulose acetate electrophoresis indicate that, with this system too, both activities have identical mobilities, and co-migrate, as well, with the major, silver-stained, protein band. Kinetic studies with the purified enzymes show that the $K_m$ value for GlcNAc, for the (1$\rightarrow$4)-transferase, is 4 mM; for the (1$\rightarrow$3)-transferase the $K_m$ value for AsOSM is 5 mM, in terms of GalNAc ($N$-acetylgalactosamine) equivalents. Both enzymes have a $K_m$ value of 25 $\mu$M for UDP-galactose.

Glycosyltransferases catalyse the synthesis of all glycosidic bonds; the total number of these enzymes is unknown, but must be very large. The transferases can clearly recognize even subtle differences among acceptor molecules (Owens & Mackenzie, 1982; Betteridge & Watkins, 1983). Some investigators have suggested that the transferases exist as families of related proteins (Rowe, 1970; Roth, 1973). Although a small, but increasing, number of membrane-bound transferases has been purified extensively (Smith & Brew, 1977; Bouchilloux, 1979; Fraser et al., 1980; Mendicino et al., 1982; Weinstein et al., 1982; Thomson, 1983; Sheares & Carlson, 1983; Strous et al., 1983; Kaplan & Hechtman, 1983), in no case have two or more functionally and biologically related galactosyltransferases been purified and compared. The present paper describes the purification to apparent homogeneity of two, initially membrane-bound, galactosyltransferases from 13-day chick-embryo livers.

Experimental

Tissue

Embryonated chicken eggs were purchased from Truslow Farms (Chestertown, MD, U.S.A.). Eggs were incubated in a commercial egg incubator at 37°C in water-saturated air. After 13 days of incubation, livers were removed immediately and

Abbreviations used: GlcNAc, $N$-acetylgalactosamine; GalNAc, $N$-acetylgalactosamine; AsAgAGP, asialoagalacto-($\alpha_1$-acid glycoprotein); AsOSM, asialo-(ovine submaxillary mucin); SDS, sodium dodecyl sulphate.
placed in a solution of ice-cold 0.9% NaCl, and
frozen at −20°C. All subsequent purification steps
were performed at 4°C. When 10 g (wet wt.) was
accumulated, the livers were thawed and rinsed
twice with 0.9% NaCl, homogenized with five or
six strokes, in a Teflon-pestled Potter–Elvehjem
homogenizer, in 10 vol. of homogenizing buffer
(0.25 M-sucrose in 10 mM-Tris/HCl buffer, pH 7.4,
containing 1 mM-EDTA). The homogenate was
filtered and centrifuged at 7700 g for 30 min. The
resulting supernatant was then centrifuged at
105 000 g for 60 min in a 50 Ti rotor. The pellets
were suspended, pooled and stirred for 20 min in
10 ml of homogenizing buffer containing 1%
Triton X-100 and 0.1 M-NaCl. The solubilized
proteins, referred to below as the microsomal detergent extract, were separated from the insoluble debris by centrifugation at 105 000 g for 30 min, again in a 50 Ti rotor.

Materials

UDP-D-[6-3H]galactose (16.3 Ci/mmole) was pur-
chased from Amersham (Arlington Heights, IL,
U.S.A.) and analysed periodically for purity by
using high-voltage paper electrophoresis in 1%
sodium tetraborate. UDP-hexanolamine–Sepha-
rose was kindly given by Dr. Robert Hill (Duke
University, Durham, NC, U.S.A.), and α1-acid
glycoprotein was generously supplied by Dr. M.
Wickerhauser (American Red Cross Blood Ser-
services Laboratories, Bethesda, MD, U.S.A.).
Unlabelled UDP-galactose, and all other reagents,
were purchased from Sigma Chemical Co. (St.
Louis, MO, U.S.A.).

Purification of galactosyltransferases

Bio-Gel A-1.5m. The concentrated detergent
extract was applied to a column (1.5 cm × 56 cm) of
Bio-Gel A-1.5m equilibrated with 50 mM-sodium
cadolate buffer, pH 7.0, containing, as do all
subsequent solutions, 0.1% Triton X-100. The
column was calibrated with Blue Dextran 2000,
bovine serum albumin and Phenol Red.

Step 1: DEAE-Sepharose. The microsomal deter-
gen extract was concentrated by ultrafiltration on
a YM-10 filter. The concentrate, generally 2 ml,
was applied to a DEAE-Sepharose CL-6B ion-
exchange column (1.3 cm × 22 cm) equilibrated
with 50 mM-sodium cadolate buffer, pH 7.0,
containing 0.1 M-NaCl. The column was eluted
with the same buffer; over 95% of the galactosyl-
transferase activities was recovered in the eluate.

Step 2: UDP-hexanolamine–Sepharose. Fra-
tions 13–23 from the DEAE-Sepharose column in
Step 1 were pooled and concentrated to less than
1 ml by ultrafiltration as above. One-ninth volume
of 250 mM-MnCl2 was added to the concentrate,
which was then applied to a UDP-hexanolamine–
Sepharose column (0.8 cm × 8.5 cm) equilibrated
with 50 mM-sodium cadolate buffer, pH 7.0,
containing 25 mM-MnCl2. Bound enzymes were
eluted with the column buffer containing 1 mM-
UDP and 1 M-NaCl, but without MnCl2.

Step 3: α-lactalbumin–Sepharose. The UDP-
hexanolamine-bound fractions were concentrated
as above to 1–2 ml. One-ninth volume of 0.1 M-
GlcNAc and 0.1 M-MnCl2 was added to the con-
centrate, which was then applied to a lactalbumin–
Sepharose column (1.6 cm × 8 cm) equilibrated
with 50 mM-sodium cadolate buffer, pH 7.0,
containing 10 mM-GlcNAc, 10 mM-MnCl2 and 1 M-
NaCl. The bound fractions were eluted with the
column buffer lacking GlcNAc and MnCl2 but
containing 0.5 M-KSCN.

Step 4: GlcNAc–Sepharose. The α-lactalbumin-
bound fractions, described in step 3, were dialysed
to remove KSCN, and again concentrated to less
than 2 ml. One-ninth volume of 10 mM-sodium
cadolate buffer, pH 7.0, containing 0.1 M-MnCl2
and 10 mM-UMP was added to the concentrate.
The solution was then applied to the GlcNAc–
Sepharose column (1.6 cm × 11 cm) equilibrated
with 10 mM-sodium cadolate buffer, pH 7.0,
containing 10 mM-MnCl2, 1 mM-UMP, and 1 M-
NaCl. The bound fractions were eluted with 10 mM-GlcNAc and 1 M-NaCl in the same cadyl-
at buffer.

Step 5: AsAgAGP–Sepharose. The fractions that
initially bound to the GlcNAc–Sepharose column,
described in step 4, were again concentrated to less
than 2 ml by ultrafiltration. One-ninth volume of
10 mM-sodium cadolate buffer, pH 7.0, contain-
ing 0.1 M-MnCl2 and 10 mM-UMP was added to the
concentrate, which was then applied to an AsAg-
AGP–Sepharose column (1.6 cm × 8 cm) equilibrated
with 10 mM-sodium cadolate buffer, pH 7.0,
containing 10 mM-MnCl2, 1 mM-UMP and 1 M-
NaCl. The bound fractions were eluted with 10 mM-sodium cadolate buffer, pH 7.0, contain-
ing 10 mM-GlcNAc and 1 M-NaCl.

Step 6: AsOMS–Sepharose. The pass-through
fractions from the GlcNAc–Sepharose and AsAg-
AGP–Sepharose columns described in step 4 and 5
were concentrated as above. One-ninth volume of
10 mM-sodium cadolate buffer, pH 7.0, contain-
ing 0.1 M-MnCl2, 10 mM-UMP, 1 M-NaCl and
0.1 M-GlcNAc was added to the concentrate. The
concentrate was then applied to an AsOMS–
Sepharose column (1.6 cm × 11 cm) equilibrated
with 10 mM-sodium cadolate buffer, pH 7.0,
containing 10 mM-MnCl2, 1 mM-UMP, 10 mM-
GlcNAc and 1 M-NaCl. The bound galactosyl-
transferase activity was eluted with 10 mM-sodium
cadolate buffer, pH 7.0, containing 0.5 M-KSCN
and 1 M-NaCl.
Chick-embryo liver galactosyltransferases

Affinity columns
The Sepharose-based affinity gels used in steps 3–6 were synthesized by using CNBr-activated Sepharose according to the method of March et al. (1974). Sialic acid and galactose were sequentially removed from the α1-acid glycoprotein by (a) mild acid hydrolysis (Spiro, 1960) and (b) treatment with jack-bean β-galactosidase partly purified in this laboratory by using the methods of Li et al. (1975). Ovine submaxillary mucin was prepared from submaxillary glands dissected from sheep supplied by a local slaughterhouse. The mucin was purified by the method described by Tettamanti & Pigman (1968), and was desialylated by mild acid hydrolysis (Spiro, 1960). The increase in galactose-acceptor activity of both compounds was tested routinely with microsomal detergent extracts and UDP-[3H]galactose.

Transferase assays
All transferase assays were conducted in a final volume of 50μl containing the following reagents: 23μM-UDP-[3H]galactose (390mCi/mmol), 3mM-AMP, 0.1% Triton X-100, 20mM-Mes, pH 6.5, the appropriate enzyme fraction, and MnCl2 and acceptors according to the galactosyltransferase being assayed. For the (1→4)-transferase, the final MnCl2 concentration was 15mM and the acceptor was GlcNAc at 10mM or AsAgAGP at 250μg. For the (1→3)-transferase, the MnCl2 concentration was 10mM and the acceptor was 10mM-GalNAc equivalents of AsOSM. Mixtures were incubated at 37°C in a shaking water bath for times that are given for each experiment. In accordance with published methods (Roth et al., 1971), reactions were terminated by the addition of 0.47mM-EDTA in 10% sodium tetraborate. The resulting mixtures were applied to Whatman 3MM paper, after which the paper was saturated with 1% sodium tetraborate and subjected to high-voltage electrophoresis (3000V/23cm) for 30min. After the electrophoretograms were dried, the area around the origin was cut from each lane and the radioactivity determined.

SDS/polyacrylamide-gel electrophoresis
Polyacrylamide gels (7.5 or 10%) were performed at a constant current of 30mA/14cm, according to the method of Weber & Osborn (1969). Gels were stained with silver by using procedures described by Willoughby & Lambert (1983).

Isoelectric focusing
The detergent extract from 2g (wet wt.) of liver was mixed with Ultradex, Ampholyte (pH 3–10), and ferritin as a marker. Flat-bed isoelectric focusing was performed until the ferritin was focused, at which time the gel slurry was sliced into 5mm strips. The strips were suspended in 10mM-sodium cacodylate buffer, pH 7.0, and centrifuged lightly to pellet the gel. The supernatant transferase activities were assayed as described above. pH values were determined with a pH-meter.

Detection of transferase activity on electrophoreograms
The coupled-enzyme method for the detection of glycosyltransferases (Pierce et al., 1980) was utilized. Transferases were subjected to cellulose acetate electrophoresis in 0.1m-sodium cacodylate buffer, pH 7.5. The electrophoretograms were overlayed with Whatman 3MM paper saturated with the coupled-enzyme mixture and incubated in a 37°C water-saturated atmosphere for 1h. In the presence of the appropriate acceptor, the coupled enzymes produce 2mol of NADH per mol of galactose transferred by a galactosyltransferase. The enzyme location was determined by drying the Whatman indicator papers and examining them under u.v. light.

Sugar and protein assays
Sialic acid was assayed by using the periodate/resorcinol reagent (Jourdian et al., 1971); galactose was assayed by using the phenol/H2SO4 reagent (Dubois et al., 1956); hexosamine was measured by using Ehrlich reagent (Svennerholm, 1956). To assay GalNAc and GlcNAc in ovine submaxillary mucin and α1-acid glycoprotein, the acid hydrolysates were subjected to amino acid analysis with a Dionex amino acid analyser. More than 95% of the hexosamine in α1-acid glycoprotein was glucosamine, whereas more than 97% of the hexosamine in ovine submaxillary mucin was galactosamine. Protein was determined by the Lowry method with bovine serum albumin in the appropriate Triton concentration as a standard.

Product characterization
Galactosylated GlcNAc, AsAgAGP and AsOSM were prepared by incubating these acceptors separately with partly purified enzymes and UDP-[3H]galactose under standard assay conditions. The product of the incubation with GlcNAc was eluted from the origin of a borate electrophoretogram, as described, and applied to a Bio-Gel P-2 column (0.8cm × 59cm) before and after treatment with either 0.1m-NaOH at 37°C for 2 days (Mayo & Carson, 1970) or with the β-(1→4)-specific galactosidase from Escherichia coli (Sheares et al., 1982). The galactosylated AsAgAGP thus prepared was treated with β-(1→4)-specific galactosidase from jack beans (Li et al., 1975). The product of the incubation with AsOSM

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was similarly isolated and treated either with the β-(1→4)-specific galactosidase from jack beans, which is active on large-\(M_r\) substrates, or with 0.1 M NaOH solution in the presence or in the absence of the reducing agent 1 M NaBH\(_4\) (Sheares et al., 1982). These reactants were also applied to the Bio-Gel P-2 column. The column was calibrated by using labelled mono- and di-saccharides.

\textit{Iodination}

Purified enzymes were radioiodinated by the chloramine-T method of Hunter & Greenwood (1962). The proteins (<1 \(\mu\)g) were oxidized at room temperature for 2 min in the presence of Na\(^{125}\)I (1 mCi). The reaction was terminated with sodium metabisulphite. The iodinated proteins were desalted with Sephadex G-25, and the three peak fractions for each of the transferases were pooled and used for analysis by SDS/polyacrylamide-gel electrophoresis.

\textit{Cleveland peptide analysis}

Radioiodinated proteins were isolated by SDS/polyacrylamide-gel electrophoresis, and the area to which bovine serum albumin migrated was sliced and transferred directly to new wells containing \textit{Staphylococcus aureus} V8 proteinase, in accordance with the method of Cleveland et al. (1977). After SDS/polyacrylamide-gel electrophoresis, the dried electrophoreograms were exposed to X-ray films to obtain radioautograms.

\section*{Results}

\subsection*{Detergent extraction of galactosyltransferases}

Four detergents (Triton X-100, Nonidet P-40, SDS and sodium deoxycholate) were used initially to extract transferases from the microsomal pellets. Each of the detergents were used at concentrations of 0.25\%, 0.5\%, 1\% and 2\%. SDS and sodium deoxycholate are very ineffective solubilizers of these activities, whereas Triton and Nonidet P-40, at 1\%, each solubilizes nearly all the transferase activities. Because the addition of 0.1 M salt enhances the extraction of both enzymes, and because Triton is slightly more effective than Nonidet P-40, 1\% Triton X-100 in 0.1 M NaCl was used throughout for the initial solubilization of the transferases.

\subsection*{Effect of Mn\(^{2+}\) and pH on galactosyltransferases}

The (1→4)- and (1→3)-transferases have different optimal requirements for Mn\(^{2+}\): the (1→4)-transferase is most active at 15 mM, whereas the (1→3)-transferase is most active at 5–10 mM. These results correspond precisely to those previously obtained with rat liver enzymes (Strous et al., 1980). Subsequent assays were therefore conducted with 15 mM MnCl\(_2\) for the (1→4)-transferase and with 10 mM MnCl\(_2\) for the (1→3)-transferase. The pH optima for both enzymes are broad and lie between pH 5 and 7. For all the following assays, the pH was maintained at 6.5 with 20 mM Mes buffer. The Mn\(^{2+}\) and pH data were obtained with solubilized microsomal pellets as an enzyme source.

\subsection*{Properties of the (1→4)- and (1→3)-galactosyltransferases}

The galactosylation of individual acceptors, as described in the Experimental section, was compared with the galactosylation obtained when acceptors were mixed in pair-wise combinations. In all cases, acceptor and UDP-galactose concentrations were saturating, and the assays were conducted for 30 min, well within the linear range of the assays. The data (Table 1) show that free GlcNAc and AsAgAGP are competitive substrates, whereas AsAgAGP and AsOSM are not. Similarly, free GlcNAc and AsOSM do not compete with one another. The results indicate that the (1→4)-transferase active site recognized both free GlcNAc and AsAgAGP, and that the (1→3)-transferase active site is different and recognizes of the acceptors tested, only AsOSM.

To determine if the two activities could be separated by using isoelectric focusing, the detergent extract was subjected to flat-bed isoelectric focusing. Fig. 1 presents the results. Although the (1→4)-transferase (pH 6.24) and the (1→3)-transferase (pH 6.40) activities migrate differently from one another on the basis of their isoelectric points, the difference is not sufficient for separation. Nevertheless, the consistent difference in pI values

\begin{table}[h]
\centering
\caption{Substrate-competition analyses of chick-embryo liver galactosyltransferases}
\begin{tabular}{l c c c c}
\hline
Acceptors & Activity (nmol of galactose transferred/30 min per \(\mu\)g of protein) & Expected for two enzymes \\
\hline
GlcNAc & 0.88 ± 0.02 & \\
AsAgAGP & 1.77 ± 0.06 & \\
AsOSM & 0.19 ± 0.01 & \\
GlcNAc + AsAgAGP & 1.45 ± 0.05 & 2.71 & \\
GlcNAc + AsOSM & 1.03 ± 0.09 & 1.07 & \\
AsAgAGP + AsOSM & 2.20 ± 0.03 & 1.97 & \\
\hline
\end{tabular}
\end{table}
Purification of galactosyltransferases

**Step 1: DEAE-Sepharose.** The elution patterns of both the (1→4)- and the (1→3)-transferases from DEAE-Sepharose are shown in Fig. 3. For both enzymes, most activity is eluted relatively late, after the elution position of free glucose. No further activities are eluted even with 2M-NaCl. Neither the (1→4)- nor the (1→3)-transferase activities in the detergent extract bind to the Sepharose used as the supporting matrix for all the affinity columns in this study.

**Step 2: UDP-hexanolamine-Sepharose.** The major peak fractions of both enzyme activities from the DEAE-Sepharose (step 1) were applied to a UDP-hexanolamine-Sepharose column. More than 95% of both activities is retained. Both the retained activities were eluted with 1mM-UDP in 1M-NaCl. The two transferases were not separated on this column by elution with linear gradients of UDP or UDP-galactose.

**Step 3: α-lactalbumin-Sepharose.** All the frac-
tions eluted with UDP and NaCl were pooled, concentrated, and applied to the lactalbumin–Sepharose column. Almost all of the (1→4)-transferase activity is retained on this column, and about 85% of the (1→3)-transferase activity is similarly retained. Both retained enzymes are eluted with 0.5M-KSCN. Kinetic analyses of the enzymes at this purification step show that both have a $K_m$ towards UDP-galactose of about 25$\mu$M, and that the $K_m$ value of the (1→4)-transferase activity toward GlcNAc is 4$nM$, whereas that of the (1→3)-transferase activity towards AsOSM is 5$nM$ (results not shown).

Although $\alpha$-lactalbumin is a demonstrated modifier protein for the (1→4)-galactosyltransferase, no previous data suggest an interaction between lactalbumin and the (1→3)-transferase. Fig. 4, however, shows that the chick-embryo liver (1→3)-transferase is as sensitive to lactalbumin as is the (1→4)-transferase.

**Step 4: GlcNAc–Sepharose.** All the activity eluted from the $\alpha$-lactalbumin–Sepharose column was applied to the GlcNAc–Sepharose column. Almost all of the applied (1→4)-transferase activity is retained, whereas less than half of the (1→3)-transferase activity is retained. Elution with 10$\mu$M-GlcNAc, as described in the Experimental section, yields 52% of the retained (1→4)-transferase activity and 72% of the retained (1→3)-transferase activity. These results are presented in Table 2.

**Step 5: AsAgAGP–Sepharose.** All the activity eluted from the GlcNAc–Sepharose (step 4) was applied to the AsAgAGP–Sepharose column. Of the applied (1→4)-transferase activity 86% is retained, whereas only 11% of the (1→3)-transferase activity is retained. Elution with 10$\mu$M-GlcNAc, as described in the Experimental section, yields only 25% of the applied (1→4)-transferase, whereas (1→3)-transferase activity represents about 2% of the (1→4)-transferase activity (Table 3).

**Step 6: AsOSM–Sepharose.** The pass-through fractions from the GlcNAc–Sepharose and AsAgAGP–Sepharose columns were pooled and applied to the AsOSM–Sepharose column. About 70% of the (1→3)-transferase activity and 9% of the (1→4)-transferase activity is retained. About 28% of the bound (1→3)-transferase activity is eluted with 0.5M-KSCN, although the (1→4)-transferase activity in this eluate represents about 20% of the (1→3)-transferase activity (Table 3). Taking into account the specific activity of the (1→4)-transferase, contamination by this enzyme is less than 10%.

Overall, as presented in Table 2, both enzymes are purified about 5000–7000-fold.

Fig. 5 shows SDS/polyacrylamide-gel-electrophoretic patterns after each purification step. For both the (1→4)- and the (1→3)-galactosyltransferases, silver staining shows the relative increase in the density of a protein band that migrates with an apparent $M_r$ of 68000. There are more than ten
Fig. 3. *DEAE*-Sepharose CL-6B chromatography of galactosyltransferase activities in detergent extracts from 2 g of liver
The concentrated extract was applied to a column equilibrated with 0.1 M NaCl. Free glucose is eluted at fraction 15, as indicated: ●, (1→4)-transferase activity; ○, (1→3)-transferase activity. A 10 μl portion of each fraction (2 ml) was used to assay each enzyme. The protein values (△) represent one-tenth the total protein per fraction. For full details see the text.

Fig. 4. Effect of α-lactalbumin on galactosyltransferase activities
Galactosyltransferase activities towards GlcNAc (○), AsAgAGP (●) and AsOSM (△) were measured in the presence of increasing concentrations of α-lactalbumin. For full details see the text.

bands in the UDP-Sepharose-bound fraction, and more than six bands in the lactalbumin-Sepharose-bound fraction. Finally, only a single band appears for each transferase activity. Because of the similarity in $M_r$ values between bovine serum albumin and both purified proteins, no bovine serum albumin was ever introduced to the purification steps.

Activity stains after cellulose acetate electrophoresis
Activity stains were performed to determine if the visible protein bands coincide with the appropriate galactosyltransferase activities, and if both activities co-migrate during cellulose acetate electrophoresis as they do during SDS/polyacrylamide-gel electrophoresis. Lactalbumin-Sepharose pass-through and retained fractions were subjected to cellulose acetate electrophoresis. The electrophoreograms were stained with the coupled-enzyme assay as described in the Experimental section, and with silver. Fig. 6 shows the correspondence between the (1→4)- and (1→3)-transferase activities when both are subject to cellulose acetate electrophoresis. The NADH fluorescence indicates galactosyltransferase activity, and for both enzymes is absolutely dependent on the presence of UDP-galactose and a galactose acceptor. In parallel experiments, silver staining shows a major protein band that coincides with the NADH fluorescence, and a minor band at the
Table 2. Purification of two galactosyltransferases from chick-embryo livers

Enzyme activities at each purification step were determined as described in the Experimental section, with AsAgAGP and AsOSM as acceptors within the linear time ranges of the assays. Endogenous activities, which are appreciable in the homogenate and the detergent extract, were determined without the addition of acceptor, and these values were subtracted in each case. Not determined.

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Activity (nmol of galactose transferred/60 min per mg of protein)</th>
<th>Purification (fold)</th>
<th>Recovery (%)</th>
<th>Activity (nmol of galactose transferred/60 min per mg of protein)</th>
<th>Purification (fold)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenate</td>
<td>0.56</td>
<td>1</td>
<td>100</td>
<td>0.06</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>Detergent extract</td>
<td>5.10</td>
<td>9</td>
<td>76</td>
<td>0.44</td>
<td>7</td>
<td>80</td>
</tr>
<tr>
<td>DEAE-Sepharose</td>
<td>32.7</td>
<td>58</td>
<td>58</td>
<td>3.55</td>
<td>59</td>
<td>55</td>
</tr>
<tr>
<td>UDP-Sepharose</td>
<td>106</td>
<td>189</td>
<td>43</td>
<td>15.9</td>
<td>265</td>
<td>38</td>
</tr>
<tr>
<td>Lactalbumin-Sepharose</td>
<td>215</td>
<td>384</td>
<td>34</td>
<td>34.3</td>
<td>571</td>
<td>30</td>
</tr>
<tr>
<td>GlcNAc-Sepharose</td>
<td>427</td>
<td>762</td>
<td>16</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>AsAgAGP-Sepharose</td>
<td>3934*</td>
<td>7025</td>
<td>4</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>AsOSM-Sepharose</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>257*</td>
<td>4280</td>
</tr>
</tbody>
</table>

* Protein values are estimated from SDS/polyacrylamide-gel-electrophoresis band intensities stained with silver and compared with dilutions of bovine serum albumin.

Table 3. Relative galactosyltransferase activities toward five different acceptors in the detergent extract compared with the purified (1→4) and (1→3)-transferases

Transferase activities were determined as described in the Experimental section. Endogenous activities (with no added acceptor) were measured and subtracted from each of the values. For the AsAgAGP-Sepharose-bound fractions that were eluted with GlcNAc, endogenous values were calculated in accordance with the competition analyses presented in Table 1. Abbreviation: N.D., not detectable.

<table>
<thead>
<tr>
<th>Acceptor</th>
<th>Activity of detergent extract (pmol of galactose transferred/60 min per μg of protein)</th>
<th>Activity of AsAgAGP-bound fractions (pmol of galactose transferred/60 min per 10 μl of 0.8 ml fractions)</th>
<th>Activity of AsOSM-bound fractions (pmol of galactose transferred/60 min per 10 μl of 0.8 ml fractions)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GlcNAc</td>
<td>2.47</td>
<td>42.3</td>
<td>3.3</td>
</tr>
<tr>
<td>AsAgAGP</td>
<td>5.70</td>
<td>204</td>
<td>4.7</td>
</tr>
<tr>
<td>GalNAc</td>
<td>1.69</td>
<td>12.9</td>
<td>N.D.</td>
</tr>
<tr>
<td>AsOSM</td>
<td>0.47</td>
<td>3.2</td>
<td>21.0</td>
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<tr>
<td>Ganglioside GM₂</td>
<td>1.07</td>
<td>0.6</td>
<td>2.3</td>
</tr>
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</table>

origin that shows no fluorescence (results not shown).

SDS/polyacrylamide-gel electrophoresis of iodinated galactosyltransferases

To obtain iodinated enzymes for Cleveland digest analysis, the proteins that were purified by using all six steps were iodinated as described in the Experimental section. Both labelled protein fractions were separated from the iodine on a Sephadex G-25 column. The peak fractions for each of the transferases contained less than 1 μg of protein, as judged by the intensity of silver staining on SDS/polyacrylamide-gel electrophoresis compared with dilutions of bovine serum albumin standards that were treated identically. At the same time, the total peak fractions for the (1→4)-transferase contained 1.2 × 10⁶ c.p.m. and those for the (1→3)-transferase contained 2.7 × 10⁵ c.p.m. When each radioiodinated transferase was applied to SDS/polyacrylamide-gel electrophoresis and the electrophoretograms were analysed radioautographically, each transferase showed a single labelled band with a mobility identical with that of bovine albumin (results not shown). When subjected to limited proteolysis with S. aureus V8 proteinase, four of the five labelled peptides from each enzyme migrated identically, as depicted in Fig. 7. Both transferases show bands b, c, d and e. Band d appears in (1→3)-transferase peptides but not in grossly over-exposed radioautograms of (1→4)-transferase peptides. Band f appears in digests of
Chick-embryo liver galactosyltransferases

Fig. 5. SDS/polyacrylamide-gel electrophoretograms, stained with silver, of the proteins present after each purification step
The gels contain: in lane 1, chick-embryo liver homogenate; lane 2, detergent extract; lane 3, fractions 13–23 from DEAE-Sepharose; lane 4, bound proteins from UDP-Sepharose; lanes 5 and 6, Mr standards; lane 7, bovine serum albumin; lane 8, α-lactalbumin-Sepharose-bound proteins; lane 9, pass-through fractions from GlcNAc-Sepharose; lane 10, bound fraction from GlcNAc-Sepharose; lane 11, bound fraction from AsAgAGP-Sepharose; lane 12, bound fraction from UDP-Sepharose; lane 13, Mr standards. The Mr standards are, in order of decreasing size, phosphorylase b (Mr 94000) (A), bovine serum albumin (Mr 68000) (B), ovalbumin (Mr 43000) (C), carbonic anhydrase (Mr 30000) (D), soya-bean trypsin inhibitor (Mr 21000) (E) and lysozyme (Mr 14300) (F). For full details see the text.

Fig. 6. Galactosyltransferase activity staining of partly purified transferases after cellulose acetate electrophoresis
The bound (A, C and E) and pass-through (B, D and F) fractions from the lactalbumin-Sepharose column were subjected to cellulose acetate electrophoresis in cacodylate buffer as described in the Experimental section. After electrophoresis, NADH fluorescence was detected under u.v. light, also as described in the Experimental section. Sheets A and B were developed with the coupled-(1→4)-transferase but not in those of the (1→3)-transferase.

Discussion

Two galactosyltransferases from chick-embryo livers have been highly purified. One of the enzymes transfers galactose to AsAgAGP and to free GlcNAc with a β-(1→4)-linkage. The other transfers galactose to AsOSM with a β-(1→3)-linkage. The two enzymes co-purify when subjected to many conventional separation methods, but complete separation could be achieved only by affinity columns of their respective immobilized large-M<sub>c</sub> acceptors.

Poor recoveries from affinity columns might be due to contamination of the subsequent transferase assays with the inhibitors (e.g. UMP and KSCN) used to wash the columns and elute the bound enzymes. The extremely low protein concentrations towards the end of the purification procedure might also contribute to enzyme inactivation and serve to lower, still further, the recovery values.

Two tentative conclusions are possible from the similarities between these galactosyltransferases. First, they behave identically on Bio-Gel A-1.5m, enzyme mixture containing GlcNAc, sheets C and D with AsOSM, and sheets E and F served as controls with no acceptor.
DEAE-Sepharose, affinity chromatography on UDP-hexanolamine-Sepharose and α-lactalbumin-Sepharose, cellulose acetate electrophoresis and SDS/polyacrylamide-gel electrophoresis. Also, they behave very similarly on flat-bed isoelectric focusing. It is therefore possible that glycosyltransferases previously purified by using protocols without acceptor affinity columns might be contaminated with other transferases.

Secondly, the galactosyltransferases may make up a family of enzymes with significant sequence homologies. Such a possibility is made likely by the similarities in $M_r$ values shown by the (1→4)- and (1→3)-transferases, as well as by their almost identical behaviour in a very wide variety of separation methods. This possibility is suggested by the results of the limited proteolysis of the purified iodinated enzymes. When incubated with $S. aureus$ V8 proteinase, both the (1→4)- and the (1→3)-transferases show five labelled polypeptides of differing $M_r$ values. Four of these five are shared. Peptide and, ultimately, sequence analyses will be required to address the possibility of structural homology in an unequivocal fashion. The extent to which all glycosyltransferases share common structures, and the extent to which this primitive and highly polymorphic class of enzymes is related to other proteins [e.g. those produced by the major histocompatibility complex, as suggested by McKenzie et al. (1977)], must await primary structure information.

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References


