Liver Galactose-l-Phosphate Uridyl Transferase: Activity in Normal and Galactosemic Subjects

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ABSTRACT The kinetic characteristics of galactose-1-phosphate uridyl transferase have been determined in homogenates of human liver biopsies obtained from control subjects and in 50-fold purified enzyme preparations from liver obtained at autopsy. A standardized assay procedure employing linear kinetics was used to assess the enzyme activity in homogenates of liver biopsy specimens from five control subjects and four patients with congential galactosemia with demonstrated absence of the enzyme activity in red blood cells. Activity of control specimens ranged from 11.8 to 17.2 mµmoles of UDPgalactose formed per min mg of protein. Liver of two galactosemic patients, both Caucasian, possessed no detectable enzyme activity (less than 1-2% of normal). The tissue of two others, both Negro, who are known to be capable of metabolizing intravenously administered galactose, contained easily detectable enzyme at approximately 10% of the controls. No alternate enzymatic activity for formation of UDPgalactose was found in the liver of Negroes with galactosemia that was as active as the residual galactose-l-phosphate uridyl transferase. The data suggest that the residual liver enzyme activity accounts for the ability of Negroes with galactosemia to metabolize limited but significantly large quantities of galactose.

INTRODUCTION

The disorder, congenital galactosemia, characterized by cataracts, liver disease, and mental retardation, is due to a deficiency of the enzyme galactose-l-phosphate uridyl transferase (UDPglucose: α -D-galactose-l-P uridyl transferase [Enzyme Commission, 2.7.7.12]) (1). This enzyme which catalyzes the reaction gal-1-P + UDPglucose \rightarrow glucose-1-P + UDPgalactose has been shown to be defective in a variety of tissues from galactosemic patients, and has been studied from a kinetic point of view in human erythrocytes (2), leucocytes (3, 4), cultured fibroblasts (4), and most recently, intestinal mucosa (5). Although the liver is the principle tissue for disposal of galactose, and despite the fact that the defect has been demonstrated in liver (6), there has been no detailed kinetic characterization of human liver transferase nor further exploration of the enzyme activity in liver of galactosemic patients.

Of particular interest has been the group of patients with congenital galactosemia who despite absent red cell gal-l-P uridyl transferase, has been shown to metabolize significant amounts of galactose (7, 8). Since all of these patients have been Negro, the term "Negro variant" of galactosemia has been applied to this group (9). Of several tissues from such a patient studied in vitro, only liver has been shown to oxidize galactose-1-⁴⁴C to ¹⁴CO₂ (10). In order to determine whether this oxidation is related to the presence of residual galactose-1-P uridyl transferase activity, we have assessed the level of this enzyme in liver biopsies from galactosemic Negroes and Caucasians after first characterizing the kinetic properties of the enzyme in liver of nongalactosemic subjects. Our results form the basis of this report.

METHODS

Subjects. Pertinent clinical data on subjects from whom liver biopsies were obtained are summarized in Table I. Six patients ranging in age from 4 months to 41 yr and without any history or stigmata of deranged galactose metabolism are considered control subjects. Specimens were obtained from two controls by punch needle biopsy (C with idiopathic hypoglycemia and K who had cystinosis with a considerable degree of renal failure). Of the other four patients from whom small wedge biopsies were surgically ob-

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¹Abbreviations used in this paper: UTP, uridine triphosphate; UDPG, TDPG, CDPG, UDPGal, nucleotide sugars.

Subject	Sex	Race	Age	Diagnosis	Type of biopsy	Transferase activity
			yr			mµmoles/min/mg protein
Controls						
В	F	С	41	Cholecystitis	Ор	11.8
S	F.	С	37	Cholecystitis	Op	17.2
С	F	С	2	Hypoglycemia	P	16.1
K	Μ	С	9	Cystinosis	Р	14.7
U	F	С	4 months	Hypoglycemia	Ор	15.0
Sm	М	Ν	42	Cerebral hemorrhage	Autopsy purified	4.4 327.0
Ba	М	С	60	Perinephric abscess	Autopsy	4.0 225.0
Galactos	emics				puincu	
W. W.	М	Ν	9		Р	1.3
M. W.	М	Ν	8		Р	1.8
C. W.	F	С	5		Р	nondetectable
F. R.	М	C	2		Р	nondetectable

 TABLE I

 Patient Clinical Characteristics and Activity of Liver Galactose-1-P-Uridyl Transferase

C, Caucasian; N, Negro.

Op, operative; P, punch.

Values are representative of at least three separate assays of liver homogenate activity.

Methods are described in the text.

tained, two were undergoing gall bladder surgery, one, patient R, was being explored for recurrent Wilms tumor after receiving actinomycin D and one, patient U, underwent pancreatic exploration for an islet-cell adenoma and subtotal pancreatectomy. None of these liver specimens was histologically abnormal.

Of the four galactosemic patients, two were Negro. All had absent red cell transferase consistent with the galactosemic genotype. The Caucasian patients F. R. and C. W. had the usual clinical syndrome in the neonatal period. An earlier clinical description of the Negro siblings M. W. and W. W. has been published (8). In more recent metabolic studies, these brothers both demonstrated substantial ability to metabolize small amounts of galactose-1-¹⁴C to ¹⁴CO₂ and belong in the group termed the "Negro variant" of subjects with galactosemia (9). Previously, liver tissue from patient W. W. had been shown in vitro to oxidize radioactive galactose to ¹⁴CO₂ (8). The liver tissue from all galactosemic patients was examined histologically and found within normal limits. All had normal liver function tests at the time of biopsy.

The galactosemic patients had been on a galactose-free diet since the postnatal period except for patient W. W. who was on a normal, milk-containing diet without symptoms for the 1st yr of life when he was found to have absent red cell transferase. The control patients were on regular hospital diets and no attempt was made to assess the galactose content.

For enzyme purification, approximately 200 g of liver was obtained at autopsy from two adult male patients within 6 hr of death. These patients had no history of liver disease and livers exhibited no significant pathology. Sm, a Negro male, age 42, died from massive cerebral hemorrhage and Ba, a 60 year old Caucasian male, died from a perinephric abscess and septicemia. Handling of tissue and assay of Gal-l-P uridyl transferase. Punch biopsies weighing between 25 and 35 mg and wedge biopsies weighing about 20 mg were kept at 4°C for 10-20 min until homogenized in 20 vol of 20 mM KCl. After spinning at 30,000 g for 30 min the supernatant was divided into 0.1 ml aliquots which were frozen in glass vials and kept at -44° C until used. The homogenate enzyme activity was stable on storage at -44° C for 6 months.

Liver obtained at autopsy was frozen and stored at -44° C until partially purified with minor modifications by the method of Mayes and Hanson (11). The steps consisted of homogenization in 20 mM KCl, precipitation with ammonium sulfate at 50% saturation, dialysis against distilled water, adsorption and elution from calcium phosphate gel, and chromatography of the gel eluate on a DEAE-cellulose column employing elution with batches of 20 and 80 mM phosphate buffer. The specific activity of the enzyme before and after purification is shown in Table I.

The determination of enzyme activity was performed by the method of Bertoli and Segal (12) except that the alkaline phosphatase step was not used prior to chromatography of the incubation mixture. The assay is based on the stoichiometric formation of UDPgalactose-14C during the incubation of the enzyme with galactose-1-P-14C and unlabeled UDPG according to the reaction Gal-1-P-¹⁴C + UDPG \rightarrow UDPGal-¹⁴C + G-l-P. After incubation the labeled gal-l-P is separated from the radioactive product by chromatography on DEAE-cellulose ion exchange paper with 0.05 M LiCl as solvent. The product radioactivity was determined by counting the area of the paper containing the UDPgalactose-14C with a liquid scintillation spectrometer employing Liquifluor (New England Nuclear Corp., Boston, Mass.) as the scintillator. The amount of product formed can be calculated using the specific activity of the radioactive substrate.

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FIGURE 1 The effect of incubation time on galactose-1phosphate uridyl transferase activity of human liver. The right ordinate refers to purified enzyme (10 μ g protein), the left to biopsy homogenates (50 μ g protein).

The standard assay procedure was as follows: The incubation mixture contained a final concentration of 0.2 mm UDPglucose, 0.25 mm galactose-1-P (0.0056 μ Ci), 10 mm mercaptoethanol, 100 mm glycine buffer pH 8.2 in a total vol of 200 μ l and a final pH of 8.2. The reaction was started by the addition of 20 μ l of homogenate containing about 50 μ g protein and carried out for 5 min. When partially purified enzyme was studied 2 μ l containing 10 μ g protein was added and incubated 2.5 min. The reaction was stopped by boiling for 90 sec and 20 μ l of the reaction mixture was spotted for chromatography. The specific activity of the enzyme is expressed as millimicromoles of UDPgalactose formed per minute per milligram soluble protein.

Other assay procedures. UDPgalactose pyrophosphorylase was assayed both by the technique of Abraham and Howell (13) and by the standard procedure reported here for transferase except that 0.25 mM UTP was added as substrate in place of UDPG. Protein was estimated by the method of Lowry, Rosebrough, Tarr, and Randall (14). *Materials.* Alpha-D-galactose-1-phosphate, UDPG, UTP, UDPGal, TDPG, and CDPG were products of Calbiochem, Los Angeles, Calif. Alpha-D-galactose-1-phosphate-¹⁴C (uni-

Los Angeles, Calif. Alpha-D-galactose-I-phosphate-^{MC} (uniformly labeled, SA 13–20 mCi/mmole) was purchased from International Chemical and Nuclear Corp., City of Industry, Calif. Whatman No. DE81 was the DEAE-cellulose ion exchange paper employed.

RESULTS

Kinetic characteristics of normal enzyme. Initial velocity studies of the partially purified enzyme and whole homogenate are shown in Figs. 1 and 2. The amount of UDPgalactose formed by the purified preparation using 10 μ g of protein was linear for about 3 min of incubation while whole homogenate was linear for 10 min when 50 μ g of protein was used. On 2.5 min of incubation purified enzyme produced UDPgalactose linearly up to 15 μ g of protein, and on 5 min of incubation whole homogenates demonstrated proportionality to protein, added up to 100 μ g of protein. Therefore, the assay procedure for purified enzyme utilized, employed 2.5 min incubation and about 10 μ g of protein, that for crude homogenate 5 min incubation and about 50 μ g of protein.

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Transferase activity measured in the pH range from 8 to 9 revealed a broad optimum between 8.2 and 8.6 for the purified preparation (Fig. 3). Essentially the same results were found with crude homogenate.

The relationship of substrate concentration to enzyme activity is shown in Fig. 4 by a typical Lineweaver-Burk plot of the data obtained with both whole homogenate and purified enzyme. Table II summarizes the K_m and V_{max} values for UDPG when Gal-l-P was 0.25 mM and for Gal-l-P when UDPG was 0.20 mM for the various enzyme preparations studied. K_m values were relatively uniform in both homogenates and purified preparation. V_{max} in the various homogenates was similar except for subject R who had been given actinomycin D. The difference in maximum velocity for purified and crude enzyme reflects the approximately 50-fold purification obtained.

Various nucleotide sugars such as TDPG, CDPG, and UDPGal were tested at 0.20 mm as substrates in place of UDPG for reaction with labeled Gal-l-P. In our standard incubation only UDPGal resulted in a labeled compound migrating as a sugar nucleotide on DEAE paper chromatography. The velocity of this reaction, Gal-l-P-¹⁴C + UDPGal \rightarrow UDPGal-¹⁴C + Gal-l-P, was concentration dependent as shown in Fig. 5. The K_m and V_{max} of UDPGal when Gal-1-P was 0.25 mm are shown in Table II. These values are essentially the same as those found for UDPG. No labeled nucleotide could be shown to be formed when UTP at 0.25 mm was substituted for UDPG as substrate with the 5 min incubation time of the standard assay. On prolonged incubations of crude homogenates for 20 min, however, small amounts of labeled nucleotide sugar presumably UDPgalactose were formed at a rate of about 1.5 mµmole/ min per mg protein.



FIGURE 2 The effect of protein concentration on enzyme activity. The right ordinate refers to homogenate W. W. activity (5 min incubation) while that on the left refers to purified enzyme (2.5 min incubation) and normal homogenate (5 min incubation).

Transferase activity in galactosemic patients. The transferase activity, measured under standard conditions, of liver biopsies obtained from five control patients and four galactosemic subjects is shown in Table I. Levels in controls ranged from 11.8 to 17.2 mµmoles/min per mg protein. In two galactosemic patients, both Caucasian, there was no detectable activity while in both Negroes with galactosemia activity was observed at approximately 10% of the control. The enzyme activity observed in the liver of the two Negro siblings was dependent on addition of UDPG to the homogenates; no detectable labeled nucleotide sugar was formed without this substrate, even on prolonged incubations of 20 min. The homogenates of liver, obtained at autopsy performed within 6 hr of death, have about a quarter of activity in fresh biopsy specimens.

The formation of labeled product in homogenates from the two Negro galactosemic patients increased linearly with time as shown in Fig. 1. 20-min incubation values not shown on the graph fell on the line if extended. Activity was proportional to protein as seen in Fig. 2. Galactosemics C. W. and F. R. revealed no detectable liver enzyme activity on incubation up to 20 min, nor was activity seen on adding larger amounts of homogenate. Insufficient homogenate was available from galactosemic patients W. W. and M. W. to assay accurately substrate concentration dependence. Liver from galactosemic patients M. W. and W. W. which had measurable transferase activity under standardized conditions also revealed similar activity when UDPGal was substituted for UDPG thus behaving qualitatively like the normal.



FIGURE 3 The pH dependence of liver galactose-1-phosphate uridyl transferase. 100 mm glycine buffer at the pH's shown was employed in the standard assay. The right ordinate refers to purified enzyme, the left to the homogenate.



FIGURE 4 A. The concentration dependence of purified enzyme (\bullet) and biopsy homogenate (\blacktriangle) on galactose-1phosphate (Gal-1-P). B. Concentration dependence of the same enzyme preparations on UDPglucose (UDPG). v is expressed as mµmoles/min per mg × 10⁻⁸ for the purified enzyme and mµmoles/min per mg × 10⁻² for the homogenate.

The possibility of the presence of alternate routes of formation of galactose containing sugar nucleotides was investigated in the liver biopsies of the galactosemic children. An attempt was made with the method of Abraham and Howell (13) to assay UDPGal pyrophosphorylase which catalyzes Gal-1-P interaction with UTP to form UDPGal plus pyrophosphate (15). The use of 50 μ g of liver protein in which transferase activity was easily detectable was insufficient to detect UDPGal pyrophosphorylase either in normal liver or in that from patient M. W. Substitution of UTP at 0.25 mm for UDPG in our assay for transferase was also employed as a UDPGal pyrophosphorylase assay. No radioactive UDPGal was formed by any of the homogenates of galactosemic or normal livers with 5 min of incubation. After 20 min of incubation, radioactive sugar nucleotide formation was detected at an average rate of 1.5 mµmole/ min per mg in three control specimens while that from patient W. W., a Negro galactosemic patient, gave a value of 0.5 mµmole/min per mg.

 TABLE II

 Km and Vmax
 Values of Human Liver Galactose-1-P-Uridyl

 Transferase

Enzyme	Substrate*								
	Gal-1-P		UDPG		UDPGal				
	K_m ‡	Vmax§	Km	Vmax	Km	Vmax			
Homoge	nate								
S	0.10	18	0.26	20	0.35	22			
В	0.11	15	0.16	22	1.0	50			
R	0.07	6	0.05	6					
С	0.11	26	0.08	31	0.20	40			
Partially	v purified	l							
Sm	0.30	1117			0.31	1111			
Ba	0.25	1000	0.20	1000					

* In homogenate experiments when Gal-1-P was the variable substrate UDPG was 0.20 mm. When UDPG or UDPGal were varied Gal-1-P was 0.25 mm. When purified enzyme was used and Gal-1-P was variable UDPG concentration was 0.25 mm and when sugar nucleotide was the varied substrate Gal-1-P was 0.35 mm.

 $\ddagger K_m$ is expressed in mM.

§ V_{max} is expressed as mµmoles/min per mg protein.

To examine the possibility that transferase in galactosemic liver may be altered thereby requiring a sugar nucleotide other than UDPG, we substituted thymidine diphosphoglucose for UDPG in the assay of galactosemic liver. No TDPGal was formed after a 5 min incubation. With a 20 min incubation, three normal livers formed detectable nucleotide sugar at a velocity of 1.2 mµmoles/ min per mg, while galactosemic liver from patient F. R. formed none and that from patient W.W. formed 2 mµmoles/min per mg.

DISCUSSION

Much of our understanding of congenital galactosemia has resulted from the study of galactose-l-phosphate uridyl transferase in peripheral blood cells wherein the deficiency of the enzyme was first noted (1). Anderson, Kalckar, and Isselbacher have shown that transferase activity was also deficient in liver (6). Until recently their data seemed sufficient for interpreting the metabolic derangement in galactosemic patients. The demonstration that certain galactosemic patients with absent red cell transferase can metabolize administered galactose (7) has prompted a reinvestigation of liver transferase activity in both normals and galactosemic patients. In the present work, we have analyzed in detail the kinetic parameters of transferase from the livers of nongalactosemic controls and have shown the similarities of partially purified enzyme to biopsy homogenates. The characteristics of transferase activity in normal

human liver are similar to those observed previously with rat liver (12).

Our values for liver transferase differ from those of Anderson et al. whose data until now were the only data available about the human enzyme (6). Although the radioactive assay used was similar in principle to that described here, no kinetic analysis of the conditions for the assay was reported. The limit of sensitivity in the assay of Anderson et al. was said to be 0.3 µmoles/gm liver per hr while that of our assay is 0.25 mµmoles/mg liver per hr), (after converting from our velocity in m#moles/mg protein per min), a difference factor of 1000. This difference is no doubt due to the nonlinear kinetics of their assay in which 7-15 mg of tissue and an incubation time of 30 min was employed. The difference is also due to the addition of UTP to the earlier assay system since we have recently found UTP to be a potent transferase inhibitor.²

Comparison of the transferase activity in liver of four galactosemic subjects with that of nongalactosemic controls has revealed that two galactosemic patients, Negro siblings, possess easily detectable amounts of enzyme in liver amounting to 10% of the normal, while two Caucasian with galactosemia had nondetectable levels (that is, less than 1-2% of the normal). Of the two liver homogenates assayed by Anderson et al. (6), one was an adult Negro male who had easily detectable enzyme activity while the other was a Caucasian infant without detectable enzyme.³ The latter investigators felt that the finding of detectable enzyme in the Negro adult was related to his older age. Subsequent studies have indicated that being a Negro may be a factor in the ability of galactosemic patients to metabolize galactose (7, 9). Enzyme activity would not be expected to "mature" with age since developmental studies of rat liver transferase have indicated that transferase has the highest specific activity in the young liver and subsequently decreases with age (12).

The Negro adult who had detectable enzyme estimated at 5% of normal (6), was later extensively studied for his metabolic capability and was found to be able to easily metabolize galactose-1-¹⁴C to ¹⁴CO₂ (7, 9). Subsequently, his liver biopsy specimen was found in vitro to oxidize galactose-1-¹⁴C to ¹⁴CO₂ at a near normal rate and extent. Patient W. W. investigated here was shown to have a galactose metabolic capability at age 3, and a

^a Unpublished observations of the authors.

⁸Although the race of the patients was not stated in the paper by Anderson, Kalckar, and Isselbacher, the race of the adult is known by one of the authors, who was at National Institutes of Health at the time, and who subsequently studied the same patient (7). The race of the infant was made known to the present authors by Dr. George Donnell of the Los Angeles Childrens Hospital, who sent the biopsy to Dr. Kalckar.

punch liver biopsy at that time was capable in vitro of galactose oxidation (8). The ability of both Negro patients W. W. and M. W. to oxidize galactose-1-4¹⁴C was tested at the time of the liver biopsy for transferase activity and both were found able to rapidly and extensively convert the radioactive substrate to ¹⁴CO₂. The observations that Negro galactosemic patients convert galactose-1-14C to blood glucose (7), have inhibition of galactose oxidation by ethanol (7, 16), and produce ¹⁴CO₂ from both C-1 and C-2 labeled galactose in a normal pattern (17), are consistent with operation of the sugar nucleotide pathway of galactose metabolism. The findings that Negroes with galactosemia have residual transferase in the liver imply that it is this enzyme, though limited in amount, which accounts for their ability to metabolize galactose. This is consistent with our observations that the capacity of the Negro galactosemic to metabolize intravenously administered galactose is limited, being nearly normal for quantities up to only 2 g (7).

An alternate pathway involving a nucleotide sugar as an explanation of the galactosemic Negro's ability to metabolize galactose could be considered. The UDPGal pyrophosphorylase reaction in which Gal-I-P + UTP \rightarrow UDPGal + PP was postulated by Isselbacher to serve this function (15). Such a reaction would circumvent the transferase step and satisfy the metabolic observations enumerated earlier. Recently, Abraham and Howell studied this enzyme activity in autopsy specimens of human liver (13). Employing a standardized assay, they showed that the specific activity of the enzyme was about one hundredth that of Gal-l-P uridyl transferase, there being no increase with age as suggested by Isselbacher's rat experiments (15). Indeed, the activity is so low as to raise the question of its functional significance. Knop and Hanson, who studied the substrate specificity of human liver crystalline UDPglucose pyrophosphorylase, reported that what has been called UDPGal pyrophosphorylase activity is in reality a small degree of reactivity of UDPG pyrophosphorylase with UDPGal (18). Ting and Hanson have suggested caution in attributing significance to the pyrophosphorylase route for metabolism of galactose (19). Nonetheless, we have looked for this activity in our biopsies of galactosemic and normal subjects. In short incubation times during which transferase is very active in normal subjects and easily detectable in galactosemic Negroes, no UDPGal pyrophosphorylase activity was seen. It is our feeling that UDPGal pyrophosphorylase activity is unlikely to explain the rapid and quite proficient galactose metabolism seen in Negroes with galactosemia. Likewise, we have observed no other transferase activity employing another sugar nucleotide such as TDPG that might adequately explain the metabolic observations.

The delineation of a difference between Negro and Caucasian patients with galactosemia by the presence or



FIGURE 5 Effect of UDPgalactose (UDPGal) concentration on enzyme activity. (\bullet) purified enzyme with v expressed as mµmoles/min per mg × 10⁻³ and homogenate (\blacktriangle) with v expressed as mµmoles/min per mg × 10⁻².

absence of liver transferase activity, appears to be paralleled by similar finding with intestinal mucosa (5). About 10% of the normal intestinal Gal-I-P uridyl transferase was found to exist in mucosal biopsies from three Negroes with galactosemia, two of whom are subjects in the present paper. This would appear to reflect the common embryonic origin of the two tissues. The exact contribution of gastrointestinal tissue to the disposition of galactose, however, in the normal individual is not known. Although the liver is thought to be the prime organ for normal galactose disposal, it may well be that the liver and intestinal mucosa together provide the transferase complement which enables the galactosemic Negro to metabolize some galactose.

The exact explanation for possession of transferase activity by visceral tissue when other tissues of the Negro patients are deficient, remains to be explained. 6 Negroes are in the group of 18 subjects with galactosemia that we have studied. All five of the six who have been tested have metabolized galactose to a significant degree. In Hsia's group of 45 patients (20), two siblings are identified as Negroes, one of whom has been without symptoms of galactose toxicity while ingesting galactose (resembling our patient W. W.). Donnell's clinic of 41 subjects with galactosemia includes two Negroes (21). None of the patients in the latter two groups has had a quantitation of galactose metabolism but there is a clinical indication that the Negro in Hsia's group has a galactose metabolic capability. Although there appears to be a racial correlation with an ability to metabolize galactose and the possession of some liver transferase, the hereditary basis for this difference from other galactosemic patients has not been established. Kalckar ⁴ has suggested the possibility a provirus may be programming the formation of transferase in liver cells of these individuals.

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⁴ Kalckar, H. M. Personal communication.

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