ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS
Vol. 216, No. 2, July, pp. 503-511, 1982

Human Galactose-1-Phosphate Uridylyltransferase: Purification and Comparison of the Red Blood Cell and Placental Enzymes[1]

VINCENT P. WILLIAMS,[2] GEORGE R. HELMER, JR.,[3] AND CLIFFORD FRIED

Department of Psychiatry, Mental Retardation Research Center, University of California Los Angeles, School of Medicine, Los Angeles, California 90024

Received November 17, 1981, and in revised form March 1, 1982

Galactose-1-phosphate uridylyltransferase (uridine diphosphoglucose: α-D-galactose-1-phosphate uridylyltransferase, EC 2.7.7.12) has been purified from human red blood cells and placental tissue. The placental enzyme was obtained as a homogeneous protein with a specific activity of about 100 units/mg of protein by a combination of previously published methods (G. R. Helmer, Jr., and V. P. Williams, 1981, *Arch. Biochem. Biophys.* **210,** 573–580) and concanavalin A-Sepharose chromatography. The properties of the two enzyme forms have been examined with respect to subunit size, electrophoretic properties, isozyme distribution, kinetic patterns, and immunological properties.

Galactose-1-phosphate uridylyltransferase (uridine diphosphoglucose: α-D-galactose-1-phosphate uridylyltransferase, EC 2.7.7.12), to be referred to as uridylyltransferase, catalyzes the second step in the Leloir pathway by which galactose is converted to glucose 1-phosphate (1). The metabolic significance of this enzyme in humans was first recognized in 1956 when Isselbacher *et al.* (2) demonstrated that red blood cells of homozygous galactosemics lacked uridylyltransferase activity. More recent studies have shown that galactosemic individuals lack this activity in white cells (3), intestinal mucosa (4), and liver (5) and genetic studies have revealed that "uridylyltransferase-deficiency galactosemia" is inherited as an autosomal recessive trait (6).

In 1972, Tedesco (7) purified uridylyltransferase from human liver to a specific activity of 1 unit/mg and reported that the native enzyme was composed of three similar subunits, each of mass 30,000. Dale and Popják (8) made a similar claim in respect of the human red cell uridylyltransferase but they concluded that the native enzyme was composed of only two 31,000 subunits. A subsequent report by Williams (9) also described the purification of uridylyltransferase from human red blood cells, but the properties of the purified enzyme differed significantly from the earlier results of Tedesco (7) and Dale and Popják (8). For instance, the specific activity of the purified enzyme was estimated to be about 40 times higher than reported by Tedesco (7) and examination of the product by SDS[4]-polyacrylamide gel electrophoresis revealed that red cell uridylyltransferase was composed of two similar subunits of mass about 44,000. Two other reports have now appeared which describe the purification and properties of

[1] This research was supported by United States Public Health Service Grant HD-06576 from the National Institute of Child Health and Human Development.

[2] To whom correspondence should be sent.

[3] Present address: Department of Clinical Pathology, City of Hope National Medical Center, Duarte, Calif. 91010.

[4] Abbreviations used: DTE, dithioerythritol; MTT, 3-(4,5-dimethyl thiazolyl-2)-2,5-diphenyl tetrazolium bromide, thiazolyl blue; SDS, sodium dodecyl sulfate.

0003-9861/82/080503-09$02.00/0

human uridylyltransferase from placenta (10) and red blood cells (11). Both confirm the findings of Williams (9) that human uridylyltransferase contains two similar subunits of mass about 45,000.

Because of the disparities described above, the demonstration of "cross-reacting material" in galactosemic cells by Tedesco (7) and Tedesco and Mellman (12) and the further isolation of a catalytically inactive form of uridylyltransferase (which contained a subunit of mass about 31,000) from galactosemic red cells by Dale and Popják (8) cannot now be taken as evidence that galactosemia results from structural gene mutations and the synthesis of an inactive uridylyltransferase protein. Thus, before any other investigation of galactosemia is made, it will be necessary first to prepare antibody against pure uridylyltransferase then use this antibody as a probe in galactosemic cells.

The task, however, of obtaining several milligrams of pure uridylyltransferase for immunization is difficult since none of the preparative methods available lead to sufficient amounts of the pure enzyme. The problem is further complicated by the finding that the specific activity of the pure enzyme is between 80 and 100 units/mg (10), and since red cells contain about 15 units[5] of activity per 250 ml of packed cells, only a few micrograms of pure enzyme would be available from this source (9). Helmer and Williams (10) recognized this problem, and developed a purification scheme to isolate the enzyme from human placenta. Unfortunately, the overall yield of the procedure was only about 4% and the method was, therefore, not suitable for the large-scale preparation of the enzyme.

We describe here an alternative purification procedure for the isolation of uridylyltransferase from human placental tissue. Our method was developed from the one described by Helmer and Williams (10) and includes a concanavalin A-Sepharose step. This modified procedure leads to pure enzyme with an overall yield of about 20% and a specific activity between 80 and 100 units/mg. The properties of the purified placental enzyme have been examined and are compared to the enzyme isolated from red blood cells.

[5] One enzyme unit is defined as the amount of uridylyltransferase which catalyzes the formation of 1 μmol of NADPH/min; enzyme specific activities are given as units/mg of total protein.

EXPERIMENTAL PROCEDURES

Materials. The source of all chemicals, enzyme substrates, and coupling enzymes used in the uridylyltransferase spectrophotometric assay and other enzymatic assays was Sigma Chemical Company, St. Louis, Missouri. Polyacrylamide gel equipment and chemicals were obtained from Bio-Rad Laboratories, Richmond, California, and preformed isoelectric focusing gels were products of LKB-Producter AB, Bromma, Sweden. DEAE Affi-Gel Blue was obtained from Sigma Chemical Company. Sephadex G-200, Sephacryl S-200, and concanavalin A-Sepharose 6B were purchased from Pharmacia Fine Chemicals, Piscataway, New Jersey. Aquacide III and Freund's adjuvant were obtained from Calbiochem-Behring Corporation, La Jolla, California. Amicon Corporation, Lexington, Massachusetts, supplied all pressure dialysis equipment.

Enzyme purification. Galactose-1-phosphate uridylyltransferase was purified from outdated human red cells and fresh human placenta by the following procedures.

The red cell uridylyltransferase was purified by two alternative methods which are described below.

Method 1. This purification procedure has been described in detail by Williams (9). It consists of two steps of chromatography on DEAE-cellulose, followed by another on hydroxylapatite eluted with phosphate buffers at pH 7.0. The final product showed some heterogeneity by SDS-polyacrylamide gel electrophoresis having bands of protein of mass 47,000, 44,000, and 22,000. Each of the enzyme preparations, obtained by this procedure, had a specific activity of at least 15 units/mg and were used for immunization as described elsewhere under Experimental Procedures.

Method 2. This purification procedure has been developed recently and is a combination of the method described by Williams (9), and that of Helmer and Williams (10) for the purification of the placental enzyme. The exact experimental procedures such as column dimensions and composition of buffer solutions have been described in detail in the report of Helmer and Williams (10). Briefly, the method involves fractionation of crude hemolyzates by chromatography on DEAE-cellulose, hydroxylapatite, ethyliminohexylagarose, and Sephacryl S-200 in the presence of UDP-glucose. The final product was recovered in an overall yield of about 20% and had a specific activity of 44 units/mg.

Placental enzyme. The purification of uridylyltransferase from fresh human placenta was performed as previously described by Helmer and Williams (10), with the following modifications. After the crude cell extract was fractionated through the DEAE-cellulose and hydroxylapatite steps of the purification protocol, the enzyme fraction obtained from the ethyliminohexylagarose column was concentrated to 8 ml in an Amicon pressure cell with a YM-10 membrane at 70 psi, then applied to a column (2.2 × 160 cm) of Sephacryl S-200 equilibrated in 50 mM Tris-HCl buffer, pH 8.5, containing 0.5 M NaCl, 0.5 mM EDTA, and 2 mM DTE. The fractions containing uridylyltransferase were combined, concentrated to 5 ml by pressure dialysis (YM-10 membrane) and dialyzed for 16 h against 2 × 500-ml portions of 20 mM Tris-HCl buffer, pH 7.4, containing 0.5 M NaCl and 10 mM 2-mercaptoethanol. This fraction was applied to a column (1 × 27 cm) of concanavalin A-Sepharose, previously equilibrated in the same Tris-HCl buffer, pH 7.4; the enzyme was recovered in the void volume of this column. The active fractions were combined and concentrated by dehydration to 2 ml in a dialysis sac suspended in a 50% (w/v) slurry of Aquacide III and 20 mM Tris-HCl buffer, pH 8.5, containing 0.5 M NaCl and 2 mM DTE.

Assay procedures. All protein measurements were made by the Coomassie blue (G-250) procedure of Bradford (13). Crystalline bovine serum albumin, prepared in 10 mM sodium phosphate buffer, pH 7.5, was used to generate a calibration curve. Uridylyltransferase was monitored routinely at 37°C by the NADPH-coupled spectrophotometric assay system, described previously (8, 9). The reaction mixture for these assays was made up in 0.1 M glycine-NaOH, pH 8.5, containing 0.6 mM $MgCl_2$, 6 mM DTE, 0.48 mM galactose 1-phosphate, 0.6 mM UDP-glucose, 0.4 mM NADP, along with catalytic amounts (10 μM) of glucose 1,6-diphosphate and nonlimiting amounts of phosphoglucomutase (2 units) and glucose-6-phosphate dehydrogenase (1 unit).

Polyacrylamide gel electrophoresis. Disc gel electrophoresis was performed on 8% polyacrylamide gels in Tris buffers at pH 8.5. The experimental details of the procedures used and the method of monitoring uridylyltransferase activity in the gels have been described in detail elsewhere (9, 10).

SDS-polyacrylamide gel electrophoresis was performed on 10% gels by the method of Weber *et al.* (14). In each experiment, a mixture of crosslinked lysozyme calibration markers were run on duplicate gels and yielded five protein bands corresponding to the monomer (M_r = 14,300) through the pentamer (M_r = 71,500).

Isoelectric focusing. Isoelectric focusing was carried out on preformed 1-mm-thick LKB Ampholine polyacrylamide gels with Ampholines of 5.0 to 6.5 pH range. The anode solution consisted of 1% (v/v) acetic acid and the cathode was 0.01 M NaOH. Constant power of 20 W was applied for 3 h and the gel was cooled to 16°C. After completion of the focusing period, the pH gradient was measured in aqueous suspensions of 10 fractions cut from one edge of the gel, and the bands of uridylyltransferase activity were monitored by the MTT tetrazolium bromide salt method of Sparkes *et al.* (15). This staining method was, however, relatively insensitive and could only be used if about 150 munits of enzyme were applied initially to the gel surface. The following was the most convenient method to apply this quantity of enzyme to the surface of the gel. Samples were first dialyzed against 10 mM Tris-HCl buffer, pH 8.5, containing 2 mM DTE and each solution was diluted with the same buffer to a concentration of 2.8 units of uridylyltransferase/ml. An aliquot (0.1 ml) was then mixed with an equal volume of 2% (w/v) aqueous agarose at 40°C and the solution was poured into a Teflon mold, 10 × 5 mm and 3 mm deep, to solidify. The agarose slabs were removed from the molds and placed on the gel surface.

Molecular-weight determinations. The molecular weights of red cell and placental uridylyltransferase were determined by gel filtration on columns (2.5 × 80 cm) of Sephacryl S-200 or Sephadex G-200 according to the method of Andrews (16). These experiments were performed with a variety of buffers. For instance, the Sephacryl S-200 column was eluted with 50 mM Tris-HCl buffers, pH 8.5, containing 0.5 mM EDTA, 2 mM DTE, and one of the following NaCl concentrations: (a) none, (b) 0.1 M, or (c) 0.3 M. The buffer used to elute the Sephadex G-200 column was 10 mM sodium phosphate, pH 7.0, containing 2.5 mM DTE, as described by Banroques *et al.* (11).

Antibody production. For injection of 2 mg of red cell uridylyltransferase, specific activity 15 to 20 units/mg (purified by Method 1, Experimental Procedures) the protein solution (2 ml) was homogenized in 2 ml of Freund's complete adjuvant. Injections were given subcutaceously in four sites (1.0 ml each) to a male New Zealand albino rabbit. Booster injections were given in the same manner with Freund's incomplete adjuvant. The formation of anti-uridylyltransferase was monitored biweekly by removing about 1 ml of blood from an ear vein and determining the titer by immunoprecipitation, i.e., volume of antiserum required to remove 50% of activity from solution. When the titer was at its highest point, 30 ml of blood was removed from an ear vein and the serum was collected and stored at −20°C. After a total of 60 ml of serum was collected, the IgG fraction was purified first by two ammonium sulfate precipitations (0–25%; 25–50% saturation) followed by chromatography on a column (21 × 2.5 cm) of DEAE Affi-Gel Blue, equilibrated in 25 mM Tris-HCl buffer, pH 8.0, containing 28 mM NaCl. A similar experiment was also performed to purify the IgG fraction from 30 ml

of serum obtained from a nonimmunized rabbit. Both IgG fractions were then dialyzed against 10 mM sodium phosphate buffer, pH 7.4, containing 0.14 M NaCl and 0.1 mM EDTA and each solution was adjusted to a final protein concentration of 2.3 mg/ml.

Initial rate measurements. The standard NADPH-coupled spectrophotometric assay system was used to measure initial rates, except that the concentrations of UDP-glucose and galactose 1-phosphate were varied between 5.4 to 17.9 μM using 8 munits of uridylyltransferase activity. The average rate data were fitted to the best straight lines by a least squares analysis which yielded correlation coefficients of at least 0.98.

Solutions of UDP-glucose and galactose 1-phosphate were prepared in water. All rate determinations were performed with the same stock solution of substrates. The concentration of UDP-glucose was estimated spectrophotometrically at 262 nm (=10,000 M^{-1} cm^{-1}) and galactose 1-phosphate concentration was determined by a "galactose 1-phosphate consumption assay" at 340 nm with the NADPH-coupled assay system containing 30 munits of placental uridylyltransferase.

RESULTS

Concanavalin A-Sepharose Chromatography

The original procedure developed by Helmer and Williams (10) for the purification of uridylyltransferase from placental tissue included four chromatographic steps: DEAE-cellulose, hydroxylapatite, ethyliminohexylagarose, and Sephacryl S-200. The gel filtration step was necessary in order to remove a glycoprotein component of subunit mass 67,000 from the enzyme. Although these chromatographic procedures yielded homogeneous uridylyltransferase, the method was not suitable for the large-scale preparation of the enzyme since the overall yield of active enzyme was only about 4%. Helmer and Williams (10) discovered, however, that if the gel filtration step was performed under identical conditions with the inclusion of 25 μM UDP-glucose in the elution buffer, over 90% of the activity applied to the Sephacryl S-200 column was recovered, but the enzyme fraction was now contaminated with a 67,000-dalton glycoprotein component.

We have repeated these experiments and confirmed that, although UDP-glucose stabilizes uridylyltransferase during chromatography on Sephacryl S-200, the enzyme fraction is eluted in the same volume as the 67,000-dalton glycoprotein component. We have found that the 67,000-dalton glycoprotein component may be separated from uridylyltransferase on a column of concanavalin A-Sepharose, as described under Experimental Procedures. The enzyme was recovered in the void volume of this column with an overall yield of about 80%. Table I lists the details of the Sephacryl S-200 and concanavalin A-Sepharose steps and the specific activity of the final products. The recovery of protein and enzyme activity from placental tissue extracts prior to the Sephacryl S-200 step was similar to that described by Helmer and Williams (10).

TABLE I

STAGE II PURIFICATION OF URIDYLYLTRANSFERASE FROM PLACENTAL TISSUE[a]

	Total protein (mg)	Total activity (units)	Specific activity (units/mg)
Hydroxylapatite	39.4	125.1 (122)[b]	3.2
Ethyliminohexylagarose	4.2	81.8 (75.4)	19.5
Sephacryl S-200 (+UDP-glucose)	1.6	67.7 (16.3)	42.3
Concanavalin A-Sepharose	0.46	42.4	92.2

[a] The data and experimental details for the first four steps of Stage I of the purification protocol have been presented in the report of Helmer and Williams (10).

[b] The figures in parentheses are the total activity units recovered in the purification procedure described by Helmer and Williams (10).

Properties of the Red Cell and Placental Uridylyltransferase

Electrophoretic properties. The purified preparations of red cell and placental uridylyltransferases were analyzed on native polyacrylamide gels at pH 8.5. In each case a protein band was found ($R_f = 0.33 \pm 0.02$) which contained uridylyltransferase activity (Fig. 1). It has not been possible, however, by either of the two methods described under Experimental Procedures to obtain homogeneous red cell uridylyltransferase. Gel A (Fig. 1) represents the best preparation of the red cell enzyme which was obtained by Method 2 and had a specific activity of 44 units/mg. Figure 2 shows the activity profiles obtained from analysis of sliced gels containing samples of red cell enzyme (A), placental enzyme (B), and a mixture of the two forms (C).

Although the activity profiles in Fig. 2 gave only one peak for both the red cell and placental forms of the enzyme, the native gel pattern of the placental enzyme (gel B, Fig. 1) shows three distinct protein bands which were coincident with uridylyltransferase activity. This pattern of protein bands has never been observed in pH 8.5 native gels of red cell preparations. One explanation for the differences observed in the native gels might be that the placental enzyme was composed of a fewer number of active isozymes which were resolved in the native gels. If this were the case, then analysis of the two enzyme forms by polyacrylamide gel isoelectric focusing would reveal whether the two enzyme forms were composed of different numbers of isozymes.

The isoelectric focusing patterns, presented in Fig. 3, confirm that the purified red cell and placental enzymes each contain different numbers of catalytically active isozymes. It would appear, therefore, that the three protein bands present in the pH 8.5 native gel of the placental enzyme (gel B, Fig. 1) correspond to the three isozymes (pI values 5.72, 5.80, and 5.86) in Fig.

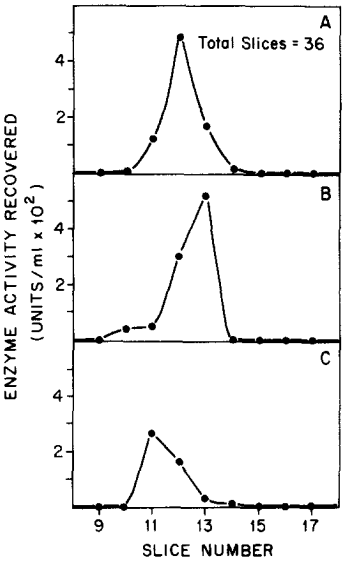

FIG. 2. Uridylyltransferase activity profiles obtained from the analysis of sliced gels of: red cell (A), placental (B), and a mixture of red cell/placental (C) enzymes. Each gel contained 500 munits of activity. Electrophoresis was performed at 3 mA/gel and continued until the bromophenol blue dye band had traveled a distance of 7.8 cm. A total of 36 slices was recovered from each gel.

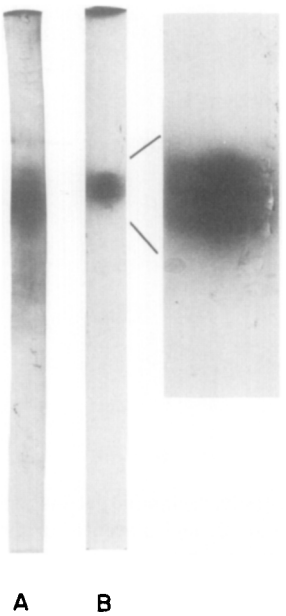

FIG. 1. Native polyacrylamide gels (pH 8.5) of uridylyltransferase purified from red cells (gel A) by Method 2, as described under Experimental Procedures, and placenta (gel B). Shown alongside gel B is a enlargement of the gel area to aid in the visualization of the three protein bands.

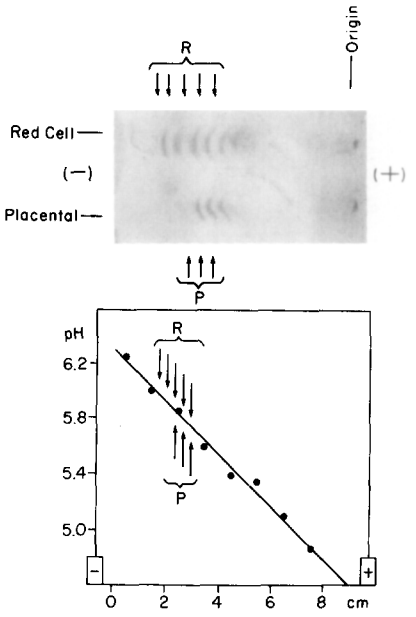

FIG. 3. Isoelectric focusing patterns of purified red cell (Method 2) and placental uridylyltransferase on LKB Ampholine polyacrylamide gel, pH range 5.0 to 6.5. Uridylyltransferase activity was monitored on the gel with the MTT stain and other details of this experiment are described in the text.

3. The presence of the same three isozymes as well as two additional cathodic isozymes in the red cell preparation probably accounts for the detection of only one diffuse protein band in the native gel (gel A, Fig. 1).

Although the gel patterns in Fig. 3 indicate that the red cell and placental enzymes contain different patterns of isozymes, analysis of the two forms by SDS-polyacrylamide gel electrophoresis showed the presence of only one protein band of mass 45,000 ± 2000, common to both forms of the enzyme, as illustrated in Fig. 4. Because of the heterogeneity of the red cell preparations, it has not been possible, however, to estimate directly from the SDS-gels the exact subunit composition of the red cell enzyme. For Instance gel A (Fig. 4) was obtained from a preparation of the red cell enzyme which had a specific activity of 44 units/mg (prepared by Method 2, Experimental Procedures) and contained two major protein bands of mass 45,000 and 22,000. In order to ascertain whether the 22,000-dalton component was associated with the enzyme, the native gel slices, which contained enzymatic activity (Fig. 2), were analyzed also on SDS-gels. In this instance only the band of protein of mass 45,000 was detected as illustrated by gel C in Fig. 4.

Steady-state kinetics. Representative initial rate data were obtained for the red cell and placental enzymes and the reciprocal plots were families of straight and parallel lines in the direction of UDP-galactose and glucose 1-phosphate formation. When substrate concentrations above 20 μM were used the plots showed nonlinearity due to product inhibition. The average K_m values for galactose 1-phosphate and UDP-glucose were calculated from intercept replots; these values were 0.021 and 0.015 mM for the red cell enzyme, and 0.011 mM and 0.011 mM for the placental enzyme, respectively. All of the rate data were measured with DTE-heat-treated fractions since the activities of these en-

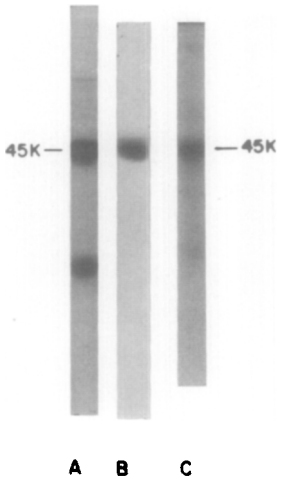

FIG. 4. SDS-polyacrylamide gel analysis of purified red cell (Method 2) and placental uridylyltransferases are represented by gel A and gel B, respectively. Each gel contained about 8 μg of total protein. Gel C was obtained from an analysis of the red cell uridylyltransferase-containing slices (slices 11-13) of the pH 8.5 native gel, shown in Fig. 3 (A). The mass of each protein band present in gels A, B, and C was estimated from duplicate gels containing a mixture of cross linked lysozyme marker proteins, as described in the text.

zyme solutions did not change over the duration of the experiments if stored at −20°C (9, 10). However, the kinetic parameters of both the purified red cell and placental fractions were examined also by the same methods and the K_m values obtained from these measurements were similar to those summarized above.

The experimental kinetic patterns were consistent with a ping-pong bi-bi reaction mechanism involving a modified form of the enzyme as an intermediate (17). Other reports have described in some detail the kinetic parameters and substrate inhibition patterns for the human red cell (18) and the *Escherichia coli* (19, 20) uridylyltransferases. In all respects the kinetic picture is consistent with a ping-pong bi-bi mechanism. Final proof of a uridylyl-enzyme intermediate on the catalytic pathway has come from studies of the different reactivities of the red cell enzyme toward iodoacetic acid before and after treatment of the enzyme with UDP-glucose (21), and the isolation of a covalent uridylyl-enzyme complex from the *E. coli* uridylyltransferase (22, 23).

Molecular-weight determinations. The molecular weights of the two enzyme forms were estimated by gel filtration on calibrated columns of Sephacryl S-200 and Sephadex G-200. The elution volumes of the two enzymes were monitored enzymatically and both were recovered from each column with similar V_e/V_0 values. The average native molecular weights for both forms of uridylyltransferase calculated from at least three separate experiments was 67,000 ± 2000. A similar value was also reported for the red cell enzyme by Dale and Popják (8) and Williams (9). However, Banroques *et al.* (11) have recently presented data to show that the native molecule weight of the red cell enzyme was 88,000 as determined by Sephadex G-200 chromatography. This agrees with the value reported earlier by Williams (9) from ultracentrifugation studies in calibrated sucrose density gradients that the molecular weight of the red cell enzyme was about 88,000.

In repeated experiments using Sephacryl S-200, equilibrated in Tris-HCl buffers at pH 8.5 and salt concentrations of (a) zero, (b) 0.1 M NaCl, and (c) 0.3 M NaCl, both the red cell and placental forms are eluted in volumes corresponding to a native molecular weight of about 67,000. Furthermore, we have repeated the gel filtration analysis on a calibrated column of Sephadex G-200, using the buffer system of Banroques *et al.* (11), and both enzyme forms were eluted in the same volume as bovine serum albumin (M_r = 68,000).

Immunological Properties

The ability of the purified red cell uridylyltransferase antibody to precipitate the two forms of uridylyltransferase were compared. Freshly prepared samples of both enzymes, containing equal amounts of uridylyltransferase activity, were incubated at 0°C with increasing amounts of antibody. After centrifugation, the supernatants were assayed for residual uridylyltransferase activity. The results presented in Fig. 5 show the percentage removal of enzyme from solution as a function of antibody concentration.

When the red cell antibody was tested by double immunodiffusion on Ouchterlony plates against the immunizing material and pure placental enzyme, precipitation lines did not form in the agar gel. The failure to demonstrate precipitation by this method was probably due to the low antibody titer since approximately 0.03 ml of antibody solution were required to precipitate 50% of activity, as shown in Fig. 5.

DISCUSSION

A method is described for the purification of milligram quantities of homogeneous galactose-1-phosphate uridylyltransferase from fresh human placental tissue. Although the method is essentially the same as reported previously by Helmer and Williams (10), the introduction of the concanavalin A-Sepharose step improves substantially the overall yield of active enzyme. It should now be possible to obtain sufficient amounts of pure human uridylyltransferase in order to elicit antibodies against the enzyme and use this

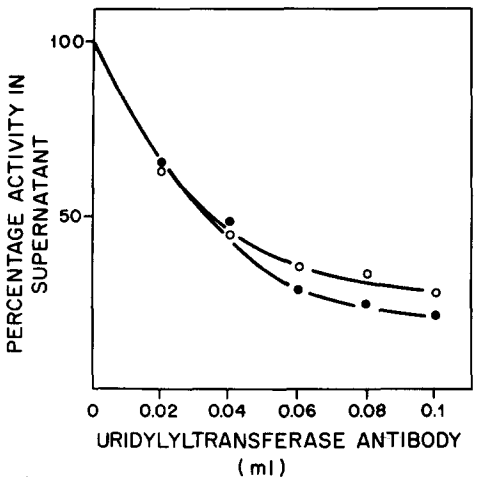

FIG. 5. Immunoprecipitation of human uridylyltransferase by increasing amounts of red cell uridylyltransferase antibody for a red cell (●) and placental sample (○). Replicate aliquots of 0.05 ml containing equal amounts of uridylyltransferase activity (0.12 unit/ml in 0.1 M Tris-HCl buffer, pH 8.5, containing 2 mM DTE) were added to solutions containing 0.15 M NaCl, 2 mM DTE, and varying amounts of control/antibody fractions, as shown in the figure, in a total volume of 0.2 ml. Mixtures were incubated for 3 h at 0°C then centrifuged for 15 min in a Beckman Airfuge using an air pressure of 30 psi (130,000g_{av}). The amount of uridylyltransferase (percentage) remaining in the supernatants was determined.

uridylyltransferase-specific antibody fraction to investigate the molecular nature of "uridylyltransferase-deficiency galactosemia."

One important characteristic of inherited galactosemia is the absence of uridylyltransferase activity in galactosemic red cells (3). Blood samples taken from galactosemics represent, therefore, an important physiological fluid by which this inborn error of metabolism may be investigated in individuals who are either heterozygous carriers or homozygous galactosemics. However, before proceeding further with these investigations, we felt that it was important to establish whether the red cell and placental enzymes shared common properties since, whereas, one form was derived from mature red cells the other was obtained from fetal tissue. From the data presented here, the two forms of the enzyme show a number of similar chemical and physical properties which indicate that the synthesis of both is directed by the same gene locus. For example, the electrophoretic properties of the two enzyme forms indicate that both forms carry similar net charges at pH 8.5 and share a common subunit of mass about 45,000. The initial rate data support also a similarity of mechanism of action except that the substrate K_m values were about two times greater for the red cell enzyme.

The most notable similarity as far as the understanding and elucidation of the galactosemic trait is concerned is the finding that each form of the enzyme is precipitated to about the same extent with antibody prepared against the red cell enzyme. As far as we can ascertain, the different isozyme patterns in the two enzyme preparations will not constitute any serious problem which may relate to the investigation of the galactosemic trait as expressed in the red cells. The two additional active isozymes in the red cell preparations may only reflect the differences which result from isolation of the enzyme from either fresh placental tissue or outdated blood in which the red cell enzyme may be altered as a function of age by deamidation of asparagine and glutamine residues.

There is, however, one property of the placental uridylyltransferase which is not fully understood. In the original report by Helmer and Williams (10), the last step of the purification procedure was chromatography of the enzyme on Sephacryl S-200. Under these conditions, a contaminating protein was eluted ahead of the enzyme. Analysis of this protein by SDS-polyacrylamide gel electrophoresis showed that it was a glycoprotein with a subunit mass of 67,000. When the chromatography was repeated in the presence of UDP-glucose to stabilize the enzyme, the glycoprotein component was eluted in the same volume as the enzyme. From the data presented it was not clear, therefore, whether it was the presence of UDP-glucose per se or the glycoprotein which stabilized the enzyme during chromatography.

In the six preparations of the placental enzyme we have made, the uridylyltransferase obtained from the Sephacryl S-200 column contained a 67,000-dalton glycoprotein component. We have examined the kinetic parameters, heat stability characteristics, and electrophoretic mobility of uridylyltransferase activity on native gels of this S-200 fraction, and find that these properties are virtually indistinguishable from the pure enzyme. It would appear, therefore, that the glycoprotein component is only a contaminant whose chromatographic properties are altered in the presence of UDP-glucose.

Our aim is now to isolate between 5 and 10 mg of pure placental uridylyltransferase by the methods described here, then elicit sufficient quantities of antibodies against the pure enzyme so Ouchterlony experiments can be performed and examine red cell lysates and fibroblasts from galactosemic individuals for the presence of a catalytically inactive form of the uridylyltransferase.

ACKNOWLEDGMENTS

We thank Dr. George Popják for his support and valuable comments in the preparation of this paper. We also thank Mr. Dennis Sullivan for his excellent technical assistance and Ms. Diane F. Hill for performing the Sephadex G-200 experiments.

REFERENCES

1. LELOIR, L. F. (1951) *Arch. Biochem. Biophys.* **33,** 186–190.
2. ISSELBACHER, K. J., ANDERSON, E. P., KURAHASHI, K., AND KALCKAR, H. M. (1956) *Science* **123,** 635–636.
3. INOUYE, T., NADLER, H. L., AND HSIA, D. Y. Y. (1968) *Clin. Chim. Acta* **19,** 169–174.
4. ROGERS, S., HOLTZAPPLE, P. G., MELLMAN, W. T., AND SEGAL, S. (1970) *Metabolism* **19,** 701–708.
5. SEGAL, S., ROGERS, S., AND HOLTZAPPLE, P. G. (1971) *J. Clin. Invest.* **50,** 500–506.
6. SEGAL, S. (1978) in Metabolic Basis of Inherited Disease (Stanbury, J. B., Wyngaarden, J. B., and Fredrickson, D. S., eds.), pp. 160–181, McGraw-Hill, New York.
7. TEDESCO, T. A. (1972) *J. Biol. Chem.* **247,** 6631–6636.
8. DALE, G. L., AND POPJÁK, G. (1976) *J. Biol. Chem.* **251,** 1057–1063.
9. WILLIAMS, V. P. (1978) *Arch. Biochem. Biophys.* **191,** 182–191.
10. HELMER, G. R., JR., AND WILLIAMS, V. P. (1981) *Arch. Biochem. Biophys.* **210,** 573–580.
11. BANROQUES, J., GREGORI, C., AND SHAPIRA, F. (1981) *Biochim. Biophys. Acta* **657,** 374–382.
12. TEDESCO, T. A., AND MELLMAN, W. J. (1971) *Science* **172,** 727–728.
13. BRADFORD, M. M. (1976) *Anal. Biochem.* **72,** 248–254.
14. WEBER, K., PRINGLE, J., AND OSBORN, M. (1972) in Methods in Enzymology (Kustin, K., ed.), Vol. 16, pp. 3–27, Academic Press, New York.
15. SPARKES, M. C., CRIST, M., AND SPARKES, R. S. (1977) *Hum. Genet.* **40,** 93–97.
16. ANDREWS, P. (1965) *Biochem. J.* **96,** 595–606.
17. CLELAND, W. W. (1970) *in* The Enzymes (Boyer, P. D., ed.), Vol. 2, pp. 1–65, Academic Press, New York.
18. MARKUS, H. B., WU, J. W., BOCHES, F. S., TEDESCO, T. A., MELLMAN, W. J., AND KALLEN, R. G. (1977) *J. Biol. Chem.* **252,** 5363–5369.
19. WONG, L.-J., AND FREY, P. A. (1974) *J. Biol. Chem.* **249,** 2322–2324.
20. WONG, L.-J., AND FREY, P. A. (1974) *Biochemistry* **13,** 3889–3894.
21. WILLIAMS, V. P., FRIED, C., AND POPJÁK, G. (1981) *Arch. Biochem. Biophys.* **206,** 353–361.
22. WONG, L-J., SHEU, K. F., LEE, S.-L., AND FREY, P. A. (1977) *Biochemistry* **16,** 1010–1016.
23. YANG, S.-L., AND FREY, P. A. (1979) *Biochemistry* **18,** 2980–2984.