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THE PRESENCE OF ISOZYMES OF HEXOSE-1-PHOSPHATE URIDYLYLTRANSFERASE FROM POSTMORTEM HUMAN LIVER, HEART AND KIDNEY

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SUMMARY

1. The distribution of hexose-1-phosphate uridylyltransferase has been studied in various human tissues obtained at postmortem. The enzyme was found to be present in human liver, heart and kidney.

2. Kinetic experiments were performed on the enzyme from the sources. The apparent K_m for galactose 1-phosphate was determined to be in the range of $2.5 \cdot 10^{-4}$–$3.3 \cdot 10^{-4}$ M for all three tissue homogenates, while the apparent K_m for UDPglucose for liver and kidney homogenates were similar at $4 \cdot 10^{-5}$–$4.5 \cdot 10^{-5}$ M. The V determinations showed the relationship liver > kidney > heart. The enzyme from the three tissues all had similar pH optima.

3. The enzyme from liver, heart and kidney homogenates were compared by electrophoresis on starch gel.

(a) Liver sample travelled as a single band with a mobility similar to that of a sample from erythrocytes.

(b) Heart sample travelled as two bands of activity, one of which was similar to the liver sample.

(c) Kidney sample travelled as five bands of activity, one of which was similar to the liver sample, another similar to the heart sample.

INTRODUCTION

Galactosemia is an inherited disorder resulting from a mutation in the enzyme, UDPglucose:α-D-galactose-1-phosphate uridylyltransferase (EC 2.7.7.12). The enzyme catalyses the reaction:

Galactose 1-phosphate + UDPglucose ⇌ UDPgalactose + glucose 1-phosphate.

Transferase has been reported to be present in various human tissues such as liver, white blood cells, red blood cells and cultured fibroblasts. The enzyme has recently been purified 1000-fold from human postmortem liver and erythrocytes [1].

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Greatly diminished transferase activity has been reported in all these tissues in galactosemia [2–5]. Variant forms of the enzyme have been described on the basis of altered electrophoretic mobility and enzymatic activity. For example, Duarte variant [6, 7], Rennes variant [8], Los Angeles variant [9].

No evidence of transferase isozymes in different tissues of an individual subject, has been published. This present report describes studies of the enzyme in various human tissues and presents evidence of isozymic variation within the tissues.

MATERIALS AND METHODS

Livers, hearts and kidneys were obtained from autopsies performed within 24 h after death, upon children dying of various causes, at the Royal Children's Hospital. The samples were stored frozen at −20 °C. Tissues were thawed, diced and homogenized in 3 vol. of 0.05 M glycine–HCl buffer (pH 8.7) using a glass Duall tissue grinder (Kontes). The homogenate was centrifuged at 6000 × *g* and the supernatant was used for assay.

Transferase assay

Samples were assayed by the use of kinetic assay measuring the NADPH produced from $NADP^+$ [10]. The reaction mixture contained 0.2 μmole galactose 1-phosphate (Sigma); 0.2 μmole UDPglucose (Sigma); 0.4 μmole $MgCl_2$ (Sigma); 0.5 μmoles dithiothreitol (Calbiochem); 0.8 unit phosphoglucomutase (Calbiochem); 0.8 unit glucose-6-phosphate dehydrogenase (Sigma, Type V, Bakers Yeast); 0.2 μmoles $NADP^+$ (Sigma); 0.025 ml 1 M glycine buffer (pH 8.7).

The volume of homogenate varied from 0.005 ml to 0.075 ml into a total final volume of 0.25 ml.

The assay was performed at 25 °C. A baseline was obtained by omitting either galactose 1-phosphate or UDPglucose. On addition of the substrate, the change in absorbance at 340 nm was monitored.

Protein determination

Protein was determined by the method of Lowry et al. [11].

Starch-gel electrophoresis

Horizontal starch-gel electrophoresis was carried out by conventional methods [2]. The starch gel was prepared by heating 45 g of starch (either Sigma, or Lot 171 Electrostarch) in 400 ml of buffer (0.1 M buffer, pH was varied from 6.8 to 8.8 by using phosphate, glycine–NaOH, or Tris–citrate [13]). The gel was poured into perspec trays (17 cm × 21 cm), then cooled for 60 min at 4 °C, prior to use. Small pieces of filter paper (Whatman 3 MM 1 cm × 0.5 cm) were saturated with the sample homogenate. The paper was introduced into the slots made in the gel by a blade. Electrophoresis was carried out at 4 °C, at 6 V/cm, for times varying from 18 to 42 h. Bovine serum albumin, stained with bromophenol blue, was used as a visible measure of the degree of mobility. At the end of electrophoresis, the gel was sliced in half and the surfaces overlayed with a gel which included the reagents required to detect activity.

The reagent consisted of: 0.3 ml 20 mM galactose 1-phosphate; 0.3 ml 20 mM

UDPglucose; 0.6 ml 20 mM $NADP^+$; 2.0 ml 10 mM dithiothreitol; 3.0 ml 1 M glycine buffer (pH 8.7); 0.02 ml phosphoglucomutase; 0.01 ml glucose-6-phosphate dehydrogenase; water was added to make a final volume of 10 ml.

Starch solution was prepared by heating 0.67 g of starch in 10 ml of glycine buffer (0.125 M). When the starch had cooled to 40 °C, it was mixed with an equal volume of the reagents and poured over the exposed cut surface. In some experiments, a solution of 1 % agar was employed instead of the starch in the reagent gel. The gels were covered and incubated at 37 or 25 °C for several hours. Transferase activity was located by viewing the gels under an ultraviolet lamp. In order to locate the bovine serum albumin for photography under these conditions, a fluorescent stain (fluorescein) was added to bovine serum albumin–bromophenol blue complexes.

RESULTS

Transferase activity was detected in homogenates of human liver, kidney and heart, but not in skeletal muscle. Conversion of $NADP^+$ to NADPH occurred in samples from skeletal muscle and heart muscle, even when the substrates UDPglucose or galactose-1-phosphate were omitted, presumably because these tissues contained either glucose 1-phosphate or glucose 6-phosphate. All homogenates were therefore chromatographed on a column of Sephadex G-25 to remove these small molecular weight contaminants. The effluent which contained protein was concentrated to the initial level of protein concentration using an Amicon ultrafilter (UMIO). After this treatment, no reduction in $NADP^+$ was observed in the absence of either glucose 1-phosphate or UDPglucose.

Substrate affinities and maximum velocities

Kinetic experiments were performed on these samples to compare the apparent K_m for both substrates and the maximum velocity (V) of homogenates from liver, kidney and heart. A Lineweaver-Burk plot in which UDPglucose was held constant at $8 \cdot 10^{-4}$ M, while galactose 1-phosphate was varied, is presented in Fig. 1. The three tissues varied in V, but the apparent K_m values all fall in the same range of $2.5 \cdot 10^{-4}$–$3.3 \cdot 10^{-4}$ M. These values for apparent K_m are compared with those obtained by other workers on transferase from various sources in Table I. Rat liver, human leukocytes and human erythrocytes all have apparent K_m values which fall in this range. A comparison of maximum velocity shows the relationship liver > kidney > heart.

Reciprocal plots of the data obtained in the alternative experiment in which galactose 1-phosphate was held constant at $1.6 \cdot 10^{-3}$ M, while UDPglucose was varied, are shown in Fig. 2. The homogenates from liver and kidney had similar apparent K_m of $1.6 \cdot 10^{-5}$ M. These values are compared with other apparent K_m determination of the enzyme from rat liver, human leukocytes and human erythrocytes in Table I. The preparations from human liver and kidney showed an apparent 3-fold greater affinity for the UDPglucose than rat liver, human erythrocytes or human leukocytes, while the homogenate from heart had a 10-fold apparent increase in affinity.

The maximum velocities from the 3 human samples show the same trend as in Fig. 1, V liver > kidney > heart.

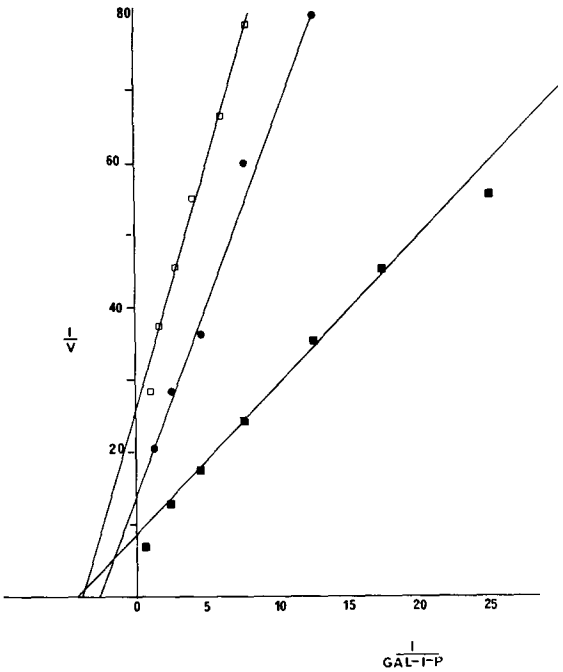

Fig. 1. The effect of varying galactose 1-phosphate concentration on the velocity of transferase catalysed reaction, UDP-glucose was held constant at $8 \cdot 10^{-4}$ M. □—□, heart; ●—●, kidney; ■—■, liver.

TABLE I

APPARENT K_m VALUES OF TRANSFERASE FROM VARIOUS SOURCES

Apparent K_m values which were obtained in Figs 1 and 2 for human liver, kidney and heart samples, are compared with apparent K_m values obtained by other workers for fibroblasts, erythrocytes, leukocytes and rat liver samples.

Source of enzyme	K_m galactose-1-phosphate (M)	K_m UDPglucose (M)
Fibroblasts		
Nadler et al. [14]	$3.2 \cdot 10^{-4}$	$8.0 \cdot 10^{-5}$
Tedesco and Mellman [15]	$1.0 \cdot 10^{-3}$	$1.2 \cdot 10^{-4}$
Erythrocytes		
Tedesco and Mellman [15]	$5.6 \cdot 10^{-4}$	$1.1 \cdot 10^{-4}$
Leukocytes		
Tedesco and Mellman [15]	$1.25 \cdot 10^{-3}$	$1.37 \cdot 10^{-4}$
Rat liver		
Bertoli and Segal [16]	$1.39 \cdot 10^{-4}$	$1.56 \cdot 10^{-4}$
Human liver		
present study	$2.5 \cdot 10^{-4}$	$4.5 \cdot 10^{-5}$
Human kidney		
present study	$3.3 \cdot 10^{-4}$	$4.0 \cdot 10^{-5}$
Human heart		
present study	$3.3 \cdot 10^{-4}$	$1.6 \cdot 10^{-5}$

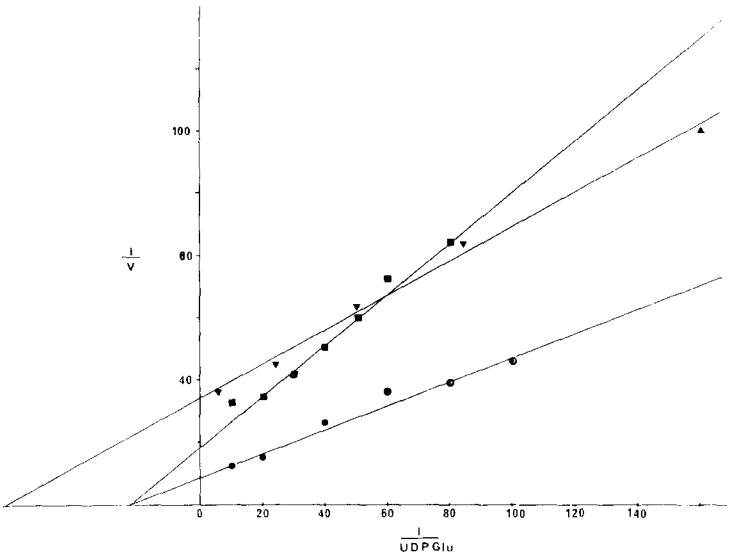

Fig. 2. The effect of varying UDPglucose concentration on the velocity of transferase catalysed reaction, galactose 1-phosphate was held constant at $1.6 \cdot 10^{-3}$ M. ▲—▲, heart; ■—■, kidney; ●—●, liver.

pH optimum

In order to study the effect of pH variation on enzyme activity, the assay was divided into two stages. In the initial stage, the reaction mixture consisted of homogenate, galactose 1-phosphate, UDPglucose, dithiothreitol, and the buffer was varied over the range of pH 6.5–9.0 using Tris–acetate, Tris–HCl and glycine–NaOH. The samples were incubated for 5 min at 37 °C and then boiled. After centrifugation, aliquots of the supernatant were removed and assayed in the second stage for glucose 1-phosphate formed utilizing the phosphoglucomutase, glucose-6-phosphate dehydrogenase system. The results obtained showed that the three human samples analysed had similar pH dependancy; the pH optimum for all three occurred at pH 8.7.

In the course of these studies, it was noted that an increase in enzyme concentration led to a non-linear reaction rate. In fact, when the homogenate volume was increased past a certain stage, the reaction was inhibited completely. Normally, the reaction rate was linear when the sample volume of homogenate was less than 60 μl. An increase of sample volume above this value resulted in an inhibition of enzymatic activity.

Sample stability

It was noted that tissues could be frozen at −20 °C for up to 1 year, with no apparent loss of transferase activity.

Electrophoretic comparison of transferase from human liver, kidney and heart

Fig. 3 shows a typical gel which has been subjected to electrophoresis at pH 8.8. A single band of activity is evident at the liver sample (Slot 1), which has similar mobility to a sample from erythrocyte (not shown). A sample from heart homogenate

is shown in Slot 2. The major band of activity travels towards the anode but has reduced mobility when compared to the liver sample. A minor band of activity was also present and this had a mobility similar to the liver sample. When the electrophoresis was performed at pH 8.0, the liver sample moved towards the anode, but the activity in the heart sample remained at the origin.

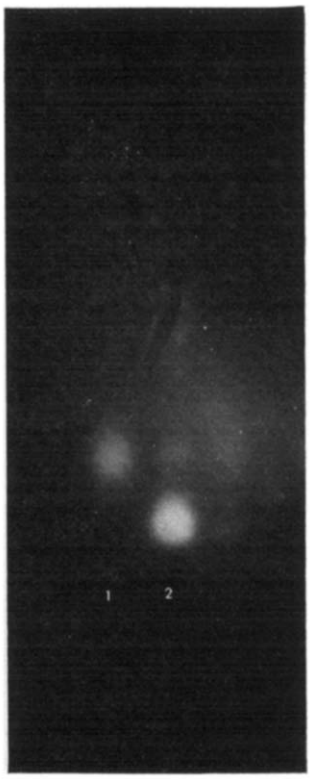

Fig. 3. Starch-gel electrophoresis of transferase from human liver and heart. The experiment was performed in Tris–citrate buffer (pH 8.8) at 250 V for 40 h, using electrostarch. Slot 1, liver; Slot 2, heart.

A mixture of the heart and liver enzyme was prepared by mixing equal volumes of the samples and then freezing and thawing the mixture in an attempt to form a hybrid molecule. Fig. 4 shows the result of such an experiment. The gel was run at pH 8.8; bovine serum albumin markers are evident at the top of the gel. Liver (Slot 2) and heart (Slot 3) travelled as in Fig. 3. The mixture of the 2 samples (Slot 4) travelled as 2 distant bands with no evidence of hybrid formation. Each of the bands had the same electrophoretic mobility as the parent enzyme.

Kidney samples are also shown on the gel in Fig. 4 (Slot 6) and compared with the mobility of another heart sample (Slot 7). A different distribution of the enzyme from kidney is evident. The sample runs as 2 distinct bands, which under these conditions appears to be very similar to those seen in the mixture of heart and liver (Slot 4).

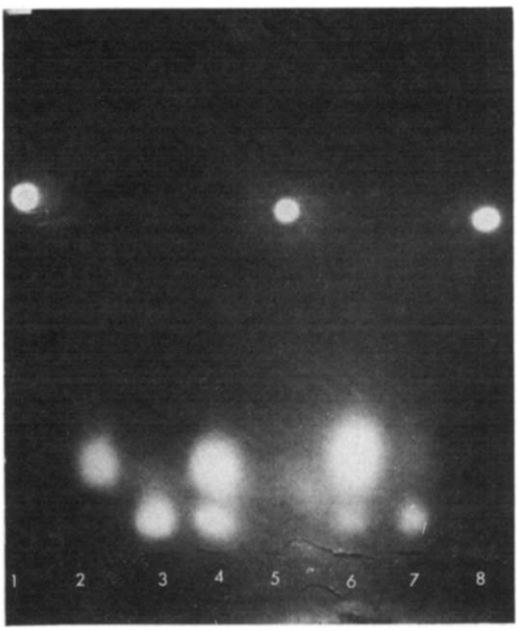

Fig. 4. Starch-gel electrophoresis of transferase from human liver, heart and kidney. The experiment was performed in Tris–citrate buffer (pH 8.8) at 250 V for 18 h, using electrostarch. Slot 1, marker; Slot 2, liver; Slot 3, heart; Slot 4, liver–heart mixture; Slot 5, marker; Slot 6, kidney; Slot 7, heart; Slot 8, marker.

When the electrophoresis was extended to run over a longer period at pH 8.8 employing electrostarch (Fig. 5), the samples from kidney appeared to separate into multiple bands (Slots 6–8) unlike the liver (Slot 2) and heart (Slots 3 and 4). The 3 different kidney samples show that there may be 5 bands of activity. This pattern was resolved by running a prolonged electrophoretograph at pH 8.8 (Fig. 6). The 2 kidney samples were run (Slots 3 and 4) and the sample in Slot 3 shows 5 well separated bands, compared to the single band obtained from heart (Slot 1). The kidney appears to have 1 band with electrophoretic mobility the same as that of the liver enzyme, and another of mobility similar to heart sample, a third band runs further towards the anode than the liver enzyme; a fourth runs to a position intermediate between liver and heart enzymes, and the fifth band remains at the origin.

DISCUSSION

The results presented above, demonstrate that transferase is more widely distributed in human tissues than had been previously reported and that differences exist between the forms of the enzyme found in different tissues.

Previous evidence indicating that the enzyme is composed of subunits can be summarized:

(i) Normal haemolysates show a single band of transferase activity when electrophoresed at either pH 7.0 [6], or pH 8.8 [7]. The Duarte variant enzyme from haemolysates travels as a single band of activity with altered mobility at pH 7.0 [6],

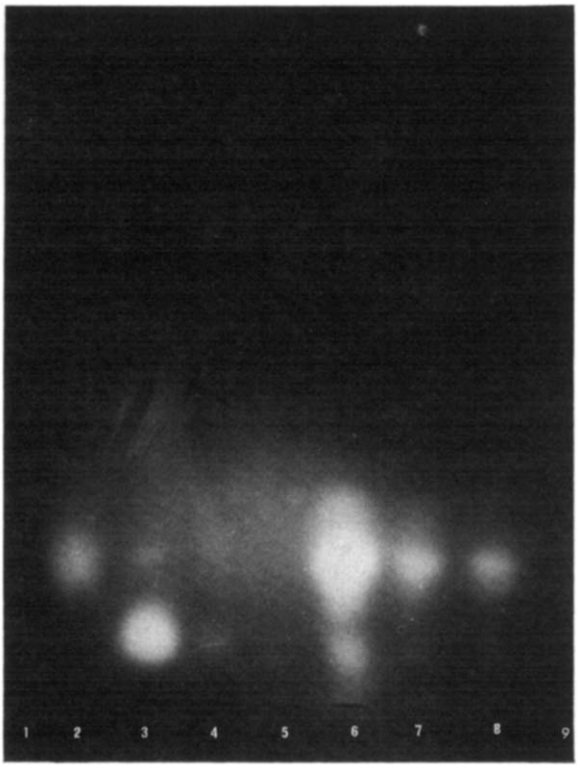

Fig. 5. Starch-gel electrophoresis of transferase from human liver, heart and kidney. The experiment was performed in Tris–citrate buffer (pH 8.8) at 250 V for 20 h, using electrostarch. Slot 1, marker; Slot 2, liver; Slot 3, heart; Slot 4, heart; Slot 5, marker; Slot 6, kidney; Slot 7, kidney; Slot 8, kidney; Slot 9, marker.

but this activity has been resolved into 3 bands by electrophoresis at pH 8.8 [7]. Another variant termed Los Angeles, can be resolved into 3 bands of activity at pH 8.8 [9].

(ii) Tedesco [1] has also presented evidence that the enzyme is probably composed of sub-units. Sodium dodecylsulfate–polyacrylamide-gel electrophoresis of the purified erythrocyte enzyme showed a single band of protein of molecular weight 31 000. The molecular weight of transferase was also estimated by gel filtration and a value of 90 000 was obtained. Tedesco postulated from this data a trimeric structure for the enzyme, but allowed for the possibility of a tetrameric structure.

The different electrophoretic patterns observed for the isozymes from liver, heart and kidney may be explained if we assume that the enzyme has a dimeric form.

The enzyme may exist in liver in the form of α,α which is similar to erythrocyte samples. The heart samples may be determined at a different locus to produce the polypeptides β,β of different electrophoretic mobility. In the experiment reported no hybrid molecule α,β could be formed by freezing and thawing, as has been done with peptidase A [17]. The minor band of activity present in heart samples may be due to contaminating amounts of α,α present in erythrocytes.

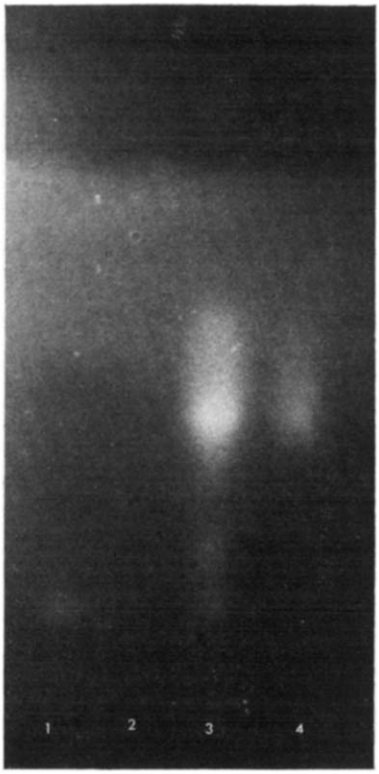

Fig. 6. Starch-gel electrophoresis of transferase from human heart and kidney. The experiment was performed in Tris–citrate buffer (pH 8.8) at 250 V for 42 h, using electrostarch. Slot 1, heart; Slot 2, marker (run off); Slot 3, kidney; Slot 4, kidney.

Kidney presumably has a locus which produces chains of $\alpha,\alpha\ \beta,\beta\ \lambda,\lambda$ and also some hybrid forms of intermediate mobility.

The studies so far performed, give no indication of any major kinetic differences present in these enzymes from kidney, liver and heart.

It would be of great interest to examine heart and kidney samples from patients with galactosemia. If the inherited disorder of galactosemia is due to a mutation in the transferase from both liver and erythrocyte, one might expect that the isozyme from heart and kidney would still continue to function. It may be important to study samples of tissues which contain activity in developmental stages, as the enzyme from heart and kidney may not be functional in neonatal stages of development, which is the crisis period for galactosemia.

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