UDP-galactose:globoside galactosyltransferase in murine kidney[1]

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Abstract There are increased levels of stage-specific embryonic antigens-3 and -1 (SSEA-3 and SSEA-1) globo-series glycolipids in male versus female DBA/2 and C57BL/6 kidneys, respectively. To determine what enzymatic steps may be responsible for these differences, the activity and properties of UDP-galactose:globoside galactosyltransferase were studied in male and female mouse kidney microsomes. This enzyme participates in the biosynthesis of galactosylgloboside, SSEA-3 glycolipid; the reaction product was identified by high performance thin-layer chromatography (HPTLC) immunostaining. In C57BL/6 mice, the specific activity of the enzyme, in the presence of CHAPS, was 2-fold greater in the male than that in the female. Optimum pH for the enzyme from both sexes was about 5.6, and Mn^{2+} was essential for maximal activity. Fifty percent of the male and female enzyme activity was lost after preincubating the microsomes for 1 min at 55°C; thereafter, the enzyme from female microsomes had a slower rate of denaturation. The K_m for globoside in presence of sodium cholate for both male and female was 0.035 mM, but it was approximately 2-fold greater for the female in presence of CHAPS. The enzyme in male and female microsomes was differentially activated by CHAPS and cholate. The results suggest the presence of an enzyme modulator in these membranes. In DBA/2 mice, the enzyme activity was about 2-fold greater in males than that in the female. The specific activity of the enzyme in the two strains was of a similar magnitude. The data presented here suggest that UDP-galactose:globoside galactosyltransferase activity is responsible for the sexual differences observed in the levels of SSEA-1- and SSEA-3-containing glycolipids in C57BL/6 and DBA/2 kidneys, respectively. —**Koul, O., M. Prada-Maluf, and R. H. McCluer.** UDP-galactose:globoside galactosyltransferase in murine kidney. *J. Lipid Res.* 1990. **31:** 2227–2234.

Supplementary key words SSEA-3 • SSEA-1 • microsomes • galactosylgloboside • HPTLC-immunoblot

The diverse carbohydrate structures of glycolipids and glycoproteins have been demonstrated to play an important role in various biological phenomena. These carbohydrate structures include globoside, stage-specific embryonic antigen-1 (SSEA-1), SSEA-3, SSEA-4, Forssman and globo-series ABH blood group antigens; many of them are developmentally regulated (1). The biosynthesis of these structures is accomplished by the sequential addition of sugar residues by specific glycosyltransferases (2). The specificity of transferases is expressed in relation to the sugar donor, acceptor residue, and the resultant anomeric linkage (2, 3). Most of the glycosyltransferases are membrane-bound and pose unique problems during their study. SSEA-3 epitope (Gb_5) is an extended globo-series carbohydrate structure present on lipids and proteins (4, 5). It is expressed in kidneys of adult DBA/2 mice (6), PC-12 cells (7), and embryonic carcinoma (EC) cells (8). Its concentration in male kidney is much higher than in female kidney and it is not detected in the kidney of C57BL/6 and BALB/c mice (6, 9). The glycolipids containing SSEA-1 structure, of which SSEA-3 is the precursor, are not expressed in DBA/2 but are present in the kidneys of C57BL/6 mice (9) and its concentration is greater in males than in females. The dual role of the SSEA-3 antigen as a cell surface molecule and a precursor for SSEA-1 antigen in the kidney makes the characterization of its biosynthesis an important model in understanding regulatory controls exercised during development. Here, we report the characterization of the key biosynthetic enzyme for extended globo-series structures: UDP-galactose: globoside galactosyltransferase (Gb_4Gal-TR) in murine kidney microsomes.

MATERIALS AND METHODS

Materials

[^{14}C]UDP-galactose (272.8 mCi/mmol) was purchased from New England Nuclear (Boston, MA). NADH was

Abbreviations: BSA, bovine serum albumin; HPTLC, high performance thin-layer chromatography; CHAPS, 3-[(3-cholamidopropyl)dimethylamino]-propane sulfonate; MES, 2-[N-morpholino]ethanesulfonic acid; MOPS, 3-[N-morpholino]propanesulfonic acid; Gb_4, globoside; Gb_5, galactosylgloboside; PBS, phosphate-buffered saline; SSEA-1, stage-specific embryonic antigen-1; SSEA-3, stage-specific embryonic antigen-3; SSEA-4, stage-specific embryonic antigen-4; Gb_4Gal-TR, UDP-galactose:globoside galactosyltransferase.

[1]Part of this work has been presented as an abstract (*Glycocon. J.* 1989. **6:** 435).

from Pharmacia (Piscataway, NJ); BSA, sodium cholate, UDP-galactose, H_2O_2, 4-chloro-1-naphthol, MES, and MOPS were from Sigma Chemical Co. (St. Louis, MO); aluminum-backed HPTLC plates were obtained from E. Merck (Darmstadt, Germany); Bond-Elut C18 cartridges were obtained from American Bioanalytical (Natick, MA); horseradish peroxidase-conjugated anti-rat IgM antibody was obtained from Boehringer-Mannheim (Indianapolis, IN); BCA* protein assay reagent was from Pierce Chemical Co. (Rockford, IL); anti-SSEA-3 antibody, MC-631, was purchased from the National Developmental Hybridoma Bank (Iowa City, IA). Globoside was prepared from human red blood cells obtained from American Red Cross. All other chemicals and reagents were purchased from Fisher Scientific Co. (Medford, MA).

Animals

Five- to 6-week-old virus-free C57BL/6 and DBA/2 mice were purchased from Charles River Animal Labs (Kingston, RI) and housed at Shriver Center animal facility for 3–4 days prior to use.

Preparation of microsomes

Kidney microsomes from male and female mice were used to study the enzyme activity throughout this work. Animals were killed with carbon dioxide. Kidneys were quickly removed, decapsulated, minced, and homogenized (10%, w/v) in 0.32 M sucrose at 4°C using a Potter-Elvehjem Teflon–glass homogenizer. The homogenate was centrifuged at 12,000 *g* in a Sorvall centrifuge for 20 min. The microsomes were isolated from the supernatant by ultracentrifugation at 100,000 *g* for 90 min in a Beckman L-2-65B centrifuge according to Koul, Chou, and Jungalwala (10). The pellet obtained was resuspended in ice-cold water using a glass homogenizer; aliquots were frozen at −70°C until the enzyme was assayed. Pierce BCA* protein assay reagent was used for the spectrophotometric determination of protein concentration (11) with BSA as a standard.

Assay of UDP-galactose:globoside galactosyltransferase (Gb_4Gal-TR)

The enzyme activity in microsomes was determined in the presence of exogenously added globoside. The incorporation of galactose into endogenous lipid acceptors was routinely determined at the same time. The assay conditions were adapted and modified from those described by Coste et al. (12). Briefly, globoside (30 μg) in chloroform–methanol 2:1 (v/v) was pipetted into glass screw-cap culture tubes and the solvent was evaporated. To ensure complete evaporation of organic solvents, the tubes were left overnight in vacuo. Detergent was added, mixed by sonication, and left to stand for 1 h. Later on, the incubation medium was added, followed by the enzyme protein. The tubes were capped and placed for 1 h in a shaking water bath at 37°C. Standard incubation medium contained 0.1% sodium cholate, MES buffer pH 5.6 (50 mM), NADH (1 mM), $MnCl_2$ (6.25 mM), UDP-galactose (0.015 mM, 100,000 cpm [^{14}C]UDP-galactose), and 150 μg microsomal protein in a total incubation volume of 100 μl. Assay conditions were appropriately altered to study the effect of temperature, pH, detergents, ions, and substrate concentration to characterize this enzyme in C57BL/6 mouse kidney microsomes. The reaction was stopped by transferring the tubes onto ice; 5 ml of Folch theoretical upper phase (13) containing 0.1 M KCl was added and the tubes were vortexed and left overnight at room temperature. The radioactive lipid reaction product was isolated by passing the mixture through a C18 Bond-Elut cartridge as described by Figlewicz et al. (14) based on the methodology of Williams and McCluer (15). The lipids were eluted from the columns in chloroform–methanol 2:1 (v/v) and methanol, 5 ml each. The fractions were dried down in plastic vials and the radioactivity was measured in an LKB scintillation counter in presence of 5 ml of scintillation fluid. The galactosyltransferase activity was expressed as pmoles galactose transferred to globoside per mg protein per hour.

Identification of the lipid reaction product

Kidney microsomes from male and female C57BL/6 and DBA/2 mice were used for these experiments. The enzyme was assayed as described above. However, 500 μg of enzyme protein and 1 μCi of [^{14}C]UDP-galactose were used in each tube. Duplicate assays were run for each sex in presence or absence of Gb_4. The tubes were incubated for 2 h at 37°C. The radioactive lipid reaction product obtained was base-treated with alkaline methanol (16) and passed through a C18 Bond-Elut cartridge. The hydrolyzed samples were subsequently passed through an Iatrobead column and the methanol fraction, that contained most of the radioactivity, was spotted on an aluminum-backed HPTLC plate. Galactosylgloboside obtained from kidneys of male DBA/2 mice was run alongside as a standard. The plate was developed in chloroform–methanol–0.25% $CaCl_2$(aq) 5:4:1 (v/v/v) and then exposed to a Kodak X-OMAT X-ray film to detect the radioactive spots. Immunostaining of the previously exposed HPTLC plate was done essentially according to Brockhaus et al. (17). Briefly, the plate was immersed for 1 min in 0.05% methacrylate in hexane, dried, and then soaked for 1 h in 1% BSA in PBS, pH 7.4. The plate was then incubated overnight with anti-SSEA-3 antibody, MC 631. Horseradish peroxidase-conjugated anti-rat IgM diluted 1:100 in 1% BSA in PBS was used as a secondary antibody. The plate was rinsed with PBS, and 4-chloro-1-napthol in presence of H_2O_2 was used as a chromophore.

Effect of pH

The microsomal enzyme was assayed in three different buffer systems to cover a pH range from 3.8 to 7.9. We used acetate buffer for pH 3.8–5.6, MES buffer for pH 5.6–6.5, and MOPS buffer for pH 6.5–7.9. The assay protocol was otherwise similar.

Determination of kinetic parameters

The effect of varying the concentration of globoside (acceptor) from 15.5 to 467.2 μM was studied at pH 5.6 and in presence of 0.1% sodium cholate. A Gb_4 concentration range from 7.78 μM to 467.2 μM, at pH 6.5 and in presence of 0.5% CHAPS was also tested. The concentration of UDP-galactose (donor) was varied from 5.4 to 266.7 μM at pH 5.6 in presence of 0.1% sodium cholate, to study its effect upon the enzyme activity. The K_m and V_{max} were calculated from Hanes-Woolf plots obtained from the data.

Heat inactivation

Aliquots of the microsomal membranes were preincubated at 55°C for up to 30 min and then assayed for enzyme activity at 37°C. The assay was carried out at pH 6.5 in presence of 0.5% CHAPS. The radioactivity in the lipid reaction product was determined as described earlier.

Effect of ions

Under standard assay conditions, the enzyme activity was determined in presence of $MnCl_2$ (6.25 mM). We replaced Mn^{2+} with other divalent cations (chlorides) at the same concentration and then measured the enzyme activity. The results thus obtained were compared to those obtained under standard assay conditions. The effect of acetate (sodium salt, 6.25 and 50.0 mM, in presence or absence of Mn^{2+}), EDTA (6.25 mM), and Cl^- (sodium salt, 6.25 mM) on the enzyme was also studied.

RESULTS

Gb_4Gal-TR specific activity in kidney microsomes

The activity of Gb_4Gal-TR was assayed in the microsomes obtained from kidneys of C57BL/6 and DBA/2 mice. The enzyme activity was linear up to 1.5 h and 300 μg enzyme protein. A comparison of the enzyme activity in these two strains is shown in **Table 1.** Irrespective of the strain, the enzyme activity was 2 to 3.6 times higher in presence of 0.1% sodium cholate than that obtained in presence of 0.5% CHAPS. Microsomes from males had higher Gb_4Gal-TR specific activity than those obtained from females.

In presence of 0.5% CHAPS (pH 6.5) the activity was 2- to 3-fold higher in the males, but in presence of 0.1% sodium cholate this difference was reduced to about 10% for C57BL/6 microsomes and to 40% in DBA/2 membranes (Table 1).

Effect of detergents

Table 1 shows the effect of different detergents on the enzyme activity in microsomes obtained from kidneys of C57BL/6 mice. Triton X-100 and Triton CF-54 proved to be ineffective in activating the enzyme. The transfer of galactose to Gb_4 increased 7- to 9-fold in presence of 0.1% sodium cholate; the enzyme was activated to a lesser extent in presence of CHAPS. Several concentrations of sodium cholate and CHAPS were used to study the range of stimulation of the enzyme (**Fig. 1**). Maximal activity

TABLE 1. Effect of detergents on mouse kidney microsomal Gb_4Gal-TR activity

	Specific Activity			
	C57BL/6		DBA/2	
Assay Condition	Male	Female	Male	Female
	pmol/mg/h			
No detergent	21.6 (15.1)	14.8 (8.2)		
CHAPS	56.0 (22.8)	24.8 (14.1)		
CHAPS (0.5%, pH 6.5)	82.9 (17.2)	49.2 (7.8)	101.0 (14.0)	33.5 (15.5)
Triton X-100	10.5 (18.3)	15.5 (13.1)		
Triton CF-54	12.0 (12.9)	17.9 (14.0)		
Sodium cholate	157.9 (38.5)	131.5 (22.1)	204.2 (30.1)	121.5 (14.0)

The enzyme was assayed in presence of added globoside as described in Methods. Detergent concentration in the incubation medium was 0.1% and pH 5.6, unless indicated otherwise. Numbers in parentheses represent galactose incorporated into endogenous lipid acceptor in the absence of added globoside. Each value is an average from a representative experiment run in triplicate, repeated at least two different times with a variability in data of less than 10% between experiments.

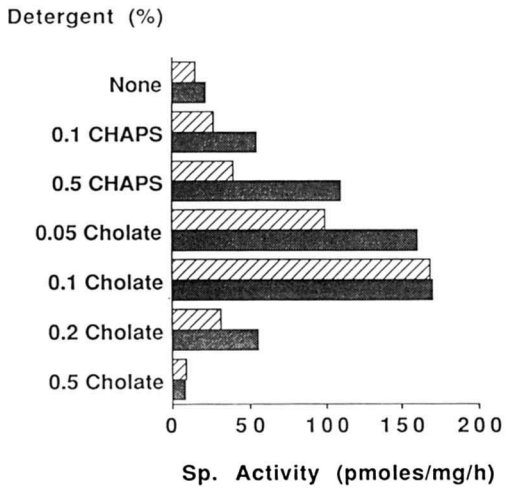

Fig. 1. Effect of sodium cholate and CHAPS at various concentrations on Gb_4Gal-TR activity from C57BL/6 mouse kidney microsomes. The enzyme was assayed as described in Methods; (■) male; (▨) female.

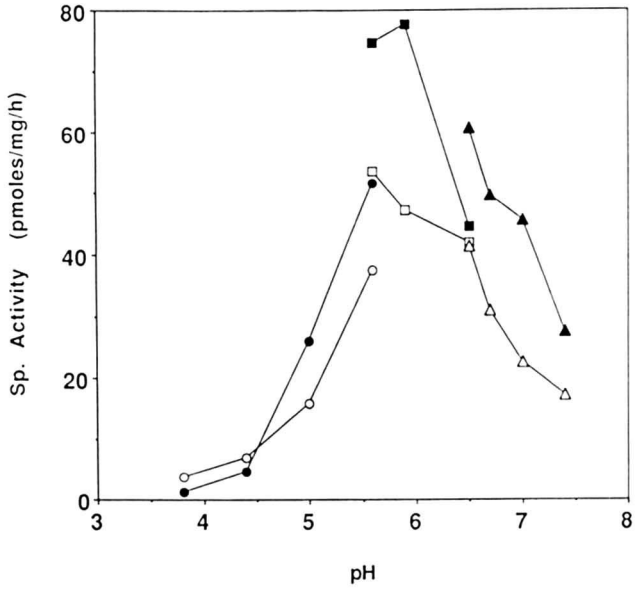

Fig. 2. Effect of varying pH conditions on Gb_4Gal-TR activity from C57BL/6 mouse kidney microsomes. The enzyme was assayed in three different buffers to cover a pH range from 3.8 to 7.9. Open symbols: female; filled symbols: male; (●) acetate; (■) MES; (▲) MOPS. Each point represents a mean value from an assay run in triplicate and repeated at least twice with less than 10% variability in the data.

was obtained in presence of 0.1% sodium cholate and higher concentrations were inhibitory. Among the concentrations of CHAPS tested, 0.5% stimulated the enzyme maximally but the values obtained were significantly lower than those obtained in presence of 0.1% sodium cholate. One percent CHAPS was inhibitory to the enzyme (data not shown). A comparison of the maximum enhancement of the enzyme activity in male and female membranes by sodium cholate versus CHAPS indicated a differential activation of the enzyme depending on the sex. The difference in maximal activation of the enzyme obtained in presence of 0.1% sodium cholate and 0.5% CHAPS was greater for females (5-fold) than that for the male microsomes (1.5-fold). The difference between male and female enzyme activity in presence of 0.1% sodium cholate was reduced dramatically as noted above. Although the incorporation of galactose into endogenous lipids was low, it was modulated by varying concentrations of detergents similarly to that observed in presence of added Gb_4.

Effect of pH

Fig. 2 shows the effect of pH on enzyme activity. At most pH values the activity of the enzyme was greater in male than in female microsomes. The enzyme activity in presence of acetate buffer was about 40% lower than that obtained in presence of MES at the same pH, irrespective of sex. The maximal activity for the enzyme in female microsomes was obtained in MES buffer at pH 5.6 whereas for males it was obtained at pH 5.9. A pH of 5.6 was routinely used for our investigations.

Heat inactivation of the enzyme

Preincubating the microsomal membranes for 1 min at 55°C resulted in about 50% decrease of enzyme activity in the membranes from male or female kidneys (**Fig. 3**). However, longer preincubations resulted in a slower rate of loss of the remaining activity in microsomes from female than that from the male kidneys. Thirty minutes of preincubation resulted in complete denaturation of enzyme activity in the membranes from either source.

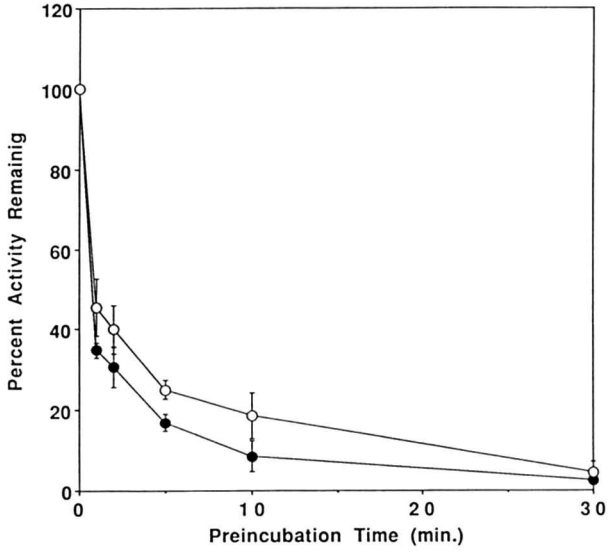

Fig. 3. Heat inactivation profile of the Gb_4Gal-TR activity from C57BL/6 mouse kidney microsomes. The microsomes were preincubated at 55°C for various times as indicated and then assayed as described in Methods; (●) male; (○) female.

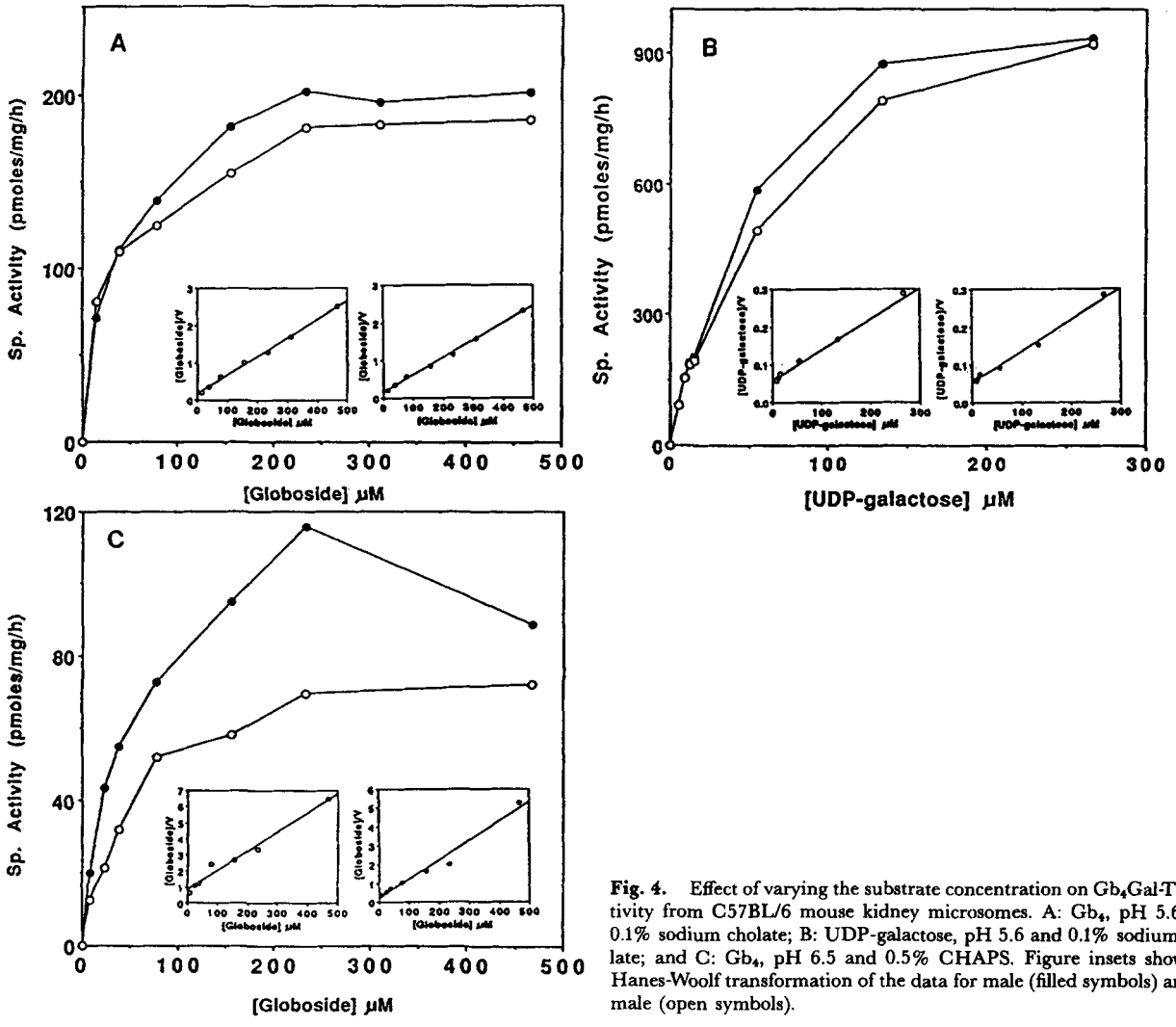

Fig. 4. Effect of varying the substrate concentration on Gb_4Gal-TR activity from C57BL/6 mouse kidney microsomes. A: Gb_4, pH 5.6 and 0.1% sodium cholate; B: UDP-galactose, pH 5.6 and 0.1% sodium cholate; and C: Gb_4, pH 6.5 and 0.5% CHAPS. Figure insets show the Hanes-Woolf transformation of the data for male (filled symbols) and female (open symbols).

Kinetic parameters

Fig. 4A shows the effect of varying the substrate concentration on enzyme activity in presence of cholate. The activity increased with increase in Gb_4 concentration up to about 30 μg and remained steady thereafter for both male and female microsomes. Fig. 4B shows the results of varying UDP-galactose concentration on the enzyme activity. The activity steadily increased up to 200 μM UDP-galactose. The apparent K_m for Gb_4 in presence of 0.1% sodium cholate (**Table 2**) was 35 μM and the K_m for UDP-galactose was 68 μM. In presence of CHAPS the enzyme activity also increased with the increase of globoside up to 30 μg (Fig. 4C), but the K_m for Gb_4 was about four times higher (64 μM) in the female than that in the male (Table 2). In both male and female microsomes the V_{max} for UDP-galactose was about 1200 pmol/mg per h in presence of cholate.

Effect of ions

The standard incubation medium used for the assays contained Mn^{2+} (6.25 mM). **Table 3** shows the effect of replacing this cation in the incubation medium. In the absence of Mn^{2+}, the enzyme activity decreased by more than 95%; and of all the divalent cations tested, only cobalt partially activated the enzyme activity. The presence of Ca^{2+}, Mg^{2+}, Zn^{2+}, and Ni^{2+}, and Cl^-, did not stimulate galactosyltransferase activity in the microsomes. Addition of EDTA in the absence of any cation did not decrease the activity any further than did the removal of Mn^{2+} from the incubation medium. In the absence of Mn^{2+}, acetate (6.25 and 50.0 mM) had no effect on the enzyme activity. However, in its presence, 50.0 mM acetate decreased the specific activity of the enzyme by about 40% (data not shown).

Characterization of enzyme reaction product: β1-3 galactosylgloboside

The identity of the enzymatic reaction product was ascertained by autoradiography and immunostaining. HPTLC autoradiogram of the purified reaction product showed a single band (**Fig. 5A**) co-migrating with standard galactosylgloboside isolated from male DBA/2 mouse

TABLE 2. Kinetic parameters of Gb_4Gal-TR in kidney microsomes from C57BL/6 mice

Assay Condition	Male		Female	
	K_m	V_{max}	K_m	V_{max}
	mM	*pmol/mg/h*	*mM*	*pmol/mg/h*
0.5% CHAPS, pH 6.5[a]				
Gb_4	0.016	95.5	0.064	84.0
0.1% Sodium cholate, pH 5.6[b]				
Gb_4	0.035	237.9	0.035	208.2
UDP-Gal	0.064	1219.3	0.068	1212.4

The enzyme was assayed as described in Methods.

[a]To determine this parameter, the concentration of Gb_4 was varied from 7.78 to 467.2 μM.

[b]To determine these parameters, the concentration of Gb_4 was varied from 15.5 to 467.2 μM, and the concentration of UDP-galactose was varied from 5.4 to 266.7 μM.

kidney, that also reacted with the anti-SSEA-3 antibody, MC 631 (Fig. 5B). This antibody recognizes the terminal β1-3 linkage of galactose to N-acetylgalactosamine in Gb_5, although it does slightly cross-react with the Gb_4 core structure. The reaction product of galactosyltransferase in microsomes from DBA/2 mouse kidney also co-migrated with standard Gb_5 (data not shown).

DISCUSSION

UDP-galactose:globoside galactosyltransferase may be a regulatory enzyme in the expression of SSEA-3, SSEA-4, and globo-series SSEA-1 and ABH blood-group antigens. Sex, strain, and age-dependent expression of some of these antigens has been well documented (6, 9, 18). SSEA-3, galactosylgloboside, is expressed in kidneys of adult DBA/2 but is not detectable in kidneys of C57BL/6 mice, which express globo-series SSEA-1 glycolipid instead. We have measured the activity of the β1-3 galactosyltransferase in kidney microsomes from both strains. The specific activity of the globoside:galactosyltransferase was higher in microsomes from males than in females. The expression of this enzyme in C57BL/6 kidney suggests an active system for the biosynthesis of SSEA-1 glycolipid from SSEA-3 and a consequent high turnover rate for the latter, that probably contributes to its undetectable amounts. In contrast, the biosynthesis of SSEA-1 in DBA/2 mice is genetically blocked, therefore SSEA-3 antigen accumulates (19). The difference between the levels of galactosyltransferase specific activity in male and female kidneys was pronounced in presence of CHAPS, a zwitterionic detergent that prevents the destruction of protein structures. In contrast, sodium cholate, the ionic analogue of CHAPS, disrupts the membrane and possibly alters the native configuration of the enzyme. The activity obtained under minimal perturbing conditions might be indicative of the native membrane organization and the in vivo status of the enzyme. The data reported here, therefore, suggest that the observed differential activity of Gb_4Gal-TR is responsible for the increased levels of SSEA-3 and SSEA-1 glycolipids in male versus female DBA/2 and C57BL/6 kidneys, respectively (9). One of the mechanisms involved in this phenomenon might be the difference in affinity of the enzyme for globoside observed in presence of nondisruptive environment of CHAPS (Table 2). This is consistent with our suggestion of a regulatory role of the enzyme in the biosynthetic pathway of globo-series glycolipids.

The Gb_4Gal-TR is differentially activated in males and females by the ionic detergent. In 0.1% sodium cholate the enzyme activity increased by approximately 70% in female microsomes and by only about 6% in male microsomes in comparison to the activity obtained in presence of 0.05% sodium cholate (Fig. 1). As a result, the difference in specific activity of the enzyme between the sexes decreased considerably in presence of 0.1% sodium cholate. The differential activation and a reduction in the difference of enzyme activity between male and female microsomes in presence of different concentrations of sodium cholate could have several explanations. We suggest that most likely, in the female microsomes, the presence of sodium cholate either releases an otherwise inaccessible activator, or an inhibitor normally bound to the enzyme is dissociated. Existence of a putative modulator in this system is indicated by our data on substrate affinity (Table 2), heat denaturation profile (Fig. 3), and a differential activation of the enzyme in male and female microsomes by CHAPS and sodium cholate (Fig. 1). An inhibitor bound to the enzyme in the female microsomes

TABLE 3. Effect of various ions on the activity of C57BL/6 kidney microsomal Gb_4Gal-TR

Condition	Specific Activity	
	Male	Female
	pmol/mg/h	
Complete	186.6 (33.2)[a]	128.2 (23.6)[a]
− Mn^{2+}	4.1	3.8
− Mn^{2+} + EDTA	3.5	4.4
+ Ca^{2+}	4.5	5.3
+ Mg^{2+}	5.9	4.3
+ Zn^{2+}	6.2	4.4
+ Ni^{2+}	24.6	23.7
+ Co^{2+}	101.3	94.9
+ Cl^-	3.8	3.9
+ CH_3COO^-	3.8	4.8
+ CH_3COO^- (50.0 mM)	6.9	4.9

The enzyme was assayed in presence of added globoside as described in Methods at pH 5.6. Various ions (6.25 mM) were used to replace Mn^{2+} (6.25 mM) in the incubation medium. Each number is a mean value from representative assays run in triplicate and repeated at least twice. The variability in data between experiments was less than 10%.

[a]Galactose incorporated into endogenous acceptor in the absence of added globoside.

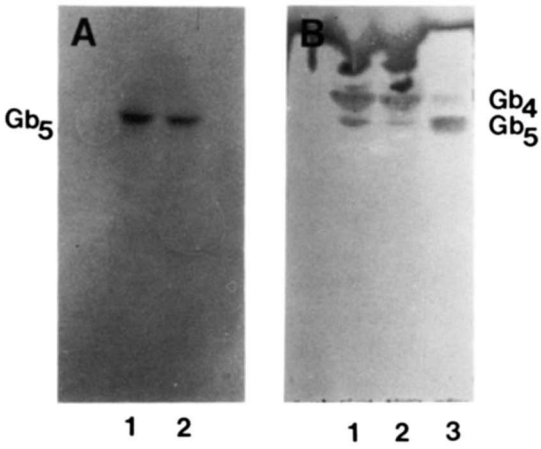

Fig. 5. Identification of C57BL/6 mouse kidney microsomal Gb_4Gal-TR reaction product. The radioactive lipid obtained and purified as described in the text was spotted on an HPTLC plate and developed in chloroform–methanol–0.25% $CaCl_2$(aq) 5:4:1 (v/v/v). Panel A: autoradiogram: lane 1, male; lane 2, female. Panel B: immunoblot of the plate shown in panel A with anti-SSEA-3 antibody (MC 631); lane 1, male; lane 2, female; and lane 3, standard Gb_5 (solvent front staining is an artifact).

may lower its affinity for the substrate and also alter its heat inactivation profile. In contrast, the absence of a bound ligand in male microsomes might facilitate the interaction with the substrate and also make the enzyme more susceptible to heat denaturation (Fig. 3). Different modulators for various glycosyltransferases have been described that affect their kinetic parameters (20–23). Moscarello, Mitranic, and Vella (24) described the decrease of K_m and increase in V_{max} for milk galactosyltransferase in presence of N-acetylglucosaminyltransferase as a modulator. Recently, an endogenous soluble thermolabile protein inhibitor has been described for an intestinal fucosyltransferase (25). Most of the modulators described so far are heat-stable, membrane-bound, or soluble proteins. However, modulation of galactosyltransferases by lipids is also a well established phenomenon (26–28). Some of the phospholipids we tested (data not shown) did modify the enzyme activity. Further studies are under way to fully characterize the putative modulator(s) in our system.

The β1-3 globoside:galactosyltransferase described by Chen et al. (29) in EC cells shows some interesting differences and similarities with the enzyme described here. The enzyme in EC cells has a neutral optimal pH, is activated by Triton CF-54, and the K_m for UDP-galactose is about 2-fold greater than the one we have observed. However, the K_m for Gb_4 is similar and the enzyme from both sources requires Mn^{2+} for expression of activity. The differences might be more a characteristic of the membrane environment than of the enzyme itself.

Most of the galactosyltransferases studied so far have an absolute requirement for a divalent cation, especially Mn^{2+}. Holmes (30) has described a lactosylceramide β1-3 galactosyltransferase from Colo 205 cells that is activated by cobalt. Our data on the Gb_4Gal-TR indicate that the kidney enzyme can be partially activated in presence of cobalt, although Mn^{2+} is essential for full activity. Most of the galactosyltransferases have been reported to express maximal activity around neutral pH, in contrast to the acidic range for the enzyme described here. Bailley, Piller, and Cartron (31) have, however, described a human kidney galactosyltransferase with an optimum pH of 5.0 and a requirement for cadmium. This would suggest a tissue specificity in the functional environment of glycosyltransferases. On the other hand, Hildebrand and Hauser (32) have described glycosyltransferases in spleen for the biosynthesis of lactosylceramide and triglycosylceramide with optimal pH in the acid range close to that observed by us for Gb_5 synthesis in the kidney. Our observation that acetate inhibited the enzyme activity in the presence of Mn^{2+} suggests a possible interference in the association of the enzyme and the cation by acetate. This interference may account for the lower activity observed in our pH experiments in presence of 50 mM acetate buffer (Fig. 2) in comparison to that in presence of MES.

It is of interest to point out that V_{max} for UDP-galactose was obtained at a higher concentration of the donor than that used in our standard incubation conditions. However, the substrate did not become limiting during our assays as suggested by linearity of the reaction.

The lipid product synthesized by this enzyme is β1-3 galactosylgloboside as deduced by its co-migration with standard galactosylgloboside from DBA/2 kidney and its reactivity to anti-SSEA-3 antibody, MC 631. This antibody recognizes the specific β1-3 linkage between terminal galactose and galactosamine that is resistant to common galactosidases (5). The reaction product from DBA/2 microsomes also behaves similarly.

We conclude that the Gb_4Gal-TR activity in the kidney may regulate the observed sex differences in the amounts of SSEA-3 and SSEA-1 in the kidney of C57BL/6 and DBA/2 mice, respectively.

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