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HUMAN INTESTINAL LACTASE-PHLORIZIN HYDROLASE: ISOLATION AND PREPARATION OF A SPECIFIC ANTISERUM

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Summary -- Human intestinal lactase-phlorizin hydrolase (lactase) was selectively isolated with monospecific polyclonal antibodies to rat lactase. In addition to their immunologic similarities indicated by this isolation, human and rat lactase have similar kinetic characteristics but different subunit structure when analyzed by gel electrophoresis under reducing conditions. Rabbits immunized by injecting human lactase complexed with anti-rat lactase produced specific antibodies to human lactase that exhibited little cross-reactivity to the rat enzyme. The simple single-step procedure allows isolation of human lactase in high purity from small biologic samples and preparation of specific antisera to the human enzyme.

Lactase-phlorizin hydrolase is the predominant digestive carbohydrase of the intestinal brush border membrane in mammalian neonates where it cleaves dietary lactose to the absorbable monosaccharides glucose and galactose. LPH undergoes rapid decline accompanying the process of weaning(1), with the resulting hypolactasia the probable cause of lactose intolerance among most human adult populations of the world(2,3). The mechanism(s) of maturational LPH decline, although of great nutritional and clinical significance to man, remains unclear. Various mechanisms have been proposed including a) shortened enterocyte life span without suppressed synthesis of LPH (4,5), b) decreased synthesis of LPH(7), c) altered processing(8) and d) combination of above(9).

The study of human LPH has been limited by the unavailability of human intestinal tissue and the ability to isolate the enzyme from small amounts of tissue. Purification of human LPH has been achieved by multi-step immunoabsorption chromatography(10) and more recently by a monoclonal antibody technique(11). The use of either isolation procedure may be restricted in many laboratories by the availability of polyclonal or monoclonal antibodies raised to the human enzyme.

In the present report, we describe a single-step procedure for isolating human LPH based on its selective immunoprecipitation by anti-rat LPH, and the

Abbreviations used: lactase-phlorizin-hydrolase, LPH; sucrase-isomaltase, SI; maltase-glucoamylase, MG; phosphate buffered saline, PBS; p-hydroxymercuribenzoate, PHMB; phenylmethylsulfonyl fluoride, PMSF.

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preparation of specific antiserum to the human enzyme. Since LPH has been widely studied in the rat and an antiserum to the rat enzyme is available to numerous laboratories, the isolation of human LPH by anti-rat LPH should allow broader participation in the study of the human enzyme.

MATERIALS AND METHODS

Materials - Cellobiose, glucose oxidase, horseradish peroxidase and papain were obtained from Sigma Chemical Company (St. Louis, MO). All reagents for SDS-PAGE were from Bio-Rad Laboratories (Richmond, CA). Suckling Wistar rats were purchased from Simonsen Labs (Gilroy, CA) and New Zealand white rabbits from Nitabell (Hayward, CA).

Preparation of Human and Rat Intestine for Analyses - Human intestine was obtained at autopsy from two adults and two infants. After flushing the intestine with cold saline, the mucosa was scraped from the jejunum and homogenized in 9 volumes of 10 mM sodium phosphate, 5 mM Tris, 1 mM EDTA, pH 6.0, containing 0.002% Triton X-100. The homogenate was centrifuged for 30 minutes at 100,000 x g and membrane-bound LPH was solubilized with 0.5% Triton X-100 or by papain digestion (6 mg papain plus 0.12 mg Cysteine-HCl per gm intestine). Solubilized and membrane-bound proteins were separated by centrifugation at 100,000 x g for 1 h.

Enzyme Assays - Lactase activity was assayed using 150 mM lactose or 15 mM cellobiose as substrate as previously described(5). Sucrase-isomaltase[1] and maltase-glucoamylase[1] activities were determined in the presence of 30 mM sucrose and 15 mM maltose respectively(5). Enzyme activity units are defined as μmols of substrate hydrolyzed per minute.

Antisera Preparation - Polyclonal antibodies to both rat and human LPH were raised in New Zealand white rabbits as previously described(5). The rabbits were injected subcutaneously with either 25 μg of rat LPH or 50 μg of human LPH complexed with anti-rat LPH.

Immunotitrations - Immunotitrations were performed by incubating solubilized LPH with anti-rat or anti-human LPH antisera for 18 hours at 4°C or 1 hour at 37°C in 50 mM NaCl, 10 mM sodium phosphate, pH 6.0, and 0.25% Triton X-100. Titration curves were obtained by reacting 10 mU of LPH activity with increasing amounts of antiserum. Immunoprecipitates formed were sedimented at 12,000 rpm for 5 minutes (microfuge) and the supernatants were assayed for remaining lactase activity.

Immunoprecipitation and gel electrophoresis - LPH was isolated by immunoprecipitation from Triton-solubilized intestinal membrane pellets from an adult human and an adult, post-weaned rat. The solubilized proteins were incubated with anti-human or anti-rat LPH for 2 hr at 37°C in PBS containing 0.25% Triton X-100 and protease inhibitors (1 mM PMSF, 1 mM EDTA, 0.1 mM PHMB). Fixed Staphylococcus aureus cells (0.03 ml of a 10% suspension in PBS) were added to bind immune complexes, and the mixture was incubated for 30 min at 4°C. The immune complexes were collected by centrifugation, washed 3 times in PBS and 0.25% Triton X-100, and resuspended in Laemmli sample buffer (62.5 mM Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 5% 2-mercaptoethanol). The samples were heated at 100°C for 3 min and analyzed by SDS-PAGE using a 4% stacking gel and a 7.5% separating gel according to the method of Laemmli(6). The gel was stained with Coomassie Brilliant Blue G250. The molecular weight standards were myosin (200 kDa), ß-galactosidase (116 kDa), phosphorylase B (97.4 kDa), bovine serum albumin (66.2 kDa), and ovalbumin (42.7 kDa).

RESULTS

Immunoprecipitation Studies - The rabbit antiserum to rat LPH exhibited strong cross-reactivity to human LPH. Figure 1 compares immunotitration

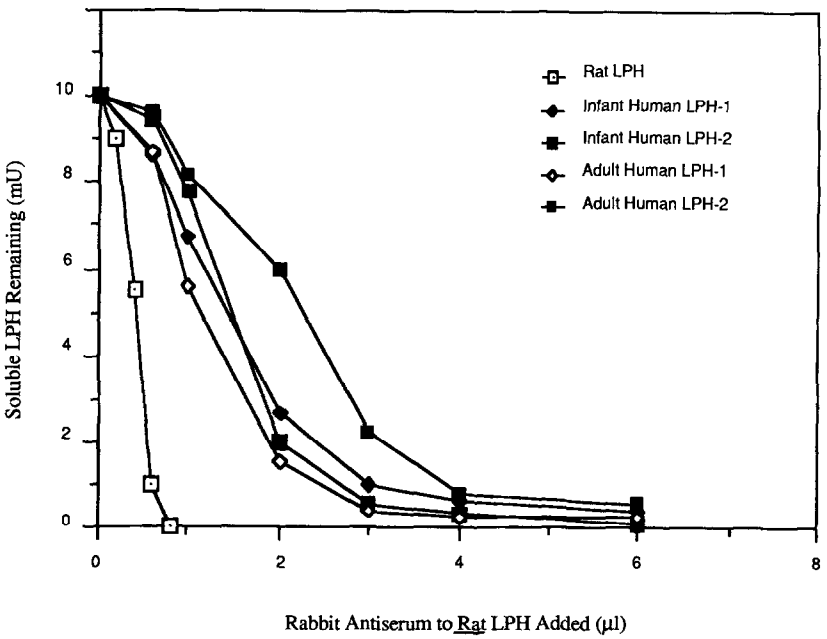

Figure 1. Comparative immunotitration curves of rat, human infant and human adult intestinal LPH by rabbit antiserum prepared to rat LPH. Aliquots of Triton X-100 extracts of rat, 2 infant human and 2 adult human intestines containing 10 mU of LPH were reacted overnight at 0°C with increasing amounts of anti-rat LPH. Resulting immunoprecipitates were sedimented and the remaining LPH in solution determined.

curves of rat LPH and infant and adult human LPH when titered against anti-rat LPH. The immunotitration curves show that similar amounts of anti-rat LPH precipitate infant and adult human LPH. However, complete precipitation of the human enzyme required 3 to 4-times greater amounts of anti-rat LPH than required for the rat enzyme. Interestingly, rabbit antisera to rat sucrase-isomaltase or rat maltase-glucoamylase did not precipitate human sucrase-isomaltase or maltase-glucoamylase, suggesting there is little antigenic similarity between the rat enzyme and its analogous human enzyme (data not shown). In contrast to anti-rat LPH which quantitatively immunoprecipitates both rat and human LPH, as shown in Fig 1, the anti-human LPH preparation quantitatively immunoprecipitates human LPH but not rat LPH (Fig.2).

Since both Triton X-100 and papain are commonly used to solubilize membrane-bound LPH, the immunoprecipitation of human LPH solubilized by these agents was compared. As shown in Figure 3, both Triton and papain solubilized human LPH was quantitatively immunoprecipitated by similar anti-human LPH concentrations.

Analysis of Human and Rat LPH by SDS-PAGE - After solubilization of human intestine with Triton, human LPH was isolated by immunoprecipitation and examined by SDS-PAGE under reducing conditions. A principal 160 kDa protein

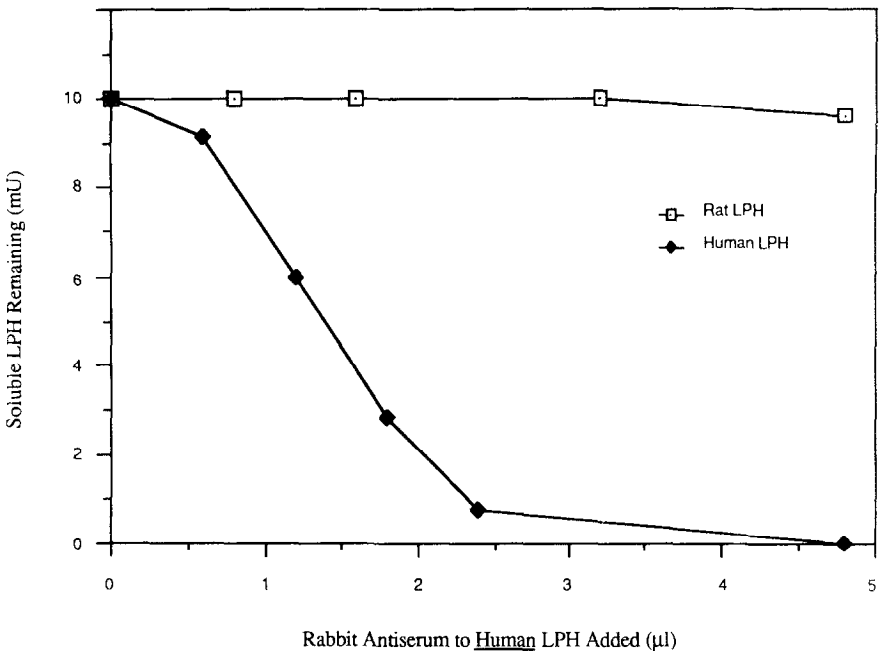

Figure 2. Comparative immunotitration curves of rat and human LPH by rabbit antiserum prepared to human LPH. Aliquots of Triton X-100 extracts of rat and human intestine containing 10 mU of LPH were reacted overnight at 0°C with increasing amounts of anti-human LPH. Resulting immunoprecipitates were sedimented and the remaining LPH in solution determined.

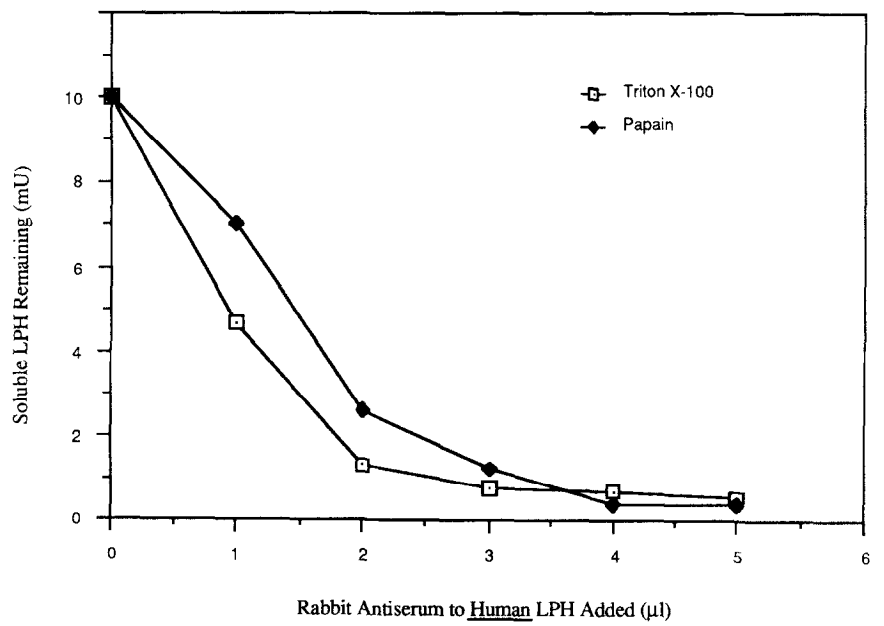

Figure 3. Comparative immunotitration curves of human LPH extracted in Triton X-100 or by papain and isolated by rabbit antiserum prepared to human LPH. Aliquots of Triton X-100 and papain extracts of human intestine containing 10 mU LPH were reacted overnight at 0°C with increasing amounts of anti-human LPH. Resulting immunoprecipitates were sedimented and the remaining LPH in solution determined.

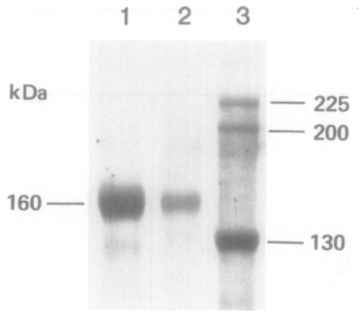

Figure 4. SDS-electrophoresis of LPH immunoprecipitates from human and rat intestine. Lanes 1 and 2 show human LPH immunoprecipitated with anti-human and anti-rat LPH, respectively. Lane 3 shows rat LPH immunoprecipitated by anti-rat LPH. Components of the resulting immunoprecipitates were separated on a 7.5% polyacrylamide gel and stained with Coomassie Blue. The mass of each major LPH species is given.

band was seen after immunoprecipitation using either anti-human or anti-rat LPH antisera (Fig.4, lanes 1 and 2). In contrast, rat LPH analyzed in identical fashion and isolated using anti-rat LPH revealed three molecular forms: a major 130 kDa species and minor bands of 225 and 200 kDa (Fig. 4, lane 3).

Selective Immunoprecipitation of Human LPH by Anti-Rat and Anti-Human LPH- Table 1 compares disaccharidase activities in Triton-solubilized human intestine after immunoprecipitation of LPH by anti-rat LPH and anti-human LPH. A constant-titered excess of either antibody was added to Triton-solubilized human intestinal membranes containing the indicated initial activities of LPH, SI, and MG. After incubation overnight at 4°C, the samples were mixed and assayed prior to and following removal of the immunoprecipitates by

TABLE 1. Selective Immunoprecipitation of Human LPH by Anti-Rat LPH and Anti-Human LPH

Enzyme	Initial Activity (mU)[1]	Activity (mU) in the Presence of Anti-LPH		Activity (mU) Remaining After Removal of Immunoprecipitates	
		Anti-Rat	Anti-Human	Anti-Rat	Anti-Human
LPH	6.7	6.7	1.9	0	0
SI	28.2	27.2	27.6	28.7	27.6
MG	223	220	216	216	201

[1] Enzyme activities in a crude Triton X-100 extract of human intestine.

TABLE 2. Comparative Kinetic and Inhibition Properties of Human and Rat LPH

	Human LPH	Rat LPH
Apparent K_m (mM)		
Lactose	12	16
Cellobiose	1.2	1.5
Apparent K_i (mM)[a]		
Tris	1.6	1.3
Imidazole	0.52	0.52
Colchicine	53	3.5

[a] K_i measurements were performed in the presence of 3 mM Tris, 2 mM Imidazole or 3 mM Colchicine and increasing concentrations of lactose as substrate.

centrifugation. All three enzymes retained complete activity in the presence of anti-rat LPH. In contrast, approximately 30% of the initial LPH activity was observed when human LPH was complexed with anti-human LPH. No LPH activity remained in the supernates after removing the immune complexes by centrifugation. However, there was little or no loss of SI and MG activity. These data indicate that both the anti-rat and anti-human LPH selectively recognize human LPH.

Kinetic Properties of Rat and Human LPH - As shown in Table 2, both human and rat LPH have similar affinities (K_m) for lactose or cellobiose. K_i measurements showed similar inhibition with Tris and Imidazole. However, human and rat LPH differ in their relative inhibition by colchicine, confirming findings described in an earlier report(12).

DISCUSSION

The present study describes a single-step procedure for the isolation of human LPH using a monospecific, polyclonal antiserum to rat LPH and the production of a specific antiserum for human LPH. Immunotitration studies showed that anti-rat LPH recognized both infant and adult human LPH. Both anti-human LPH and anti-rat LPH selectively recognized human LPH but not other disaccharidases. Human and rat LPH exhibited similar kinetic properties and inhibition with Tris and imidazole but differed in subunit structures.

The antigenic similarities described for human and rat LPH in this study are supported by a recent finding that the cDNA sequences of these proteins show 79% nucleotide homology (16). In addition, the primary amino acid structures of human and rabbit LPH show 85% homology, as deduced from their

cDNA sequences (13). Although the cDNA and presumably amino acid sequences of human and rat LPH are similar, they differ in subunit structure (Fig. 4). This and previous studies of human LPH indicate that the mature brush border form has a molecular mass of 160 kDa (13,17). In contrast, analysis of the structure of rat LPH revealed several protein subunits, the most predominant being a 130 kDa species (5,7).

Both anti-human and anti-rat LPH antisera recognized human LPH. SDS-PAGE analysis of human LPH isolated by either antibody revealed an identical molecular form of 160 kDa. In contrast, the anti-human LPH obtained by immunizing rabbits with human LPH complexed with anti-rat LPH showed little cross-reactivity with rat LPH (Fig.2). This finding suggests that the antibodies produced using the procedure described in this report are directed to a set of epitopes on the human LPH molecule that are not recognized by anti-rat LPH. The finding of similar immunotitration curves among infant and adult human LPH samples suggests little antigenic variability between the infant and adult enzymes.

The strong homology between the antigenic and structural features of human and rat LPH are reflected further in their similar kinetic properties and inhibition characteristics. Both enzymes have similar affinities for lactose and cellobiose and similar inhibition with Tris and imidazole. However, the rat enzyme is more sensitive to inhibition with colchicine. This difference has been described previously (12).

Despite the restricted availability of human intestinal tissue, important information on the biosynthesis, precursor forms, and primary structure of human LPH has been accumulating (11,13,14). However, major areas of study such as characterization and regulation of intracellular synthesis and maturational processing of the enzyme, remain largely unexplored. The simple single-step procedure described in this report results in production of polyclonal antisera highly specific for human LPH which can be used for the isolation and study of the various antigenic forms of the enzyme at high sensitivity. This should permit optimal utilization of the limited amounts of human intestinal tissue available for investigation as well as encourage wider participation in the study of human LPH.

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